BIOCHEMISTRY AND MOLECULAR BIOLOGY

(DBT02)

(PG DIPLOMA)

ACHARYA NAGARJUNA UNIVERSITY

CENTRE FOR DISTANCE EDUCATION

NAGARJUNA NAGAR,

GUNTUR

ANDHRA PRADESH

Lesson 2.1.1

CARBOHYDRATES

Objective

Carbohydrates are the compounds containing carbon, hydrogen and oxygen constituting an important class of food molecules of many animals and man. In this chapter the classification and properties of all types of carbohydrates were explained.

2.1.1.1 Introduction

The name carbohydrates or “Saccharides” is derived from a Greek Work Sacharan meaning sugar. Carbohydrates are essential components of all living organisms and constitute the most abundant class of biological molecules.

They are the precursors for the synthesis of all organic compounds found in plants and animals. Plants synthesize carbohydrates by photosynthesis using CO_2 and water. Plants use carbohydrates as the precursors for the synthesis of proteins, lipids and other organic compounds. Animals obtain their carbohydrates from plants.

They are the main components of food, they are present in grains, tubers, roots, fruits, milk, egg, meat etc. Carbohydrates are used as raw materials in textiles, paper, plastics, fermentation industries and in alcohol production.

Cellulose of wood and paper, starches present in cereals, roots and tubers, cane sugar and milk sugar are all examples of carbohydrates. Animal tissues contain glycogen and body fluids contain glucose both of which are carbohydrates.

2.1.1.2 Definition

Chemically carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones or substances that yield such compounds upon hydrolysis.

The term carbohydrate literally means "Carbon hydrate" or hydrates of carbon in which the ratio of carbon to hydrogen and to oxygen atoms is 1:2:1. Hydrogen and oxygen are combined in the same proportion as in water (H_2O). The general formula $C_n(H_2O)_n$ represents most of the carbohydrates. For example glucose has the molecular formula $C_6H_{12}O_6$.

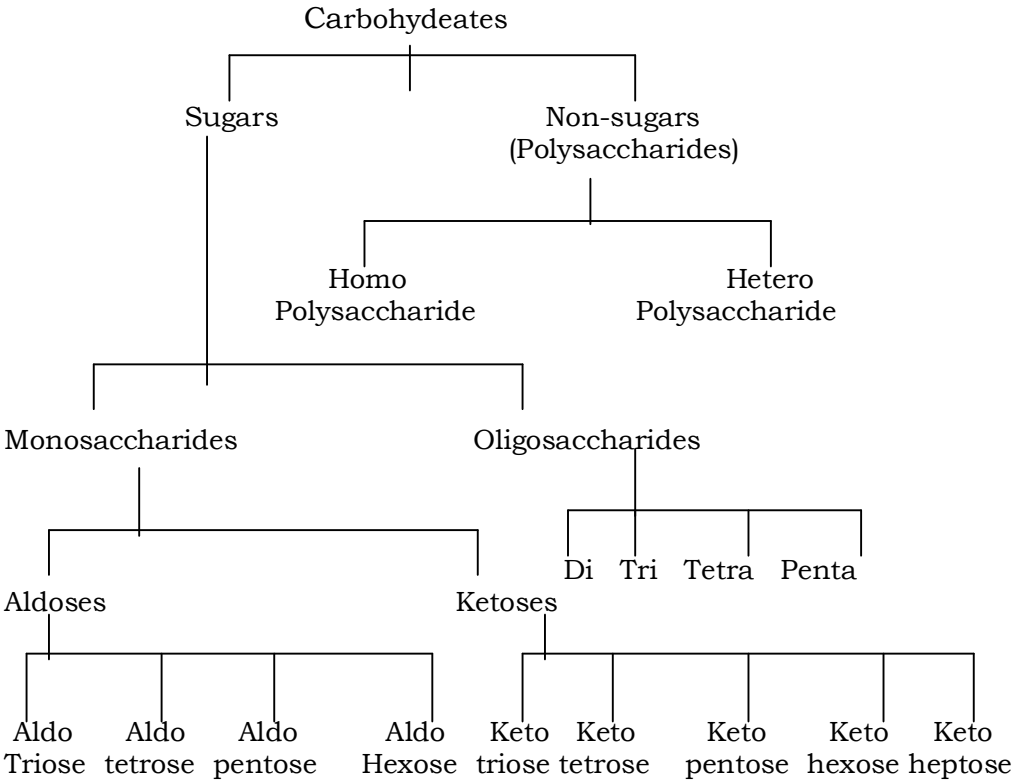
A characteristic feature of carbohydrates is the occurrence in them of a carbonyl group (aldo or keto) and at least two hydroxyl groups.Therefore glycoldehyde and dihydroxyacetone may be referred as simplest carbohydrates.

2.1.1.3 Classfication of Carbohydates

Carbohydrates are classified into three groups :

1. Monosaccharides
2. Oligosaccharides
3. Polysaccharides

Monosaccharides and oligosaccharides are called sugars due to their sweet taste polysaccharides are called non sugars as they are tastless.



2.1.1.4 Monosaccharides

Monosaccharides are defined as polyhydroxy aldehydes or ketones which cannot be further hydrolysed to simple sugars. (Mano – single, Saccharide – sugar).

Monosaccharides are simple sugars. They are sweet in taste.

They contain 3 to 10 carbon atoms, 2 or more hydroxyl (OH) groups and one aldehyde (CHO) or one ketone (CO) group.

They are represented by the general formula $(CH_2O)_n$.

The most important property of monosacharides is that they readily reduce oxidising agents. Glucose and other sugars capable of reducing oxidising agents are called reducing sugars so monosaccharides are reducing agents.

Based on the number of carbon atoms present the monosaccharides are named as trioses, (3c) tetroses, (4c) pentoses, (5c) hexoses (6c) and so on.

Based on the type of carboxyl group present, the monosaccharides are divided into two groups namely aldoses and ketoses.

Aldoses have an aldehyde group (CHO) and ketoses have a ketone group.

Classification of Monosaccharides

Monosaccharides are classified in two ways. First, based on the number of carbon atoms present in them, monosaccharides are classified into

Trioses are monosaccharides containing 3 carbon atoms $C_3H_6O_3$ The triose may contain an aldehyde or a ketone group, the triose containing a ketone group is called a ketotriose or triulose. The triose containing an aldehyde group is called aldotriose.

The common aldose occurring in organisms is called glucuose or glyceroldehyde.

The common ketose occurring in organisms is called Dihydroxy acetone.

Glyceroldehyde and dihydroxy acetone occur as intermediate products in the metabolism of glucose.

They are also found to be precursors of glycerol

Asymmetric Compound

The carbon atom to which four different atoms or groups are attached is called asymmetric carbon. The compound containing an asymmetric carbon atom is called an asymmetric compound. In aldotriose of glycerose, four different atoms or groups are attached to the carbon atom number 2, the four different groups are CHO, CH_2OH, H and OH. So glyceraldehydes is an asymmetric compound.

Isomers

Isomers are different compounds with the same molecular formula and the phenomenon is called isomerism. The presence of asymmetric carbon atoms in carbohydrates produces isomers.

When a compound contains an asymmetric carbon atom, the compound exists in two isomers. When a compound contains 2 asymmetric carbon atoms, it exists in 4 isomers.

The number of possible isomers of any given compound thus depends on the number of asymmetric carbon atoms present in the molecule.

The total number of isomers of a compound will be equal to 2^n where "n" represents the number of asymmetric carbon atoms present in the molecule.

As glyceraldehyde has one asymmetric carbon atom, it exists in two isomers ($2^n = 2$). In one form H atom at carbon 2 is projected to the left side and OH group to the right side. This molecule is called D-form or D-glycerose. In the other form H-atom is projected to the right side ad the OH group to the left side. This molecule is called L-form or L-glycerose, D-glycerose is the mirror image of L-glycerose or vice versa.

In the case of ketotriose, there is no asymmetric carbon atom. So dihydroxy acetone has no optical isomer. So dihydroxy acetone is optically inactive.

2. Tetroses

Tetroses are monosaccharides containing 4 carbon atoms. The molecular formula of a tetrose $C_4H_8O_4$. The tetroses may contain an aldehyde group or a ketone group called an aldotetrose or ketotetrose respectively. The common aldotetrose occurring in organisms in erythrose and the common tetrulose is erythrylose.

Erythrose has two asymmetric carbon atoms namely carbon atom number 2 and 3. So erythrose exists in four isomers namely D-erythrose, L-erythrose, D-throse and L-throse. In keto tetrose the carbon atom number 3 is asymmetric. So it has 2 isomers namely D-erythrulose and l-erythrulose.

3. Pentoses

Pentoses are the monosaccharides containing 5 carbon atoms. The molecular formula of pentose is $C_5H_{10}O_5$. The pentose may contain an aldehyde group or a ketone group. The pentose containing an aldehyde group is called an aedopentose and that containing a ketone group is called a ketopentose or pentulose.

Pentoses are important components of nucleic acids both in animal and plant kingdom. The biologically important pentoses are ribose, deoxyribose, L-xylulose, xylose ribulose etc.

Ribose sugar occurs in RNA and deoxy ribose sugar occur in DNA.

The aldopentose contains 3 asymmetric carbon atoms, so it can exist in 8 isomers. Th ketopentose contain 2 asymmetric carbon atoms at positions 3 and 4. So they exist in 4 isomeric forms ($2^4 = 2^2 = 2x2=4$).

4. Hexoses

Hexoses are monosaccharides containing 6 carbon atoms. The molecular formula of hexose is $C_6H_{12}O_6$. The hexose may contain an aldehyde group or a ketone group the hexose containing an aldehyde group is called an aldohexose and that containing a ketone group is called a ketohexose .

Hexoses are biologically important sugars.The common hexoses are glucose, galactose, mannose and fructose.

The aldohexoses contain 4 asymmetric carbon atoms at positions 2,3,4 and 5. Hence aldohexoses exist in 16 isomericforms ($2^4 = 2^4 = 16$).

The ketohexoses contain 3 asymmetric carbon atoms at positions 3,4 and 5. Hence ketohexoses exist in 8 isomeric forms ($2^4 = 2^3 = 8$).

Epimers

Two sugars which differ from one another only in the configuration of around one specific carbon are called epimers of each other. Thus D-glucose and D-mannose are epimers with respect to carbon atom number 2. Similarly D-glucose and D-galactose are epimers with respect to carbon atom number 4.

Structue of Monosaccharides

The monosaccharides may be represented by two structures.

1. Straight chain structure or open chain structure.
2. Cyclic structure or ring structure.

1. Straight chain structure of glucose

In straight chain structure, the 6 carbon atoms of glucose are arranged in a straight line. It is also called open chain structure because the two ends remain separate and they are not linked.

Fitting and Bayer proposed a straight chain structure. According to this the aldehyde group is at one end, CH_2OH group at the other end and four CHOH groups in between.

Fisher proposed another straight chain structure. The straight chain structure proposed by fisher is called Fisher's projection formula. According to Fisher the aldehyde group is placed at one end and the remaining carbon atoms

are arranged one behind the other the carbon atom of the aldehyde group is the carbon atom number 1 and carbon atom containing H_2OH is the carbon atom number 6 (in hexoses).

Fischer's straight chains structure explains the existence of 4 symmetric carbon atoms 2,3,4 and 5. As there are 4 asymmetric carbons, glucose exists in 16 isomers.

The characteristic feature of straight chain structure is the presence of free aldehyde group.

Objections to straight chain structure

The open chain structure stresses the presence of a free aldehyde group in glucose. But chemical reactions indicate that glucose does not contain free aldehyde group.

1. Glucose does not restore schiff's reagent colour in schiff's test. When aldehyde is added to this solution, the pink colour is restored. But the addition of glucose does not restore the colour.

2. Glucose does not form additive compounds with sodium bisulphate and ammonia, where as aldehydes do form such additive compounds.

3. **Mutarotation :** A freshly prepared solution of glucose crystallized from water or alcohol at low temperatures exhibits a specific rotation of $+112^O$. But a solution of glucose crystallized from water above 98^O or from boiling pyridine has a specific rotation of $+19^O$ only. When either of the solutions is kept for some time, the rotation gradually changes to 52.5^O and remains constant there. This change in optical rotation is known as mutarotation. It is explained by the existence of 2 optical isomers of glucose namely α-D-glucose with $+112^O$ rotation and β-D-glucose with $+19^O$ rotation this is made, by the –CHO group taking on a H from the fourth or fifth carbon and forming a ring structure instead of a straight chain.

The above reactions clearly show that glucose does not exist in straight chain structure. The straight chain structures are convenient to write, but they do not represent the actual molecular structure of the sugars. However such structures are correct for the trioses and tetroses. But pentoses and hexoses usually occur in solution as cyclic or ring structures, in which the carboxyl group is not free.

2. Cyclic structure or ring structure

In cyclic structure, the atoms are arranged in the form of a ring. Haworth devised the ring structure. Hence the ring structure is referred to as Haworth's projection formula. The sugar molecules exist in two types of rings. They are furanose ring and pyranose ring.

1. Furanose ring

Furanose is a 5-membered ring. It is a pentagonal ring. It resembles the ring of a compound called furan and hence is called furanose ring. Sugars containing the furanose ring are called furanoses. The furanose ring is formed of 4 carbon atoms and one oxygen atom.

2. Pyranose ring

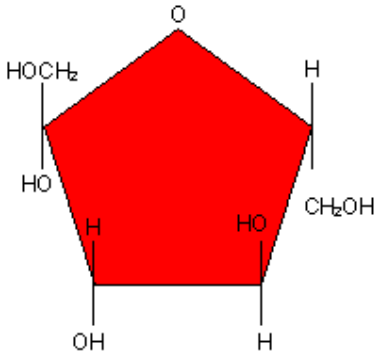
Pyranose is a 6-membered ring The ring resembles the ring of a compound called pyran. The sugars containing pyranose ring are called pyranose sugars. It is more common among hexoses. It is formed of 5 carbon atoms and one oxygen atom.

Anomers are isomers differing in configuration of a particular carbon atom alone.

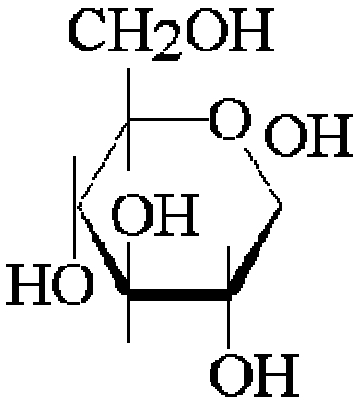
An anomer is a stereo - isomer of glucose. In glucose the 1^{st} carbon atom next to the ring oxygen is asymmetric and it is called anomeric carbon atom. This asymmetric carbon atom produces two forms of glucose namely α and β forms. They are called anomers.

The α and β glucopyranoses differ only in the configuration H and OH of the carbon atom number 1. So the two forms of glucose are called anomers and the first carbon atom is called anomeric carbon atom. When the hydroxyl group of the carbon atom number 1 is attached below the plane of the ring, the pyranose glucose is called α-glucopyranose, when the hydroxyl group is alone the plane of the ring in the C-1, the pyranose glucose is called β-glucopyranose.

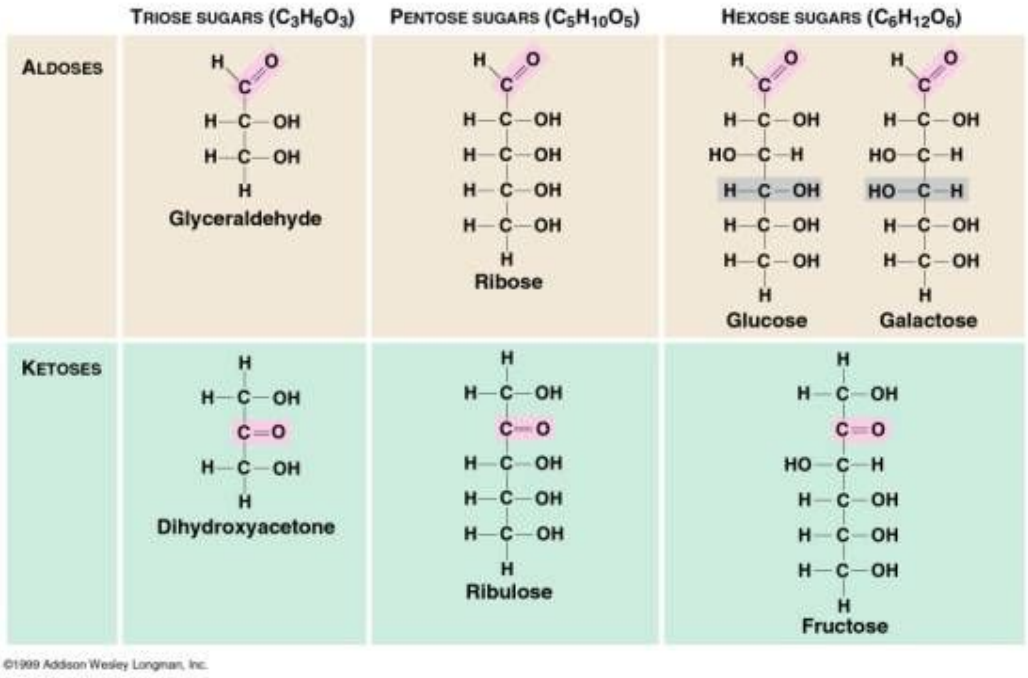
The characteristic feature of the cyclic structure is absence of a free aldehyde group in glucose. So glucose does not answers the aldehyde reactions. Fructose exists in the pyranose from in free solution. But it is in the furanose form in sucrose.



(Fructose, a monosaccharide)
(The pentagon structure represents five carbon atoms.)



D-glucose



Properties of Monosaccharides :

Physical Properties :

1. Colour and shape

Monosaccharides are colourless and crystalline compounds.

2. Solubility

They are readily soluble in water.

3. Optical Activity

They are optically active they rotate the plane polarized light. When a monosaccharide rotates the plane polarized light in the clock – wise direction or to the right (dextro rotatory) the monosaccharide is called `d' form. When a monosaccharide rotates the light in the anticlock wise direction or to the left (levo rofactory) the monosaccharide is called `l' form.

4. Mutarotation

Monosaccharides exhibit mutarotation. The change in specific rotation of an optically active compound is called mutarotation.

Chemical Properties

Carbohydrates contain active groups. The active groups are responsible for their chemical properties. There are three types of active groups in monosaccharides. They are :

1. Glucosidic OH group
2. Alcoholic OH group
3. Aldehyde (CHO) or Ketone (CO) group.

1. Glucoside Formation

Glucose reacts with methyl alcohol in the presence of hydrogen chloride gas to give α and β glucosides. Glucoside formation is due to the reaction of alcohol with the glucosidic OH of monosaccharides.

Similarly β-D-glucose gives the β-D-methyl glucosides. In the same way, fructose forms fructosides.

2. Esterification

Glucose reacts with 5 molecules of acetic anhydride to form acetyl derivatives called esters. The ability of sugars to form esters indicates the presence of alcohol groups. As glucose yields a penta acetate derivative on acetylation, it obviously contains five OH groups.

3. Reduction

Monosaccharides can be reduced by various reducing agents. The reduction is due to the presence of CHO or CO group. On reduction they yield alcohols. They are reduced by many reducing agents.

4. Formation of osazone

Aldoses and ketoses react with phenylhydrazine. Glucose consumes 3 molecules of phenyl hydrazine and produces osazones, aniline and ammonia.

Reaction with phenylhydrazine involves only 2 carbon atoms, namely the carbonyl carbon atom (the aldehyde or ketone group) and the adjacent one.

First of all one molecule of phenyl hydrazine reacts with one molecule of aldose or ketose to form a molecule of hydrazone.

With a second molecule of phenyl hydrazine the hydrazone is oxidized to aldohydrazone and the phenylhydrazine itselfis reduced to aniline and ammonia. Finally a third molecule of phenyl hydrazine reacts with the aldohydrazone to produce osazone.

Ketose also will give the same series of reactions with phenyl hydrazine.

Disaccharide also exhibit osazone formation. But sucrose does not form osazone since it does not contain functional carboxyl groups.

The hydrazone, formed in the reaction may be regarded as a special type of schiff's base and the osazone as a double schiff's base.

Fig-1

5. Formation of oximes

Aldoses and ketoses react with hydroxylamine to form oximes.

Glucose + Hydroxyl amine → Aldoxime + water.

Fig-2

6. Enolization

Glucose, Fructose and mannose are interconvertable in weak alkaline solutions sucha s $Ca(OH)_2$ and $Ba(OH)_2$ at low temperature.

7. Phosphorylation of Hexoses

The formationof a phosphoric acid derivative of hexoses is called phosphorylation.On phosphorylation, the hexoses are converted into phosphoric acid esters.

Phosphorylation is an important step in metabolism such as glycolysis, krebs cycle glycogenesis, glycogenolysis etc.

8. Fermentation

Fermentation is the process of converting a larger complex molecule into simple molecules by means of enzymes in an anaerobic condition. It releases energy as well as CO_2.

9. Reducing Agents (Oxidation)

Monosaccharides act as best reducing agents they readily reduce oxidizing agents such as ferricyanide, hydrogen peroxide or cupric ion. In such reactions the sugar is oxidized at the carboxyl group and the oxidising agents becomes reduced.

Glucose and others capable of reducing are called reducing sugars.

Glucose reduces Tollen's reagent, Fehling's solution, Benedict's reagent etc. Glucose is oxidized to gluconic acid.

Glucose + Tollen's reagent → Gluconicacid + silver mirror

Monosaccharides of Biological Importance

Among monosaccharides, the pentoses and hexoses are biologically important. Some biologically important monosaccharides are the following :

1. D-glucose
2. Fructose

3. Mannose
4. Galactose
5. Ribose
6. Deoxyribose

1. D-Glucose or Dextrose

(a) D-glucose is a simple sugar. It cannot be hydrolysed further.

(b) It is sweet in taste.

(c) It is also called fruit sugar, corn sugar, grape sugar etc.

(d) Human blood contains 14mg per100 ml of blood.

(e) It serves as the major metabolic fuel of cells and tissues. Hence glucose is described as the carbohydrate currency of the body.

(f) Glucose contains 6 carbon atoms and has a free aldehyde group. Hence it is called an aldohexose.

(g) Of the 5 hydroxyl groups, one OH is called primary alcoholic group and the remaining 4 are called secondary alcoholic groups.

(h) The structure of glucose can be represented by straight chain structure as well as ring or cyclic structure. D-glucose is optically active and it is a dextra-rotatory compound and hence the name D-glucose. It shows mutarotation. D-glucose exists in α form and β form

2. Fructose

(a) It is a monosaccharide and a ketohexose.

(b) It is a sugar with sweet taste and soluble in water.

(c) It occurs in plants and honey in the form of digosaccharides and polysaccharides like inulin. Inulin and other digoseccharides containing fructose on hydrolysis give fructose.

(d) It is a reducing sugar. It reduces Tollens reagent, Fihing's solution.

(e) It is an optically active compound. It is levorotatory. Its specific rotation is – 92^{O}.

(f) It has straight chain structure and cyclic or ring structure. In straight chain structure. Six carbon atoms are in a chain the two and carbons have primary alcoholic groups. The 2^{nd} carbon is a keto group. The other 3 carbon 3,4,5 have secondary alcoholic groups.

3. Galactose

(a) It is a monosaccharide (hexose). It is a reducing sugar.

(b) It is a component of milk sugar (lactose).

(c) It is synthesized in the mammary glands from glucose.

(d) Lactose on hydrolysis gives galactose

(e) In lives, galatose is changed into glucose and thus used in the body.

2.1.1.5 Oligosaccharides

Oligosaccharides are sugars which yield 2 to 10 monosaccharide molecules on hydrolysis. In oligosaccharides the monosaccharides are linked by a glycosidic bond.

Glycosidic linkage

Glycosidic linkage is a bond formed between one carbohydrate with another carbohydrate. The glucosidic linkage is commonly formed when two glucose molecules are liked to form a maltose.

The glucosidic bond is formed between the first carbon atom of one glucose and the 4^{th} carbon atom of second glucose. During this bond formation the hydroxyl group (OH) of the first carbon atom is eliminated along with the hydrogen atom of the 4^{th} carbon atom. The remaining oxygen atom forms the glycosidic bond between the 1^{st} carbon atom and the fourth carbon atom. The eliminated OH and H join together to form a water (H_2O) molecule.

When two glucose molecules are united one glycosidic bond is formed with the elimination of one water molecule and the resulting compound is called a disaccharide.

When three glucose molecules are united two glycosidic bonds are formed with the elimination of two molecules of water and the resulting compound is called a trisaccharide.

Classification of oligosaccharides

Oligosaccharides are classified into three or more types based on the number of monosaccharide molecules released on hydrolysis. They are

1. Disaccharides
2. Trisaccharides
3. Tetrasaccharides

1. Disaccharides

Disaccharides are sugars which yield 2 molecules of monosaccharides on hydrolysis. They are sweet in taste and soluble in water. Among oligosaccharides, disaccharides are more important in the biological world. The important disaccharides are sucrose (cane sugar), lactose (milk sugar) maltose (malt sugar), cellobiose, trehalose, gentibiose and melibiose.

1. Sucrose

It is a disaccharide. It is commonly called cane sugar. On hydrolysis an equi molar mixture of α-D glucose and β-D fructose are formed.

2. It is founding sugar cane, beet, apple, pine apple, carrot and ripen fruits.
3. It is the only food stuff used in the crystalline form.
4. Sucrose on hydrolysis by dilute acids or the enzyme invertase (sucrase) gives invert sugar. It is a mixture of glucose and fructose. Glucose is dextro rotatory. Fructose is levorolatory. There is inversion of the sign of rotation. This process is called inversion and the mixture is called invert sugar.
5. Sucrose is formed by the elimination of a molecule of water from glycosidic hydroxyl group of α-D glucose and β-D-fructose.
6. Sucrose does not posses mutarotation and is not a reducing sugar. It is not reactive.

2. Lactose

(a) It is a disaccharide purely of animal origin.

(b) It is commonly called milk sugar. It is present in milk of mammals.

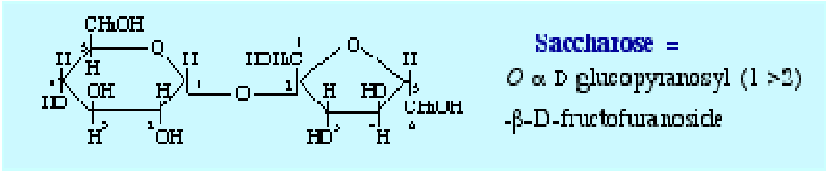
(c) It is present in the mammary gland. It is also found in urine during pregnancy.

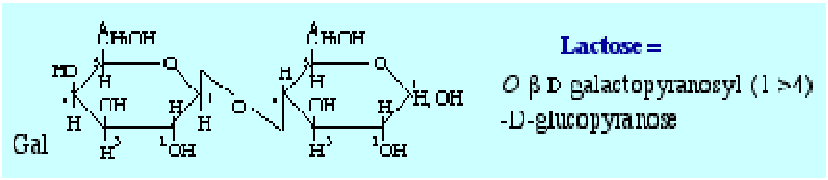
(d) It is formed by the elimination of a molecule of water from the glucosidic – OH group of β-D galactose and the alcoholic OH group of the carbon atom 4 of D-glucose.

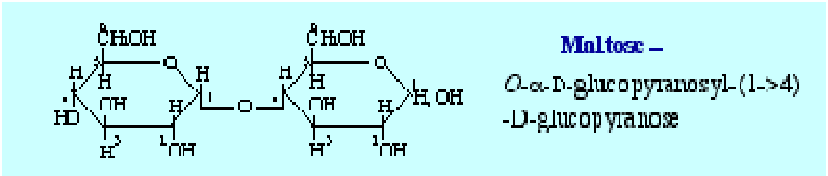
(e) It exhibits mutarotation due to the presence of a carbonyl group on the carbon atom 1 of glucose unit.

(f) The enzyme lactase hydrolyses lactose to glucose and galactose.

(g) The intestine of milk sucking infants has the enzyme lactase. This converts lactose to glucose and galactose. Then only it is absorbed in the body. Excess of lactose in the milk injected into the body causes watery diarrhoea, abnormal intestinal flow and colic pains.







3. Maltose

(a) It is a disaccharide. It is commonly called malt sugar. Malt from sprouting barley is the major source of maltose.

(b) It is produced during the digestion of starch by the enzyme α-amylase. α-amylase converts starch to dextrins and maltose.

(c) Maltose on hydrolysis by maltase gives two molecules of D-glutose.

4. 1.4-Glycosidic linkage

In maltose the glycosidic linkage is formed between the 1st carbon atom of one glucose and the 4th carbon atom of the second glucose. Hence this glycosidic linkage is called 1,4-glycosidic linkage.

Again in the 1st carbon atom the hydroxyl group is situated below the ring and hence this glucose is called α-glucose. Hence the glycosidic linkage is called α,1,4-glycosidic linkage.

5. It is a reducing sugar. It reduces Fehling's solution and Tollen's reagent this shows the presence of a free aldehyde group.

4. Cellobiose

It is a disaccharide. It is identical with maltose. In cellobiose, β 1,4 glucosidic linkage is present. In maltose the linkage is α 1,4 glucosidic.

β 1,4 glycosidic linkage

Here the glycosidic bond is a 1,4 – glycosidic linkage because it is formed between the 1st carbon atom of one glucose and the 4th carbon atom of the second glucose.

But the glucose molecules are β-forms because the hydroxyl (OH) group of the 1st carbon atom is located alone the ring. Hence the glycosidic linkage is called β 1,4-glycosidic linage.

2. It is released during the digestion of the polysaccharide cellulose by cellulases.

3. On hydrolysis it gives glucose units only.

Trisaccharides

When an oligosaccharide contains three monosaccharide units, it is called a trisaccharide. Ex: Rhamnose.

When three glucose molecules are united two glycosidic bonds are formed with the elimination of two molecules of water and the resulting compound is called a trisaccharide.

3. Tetrasaccharides

When an oligosaccharide contains four monosaccharide units it is called tetrasaccharide.

Some oligosaccharides are found in combination with proteins. Such oligosaccharides are called glycoproteins. Commonly they are found as side chains of polypeptides.

Functions of oligosaccharides

1. Cell Recognition

The oligosaccharides and glycoproteins present on the surface of cell membrane serves as identifies that assist the cells in their recognition and cell – cell adhesion.

2. Recognition of Immunoglobulins

Liver cells recognize immunoglobulins with the help of oligosaccharides.

3. Nitrogen fixation

The oligosaccharides present on the well of nitrogen fixing bacteria help in the binding of bacteria to the root hairs of leguminous plants.

2.1.1.6 Polysaccharides

Polysaccharides are non sugars. Several monosaccharide molecules combine to form polysaccharides. They are linked by glycosidic linkages. Polysaccharides give more than 10 monosacharide molecules on hydrolysis. They form linear chain or branched chain molecules.

They do not have sweet taste. They have high molecular weight and insoluble in water, they do not exhibit any of the properties of aldehyde or keto groups.

Polysaccharides are classified into 2 types they are homopolysaccharides and heteropolysaccharides.

Homopolysaccharides are composed of only one type of monosaccharides. On hydrolysis they yield only one type of monosaccharides.

Ex: Starch, glycogen, cellulose, chitin etc.

Hetoropolysaccharide

They are composed of a mixture of monosaccharides. On hydrolysis they yield a mixture of monosaccharides.

Ex: Hyalurnic acid, chondriotin, heparin, agar – agar etc.

Heteropolysaccharides include neutral sugars and mucopolysaccharides.

1. Homopolysaccharides

(a) Starch: It is a homopolysaccharide. It yields glucose on complete hydrolysis. So it is a glucan.

(b) It is a reserve food material in plants and founding cereals, legumes, potatoes and other vegetables.

(c) It is insoluble in cold water, alcohol and ether but forms a colloidal solution on boiling with water.

(d) It gives dextrins during incomplete hydrolysis.

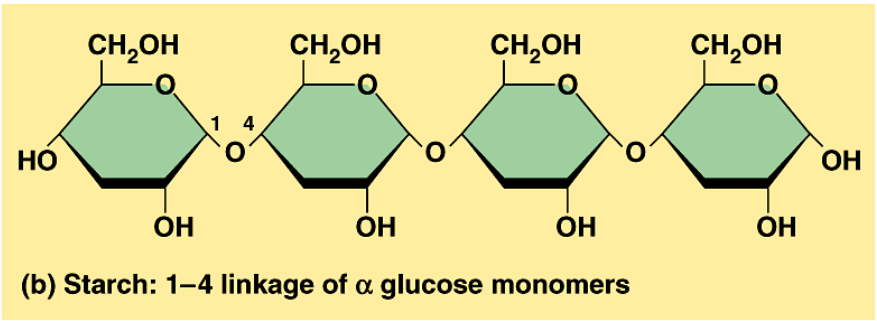
It is a mixture of glucons that plants synthesize as their principal food reserve and which is deposited in the cytoplasm of plant cells starch is made up of two structurally different homopolysaccharide units. They are amylose and amylopectin the percentage of amylose is 20% and that of amylopectin is 80%.

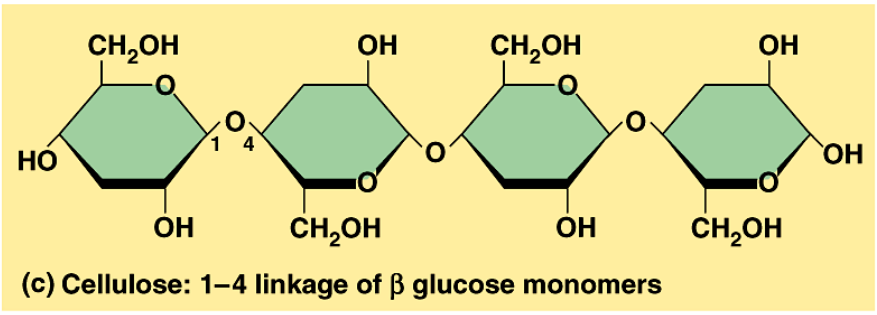
Amylose

1. It is a homopolysaccharide with a molecular weight ranging from 10,000 to 50,000.
2. It has 1,4 glycoside linkage. The glycoside OH group of one glucose unit is joined to the 4^{th} carbon of the next unit.
3. It is water soluble and has a long un branched straight chain.
4. It gives maltose during enzymatic hydrolysis. The enzyme used is amylase.
5. It is considered to be an anhydride of α-D glucose units.
6. It gives intense blue colour with iodine.

Amylopectin

1. It is also homopolysaccharide with high molecular weight of 50,000 to 1,000,000.
2. It is a branched chain polysaccharide in starch.
3. It is insoluble in water.
4. It gives isomaltose during incomplete hydrolysis.
5. In addition to α-1,4 glycoside linkage it has α-1,6 glycoside linkage.
6. The glucose unit present at each point of branching has substituents at carbon atoms 1,4 and 6 i.e., glucose unit has three points of attachment.
7. The average chain length in amylopectin is about 24 glucose units.
8. Haworth proposed a laminated structure.
9. It gives purple colour with iodine.





Reaction with Iodine

Both the graceless and the colleridal solutions react with Iodine to give a blue colour. This is chiefly due to Amylose which forms a deep blue colour, which dissociates on heating. Amylopectin solutions are colour blue – violet or purple.

Ester Formation

Starches are capable of forming esters with either organic or inorganic acids some starch esters (synthetic) are useful as plastics. Where as starch nitrates are violent explosures.

Hydrolysis

Starch on hydrolysis yields the following course of hydrolysis : Starch (Blue) → Soluble starch (Blue) → Amylodextrin (Purple) → Erythrodextrin (Red) →

Starch granules appear under microscope as particles made up of concentric layers of materials. They differ in shape, size and markings according to the source.

Achrodextrin (Colourless) → Maltose → Glucose

Enzyme hydrolysis ends at Maltose. It is not quantitative conversion, traces of dextrins are also formed. For formation of glucose it requires the enzyme maltase.

Types of Amylases

Two broad classes of amylases exist as

(a) α-amylase is present in saliva and pancreatic juice.

(b) β-amylase is present in sprouted grains and malts.

$$\text{Starch} \xrightarrow{\text{Amylase}} \text{Maltose} + \text{Dextrins} \xrightarrow{\text{Maltase}} \text{Glucose}$$

$$\text{Starch} \xrightarrow{\text{Acid}} \text{Glucose}$$

Hydrolysis

Both of them hydrolyse only α-glycosidic linkage.

α-amylase produces a random cleavage of glycosidic bonds well inside the starch molecules yielding a mixture of maltose and some fragments lager than maltose (dextrins) where as, β amylase split off maltose moieties liberating successive maltose units commencing at the non-reducing end of the starch molecule and ends in limit dextrin.

Difference between Amylose and Amolypectin

S.No.	Amylose	Amylopectin
1	It has a simple unbranched structure	It has branched chain structure
2	It is soluble in water	It is insoluble in water
3	It has α 1,4 – glycosidic linkage	It has α 1,4 – glycosidic and α 1,6 glycosidic linkage
4	It is easily dispersed in water	It is not easily dispersed in water
5	It gives blue colour with iodine	It gives purple colour with I_2 (Iodine)

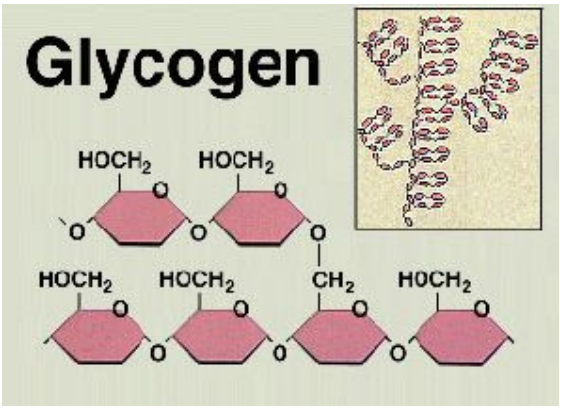
Glycogen

1. Glycogen is a homopolysaccharide, since it gives glucose on complete hydrolysis. It is a glucon.
2. It is a major reserve carbohydrate in animals. So it is called as animal starch.
3. It is stored mainly in the lives and muscles of all animals. Among plant it is found in fungi and yeast.
4. It is a white powder. It readily dissolves in water. The solution gives reddish brown colour with iodine.

5. It occurs as cytoplasmic granules in liver and skeletal muscle.

6. Structurally glycogen resembles amylopectin but it is much more highly branched with branch points occurring after every 8 to 12 glucose residues. Its degree of polymerization is nevertheless. Similar to that of amylopectin. In the cell, glycogen is degraded for metabolic use by the enzyme glycogen phosphorylase which phosphorolytically cleaves the α-(1→4) bonds sequentially inward from its reducing ends to yield glucose 1-phosphate units rather than free glucose Glycogen thus has a highly branched structure with many non-reducing ends this permits the rapid mobilization of glucose in times of metabolic need, since hydrolysis starts from the non-reducing ends. The α -(1→6) branches of glycogen are cleaved by a debranching enzyme α (1→6) glucosidase, which play important role in glucose metabolism.

Formation of glycogen from glucose is called as Glycogenesis and breakdown of glycogen to form glucose is called Glycogenolysis.



Solubility

Glycogen is not readily soluble in water and it form an opalascent solution.

It can be precipitated from opalascent solution by ethyl alcohol and drying it forms a pure white powder.

Action of Alkali

Glycogen is destroyed by a hot strong KOH or NaOH solution. This property is made use of in the method for determining it quantitatively in tissues.

Cellulose

1. It is a non-sugar and homopolysaccharide since it is composed of glucose units. It is not easily hydrolysed. It is hydrolysed to glucose by concentrated H_2SO_4 or concentrated NaOH.

2. It is the primary structural component of cell walls of plants.

3. Although it is predominantly of vegetable origin, it also occurs in marine invertebrates known as tunicates.

Properties

Cellulose is a fibrous, tough water insoluble substance, a linear polymer of upto 15,000 D-glucose residues (a glucan) linked by β-(1→4) glycosidic bond.

1. It is not hydrolysed readily by dilute acid but heating with fairly high concentration of acid yields the disaccharide cellobiose and D-glucose.

2. Water insoluble, soluble in acids and basic solutions.

3. Cellulose may be nitrated to form cellulose nitrates or nitro celluloses which are of much importance in the manufacture of explosives etc.

4. Cellulose does not give a characteristic reaction with iodine.

5. Cellulose is formed by the linking of glucose units. Here also the glycosidic bond is a β1,4 – glycosidic linkage because it is formed between the 1^{st} carbon atom of one glucose and the 4^{th} carbon atom of the second glucose.

   But the glucose molecules are β-forms because the hydroxyl (OH) group of the 1^{st} carbon atom is located alone the ring. Hence the glycosidic linkage is called, β 1,4-glycosidic linkage.

6. Its molecular weight ranges between 200,000 and 2,000,000 i.e., it may contain 1,200 to 12,500 glucose units per molecule.

7. It occurs in lignin and cotton.

8. It is not nutritive because of its inertness towards chemical reaction.

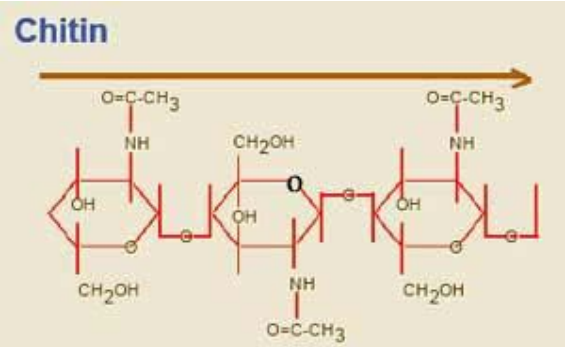
9. Cellulose is not digested by man.

10. Ruminants like cattle, sheep, goats etc. are able to digest cellulose due to the presence of the digesting enzyme cellulase.

11.It is used in the manufacture of paper , cellulase acetate etc.

Chitin

Chitin is the principal structural component of the exoskeletons of invertebrtes such as Crustacean, insects and spider. It is also present in the cell walls of most fungi and many algae. It is therefore almost as abundant as cellulose. Chitin is a homopolymer of β (1→4) linked N-acetyl glucosamine residues. It is different chemically from cellulose only in that each C_2 – OH group is replaced by an acetamide group.



Chitin is decomposed to N-acetyl glucosamine by chitinase present in the gastric juice of snails or from bacteria.

Inulin

1. It is the major reserve carbohydrate of many plant like tubers and roots of dahlias, artichokes etc.

2. It is a homopolysaccharide, non sugar with 30 to 35 fructose units per molecules with molecular weight of about 5000. It may be described as fructosin.

3. The six carbon atoms are situated in the five membered furanose ring.

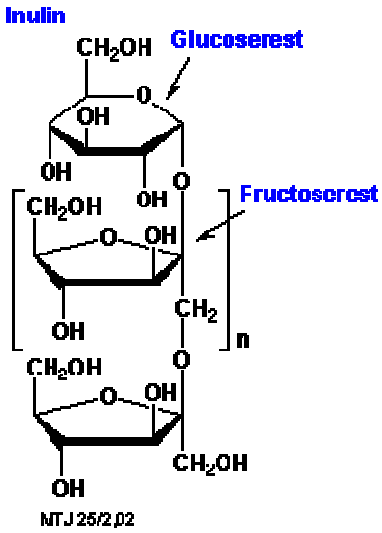
4. It is formed by eliminating water molecule from the glycosidic group on the carbon atom 2 of one β-D fructose unit and the alcoholic OH group on carbon 2 of the adjacent β-D fructose units (1,2 – linkage).

5. On hydrolysis inulin gives a small amount of glucose in addition to fructose molecules.

6. It is a white powder, insoluble in cold water but soluble in warm water.

7. It does not give any colour with iodine.

8. It is used in the physiological investigation for determining the rate of glomerular filteration.

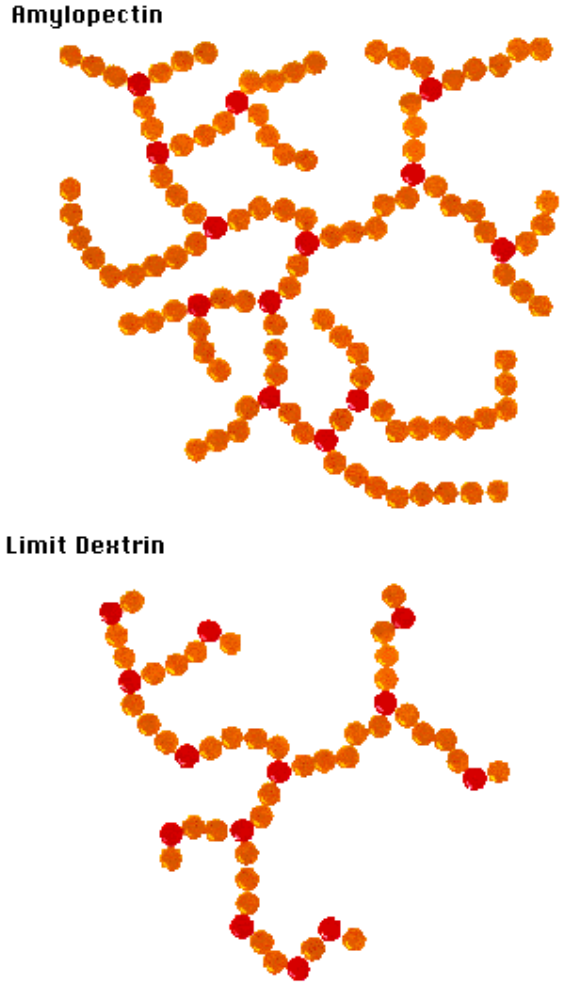


Dextrins

When starch is partially hydrolysed by the action of acids or enzymes, it is broken down into a number of products of lower molecular weight known as "dextrins".

Soluble starch forms a clear and colourless solutions, not at all "starchy in appearance but gives a `blue' iodine reaction.

The other dextrins are water soluble and react with iodine. They resemble starch by being opted by alcohol, forming sticky, gummy substances.



Medical Importance

1. Dextrin solutions are often used as “mucilages” (mucilages on the back of the postage stamp).

2. Starch hydrolysates consisting largely of dextrins and maltose are widely used in infant feeding.

Limit Dextrin

It is a well defined dextrin. This is the product remaining after the β-Amylase has acted upon starch until no further action is observed.

Dextrans

It is a polymer of D-Glucose. It is synthesized by the action of Leuconostoc mesenteroides, a non pathogenic Cocci in a sucrose medium. Exocellular enzyme produced by the organism bring about polymerization of glucose moiety and forms the polysaccharide known as Dextrans.

They differ from dextrin in structure they are made up of units of a number of D-glucose molecules having α 1→6, α 1→4 or α 1→3 glycosidic linkages, within each unit and the units are joined together to form a network.

Biomedical Importance

Dextran solution, having M.W. approximately of 75,000 have been used as plasma expander. When given I.V., in cases of blood loss (hemorrhage), it increases the blood volume.

Disadvantage

Only disadvantage is that it can interfere with grouping and cross matching, as it forms false agglutination (Rouleux formation). Hence blood sample for grouping and cross matching should be collected before administration of dextran in a case of hemorrhage and blood loss, where blood transfusion may be required.

Starch Inulin Glycogen	Storage Polysaccharides	Cellulose Chitin	Structure Polysaccharides

Heteropolysaccharides (Heteroglycans)

Heteropolysaccharides are composed of a mixture of monosaccharides. On hydrolysis they yield a mixture of monosaccharides.

Ex: Neutral sugars such as hemi cellulose, gums etc.

Mucopolysaccharides such as hyaluronic acid, chondriotin, chondriotin sulphate A,B and C Keratosulphate, heparin etc.

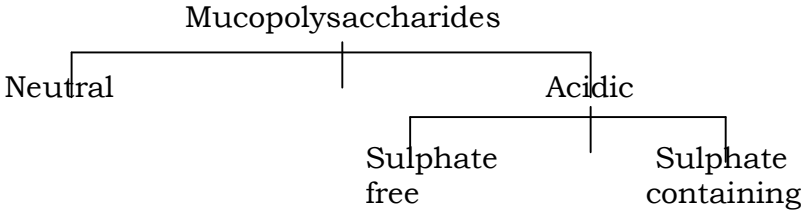
Heteropolysaccharides are classified into two types namely neutral sugars and mucopolysaccharides.

The Neutral sugars give more than one type of sugar units on hydrolysis and sometimes non sugar components also. This group includes some hemicelluloses some gums, mucilages and pectic substances.

Mucopolysaccharides

The Mucopolysaccharides are essential components of tissues where they are generally present, atleast in part combined with protein as mucoproteins or mucoids.

Although there is no agreement on classification, the mucopolysaccharides are classified as



The mucopolysaccharides such as Hyaluromic acid, Heparin and chondriotin sulphate which are acidic in character are called Acidic Mucopolysaccharides. The acid Mucopolysaccharides also called Glycosamino glycans.

1. Mucopolysaccharides derive their name from their slime like consistency. They are gelatinous substances.

2. They are composed of a mixture of sugars as well as derivatives of sugars such as aminosugars, urinic acids etc.

3. They have high molecular weight upto 5 million.

4. Most of them act as structural support material for connective tissue or mucous substances of the body.

5. They serve as lubricants and biological cements. They cement the adjacent cells.

6. They are not found in free state but bound with proteins. Such heteromacero molecules are called proteoglycans.

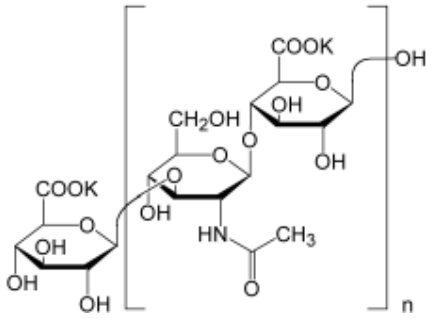
7. They are made up of disaccharide units in which an uronic acid is bound by a glycosidic bond to the C_3 of an acetylated amino sugar (1→3 linkage) these disaccharide residues are polymerized by 1→4 linkages to give a linear macromolecule.

The common examples of mucopolysaccharides are :

Hyaluronic acid	---	Heparin
Chondriotin	---	Blood group substances
Dermatan sulphate	---	Bacterial polysaccharides
Keratosulfate	---	Vegetable gums, Agar – Agar

Hyaluronic Acid

1. It is a heteropolysaccharide.
2. It is found in the skin, vitreous body of the eye, the umbilical cord, as a coating around ovum, synovial fluid of joints and in some bacteria.
3. It is a highly viscous substance and it has a molecular weight of 50,00,000.
4. It acts a lubricant and act as a biological cement in connective tissues.
5. It is a straight chain polymer of disaccharide which form the repeating unit. Each disaccharide is linked to the next by a β-1,4-glycosidic bond. Each disaccharide unit is formed of a D-glucuronic acid and N-acetyl D-glucosamine linked by β-1,3 linkage.
6. On hydrolysis, hyaluronic acid yields an equimolar mixture of D-glucuronic acid D-glucosamine and acetic acid.
7. Hyaluronic acid is split by the enzyme hyaluronidase. The spleen is rich in hyaluronidase and hence can advance better in the cervical canal and finally fertilize the ovum. It is a sulphate free mucopolysaccharide.



Chondroitin

1. It is a mucopolysaccharide.
2. It is found in cartilages and is also a component of cell coats.
3. It is the parent substance for chondroitin sulphate A,B and C.
4. It is a straight chain polymer of disaccharides which form the repeating units. Each disaccharide is linked to the next by a β-1,4-glucosidic bond.

Each disaccharide unit is formed of a D-glucuronic acid and N-acetyl D-galactosamine joined by β-1,3 linkages.

Thus chondroitin is similar to hyaluronic acid. In chondroitin galactosamine is present instead of glucosamine.

Heparin

1. It is a heteropolysaccharide because it yields mixtures of monosaccharides and their derivatives.
2. It is a mucopolysaccharide because it has gel like consistency.
3. It contains uronic acid and sulphuric acid and is acidic in nature. Hence it is also called acidic heteropolysaccharide.
4. It is present in liver, lungs, arterial wells etc. It is secreted by the most cells of connective tissue.

5. It functions as an anticoagulant because it prevents coagulation or clotting of blood. It prevents the conversion of prothrombin into thrombin and thus eliminates the effect of thrombin on fibrinogen

6. It is a straight chain polymer composed of D-glucuronic acid and D-glucosamine N-sulphate. The two molecules are alternating in the chain. It contains an additional O-sulfate group at C_6.

7. The two molecules are linked by 1→4 linkage only and 1→3 linkage is absent.

8. The D-glucuronic acid is esterified at carbon atom number 2.

Agar – Agar

1. Agar – Agar is a heteropolysaccharide because it yields a mixture of monosaccharides on hydrolysis.

2. It is a mucopolysaccharide because it has a gel like consistency.

3. It is acidic in nature. Hence it is acidic heteropolysaccharide. The acidity is due to the presence of sulfuric acid.

4. In consists of D and L galactose in a ratio of 9:1.

5. It is insoluble in cold water but soluble in hot water.

6. Agar – Agar is a commercially important product. It is used extensively in biology laboratories for the preparation of culture media for bacteria, fungi etc.

7. It is used as a solidifying agent in deserts, as a laxatine.

2.1.1.8 Functions of carbohydrates

The most important function of carbohydrates is to provide energy to the body. They are also structural components of tissues. Carbohydrates are important in the regulation of fat metabolism, protein sparing functions and function in the digestive tract.

1. Structural components of cells

Carbohydrates serve as an important structural material in some animals and plants. Carbohydrates constitute the cellulose frame work.

2. Major source of energy

Almost all animals uses – them as respiratory fuels. Breakdown of a gram of carbohydrate yields 45 Kilo calories of energy. Glucose supplies the immediate energy needed by tissues Glucose is the sole form of energy for the brain and other nervous tissue.

3. Storage substance of potential energy

Carbohydrate is stored in the body in the form of glycogen in liver and muscles. In plants starch is the storage substance.

4. Protein sparing function

As long as carbohydrates are present in required amount in the body, it is used as a source of energy. If it is not supplied in required amount, fat and then protein will be utilized as a source of energy this is known as protein sparing function.

5. Key role in metabolism

Carbohydrates play a key role in the metabolism of aminoacids and fatty acids.

6. Supportive Function

Cellulose in plants and chondroitin sulphate in bone tissue offer supportive function.

7. Heparin exhibits an anticoagulative function.

8. Acidic heteropolysaccharides such as hyaluronic acid function as intercellular cementing substances.

2.1.1.8 Summary

1. Carbohydrates are the polyhydroxy aldehydes or ketones or compounds which produce them on hydrolysis. The term sugar is applied to carbohydrates soluble in water and sweet to taste. Carbohydrates are the major dietary energy sources, besides their involvement in cell structure and various other functions.

2. Carbohydrates are broadly classified into 3 groups – monosaccharides, oligosaccharides and polysaccharides. The monosaccharides are further

divided into different categories based on the presence of functional groups (aldoses or ketoses) and the number of carbon atoms (trioses, tetroses, pentoses, hexoses and heptoses).

3. Glyceraldehyde (triose) is the simplest carbohydrate and is chosen as a reference to write the configuration of all other monosaccharide (D and L forms). If two monosaccharides differ in their structure around a single carbon atom, they are known as epimers. Glucose and Galactose and C_4 – epimers.

4. Glucose exists as α and β anomers with different optical rotations. The inter conversion of α and β anomeric forms with change in the optical rotation is known as mutarotation.

5. Monosaccharides participate in several reactions. These include oxidation, reduction, dehydration, osazone formation etc. Formation of esters and glycosides by monosaccharides is of special significance in biochemical reactions.

6. Among the oligosaccharides, disaccharides are the most common. These include the reducing disaccharides namely lactose (milk sugar) and maltose (mlat sugar) and the non-reducing sugar (cane – sugar). Sucrose is dextrorotatory, but on hydrolysis it becomes levorotatory this phenomenon is known as inversion.

7. Polysaccharides are the polymers of monosaccharides or their derivatives.

Ex: Starch, glycogen etc. are homopolysaccharides. Mucopolysaccharides are the heteropolysaccharides.

2.1.1. 9 Model Questions

1. What is isomerism? Give examples.
2. Describe the structure of monosaccharides.
3. What is asymmetric compound? Give examples.
4. Describe the properties of monosaccharides.
5. What is mutarotation.
6. What is glycosidic linkage.
7. Describe the structure, properties and importance of D-glucose.

8. Give an account of the classification of monosaccharides with examples.
9. Describe the structure and importance of starch.
10. Glycogen is the animal starch – Discuss.
11. What is a polysaccharide? Give examples.
12. How do you say that glycogen is a polysaccharide.
13. Give an account of the outline classification of carbohydrates with examples.
14. Give an account of the biological importance of carbohydrates.
15. What is a mucopolysaccharide? Give examples.
16. Describe the structure and importance of any one mucopolysaccharide.

2.1.1.10 Reference Books

Biochemistry – Saras Publication

Biochemistry – U.Satyanarayana.

Lesson 2.1.2

AMINO ACIDS

Objective

objective

The aminoacids are the building blocks of proteins which are essentially organic molecules consisting of amino as well as acidic groups. This chapter deals with the classification, physical and chemical properties of aminoacids.

2.1.2.1 Introduction

Aminoacids are compounds containing amino groups (-NH_2) and carboxyl (COOH) groups. So aminoacids are also called amino carboxylic acids.

Aminoacids are the essential components of all living cells. They are the building blocks of proteins. They are the monomers of the protein polymers.

Animals and plants contain about 200 aminoacids. But human body contains only about 60 aminoacids of these only 20 aminoacids are used as

building blocks for the synthesis of proteins. These 20 aminoacids are repeatedly used to synthesize millions of proteins.

2.1.2.2 Number of Amino acids

Already more than 200 aminoacids have been isolated and identified. But human body contains only about 60 aminoacids. However proteins on hydrolysis yield only 25 aminoacids. That is, only 25 aminoacids are used for the synthesis of proteins. These 25 aminoacids are called proteogenic aminoacids. The proteogenic aminoacids are of two types. They are major aminoacids and rare aminoacids.

The major aminoacids are 20 in number and are specified by the genes. The rare aminoacids are the derivatives of the 20 major aminoacids.

Ex: Hydroxy proline, hydroxyl lysine, amino citric acid, aspargine and glutamine.

Out of the 25 aminoacids only 11 aminoacids are essential amino acids and the others are non-essential amino acids.

Human cells can synthesize the non-essential aminoacids from essential aminoacids and other compounds.

Out of the 25 aminoacids, 23 aminoacids are α-aminoacids and the remaining 2, proline and hydroxy proline are imino acids.

Other aminoacids appear as intermediate products in metabolism. Some other occur in unusual sources. These aminoacids are not incorporated in protein. But they occur either in a free state in the cell as metabolite or in combination with other compounds.

2.1.2.3 Structure of Aminoacids

Amino acid is an amino carboxylic acid. They are the building blocks of proteins. An amino acid is made up of five components namely.

1. A Carbon atom C
2. A Hydrogen atom H
3. An amino group NH_2
4. A carboxyl group COOH
5. A side chain or residue R

R is the side chain or residue and it represents the group other than NH_2 and COOH. It may be a hydrogen atom (H) or a methyl group (CH_3) or an aliphatic group or an aromatic group or a heterocyclic group. In glycine the simplest amino acid R represents a H atom. In alanine it is a methyl group. In serine it is a CH_2OH.

1) α, β and γ – Aminoacids

In aminoacids, the numbering of carbon atoms is made from the carbon atom situated next to the COOH group is carbon atom number 1 and is called a α - carbon atom. The second carbon atom is called β-carbon atom and the third carbon atom is called γ carbon atom.

The aminoacids are named as α, β and γ aminoacids according to the position of amino group. When the amino group is attached to the α-amino acid.

Ex: Isoleucine when the amino group is attached to the β-carbon atom the amino acid is called β-amino acid. Ex: cysteine. When the amino group is attached to the γ carbon atom, the amino acid is called γ- amino acid.

2) Imino acids

Some aminoacids contains imino group (NH) in the place of amino group (NH_2). These aminoacids are called imino acids.

Ex: Proline, hydroxy proline etc.

3) Name of amino and acid groups

Some aminoacids contain only one amino group and only one acid group. These amino acids are called mono amino mono carboxylic acids. Ex: Glycine. Some amino acids contain one amino group and two acid groups. These aminoacids are called mono amino di carboxylic acids. Ex: Aspartic acid. Another group of amino acid contains two amino groups but only one acid group. These aminoacids are called di amino – mono carboxylic acids. Ex: Lysine. The aminoacids containing two amino groups and two acid groups are called diamino dicarboxylic acids. Ex: Cystine.

4) Nature of R group

The amino acid containing a straight chain as the side chain is called an aliphatic amino acid. Ex: Isoleucine. The amino acid containing an aromatic ring as the side chain is called aromatic amino acid. Ex: Phenylalanine. The amino

acid containing a heterocyclic ring as the side chain is called a heterocyclic amino acid. Ex: Histidine.

5)Asymmetry in Amino acids

In all aminoacids (α-aminoacids) the α carbon atom is asymmetric because it is attached to four different groups. Hence they exhibit optical activity and they rotate plane – polarized light to the right or left. As a result, they exist in two optical isomers.

One isomeric amino acid rotates the plane polarized light to the right and it is called dextrorotatory form and this amino acid is represented by the symbol (+). The second isomeric amino acid rotates the plane polarized light to the left and it is called levorotatory form and this amino acid is represented by the symbol (-).

While most of the aminoacids have only one asymmetric carbon atom, the two aminoacids, threonine and isoleucine have two asymmetric carbon atoms each so they exist in 4 optical isomers ($2^4 = 2^2 = 2x2 = 4$).

6) D and L Amino acids

Based on the configuration, two types of aminoacids exist. They are D and L aminoacids. The amino acid having the NH_2 group on the right is called D-amino acid. The amino acid having the NH_2 group on the left is called L-amino acid. These isomers are related to the mirror images of each other.

It may be remembered that D and L do not refer to optical rotation, but to the steric configuration of NH_2 group to the right and left side of the COOH group.

The steric configuration and optical rotation of an amino acid may be simultaneously expressed as D(+) amino acid or D(-) amino acid or L(+) amino acid or L(-) amino acid. In nature L-aminoacids are more common. Therefore L-aminoacids are called natural aminoacids.

2.1.2.4 Classification of Aminoacids

Aminoacids are classified in various ways.

1. Classification based on their incorporation in proteins.
2. Classification based on the structure of the side chain.
3. Classification based on the position of amino group.
4. Classification based on reaction in solution.

5. Classification based on polarity.

6. Classification based on biological importance.

1. Classification of Amino acids based on their incorporation in proteins

At present more than 200 aminoacids have been identified in animals and plants. Still not all of them serve as constituents for proteins. Thus based on their incorporation in proteins, aminoacids are classified into two groups. They are proteogenic aminoacids and non-proteogenic aminoacids.

Proteogenic Amino acids

The aminoacids which are used to the synthesis of proteins are called proteogenic aminoacids. They are usually components of proteins. The proteogenic aminoacids are of two types namely major aminoacids and rare aminoacids.

The major aminoacids are widely involved in the synthesis of a vast number of proteins irrespective of the species concerned.

The rare aminoacids are the derivatives of the 20 major aminoacids.

Ex: Hydroxy proline, hydroxylysine, amino citric acid, aspargine and glutamine.

Non-proteogenic Amino acids

A majority of aminoacids are not incorporated into the proteins. These aminoacids are called non-proteogenic aminoacids. They do not participate in protein synthesis. They occur in the cells either in a free state as metabolites or as part of non-protein compounds.

Ex: Ornithine, citruline , γ-aminobutyricacid, β-alanine, pantothenic acid, thyroxin and many others.

Certain non-proteogenic aminoacids such as canavarine, and β-cyano alanine isolated from plants are toxins for man.

2. Classification based on the structure of side chain

On the basis of the structure of the side chain (R group). Aminoacids are classified in to following 3 types.

1. Aliphatic aminoacids

2. Aromatic aminoacids

3. Heterocyclic aminoacids

1. Aliphatic aminoacids

The amino acids containing straight chain the R-group are called aliphatic aminoacids.

The aliphatic aminoacids are further classified into four types based on the number of amino and carboxyl groups in the side chain.

1. Monoamino monocarboxylic acids

2. Monoamino dicarboxylic acids

3. Diamino monocarboxylic acids

4. Diamino dicarboxylic acids

1. Monoamino Monocarboxylic acids

These aminoacids have one amino group and one carboxylic group in the structure. These are neutral amino acids.

Ex: Glycine alanine, serine, cysteine, threonine, methionine, valine, leucine and isoleucine.

2. Monoamino Dicarboxlylic acids

These aminoacids contain one amino group and two carboxylic groups. These are acidic aminoacids.

Ex: Aspartic acid and glutamic acid, and their derivatives asparagines and glutamine.

3. Diamino Monocarboxylic acids

These aminoacids contain two amino groups and one carboxyl group. These are also called basic aminoacids.

Ex: lysine, hydroxylysine and arginine.

4. Diamino Dicarboxylic acids

These aminoacids contain two amino groups and two carboxyl groups.

Ex: Cystine or dicysteine.

2. Aromatic aminoacids

The aromatic aminoacids contain aromatic rings. They are mono amino mono carboxylic acids and are neutral in reaction.

Ex: Phenyl alanine and tyrosine.

3. Heterocyclic Amino acids

These aminoacids contain a heterocyclic ring in the structure. The ring may be an imidazole ring or an indole ring.

Ex: Proline, hydroxy proline, histidine and tryptophan.

Based on the position of the $-NH_2$ group

According to the position of the NH_2 group aminoacids are classified into three groups.

1. α-Amino acids

The carbon atom next to the acid group is called α carbon atom. If the amino group is attached to the αcarbon atom, the amino acid is called α-amino acid. Ex: Alanine.

2. β-Amino acids

The second carbon atom from the acid group is called β carbon atom. When the amino group is attached to the β carbon atom the amino acid is called β-amino acid. Ex: β amino propionic acid.

3. γ-Amino acid

The third carbon atom from the acid group is called γ carbon atom when the amino group is attached to the γ carbon atom the aminoacid is called γ-amino acid.

Classification of Aminoacids on the basis of the polarity of the side chain

There are four main classes of amino acids: those with (1) non polar or hydrophobic R groups (2) neutral (uncharged) polar R groups (3) positively charged R groups and negatively charged R groups.

Aminoacids with Non-polar (Hydrophobic) R groups

This family includes five aminoacids with aliphatic hydrocarbon R groups (alanine, leucine, isoleucine, valine and proline), two with aromatic rings (phenyl alanine and tryptophan) and one containing sulphur (methionine). As a group, these aminoacids are less soluble in water than the aminoacids with polar R groups. The least hydrophobic member of this class is alanine, which is thus near the border line between non-polar aminoacids and those with uncharged polar R groups.

Proline differs from all the other standard aminoacids in actually being an α-imino acid; it may be regarded as an α amino acid in which the R group is a substituent in the amino group.

Amino acids with uncharged (Hydrophilic) polar R groups

These aminoacids are relatively more soluble in water than those with non polar R groups their R groups contain neutral (uncharged) polar functional groups which can hydrogen bond with water. The polarity of serine, threonine and tyrosine is contributed by their hydroxyl groups; that of aspargine and glutamine by their amide groups; that of cysteine by its sulphydryl (-SH) group. Glycine, the borderline member of this group is sometimes classified as a non-polar amino acid, but its R group, a single hydrogen atom, is too small to influence the high degree of polarity of the α-amino and α-carboxyl groups.

Cysteine and tyrosine have the most polar substituents of this class of aminoacids namely, the thiol and phenolic hydroxyl groups respectively. These groups tend to loose protons by ionization far more readily than the R groups of other amino acids of this class although they are only slightly ionized at pH 7.0. Cystine often occurs in proteins in is oxidized form cysteine, in which the thiol groups of two molecules of cysteine have been oxidized to a disulfide group to provide a covalent cross – linkage between them.

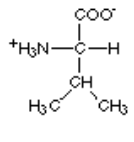
Amino acids with positively charged (basic) R groups

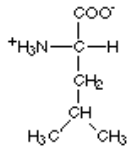
The basic aminoacids in which the R groups have net positive charge at pH 7.0 all have six carbon atoms. They consist of lysine which bears a positively

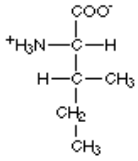
charged amino group at the t position on its aliphatic chain. Arginine, which bears the positively charged guanidinium group; and histidine, which contains the weakly basic imidazolium function. Histidine is borderline in its properties.

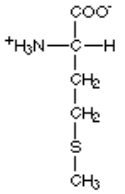
Amino acids with Negatively charged (Acidic) R Groups:The two members of this class are aspartic acid and glutamic acid, each with second carboxyl group which is fully ionized and thus negatively charged at pH 6 to 7.

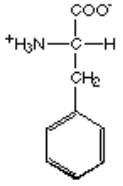
Amino acids with hydrophobic side groups


Valine (val)

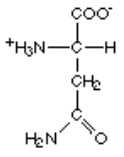

Leucine (leu)

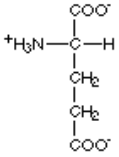

Isoleucine (ile)

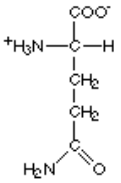

Methionine (met)

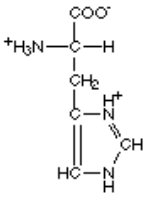

Phenylalanine (phe)

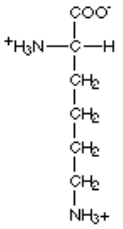
Amino acids with hydrophilic side groups

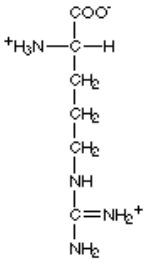

Asparagine (asn)

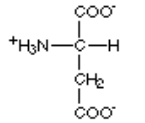

Glutamic acid (glu)


Glutamine (gln)

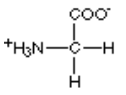

Histidine (his)

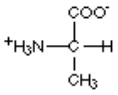

Lysine (lys)

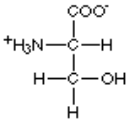

Arginine (arg)

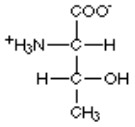

Aspartic acid (asp)

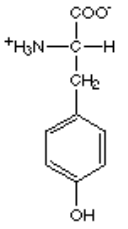
Amino acids that are in between

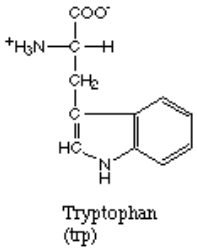

Glycine (gly)

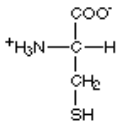

Alanine (ala)

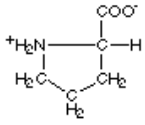

Serine (ser)


Threonine (thr)


Tyrosine (tyr)


Tryptophan (trp)


Cysteine (cys)


Proline (pro)

Classification based on reaction in solution

On the basis of the chemical reaction in solution aminoacids are classified into three groups, namely, neutral aminoacids, acidic aminoacids and basic aminoacids.

1. Neutral amino acid

The amino acids which do not contain any amino group or carboxyl group in the side chain or R are called neutral aminoacids. They contain one carboxyl group and one amino group and hence they are neutral in character.

Ex: Glycine, alanine, serine, threonine, valine, leucine, isoleucine, phenyl alanine, tyrosine, cysteine, cystine, methionine tyrosine, tryptophan, proline, hydroxy proline etc.

2. Acidic Aminoacids

The aminoacids containing additional carboxylic groups in the side chain are called acidic aminoacids. As they contain an addition carboxyl group, they impart acidic properties.

Ex: Aspartic acid, glutamic acid, asparagine, hydroxy glutamic acid etc. glutamine etc.

3. Basic Aminoacids

The aminoacids carrying an additional amino group in the side chain are called basic aminoacids. They impart basic properties.

Ex: Lysine, arginine, histidine, ornithine etc.

Classification of amino acids on the basis of biological importance

Based on their inclusion in the diet aminoacids are classified into two groups, namely essential aminoacids and non-essential aminoacids.

1. Essential aminoacids

Certain aminoacids cannot be synthesized by organisms. They must compulsorily included in the diet for normal health. Hence these aminoacids are called essential aminoacids. For human beings about 11 aminoacids are essential.

2. Non-essential aminoacids

Certain aminoacids can be synthesized in the cells from essential aminoacids or other compounds. Hence these aminoacids need not be included in the diet. These aminoacids are called non-essential aminoacids.

2.1.2.5 Properties of Aminoacids

Aminoacids have the following physical and chemical properties.

1. Solids

Most of the naturally occurring aminoacids are solids. They are in the form of crystals. The crystal forms vary from slender needles (tyrosine) to thick hexagonal plates (cystine).

2. Colour

The aminoacids are colourless.

3. Taste

Aminoacids may be tasteless (tyrosine) or sweet in taste (alanine and glycine) or bitter in taste (arginine).

4. Solubility

In general, the aminoacids are readily soluble in water, slightly soluble in alcohol and insoluble in ether. Tyrosine is only slightly soluble in cold water but more soluble in hot water. Cystine is insoluble in cold water and hot water. Proline and hydroxy proline are soluble in alcohol insoluble in ether.

The aminoacids are generally soluble in dilute acids and bases in which they form aminoacid salts. Tyrosine in only moderately soluble in dilute acids and bases. Cystine is soluble in solutions of strong mineral acids such as Hcl, but only slightly soluble in solutions of acetic acid.

5. Melting points

The aminoacids possess high melting points. In general melting points are above 200^{O}C. Certain aminoacids melt above 300^{O}C.

Most of the aminoacids undergo decomposition at or near the melting point.

6. Optical activity

All aminoacids (except glycine) are optically active. Optical activity is the property of the molecules by virtue of which they can rotate the plane polarized light. The amino acids which rotate the polarized light to the left are called levorotary aminoacids and are designated by the symbol (L or T). The aminoacids which rotate the plane polarized light to the right are called dextrorotatory aminoacids and are designated by the symbol (+) or (D). The property of optical rotation is due to the existence of asymmetric carbon atoms in aminoacid molecule. As glycine has no asymmetric carbon atom, it cannot rotate plane polarized light.

7. Ampholytes or Amphoteric Nature

Aminoacids behave both as weak acids and weak bases because they contain atleast one carboxyl group (COOH) and one amino group (NH_2). Such compounds are called ampholytes or amphoteric compounds. These are capable of both donating and accepting protons.

8. Zwitter ions

Aminoacids behave as zwitter ions. A zwitter ion is a dipolar ion containing negative and positive charges.

The aminoacids possessing both positive and negative charges are called zwitterions when the aminoacid is in the zwitter ion state.

As aminoacids contain both acidic (COOH) and basic (NH_2) groups, they can react with both alkalies and acids to form salts. In acid solution (low pH) aminoacids carry positive charges and hence they move towards cathode in an electric field.

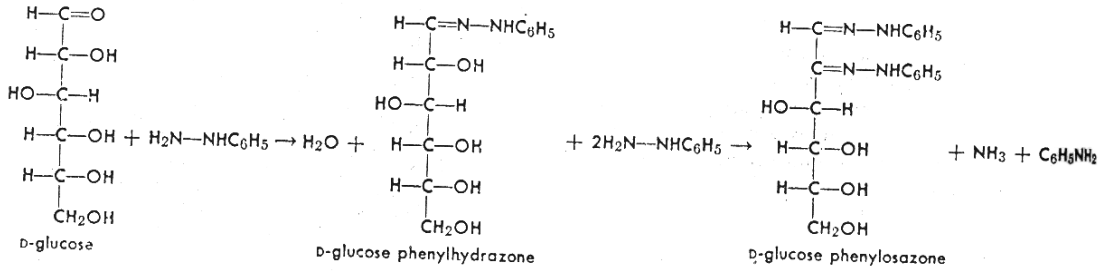
In an alkaline solution (increasing pH), The aminoacids carry negative charges and therefore move towards anode. But at a certain pH, the aminoacid molecule is electrically neutral, where the number of positive charges equals the number of negative charges.

When the aminoacid is in the zwitter ion state it will not move to the electrodes in an electric field. Hence they are electrically neutral. The pH, at

which an aminoacid has no tendency to move either to the positive or negative electrode is called isoelectric point or isoelectric pH. At this pH the aminoacid molecule bears a net charge of zero. The isoelectric point is symbolized by pI. The isoelectric point varies from one aminoacid to another.

When an acid is added to a zwitter ion amino acid, the aminoacid becomes positively charged and it moves to a cathode. The carboxylate ion of (-coo) of the zwitter ion aminoacid combines with the proton (H^+) of the acid (Hcl) to produce an acid (COOH) bearing a net positive charge. Hence it moves to the cathode.

When an alkali is added to a zwitter ion aminoacid, the aminoacid becomes negatively charged and it moves to an anode. The NH_3 (ammonian ion) of the zwitter ion aminoacid liberates a proton (H^+) and the aminoacid becomes an organic salt bearing a negative charge.



2.1.2.6 The chemical reactions of amino acids

The characteristic organic reactions of aminoacids are those of their functional groups in the carboxyl groups, the α amino groups and the functional groups present in different side chains. Knowledge of these reactions is useful in several important aspects of protein chemistry.

1. Identification and analysis of aminoacids in protein hydrolysates.
2. Identification of aminoacid sequence in protein molecules.
3. Identification of the specific aminoacid residues of nature proteins that are required for their biological function.
4. Chemical modification of aminoacid residues of nature proteins that are required for their biological function.
5. Chemical synthesis of polypeptides.

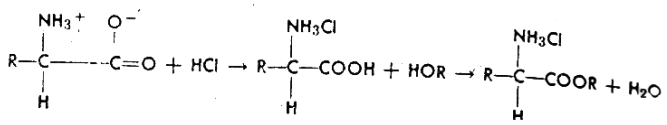
1) Reactions of carboxyl groups

The carboxyl groups of all aminoacids well known organic reactions leading to the formation of amides, esters and acid halides.

a) Formation of esters

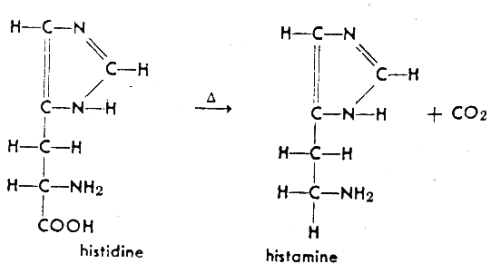
Aminoacid reacts with alcohol in the presence of Hcl to form esters.

In the reaction fist, Hcl breaks up zwitter ion structure to form the aminoacid hydroxychloride which then reacts with the alcohol (ROH) to form the ester hydrochloride.



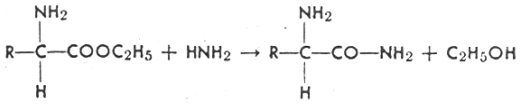
b) Decarboxylation and the formation of amines

When aminoacids are heated in the presence of barium hydroxide, CO_2 is lost and an amine is formed. Here the carboxyl group is removed and hence the process is called decarboxylation.



c) Amide formation

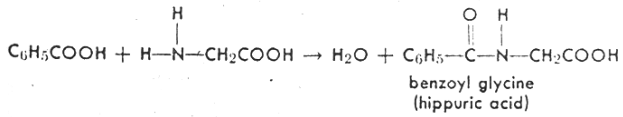
Aminoacid esters form amides when treated with anhydrous or alcoholic ammonia.



2)Reactions of Amino groups

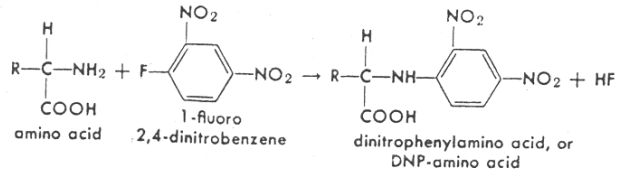
a) Benzoylation of Aminoacid

Aminoacids yield benzoyl derivatives with benzoic acid. For example glycine reacts with benzoic acid to form Benzoyl glycine. In this reaction the amino groups condense with benzoic acid to form hippuric acid. This process occurs in man as a process for detoxication of benzoic acid.



c) Reaction with Sanger's reagent

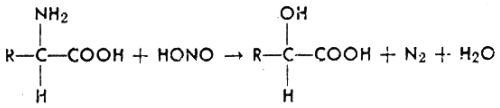
The amino groups of aminoacids react with Sanger s reagent 1-glouco – 2,4-dinitrobenzene (FDN 13). In this reaction, the reagent (FDNB) condenses with free amino groups in cold mild alkaline solution (bicarbonate) to give dinitrophenyl amino acid.



With the help of this Sanger determined the sequence of aminoacids in insulin.

d) Reactions of Amino acids with Nitrous Acid

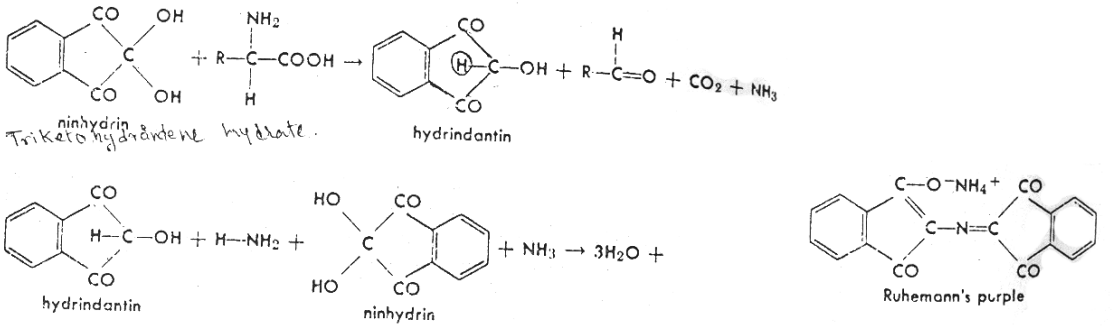
Aminoacid reacts with Nitrous acid to form the corresponding hydroxy acids with the liberation of N_2.



e) Ninhydrin Reaction (Oxidative Deamination)

The amino group of an amino acid may be easily removed by oxidation. This process is called oxidative deamination. When amino acid is heated with

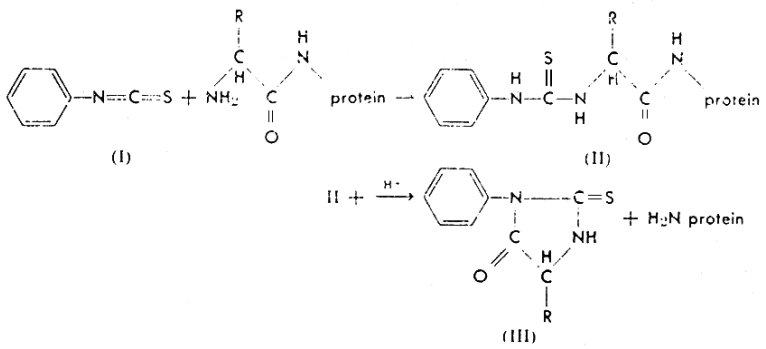
Ninhydrin (triketo hydrindene hydate, the aminoacid is deaminated to NH_3 and ketoacid by oxidative deamination. The reaction occurs in two stages.



The ninhydrin reaction is used for the quantitative estimation of aminoacids, peptides and proteins.

f) Edmann Reaction

Phenylisothiocyanate reacts with amino group of aminoacid under alkaline condition to form phenyl isothiocarbamyl peptides.



G_ Siegfried's carbamino reaction

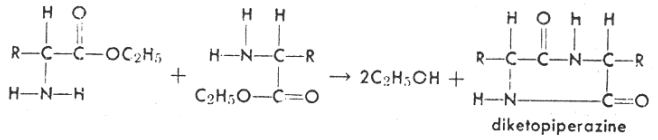
The free amino group of aminoacid can condense with CO_2 to form carbamino compounds. This reaction is called Siegfried's carbamino reaction.

$$R\text{-}NH_2 + CO_2 \rightarrow R - N - Coo^- + H^+$$

Carbamino reaction

h)Formation of Diketo piperazines

The esters of the aminoacids condense to form anhydride ring structures containing two amino acid groups. These substances are called diketopiperazines.



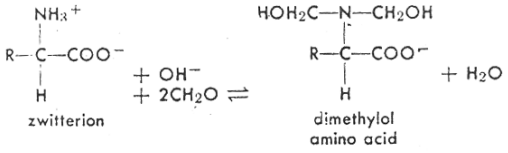
i) Sorensen's Formal Titration

The method of titrating aminoacids with alkalis is termed as the Sorensen formal titration.

The monoamino mono carboxylic aminoaids (aminoacids having one NH_2 group and one carboxyl group) behave both as an acid and as a base. Hence the carboxyl group of α-aminoacids cannot be accurately titrated in water solution with alkali because it reacts with the basic amino group to form zwitter ions that are not decomposed completely at the end point of alkaline indicators (phenol phthalein) Sorensen observed that if aminoacid solutions are neutralized to phenolphthalein and treated with a large excess of neutralized formaldehyde solution, the mixture becomes acid and can be titrated sharply to phenolphthalein with standard alkali. The amount of alkali required for this titration was found to correspond to the complete titration of the carboxyl group of the aminoacid.

The reaction of formaldehyde with aminoacids in the Sorensen titration is complex. The amino group combines with formaldehyde to form dimethylol aminoacid which is an aminoacid formaldehyde complex.

In this way, the existence of all the amino groups are eliminated resulting in an acidic solution due to the presence only of the carboxyl groups. This solution can be titrated with standard alkali, using phenolphthalein as indicator. The amount of alkali required indicates the amount of carboxyl groups present.



2.1.2.8 Summary

1. Proteins are polymers composed of L-α aminoacids. They are 20 in number and classified into different groups based on their structure, chemical nature, nutritional requirement and metabolic fate.

2. Aminoacids posses two functional groups namely carboxy (-COOH) and amino (-NH_2). In the physiological system, they exist as dipolar ions commonly referred to as zwitter ions.

3. Besides the 20 standard amino acids present in proteins, there are several non-standard aminoacids. These include the amino acid derivatives found in proteins.

Ex: Hydroxy proline,hydroxy lysine), non –protein aminoacids (Ex: ornithine, literlline) and D-aminoacids.

2.1.2.8 Model Questions

1. Give an account of classification of aminoacids?

2. Comment on essential and non-essential aminoacids.

3. Give an account of structure and significance of aminoacids.

4. Give an account of properties of aminoacids?

2.1.2.9 Reference Books

Biochemistry – Saras Publication

Biochemistry – U.Satyanarayana.

Lesson 2.1.3

STRUCTURE AND BIOLOGICAL SIGNIFICANCE OF LIPIDS

Objective

Objective

Lipids are a diverse group of compounds, present as structural components of cell membranes. In this chapter the structure, classification and properties of lipids were clearly explained

2.1.3.1 Introduction

The lipids are organic substances insoluble in water but soluble in organic solvents like chloroform, ether and benzene. They are esters of fatty acids or substances capable of forming such esters and are utilizable by living organisms. They form important dietary constituents on account of their high calorific value and the fat soluble vitamins and the essential fatty acids contained in them. In the body they are present in the cytoplasam as well as the cell wall and are also in specialized areas in the body as deposits of fat in which form energy is stored. Nervous tissues are particularly rich in lipids which appear to serve an important role in their function. The subcuntaneous fat serves the role of insulating against atmospheric heat and cold and also helps in rounding off the counters of the body.

2.1.3.2 Classification :

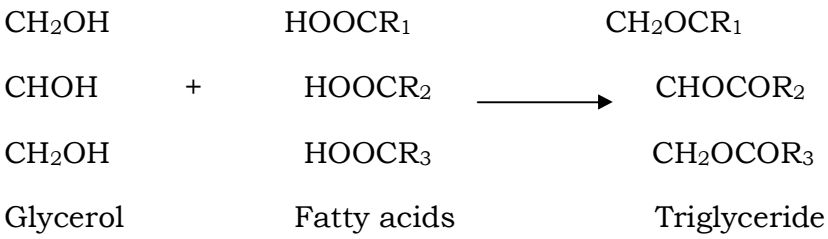
Lipids are classified as follows.

1. Simple lipids : (i) Neutral fats (ii) Waxes
2. Compound lipids : (i) Phospholipids (ii) Glycolipids (iii) Lipoprotiens and others.
3. Derived lipids : (i) Fatty acids (ii) Sterols

2.1.3.3 Simple lipids

1. Neutral fats

Neutral fats are the fatty acid esters of the trihydric alcohol, glycerol. They are also known as the triglycerides. They are also known as the triglycerides or triacylglycerols. Some are solids at room temperature while others are liquids. The latter are sometime referred to as oils.



Glycerol is optically inactive. But when it is etherified by two different fatty acids in positions 1 and 3, the carbon in position 2 becomes asymmetric and may be considered to be derived from L-glyceraldehyde.

R_1, R_2 and R_3 may be same or different fatty acids. Depending on the fatty acids present, they are called tripalmitin, tristearin, palmito-oleio-stearin, etc.

a. Physical properties

1. Neutral fats are colorless, odorless, tasteless substances.
2. They are insoluble in water, but soluble in organic solvents.
3. They have well defined melting points and solidifying points.
4. They have low specific gravity and float on water. Oils spread on water to form thin monomolecular layers.

2. Chemical properties

1. Hydrolysis : Heating with super heated steam or boiling with acids or alkalies will produce hydrolysis of the neutral fat into glycerol and fatty acids.

2. Additive reactions : The unsaturated fatty acids present in the neutral fat will exhibit all the additive reactions (hydrogenation, halogenation). Oils which are liquid at ordinary temperatures on hydrogenation become solidified. This is the basis for vanaspathi manufacture, where inedible and cheap oils like cotton seed oil are hydrogenated and converted to edible fat.

3. Oxidation : Fats are very rich in unsaturated fatty acids such as lime seed oil undergo spontaneous oxidation at the double bond forming aldehydes, ketones and resins which form thin transparent coating on the surfaces to which the oil is supplied. These are called drying oils and are used in the manufacture of paints and varnishes.

4. Rancidity : Naturally occurring fats, partially those from animal sources, are contaminated with enzymes like lipase. The action of enzymes and also atmospheric moisture and temperature bring about partial hydrolysis of the fat and some degree of oxidation of the unsaturated fatty acids at the double bond. The fats will develop a characteristic taste and odor. The process is called rancidity and the fat is said to have become rancid. Vegetable fats contain substances like vitamin E, phenols, hydroquinone, tannins and others which are antioxidants and therefore prevent development of rancidity. Hence vegetable fats preserve for longer periods than animal fats.

2. Waxes

They are of the nature of insect secretions or protective coating an animal furs and leaves. Chemically they are esters of higher fatty acids with higher monohydroxy alcohols. Free fatty acids, alcohols and some other hydrocarbons are also present mixed with ester. A few of the waxes are listed below.

Bees wax : Palmitic acid ester of myricyl alcohol ($C_{30}H_{61}OH$).

Lanoline or wool fat : Palmitic, oleic or stearic acid ester of `cholesterol', a complex structure which will be dealt with in detail under `sterols'.

Lanoline is useful in the manufacture of cosmetic creams, ointments etc., since it closely resembles sebaceous secretion.

Spermaceti: Palmitic acid ester of cetyl alchohol ($C_{16}H_{33}OH$).

It is an oil from the head of the sperm whale and is useful in the manufacture of polishes, ointments, candles, etc.

2.1.2.4 Compound lipids

Besides fatty acids and alcohol, they also contain other groupings.

1. Phospholipids

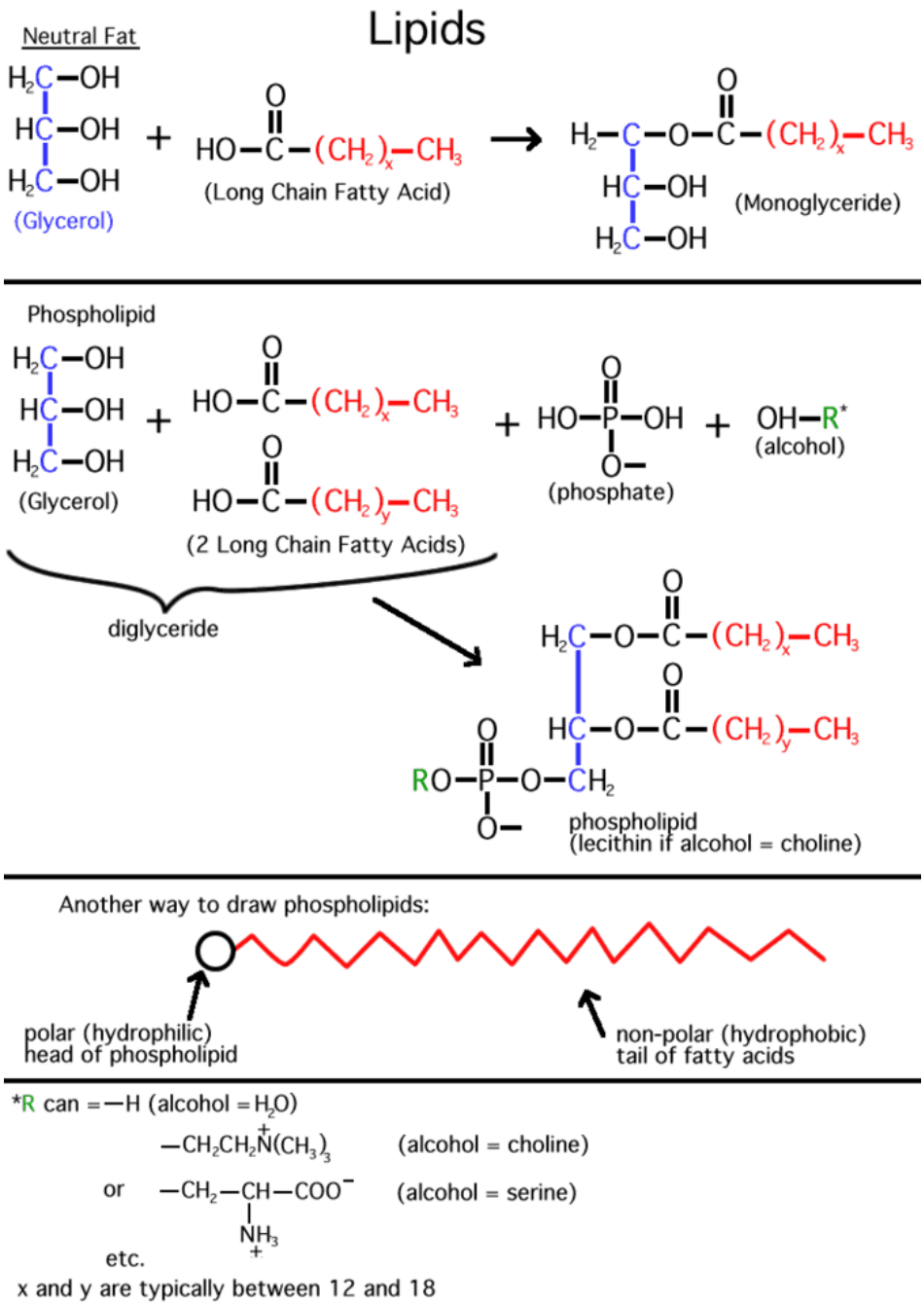
Phospholipids are insoluble in dry acetone. They are present in abundance in brain and nervous tissues. Varying amounts are present in every living cell of plant or animal origin. They are present both in cytoplasm as well as cell membranes and serve important functions in both cell activity and cell permeability. They are of importance (like the rubber or plastic covering around an electric wire) from the surrounding structures and in channeling out the enzymes into distinct groups. They also form important intermediate substances in the transport of lipids from and to the liver. They are made up of fatty acid, glycerol or other alcohol, nitrogenous base and phosphoric acid.

a. Classification of phospholipids

Phospholipids are classified based on the alcohol moiety of the phospholipid.

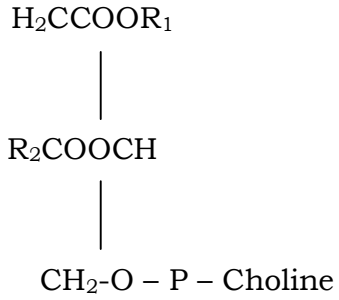
a. Glycerophosphatides

Glycerol is the alcohol in this group. This include lecithins, cephalins (phosphotidyl ethanolamine), phosphatidyl serine, plasmalogens and diphosphatidyl glycerols.



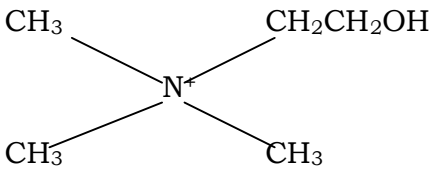
i. Lecithins or phosphatidyl cholines

They are widely distributed in the body, but particularly rich in liver. They are also present in plasma. They contain glycerol, fatty acid, phosphoric acid and a quaternary base `choline'. The structure is given below.



The structure shown is that of an alpha lecithin. In beta lecithin, the two fatty acids occupy the alpha positions while the base and phosphoric acid occupy the beta position. A large number of lecithins exist differing in their fattyacid moiety.

The base `choline' which has the following structure is itself a very important substance in the prevention of accumulation of abnormal amounts of fat in the liver (lipotropic action of choline) and also as a constituent of acetylcholine, so important in the transmission of nerve impulses.



Choline

Snake venom owes part of its toxicity to the presence of lecithinases – enzymes which hydrolyze the lecithins.

Lecithins lower the surface tension of water and aid in emulsification of lipid water mixtures, a prerequisite in the digestion as well as absorption of lipids from the gastrointestinal tract. In the plasma, they serve the very useful function of keeping cholesterol and its ester in the dissolved state. If the base, choline, is removed, the resulting structure is called `phosphatidic acid'.

ii. Phosphatidyl ethanolamines (cephalins) : They are structurally similar to lecithins except that the base is `ethanolamine' (NH_2 CH_2 CH_2OH). They occur together with lecithins and are particularly concentrated in the brain. They are also present in the erthrocyte.

iii. Phosphatidyl serine : Similar in structure to lecithins, but the base is an amino acid serine ($OHCH_2CH$ NH_2 COOH). Occurrence and functions are also similar to lecithins.

iv. Plasmalogens : They form about 10% of the total phosphatides of muscle and brain. One of the fatty acids is replaced by a long chain aliphatic aldehyde. The aldehyde is in the enolic form (R CH = CH OH) and combines with the alfa carbon of glycerol by an ether linkage to form $CH_2OCH = CHR$. The remaining two carbons of glycerol have similar structure as in cephalins. The base is choline or ethanolamine.

v. Diphosphatidyl glycerols : These are two molecules of phosphatidic acid combined by a bridge of glycerol. Cardiolipin, initially isolated from heart muscle, is an example. It forms the basis for a serological test for the diagnosis of syphilis.

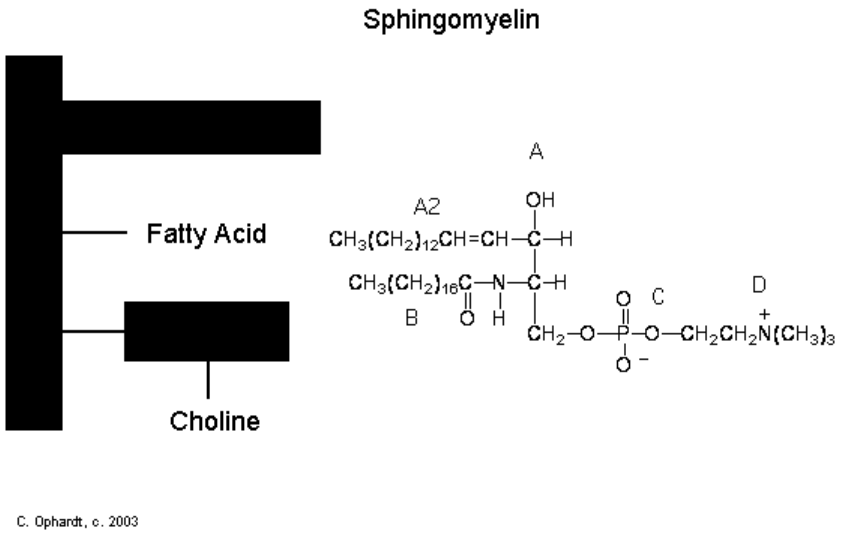
B. Phosphoinositides

In these the cyclic hexahydric alcohol `mositol' replaces the base. Depending on whether there is only one phosphate (in alpha position) attached to inositol (in which case there are two fatty acids attached to other two carbons) or two phosphates in the two alpha positions joined together by the inositol as bridge (in which case there is only one fatty acid esterifying the beta position), they are called a `monophosphoinositide' or `diphosphoinositide'.

C. Phosphosphingosids or sphingolipids

In this group of substances the trihydroxy alcohol, glycerol, is replaced by a complex amino alcohol, `sphingol'. Fatty acid, choline and phosphoric acid are the other constituents.

The structures of sphingol an sphingomyelin are given below.



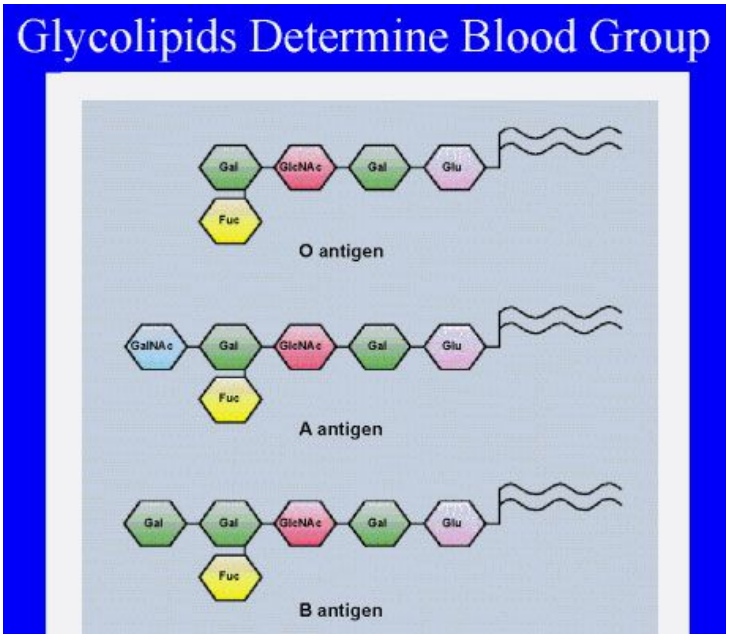
2. Glycolipids, Glycosphingosides or Cerebrosides

These contain sphingol, a carbohydrate – galactose, and fatty acid. They do not contain phosphoric acid. Hence they are not phospholipids, but are called galactolipids or glycolipids. They are present in large amounts in the white matter of the brain and in the myeline sheathes of nerves. In Gaucher's disease they accumulate in large amounts in the liver and spleen.

Gangliosides

They contain 6% of total lipids in gray matter. They are glycosphingolipids. The carbohydrate moiety contains one or more residues of sialic acid. In human brain, the gangliosides contain N-acetylneuraminic acid as the sialic acid component. It is made up of a molecule of N-acetyl, D-mannosamine combined with a molecule of pyruvic acid. The glycosphingo lipids have important functions. As constituents of cell membranes, they are responsible for the blood group specificity and tissue and organ specificity. They are also responsible for tissue immunity and for cell-cell recognition sites. Gangliosides are particularly abundant in nerve endings. They may function in transmission of nerve impulses across synapses. They are also present in the receptor sites for acetylcholine and other neurotransmitter substances.

Gangliosides accumulate in brain in Tay-sach's disease due to genetic lack of the enzyme required for its degradation



Terpenes

They are polymers of the five-carbon hydrocarbon "Isoprene". The side chains in vitamins A, E and K, beta carotene and squalene are examples of terpenes. Natural rubber is a polyterpene containing hundreds of isoprene units in regular linear order.

2.1.2.5 Derived lipids

This group consists of (1) fatty acids and (2) sterols.

1. Fatty acids

They contain the elements C, H and O. Most of the naturally occurring fattyacids are straight chain derivatives and have an even number of carbon atoms. They may be saturated or unsaturated. A few are cyclic.

Saturated fatty acids : They can be represented by the general formula $CH_3(CH_2)_nCOOH$. The simplest is acetic acid (n=o). Some of the acids in the series are listed below.

Acetic acid	CH_3COOH	2
Butyric acid	$CH_3(CH_2)_2COOH$	4
Caproic acid	$CH_3(CH_2)_4$ COOH	6
Caprylic acid	$CH_3(CH_2)_6$ COOH	8
Capric acid	$CH_3(CH_2)_8$ COOH	10
Lauric acid	CH_3 $(CH_2)_{10}$ COOH	12
Myristic acid	$CH_3(CH_2)_{12}$ COOH	14
Palmitic acid	$CH_3(CH_2)_{14}$ COOH	16
Stearic acid	$CH_3(CH_2)_{16}$ COOH	18
Arachidic acid	$CH_3(CH_2)_{18}$ COOH	20
Lignoceric acid	$CH_3(CH_2)_{22}$ COOH	24

Unsaturated fatty acids

They contain one or more double bonds. Some of the important members of this series are listed below.

Palmitic acid

A derivative of palmitic acid with a double bond between the 9th and 10th carbons. The carbons are counted from the carboxylic end.

Elaidic acid is the trans-isomer of oleic acid. It is not present in naturally occurring fats, but is formed during the hydrogenation of fats (eg. In vanaspathi). Consumption of these hydrogenated fats may cause the appearance of elaidic acid in human fat also.

Linoleic acid

It is derived from stearic acid with two double bonds, one between C_9 and C_{10} and a second one between C_{12} and C_{13}.

Linolenic acid

It is derived from stearic acid with three double bonds between C_9 and C_{10}; C_{12} and C_{13}; C_{15} and C_{16}.

Arachidonic acid

Derivative of arachidic acid with four double bonds between C_5 and C_6; C_8 and C_9; C_{11} and C_{12}; C_{14} and C_{15}.

Linoleic and linolenic acids are present in plant sources. Archidonic acid is present only in mammalian tissues. It is synthesized from linoleic acid. Prostaglandins are synthesized from archidonic acid.

Cyclic fatty acids

Chaulmoogric oil, once used in the treatment of leprosy, contains cyclic fatty acids called chaulmoogric and hydrocarpic acids.

a. Physical properties

1. The lower fatty acids are liquids at room temperature some of them are soluble in water and are steam volatile.

2. The higher fatty acids are solids at room temperature and are insoluble in water but soluble in organic solvents. Except acetic acid, all other fatty acids are lighter than water.

b. Chemical properties

1. Formation of esters with alcohols : The esters of fatty acids with the trihydric alcohol, glycerol, are called neutral fats or triglycerides. Esters with some higher monohydroxy alcohols are called waxes.

$$R\ COOH + C_2H_5OH \longrightarrow R\ COOC_2H_5 + H_2O$$

2. Formation of soaps with alkalies

$$RCOOH + NaOH \longrightarrow R\ COO\ Na + H_2O$$

The soaps of sodium and potassium are useful in daily life. Sodium soaps are hard. Potassium soaps are soft but costly. To make the sodium soaps usable as toilet soaps, sodium carbonate or silicate is added in small amounts. This will make soap lather even with hard water.

Shaving soaps are usually potassium soaps using coconut oil or palm oil as the source of fatty acids. To make the soap less alkaline and more smooth to the skin, excess of fatty acids are used.

Zinc stearate is a soft powder which is non-irritant to the skin and water repellent. It is commonly used in dusting industries. Calcium and magnesium soaps are insoluble in water and do not lather. Hard water which contains salts of calcium and magnesium is hence unsuitable for washing purposes.

Detergents : They are non-soap cleaning agents. They contain sodium salts of lauryl sulfuric acid, sulfated lauryl monoglyceride or salts of long chain fatty acids with quaternary ammonium as ingredients. Like soaps, they are also good wetting agents and emulsifiers. They have the advantage that they can lather equally well in hard water.

3. Reactions due to double bond of unsaturated fatty acids :

i. Hydrogenation

Unsaturated fatty acids can under suitable conditions of temperature and pressure and in the presence of catalysts, take up hydrogen at the double bond to form the corresponding saturated fatty acid.

$$RCH = CH \ldots\ldots COOH + 2H \longrightarrow R\ CH_2\ CH_2 \ldots\ldots COOH$$

(Oleic acid) (Stearic acid)

ii. Halogenation

The double bond can also be saturated by taking up Cl, Br or I under appropriate conditions.

RCH = CH ……. COOH + Br_2 ⟶ R CH Br CHBr ….. COOH

iii. Oxidation at the double bond

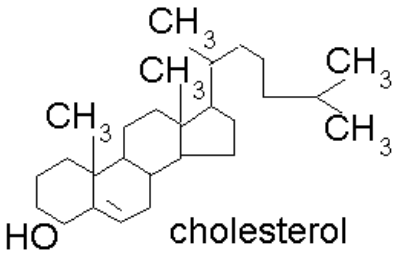
The carbons at the double bond are readily oxidizable. A number of intermediate products like hydroxy acids, aldehydes and peroxides are formed and finally, the fatty acid is broken down at the double bond to form a smaller fatty acid and a dicarboxylic acid.

R CH = CH ……. COOH + O ⟶ R COOH + HOOC … COOH

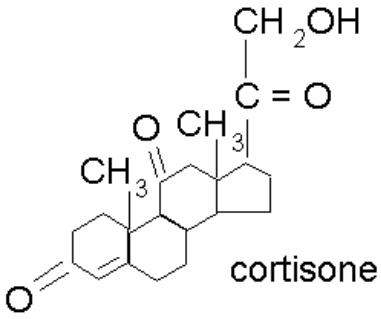
2. Sterols

These are derivatives of a complex ring system called ` Cyclopentanoper hydrophenanthrene' ring system. The meaning of the name and the method of naming the individual compounds of system as A, B, C, D rings and numbering of the seventeenth positions in the ring system are depicted in figure below.

Hormones



cholesterol

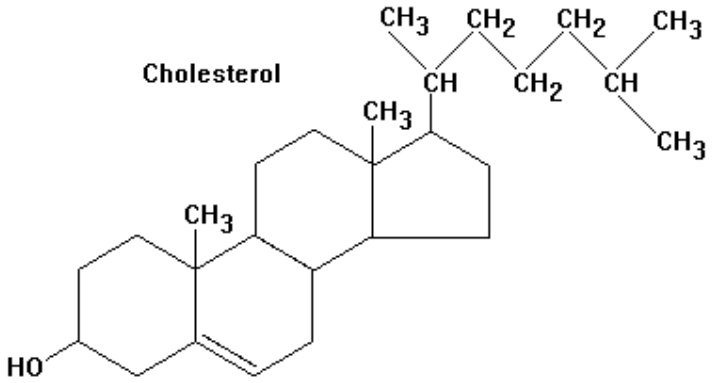


cortisone

Cholesterol, ergosterol, bile acids, sex harmones, adrenal cortical hormones and the D-vitamins are some of the important sterol derivatives. The term sterol means `solid alchohol'.

Cholesterol

Since it was first isolated from gall stones, it was called cholesterol which means `solid alcohol from bile'. Brain, nervous, tissues, adrenal glands and egg yolk are rich sources. White matter contains as much as 14%, gray matter 5%, spinal cord 12% and liver about 1% cholesterol. It has a molecular formula $C_{27}H_{45}OH$ and has a structure shown above.



Saturation of the double bond between C_5 and C_6 will give dihydrocholesterol.

Coprosterol is the cis isomer of dihydrocholesterol and is found in feces.

While the ring structure shown in fig above is in common use, the three dimensional structure of the steroid molecule is more correctly represented if the rings are replaced by chair or boat forms. Between the two, the chair form is more stable.

Further, the CH_3 in position 10 is considered to be in the beta position, i.e projects above the plane of paper. If it in position 5 is in the same plane as the CH_3, the rings are in the cis-configuration and the steroid is said to belong to the beta series, and the bond linking H is shown as an unbroken line.

If H in position 5 is in the opposite plane to that of CH_3 (i.e, below the plane of the paper), the bond is represented by a broken line and the steroid belongs to

the alfa series. Rings A and B are in the trans position to each other. These concepts and structures are illustrated in fig. To cholesterol. (in which the double bond of cholesterol between C_5 and C_6 is saturated).

Cholesterol belongs to the alfa series. The H at position 5 is in the transposition to the CH_3 at position 10. The OH at position 3 is however is in c is relationship with the CH_3 at position 10. It has double bond between C_5 and C_6, in the ring B.

a. Physical properties of cholesterol

1. It is a white crystalline substance showing the usual solubility properties of the lipids. The crystals are rhombic plates with one of the angles broken.

2. It has a melting point of 149^o.

3. It is usually prepared in the laboratory by extraction from brain or spinal cord with acetone. It is a poor conductor of electricity and functions probably as an insulating mechanism for the nerve impulses. As a precursor of bile salts, steroid hormones and vitamin D_3 it is of great significance. It is present in blood to the extent of 150-250 mg per 100 ml and its variations are of considerable clinical significance.

b. Chemical properties

1. The double bond can be saturated by addition of hydrogen to form the dihydroderivative.

   It can also be halogenated.

2. The OH group in position 3 can be etherified with fatty acids to form cholesterol esters.

3. Three fourths of the cholesterol of plasma exists as ester.

4. The OH combines with digitonin to form an insoluble digitonide. This property is used to separate cholesterol from its esters which do not react with digitonin.

Color reactions

i. Salkowski reaction : When a solution of cholesterol in chloroform is shaken with an equal volume of concentrated sulfuric acid and the layers are allowed to separate, the chloroform layer turns red and the acid layer shows a greenish fluorescene.

ii. Lieberman-Burchard reaction : To a solution of cholesterol in chloroform, if a few drops each of acetic an hydride and conc. Sulfuric acid are added, a rose-red color develops and rapidly changes to blue and finally green. A modified reagent containing ferric chloride, glacial acetic acid and the sulfuric acid gives a purple color.

3. Other sterols

Ergosterol (C_{28} H_{43} OH)

Ergo sterol present in fungi, yeast and other vegetable matter. Besides differing in the side chain from position 17, it has also an additional double bond between C_7 and C_8. This is the precursor for vitamin D_2 (calciferol) while 7-dehydrocholesterol is the precursor for vitamin D_3.

Bile acids

The parent structure is `cholanic acid'. The side chain at position 17 is oxidized to end in a COOH. Three different bile acids occur in nature. They differ only in the number of ring carbons oxidized to form an OH.

3-hydroxy cholanic acid is lithocholic acid.

3, 12 – dihydroxy cholanic acid is disoxycholic acid.

3,7,12 – trihydroxy cholanic acid is cholic acid.

They are conjugated in the liver with `glycine' or `taurine' to form `glycocholic' or `taurocholic' acids which combine with sodium to form the bile salts. The bite salts have a remarkable ability to lower surface tension and thus help in emulsification of fats.

Sex hormones and adrenal cortical hormones have also a steroid structure.

2.1.2.6 Summary

The lipids are organic substances insoluble in water but soluble in organic solvents like chloroform, ether and benzene. Lipids are classified as follows.1.Simple lipids (Neutral fats and Waxes)2.Compound lipids (Phospholipids ,Glycolipids ,Lipoproteins and others.)3.Derived lipids (Fatty acids and Sterols). Neutral fats or triglycerides are the fatty acid esters of the trihydric alcohol, glycerol. Waxes are esters of higher fatty acids with higher monohydroxy alcohols. Besides fatty acids and alcohol, compound lipids also contain other groupings. Depending on the group attached they are named as phospholipids (if phosphate

is attached)glycolipids(if carbohydrate is present).where as the derived lipids are the products of simple and compound lipid hydrolysis.

2.1.2.6 Model questions

1) Classify the lipids based on their structure

2) Write a detailed account on Sterols.

2.1.2.7 Reference books

Biochemistry – Saras Publication

Biochemistry – U.Satyanarayana.

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Lesson 2.1.4

NUCLEIC ACIDS

Objective

Types of Nucleic Acids

Components of Nucleic Acids

A. Phosphoric acid

B. Pentose sugar

C. Nitrogenous bases

D. Nucleosides

DNA

A. Chargaff's rule

B. Double helix structure of DNA

C. Characteristics and properties of DNA

i. Molecular weight

ii. Length of DNA

iii. Shape and size

iv. Antiparallelity

v. Base composition

vi. Denaturation and Renaturation

vii. Effect of pH

viii. Effect of temperature

D. Different forms of DNA

a. B-DNA

b. A – DNA

c. C – DNA

d. D – DNA

e. Z – DNA

2.1.4.4. Ribonucleic Acid (RNA)

A. r RNA

B. t RNA

C. m RNA

2.1.4.5. Biological Significance of Nucleotides

1. Building blocks of nucleic acids
2. Chemical energy carriers
3. Coenzyme components
4. Signal transduction

2.1.4.6 Summary

2.1.4.7 Model Questions

2.1.4.8 Reference Book

Objective

The structure of every protein and ultimately every cell constitutes, is a product of information programmed into the nucleotide sequence of a cell's nucleic acid. The nuclei of pus cells was first isolated by Friedrick Miescher in 1869. The purine and pyrimidine bases were first discovered by Fischer in 1880's.

Two types of nucleic acids are DNA and RNA. Nucleic acids are the biopolymers of mononucleotides. These nucleotides will be linked with phosphodiester linkage. The objective of the lesson is to study the detailed structure of the nucleic acids and their significance.

2.1.4.1. Types of Nucleic Acids

There are two kinds of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Both types of nucleic acids are present in plants, animals and prokaryotes except in viruses. The viruses will possess either RNA or DNA, but not both. The DNA is found mainly in the chromatin of the cell nucleus, where as most of the RNA is present in the cell cytoplasm and a little amount in the nucleolus. The extranuclear DNA also exists as mitochondrial DNA and chloroplasts DNA in animals and plants. The extra chromosomal DNA in bacteria and yeast, which has autonomous replication characters is called plasmid.

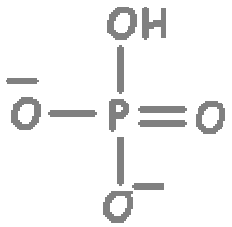
2.1.4.2. Components of Nucleic Acids

In the structure of nucleic acids sugar and nitrogen bases and phosphoric acid are components. The compounds in which nitrogen bases are conjugated to the pentose sugars are called nucleosides. The nucleosides conjugated with phosphoric acid are called nucleotides. The nucleotides are the building blocks of nucleic acids.

In the case of DNA and RNA phosphoric acid is common. The sugar moiety varies in DNA it is deoxy ribose and in RNA sugar molecule is ribose. The common nitrogen bases in DNA are adenine, guanine, cytosine and thymine. In case of RNA instead of thymine uracil is the component.

A. Phosphoric acid

The phosphoric acid contains three monovalent hydroxyl groups and a divalent oxygen atom, all linked to the entavalent phosphorus atom.



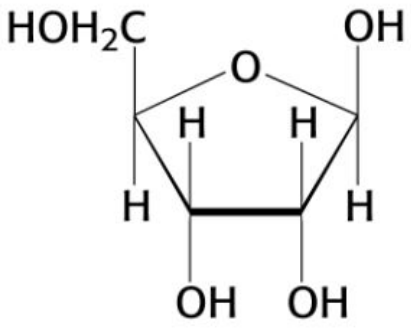
Phosphoric acid

The phosphoric acid of one nucleotide forms phosphodiester bond with 3^1 hydroxyl group of its succeeding nucleotide. The phosphodiester bond forms the backbone structure of nucleic acids.

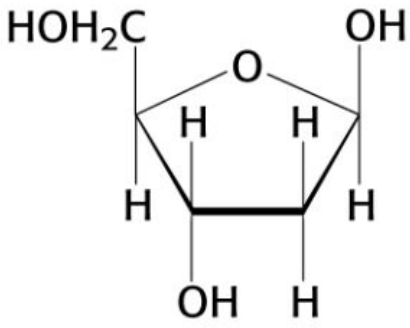
B. Pentose sugar

The two types of nucleic acids are distinguished primarily on the basis of pentose they posses. The nucleic acid having D-2-deoxyribose is called DNA and that having D-ribose is called RNA. Both these sugars in nucleic acids are present in the furanose form and are of B-configuration.

The important property of the pentoses is their ability to form esters with phosphoric acid. In the formation of phospho diester bond C_3 and C_5 of sugar are involved.



D-Ribose



2-Deoxy-D-ribose

C. Nitrogenous bases

Two types of nitrogeneous bases are seen in the nucleic acids. These are derivatives of purine and pyrimidine compounds. Depending on this the nitrogeneous bases are of two types.

i.purine

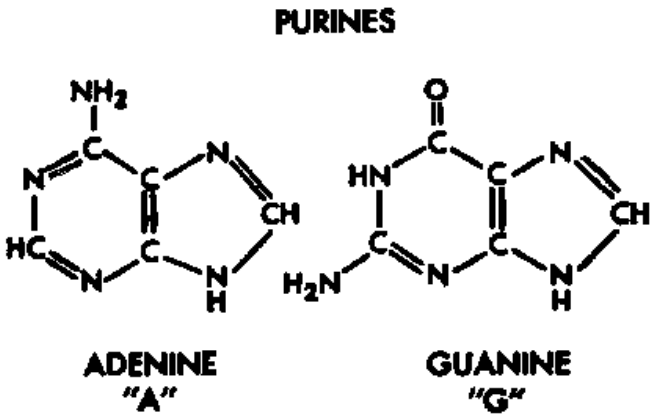
ii.pyrimidines.

sometimes these purine and pyrimidine derivatives are modified with some reactions like methylation. Those are modified nitrogeneous bases.

i. Purines

These are derived from their parent compound purine, which contains a six membered pyramidine ring fused to the five membered imidazole ring.

The prevalent purine derivatives found in nucleic acid are adenine and guanine.



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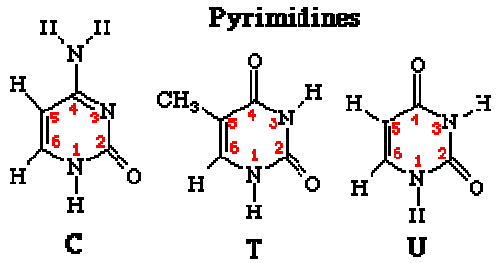
ii. Pyrimidines

These are derived from the parental heterocyclic compound pyrimidine.

The common pyrimidine derivatives found in nucleic acids are uracil, thymine and cytosine.



iii. Modified nitrogenous basis

Apart from the major 5 bases, some other minor bases called modified nitrogenous bases also occur in polynucleotide structures.

Modified pyrimidines are 5,6 – dihydrouracil, 5-methyl cytosine, 5-hydroxymethyl cytosine, 4-thiouracil, pseudouracil, etc., the 5-methyl cytosine is a common component of plant and animal DNA. 25% of cytosol in plant genome are methylated. The DNA of T-even bacteriophages of E.coli has cytosine in the form of 5-hydroxymethyl cytosine.

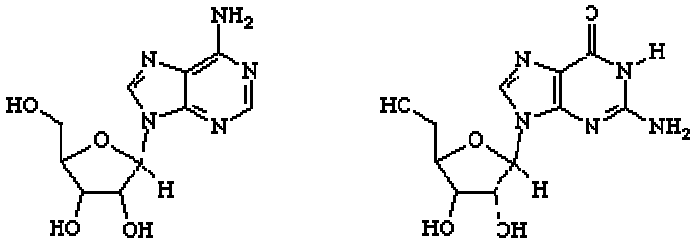
Modified purines are mostly methylated forms. Methylation of purines in DNA is now known to occur in the genetic material of microorganisms. 6-methyl adenine is found in bacterial DNA.

D. Nucleosides

The nucleosides are compounds in which nitrogenous bases are conjugated to the pentose sugar by a β-glycosidic linkage. The β-glycosidic linkage involves the C' – 1 of sugar and the hydrogen atom of N-9 of purines or N-1 of pyrimidines, thus eliminating a molecule of water. So the purine nucleosides are N-9 glycosides and the pyrimidine nucleosides are N-1 glycosides.

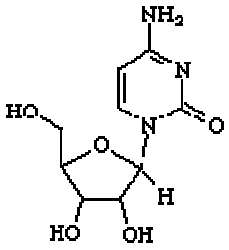
The nucleosides are generally named for the particular purine or pyrimidine present. Nucleosides containing ribose are ribonucleosides, while those possessing deoxyribose are called deoxyribose nucleosides.

The nucleoside analogues 3^1 – azo deoxy thymidine and 2^1, 3^1 – dideoxy cytidine have been therapeutically used for the treatment of acquired immune deficiency syndrome.

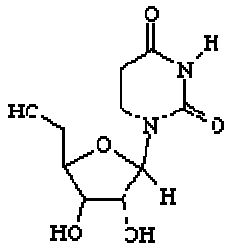


Adenosine

Guanosine



Cytidine



Uridine

E. Nucleotides

Nucleotides are the phosphoric acid esters of nucleosides. The phosphorylated forms of nucleosides are called nucleotides. Ex. AMP, ATP.

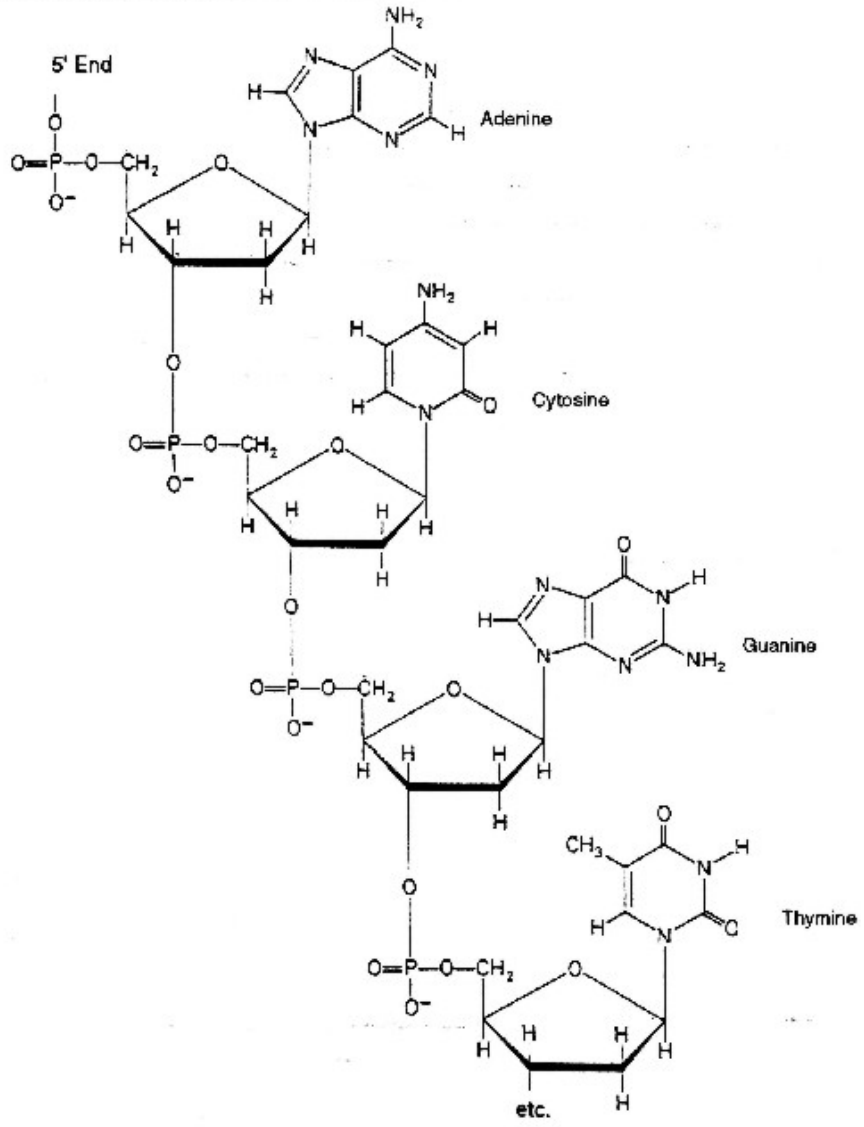
2.1.4.3. Deoxyribonucleic Acid (DNA)

DNA is the polymer of mononucleotides with phosphodiester linkage. The purine and pyrimidine bases of DNA carry genetic information where as the sugar and phosphate groups perform a structural role.

With the primary, secondary and tertiary structural organization of nucleic acid structure the DNA had a complex organization. The primary structure is the order of the nucleotides in the DNA chain with phosphodiester linkage. The secondary structure is double helix of DNA and the tertiary structure is the overall conformation.

FIGURE A–4

A PIECE OF DNA CONTAINING FOUR NUCLEOTIDES.



From Conn, E. E., Stumpf, P. K., Bruening, G., and Doi, R. H. (1987) *Outlines of Biochemistry*, 5th ed., John Wiley & Sons, New York, fig. 6.1. Reproduced with permission.

A.Chargaff's rules

The important rules proposed by Chargaff are :

i. The sum of purines = sum of pyrimidines

A+G = T + C

ii. The ratio of A/T or G/C is one

iii. The ratio of A+T/G+C is called dissymmetry ratio

When the dissymmetry ratio exceeds one the DNA is called AT type and when the ratio is less than one is called GC type

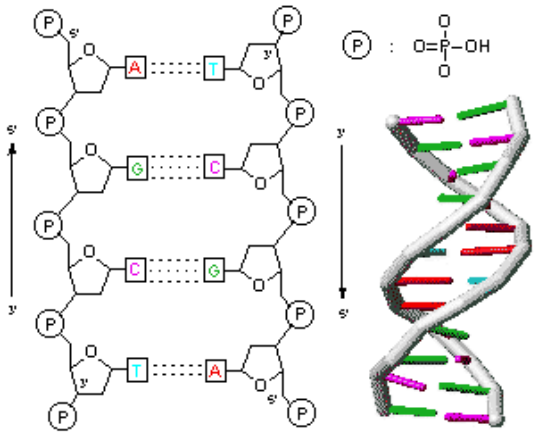
B. Double helix structure of DNA

Watson and Crick proposed the double helical DNA structure in 1953 for this discovery they got Nobel prize in 1962. The Salient features of the Watson – Crick model are :

i. The DNA double helical structure consists of two helical polynucleotide chains running anti parallel to one other i.e., one strand runs in $5^1 - 3^1$ direction and other strand runs in $3^1 - 5^1$ direction. The end of DNA having free 3^1 hydroxyl group without participating in phosphodiester bond is called 3^1 end. The end of DNA having free phosphate without participating in phosphodiester bond is called 5^1 end.

ii. The two helical chains of DNA are wound with one other producing two types of grooves – a major groove with 12A^o and a minor groove with 6A^o width. The two grooves arise as the glycosidic bonds of a base pair are not diametrically opposite each other.

iii. The two strands of DNA has no same sequence of bases because of base pairing is complementary to each other. i.e., if one strand of DNA contains adenine, the nucleotide of the second strand base paired with this must be thymine and vice versa. The G in one strand base pairs with C in other strand and vice versa.



iv. The strands in the double helix cannot be pulled apart. These can be separated only by an unwinding process. These coils like the DNA, which are interlocked about the same axis are called plectonemic coils.

v. The phosphate and sugar units are found on the periphery of the helix, where as nitrogenous bases occur in the centre.

vi. The diameter of helix is 20A^{o}. The distance between two successive bases is 3.4A^{o}. Each turn of helix contains 10 nucleotides.

vii. The entire structure of DNA molecule resembles a winding stair case, with the sugar and phosphate molecules of the nucleotides forming the rings and the linked nitrogen base pairs forming steps.

C. Characteristics and properties of DNA

i. Molecular weight

DNA molecules are largest biomolecules. The eukaryotes have high molecular weight DNA than the prokaryotes. There will be about 3000 nucleotides per million molecular weight of DNA. For example the molecular weight of *E.Coli* DNA is 2.6 x 10^6.

ii. Length of DNA molecule

The amount of DNA has been found to be constant from cell to cell and species. 31cm length DNA weigh ll picogram. For example human diploid cells contain 5.6 pg of DNA.

iii. Shape and size

The DNA molecules are characteristically highly elongated structures. The E.Coli chromosome is a double stranded circular DNA. It contains 4 x 10^6 bp. The length of E.Coli DNA is 1.4 mm and diameter is 20A^{o}. In humans 23 pairs of chromosomes with 3 billion base pairs and 1000 mm length per cell are present.

iv. Antiparallelity of the Chain

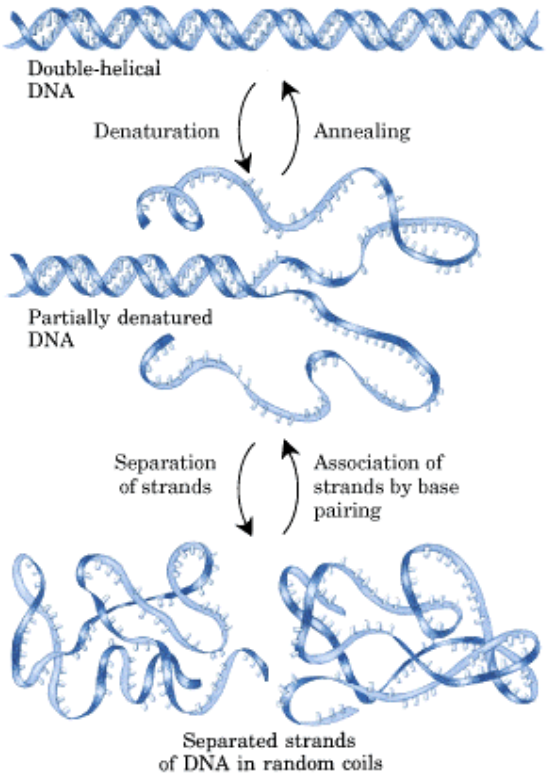
One strand of DNA runs in 5' – 3' direction and the other in the 3'-5' direction. Thus of the two chains one ascends and other descends.

v. Base complementality

Pairing between the nitrogenous bases of two strands always occurs between adenine and thymine and between cytosine and guanine.

vi. Denaturation and Renaturation

Denaturation of DNA is a loss of biological activity and is accompanied by cleavage of hydrogen bonds holding the complementary sequences of nucleotides together. This results in the separation of the double helise into the two strands. After denaturation DNA is converted to a flexible, single stranded denatured state.



The denaturation process brings some changes in DNA like

a. Increase in absorption of UV light.

b. Decrease in specific optical rotation

c. Decrease in viscosity.

Renaturation of DNA is regaining of the original conformation by rejoining of the two strands of DNA, which are separated by the denaturation.

Denaturation and renaturation depends on the concentration of AT and GC pairs.

vii. Effect of pH

Denaturation of DNA helix also occurs at acidic and alkaline pH values at which ionic changes of the substituents on the purine and pyrimidine bases can occur. Both at extreme acidic and basic pH conditions DNA denatures.

viii. Effect of temperature

The unwinding of the double helix is called melting, as it occurs at melting temperature. The melting temperature (T_m) is the temperature at which half the helical structure is unwinded. Since GC base pairs has 3 hydrogen bonds as compared to two for AT the DNA with high concentrations of G and c might be more stable and have a higher T_m, than those with DNA of high concentrations of A and T. The normal T_m for most organisms DNA is 77 to 100^o C.

D. Different forms of DNA

Under physiological conditions most of the DNA in bacteria or eukaryotes gonome is in classic Watson – Crick form which is called B-DNA. This is the reference form of DNA for any study of properties of DNA.

The DNA is remarkably flexible molecule because of its glycosidic linkage and phospho diester bridge. The DNA can also be kinked i.e., bent at discrete sites, where more than 4 adenine molecules successively. Because of these bending, stretching and unpairing of DNA is possible.

The structural variations do not affect the key properties of DNA molecule. Infact, adopt several 3-D conformations.

a. B-DNA

All the characters we have seen are of B-DNA.

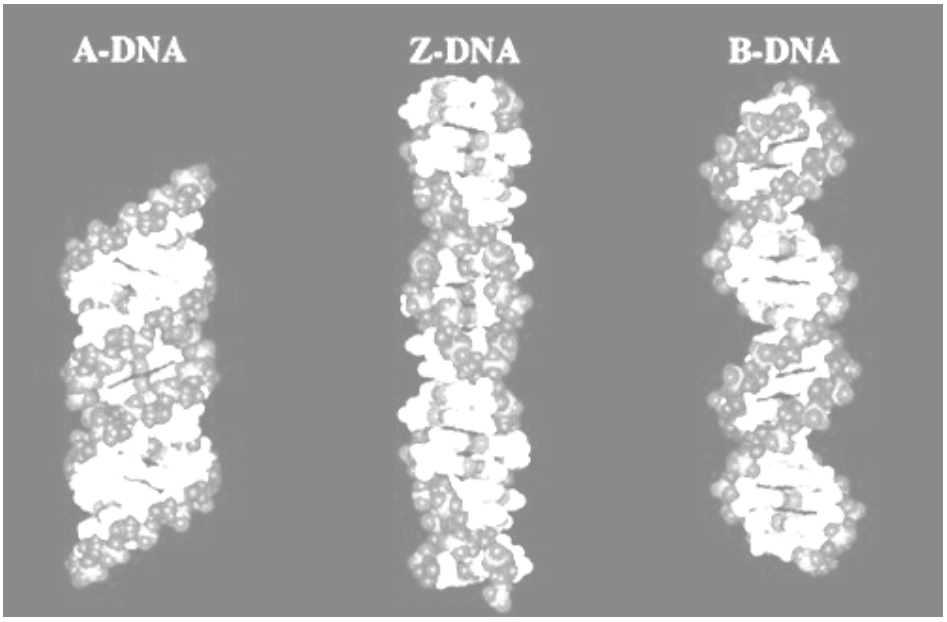
b. A-DNA

A-DNA appears when the B-DNA is dehydrated when humidity is only 75% and Na^+, IC^+, and Cs^+ ions present in the medium. This is because the phosphate groups in the A-DNA bind fewer water molecules than do phosphates in B-DNA. The difference of A-DNA from B-DNA are vertical rise of chain per base pair is only 2.3 A^o, number of base pairs per turn are 11, and diameter is 25.5 A^o. so the helix of A-DNA is wider and shorter than that of B-DNA.

e. Z-DNA

Z-DNA is the more radical departure from B-DNA and is characterized by a left – handed helical rotation instead of right – handed helical rotation in all other

forms. The phosphates in the DNA backbone are in a zig- zag manner hence the term z-DNA. 12 base pairs per turn in Z-DNA. The Z-form occurs in short oligonucleotides that have sequences of attending pyrimidine and purine bases.



c. C-DNA

C-DNA is formed at 66% humidity in the presence of Li^+ ions. The differences of C-DNA from B-DNA are the – axial rise is 3.32 A^o per base pair, pern turn 9.33 base pairs, diameter is 19 A^o.

d. D-DNA

D-DNA is extremely rare variant with only 8 base pairs per helical form. This form of DNA is found in guanine devoid DNA molecules.

2.1.4.4. Ribonucleic Acid (RNA)

RNA like DNA is long unbranched macromolecule consisting of nucleotides joined by phosphodiester bonds. The RNA contain ribose sugar instead of deoxyribose in DNA. The nitrogenous bases adenine, guanine, cytosine are common in both DNA and RNA but in RNA instead of DNA's thymine uracil is

present. The RNA acts as genetic material only in some viruses like Human Immunodeficiency Viruses (HIV).

RNA main functions are as ribosomal structural component, in transferring amino acids to the growing polypeptide chain in transferring message on DNA to the expressible form. Depending on functions, structure and stability the RNA are basically three types – ribosomal RNA (rRNA), transfer RNA (tRNA), and messenger RNA (mRNA). Each of these polymeric forms serves as extremely important informational links between DNA and proteins.

A. r RNA

The r RNA is the most insoluble form of RNA, so also called insoluble RNA. This is found in ribosomes. In prokaryotes these are of three kinds – 23s, 16 s and 5 s RNA depending on sedementation behaviour. r RNA is the most abundant of all types of RNA s and make up about 80% of the total RNA of a cell. The ribosomes of eukaryotic cells contain 5S, 5.8 S, 16 S and 28 S r RNA.

The remarkable property of r RNA is all sources of r RNA has G-C content more than 50%. At low ionic strength, the molecule shows a compact rod structure with random coiling. But at high ionic strength, the molecule reveals the presence of compact helical regions with complementary base pairing and looped outer regions. The function of r RNA molecules in the ribosomes is not yet fully understood, but they are necessary for ribosomal assembly and seems to provide a specific sequence to which the m RNA molecule bind in order to be translated.

B. t RNA

The t RNA or soluble RNA is the smallest polymeric form of RNA. This seems to be generated by the nuclear processing of precursor molecule. tRNA amounts to 15% of total cellular RNA.

The cloverleaf model structure of t RNA was proposed by W. Holley and co workers.

Common structural features of tRNS

i. All tRNA molecules have a common design and consists of 3 folds giving it a shape of the cloverleaf with four arms.

Fig 14. Structure of tRNA (Jain 15-32)

ii. All tRNA molecules are unbranched chains containing from 73 to 93 ribonucleotides.

iii. tRNAs contain 7 to 15 unusual bases. Many of these unusual bases are methylated or dimethylated derivatives of A, U, G and C. For example pseudouridine, thymine, 5-methyl cytosine, etc.

iv. Phosphorylated 5^1 end residue is usually guanine.

v. The base sequence at 3^1 and 5^1 end of all tRNA s is CCA. All amino acids bind to this via the 3^1 -OH of ribose.

vi. Except at 5^1 sites all the tRNA structure will be in double stranded forms. These five nonbase paired sites are:

a. 3^1 CCA terminal region.

b. TψC loop

c. Extra arm

d. Dihydrouracil loop

e. Anticodon loop

vii. The tertiary structure of tRNA is L-shaped.

The important function of tRNA is to act as specific carriers of activated amino acids to specific sites on the protein synthesizing templates. As the function of tRNA is to bind to the specific amino acids, one might think that there are 20 types of tRNAs. Since the code is degenerative there may also be more than one tRNA for a specific amino acid. Infact the total number for exceeds than 20. In a bacterial cell, there are more than 70 tRNAs.

C. mRNA

The mRNA is also called tem-plate RNA. The abundance of RNA in the cytoplasm and its role in protein synthesis suggested that the genetic information of nuclear DNA is transcripted to an RNA, which functions as the template for protein synthesis.

The properties of mRNA are

1. The mRNA should be a polynucleotide

2. The base composition of the mRNA should reflect the base composition of the DNA that species it.

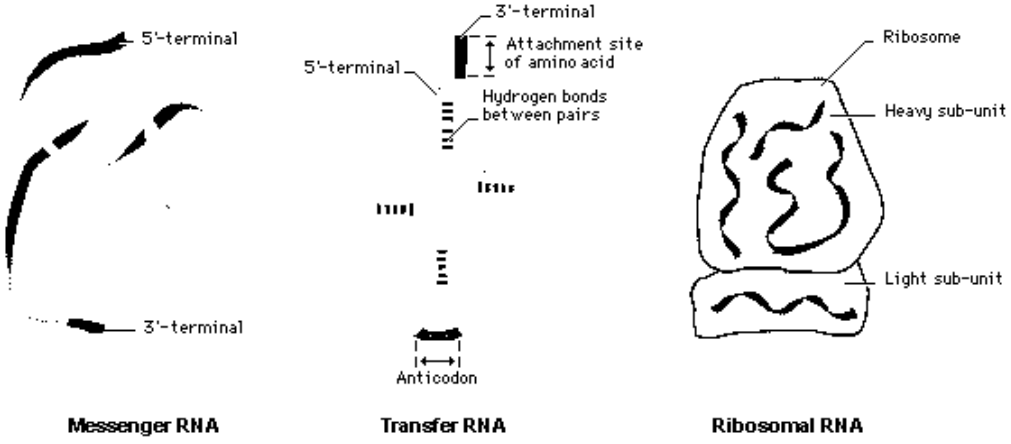
3. The mRNA should be very heterogenous in size as genes or group of genes vary in length.

   Three nucleotides code for one amino acid.

4. The mRNA associates with ribosomes during protein synthesis.

5. The mRNA half life is short.

The mRNA's are unstable in the bacterial systems, with a half life from a few seconds to about 2 minutes. In mammalian systems mRNA molecules are more stable with a half life ranging from a few hours to one day. This is because 5^1 end of mRNA is capped by a 7-methyl guanosine triphosphate. The 3^1 end is polyadenylated. These probably serves to maintain the intracellular stability of the specific mRNA.



2.1.4.5. Biological Significance of Nucleotides

Both the ribonucleotides and deoxyribonucleotides have very significant biological roles:

1. Building blocks of nucleic acids

The nucleotides primary role in cellular activities is in synthesis of nucleic acids. These nucleic acids inturn serve very important functions like transmission of genetic information, ribosomal synthesis, transcription, translation, etc.

2. Chemical energy carriers

Among the common types of nucleotides ATP is the mostly widely used energy source. The remaining types of nucleotides UTP, GTP and CTP are used in specific reactions.

3. Coenzyme components

Some coenzymes like NAD, FAD and COA contain adenosine as part of their structure.

4. Signal transduction

The signals of hormones to the cells will be mediated by second messengers like CAMP, CGMP.

2.1.4 .6 SUMMARY

1. Nucleotides are the building blocks of nucleic acids.
2. Phosphoric acid plays very important role in joining the nucleotides.
3. The complex double helical structural generally existing form in cells is B-DNA.
4. mRNA acts as messenger to carry genetic information.

2.1.4.7 Model Questions

1. What is Denaturation
2. Describe in detail about the structure of DNA.
3. Write about the RNA acting as messenger of genetic information

2.1.4.8 Reference Books

1. Biochemistry – S.C. Rastogi
2. Principles of Biochemistry – Lehninger, Nelson and Cox.

3. Fundamentals of Biochemistry – J.L. Jain.

Mrs. G.V. Padmavathi,

P.G.Dept. of Biochemistry

T.J.P.S. College (P.G.Center).

Lesson 2.1.5

VITAMINS

Objective

2.1.5.1. Characteristics of Vitamins

2.1.5.2. Classification of Vitamins

a. Fat-soluble

b. Water-soluble

2.1.5.3. Fat Soluble Vitamins

1. Vitamin A
2. Vitamin D
3. Vitamin E
4. Vitamin K
5. Coenzyme Q

2.1.5.4. Water Soluble Vitamins

1. B Complex Vitamins
   - A. Vitamin B_1
   - B. Vitamin B_2
   - C. Vitamin B_3
   - D. Vitamin B_5
   - E. Vitamin B_6
   - F. Vitamin B_7

G. Vitamin B_9

H. Vitamin B_{12}

2. Vitamin C
3. Other water soluble vitamins

2.1.5.5 Summary

2.1.5.6 Model Questions

2.1.5.7 Reference Books

Objective

The term vitamin refers to the substances distinct from major components of food required in minute quantities and whose absence causes specific deficiency diseases. The animals cannot synthesize most of the vitamins, so a steady supply of them is an indispensable condition. The ultimate source is the plant and bacterial world.

In 1906, Frederick Hopkins ascribed the diseases such as scurvy and rickets to the lack of some dietary factors. Funk suggested the vitamin theory that specific diseases such as beri-beri, scurvy and rickets are each caused by the absence from the diet of a particular nutritional factor. The term vitamine (vita=life).

2.1.5.1. Characteristics of Vitamins

The general characters of a vitamin are ;

1. Vitamins are of widespread occurrence in nature both in plant and animal worlds.
2. All common food stuffs contain more than one vitamin.
3. The plants can synthesize all the vitamins whereas only a few vitamins are synthesized in animals.
4. Human body can synthesize some vitamins, e.g., vitamin A is synthesized from its precursor carotene and vitamin D from UV irradiation of ergosterol

and 7-dehydrocholesterol. Some members of the vitamin B complex are synthesized by microorganisms present in the intestinal tract. Vitamin C is also synthesized in some animals such as rat.

5. Most of vitamins have been artificially synthesized.

6. All the cells of the body have vitamins to some extent.

7. Vitamins are non antigenic.

8. Vitamins carryout functions in very low concentrations. Hence, the total daily requirement is very small.

2.1.5.2. Classification of Vitamins

In 1913 MC collum and Davis described a lipid soluble essential food factor in butter fat and egg yolk. In 1915, a water soluble factor in wheat germ necessary for the growth of young rats was also described. Since then, two categories of vitamins are usually recognized: (a) fat-soluble and (b) water – soluble.

a. Fat-soluble vitamins

These are oily substances, not readily soluble in water and their biochemical functions are not well understood. These are vitamins A, D, E and K. They play more specialized roles in certain group of animals and in particular type of activities. They function in the formation of visual pigment, in the absorption of calcium and phophorus from the vertebrate intestine, in protecting mitochondrial system from inactivation or in the formation of a blood clotting factor in vertebrates. The individual fat soluble vitamins bear a closer resemblance to each chemically. Unlike the water soluble vitamins, fat-soluble vitamins can be stored in the body. An adults liver can store enough vitamin A to last several months or longer. As fat-soluble vitamins are storable, their excessive intakes can result in toxic conditions.

b. Water-soluble vitamins

These are catalytic factors and as such form vital links in the chains of biochemical reactions characteristic of living objects. For instance, thiamine is required whenever sugars are oxidized aerobically to release energy. The individual vitamins bear no closer resemblance to each other chemically. The biochemical or coenzyme functions of nearly all vitamins is known. The common water soluble vitamins are vitamins of B complex such as B_1, B_2, B_3, B_5, B_6, B_7,

B_9, and B_{12} and the vitamin C. Choline, inositol, p-amino benzoic acid are frequently included in this category. Many nutritionists, however, do not consider them as true vitamins. The B-series of vitamins, being water – soluble and excretable, are required daily in meager amounts for the normal growth and good health of humans and many other organisms.

2.1.5.3. Fat Soluble Vitamins

The different types of fat soluble vitamins are

1. Vitamin A
2. Vitamin D
3. Vitamin E
4. Vitamin K
5. Co enzyme Q

1. Vitamin A

A. History

It was first recognized as an essential nutritional factory b_y MC collum in 1915 and then isolated from fish-liver oil by Holmes in 1917. On account of its established role in the visual process, it is often called as anti xerophthalmic factor.

B. Occurrence

Liver oils of various fishes are the richest natural sources of vitamin A. Other sources are butter, milk and eggs and to lesser extent kidneys. In its provitamin form it is supplied by all pigmented vegetables and fruits such as carrots, pumpkins, peas, papayas, tomatoes, etc. Yellow corn is the only cereal containing significant amounts of carotene. The milk of well-fed mothers contains sufficient amounts of vitamin A for the infant's need.

C. Structure

Vitamin A is found in two forms A_1 and A_2. The carotenoids that give rise to vitamin A in animal body is named as provitamin A. These include, β and γ-carotenes and cryptoxanthin. β carotene is the most potent of all these forms. Vitamin A, is a complex primary alcohol called retinal, having the empirical formula, $C_{20}H_{29}OH$.

Another form of vitamin A present in fresh water fishes is known as vitamin A_2. It differs from vitamin A_1, which is found in salt – water fishes, in possessing an additional conjugate double bond between carbon atom 3 and 4 of the β-ionone ring.

D. Functions

1. Vitamin A and vision

The retina of the human eye contains 2 types of receptor cells, rods and cones. Animals which have vision only in bright light like pigeons have only cones while animals which can see in night or dim light like owls have only rods. Thus the rods are concerned with seeing at low illumination and the cones are responsible for colour vision.

2. Maintaining epithelial cells

Vitamin A helps in maintaining the epithelial cells of the skin and the linings of the digestive, respiratory and genito urinary systems. These linings play a protective role against cancer-causing agents, viruses and bacteria and are rendered vulnerable by a deficiency of vitamin A.

3. Vitamin A guards against cancer by protecting cell walls from undesirable oxidation, and scavenging the products of oxidation – free radicals which are linked to the development of cancer.

E. Deficiency

Vitamin A is perhaps the most important as it effects the various metabolic processes in the body. It has profound effect on epithelial structures, in general. Its deficiency leads to the onset of many diseases like nyctalopia or night blindness, to see in night they are unabled. Xerophthalmia, phyrenoderma or toad skin and stunted growth.

i. Xerophthalmia or Xerosis

This is a major cause of blindness in childhood. The disease affects large number of children but few adults. Xerophthalmia is characterized by drying of the eyes and hence so named. The lacrymal glands become stratified and keratinized and cause to produce tears. This makes the external surface dry and dull. The ulcers develop. The bacteria are not washed away. The eyelids swell and become sticky. This results in frequent exudation of blood, causing severe infection to the eye. If left untreated, blindness results.

ii. Keratomalacia

This is a corneal disease, occurring maximally in pre-school children of 3-4 years. This usually happens suddenly in young children with Kwashiorkor esp., after an episode of diarrhoea or infection. At first the cornea loses its luster, undergoes a necrosis and develops a few-pin-point ulcers, which later coalesce to form a large, white ulcerative area.

iii. Phrynoderma

This is a skin lesion and is characterized by follicular hyperkeratosis. In it, the skin, esp., on the outer aspects of forearms in the regions of the elbows and of the thighs and buttocks becomes rough and spiky. In severe cases, the trunk is also affected.

iv. The damaged epithelial structure in diverse organs such as the eyes, kidneys or the respiratory tract often become infected. It is for this reason that vitamin A has been called an anti infection vitamin. Deficiency of this vitamin also causes kidney stones, probably due to infection in the renal pelvis.

v. Vitamin A is an important factor in tooth formation. In its deficiency, there is a defective formation of enamel so that the dentin is exposed. Evidently, sound tooth formation does not occur.

vi. Other deficiency diseases that have been attributed to vitamin A are atrophy of the testes and disturbances of the female genital organs.

F. Requirements

The recommended dietary allowances of vitamin A is about 5,000 International Units (I.U). Growing children, pregnant and lactating mothers require high doses of upto 8,000 I.U. It is also possible that some individuals require more than the minimal requirement due to either faulty absorption or some other reasons.

2. Vitamin D

A. History

The first demonstration of the co existence of vitamin D was shown by Elmer MC Collum in 1922, who found that cod liver oil was effective in preventing rickets, a disease induced in rats by providing low calcium diet on account of its preventive action on vitamin D is often called as antirachitic factor. It is also known as “sunshine vitamin”, as its provitamin form present in human skin is easily converted to the active form by irradiation with UV light.

B. Occurrence

The best natural sources of vitamin D are the liver oils of many fishes such as cod and halibut. The flesh of oil fishes is also excellent source. Egg yolks are fairly good but milk, butter and mushrooms are poor. Like vitamin A, vitamin D is absent from vegetable fats and oils vitamin D_3 is of animal origin and can be produced from 7-dehydrocholesterol also by irradiating with ultraviolet light.

C. Structure

The two vitamins D_2 and D_3 and also D_4 and D_5 differ only in their side chains attached to C_{17}. The biologically active form of vitamin D_3 present in animals such as rat, is 25-hydrocholesterol.

D. Functions

i. Vitamin D plays an important role in calcification of bones and teeth. It encourages the absorption, into the blood, of calcium salts and phosphates. Calcium passage across duodenum occurs mainly by diffusion and active transport of Ca^{2+} occurs across the ileal mucosa. Both these processes are related in deficiency of vitamin D. The subsequent release of bound calcium is also markedly stimulated by vitamin D but only in the presence of parathyroid hormone. So, the function of vitamin D is to cause increased absorbtion, longer retention and their utilization of calcium and phosphors in the body.

ii. Vitamin D_2 is a powerful antirchitic agent for man and for the rat but not for the chicken. Vitamin D_3 on the contrary, is much more potent for the chicken than either for man or the rat.

E. Deficiency

The most characteristic symptom of vitamin D deficiency is the childhood disease known as rickets. Deficiency of it in human adults leads to osteomalacia, a condition that might also be terms adult rickets.

i. Rickests

This is primarily a disease of growing bones. It is, the deposition of inorganic materials on the matrix of bones fail to occur. As a result bones may become soft, flexible and miss happen leading to enlarged skulls, swollen joints,

knock-knees or bow legs, hunched backs, beaded ribs and protruding chest. Contracted pelvis. Pot bellies and muscular impairment are other symptoms. The first tooth in such babies appears between 6^{th} and 9^{th} month, at which time it has appeared in half of the normal babies. In deficiency of vitamin D, the formation of teeth becomes defective and leads to the development of dental caries. In the blood serum, there is usually a normal content of calcium but the phosphate is decreased. Rickets is most prevalent where climate prevents individuals from exposure to sun, whereby checking vitamin D production by irradiation the skin.

ii. Osteomalacia

In this action of bones is essentially like that in rickets. The bones become softer than the rachitic bones and the Ca/P ratio does not remain constant. The loss of calcium is greater than that of phosphrous and there is a relative gain in magnesium content. The disease is more prevalent among women in some countries, because of custom that keeps them indoor and also prevents them from exposure to sun.

iii. Vitamin D_3 also leads to disease called idiopathic steatorrhea and celiac disease. Like osteomalacia, the disease is characterized by demineralization of the bones which may result in deformities or dwarfism.

F. Requirements

Vitamin D requirement is greatly influenced by the amount of UV light to which the individual is exposed. Half an hour of direct sunlight on the cheeks of a baby each day is sufficient to generate the minimal daily requirement of vitamin D. For adults also exposure to sunlight for 30 min a day is believed to satisfy the daily requirement, 10 ug, of vitamin D.

3. Vitamin E

A. History

The presence of this active principle was first demonstrated in vegetable oils. This was designated as vitamin E or antisterility factor on account of the development of sterility in animals in its absence. In 1936, two compounds with vitamin E activity were isolated from wheat germ oil by Evans and his associates and given the name, α and β – tocopherol.

B.Occurrence

The tocopherols are of widespread occurrence in many plant oils such as wheat germ, rice, corn, cottonseed, soybean, and peanut but not olive oil. These are also present in

small amounts in meat, milk, eggs, leafy plants and some fruits. Fish liver oils, so abundant in vitamin A and D, are devoid of vitamin E.

C. Structure

Vitamin E is the collective name for a group of closely related lipids called tocopherols. The tocopherols are derivatives of 6-hydroxychroman bearing an isoprenoid side chain at carbon 2.

The various tocopherols differ from each other in substituents on carbons 5, 7 and 8. These substituents are methyl groups and hydrogen atoms.

D. Functions

1. Tocopherols are antioxidants i.e., they can prevent the oxidation of various other easily oxidized substances such as fats and vitamin A. It is for this reason that they are commercially added to foods to retard the spoilage.

2. Vitamin A is essential for reproduction.

3. Where as the beneficial action of vitamin A is mainly on the ectoderm and endoderm, that of vitamin E is on the mesodermal tissue. Vitamin E influences all the three germinal layers of the embryo by preventing the too rapid destruction of vitamin A.

4. The biochemical activity of tocopherol lies in its capacity to protect mitochondrial system from inactivation by fat peroxides.

5. It has been observed that tocopherol – deficient muscles show a high oxygen uptake. Administration of tocopherol brings down the oxygen consumption to normal.

E. Deficiency

The characteristic symptoms of experimentally induced vitamin deficiency vary from animal to animal. In mature female rats, sterility develops because of reabsorption of fetus after conception while in males, the germinal epithelium of the testes degenerates and the spermatozoan become non motile.

F. Requirements

Vitamin E is not a problem in human nutrition because it is ubiquitous in foods. However the minimum daily requirement of vitamin E for adults is 30 IU for men and 25 IU for women. The infants and children require 1 to 25 IU/per kg body weight.

4. Vitamin K

Henrick Dam a Danish investigator found that newly hatched chicks, fed on artificial diets, develop hemorrhage, a fatal disease. Characterized by prolonged blood – clotting period. The term vitamin K was then proposed by Dam himself to designate the active factor which cured or prevented this disease. On account of its blood – clotting power, it is also called as antihemorrhagic factor or coagulation vitamin.

B. Occurrence

Vitamin K, occurs in green vegetables like spinach, alfalfa, cabbage, etc. Fruits and cereals are poor sources. Vitamin K_2 is found in some intestinal bacteria. A rich source of K_2 is putrefied fish meal.

C. Structure

Chemically, the two forms of vitamin K are derivatives of quinones and differ from each other in the composition of their side chain present at carbon 3 of the naptho quinone ring. It is a phytol radical in vitamin K_1 and a difarnesyl radical in vitamin K_2.

D. Functions

i. Vitamin K plays an essential role in the biosynthesis of prothrombin – a blood plasma protein needed in the process of blood clotting and produced in liver.

ii. The vitamin K has also been ascribed a role in electron transport system and oxidative phosphorylation in mitochondria. The vitamin K_1 and K_2 both activate electron transport in the succinate oxidase of cardiac muscle preparations that have been made inactive by treating with isooctane.

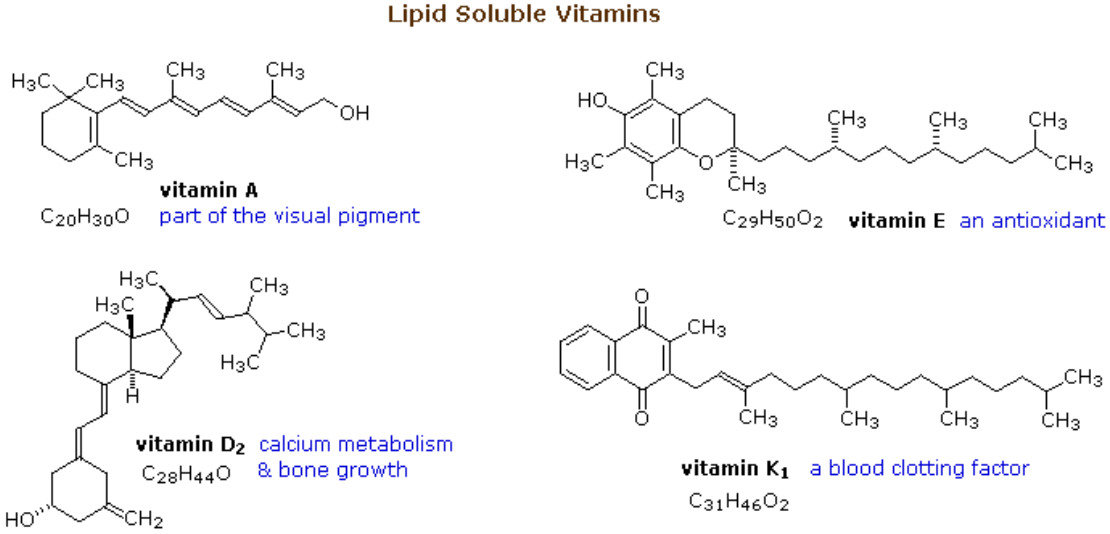
E. Deficiency

Deficiency of vitamin K causes loss of blood-clotting power. The infants may also show signs of vitamin K deficiency by developing hemorrhage. This disease

persists by the time the bacteria develop in the intestine. Administration of this vitamin to pregnants before parturation decreases the onset of this disease.

F. Requirements

There is seldom a lack of sufficient vitamin K in human beings. As such, no standard requirement has been set.



5. Coenzyme Q

Coenzyme Q serve as electron transport agents and are involved in the formation of ATP at a cytochrome a. Roles has classified the coenzyme Q group as vitamins because of their ability to cure vitamin E deficiency in several animal species.

2.1.5.4. Water Soluble Vitamins

The common features of vitamins are :

i. All of them except lipoic acid are water soluble

ii. Most are components of co enzymes that play vital roles in metabolism.

iii. The common source are liver and yeast.

iv. Most of them can be synthesized by the intestinal bacteria.

Types of water soluble vitamins are :

1. B complex vitamins
2. Vitamin C]
3. Other water soluble vitamins

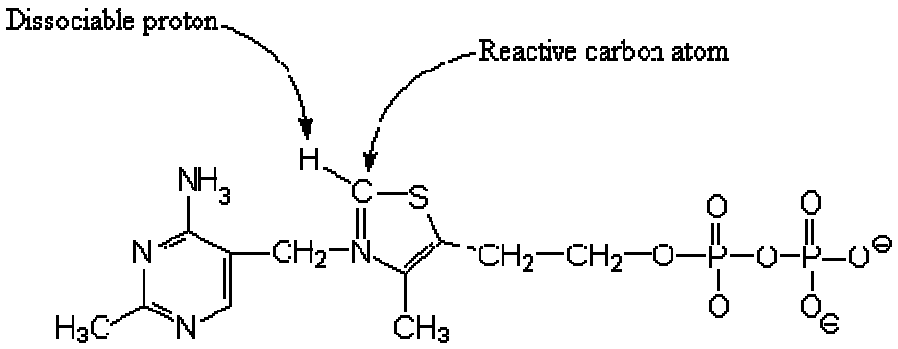
1. B complex vitamin

At present, the vitamin B complex is known to consist of a group of at least 13 components usually named as B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}.

A. Vitamin B_1

i. History

Thiamine was the first member of the vitamin B group to be identified and hence given the name vitamin B_1. Thiamine was first isolated by Jansen on account of its curing action against beriberi, it is commonly known as antiberi beri factor.



Thiamine pyrophosphate (TPP)

ii. Occurrence

Thiamine is found practically in all plant and animal foods. Cereals, heart, liver and kidney are excellent sources of it. In cereals, the outer layers of seeds are especially rich in thiamine content. Milk also contain thiamine, but in low amounts.

iii. Structure

Thiamine is 2,5 – dimethyl – 6- amino pyrimidine bonded through a methylene linkage to 4-methyl-5-hydroxyethyl – thiazoles. Thus, pyrimidine and diazoles are the two moieties present in its molecule.

iv. Functions

Thiamine is phosphorylated with ATP to form TPP. TPP participates in many reactions, such as decarboxylation of ---- ketoacids, notably pyruvic and α-ketoglutaric and transketolation. TPP in association with lipoic acid forms the prosthetic group, co carboxylase for the enzyme carboxylase.

v. Deficiency

Vitamin B_1 deficiency leads to polyneuritis in animals and beri beri in human beings.

A. Poly neuritis

In birds renders them unable to fly, walks or even stand.

B. Beriberi

It is a disease of the nervous system and is characterized by poly neuritis leading to partial paralysis of the extremities, muscular atrophy, cardiovascular changes and gastrointestinal disorders. At first there is weakness and fatigue. This is followed by symptoms like anorexia, insomnia, anesthesia and tachycardia.

The final symptoms may follow one of three :

- Symptoms involving nervous system (dry beriberi)
- Symptoms associated with odema and effusions (Wet beriberi)
- Symptoms involving heart

  (acute pernicious beriberi)

vi. Requirements

The daily recommended dietary allowances are 1.2-1.4 mg for men and 1.0 mg for women. Pregnant and lactating mothers, however, require up to 1.5mg daily. The thiamine requirement for infants is between 0.2 and 0.5 mg daily.

B. Vitamin B_2

i. History

Riboflavin or vitamin B_2 was first isolated from milk whey, so it is also called lactoflavin.

ii. Occurrence

In nature, it occurs almost exclusively as a constituent of one of the two flavin co enzyme namely, Flavin mono nucleotide (FMN) and Flavin adenine dinucleotide (FAD). Milk, liver, kidney and heart are excellent sources of this vitamin. Leafy vegetables are good sources. Fruits and most root vegetables contain moderate quantities.

iii. Structure

A molecule of thiamine consists of a sugar alcohol, D-ribitol, attached to a chromogenic dimethyl isoalloxazine ring at position number 9.

iv. Functions

Riboflavin is synthesized by all green plants, most bacteria, yeasts and moulds. When riboflavin is phosphorylated in the presence of an enzyme flavokinase, it gets converted to FMN which is essential in the biosynthesis of fats. FMN may undergo a further reaction with ATP, in the presence of an enzyme found in yeast and animal tissues, to produce FAD. These two flavo enzymes play a key role in cell metabolism . They function in accepting hydrogen atoms from reduced pyrimidine nucleotides. They have been shown to participate in the enzymatic oxidation of glucose, fatty acids, amino acids and purines.

v. Deficiency

Persons deficient in vitamin B_2 may show glossitis, cheilosis, corneal vascularization and a typical dermatitis. But these symptoms are not specific to ariboflavinosis since similar symptoms may also develop in the absence of nicotinic acid and iron.

vi. Requirements

The minimum daily requirement of riboflavin varies from 0.6 to 1.7 mg for children and adults. During pregnancy and lactation, the women require upto 2.0 mg daily.

C. Vitamin B_3

i. History

This was first isolated by Roger J. Williams from yeast and liver. This is also called pantothenic acid, on account of its wide distribution. This vitamin is sometimes called filtrate factor or the yeast factor.

ii. Occurrence

Yeast, liver and eggs are the richest sources of it. The vegetables and fruits and also the skimmed milk. Wheat bran, whole milk and canned salmon are also sources of vitamin B_3.

iii. Structure

Pantothenic acid is an amide of pantoic acid and β – alanine.

iv. Functions

The coenzyme form of pantothenic acid is coenzyme A. The coenzyme A functions in acetylation reactions. In order to be effective, Co A must be present in the form of acetyl – CoA.

Acetyl COA + Choline → Acetylcholine + COA

v. Deficiency

A deficiency of patothenic acid leads to depigmentation of the hair in rats, pigs and dogs and to depigmentation of feathers in chicks. Atrophy of the adrenal cortex with necrosis and hemorrhage may also occur in animals including rat. Corneal changes consisting of vascularization, thickening and opacity may be seen.

In human beings, no definite deficiency syndrome has been ascribbed to pantothenic acid.

vi. Requirements

The dietary allowance does has not been officially worked out yet 5-10 mg per day of vitamin B_3 has been suggested.

D. Vitamin B_5

i. History

Vitamin B_5 refers to nicotinic acid and was named as pellagra preventive factor, antiblack tongue factor. It was first isolated by Funk in 1911.

ii. Occurrence

Nicotinic acid is widely distributed in nature in plant and animal tissues mainly as its amide called nicotinamide. It is most abundantly found in yeast. Liver, meat, and poultry are also good sources. Most vegetables and fruits are poor sources of it. Milk and eggs are usually devoid of niacin.

iii. Structure

Niacin is simplest of all the known vitamins. It is a pyridine derivative.

iv. Functions

Nicotinic acid and its amide both are necessary for the growth of various microorganisms. The two co enzyme forms of this vitamin, NAD and NADP, carry out two important functions in the tissues.

a. Oxidation of alcohols, aldehydes, amino acids and hydroxy-carboxylic acid.

b. Reduction of the flavin coenzymes.

v. Deficiency

A deficiency of niacin causes pellagra in man and black tongue in dogs. Pellagra is characterized by 3D's, namely dermatitis of the exposed parts, diarrhoea and dementia. In it the weight is lost rapidly. The tongue becomes magenta-coloured and the neural symptoms appear leading to insanity and death. As coffee is particularly rich in niacin, the heavy coffee drinkers usually do not develop pellagra.

The canine black tongue disease leads to complete loss of appetite. The inner surfaces of the lips and cheeks develop pustules.

vi. Requirements

The recommended daily allowances of nicotinic acid is between 8 and 15 mg for children, between 15 and 20 mg for men and between 13 and 15 mg for women. Pregnant and lactating mothers may require upto 20 mg daily.

E. Vitamin B_6B

i.History

The name vitamin B_6 was suggested by Albert Szent-Gyorgyi to designate substances, other than thiamine and riboflavin, which cured a dermatitis in rats. It was also named as adermin or antidermatitis factor. Vitamin B_6 group includes 3 compounds:

Pyridoxine, pyridoxal and pyridoxamine.

ii. Occurence

The B_6 vitamins are widely distributed in nature in plant and animal tissues, e.g., cereal grains, yeast, egg yolk, and meat.

iii. Structure

All the three forms of vitamin B_6 are derivatives of pyridine and differ from each other in the nature of substituent at position 4 of the ring. All the three forms are readily interconvertable biologically.

iv. Functions

The various forms of vitamin B_6 serve as growth factors to a number of bacteria. In addition, pyridoxal phosphate plays a key role in various enzymatic reactions involved in amino acid metabolism such as transamination, decarboxylation, racemization, etc. pyridoxal phosphate and pyridoxamine phosphate are the coenzyme forms. Pyridoxal or its phosphate derivative also possibly acts as a carrier in the active transport of amino acids across cell membranes.

v. Deficiency

Vitamin B_6 deficiency or apyridoxosis in rats leads to the development of acrodynia, a disease of dermatitis on ears, mouth and tail and accompanied by edema and scaliness of these structures. Dogs and chick develop anemia and nervous lesions in apyridoxogis.

In human infants, vitamin B_6 deficiency results in convulsions, anemia, dermatitis and gastrointestinal disorders such as nausea and vomiting. In adults the vitamin B_6 deficiency is normally not found because the intestinal bacteria are capable of synthesizing vitamin B_6.

vi. Requirements

The minimum dietary allowance of vitamin B_6 is between 0.2 and 1.2 mg for infants and children and around 2.0 mg for men and women per day. During pregnancy and lactation, the recommended daily dose is 2.5 mg.

F. Vitamin B_7

i. History :

In 1935, Kritz Kogl isolated in crystalline form from 250 kg of dried egg yolvs about 1 mg of a growth promoting factor is named as "biotin".

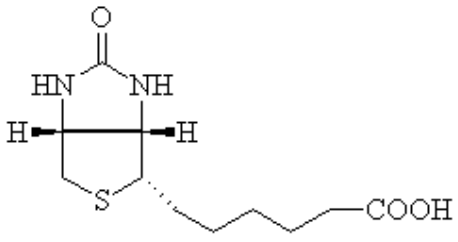
The raw egg white contains a protein, avidin, which combines with biotin in a firm linkage to form a compound that cannot be absorbed by the intestine and is therefore, excreted.

ii. Occurrence

Biotin has a wide range of distribution both in animal and the vegetable kingdoms. Yeast, kidney, milk and molasses are among the richest sources: peanuts and eggs have lesser amounts. Biotin occurs in nature usually in combined state as biocytin.

iii. Structure

Biotin has an unusual structure and consists of a fused inidazole and thiophene ring with a fatty acid side chain. Two forms of biotin can exist, allobiotin and epibiotin. Biotin is optically active, only the (+) biotin is active. Only the (+) biotin is active; the DL – biotin is half as active as the naturally occurring biotin. The oxybiotin, in which S-atom of biotin is replaced with an O-atom, has some activity. Biotin and thiamine are the only sulfur containing vitamins isolated to date.



iv. Functions

This vitamin serves as a prosthetic group for many enzymes. These biotin – containing enzymes catalyze the fixation of CO_2 into organic molecules, thus bringing about carboxylation. The carbon dioxide is carried as a carboxyl group attached to one of the ureido nitrogen atoms of biotin, forming N-carboxy biotin complex. They also bring about synthesis of fatty acids such as oleic acid.

v. Deficiency

In most animals including man, intestinal bacteria synthesize appreciable amounts of biotin. Avidin, the egg white protein, inactivates biotin by eliminating it from an otherwise complete diet. Such a deficiency in man leas to dermatitis, loss of hair, decrease in weight and edema. The lesions on skin appear with changes in posture and gait. These disorders may lead to death. Heating egg white destroys the avidin and prevents the so called egg white injury.

vi. Human requirements

The intestinal bacteria synthesize biotin in such appreciable amounts that the amount excreted in urine exceeds the intake. About 10 μg per day of biotin is sufficient for an adult.

G. Vitamin B_9

i. History

The potent factor was obtained from spinach leaf and this led to its nomenclature as folic acid. The official name of this vitamin is folacin.

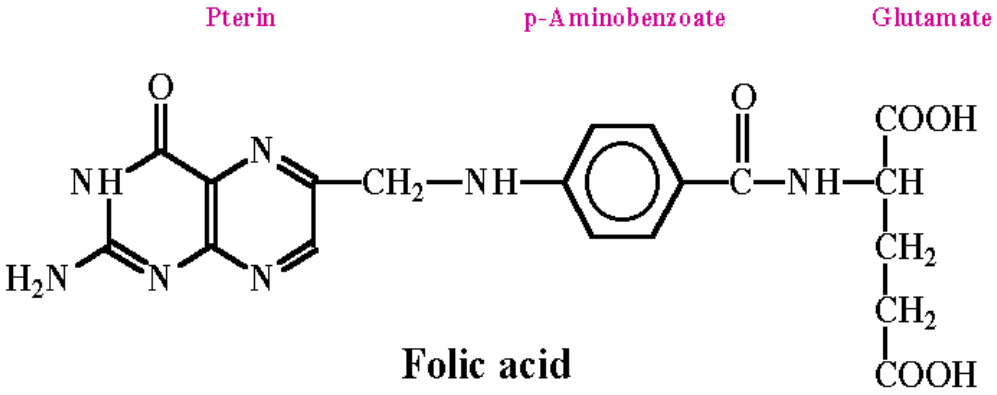
This is also known as liver lacto bacillus casei factor as it was isolated from liver and was shown as necessary for the growth of lactic acid bacteria.

ii. Occurrence

Folic acid and its derivatives are widely distributed in biological world. A few important sources are liver, kidney, yeast and wheat. Root vegetables, sweet potatoes, rice, corn, tomatoes, bananas, pork and lamb contain little folic acid.

iii. Structure

A molecule of folic acid consists of 3 units : glutamic acid, p-aminobenzoic acid and a derivative of the heterocyclic fused – ring compound pterin.



Folic acid

iv. Functions

The reduction products of folic acid act as coenzymes. An enzyme, folic reductase, reduces folic acid to dihydrofolic acid, the latter compound is further reduced by dihydrofolic reductage to 5,6,7,8 – tetrahydro folic acid (THFA). The vitamins of B_9 group are involved in one – carbon metabolism in a way similar to the two carbon metabolism in which COA is involved. THFA acts as an acceptor of a one – carbon unit either from formate or from formaldehyde. THFA is also involved in the transfer of the methyl group and in the utilization of single carbons in some anabolic reactions.

v. Deficiency

In chicks, lack of this factor leads to anemia. Rats develop achromotrichia. The monkeys show macrocytic anemia, leukopenia, diarrhoea and edema. In man, the folic acid deficiency leads to megaloblastic anemia, glossitis and gastrointestinal disorders. Pregnant women and infants are also particularly vulnerable. Folic acid deficiency is a major feature of tropical sprue, in which there is a general deficiency in absorption of many nutrients from the small intestine.

vi. Requirements

The daily dietary allowance of folic acid is 0.1 mg for infants, 0.2 mg for children and 0.4 mg for adult men and women. Pregnant mothers may require upto 0.8 mg per day.

H. Vitamin B_{12}

i. History

In 1926, two American physicians Minot and Murphy discovered that patients suffering from pernicious anemia could be cured by feeding them with about half a pound of liver a day. This land mark in medicine brought them Nobel Prize in 1934.

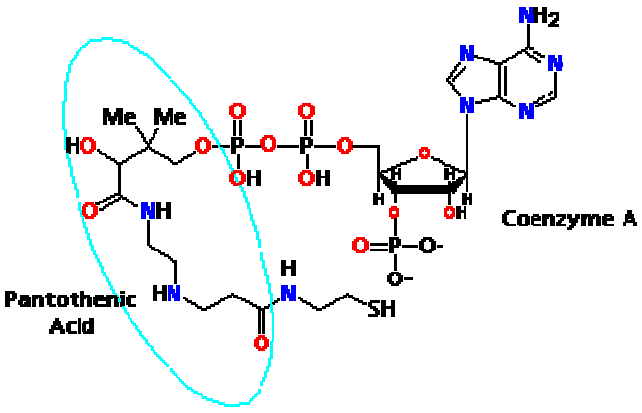
ii. Occurrence

Vitamin B_{12} has been found only in animals: the chief source is liver, although it is also present in milk, meat, eggs, fish, oysters and clams. Cyanocobalamin is not present in plant foods except in spirulina, a blue – green algae. Cyanocobalamin is unique in that it appears to be synthesized only by microorganisms especially anaerobic bacteria.

iii. Structure

A unique feature of this vitamin is the presence, in its molecule, of an a tom of a heavy metal cobalt in the trivalent state. No other cobalt – containing organic compound has been found in nature. The cobalt atom is centrally – situated and is surrounded by a macro cyclic structure of 4 reduced pyrrole rings collectively called as corrin. It may be noted from the structural formula that the six coordinate valences of the cobalt atom are satisfied by the 4 nitrogens of the reduced tetrapyrrole, a nitrogen atom of 5,6 – dimethyl benzimidazole and a cyanide ion. Two of the pyrrole rings namely A and D are directly linked to each other and the corrin has lower degree of unsaturation with only 6 double bonds. The other two pyrrole rings, namely B and C are joined through a single methane

carbon. Another distinct feature of the vitamin B_{12} molecule is the presence of a loop of the isopropanol, phosphate, ribose and 5,6 – dimethyl – benzimidazole in that order, the end of the loop being attached to the central cobalt atom. Vitamin B_{12} co enzyme is the only known example of a carbon – metal bond in a biomolecule.



iv. Functions

Vitamin B_{12} is converted to coenzyme B_{12} by extracts from microorganisms supplemented with ATP. The coenzyme B_{12} is associated with many biochemical reactions are

a) 1,2 shift of a hydrogen atom

b) Carrier of a methyl group

c) Isomerization of dicarboxylic acids

d) Dismutation of vicinal diols

e) Biosynthesis of methyl groups from 1-carbon precursors.

v. Deficiency

A nutritional deficiency of this vitamin is usually not observed on account of its ubiquitous nature in food stuffs. However, deficiency may by observed in individuals who obtain from all animal products, i.e., those who are strict vegetarians. The deficiency disease, pernicious anemia is characterized by R.B.C.'s becoming abnormally large and fewer in number. The patient weakens, loses its weight and the nervous system is also gradually affected because there

occurs demyelinization of the large nerve fibres of the spinal cord. All these changes ultimately lead to death.

vi. Requirements

The recommended daily allowance of vitamin B_{12} is 2 to 4 μg for children and 5 μg for men and women. Pregnant and lactating mothers require 8 μg and 6 μg respectively daily.

2. Vitamin C

i. History

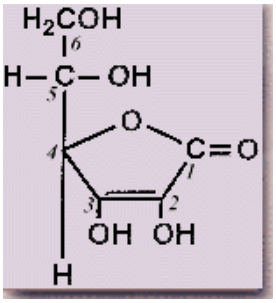
No other vitamin, with the possible exception of vitamin E, is as generally misunderstood as is vitamin C. It is ironic that the oldest therapeutically – used vitamin, furnished in 1750's in the form the lemons to British sailors to prevent scurvy, is still a subject of controversy. It is also called C-vitamin or antiscorbutic factor.

ii. Occurrence

In general ascorbic acid is not as widely distributed as other vitamins. Among plants, it is present in all fresh fruit. The fruit yields 1,000 – 4,000 mg of ascorbic acid per 100 gm of edible matter. Human milk is 3 to 4 times richer in vitamin C contents than cow's milk.

iii. Structure

The structure of ascorbic acid was established mainly by Haworth. It is a derivative of a hexose called 2-gulose. Chemically, it is 1 – threo - 2,4,5,6 – penbtoxy hexen – 2 – carboxylic acidlactone.



iv. Functions

The reduced form predominates in the plasma and also apparently in tissues at a ratio of about 15: of the oxidized form. Both of these are biologically active and are equally potent in carrying out their metabolic functions.

A major function of ascorbic acid is the formation of tissue collagen or intracellular cement substance. Infact, ascorbic acid appears to be essential to the activity of the enzyme collagen proline hydroxylase, which catalyzes the conversion of proline to hydroxyproline. Hydroxy proline is found exclusively in collagen and is vital in maintaining the tertiary structure of this major vertebrate protein collagen.

Ascorbic acid play a role in the conversion of folicacid to a physiologically active form, tetra hydrofolicacid. Ascorbic acid plays a key role in tyrosine metabolism. Ascorbic acid is also involved in electron transport in the microsomal fraction.

v. Deficiency

A vitaminosis C leads to scurvy. A disease characterized by petechial hemorrhages in the skin, mucus membrane and degenerative changes in the cartige and bone matrices. In it limbs swell, gums bleed and teeth drop out. Capillaries become brittle and burst, thus giving rise to red and purple spots over the body. The wounds fail the heal because of the failure of the cells to deposit collagen fibrils. Also, the bones cease to grow.

Infants 6-12 months of age, who are fed on processed milk only are very susceptible to this disease. Adult cases appear less frequently. Elderly bachelors and widowers who have to cook their own foods are especially prone to the development of vitamin C deficiency – a syndrome termed bachelor scurvy.

vi. Requirements

Since vitamin C is continuously oxidized in the body, the daily requirement of this vitamin is rather high. The recommended daily dose for children is 40 mg and for men and women 50-60 mg.

3. Other water soluble vitamins

A part from B complex vitamins and vitamin C some other compounds are also included in this group.

a) Choline

b) Inositol

c) Para aminobenzoic acid

d) Alpha – lipoic acid

e) Carnitine

f) Bioflavinoids

g) Vitamers

2.1.5.5Summary

- The term vitamin refers to the substances distinct from major components of food required in minute Quantities and whose absence causes specific deficiency diseases.
- Vitamins are mainly two types – fat soluble and water soluble vitamins.
- The functions of fat soluble vitamins are the formation of visual pigment (vitamin A), in the absorption of calcium and phosphorus from the vertebrate intestine (vitamin D), in protecting mitochondrial system from inactivation (vitamin E), or in the formation of blood clotting factor in vertebrates (vitamin K).

2.1.5.6 Model Questions

1. Write in detail about the vitamin playing its role in formation of visual pigment and maintain the epithelial cells.
2. What is anti – pernicious anemia factor – discuss.

2.1.5.7 Reference Books

- Fundamentals of Biochemistry – J.L. Jain.
- Biohemistry – A.V.S.S.Rama Rao

Mrs. G. V. Padmavathi

Lesson 2.2.1

GLYCOLYSIS, GLUCONEOGENESIS, GLYCOGEN METABOLISM

Objective

2.2.1.1Introduction

2.2.1.2 Glycolysis

(a) Pathway

(b) Energetic of Glycolysis

(c) Inhibitors of Glycolysis

(d) Regulation of Glycolysis

(e) Significance of Glycolysis

2.2.1.3 Gluconeogenesis

(a) Thermodynamic barriers

(b) Reactions of Gluconeogenesis

(c) Regulation of Gluconeogenesis

(d) Signficance of Gluconeogenesis

2.2.1.4 Glycogen metabolism

(a) Glycogenesis (or) Glycogen Synthesis

(b) Glycogen Synthase

(c) Branching enzyme

(d) Energy Expenditure

(e) Signficance of Glycogenesis

2.2.1.5 Glycogenolysis

(a) Glycogen phosphorylase

(b) α 1, 4 → α 1,4 glucan transferase

(c) Phosphoglucomutase

(d) Signficance of Glycogenolysis

(e) Regulation of Glycogen metabolism

2.2.1.6 Summary

2.2.1.7 Model Questions

2.2.1.8 Reference Books

Objective

This lesson describe some important carbohydrate catabolic pathways and anabolic pathways and their importance they are:

Glycolysis

Gluconeogenesis

Glycogen Metabolism

2.2.1.1 Introduction

Metabolism is highly coordinated cellular activity in which many multienzyme systems involved. Metabolism the sum of all chemical transformations taking place in a living cell occurs through a series of enzyme catalyzed reactions that constitute metabolic pathways. The term intermediary metabolism often applied to the combined activities of all the metabolic pathways that interconvert precursors, metabolites and products of low molecular weight. It is convenient to consider the metabolism into two separate processes namely anabolism and catabolism. Catabolism is the degradative process of metabolism in which organic nutrient molecules degraded into simple molecules like H_2O, CO_2, NH_3 etc. Catabolic pathways release energy some of which is conserved in the formation of ATP and reduced electron carriers ($NADH_2$, $NADPH_2$ and $FADH_2$) the

rest is lost as heat. In anabolism small, simple precursors are built up in to larger and complex molecules like proteins, lipids carbohydrates and nucleic acids.

In glycolysis, the glucose is converted into pyruvate Glycolysis is the major pathway for the utilization of glucose and is found in the cytosol of all cells. It is unique pathway since it can utilize oxygen if available via the respiratory chain in mitochondria (aerobic) or it can function in the total absence of oxygen (anaerobic). Gluconeogenesis is the term used to include all mechanisms and pathways responsible for converting non carbohydrates to glucose (or) glycogen. The major substrates for gluconeogenesis are the gluconeogenic amino acids, lactate, glycerol, and propionate. Glycogen is the major storage form of carbohydrate in animals and corresponds to starch in plants. It occurs mainly in liver and muscle. Starch it is branched polymer of α-D glucose. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of the blood glucose.

2.2.1.2 Glycolysis

The term glycolysis is derived from Greek words glycos -, sugar (or) sweet and the word lysis means dissolution (or) splitting. Glycolysis is the sequence of reactions that convert (or) oxidize glucose in to two molecules of three carbon compound pyruvate with the production of ATP. It is a universal pathway in biological system. Glycolysis was the first metabolic pathway to be elucidated. It is the principal energy generating pathway in erythrocytes, white striated muscle fibers, brain, skin, renal medulla, retina, lens, cornea and gastrointestinal tract. It is frequently referred to as Embden – Meyerhoff – Parnas (or) EMP pathway, in honour of these pioneer workers in the field of biochemistry.

There are two types of glycolysis depending upon the condition of presence (or) absence of oxygen. In aerobic glycolysis the pyruvate formed by glycolysis is further oxidized by citric acid cycle and oxidative phosphorylation. In this the pyruvate is oxidized to CO_2 and H_2O.

In Anaerobic conditions pyruvate is converted to reduced end product which is lactate in muscle which is called homolactic fermentation and ethnol and CO_2 in yeast which is called alcoholic fermentation.

(a) Pathway

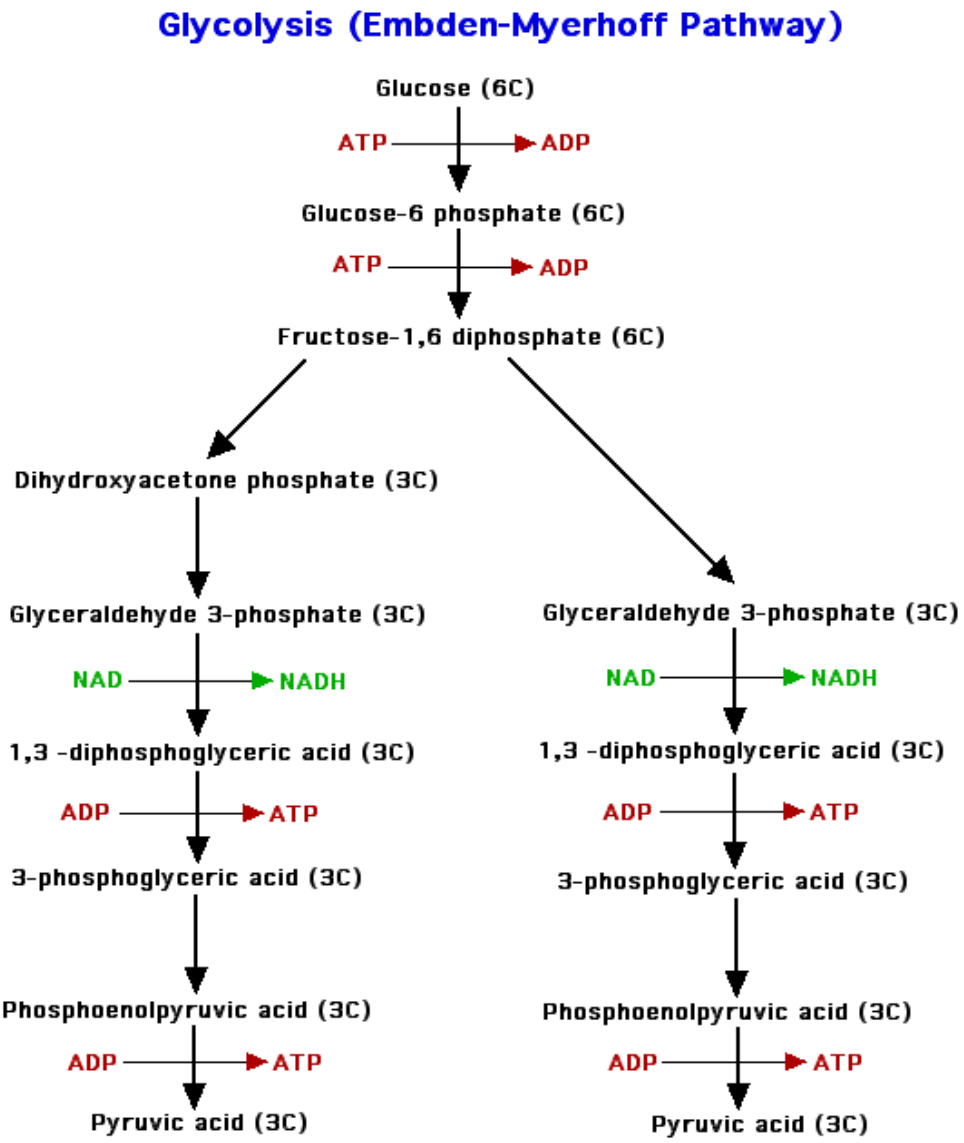
In these pathway 10 types of enzymes are involved. Some enzymes need metal ions and coenzymes. All the enzymes of he pathway are found in cytosol. Which is extra mitochondrial fraction Glycolysis can be divided into two stages.

Stage I (Preparatory phase)

In this stage glucose is phosphorylated and cleaved to yield two molecules of glyceraldehydes 3 phosphate (C_3). This stage consists of five enzyme – catalyzed steps. This process utilizes two ATP'S in a kind of energy investment.

Stage II ("Pay – Off" phase)

This stage catalyzes the oxidation of glyceraldehyde 3-phosphate to pyruvate. It consists of five enzyme – catalyzed reactions. In this stage, 4 ATPS are generated. Glycolysis therefore has a net profit of 2 ATPS per molecule of glucose.



Stage – I

(1) Phosphorylation of Glucose

The first step of glycolysis is phosphorylation of glucose in to glucose 6 phosphate. This reaction is catalyzed by enzyme Hexokinase. Kinases are enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor. Hexokinase catalyzes the transfer of a phosphoryl group from ATP to a variety of hexoses such as glucose, D-fructose and D-mannose Hexokinase requires the

metal ion Mg^{2+} for activity. The reaction is irreversible. Hexokinase is an allosteric enzyme inhibited by its own product glucose 6-pho.

(2) Conversion of Glucose 6 phosphate to fructose 6 phosphate

The enzyme phosphohexo isomerase catalyzes the reversible isomerization of glucose 6 phosphate, an aldose, to fructose 6 phosphate, a ketose.

(3) Phosphorylation of fructose 6 (P) to fructose 1,6 bisphosphate

Fructose 6(P) is phosphorylated by ATP to fructose 1,6 bis phosphate. This reaction is catalyzed by the enzyme phosphofructokinase, an allosteric enzyme. This enzyme requires metal ion Mg^{2+}. This is irreversible reaction. It is major point of regulation in glycolysis.

(4) Cleavage of fructose 1,6 bis phosphate

Fructose 1,6 bisphosphate is cleaved to yield two different triose phosphates, a glyceraldehydes 3-phosphate and dihydroxy acetone phosphate.

(5) Interconversion of triose phosphates

Dihydroxyacetone phosphate readily converts into Glyceraldehyde 3(P). The reaction is catalyzed by the enzyme Triose phosphate isomerase.

By this conversion two molecules of GAD 3 (P) are formed from one molecule of fructose 1,6 bisphosphate by the sequential action of aldolase and Triose phosphate isomerase.

Stage – II

(6) Oxidation of GAD 3(P) to 1,3 bis phosphoglycerate

This reaction is catalyzed by the enzyme glycerol–dehyde 3 phosphate dehydrogenase. In this step glyceraldehyde (P) oxidized and phosphorylated to produce 1,3 bis phosphoglycerate. In the process, a molecule of NAD^+ is reduce to NADH. This reaction generates a compound 1,3 bis phospho glycerate has a high phosphoryl – group potential than ATP.

(7) Formation of ATP from 1,3 bis phosphoglycerate

The enzyme phosphoglycerate kinase catalyzes the transfer of phosphoryl group from 1,3 bisphosphoglycerate to ADP, forming ATP and 3 phosphoglycerate.

The formation of ATP by phosphoryl group transfer from a substrate such as 1,3 bisphosphoglycerate to ADP is referred to as a substrate – level phosphorylation.

(8) Conversion of 3 phosphoglycerate to 2-phosphoglycerate

3 phosphoglycerate by the interchange of phosphoryl group converted to 2-phosphoglycerate. This reaction is catalyzed by the enzyme phosphoglyceromutase.

(9) Dehydration of 2 phosphoglycerate to phosphoenol pyruvate

Phosphoenol pyruvate is formed by the dehydration of 2 phosphoglycerate. This reaction is catalyzed by the enzyme enolase. Phosphoenol pyruvate has a high phosphoryl group transfer potential.

(10) Formation of pyruvate and the generation of a second ATP

The enzyme pyruvatekinase catalyzes the conversion of phosphoenol pyruvate to pyruvate. It is irreversible reaction. In this substrate level phosphorylation phosphoryl group transfer from substrate phosphoenol pyruvate to ADP.

Pyruvatekinase is regulated by allosteric modulators.

The stochiometry of glycolysis of glucose in to pyruvate is as follows:

Glucose + 2 Pi + 2 NAD^{+} + 2 ADP → 2 pyruvate + 2H_2O + 2 NADH + 2H^{+} + 2 ATP

In the stage I glycolysis 2 ATP molecules are consumed and in the second stage of glycolysis 4 ATP molecules are formed. Thus glycolysis has a net yield of two molecules of ATP per molecule of glucose.

(b) Energitics of Glycolysis

Under Anaerobic conditions

S.No.	Substrate	Enzyme	Product	ATP
1	Glucose	Hexokinase	Glucose 6(P)	-1

		→		
2	Fructose 6(P)	Phosphofructokinase →	Fructose 1,6 bis phosphate	-1
3	1,3 bis phospho glycerate	Phosphoglycero → Kinase	3 phosphoglycerate	+2
4	Phosphoenol pyruvate	Pyruvate → Kinase	Pyruvate	+2

Under Aerobic conditions

S.No.	Substrate	Enzyme	Product	ATP
1	Glucose	→ Hexokinase	Glucose 6(P)	-1
2	Fructose 6(P)	Phosphofructokinase →	Fructose 1,6 bis phosphate	-1
3	1,3 bis phospho glycerate	Phosphoglycero → Kinase	3 phosphoglycerate	+2
4	Phosphoenol pyruvate	Pyruvate → Kinase	Pyruvate	+2
5	Glyceraldehyde 3 phosphate	GAD 3(P) → Dehydrogenase ↗ NAD NADH+H^+ ↘	1,3 bis phosho glycerate	+6

NeT +8

In the conversion of two molecules of GAD 3(P) to 2 molecules of 1,3 bis (P) glycerate. 2 NADH are formed. Each NADH is equal to three molecules of ATP in Electron Transport chain. Thus 6 ATPS are from NADH and two actual ATP are generated. So total 8 molecules of ATP are formed in aerobic glycolysis.

In aerobic glycolysis NADH may be thought of as a "high energy" compound. Where as in anaerobic glycolysis its free energy of oxidation is dissipiated as heat.

(c) Inhibition of Glycolysis

Several inhibitors have been used with yeast and animal tissue preparations to elucidate the steps of glycolysis.

Iodoacetate: It is the inhibitor of glyceraldehyde 3 phosphate dehydrogenase involved in the conversion of GAD 3 (P) to 1,3 bis phosphoglycerate.

Arsenate: It acts as a competitive inhibitor of inorganic phosphate to bind at the active site of the glyceraldehyde 3-(P) dehydrogenase. This results in producing highly labile 1-arseno – 3- phosphoglycerate instead of 1,3 bis phospho glycerate. 1-Arseno – 3 phospho glycerate is hydrolyzed spontaneously into 3 phosphoglycerate without any ATP formation. Thus, arsenate decreases the number of high energy bonds generated by glycolysis.

Fluoride: Flouride inhibits enolase involved in the conversion of 2 phosphoglycerate to phospho enol pyruvate. Flouride is frequently added to blood samples, meant for estimation of blood glucose, so as to prevent any loss of glucose due to glycolysis by blood cells.

Bromohydroxy acetone phosphate

Bromohydroxy acetone phosphate blocks glycolysis by irreversibly inhibiting triose isomerase. Because of its structural similarity with dihydroxy acetone phosphate. Addition of bromohydroxy acetone phosphate to a fermentation mixture leads to the accumulation of dihydroxy acetone phosphate and fructose 1,6 bis phosphate.

(d) Regulation of Glycolysis

Most of the glycolysis reactions are reversible except three reactions. These three reactions are irreversible that are catalyzed by the enzyme hexokinase, phospho fructokinase and pyruvate kinase.

Herokinase: It catalyze the reaction in which glucose is converted to glucose 6(P). Hexokinase is allosterically inhibited by glucose 6 phosphate.

The hexokinase of myocytes has a high affinity for glucose. Glucose entering myocytes from the blood produces an intracellular glucose concentration high to saturate hexokinase so that it normally acts at its maximum rate.

Muscle hexokinase is allosterically inhibited by its product glucose 6(P) whenever the concentration of glucose 6(P) in the cell rises above its normal level, hexokinase is temporarly and reversibly inhibited.

Phosphofructokinase

It is a complex allosteric enzyme with many stimulatory (or) prositive and inhibitory (or) negative modulators. In skeletal muscle phosphofructokinase rate is regulated by the concentration of its substrate, ATP, fructose 6(P) and also its products fructose 1,6 bis phosphate and ADP. ATP in higher amounts acts as a negative modulators. So inhibition of reaction occur. ADP and fructose 1,6 bis phosphate acts as a positive modulators so reaction proceeds.

If concentration of ATP falls during muscular contraction and more energy is required for the body. The phosphofructokinase is acclerated to proceed the glycolytic pathway.

Pyruvate kinase

Pyruvate kinase is inhibited by ATP. In vertebrates there are atleast three isoenzymes of pyruvate kinase differing some what in their tissue distribution and in their response to modulators.

The substrate for pyruvate kinase is phosphoenol pyruvate and ATP. High concentration of ATP inhibits pyruvate kinase allosterically, of decreasing the affinity of the enzyme for its substrate phosphoenol pyruvate.

Pyruvate kinase is also inhibited by acetyl co A and by long chain fattyacids both are important fuels for the citric acid cycle.

(e) Significance of Glycolysis

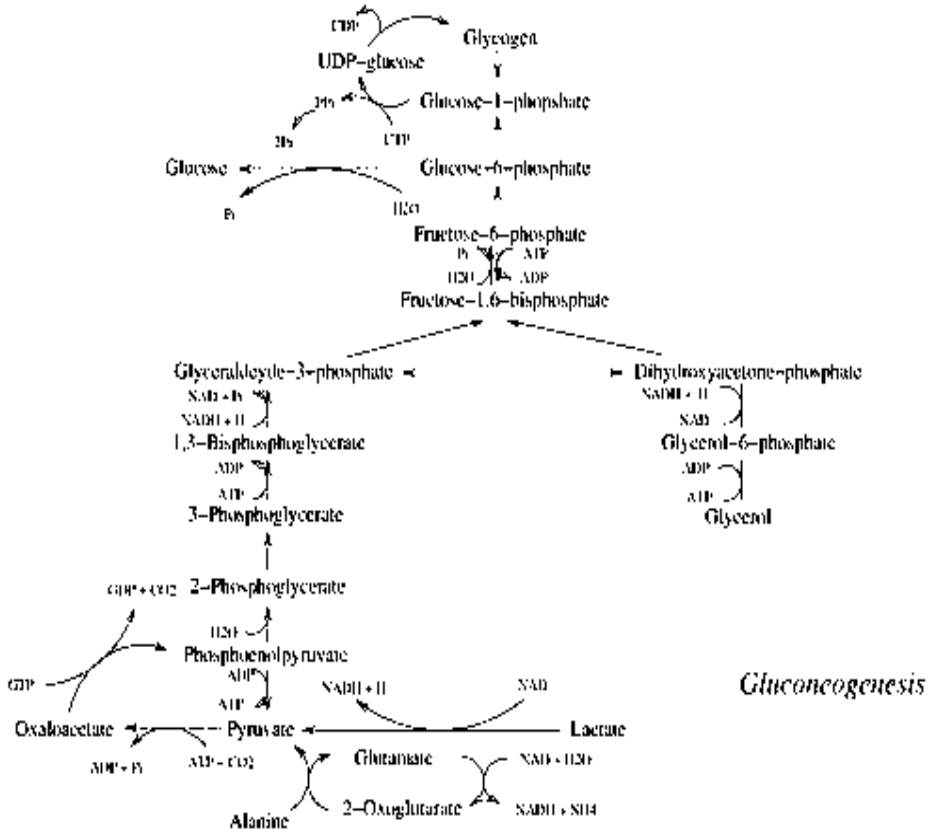
- ➔ Glycolysis is the shortest anaerobic pathway that gives energy instantaneously.
- ➔ Some of the tissues that have to work even under hypoxic conditions prefer this pathway.
- ➔ Ex: skeletal muscles, white fibres, RBC.
- ➔ Some of the tissues and organs that depend upon glycolysis include retina, skin, Gastro intestinal tract brain etc. try to derive energy by utilizing glucose.

2.2.1.3 Gluconeogenesis

Gluconeogenesis is the formation of glucose from non carbohydrate materials. It is carried in the liver and the renal cortex. The liver normally synthesize 9-10 times more glucose by gluconeogenesis than the kidneys. Non carbohydrate precursors like pyruvate, lactate, glycerol and certain amino acids participates in gluconeogenesis. When carbohydrates are not available in sufficient amounts from the diet then gluconeogenesis occur.

In glycolysis pyruvate is formed from glucose but in gluconeogenesis glucose is formed from pyruvate. Although gluconeogenesis is not the reversal of glycolysis.

Some steps of glycolysis are reversible and the same enzymes that catalyze their forward reaction during glycolysis can also catalyze the respective reverse reactions during gluconeogenesis.



(a) Thermodynamic barriers

There are three essential irreversible steps in glycolysis. These reactions in glycolysis are called thermodynamic barriers. Synthesis of glucose from pyruvate is possible only when these thermodynamic barriers are bypassed. These reactions are thermodynamic barriers at three steps prevents the simple reversal of glycolysis. These steps are:

1. Conversion of pyruvate to phosphoenol pyruvate.
2. Conversion of fructose 1,6 bis (P) to fructose 6(P).
3. Conversion of glucose 6(P) to glucose.

(b) Reactions of Gluconeogenesis

The enzymes of gluconeogenesis are present partly in the mitochondrial matrix and partly in the cytosol.

1st thermodynamic barrier

Conversion of pyruvate to phosphoenol pyruvate

Phosphoenol pyruvate is formed from pyruvate by the way of formation of oxaloacetate. Pyruvate is first carboxylated to oxaloacetate by consuming ATP. This reaction is catalyzed in the presence of pyruvate carboxylase, CO_2, ATP and Biotin.

In this the enzyme combines with carboxy biotinyl group that means the biotin is place an important role to transfer the carboxyl group. First enzyme combines with biotin and then biotin and then biotin combines the carboxyl group from bicarbonate in the presence of ATP.

E – biotin + HCO_3^- → E – carboxyl biotin + ADP

Enzyme carboxyl biotin transfers the carboxyl group to pyruvate then pyruvate converts into oxaloacetate.

E – carboxyl biotin + Pyruvate → oxaloacetate + E – Biotin

Phosphoenol pyruvate carboxy kinase : (PEPCK)

PEPCK, a monomeric enzyme, catalyzes the GTP – driven decarboxylation of oxaloacetate to form phosphoenol pyruvate and GDP.

The cellular location fo PEPCK varies with the species. In mouse and rat liver it is located almost exclusively in the cytosol. Which creates a problem because oxaloacetate does not diffuse through the mitochondrial inner membrane. This is overcome by conversion of malate. Malate is transported to cytoplasm where it is reconverted to oxalocacetate by extra mitochondrial malate dehydrogenase.

In pigeon and rabbit liver phosphoenol pyruvate carboxykinase is a mitochondrial enzyme and phosphoenol pyruvate is transported in to the cytosol for conversion into fructose 1,6 bis phosphate by reversal of glycolysis.

In humans, guinea pig and the cow the phosphoenol pyruvate carboxykinase is equally distributed in mitochondria and cytoplasm.

Conversion of phosphoenol pyruvate to 2 phosphoglycerate

Phosphoenol pyruvate is hydrated to 2 phosphoglycerte by enolase.

Conversion of 2 phospho glycerate to 3 phospho glycerate

Phospho glycerate causes the intramolecular rearrangement of 2 phospho glycerate to 3 phospho glycerate.

Formation of 1,3 bis Phospho glycerate

Phospho glycerol kinase adds a phosphate moiety to 3 phospho glycerate to form 1,3 bis phospho glycerate.

Reduction and dephosphorylation of 1,3 bis phosphoglycerate

Glyceradehyde 3 phosphate dehydrogenase is NAD dependant enzyme causes the reductive dephosphorylation of 1,3 bis phospho glycerate to glyceraldehyde 3 phosphate one NADH is used in this reaction.

Isomerisation

Triose phosphate isomerase isomerises glyceradehyde 3 phosphate to dihydroxy acetone phoshate.

Aldol condensation

Glyceraldehyde 3 phosphate and dihydroxy acetone phosphate are condensed to form fructose 1,6 bis phosphate in the presence of aldolase.

Formation of fructose 6 phosphate from fructose 1,6 bis phosphate:

Second Thermodynamic barrier:

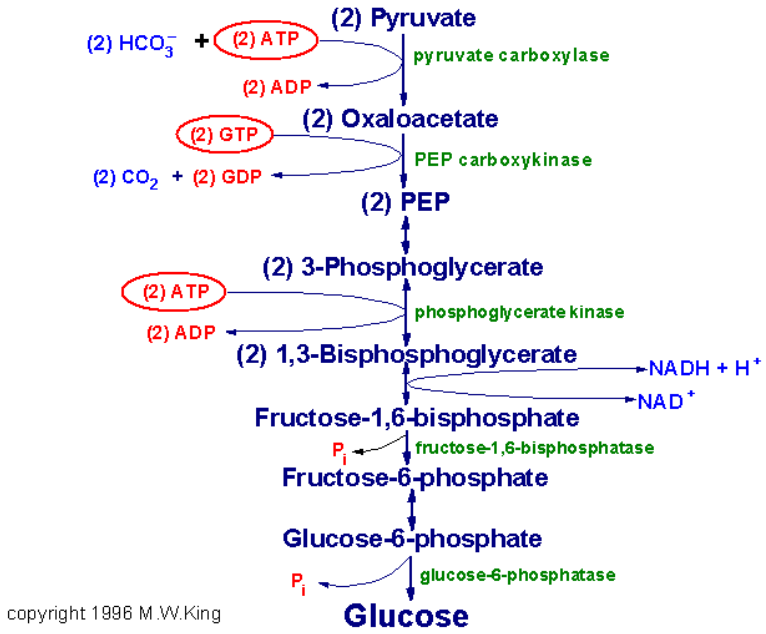
Fructose 1,6 bis phosphatase converts fructose 1,6 bis phosphate to fructose 6 (P). Here the phosphate moiety at the first position is hydrolysed in the presence of water.

Isomeristion

Fructose 6 phosphate is isomerised to glucose 6 – phosphate in the presence of phospho glucoisomerase.

Formation of Glucose 6 phosphate to Glucose third thermodynamic barrier

Glucose 6 phosphotase hydrolyses the phosphate moiety from the 6th position of glucose 6 phosphate to form glucose.



Major substrates for gluconeogenesis entry of fats

The fattyacids of the fat when undergo β-oxidation (fattyacid oxidation) liberate acetyl CoA residues. This can enter TCA cycle and produce the end product oxaloacetate Oxaloacetate can be converted to phospho enol pyruvate by

phosphoenol pyruvate carbooxykinase phosphoenol pyruvate enters in to gluconeogenesis and produce glucose.

Entry of glycerol in to gluconeogenesis

Glycerol, a metabolic product of fat hydrolysis can enter gluconeogenesis as follows :

Glycerol is converted in to glycerol 3 phosphate in the presence of enzyme glycerol kinase.

Glycerol 3 (P) is converted in to dihydroxy acetone phosphate in the presence of enzyme Glycerol 3(P) dehydrogenase. NAD is reduced to NADH.

Dihydroxy acetone phosphate is converted in to glycerol dehyde 3 phosphate in the presence of enzyme Triose phosphate isomerase.

GAD 3(P) and DHAP condenses to form fructose 1,6 bis phosphate in the presence of enzyme Aldolase.

Fructose 1,6 bis phosphate converts fructose 6 phosphate and then Glucose 6 (P).

Finally Glucose 6(P) converts in to Glucose.

Entry of propionate in to gluconeogenesis

Propionate is the source of gluconeogenesis. It happens in the case of ruminants. It enters the gluconeogenesis pathway via TCA cycle after conversion to acetyl coA.

Propionate is converted in to propionyl coA in the presence of enzyme acetyl coA synthase.

Propionyl coA is converted in to D – methyl malonyl coA in the presence of enzyme propionyl-coA carboxylase. Biotin requiring enzyme.

D-methyl malonyl coA is converted in to Carboxy-methyl malonyl coA in the presence of enzyme Racemase.

L-methyl malonyl CoA is converted in to succinyl coA in the presence of enzyme isomerase.

Succinyl CoA enters in to TCA cycle to form oxaloacetate.

Oxaloacetate is converted in to Glucose when enters in to gluconeogenesis pathway.

Gluconeogenic amino acids

Gluconeogenic amino acids Intermediate through which they enter gluconeogenesis

Glycine, alanine, cysteine

Serine, Methionine → pyruvic acid →Glucose Lysine, Arginine, Ornitine

Proline, Histidine, Tryptophan → α Ketoglutaric acid → Glucose Citruline

Valine, isoleucine → succinyl coA → Glucose

Aspartic acid → oxaloacetate → Glucose

All these amino acids breakdown to produce TCA cycle intermediates. These intermediates enters in to gluconeogenesis to produce glucose.

(c) Regulation of Gluconeogenesis

Pyruvate carboxylase, phosphoenol pyruvate carboxy kinase, fructose 1,6 bis phosphatase and glucose 6. Phosphatase ae the key regulated enzymes of this pathway.

Fattyacid oxidation promotes gluconeogenesis from pyruvate because acetyl coA from fattyacids serves energy purposes and spares pyruvate for gluconeogenesis. Acetyl coA as well as NADH from β-oxidation inhibits pyruvate dehydrogenase to reduce the aerobic oxidation of pyruvate. Glucagon, adrenaline and glucocorticoids induces the synthesis of pyruvate carboxylase and increase gluconeogenesis. Insulin represses the enzyme and reduces gluconeogenesis.

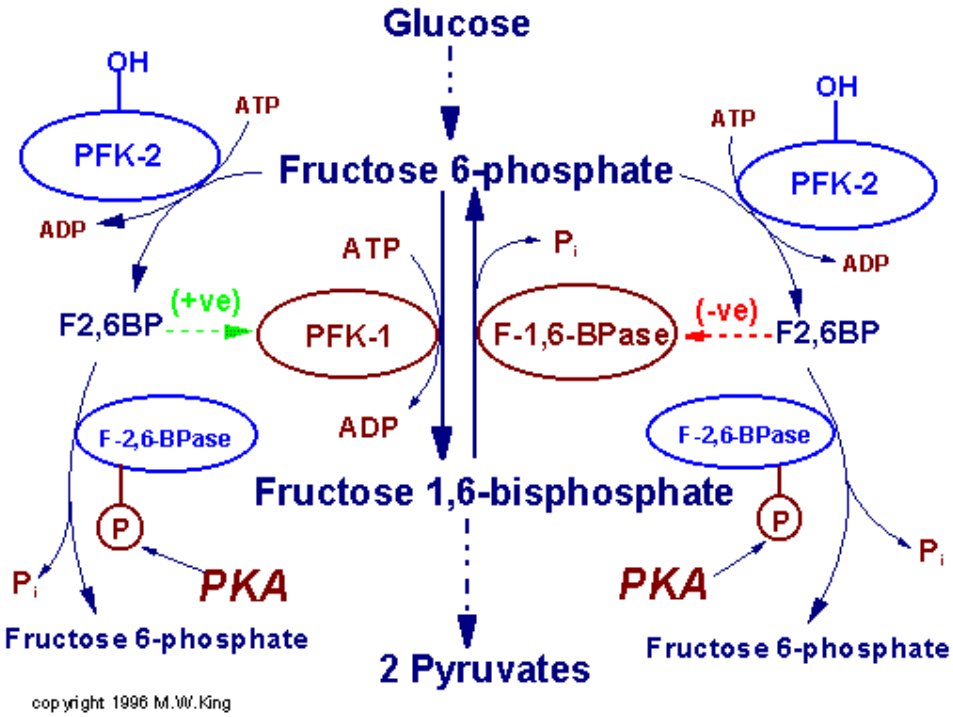
Phospho enol pyruvate carboxy kinase is induced by glucagons during starvation leading to increased gluconeo- genesis. Glucagon increases the intracellular concentrations of CAMP which inturn increases the transcription of the phospho enol pyruvate carboxy kinase gene in to the mRNA meant for translating PEPCK. Insutin reduces gluconeogenesis and repress PEPCK by lowering the transcription rate of its gene.

Fructose 1,6 bisphosphotase is strongly and allosterically inhibited by AMP. But it is activated by citrate. So gluconeogenesis is promoted due to its activation when liver cells are rich in ATP and in fuel molecules such as citrate. Gluconeogenesis is reduced through the inhibition of this enzyme when liver cells are poor in citrate and rich in AMP.

Glucagon, adrenaline the glucocorticoids also induce the synthesis of fructose 1,6 bis phosphotase to enhance gluconeogenesis.

Glucose 6 phosphotase is induced by glucagons and cortisol secreted during starvation. This enhances gluconeogenesis. Insulin represses the enzyme.

High carbohydrate diet reduce gluconeogenesis by raising the insulin/glucagons ratio and thereby reducing the activities of all four key gluconeogenic enzymes.



(d) Significance of gluconeogenesis

- ➔ When carbohydrates insufficient in the diet, gluconeogenesis meet the needs of the body glucose.
- ➔ A continued supply o glucose is necessary as a source of energy especially to the nervous system and RBC.
- ➔ Glucose is the only fuel which supplies energy to the skeletal muscles under anaerobic conditions.

- ➔ Kidneys – Energy is continuously required by the tubular cells of nephron for active reabsoption of several solutes from the lumen of tubule in to tubular cell. The process of active reabsorption requires ATP which is supplied by gluconeogenesis.

- ➔ It serves as the major metabolic pathway for propionate. Genetic failure of gluconeogenesis from propionate produces fatal methylmalonoic aciduria in human infants. Gluconeogenesis from propionate is important in maintaining the blood sugar in ruminants.

2.2.1.4 Glycogen Metabolism

Glycogen is the readily metabolized form of glucose. It is a very large polymer of glucose residues. Most of the glucose residues are linked by (α1,4) bonds and the branches are created by (α 1,6) glycosidic linkages. The two major sites of glycogen storage are the liver and skeletal muscle.

The synthesis and degradation of glycogen is important because they regulate blood glucose level and provide a reservoir of glucose for muscular activity.

Glycogen metabolism shows two phases Glycogenesis, Glycogenolysis.

(a) Glycogenesis (or) Glycogen Synthesis

The formation of glycogen from glucose is called glycogenesis.

Glycogen is the major storage form of carbohydrates in animals and corresponds to starch in plants. It occurs mainly in the liver and muscle. Brain and kidneys carry out some glycogenesis and store some glycogen.

Reaction sequence

Glucose is phosphorylated to glucose 6 phosphate with the help of ATP, Mg^{2+}. This reaction is common to the first reaction in the pathway of glycolysis from glucose. This reaction is catalyzed by hexokinase in the muscle and glucokinase in liver.

$$\text{Glucose} + \text{ATP} \xrightarrow[\text{Hexokinase}]{\text{Glucokinase}} \text{Glucose 6(P)} + \text{ADP}$$

Hexokinase are relatively less specific for the substrate and can phosphorylate α and β-D glucose, other D-hexoses.

Glucokinase, an inducible enzyme acts specifically on glucose. Glucokinase predominates over hexokinase in the liver. Hexokinase predominate in extrahepatic tissue.

Glucose 6(P) is converted in to glucose 1 phosphate in a reaction catalyzed by Phosphoglucomutase.

Glucose 1 phosphate is more energetic form than glucose 6 phosphate.

In the next reaction glucose 1 phosphate is reacts with Uridine Tri Phosphate (UTP) to form UDP glucose. This reaction is catalyzed by UDP glucose pyro phosphorylase. In this the two terminal phosphate groups of UTP are released as inorganic pyrophosphate. An inorganic pyro phosphatase immediately hydrolyzes inorganic pyrophosphate. UDP glucose is a highly reactive complex of glucose. It serves as a donor of glucose. UDP glucose is an activated form of glucose just as ATP and the activated forms of orthophosphate and acetate.

(b) Glycogen Synthase

This enzyme is active in the dephosphorylated form. It requires primer to initiate the catalysis. Glycogen synthase can transfer glucose from UDP glucose to the non reducing end of a preexisting oligosaccharide but not to another free glucose molecule. Initiation of glycogenesis, the enzyme uses a protein called glycogenin as the initiation primer and transfers the glucose from a UDP glucose to the OH group of a tyrosine residue of glycogenin. The first carbon of the glucose gets linked with the Tyr – OH by a O-glycosidic bond. A second glucose molecule is next transferred from another UDP – glucose molecule to the fourth carbon of the glycogenin – bound glucose. This is followed by similar additions of successive glucose molecules to the non reducing end of the growing oligosaccharide, held by glycogenin. The first carbon of each added glucose binds to the fourth carbon of the pre existing terminal glucose residue of the oligosaccharide chain by an α 1,4 glycosidic bond. That is acted upon by glycogen synthase. On each one glucose addition releasing one UDP molecule at each step. Nucleoside diphosphokinase may rephosphorylate the released UDP to UTP, utilizing a phosphate group of ATP.

(c) Branching enzyme

After repeated glycogen synthase action has extended the chain length by 11 (or) more glucose residues, branching enzyme that is amylo α 1,4 → α 1,6 trans glycosylase transfers a part of α 1,4 chain to a neighbouring chain to form a new 1,6 branching. This gives a branch point in the molecule. The branches grow a

further addition of α 1,4 glycosyl units and further branching enzyme a complex polymer that is glycogen is formed with an extensive branching and linear growth.

(d) Energy Expenditure

During glycogenesis one high energy phosphate bond of ATP is spent in phosphorylating glucose to glucose 6 phosphate. UTP loses two phosphate bonds as inorganic pyrophosphate (PPi) in forming UDP glucose but regains one phosphate bond when it is released as UDP during glycogen synthase action. Finally UDP regains another phosphate bond also from ATP which however loses a phosphate bond during this reaction.

$$\text{Glucose} + (\text{Glycogen})_{n-1} + 2\ \text{ATP} + H_2O \rightarrow (\text{Glycogen})_{n+1}\ 2\ \text{ADP} + 2\ \text{Pp}$$

Hence two phosphate bonds of ATP are spent in incorporating each glucose molecule in to glycogen.

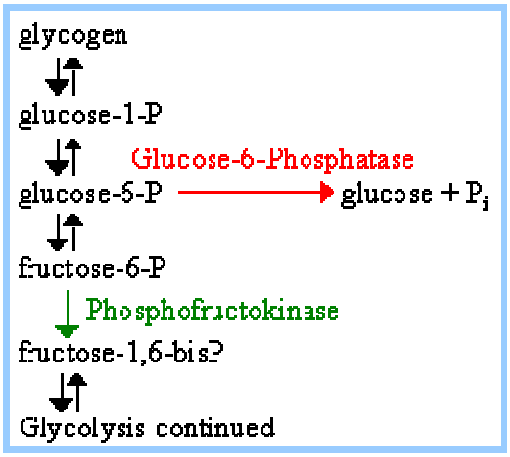
(f) Significance of Glycogenesis

Glycogenesis form glycogen as the storage polysaccharide. It helps to bring down the blood sugar level after a carbohydrate meal.

- ➔ The polymerization of glucose to glycogen reduces the bulk of the stored nutrients by eliminating water molecules from glucose.
- ➔ Extensive branching of the glycogen molecule help the actions of glycogen synthase and glycogen phosphorylase because both act only at the non reducing ends of the branches.
- ➔ It also contributes to energy production during intense activity.
- ➔ Glycogenesis help the cell to form and store a readily mobilizable polysaccharide.
- ➔ Glycogen has for larger molecules than glucose and is stored mainly as electron dense particulate cytoplasmic granules.
- ➔ Glycogenesis enables the cell to store large amounts of glycogen without any significant rise in the intracellular osmotic pressure.

2.2.1.4 Glycogenolysis

Glycogenolysis is the breakdown of glycogen to glucose mainly in the liver. The liver has tremendous capacity for storing glycogen. In the well fed humans liver glycogen content can account for as much as 10% of wet weight of this organ. The pathway of glycogen degradation was elucidated by Carl Cori and Gerty Cori. The glucose is produced from glycogen polymer. The glucose that is produced can be used as fuel. Glycogen break down requires the actions of three enzymes.



Reaction sequence

This process takes place on smooth endoplasmic reticulum and cytosol.

(a) Glycogen phosphorylase

Glycogen phosphorylase catalyzes the phosphorolysis of the α 1,4 glycosidic bonds in the stored glycogen with the help of inorganic phosphate. This breaks the α 1,4 bonds between successive glucose residues starting from the non reducing end of an outer chain of glycogen molecule and releases one glucose 1 phosphate molecule at each step.

The enzyme however cannot cleave the α 1,6 glycosidic bonds at the branching points of glycogen chains. Its repeated action on successive α 1,4 bonds in a branch of the glycogen molecule progressively shortens the branch. Glycogen posphorylase stops acting on a branch when only four glucose residues are left in it. It is the rate limiting step of glycogenolysis.

Glycogen de branching enzyme acts on the shortened branch, left after glycogen phosphorylase action, and removes that branch. The de branching enzyme shows two enzyme activities located at different loci of its molecule via α 1,4 → α 1,4 glucan transferase and amylo α 1,6 glucosidase activities.

(b) α 1,4 → glucan transferase

α 1,4 → α 1,4 glucan transferase activity cleaves the last α 1,4 glycosidic bond before the α 1,6 glycosidic bond at the branching point of the shortened branch, transfers the trisaccharide thus released from that branch to another branch and joins the trisaccharide to the latter by a new α 1,4 glycosidic bond.

The α 1,6 glycosidic bond, holding the last glucose residue of the shortened branch, is next hydrolyzed by amylo 1,6 glucosidase activity releasing that glucose residue as free glucose.

About 90% of the glucose residues of glycogen are release as glucose 1 – phosphate by glycogen phosphorylase while the remaining 10% are released as glucose by the de branchig enzyme.

(c) Phosphoglutomutase

Phosphoglucomutase isomerizes glucose 1 phosphate to glucose 6 phosphate in the presence of Mg^{2+}. The enzyme transfers a phosphate group from its phospho serine residue to the C-6 hydroxy methyl group of glucose 1 – phosphate changing the latter to a glucose 1,6 bis phosphate intermediate. A phosphate group is next transferred from the C-1 of the intermediate to the serine residue of the enzyme, reconverting that residue to phospho serine and producing glucose 6 phosphate.

Glucose 6 phosphate cannot pass in to the blood through the plasmamembrane. Hepatocytes possess glucose 6 phosphatase in their microsomal membrane, this enzyme hydrolyzes glucose 6 phosphate in to glucose. Glucose latter passing out from the cell in to the blood.

(d) Significance of glycogenolysis

Hepatic glycogenolysis adds glucose to the blood for maintaining the blood sugar level inspite of variations in the food intake and during the first 24 hours of fasting. But if fasting continues longer, glycogenolysis loses its importance.

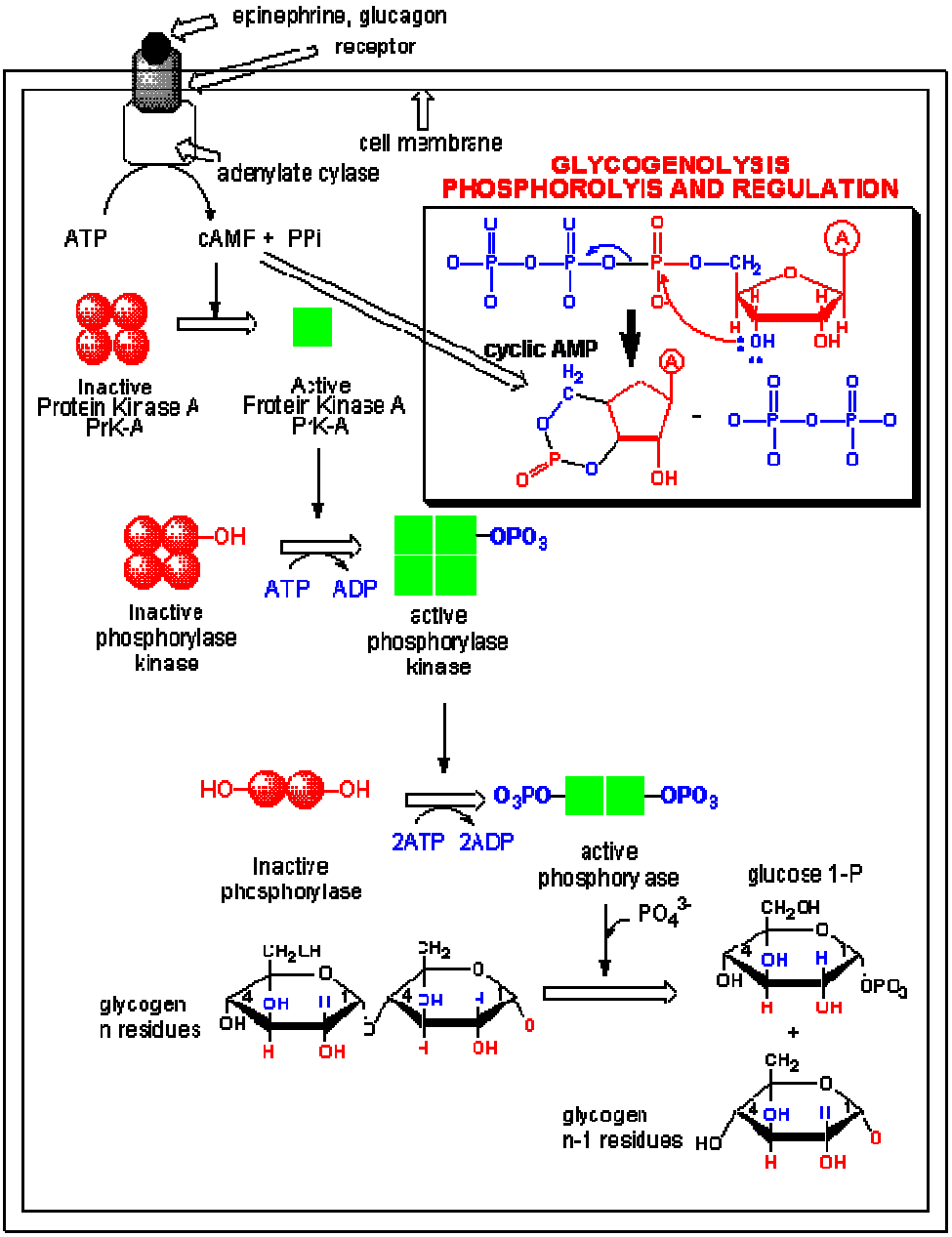
(e) Regulation of glycogen metabolism

Glycogen metabolism is regulated through hormonal control via cyclic AMP. Glycogen synthesis and degradation are coordinated so that glycogen synthase is nearly inactive when phosphorylase is fully active and vice-versa.

Glycogen metabolism is profoundly effective by a specific hormones. Insulin increases the capacity of the liver to synthesize glycogen. Epinephrin and glucagons have effects antagonistic to that of insulin. Epinephrin markedly stimulate glycogen breakdown in muscle and to a lesser extent in liver. The liver is more responsive to glucagons which is secreted by α cells of pancreas when blood sugar level is low.

The action of epinephrin and glucagons is mediated by cyclic AMP. Synthesis of CAMP from ATP is catalysed by adenyl cyclase, an enzyme associated with plasmamembrane.

The increase in intracellular level of cyclic AMP triggers a series of reaction that activate phosphorylase and inhibit glycogen synthase. Phosphorylase is active in its phosphorylated form where as glycogen synthase is active in its dephosphorylated form. This phosphorylation is brought about by a proteinkinase stimulated by cyclic AMP and dephosphorylation is brought about by an enzyme phosphatase. So glycogen metabolism in liver regulates blood glucose level.



2.2.1.6 Summary

Glycolysis is the pathway found in the cytosol of all mammalian cells for the metabolism of glucose to pyruvate and lactate. It can function aerobically by regenerating oxidized NAD^+ required in the glyceraldehyde 3 phosphate reaction by coupling this reaction to the reduction of pyruvate to lactate. Lactate is the end product of glycolysis under anaerobic conditions (or) when the metabolic

machinery is absent for the further oxidation of pyruvate. Glycolysis is regulated by the three enzymes catalyzing non equilibrium reactions namely hexokinase phospho fructo kinase and pyruvatekinase pyruvate is oxidized to acetyl coA by a multienzyme complex known as pyruvate dehydrogenase that is dependant on the vitamin cofactor thiamin pyrophosphate.

Gluconeogenesis is the mechanism for converting non carbohydrates to glucose (or) glycogen. It provides the body with glucose when carbohydrate is not available from the diet. Important substrtes are glucogenic amino acids lactate, glycerol and propionate. The pathway of gluconeogenesis found in the liver and kidney utilizes those reactions in glycolysis which are reversible plus four additional reactions the circumvent the irreversible non equilibrium reactions. The enzymes catalyzing the additional reactions are pyruvate carboxylase, phosphoenol pyruvate carboxy kinase, fructose 1,6 bis phosphotase and glucose 6 phosphotase. Lactate forms pyruvate which enters the mitochondrion for carboxylation to oxaloacetate before conversion to phosphoenol pyruvate followed by biosynthesis of glucose in the cytosol. Since glycolysis and gluconeogenesis share the same pathway but operate in opposite directions their activities must be regulated reciprocally. This is achieved by three main mechanisms affecting the activity of key enzymes induction to enzyme synthesis, covalent modification by reversible phosphorylation and allosteric effects. Defective enzymes of glyconeogenesis lead to hypoglycemia and lactic acidosis. Blocks in fatty acid oxidation are an additional cause of impaired gluconeogenesis and hypoglycemia. A deficiency in Insulin secretion results in type I diabetes mellitus. Insulin is secreted as a direct response to hyperglycemia, it assists the liver to store glucose as glycogen and facilitates uptake of glucose in to extrahepatic tissues. Glucagon is secreted as s response to hypoglycemia and activates both glycogenolysis and gluconeogenesis in the liver causing release of glucose in to the blood.

Glycogen represents the principle storage form of carbohydrate in the mammalian body present mainly in the liver and muscle. In the liver the major function is to service the other tissues via formation of blood glucose. In muscle it serves the needs of that organ only at as a ready source of metabolic fuel. Glycogen is synthesized from glucose and other precursors by the pathway of glycogenesis. It is broken down by a separate pathway known as glycogenolysis. Glycogenolysis leads to glucose formation in liver and lactate formation in muscle owing to the respective presence (or) absence of glucose 6 phosphotase. Cyclic AMP integrates the regulation of glycogenolysis and gycogenesis by promoting the simultaneous activation of phosphorylase and inhibition of glycogen synthase, insulin acts reciprocally by inhibiting glycogenolysis and stimulating glycogenesis. Inherited deficiencies in specific enzymes of glycogen metabolism in both liver and muscle are the causes of glycogen storage diseases.

2.2.1.7 Model Questions

1. Describe the glycolysis and its importance.
2. How blood glucose level is maintained by using gluconeogenesis.
3. Write about glycogenesis and its importance.
4. Explain about glycogenolysis.
5. In what way non carbohydrate source is converted to carbohydrate source.

2.2.1.8 Reference Books

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(Author - **Tulasi)**

Lesson 2.2.2

CITRIC ACID CYCLE ELECTRON TRANSPORT CHAIN, OXIDATIVE PHOSPHORYLATION

Objective

2.2.2.1 Introduction

2.2.2.2 Citric acid cycle

(a) Reactions of the TCA cycle
(b) Energitics of TCA cycle
(c) Inhibitors
(d) Regulation of TCA cycle
(e) Significance of TCA cycle

2.2.2.3 Electron Transport chain

(a) Reaction sequence
(b) Electron shuttles

2.2.2.4 Oxidative phosphorylation

(a) Chemical coupling hypothesis
(b) Confirmational coupling hypothesis
(c) Chemiosmatic theory
(d) Regulation
(e) Energy captue of the respiratory chain
(f) Uncouplers

2.2.2.5 Summary

2.2.2.6 Model Questions

2.2.2.7 Reference Books

Objective

This lesson mainly deals with the

Citric acid cycle

Electron transport chain

Oxidative phosphorylation

2.2.2.1 Introduction

In glycolysis the glucose is converted in to pyruvate under aerobic conditions the next step in the generation of energy from glucose is the oxidative decarboxylation of pyruvate to form acetyl COA. This activated acetyl COA is then completely oxidized to CO_2 by the citric acid cycle. The citricacid cycle is the final common pathway for the oxidation of fuel molecules aminoacids, fattyacids and carbohydrates. Reducing equivalents NADH, $FADH_2$ formed in glycolysis, fattyacid oxidation, and the citric acid cycle are finally oxidized by the electron transport chain large amount of energy is liberated during the oxidation of reducing equivalents. The system in mitochondria that couples respiration to the generation of the high energy intermediate. ATP, is termed oxidative phosphorylation. The electrons traveling from NADH or $FADH_2$ to O_2 through electron carriers of electron transport chain leads to the pumping of protons across the membrane. A proton motive force is generated consisting of a pH gradient and transmembrane electric potential. Thus oxidation and phosphorylation are coupled by proton gradient across the membrane.

2.2.2.2 Citric acid cycle

Citric acid cycle is the series of reactions in the mitochondria which catabolizes the oxidation of acetyl COA to CO_2 and H_2O in aerobic conditions. This cycle is also known as Tri carboxylic acid cycle Hans krebs completely elucidate this cycle that's why this cycle is also called as krebs cycle.

Oxidation of pyruvate to acetyl-COA

Pyruvate is oxidatively decarboxylated to acetyl COA before entering the citric acid cycle.

The reaction is catalyzed by the multienzyme complex consisting of several different enzymes. This complex is known as pyruvate dehydrogenase complex.

- ➔ Pyruvate dehydrogenase complex contains three enzymes pyruvate dehydrogenase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase.

- ➔ Pyruvate is decarboxylated in the presence of Thiamin pyrophosphate (TPP) to a hydroxy methyl derivative which reacts with oxidized lipoate to form S-acetyl lipoate being catalyzed by the enzyme pyruvate dehydrogenase.

- ➔ S-acetyl lipoate reacts with coenzyme A to form acetyl coA and reduced lipoate in presence of dihydrolipoyl transacetylase.

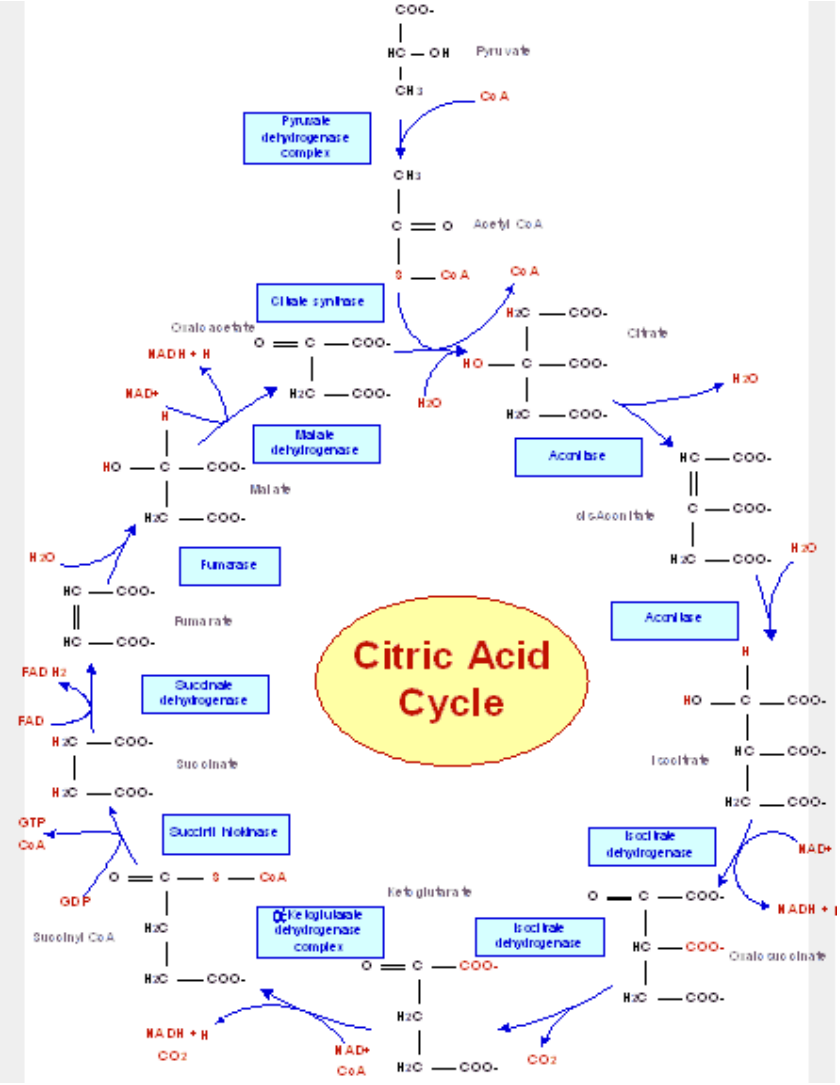
- ➔ The reduced lipoate is reoxidized by FAD in presence of dihydrolipoyl dehydrogenase.

- ➔ Finally the reduced FAD is oxidized by NAD^+ the reduced NAD (NADH $+H^+$) enters the respiratory chain producing 3 ATP.

- ➔ Arsenate inhibits pyruvate dehydrogenase and dietary deficiency of thiamin also allows pyruvate to accumulate.

(a) Reactions of the TCA cycle

This is the final common pathway for the oxidation of the end products of glycolysis, β-oxidation and catabolism of many amino acids. The cycle operates in the mitochondrial matrix and is highly operative in cardiac muscle liver and restricted muscle fibers but is less operative in white striated muscle fibers. TCA cycle operates in the brain also but not in erythrocytes devoid of mitochondria.



Citrate synthetase is the gateway to the TCA cycle

The first step of TCA cycle is the reaction catalyzed by citrate synthetase, in which acetyl coA enters the cycle and citrate is formed this reaction is an aldol condensation. In which C-2 of the acetyl group ads to the carboxyl group of oxaloacetate. CoA is released from the product.

Aconitase catalyzes the isomerization of citrate to isocitrate

Citrate is converted into isocitrate in the presence of enzyme aconitase. Cis isomerization of citrate includes the formation of an intermediate compound cis

aconitate by the removal of water molecule. Cis aconitate converts into isocitrate in addition of water molecule. So this step is accomplished by dehydration and followed by hydration.

The three compounds may be considered as belonging to the same metabolic pool.

Isocitrate dehydrogenase catalyzes the first oxidation in the TCA cycle

Isocitrate undergoes dehydrogenation in the presence of the enzyme isocitrate dehydrogenase to form oxalosuccinate. In this step NAD will be reduce to NADH + H^+.

Isocitrate dehydrogenase catalyzes the first decarboxylation in the TCA cycle

Oxalosuccinate converts into α ketoglutarate by decarboxylation reaction in the presence of isocitrate dehydrogenase.

α-ketoglutarate dehydrogenase catalyzes the decarboxylation of α-ketoglutarate to succinyl coA

The oxidative decarboxylation reactions of the TCA cycle is catalyzed by α-ketoglutarate dehydrogenase. α-ketoglutarate converts into succinyl coA. The coenzyme NAD will be reduced to NADH + H^+.

Succinate Thiokinase couples the conversion of succinyl coA to succinate with the synthesis of GTP

The succinyl coA is converted into succinate by the action of succinate thiokinase. In this step GDP will be phosphorylated to GTP.

Succinate dehydrogenase catalyzes the oxidation fo succinate to fumarate

Succinate will be oxidized to fumarate in the presence of the enzyme succinate dehydrogenase. In this step FAD is reduced to $FADH_2$.

Fumarase catalyzes the addition of water to fumarate to form malate

In this step hydration of fumarate occurs for the formation of malate. This reaction is catalyze by the enzyme fumarase.

Malate Dehydrogenase catalyzes the oxidation of malate to oxaloacetate

Finally malate is oxidized to oxaloacetate by the enzyme malate dehydrogenase. Here coenzyme NAD will be reduced to NADH + H^+.

(c) Energitics of TCA cycle

Reaction	No. of ATP formed
Isocitrte → oxalosuccinate	3
α ketoglutarate → sucinyl coA	3
Succinyl coA → succinate	1
Succinate → Fumarate	2
Malate → oxaloacetate	3
Total :	12

Total number of ATP in the complete oxidation of one molecule of glucose

One molecule of glucose forms 2 molecules of pyruvic acid by glycolysis.

Number of ATP formed in glycolysis - 8

Number of ATP formed in the oxidation of pyruvate to aceytyl coA - 3 x 2 = 6

Number of ATP formed in the citric acid cycle - 12 x 2 = 24

Total: 38

(c) Inhibition

Different inhibitors have been used with animal tissue preparations to study the course of the TCA cycle.

Fluoroacetate

Fluoroacetate inhibits the enzyme aconitase and prevents the conversion of citrate to isocitrate.

Arsenite

Arsenite inhibits α-ketoglutarate dehydrogenase and causes α ketoglutarate to accumulate.

Malonate

Malonate is structurally similar to succinate and consequently inhibits succinate dehydrogenase competitively. This blocks the TCA cycle causing accumulation of succinate.

(d) Regulation of TCA cycle

The rate of citric acid is regulated to meet the needs of cells for ATP.

Pyruvate dehydrogenase

Pyruvate dehydrogenase system is regulated mainly by reversible covalent modifications through phosphorylation and dephosphorylation of its pyruvate dehydrogenase complex. A rise in ATP/ADP ratio, NADH/NAD^+ ratio, acetyl coA concentration (or) CAMP concentration in the cell activates pyruvate dehydrogenase kinase to enhance the phosphorylation and inactivation of pyruvate dehydrogenase. Thus in starvation and diabetes, acetyl coA and NADH produced by enhanced β-oxidation activates pyruvate dehydrogenase kinase to inhibit pyruvate dehydrogenase. NADH and acetyl coA also inhibit pyruvate dehydrogenase competitively by occupying its NAD^+ and coA binding sites. A high carbohydrate diet increase pyruvate through enhanced glycolysis. Pyruvate increases pyruvate dehydrogenase activity by inhibiting pyruvate dehydrogenase kinase.

Insulin activates pyruvate dehydrogenase phosphatase and consequently causes dephosphorylation and activation of pyruvate dehydrogenase.

α ketoglutarate dehydrogenase, citrate synthetase and isocitrate dehydrogenase are considered as the enzymes or the rate – limiting steps of the TCA cycle.

Citrate synthetase

Citrate synthetase is activated by the rise in the concentrations of its substrates, acetyl coA and oxaloacetate, and is competitively inhibited by its product citrate and by succinyl coA. Citrate and acetyl coA compete respectively with oxaloacetate and acetyl coA to occupy their binding sites on the enzyme. It is also inhibited by ATP, NADH and long chain acyl – coA like palmitoyl – coA.

(e) Significance of TCA cycle

- ➔ The major function of TCA cycle is to act as the final common pathway for the oxidation of CHO, lipids and proteins. This is because fatty acids many amino acids and carbohydrates are metabolized to acetyl coA (or) intermediates of TCA cycle.
- ➔ It plays a major role in gluconeogenesis, transamanation, deamination and lipogenesis.

The Amphobolic Nature of the citric acid cycle

Generally metabolic pathway as being either catabolic with the release of free energy (or) anabolic with a requirement for free energy. The citric acid cycle is, of course, catabolic because it involves degradation and is a major free energy conservation system in most organisms. Cycle intermediates are only required in catalytic amounts to maintain the degradative function of the cycle. However several biosynthetic pathways utilize citric acid cycle is therefore amphibolic (both catabolic and anabolic).

α - ketoglutarate dehydrogenase

α - ketoglutarate is regulated allosterically. It is inhibited by a rise in the ATP/ADP ratio (or) $NADH/NAD^+$ ratio (or) succinyl coA/coA ratio, all of which indicate a high energy – status of the cell this results in a decline of the overall rate of TCA cycle. Ca^{2+}, ADP (or) AMP activates the enzyme to enhance the operation of the cycle.

Isocitrate dehydrogenase

Isocitrate dehydrogenase is activated by the rise in intracellular concentration of Ca^{2+} and of its substrte isocitrate, but is inhibited by ATP (or) NADH. ADP is a strong allosteric activator of isocitrate dehydrogenase.

Thus, a fall in intracellular ATP and a consequent rise in ADP may increase the rate of TCA cycle by activating all three rate – controlling enzymes of the cycle so as to replenish the ATP stock. With sufficient ATP and little ADP in the tissue, these enzymes are inhibited and TCA cycle declines.

All the biosynthetic pathways that utilize citric acid cycle intermediates also require free energy.

Pathways that utilize citric acid cycle intermediates glucose biosynthesis

(Gluconeogenesis) which occurs in the cytosol. Utilizes oxaloacetate, oxaloacetate is not trans. Ported across the mitochondrial membrane but malate that has been transported across the mitochondrial membrane is converted to oxaloacetate in the cytosol for gluconeogenesis.

Lipid Biosynthesis

Which includes fatty acid biosynthesis and cholestrol biosynthesis is a cytosolic process that requires acetyl coA. Acetyl coA is generated in the mitochondria and is not transported across the mitochondrial membrane. Cytosolic acetyl coA is therefore generated by the breakdown of citrate, which can cross the inner mitochondrial membrane, in a reaction catalyzed by ATP citrate lyase.

ATP + citrate + coA → ADP + Pi + Oxaloacetate + Acetyl coA

Amino acid Biosynthesis

Utilisation citric acid cycle intermediates in two ways. α ketoglutarate is used to synthesize glutamate in a reductive amination reaction involving either NAD^+ or $NADP^+$ catalyzed by glutamate dehydrogenase.

α ketoglutarate + NAD(P)H + NH^+_4 → Glutamate + $NAD(P)^+$ + H_2O

α ketoglutarate and oxaloacetate are also used to synthesize glutamate and aspartate in transamination reactions.

α ketoglutarate + Alanine → glutamate + pyruvate

Oxaloacetate + Alanine → Aspartate + pyruvate

Porphyrin Biosynthesis

Utilizes succinyl – coA as a starting material.

Reactions that replenish citric acid cycle intermediates

Reactions that replenish citric acid cycle intermediates are called anaplerotic reactions (filling up, Greek: ana, up + plerotikos, to fill). The main reaction of this type is catalyzed by pyruvate carboxylase, which produces oxaloacetate.

Pyruvate + CO_2 + ATP + H_2O → Oxaloacetate + ADP + Pi

Degradative pathways generate citric acid cycle interme- diates

- ➔ Oxidation of add chain fatty acids leads to the production of succinyl coA.
- ➔ Breakdown of amino acids isoleucine methionine and valine also leads to the production of succinyl coA.
- ➔ Transamination and deamination of amino acids leads to the production of α-ketoglutarate and oxaloacetate. These reactions are reversible and depending on metabolic demand, serve to remove (or) replenish these citric acid cycle intermediates.

The citric acid cycle is truly at the center of metabolism. Its reduced products NADH and $FADH_2$ are reoxidized by the electron transport chain during oxidative phosphorylation and the free energy released is coupled to the biosynthesis of ATP. Citric acid cycle intermediates are utilized in the biosynthesis of many vital cellular constituents.

2.2.2.3 Electron transport chain

An electron transport chain consists of a properly arranged and oriented set of electron – carriers transporting electrons in a specific sequence from reduced nicotinamide coenzymes (NADH (or) NADPH) and flavoproteins to molecular oxygen.

The chain actually consists of a series of redox couple, having a lower (more negative (or) less positive) redox potential to the oxidant of another redox couple possessing a higher (less negative (or) more positive redox potential.

The inner mitochondrial membrane carries an electron transport chain called the mitochondrial respiratory chain. This forms the final path for the flow of electrons from tissue substrates to molecular O_2. The free energy liberated during the transfer of electrons along this chain, is used in forming high energy

bonds of ATP. The respiratory chain has the following principle electron carriers. NADH dehydrogenase, succinate dehydrogenase, coenzyme Q, cytochromes b, c_1, c and a.a_3, iron sulfur proteins. Each of them functions as a redox system and changes alternately to its reductant and oxidant forms while transporting electrons.

Various NAD dependant dehydrogenases of the oxidative metabolic pathway transfers the electrons from various substrates to NAD^+ and reduces it to NADH.

(a) Reaction sequence

NADH dehydrogenase

NADH dehydrogenase, a large enzyme consisting of at least 34 polypeptide chains. Most of the electron pairs that enter the respiratory chain arise from the action of dehydrogenase. These enzymes use the action of dehydrogenase. These enzymes use the coenzyme NAD^+ as electron acceptor. They are designated as the NAD link dehydrogenases.

Ex: Glucose 6 phosphate dehydrogenase

Lactate dehydrogenase.

Reduced substrate + NAD^+ → oxidized substrate + NADH + H^+

Most dehydrogenases in cells transfers hydrogen atoms from their substrate to NAD^+. This coenzyme NAD^+ collects a pair of reducing equivalent (electron) and converts to NADH.

Flavin linked dehydrogenases

These enzymes transfers two reducing equivalents from NADH to FMN. Here FMN converts to $FMNH_2$.

NADH + H^+ FMN → $FMNH_2$ + NAD

One ATP is produced during the transfer of electrons from NADH to FMN.

Iron – sulphur centers

These are non heme iron atoms which are grouped in to several clusters. These iron atoms undergo oxidoreduction. The reduced flavin ($FMNH_2$ (or) $FADH_2$) next transfers its electrons to Fe^{3+} ions of iron – sulfur clusters, reducing them to Fe^{2+} ions. Finally Fe^{2+} ions get reoxidized to fe^{3+} by the flow of electrons to coenzyme Q.

Ubiquinone (or) coenzyme Q

The next electron carrier in the chain is ubiquinone. Which is a fat soluble quinone. By accepting reducing equivalents ubiquinone reduces to ubiquinol (QH_2).

$FMNH_2 + Q \rightarrow FMN + QH_2$

Cytochromes

Cytochromes are iron containing heme proteins in which the iron action oscillates between the ferrous and ferric states during oxido-reduction. The electrons pass from coenzyme Q to cytochrome b and then to cytochrome C_1 cytochrome C and cytochrome $a.a_3$ one ATP is produced when the when the electrons are transferred from cytochrome-b to cytochrome C_1.

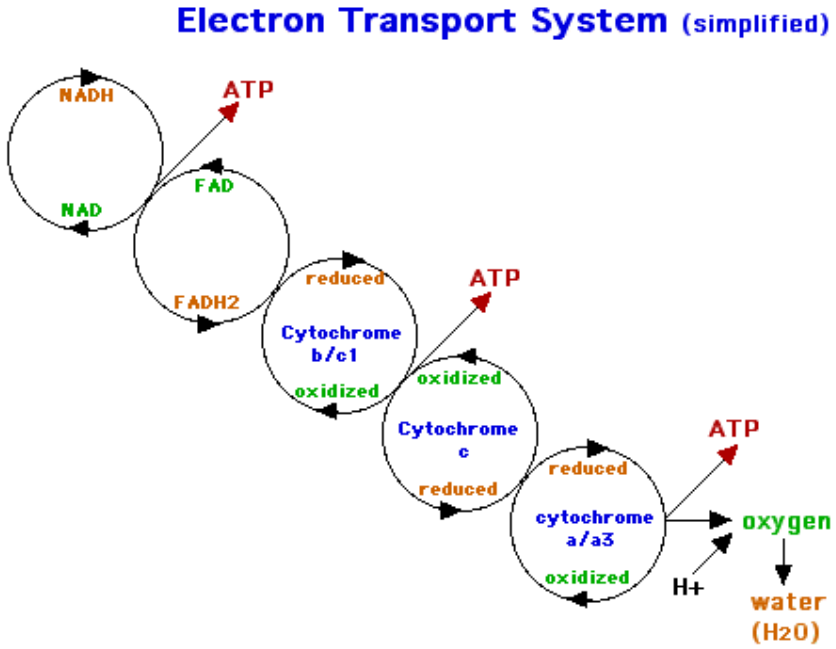
Cytochrome reductase participates in the transport of electrons from cytochrome b to C_1 and then C.

The last electron carrier cytochrome $a.a_3$ (or) cytochrome oxidase. Which donates electrons directly to molecular oxygen. Which will be reduced to H_2O_2. Cytochrome $a.a_3$ complex contains two molecules of heme each having one iron atom that oscillates between ferric and ferrous states and two atoms of copper are also seen oscillated with this enzyme. One ATP is produced when electrons are transferred from cytochrome $a.a_3$ to O_2 molecule.

By this way the process of electron transport completes.

Specific inhibitors acting at certain points in the chain have help for the study of electron transport chain.

Ex: Rotenon, antimycin-A cyanide etc.



(b) Electron shuttles

The mitochondrial electron transport chain can accept reducing equivalents (H^+ and electrons) from the mitochondrial matrix, but not from the cytoplasm. On the other hand, the inner mitochondrial membrane is impermeable to NADH and NAD^+ so the cytoplasmic NADH produced by glycolysis has to transfer its electrons to the mitochondrial matrix through some substrates shuttling as electron carriers between the cytoplasm and the mitochondria.

Malate – Aspartate Shuttle

This function in many tissues including cardiac and red fibres and hepatocyles. It consists of the malate – oxaloaccetate redox couple and involves the cytoplasmic and mitochondrial malate dehydrogenase isoenzymes . For running the shuttle oxaloacetate is provided in the cytoplasm in two ways.

(i) mainly by the transamination of aspartate.

(ii) Cleavage of citrate in to oxaloacetate and acetyl coA by the ATP citrate lyase of cytoplasm.

Cytoplasmic malate dehydrogenase transfers reducing equivalents from NADH to oxaloacetate reducing the latter to malate. A malate - α - Ketoglutarate transporter of inner membrane transports the cytoplasmic malate into the mitochondrion in exchange of mitochondrial α ketoglutarate brought out. A tricarboxylate transporter of the inner membrane may also transfer some cytoplasmic malate to the mitochondrion in exchange of mitochondrial citrate.

Mitochondrial malate dehydrogenase next re-oxidizes malate to oxaloacetate transferring its reducing equivalents to mitochondrial NAD^+. NADH thus produced donates its reducing equivalents to FMN of NADH dehydrogenase of the electron transport chain.

Glycerophosphate shuttle

The function in a few tissues such as neurons, white muscle fibres and insect flight muscle fibres. It consist of the glycerol 3 phosphate – dihydroxy acetone phosphate redox pair and involves glycerol 3 phosphate dehydrogenase of the cytoplasm and flavoprotein dehydrogenase of the inner mitochondrial membrane. The cytoplasmic enzyme transfers reducing equivalents from cytoplasmic NADH to dihydroxy acetone phosphate reducing the latter to glycerol 3 phosphate. Glycerol 3 phosphate diffuses through the outer mitochondrial membrane to reach the outer surface of the inner membrane. It is reoxidised there to dihydroxy acetone phosphate by a transfer of its reducing equivalents to FAD under the action of the flavoprotein dehydrogenase located on the outer surface of the inner membrane $FADH_2$ thus produced donates its reducing equivalents to coenzyme Q of the electron transport chain through an iron – sulfur protein. Dihydroxy acetone phosphate on the other hand diffuses back in to the cytoplasm through the outer membrane.

2.2.2.4 Oxidative phosphorylation

Oxidative phosphorylation is the formation of ATP by combining ADP and inorganic phosphate with the energy released by the transfer of electrons from different substrates to molecular oxygen through the mitochondrial respiratory chain.

The transport of electrons from one redox couple to another is accompanied by change in free energy. Which depends on the difference in redox potential between the redox couples.

Oxidative phosphorylation is carried out by respiratory chain that is located in the inner membrane of the mitochondria. The citric acid cycle and the pathway of fatty acid oxidation which supply most of NADH (or) $FADH_2$ are present in the adjacent mitochondrial matrix. The oxidation of NADH yields three ATP. Where as oxidation of FADH yields the two ATP molecules. So oxidation occurring in the

electron transport chain (or) respiratory chain and phosphorylation are coupling process.

The P:O. ratio is an index of oxidative phosphorylation. It is the number of phosphate groups esterified into ATP per atom of oxygen used (or) per pair of electrons transferred to oxygen.

$$\text{P:O ratio} = \frac{\text{Phosphate groups esterified}}{\text{Electron pairs transferred}}$$

ATP synthase, is an enzyme for ATP synthesis by oxidative phosphorylation occurs in the inner membrane particles projecting into the mitochondrial matrix from the inner membrane and its cristae.

The different mechanisms have been proposed from energy transfer between electron transport chain and ATP synthesis.

(a) Chemical coupling hypothesis

This theory proposes that electron transport is coupled to ATP synthesis by a sequence of reactions in which high energy covalent intermediate is formed by electron transport. This intermediate will be cleave and donates its energy for the formation of ATP.

(b) Conformational coupling hypothesis

It postulates that transfer of electrons along the respiratory chain causes a conformational change in the protein components of the inner membrane to yield a high energy. The conformational changes produced is transmitted to the ATP synthetase molecule causing it to become energized. Relaxation of energized ATP synthetase to its normal conformation provides energy for the synthesis of ATP and its release from the enzyme.

(c) Chemiosmotic theory

It was proposed by the British biochemist Peter Mitchell. This theory proposes to explain the coupling of mitochondrial electron transport with oxidative phosphorylation. According to this theory the fee energy released during the electron transport along the mitchondrial respiratory chain, is used in translocating protons actively from the matrix to the cytosolic side of the membrane. This creates an inward electrochemical gradiant of protons across the innermembrane. The electrochemical potential of this proton gradiant is utilized by ATP synthase is phosphorylating ADP to ATP.

The translocation of protons during the electron transport maintains a higher H^+ concentration outside the innermembrane. This generates a pH gradiant with the outside pH lower than the inside pH and a membrane potential of 0.14 volt with electro negativity on the inner side of the membrane. Thus, the total electro chemical potential resulting from the proton gradiant and used in ATP synthesis.

Experimental findings support the chemiosmotic theory

Addition of protons to the external medium of intact mitochondria leads to the generation of ATP. Oxidative phosphorylation does not occur in soluble systems where there is no possibility of a vectorial ATP synthase. A closed membrane must be present in order to obtain oxidative phosphorylation. The respiratory chain contains components organized in a sided manner as required by the chemiosmotic theory.

(d) Regulation

The rates of mitochondrial respiration and oxidative phosphorylation are largely controlled by the ATP mass action ratio and the $NADH/NAD^+$ ratio in mitochondria. A fall in the ATP mass action ratio (or) a rise in the $NADH/NAD^+$ ratio in mitochondria is accompanied by a rise in the mitochondrial concentration of reduced cytochrome C. Because cytochrome oxidase activity increases with the rise in cytochrome C concentration, a rise in mitochondrial ADP concentration (or) a fall in mitochondrial ATP concentration enhances cytochrome oxidase activity. This increases the rate of mitochondrial respiration leading to an enhanced rate of oxidative phosphorylation. Mitochondrial ADP concentration is of importance in controlling the rate of oxidative phosphorylation. Increased utilization of ATP raises the ADP level which enhances the rates of mitochondrial O_2 consumption and oxidative phosphorylation. This regulatory action of ADP is called the respiratory control.

(e) Energy capture in the respiratory chain

When the substrates are oxidized by NAD^+ linked dehydrogenase 3 molecules of inorganic phosphate are incorporated in to 3 molecules of ADP to form 3 molecules of ATP. When the substrate is oxidized through a flavin linked dehydrogenase only two ATP molecules are formed. These reactions are known as oxidative phosphorylation at the respiration chain. There are three energy coupling sites in electron transport chain.

NADH → FAD

cytb → $cytc_1$

cyt $a.a_3$ → O_2

(f) Uncouplers

Some lipid soluble weak acids such as 2,4 dinitrophenol (DNP), pentachlorophenol and dinitrocresol uncouple mitochondrial respiration and oxidative phosphorylation. Most of them function as carrier ionophores transporting protons across the innermembrane. When the cytosol has a higher H^+ concentration than the mitochondrial matrix, the mobile ionophores binds with H^+ in the cytosol diffuses through the innermembrane to the matrix and ionizes there to release the H^+ ion. Uncouplers thus reduce (or) nullity the proton gradiant created across the inner membrane by electron transport along the respiratory chain. Consequently they inhibit the synthesis of ATP by ATP synthase. They may simultaneously promote the ATPase activity of the F_1 unit by enhancing the H^+ concentration in the mitochondrial matrix. The decreased ATP synthase activity and the enhanced ATPase activity of the F_1 unit result in a rise in the mitochondrial ADP concentration. This in turn brings about a manyfold increase in the respiratory chain activity. The energy released there by is dissipated as heat, instead of being utilized in ATP synthesis.

Thyroid hormones, dicumarol may also act as uncouplers.

2.2.2.5 Summary

The citric acid cycle is the final pathway for the oxidation of carbohydrate lipid and protein it catalyzes the combination of their common metabolic acetyl coA with oxaloacetate to form citrate. By a series of dehydrogenations and decarboxylations citrate is degraded releasing reducing equivalents and $2CO_2$ and generating oxaloacetate. The reducing equivalents are oxidized by the respiratory

chain with the release of ATP. Thus, the cycle is the major route for the generation of ATP and is located in the matrix of mitochondria adjacent to the enzymes of the respiratory chain and oxidative phosphorylation. The citric acid cycle is amphibolic since it has other metabolic role in addition to oxidation. It takes part in gluconeogenesis transamination deamination and synthesis of fatty acids.

Virtually all energy released from the oxidation of carbohydrates fat and protein is made available in mitochondria as reducing equivalents. These are funneled in to the respiratory chain where they are passed down a redox gradiant of carriers to their final reaction with oxygen to form water. The redox carriers are grouped into respiratory chain complexes in the inner mitochondrial membrane these use the energy released in the redox gradiant to pump protons to the outside of the membrane creating an electrochemical potential across the membrane spanning the membrane are ATP synthase complexes that use the potential energy of the proton gradiant to synthesize ATP from ADP and inorganic phosphate. In this way oxidation is closely coupled to phosphorylation to meet the energy needs of the cell. The inner mitochondrial membrane is impermeable to protons and other ions special exchange transporters span the membrane to allow passage of ions such as OH^-, Pi, ATP, ADP/Pi and metabolites. Without discharging the electrochemical gradiant across the membrane. Many well known poisons such as cyanide arrest respiration by inhibition of the respiratory chain.

2.2.2.6 Model Questions

1. Describe the TCA cycle?
2. Write about the significance of TCA cycle.
3. Explain about electron transport chain and its importance.
4. Write about the ATP synthesis (or) oxidative phosphorylation.
5. In what way pyruvate is converted to acetyl coA.

2.2.2.7 Reference Books

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(Author-tulasi)

Lesson 2.2.3

BIOSYNTHESIS AND DEGRADATION OF FATTYACIDS AND KETONE BODIES

Objective

2.2.3.1 Introduction

2.2.3.2 Fattyacid biosynthesis

(a) Reactions of fattyacid biosynthesis
(b) Fattyacid synthase
(c) Reaction sequence of fattyacid synthase
(d) Regulation of fattyacid synthesis
(e) Energitics

2.2.3.3 Fattyacid oxidation (or) β-oxidation

(a) Reactions of β-oxidation
(b) Energitics of β-oxidation
(c) Regulation
(d) Oxidation of unsaturated fatty acids
(e) Oxidation of fattyacids with add number of carbon atoms

2.2.3.4 Kenote bodies

(a) Formation of acetoacetate (or) aceto acetyl coA
(b) Formation of β-hydroxy butyrate
(c) Formation of acetone
(d) Ketone body utilization
(e) Regulation
(f) Significance

2.2.3.5 Summary

2.2.3.6 Model Questions

2.2.3.7 Reference Books

Objective

This chapter mainly deals with the Fattyacid synthesis

Fattyacid oxidation and Ketone bodies

2.2.3.1 Introduction

Many other degradative and synthetic processes fattyacid synthesis was formerly considered to be merely the reversal of oxidation with in the mitochondria. It is mainly occurs in extra mitochondrial system is responsible for complete synthesis of palmitate from acetyl coA. There are wide variations among species both in deposition of the principle lipogenic pathways between the tissues and in the main substrates for fattyacid synthesis. In the rat lipogenesis pathway is well represented in adipose tissue and liver. In humans adipose tissue may not be an important site and liver has only low activity. In birds lipogenesis is confined to the liver. In mammals glucose is the primary substrate for lipogenesis. Fattyacids are both oxidized to acetyl coA and synthesized from acetyl coA. Fattyacid oxidation is not the simple reverse of fattyacid biosynthesis but an entirely different process taking place in a separate compartment of the cell. Fattyacid oxidation takes place in mitochondria. Each step involves acetyl coA derivatives catalyzed by separate enzymes. Utilizes NAD^+ and FAD as coenzymes and generates ATP. Fattyacid oxidation is an aerobic process requiring the presence of oxygen. Increased fattyacid oxidation is characteristic of starvation and of diabetes mellitus leading to ketone body production by the liver ketone bodies are acidic and when produced in excess over long periods as in diabetes cause keto acidosis, which is ultimately fatal. Gluconeogenesis is dependant upon fattyacid oxidation. Any impairment in fattyacid oxidation leads to hypoglycemia. This occurs in various stages of carnitine deficiency (or) deficiency of enzymes in fattyacid oxidation.

2.2.3.1 Fattyacid biosynthesis

Fattyacid biosynthesis occurs through condensation of C_2 units, the reverse of β-oxidation process. The pathway of fattyacid synthesis differs from that of fattyacid oxidation. The reductive synthesis process of lipids takes place in the cytosol. This system is present in many tissues including liver, kidney, brain, lung, mammary gland and adipose tissue. Cofactor requirements include NADPH, ATP, biotin, Mn^{2+} and HCO^-_3.

- Acetyl coA is the substrate and palmitate is the end product.

(a) Reactions of fattyacid synthesis

Formation of malonyl coA

Acetyl coA is formed by pyruvate oxidation, fattyacid oxidation, amino acid degradation in mitochondria. It is transported into cytosol by a acetyl group shuttle via citrate formation.

Acetyl coA reacts with oxalo acetate to form citrate in a reaction catalyzed by citrate synthetase.

➔ Citrate passes into the cytosol and reacts with cytosolic coA and ATP in the presence of citrate lyase to form acetyl coA and oxaloacetate.

Acetyl coA is carboxylated to malonyl coA in the presence of Acetyl coA carboxylase and ATP is the source of energy. Biotin is the prosthetic group for this enzyme. Malonyl coA is the initial and controlling step of fattyacid synthesis.

(b) Fattyacid synthase

The enzyme involved in fattyacid biosynthesis is a multienzyme complex called fattyacid synthase system. This enzyme is located in cytosol. It is of 440 kilo dalton molecular wt which contains 7 different enzymes and one acyl carrier protein (ACP) that catalyzes seven reactions in a sequential manner.

The tertiary structure of each monomer possess three domains called the condensation unit (domain I), reduction unit (domain II), and palmitate release unit (domain III) respectively. β-ketoacyl synthase, malonyl trans acylase and acetyl transacylase are located in domain 1. Similarly domain 2 consists of 3-hydroxy acyl dehydratase, enoyl reductase, 3 keto acyl reductase and an ACP (Acyl carrier protein) having a prosthetic group of 4-phosphopantetheine. Domain 3 bears the site for thioesterase activity. Each monomer consists of seven enzymes and one ACP. The two monomers are non covalently held together in a head to tail manner with the domain, of one monomer opposed to the domain 3 of the other and vice versa. The individual monomers isolated from one another are inactive but when held together two ends of the dimer participates in synthesizing two palmitate molecules, simultaneously.

(a) Ketoacyl synthase

(b) Malonyl trans acylase

(c) Acetyl trans acylase

(d) 3-hydroxy acyl dehydrogenase

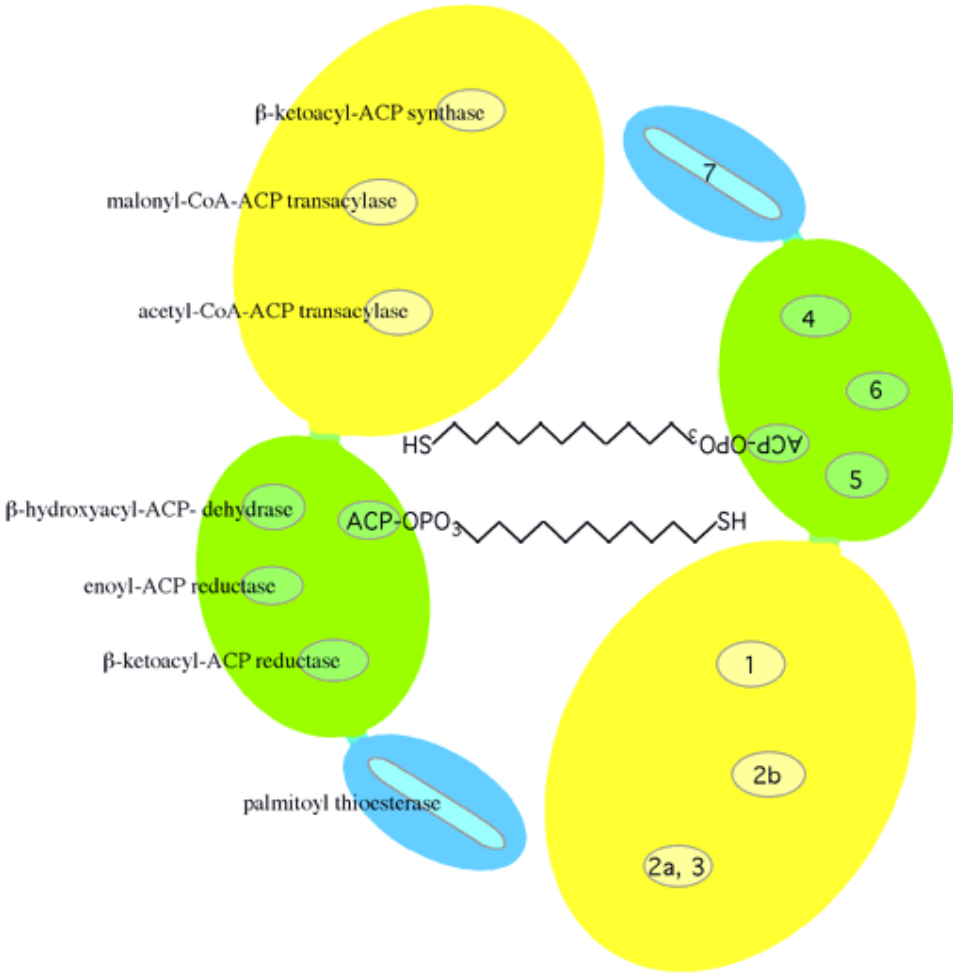
(e) Enoyl reductase

(f) 3 keto acyl reductase

(g) Acyl carrier protein (ACP)

(h) Thioesterase

Mammalian Fatty Acid Synthase



(c) Reaction sequence of fattyacid synthase

Binding of acetyl coA and malonyl coA

Acetyl trans acylase of domain 1 of the multienzyme complex transfers the acetyl group from an acetyl coA molecule to the sulfhydryl group of cysteine. CYS – SH of 3 keto acyl synthase of the same domain, simultaneously malonyl transacylase of the same domain I, transfers the malonyl group from a malonyl coA molecule to the adjacent sulfhydryl group of phosphopantetheien of ACP on domain 2 of the other monomer. This forms an acyl- malonyl enzyme.

Condensation

The acetyl group on the cystein SH group of one monomer attacks the methylene group of malonyl residue catalyzed by 3 keto acyl synthase

The cystein SH group is free now.

Reduction

3 ketoacyl reductase transfers reducing equivalents from NADPH to the 3 ketoacyl moiety to form 3 hydroxy acyl moiety.

Dehydration

3 Hydroxy acyl dehydratase removes water from 3 hydroxy acyl group changing to Δ^2 trans enoyl group or 2-3 unsaturated to an acyl group still held by 4-phosphopantetheine of ACP.

The acyl group held by the pan-SH is next transferred to the adjacent cys – SH of the other monomer. Malonyl transacylase then transfers a malonyl group from another malonyl coA molecule to the vacated pan – SH of ACP. This forms a new acyl – malonyl enzyme. The fatty acid chain is separated from the fatty acid synthase complex by thioesterase.

(d) Regulation of fattyacid synthesis

Acetyl coA carboxylase is the allosteric regulator of fattyacid synthesis. Its activators are :

Citrate: Its concentration increases in the well fed state.

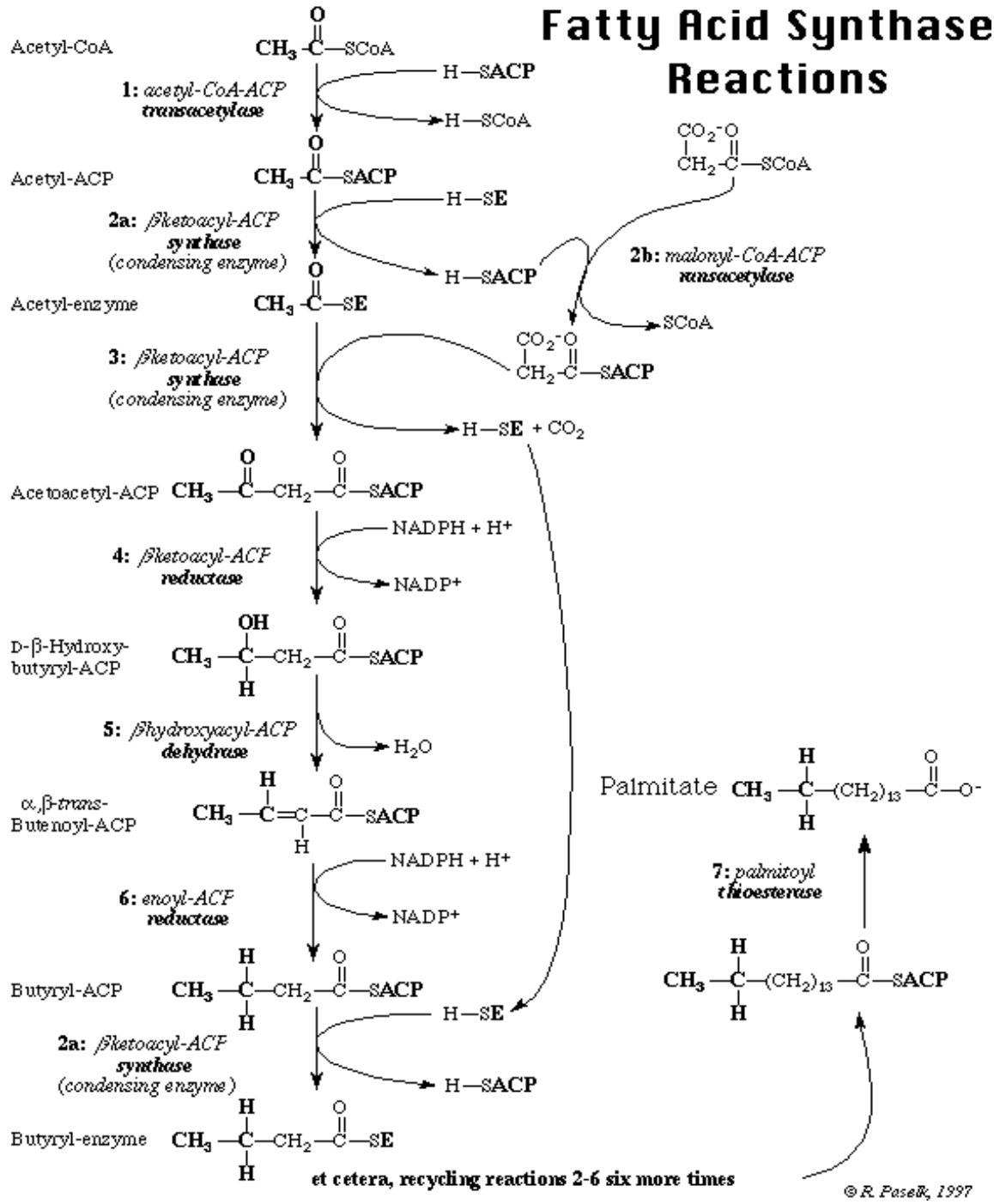
Insulin: It increases aerobic oxidation of glucose to acetyl coA which can enter fattyacid synthesis.

Inhibitors: A long chain fattyacid molecules.

(e) Energitics

One high energy bond of ATP is spent in producing each acetyl – coA molecule from citrate by ATP citrate lyase in the cytoplasm so 8 high energy phosphate bonds are spent in providing 8 acetyl coA molecules for synthesizing one palmitate molecule.

- ➔ Seven of the acetyl coA molecules have to be carboxylated to seven malonyl coA molecules have to be carboxylated to seven malonyl coA molecules by acetyl – coA carboxylase, each involving the expenditure of one high energy bond of ATP so seven high energy phosphate bonds are spent in providing seven malonyl coA molecules for one palmitate.


Fatty Acid Synthase Reactions
Acetyl-CoA
1: acetyl-CoA-ACP transacetylase
H—SACP
H—SCoA
Acetyl-ACP
2a: βketoacyl-ACP synthase (condensing enzyme)
H—SE
H—SACP
2b: malonyl-CoA-ACP transacetylase
SCoA
Acetyl-enzyme
3: βketoacyl-ACP synthase (condensing enzyme)
H—SE + CO2
Acetoacetyl-ACP
4: βketoacyl-ACP reductase
NADPH + H+
NADP+
D-β-Hydroxy-butyryl-ACP
5: βhydroxyacyl-ACP dehydrase
H2O
α,β-trans-Butenoyl-ACP
6: enoyl-ACP reductase
NADPH + H+
NADP+
Butyryl-ACP
2a: βketoacyl-ACP synthase (condensing enzyme)
H—SE
H—SACP
Butyryl-enzyme
et cetera, recycling reactions 2-6 six more times
Palmitate
7: palmitoyl thioesterase
© R. Paselk, 1997

2.2.3.3 Fattyacid oxidation (or) catabolism of fattyacids (or) β-oxidation

Fattyacids are oxidized aerobically by β-oxidation in the mitochondrial matrix of liver, muscles, heart, renal cortex and adipose tissue. This serves much of the energy need during starvation, cold thermogene. Sis and sustained muscular work such as marathon runs, prolonged flights of birds and insects and prolonged swims of whales and migratory fishes.

The natural fat is hydrolyzed to fattyacids and glycerol in the adipose tissue and liberated into the blood. The fattyacids are transported as albumin fattyacid complex. The glycerol may be glycerol phosphate by glycerokinase and later it may be converted to dihydroxy acetone phosphate that enters glycolysis.

Knoop proposed β-oxidation theory for the degradation of fats. This original β-oxidation theory was subsequently modified by Embden, Dakin & others. In β-oxidation two carbons are cleaved at a time form acetyl coA molecule starting at the carboxyl end.

The chain is broken between the α and β Carbons hence the name β-oxidation. The two carbon units formed as acetyl coA. After each turn of the β-oxidation cycle fattyacid will loose two carbons and will be two carbons less than the previous molecule. It has been demonstrated that the enzymes for β-oxidation occur in mitochondria, fattyacids collected or synthesized in the cytoplasm must be activated as well as transferred in to mitochondria for β-oxidation.

(a) Reactions of β-oxidation

Activation of fattyacid

It takes place in the cytoplasm in the presence of fattyacyl coA synthase.

Fattyacid combines with coA and forms fattyacyl coA. In this reaction the needed energy is supplied by ATP hydrolysis to AMP and pyrophosphate.

The enzymes which are involved in the further β-oxidation reactions are present in the mitochondrial matrix. Fatty acyl coA is impermeable to mitochondrial matrix. Here carnitine plays an important role in the transport of fattyacyl coA in to mitochondrial matrix.

Role of carnitine

Long chain acyl groups are carried across the inner mitochondrial membrane by a mobile carrier known as carnitine. In the presence of carnitine

acyl transferase, fatty acyl coA reacts with carnitine that is present in the mitochondrial membrane to form fattyacyl carnitine and coA is liberated in to cytoplasm.

Now the fattyacyl carnitine reacts with coA of mitochondrial matrix and fattyacyl coA is reformed liberating carnitine.

(a) Formation of unsaturated fattyacyl coA

A flavoprotein called acyl – coA dehydrogenase oxidizes fattyacyl coA to Δ^2 trans enoyl coA (or) α, β unsaturated fattacyl coA. FAD is there by reduced to $FADH_2$. $FADH_2$ enters in to electron transport chain and produces two high energy phosphate bonds (ATP) are produced.

(b) Formation of β-hydroxy fattyacyl coA

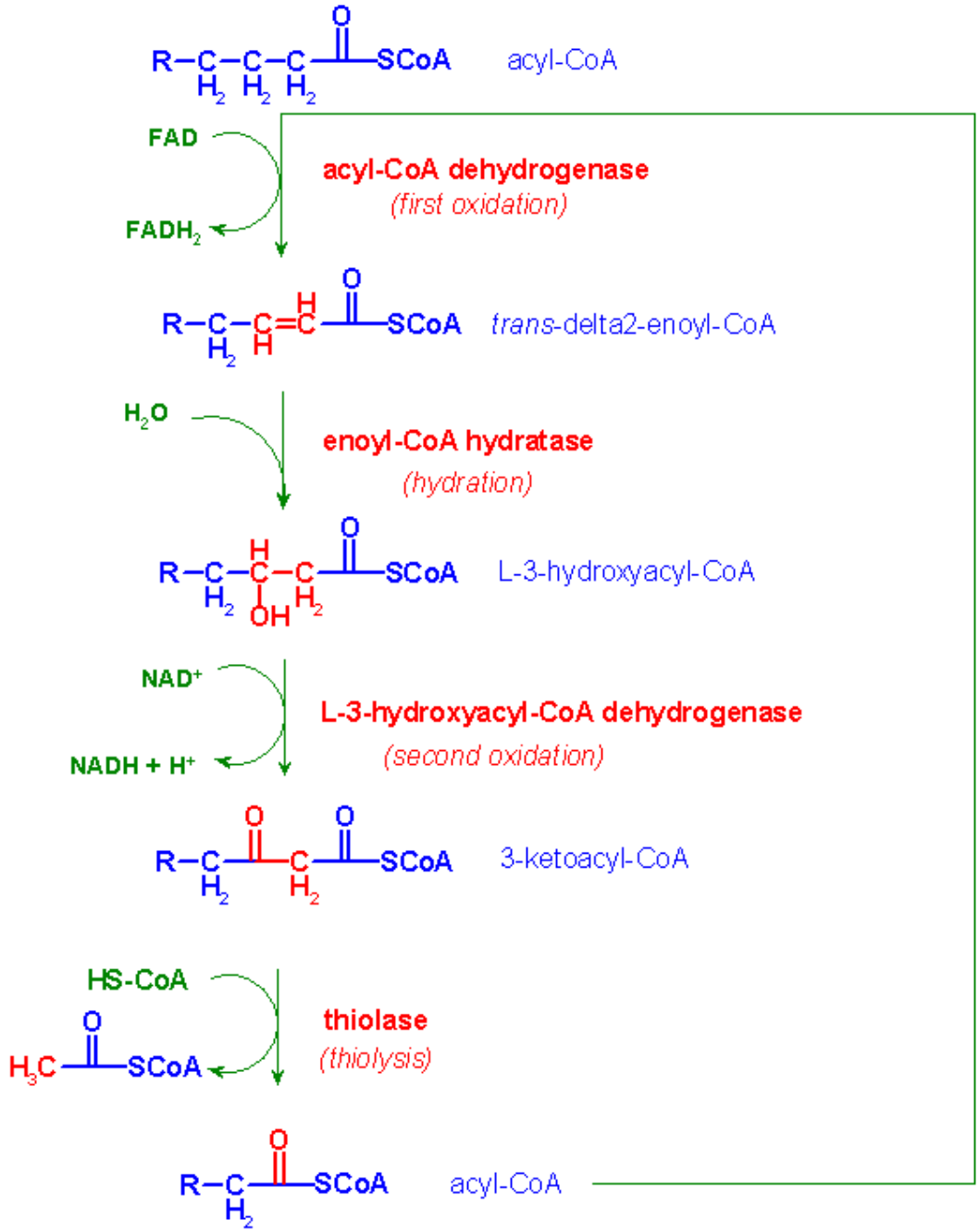
α, β unsaturated fattyacyl coA in the presence of Δ^2 enoyl coA hydrotase takes one water molecule and converts in to L(+) 3 OH acyl coA (or) β-OH acyl coA. The enzyme can hydrate acyl groups, varying largely in chain length.

(c) Formation of β-keto acyl coA

In the presence of β-OH acyl coA dehydrogenase an NAD dependant enzyme, β-OH acyl coA undergoes dehydrogenation to form β-keto acyl coA. NAD^+ is reduced to $NADH_2$.

(d) Cleavage of β keto acyl coA

In the presence of thiolase β keto acyl coA is cleaved into acetyl coA and a fattyacid with two carbon loss. The β-oxidation cycle is explain in Figure.



The cycle of reactions (a) to (d) is then repeated with the new fattyacyl coA. The mitochonrial thiolase has no chain length specificity and can therefore act on progressively shorter acyl – coA molecules until the recurrence of the cycle finally breaks an even carbon fattyacid into many acetyl – coA molecules. Palmitic acid

(C_{16}) is thus oxidized into 8 acetyl coA molecule through 7 cycles of β-oxidation following is the stichometry of palmitate oxidation.

Palmitoyl coA + 7 NAD^+ + 7 FAD + 7 H_2O + 7 coA → 8 acetyl coA + 7 NADH + H^+ + 7 $FADH_2$

Acetyl coA molecules are normally oxidized to CO_2 and H_2O in the TCA cycle. But if the TCA cycle fails to oxidize all the acetyl coA molecules they are condensed in pairs to produce acetoacetyl coA which rise to ketone bodies.

Repeated β-oxidation of an odd carbon fattyacid releases an acetyl coA at each step and finally yields a propionyl coA molecule, the latter is converted to succinyl coA which is metabolized through the TCA cycle.

(b) Energitics of β-oxidation cycle

Each β-oxidation cycle has only two oxidative steps catalyzed respectively by acyl – coA dehydrogenase and β hydroxy acyl coA dehydrogenase. $FADH_2$ produced in the first case donates its electrons to coenzyme A of mitochondrial respiratory chain. Only 2 high energy phosphate bonds are produced. In the second case NADH produced donates its electrons to the NADH – Q reductase complex of the chain. This oxidative step yields three high energy phosphates. So each cycle of β-oxidation produces 5 high energy bonds of ATP. In addition each acetyl – coA molecule is oxidized through the TCA cycle producing 12 ATP.

For example oxidation of even C-fattyacid palmitic acid contains 16 carbon atoms. It runs through β-oxidation 7 times.

Each cycle produces 5 ATP

7 cycles produces 7 x 5 = 35 ATP

The acetyl moiety when enters TCA cycle yields 12 ATP. No. of acetyl coA produced from palmitic acid C16/2 = 8.

Therefore 8 acetyl coA produces 8 x 12 = 96 ATP.

One complete oxidation of palmitic acid to CO_2 and H_2O. The energy only by β-oxidation is 35 ATP.

β-oxidation	–	35 ATP
Acetyl coA – TCA cycle	-	96 ATP

Net :		131 ATP

On complete action of palmitic acid 131 ATPS are produced. But two high energy phosphate bonds are utilized for the activation of fattyacid. There fore net ATP production is 131-2 = 129 ATP. The net yield is 129 ATP for palmitic acid oxidation.

(c) Regulation

The rate of β-oxidation is largely regulated by changes in the circulating level of free fattyacids Glucagon, adrenaline cortisol and insulin regulate β-oxidation by changing the serum free fattyacids.

- ➔ Starvation enhances glucagons secretion while exercise and stress stimulate adrenaline secretion. Both glucagons and adrenaline increases intracellular cyclic AMP in adipocytes this leads to an activation of the hormone sensitive lipase in those cells.

- ➔ A high cellular energy status is associated with a rise in the mitochondrial ATP level which reduces the rate of the TCA cycle to accumulate acetyl – coA in mitochondria. The inner membrane being impermeable to coenzyme A, mitochondrial accumulation of acetyl coA decreases the availability of free coenzyme A for running the β-oxidation.

- ➔ A high ATP level is associated with a rise in cellular citrate level. Citrate provides more acetyl coA in the cytoplasm with the help of ATP citrate lyase and also allosterically activates acetyl – coA carboxylase. So acetyl coA carboxylase carboxylates more acetyl coA to malonyl coA which inhibits carnitine palmitoyl transferase needed for the entry of long – chain acyl groups into mitochondria. The consequence decline in the mitochondrial availability of fattyacids decreases β-oxidation.

➔ Rise in mitochondrial ATP is accompanied by rise in mitochondrial NADH/NAD^+ ratio, NAD^+ is consequently less available for β hydroxyacyl coA dehydrogenase action. This also decreases β-oxidation.

(d) Oxidation of unsaturated fattyacids

Most of the fattyacids found in triacyl glycerols and phospholipids of animals (or) fats are unsaturated. They have one or more double bonds which are in cis configuration unsaturated fattyacids linoleic, oleic acid etc. Are oxidized by the same general pathway as saturated fattyacids but two special problems arise.

➔ The double bond of naturally occurring unsaturated fattyacids are in cis configeration where as the unsaturated fattyacyl coA intermediates in the oxidation of saturated fattyacids are trans.

➔ The double bonds of the most unsaturated fattyacids occur at such positions in the carbon chain that successive removal of two carbon fragments given Δ^3 unsaturated fattyacyl coA rather than Δ^2 trans unsaturated fattyacyl coA.

These problems are solved with the help of some special enzyme like enoyl coA isomerase that catalyzes the shift of the double bond from Δ^3 cis to Δ^2 trans which can be acted upon by the enzymes of β-oxidation cycle unsaturated fattyacid oxidation is shown in Figure.

Sequence of reactions in the oxidation of unsaturated fattyacids

The fattyacyl coA molecule is degraded by β-oxidation enzymes until either a Δ^3 cis acyl coA Δ^4 cis acyl coA compound is formed.

(a) If it is Δ^3 cis compound that it is isomerized to Δ^2 trans coA stage of β-oxidation by enoyl coA isomerase and undergoes further oxidation of β-oxidation pathway.

(b) If it is Δ^4 cis acyl coA then oxidation takes place as follows.

Acyl coA dehydrogenase

It catalyzes the formation of Δ^2 trans Δ^4 cis dienoyl coA from Δ^4 cis enoyl coA.

Δ^2 trans - Δ^4 cis dienoyl coA reductase

An NAD^+ dependant enzyme converts Δ^2 trans - Δ^4 cis dienoyl coA to Δ^3 trans enoyl coA.

Δ^3 cis (or) Δ^3 trans - Δ^2 trans enoyl coA isomerase

It attacks the Δ^3 trans double bond to produce Δ^2 trons encyl coA, an intermediate in β-oxidation.

(e) Oxidation of fattyacids with odd number of carbon atoms

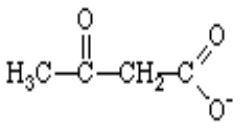
Most fatty acids have even number of carbon atoms and are therefore completely converted to acetyl coA. Some plants and microorganisms however synthesize fattyacids with an add number of carbon atoms. The final round of β-oxidation of these fattyacids forms propionyl coA which is three carbon compound. Propionyl coA is converted to succinyl coA for entry into the citric acid cycle.

Propionyl coA undergoes enzymatic carboxylation in an ATP dependant process to form D-methyl malonyl coA catalyzed by propionyl carboxylase. This enzyme contains biotin as the prosthetic group. D-methyl malonyl coA under goes enzymatic racemisation to L-methyl malonyl coA catalyzed by methyl malonyl coA racemase.

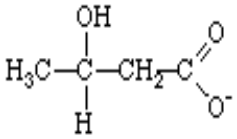
L-methyl malonyl coA is isomerized to succinyl coA by methyl malonyl coA mutase. Succinyl coA enters citric acid cycle.

2.2.3.4 Ketone bodies (or) ketogenesis

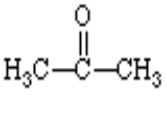
Acetyl coA produced by oxidation of fattyacids in liver nitochondria can be further oxidized via the citric acid cycle. A significant fraction of this acetyl coA has another fate, how ever by a process known as ketogenesis. Which occur primarily in liver mitochondria acetyl coA is converted to acetoacetate (or) β-OH butyrate. These compounds which to gether with acetone are referred to as ketone bodies.



Acetoacetate



D-β-Hydroxybutyrate



Acetone

(a) Formation of acetoacetate (or) aceto acetyl coA

Two molecules of acetyl coA are condensed to form acetoacetyl coA by thiolase.

(b) Formation of β-hydroxy butyrate

Acetoacetyl coA when condenses with another molecule of acetyl coA to form 3 hydroxy 3 methyl Glutaryl coA catalyzed by HMG coA synthase.

HMG coA lyase

HMG coA lyase causes the removal of one molecule of acetyl coA from hydroxy methyl glutaryl coA and a molecule of acetoacetate forms.

Hydroxy butyrate dehydrogenase, an NAD dependant enzyme reduces acetoacetate to form β-OH butyrate.

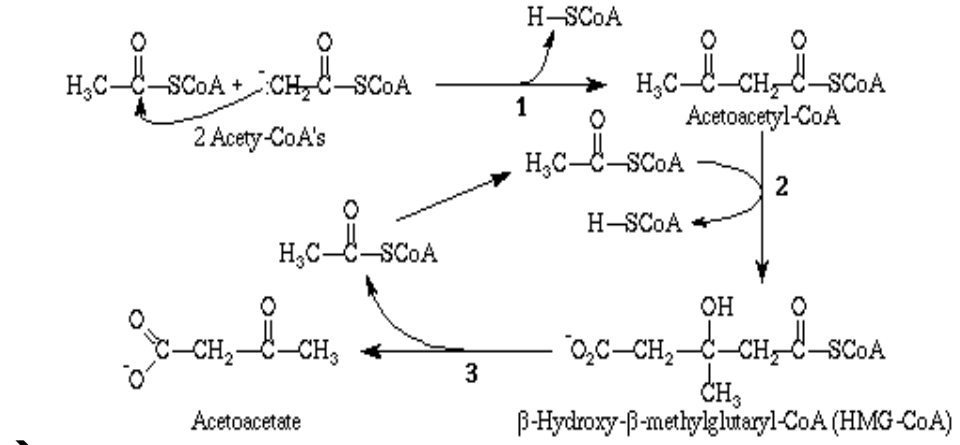
(c) Formation of Acetone

It is formed by spontaneous decarboxylation of acetoacetate.

(d) Ketone body utilization

Ketone bodies serves as a utilization fuel for extrahepatic tissues.

- ➔ In the extrahepatic tissues acetoacetate is formed from 3 hydroxy butyrate by 3-hydroxy butyrate dehydrogenase one molecule of NADH is formed in this reaction.
- ➔ Aceto acetate reacts with one molecule of succinyl coA to produce succinate and acetoacetyl coA by coA transferase
- ➔ Acetoacetyl coA is cleaved to two molecules of acetyl coA by Thiolase.
- ➔ The acetyl coA combines with oxaloacetate and enters the citric acid cycle and then the products of TCA cycle that is NADH and $FADH_2$ enters electron transport chain gives energy.



➔

(e) Regulation

Ketogenesis is regulated mainly by controlling the activity of HMG coA synthase. In starvation HMG coA synthase activity is directly enhanced by the heightened mitochondrial concentration of its substrate acetyl coA from increased β-oxidation. This enhances ketogenesis.

On the contrary carbohydrate oxidation increases the mitochondrial concentration of succinyl coA which succinylates the acetate biding site of HMG coA synthase to inhibit the latter, this decreases ketogenesis.

Glucagon, secreted in starvation reduces the hepotic mitochondrial concentrations of oxaloacetate and succinyl coA by directly stimulating hepatic gluconeogenesis. Glucagon also increases adipose tissue lipolysis to enhance the amount of fattyacids reaching the liver from adipose tissue this leads to increased β-oxidation and higher mitodrondrial concentration of Acetyl – coA. Enhanced β-oxidation also increases the mitochondrial NADH/NAD^+ ratio and consequently increases the NADH mediated reduction of oxaloacelate to malate. The resulting non availability of oxaloacetate for condensation with acetyl coA prevents the entry of the latter in the TCA cycle and enhances its accumulation in mitochondria. The simultaneous decline in mitochondrial succinyl coA enable the acetyl group to replace the succinyl group bound to HMG coA synthase and consequently reverse the inhibition of the latter. This increases ketogenesis.

Diabetes ketosis results largely from a lack of insulin action. Increased gluconeogenesis reduces the metochondrial concentration of oxaloacetale in

diabetes. The rate of tricarboxylic acid cycle falls. There is a simultaneous rise in adipose tissue lipolysis, β-oxidation of large amount of fattyacids in the liver. Ketosis results from the failure to oxidize the accumulated acetyl coA.

(f) Significance

- ➔ In starvation (or) diabetes or on a high fat (or) low carbohydate diet mainly fattyacids are oxidized for energy purposes. But the rate of the TCA cycle falls in these states due to a decline in mitochondrial oxaloacetate in consequence of increased gluconeogenesis and reduced carbohydrate metabolism so acetyl coA from enhanced β-oxidation accumulates in hepatic mitochondria. Because the inner membrane is impermeable to coenzyme A. A rise in mitochondrial acetyl coA would tend to reduce the amount of free coenzyme A available for β-oxidation. By forming acetoacetate from acetyl coA the liver endeavours to regenerate free coenzyme A in mitochondria for continuing β-oxidation.

- ➔ During starvation liver depends largely on the β-oxidation of fattyacids for its energy source and consequently produces plenty of ketone bodies. These are supplied to the brain, heart muscles and renal cortex as their major fuel for energy production during starvation as well as diabetes. Ketosis consists of this shift towards the metabolism of ketone bodies.

2.2.3.5 Summary

The synthesis of long chain fattyacids is carried out by two enzyme systems present in the cytosol of the cell acetyl coA carboxylase ad fattyacid synthase. The pathway converts acetyl coA to palmitate requires NADPH, ATP, Mn^{2+} biotin pantothenic acid and HCO_3^- as cofactors. Acetyl coA carboxylase is required to convert acetyl coA to malonyl coA. Inturn, fattyacid synthase, a multienzyme complex of one polypeptide chain with seven separate enzymatic activities catalyzes the assembly of palmilate from one acetyl coA and seven malonyl – coA molecules. Lipo genesis is regulated at the acetyl coA carboxylase step by allosteric modifiers, covalent modification and induction and repression of enzyme synthesis. Citrate activates the enzyme and long chain acyl – coA inhibits its activity. Insulin activates acetyl coA carboxylase in the short term by dephosphorylation and in the long term by induction of synthesis. Glucagon and epinephrine have opposite action to insulin. Lengthening of long chain fatty acids

takes place in the endoplasmic reticulam catalyzed by a microsomal elongase enzyme system.

Fattyacid oxidation in mitochondria leads to the generation of large quantities of ATP by a process called B-oxidation that cleaves acetyl COA units sequentially from fattyacyl chains. The acetyl COA is oxidized on the citric acid cycle generating further ATP. Oxidation of odd numbered carbon fattyacids yields acetyl COA plus one molecule of propionyl COA. Peroxisomes are capable of oxidizing very long chain fattyacids. The ketone bodies (acetoacetate, 3 hydroxy butyrate, and acetone) are formed in hepatic mitochondria when there is a high rate of fattyacid oxidation. The pathway of ketogenesis involves synthesis and breakdown of 3 hydroxy-3 methyl glutaryl COA (HMG COA) by two key ketogenic enzymes. HMG COA synthase and HMG COA lyase. Ketone bodies are important fuels in extrahepatic tissues. Ketogenesis is regulated in control of free fattyacid mobilization from adipose tissue and partition of acetyl COA between the pathway of ketogenesis and the citric acid cycle. Diseases associated with impairment of fatty acid oxidation lead to hypoglycemia fatty infiltration of organs and hypo ketonemia. Ketosis is made in starvation but severe in diabetes mellitus.

2.2.3.6 Model Questions

1. Describe the fattyacid biosynthesis
2. Write about catabolism of fattyacids (or) B-oxidation.
3. Describe the oxidation of odd chain fattyacids
4. Write about the oxidation of unsaturated fattyacids
5. Write about ketone bodies and explain its synthesis and utilization in the body.

2.2.3.7 Reference Books

1. David L. Nelson and Michae M. Con (2003) Lehanger Principles of Biochemistry 3rd edition Mac Millan Worth Publishers.

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(Author-**Tulasi)**

Lesson 2.2.4

CHOLESTEROL METABOLISM CHOLESTROL BIOSYNTHESIS & DEGRADATION

Objective

2.2.4.1Introduction

2.2.4.2 Cholesterol biosynthesis

(a) Reaction sequence
(b) Acetyl CoA forms HMG coA and mevalonate
(c) Mevalonate forms active isoprenoid units
(d) Six isoprenoid units form squalene
(e) Squalene is converted to lanosterol
(f) Lanosterol is converted to cholesterol

2.2.4.3 Degradation of cholesterol (or) biosynthesis of bile acids

(a) Formation of primary bileacids
(b) Formation of secondary bileacids
(c) Regulation of cholesterol biosynthesis
(d) Transport of cholesterol
(e) Biomedical importance

2.2.4.4 Summary

2.2.4.5 Model Questions

2.2.4.6 Reference Books

Objective

This lesson mainly deals with

Cholesterol biosynthesis

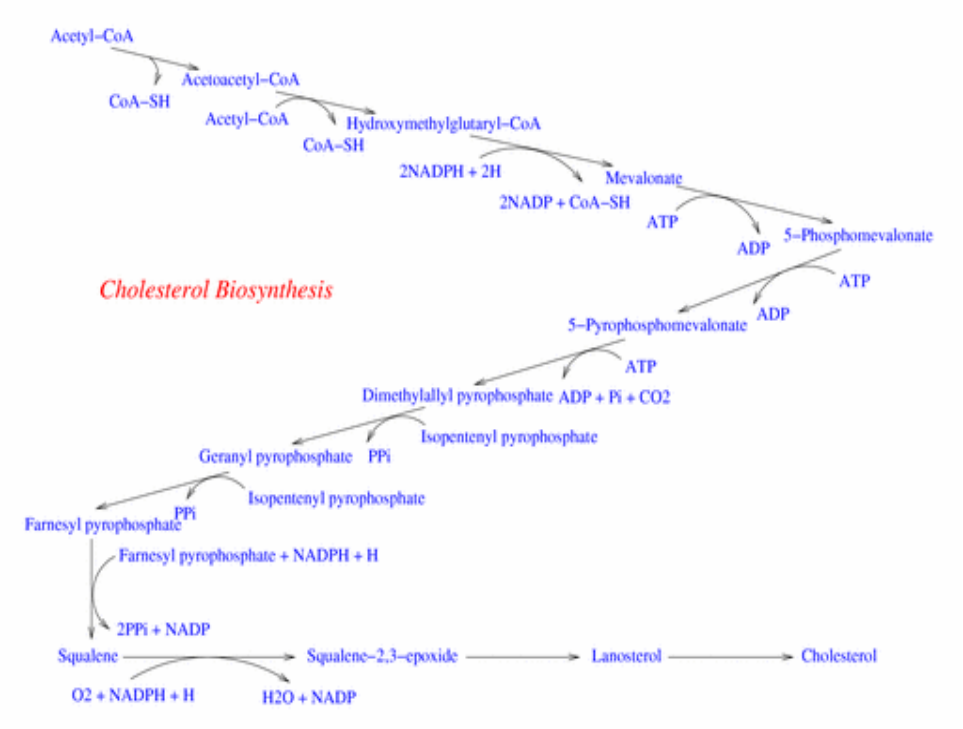
Cholesterol degradation

2.2.4.1 Introduction

Cholesterol if present in tissues and in plasma lipoproteins either as cholesterol (or) combined with a long chain fattyacids, as cholesterol ester. It is synthesized in many tissues from acetyl coA and is ultimately eliminated from the body in the bile as cholesterol (or) bile salts. Cholesterol is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids and vitamin D. It is typically α product of animal metabolism and therefore occurs in foods of animal origin such as egg yolk, meat, liver and brain.

2.2.4.2 Cholesterol biosynthesis

This extremely difficult pathway was elucidated by Konarad Bloch, Feoder Lynen, John Kornforth and George Popjack in the late 1950's. Cholesterol is an essential molecule in many animals including man. Liver and intestinal mucosa synthesizes respectively about 250 and 75 mg cholesterol in 24 hrs normally, the rest is formed largely in the skin and to a lesser extent in the adrenal cortex and gonads. The acetyl co A is the precursor and cholesterol is the and product of cholesterol synthesis. Virtually all tissues containing nucleated cells are capable of synthesizing cholesterol. The microsomal (Endoplasamic reticulum) and cytosol fractions of the cell is mostly responsible for cholesterol biosynthesis.



(a) Reaction sequence

The biosynthesis of cholesterol may be in to five stages.

(1) Mevalonate a six carbon compound, is synthesized from acetyl coA.

(2) Isoprenoid units are formed from mevalonate by loss of CO_2.

(3) Six isoprenoid units condenses to form the intermediate, squalen.

(4) Squalene cyclizes to give rise to the parent steroid lanosterol. Cholestrol is formed from lanosterol after several further steps, including the loss the three methyl groups.

Step I

(b) Acetyl coA forms HMG coA and mevalonate

Two molecules of acetyl coA condenses to form acetoacetyl coA catalyzed by a cytosolic thiolase enzyme. Alternatively in liver, acetoacetate made inside the mitochondria in the pathway of ketogenesis diffuses into the cytosol and may be activated to acetoacetyl coA by acetoacetyl coA synthase, requiring ATP and coenzymes A.

- ➔ Acetoacetyl coA condenses with a further molecule of acetyl coA catalyzed by HMG coA synthase to form HMG coA.

- ➔ HMG coA is converted to mevalonate by HMG coA reductase. Here NADPH is converted to NADP. HMG coA reductase, a microsomal enzyme considered to catalyze the rate limiting step in the pathway of cholesterol synthesis. The drugs used in cormany diseases like mevastatilen, lovastatine exhibit allosteric regulation of this enzyme.

Step 2

(c) Mevalonate forms active isoprenoid units

Mevalonate is phosphorylated by ATP to form several active phosphorylated intermediates.

Mevalonate is phosphorylated by ATP to form mevalonate S(P) in the presence of enzyme Mevalonate kinase.

- ➔ Mevalonate 5(P) is converted to mevalonate 5 pysophosphate in the presence of enzyme Phosphomevalonate kinase.

- ➔ Mevalonate 5 pysophosphate is converted to mevalonate 3 phosphate 5 pysophosphate in the presence of enzyme Diphopho mevalonate kinase.

- ➔ Mevalonate 3 phosphate 5 diphosphate is converted to isopentinyl diphosphate in the presence of enzyme Diphospho mevalonate decarboxylase. In this reaction one CO_2 is removed.

Step – 3

(d) Six isoprenoid units form squalene

- ➔ Isopentinyl pyrohosphate is isomerised to 3,3 dimethyl allyl diphosphate in the presence of the enzyme isomerase.

- ➔ 3,3 dimethyl allyl diphosphate condenses with isopentinyl diphosphate to form Geranyl diphosphate in the presence of enzyme cisprenyl transferase.

- ➔ Genranyl diphosphate condenses with isopentinyl pyro phosphate to form farnesyl diphosphate in the presence of enzyme cis prenyl transferase.

- ➔ Two molecules of farnesyl diphosphate condenses at the diphosphate end in a reaction involving first an elimination of inorganic pyrophosphate to form presqualene diphosphate, followed by a reduction with NADPH with elimination of the remaining inorganic phosphate radical. The resulting compound is squalene. This reaction is catalyzed by squalene synthetase

Step 4

(e) Squalene is converted to lanosterol

Squalene has a structure that closely resembles the steroid nucleus. Squalene is converted to squalene epoxide by a mined function oxidase in the endoplasmic reticulum, squalene epoxidase.

- ➔ The methyl group on C_{14} is transferred to C_{13} and that on C_8 to C_{14} as cyclisation occurs, catalyzed by Oxidosqualene lanosterol cyclase.

Step 5

(f) Lanosterol is converted to cholesterol

In this stage the formation of cholesterol from lanosterol takes place in the membranes of the endoplasmic reticulum and involves changes in the steroid nucleus and side chain.

- ➔ The methyl group of C_{14} are removed to produce zymosterol. This reaction is catalyzed by the enzyme oxidase.

- ➔ By the shift of double bond between C_8 and C_9 to a position between C_8 and C_7 to form $\Delta^{7,24}$ cholesteradienol in the presence of enzyme isomerase.

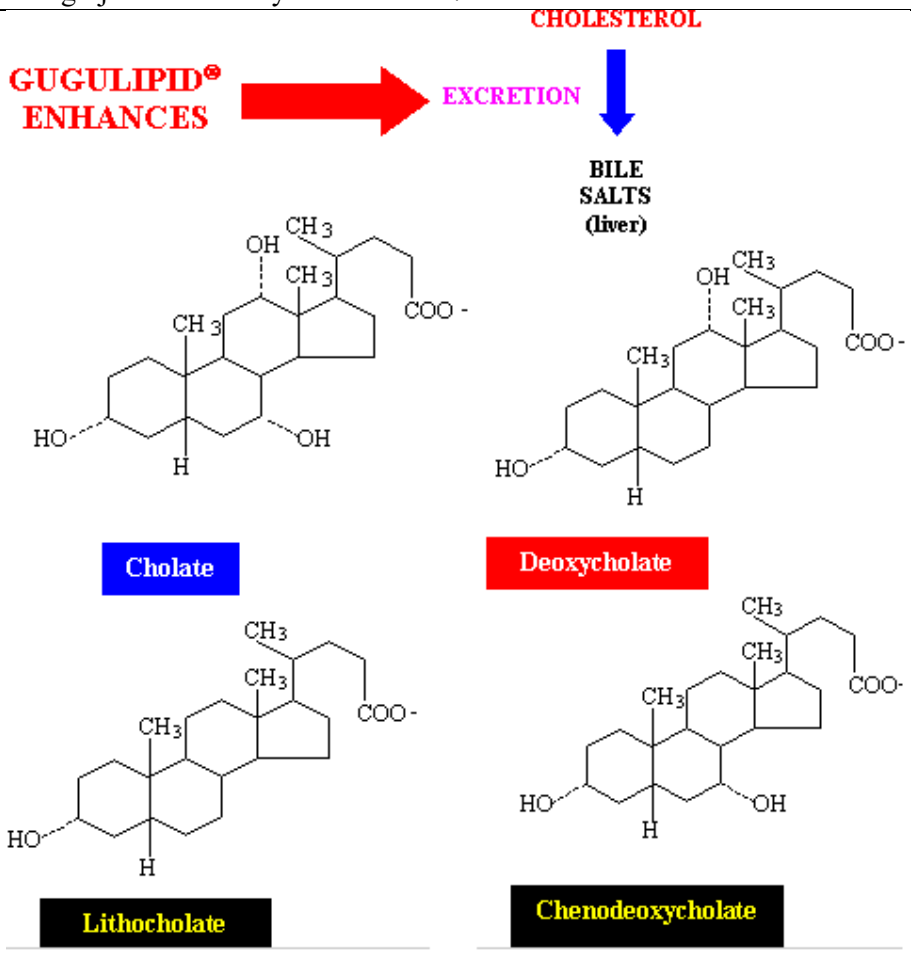
- ➔ Desmosterol is formed by a further shift in the double bond between C_8 and C_7 to a position of C_5 and C_6. This reaction is catalyzed by the enzyme isomerase.

- ➔ By reduction of double bond of side chain at 24^{th} carbon by the action of enzyme Δ^{24} reductase. The final product cholesterol is formed.

2.2.4.3 Degradation of cholesterol (or) catabolism of cholesterol (or) biosynthesis of bile acids

Cholesterol is mainly excreted in the bile, its esters are carried in the non aqueous central core of mined (or) discoid micelles formed by bile salts and some phosphotidyl cholines in the bile. Most bile salts and some cholesterol of bile are reabsorbed from the intestine, the rest appearing in the feces.

Liver occupies the central position in the metabolism of cholesterol. Cholesterol is excreted in the bile as cholesterol (or) bile acids (salts). About 1g of cholesterol is eliminated from the body per day. Approximately half is excreted in the feces after conversion to bile acids. The remainder is excreted as cholesterol. Much of the cholesterol secreted in the bile is reabsorbed. Caprosterol is the principal sterol in the feces. It is formed from cholesterol by the bacteria in the lower intestine. A large proportion of the biliary excretion of bile salts is reabsorbed in to the portal circulation taken up by the liver, and excreted in the bile.



Bile acids are formed from cholesterol

50% of cholesterol is excreted as bile acids. The primary bile acids are synthesized in the liver from cholesterol. These are choic acid and chemodeoxy cholic acid both formed from a common precursor itself derived from cholesterol.

A portion of primary bile acid is subjected to further changes by intestinal bacteria and produce secondary bile acids like deoxy cholic acid and litho cholic acid.

(a) Formation of primary bile acids

Cholesterol is hydroxylated the 7^{th} position by 7 α hydroxylase, a microsomal enzyme. It requires O_2, NADPH and vitamin C.

Vitamin C deficiency interferes with bile acid formation at the 7 α hydroxylation step and leads to cholestrol accumlation and atherosclerosis in guinea pigs.

The pathway of bile acid biosynthesis divides early into one sub pathway leading to cholyl coA characterized by an extra α-OH group on position 12 and another pathway leading to chemodeoxy cholyl coA.

Both pathways involve similar hydroxylation reactions and shortening of the side chain to give the typical bile acid structure of α-OH groups on position 3 and 7 and full saturation of the steroid nucleus.

These primary bile acids enter the bile as glycine (or) Taurine conjugates to form glycocholic acid (or) Taurocholic acid. Bile contains significant amounts of sodium and potassium and the pH is alkaline. It is assumed that the bile acids and other conjugates are actually in a salt form hence the term "bile salts".

(b) Formation of secondary bile acids

Primary bile acids in the intestine is subjected to further changes by the activity of the intestinal bacteria. These include deconjugation and 7 α dehydroxylation which produce the secondary bile acids, deoxycholic acid from cholic acid and lithocholic acid from chemodeoxy cholic acid. The cholesterol degradation pathway shown in Figure.

(c) Regulation of cholesterol biosynthesis

In fasting there is a marked decrease in the activity of HMG coA reductase.

HMG coA reductase is inhibited by mevalonate and also by the cholesterol the end product of the pathway.

- ➔ Synthesis is also regulated by low density lipoproteins.
- ➔ Insulin and thyroid hormones increase the activity of HMG coA reductase and glucogon, glucocorticods decrease the activity.

HMG coA reductase exists in both active and inactive form that can be reversible modified by phosphorylation and dephosphorylation. The active enzyme is the dephosphorylated form. The phosphorylated form is inactive.

- ➔ Bie acid synthesis is regulated by 7 α hydroxylase which is the rate limiting step of bile acid synthesis.

Hormones will exhibit this control by covalent phosphorylation and dephosphorylation. 7 α hydroxylase is active in the phosphorylated form.

(d) Transport of cholesterol

Each dl (deci litre) of human plasma normally contains a total of ISO 26s mg of cholesterol free and esterified cholesterol are both transported in plasma lipoproteins and constitutive respectively of total plasma cholesterol.

Cholesterol is esterified mainly by lecithin – cholesterol acyl transferase in the plasma and by acyl – coA cholesterol acyl transferase in tissue cells. LCAT transfers a poly unsaturated acyl group from phosphotidyl choine to cholesterol. While ACAT transfers a monounsaturated acyl group from acyl coA to cholestrol. Cholesterol is trans ported from liver to extrahepatic tissues mainly in low density lipoproteins. LOL particles bind to LDL receptors on the plasma membrane of extrahepatic tissue cells and are subsequently pinocytized by these cells. LDL is then hydrolyzed by lysosomal cholesterol esterase to give free cholesterol which is either incorporated in cellular membranes or used in steroid synthesis (or) re esterified to cholesteryl oleate (or) palmitoleate by ACAT and stored in the cell. Cholesterol is transported from extrahepatic tissues to the liver mainly by HDL and either used in bile acid synthesis or excreted in the bile or recycled to very low density lipoproteins and low density lip proteins (LDL).

Plasma cholestrol (or) LDL cholestrol may rise abnormally due to a continued intake of diets rich in cholesterol and saturated fatty acids (or) an inherited deficiency of LDL – receptors, the latter resulting in a failure of extrahepatic removal of plasma LDL. Hyper cholesterolemia also accompanies diabetes mellitus and hypothyroidism very frequently. Hyper cholesterolemia may cause atherosclerosis due to the deposition of cholesterol on arterial wall.

(f) Biomedical importance

Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. Lipoproteins transport free cholesterol in the ciruculation where it readily equilibrates with cholestrol in other lipoproteins and in membranes. Cholesteryl ester is a storage from of cholesterol found in most tissues.

- Low density lipoproteins is the mediator of cholesterol and cholesteryl ester uptake in to many tissues.

➔ Cholesterol is a major constituent of gall stones.

- ➔ Free cholesterol is removed from tissues by HDL and transported to the liver for conversion to bile acids in the process known as reverse cholesterol transport.

- ➔ Its chief role in pathologic processes is as a factor in the genesis of atherosclerosis of vital arteries causing cerebrovascular coronary and peripheral vascular disease.

2.2.4.4 SUMMARY

Cholesterol is the precursor of all other steroids in the body Ex: corticosteroids sex hormones bileacids and vitamin D. It is also an important amphipathic lipid which allows it to play a structural role in membranes and in the outer layer of lipoproteins. Cholesterol is synthesized in the body entirely from acetyl Coa via a complex pathway. Three molecules of acetyl COA form mevalonate via the important rate limiting reaction for the pathway, catalyzed by HMGCOA reductase. A five carbon isoprenoid unit is formed from mevalonate and six isoprenoid units condence to form squalene. Squalene undergoes cyclization to form the parent steroid lanosterol, which after the loss of three methyl groups form cholesterol. Cholesterol synthesis in the liver is regulated partly by the influx of dietary cholesterol in cholesterol rich chylomicron. In tissues in general a cholesterol balance is maintained between the factors causing loss of cholesterol ester formation. Many others having conditions such as diabetes mellitus, hypothyroidism kidney diseases and atherosclerosis occurs during hyper cholesterol level in blood. Bile acids are formed from cholestrol. Cholestrol is converted into 7α Hydroxy cholesterol. 7α hydroxy cholesterol is converted to cholyl COA and chenm deoxycholyl COA. Cholyl COA and chemodeoxycholyl COA conjugates with glycine and taurine to form Tauro cholic acid, glycocholic acid, Tauro and glyco chemodeoxycholic acids. These acids are called primary bile acids. Primary bile acids is converted to secondary bile acids like deoxycholic acid and lithocholic acid. In this deconjugation occurs.

2.2.4.5 Model Questions

1. Write about cholesterol biosynthesis ?
2. Describe the formation of bile acids (or) bile salts

2.2.4,.6 Reference Books

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(Author-**Tulasi)**

Lesson 2.3.1

METABOLISM OF AMINO ACIDS

BIOSYNTHESIS OF AMINOACIDS

Objective

objective

Amino acids are the building blocks of proteins. Among twenty amino acids which are required for the synthesis proteins our body can synthesize only few amino acids and remaining must be supplied by through diet. The present chapter deals with the synthesis of all these amino acids.

2.3.1.1 Introduction

Although amino acids are of central importance in the metabolism of all organisms, principally because they are the precursors of proteins, different organisms vary considerably in their ability to synthesize amino acids and in the forms of nitrogen utilized for this purpose vertebrates are not able to synthesize all the common amino acids for example, man and the albino rat can make only 10 of the 20 amino acids required as the building blocks of proteins. The remaindes the essential or nutritionally indispensable amino acids must be obtained from plants or bacteria. Higher animals can utilize ammonium ions as the nitrogen source for the synthesis of non-essential amino acids but they are unable to use nitrite,

nitrate more versatile; they can make all the amino acids required for protein synthesis and can utilize ammonia, nitrite or nitrate as nitrogen source.

Micro-organisms differ widely in their capacity to synthesize amino acids. For example the bacterium *Leuconostoc mescneteroides* cannot grow unless it is supplied with a total of 16 different amino acids other bacteria such as *E.coli* can manufacture all their amino acids starting from ammonia.

The 20 different amino acids are synthesized by 20 different multi enzyme sequences, some of which are exceedingly complex. As with most biosynthetic routes, the pathways of amino acid synthesis are for the most part different from those employed in their degradation biosynthesis of most of the amino acids is under feed back control through the action of regulatory enzymes. Moreover the biosynthesis of the enzymes catalyzing the formation of amino acids is also under regulation.

2.3.1.2 Biosynthesis of Nonessential Amino acids

The nonessential or dispensable, amino acids are those which can be synthesized by more and the albino eat, organisms which have identical amino acid requirements. It is convenient to consider this group of amino acids first because in most organisms their biosynthesis can be brought about from other precursors. These amino acids have relatively short biosynthetic pathways Alanine, Aspargine, Aspartic acid, cystine Glutamic acid, Glutamine, Hydroxy glutamic acid Glycine, Hydroxy proline, Proline and serine are the amino acids which are included in the nonessential amino acids.

1)Biosynthesis of Glycine

It is the simplest amino acid and is glycogenic. Glycine was isolated by Braconnot in 1820.

1. Glycine is a nonessential amino acid and can be formed from serine.

The beta carbon of serine is taken to the one carbon pool carried by tetra hydrofolic acid (THFA). The alpha carbon of serine becomes the alpha carbon of glycine. The transfer of β carbon atom of serine to tetra hydrofolate is catalysed by the enzyme serine hydroxy methyl transferase. Pyridoxal phosphate is the prosthetic group of the enzyme since this reaction is reversible it also provides a pathway for the biosynthesis of serine.

2. Glycine may also be formed from threonine by the activity of threonine aldolase.

3. Glycine is also formed from carbon dioxide and ammonia by the action of the enzyme Glycine synthase, a pyridoxal phosphate enzyme, which catalyses the reversible reaction. This appears to be the major pathway in the lives of vertebrates.

$CO_2 + NH_3 + N^5, N^{10}$ – methylene tetrahydrofolate + NADH + H^+ glycine + tetrahydrofolate + NAD^+

Special metabolic functions of Glycine

Glycine may be used for the biosynthesis of several compounds. These are (1) haeme (2) gratine (3) purine nucleotides (4) Glutathione and it is used also as a conjugating agent.

Synthesis of Haeme

Glycine condenses with succinyl coA to form delta amino levulinic acid (ACA) which is the first step and the key reaction in heame synthesis. The enzyme is called ALA synthase which is pyridoxal dependant.

2) Synthesis of Serine

1. The major source of serine in the body is 3-phosphoglycerate. The steps involve dehydrogenation, transamination and removal of phosphate group.

3-phosphoglycerate an intermediate of glycolysis is oxidized by phospho glycerate dehydrogenase at the expense of NAD to yield 3-hosho hydroxy pyruvate.

Transamination from Glutamate yields 3-phospho serine which forms free L-serine on hydrolysis by phospho serine phosphatase.

2. The alternate pathway involves removal of phosphate group followed by dehydrogenation and transamination.

3. In an alternate route to serine, hydroxy pyruvate formed from D-glycerate undergoes transmination with glycine or alanine to yield serine and either glyoxylate or pyruvate.

Hydroxy pyruvate + glycine $\underset{\text{amino transferase}}{\overset{\text{Serine glyoxalate}}{\rightleftharpoons}}$

4. Serine + glyoxalate
5. Another source of serine is glycine by the reversal of serine hydroxy methyl transferase reaction.

Glycine + N^5, N^{10} – methylene THFA $\xrightarrow[\text{methyl transferase}]{\text{Serine hydroxy}}$ Serine + THFA

Functions of serine

1. Serine is used in the formation of Glycine cystine.
2. Serine may also be converted to alanine by dehydration followed by transamination.
3. Serine is decarboxylated to ethanolamine by a pyridoxal phosphate dependent alpha decarboxylase.

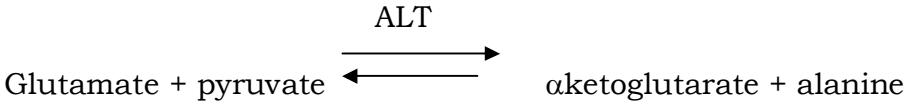
Serine is used in the synthesis of the phospholipid phosphotidyl serine and amino alcohol, sphingosine.

3) Alanine (Ala)(A)

Alanine is alpha amino propionic acid. It is non-essential glucogenic amino acid Alanine was isolated by Stracker in 1849.

Formation of Alanine

In most organisms alanine arise from pyruvate by transamination from L-glutamate.



The enzyme is alanine amino transferase (ALT).

Alanine may also be formed from other amino acids like serine, glycine and cystine which may be converted to pyruvate. The side chain of tryptophan is split into alanine during its catabolism.

Functions

The major metabolic role of alanine is to provide substrate for Gluconeogenesis under conditions of starvation, the glucose alanine cycle is of special metabolic significance. It is the most important amino acid taken up by the liver from peripheral tissues. During starvation alanine released from the muscle provides the major substrate for gluconeogenesis. In the lives, alanine is transaminated to pyruvate which undergoes gluconeogenesis.

Beta-Alanine

Here the amino group is attached to the beta carbon atom.

$$H_2N - CH_2 - CH_2 - COOH$$

Beta alanine is formed during the catabolism of the pyrimidine bases, cytosine and uracil. It is mainly used for the synthesis of co-enzyme A.

4)Aspargine

Vauquelin and Robiquetin 1906 isolated aspargine from asparagus. Aspargine was shown to be a member of all proteins.

Aspartate can react with ammonia to form its beta amide aspargine. Aspartic acid is the direct precursor of asparagines in reaction catalysed by aspargine synthetase.

Aspargine synthetase

NH_3 + aspartate ⟶ Aspargine

ATP ADP+P

In some organisms an alternative pathway may occur in which the amide amino group in transferred to the β-carboxyl group of aspartic acid by the action of asparagines synthetase.

ATP + glutamine + aspartate ⇌ glutamate + asparagines +

AMP + Ppi

5)Aspartic acid (ASP)

It was isolated by Krensler in 1869. It is nonessential and glucogenic. It is alpha amino beta carboxy butyric acid.

In most organisms aspartate arise from oxaloacetate by transamination from L-glutamate (amino donor).

Glutamate + oxaloacetate ⇌ α Keto glutarate + aspartate

In some plants aspartate may be formed by reductive amination with ammonia in reactions analogous to the glutamate dehydrogenase reactions.

6) Cystine

Cystine is a non-essential and glucogenic dicytine or cystine is not found free but only as a constituent of body proteins. Cystine is present in large quantity in Keratin of hair and oils.

Formation of Cystine

Cystine may be formed during the degradation of methionine. In this pathway the sulphur atom of methionine is transferred to replace the hydroxyl oxygen atom of serine thus converting serine into cystine. This process is often called trans sulfuration. In the first step of this sequence, methionine loses the methyl group from its sulphur atom to become homocystine. This conversion which requires ATP to convert methionine into an activated form, S-adenosyl methionine, in every unusual reaction, in which the adenosyl group of ATP is transferred to methionine.

L-methionine + ATP → S-adenosyl methionine + Ppi + Pi

S-Adenosyl methionine is an important biological methylating agent. Its methyl group which is attached in a sulfonium linkage having high energy characteristics, may be donated to any of a large number of different methyl group acceptors in the presence of the appropriate enzyme, leaving S-adenosyl homocystine, as the demethylated product S-Adenosyl homocystine then undergoes hydrolysis to yield free homocystine.

In the second stage of cystine synthesis homocystine reacts with science in a reaction catalysed by cystathionine β-synthase to yield cystathione.

Homocystine + serine → cystathoinine + H_2O

In the last step, cystathionine γ-lyase, a pyridoxal phosphate enzyme, catalyses the cleavage of cystathionine to yield free cysteine, with α-ketobutyrate and NH_3 as the other products.

Cystathionine → α-ketobutyrate + NH_3 + cystine

Cystine is an allosteric inhibitor of cystathionine Y-lyase. The overall equation of cystine synthesis is thus.

Methionine + ATP + methyl group acceptor + H_2O + serine → cystine + methylated acceptor + adenosine + α ketobutyrate + NH_3 + Ppi + Pi

The only function of cystathionine, the key intermediate in these reactions in mammals is to serine as an intermediate in the transfer of sulphur from methionine to form cystine cystathionine is present in rather high concentrations in the brain of man, but lesser amounts are found in lower vertebrates people with genetic defects in cystathionine synthase have mental deficiencies, in one such genetic disorder the unused homocystine is excreted as homocysteine in the condition known as homocystinuria. In another genetic disease involving defective cystathionine – Y lyase cystathionine is excreted in the urine (cystathionuria).

In some micro-organisms cystine is made from science by a different pathway, catalysed by cystine synthase a pyridoxal phosphate enzyme.

L-serine + H_2S ⟷ L-cystine + H_2O

7)Glutamic acid, Glutamine

Formation of Glutamic acid

1. Most aminoacids transfer their amino group to α ketoglutaric acid to form glutamic acid.

Amino tansferase

Amino acid + ⟶ α keto acid + α keto glutarate Glutamic acid

2. Glutamate is also formed in most organisms through the amination of α ketoglutarate by the action of L-glutamate dehydrogenase which in animal tissues can utilize either NAD or NADP. In plants the enzyme is often specific for NADP the reaction is

NH_3 + α-ketoglutarate + NAD(P)H + H^+ ⇌ L-glutamate + $NAD(P)^+$ + H_2O

Glutamine is formed from glutamic acid by the action of glutamine synthetase.

NH_3 + glutamate + ATP ⟵ glutamine + ADP + Pi

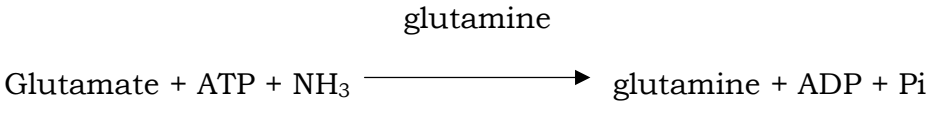
It has been shown that Y-glutamyl phosphate functions as an enzyme bound intermediate.

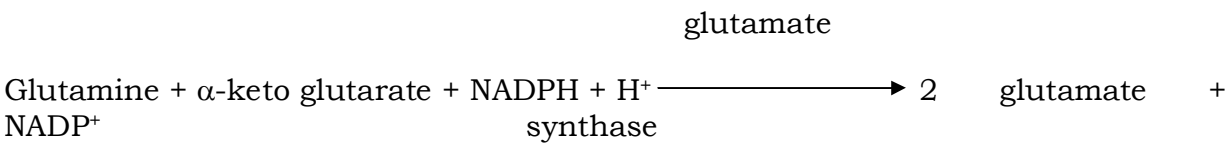
ATP + glutamic acid ⇌ ADP+(Y-glutamyl phosphate

(Y-Glutamyl phosphate) + NH_3 ⇌ glutamate + Pi

Glutamine synthetase is a structurally complex enzyme of molecular weight 350,000 to 600,000 depending on some species in some cells it is a regulatory enzyme.

The glutamine synthetase reaction also participates in the conversion of free ammonia into the α-amino groups of amino acids, since it may co-operate with the reaction catalyzed by the flavin enzyme glutamate synthase to convert free ammonia into the α-amino group of glutamate.





8) Proline (and Hydroxy Proline)

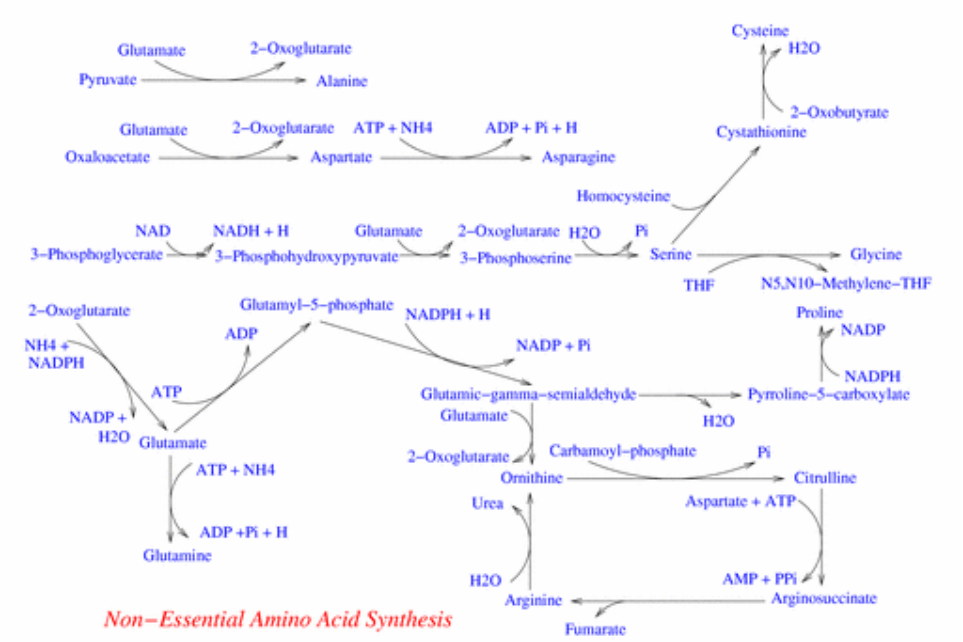
Proline is synthesized from glutamic acid by the pathway shown in the Figure the Y-carboxyl group of glutamate presumably is first phosphorylated by ATP. Then it is reduced by NADH or NADPH to yield glutamic –Y semialdehyde, which spontaneously cyclizes to yield a five membered ring compound Δ^1 – pyrroline – 5 carboxylate. A second reduction then yields proline. The end product of this sequence L-proline is an allosteric inhibitor of the first reaction step.

Hydroxy proline residues found in collagen and a few other fibrous proteins are formed from proline as a post translational modification by the enzyme proline – 4 – mono oxygenase this mixed function oxygenase utilizes α-ketoglutarte as coreductant.

Proline residue + O_2 + α ketoglutarate + coA → 4-Hydroxy proline residue + succinyl coA + coA + CO_2 + H_2O Fe^{+2} and ascorbic acid are required in this reaction as cofactors.

2.2.3.2 Biosynthesis of Essential Aminoacids

The biosynthetic pathways to the essential aminoacids are more complex and longer than those leading to the nonessential aminoacids.



1) Threonine and Methionine

These two essential aminoacids have a common denominator; their four – carbon skeletons arise from homoserine, a four – carbon analogue of serine. The carbon chain of homo serine is in turn derived from aspartic acid in a series of reactions that does not take place in mammals. The reaction pathway occurs by the reduction of the β-carboxyl group of aspartic acid to the aldehyde via an acyl phosphate (intermediate). Homoserine formed by the two reduction steps is then phosphorylated to homoserine phosphate in an ATP requiring reaction. Homo serine phosphate is now converted into threonine by threonine synthase a pyridoxal phosphate enzyme . This complex reaction, which proceeds in several steps, is believed to take place with the α-amino group of the substrate linked as a Schiff's base to the aldehyde group of the pyridoxal phosphate of the enzyme, in this complex the α hydrogen atom is labile .Threonine, the end product of the sequence is an inhibitory modulator of one of the three isozyme forms of aspartate kinase (Figure).

An alternate pathway to threonine is provided by the pyridoxal phosphate enzyme serine hydroxymethyl transferase, which can catalyze the reaction.

Acetaldehyde + glycine → L-threonine

The conversion of homoserine into methionine begins with the enzymatic formation of O-succinyl homoserine by transfer of the succinyl group of succinyl – coA to homoserine. In the next reaction cystine displaces succinate frin O-succinyl homoserine, resulting in the formation of cystathionine. Cystathionine is then cleaved hydrolytically to yield homocystine, pyruvic acid and NH_3 by the action of cystathionine. β-lyase. Since homocystine can be methylated to form methionine, it is apparent that cystathionine can serve as an intermediate in the conversion of cystine to methionine in plants and bacteria and in the conversion of methionine to cystine in mammals.

The methylation of homocystine to methionine in *E.coli* takes place by transfer of the methyl group from N^5 – methyl tetrahydrofolate. This is probably the only reaction in which N^5 – methyl tetrahydrofolate donates a methyl group other direct methyl group donors for methionine biosynthesis are befaine and dimethylthetin.

2) Lysine

There are tow major routes of synthesis of lysine, one proceeding via diaminopimelic acid, which is the major route in bacteria and higher plants, and the other via α-amino adipic acid, the route in most fungi.

The diaminopimelic route begins with aspartic semialdehyde and pyruvate which undergo an aldol condensation and loose water to yield a cyclic intermediate 2,3 dihydropicolinic acid. At a later stage, L,L - α, E-diaminopimelic acid is formed which is converted to its mesoform and then decarboxylated to yield 2-lysine. Lysine biosynthesis by this pathway is subject to feedback inhibition of one of the isozymes of aspartate kinase. The synthesis of the entire set of enzymes leading from aspartate semialdehyde to lysine is co-ordinately repressed by lysine.

The aminoadipic acid pathway begins with acetyl – coA and α-keto glutarate and proceeds via homoisocitric acid to α-ketoadipic acid, in reactions analogous to the tricarboxylic acid cycle reactions from citric to α-ketoglutaric acids. The α-keto adipic acid is aminated to α-aminoadipic acid, which is ultimately convertd into L-lysine.

3) Valine, Isodencine and leucine

These three aminoacids, which have branched aliphatic R groups, are made by similar pathways. The pathways to valine and isoleucine, which are catalyzed by the same set of enzymes, begin with the formation of the α-keto acids pyruvate and α-ketobutyrate, respectively to which an active acetaldehyde group, derived

from pyruvate in the form of α-hydroxy ethyl thianein pyrophosphate is added the products are the corresponding α-aceto - α-hydroxy acids (Figure). These undergo reduction with simultaneous migration of a methyl or ethyl group. The products are then dehydrated to yield the α-keto analogues of isoleucine and valine, which are aminated by transaminases .

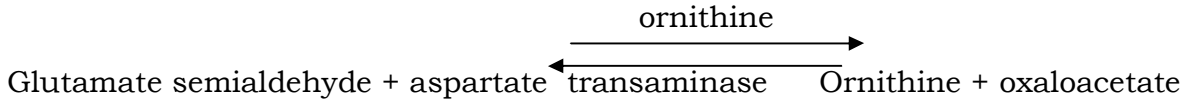
The formation of leucine by condensation of α-keto isovaleric acid (which is also the precursor of valine) with acetyl coA to yield α-iso propylmalic acid. The subsequent steps are similar to those leading from citric acid to α-keto glutaric in the tricarboxylic acid cycle.

In bacteria the biosynthesis of valine isoleucine and leucine are subjected to feed back inhibition at the first step by the end products.

4) Ornithine and Arginine

Ornithine is the precursor of arginine in many species. Although ornithine can be converted to arginine in mammals during the operation of kreb's urea cycle, arginine is so rapidly broken down by arginase to form urea and ornithine that insufficient amounts are available for protein synthesis. For this season arginine is an essential amino acid in mammals. Ornithine is formed in bacteria and plants and glutamic acid by two general routes; the major pathway is that shown in the Figure.

The final step in this route depends on the species. In *E.coli*, N-acetyl ornithine is hydrolyzed to yield ornithine and free acetic acid, whereas in other microorganisms and plants, N-acetyl ornithine donates its acetyl group to glutamic acid to yield free ornithine and ultimately N-acetyl glutamic Y-semialdehyde. The N-acetyl group appears to prevent glutamic acid semialdehyde from spontaneous cyclization. This reaction sequence constitutes the N-acetyl ornithine cycle in some organisms ornithine is made by transamination from different aminoacids to L-glutamate Y-semialdehyde.



The ornithine formed by these pathways is converted into arginine by the following reactions of the urea cycle.

Ornithine + carbamoyl phosphate → liteulline + Pi

Liteulline + aspartate + ATP → arginosuccinate + AMP + Ppi

Arginosuccinate → arginine + fumarate

Since arginase is lacking in many bacteria, this pathway yields net synthesis of arginine.

5) Histidine

The pathway of histidine biosynthesis has been studied primarily in a few bacteria, Neurospora and yeast. The pathway of histidine formation is given in the Figure. It contains a number of unusual and complex reactions. The first step is extraordinary; 5-phosphoribosyl 1-pyrophosphate reacts with ATP in such a manner that the pyrophosphate group of the former is lost and the 5-phosphoribosyl moiety forms an N-glycosyl linkage with Nitrogen atom 1 of the purine ring of ATP. The three carbon side chain and two carbon atoms of the imidazole ring of histidine arise from the 5-phosphoribosyl moety. One of the –N = C- structures of the imidazole ring arises from the adenine of the ATP and the other nitrogen atom of the ring from the amide nitrogen of glutamine, after an unusual enzymatic fragmentation of the adenine ring of ATP. The remaining fragment of the adenine ring is in fact salvaged and reused as a precussor of purines, evidentl, by-products are wasted during the biosynthesis of histidine. In fact the biosynthesis of histidine and of the purine ring are linked. Another note worthy point is that the carboxy group of histidine is formed by a two step oxidation of the corresponding α-amino alcohol histidinol, apparently by a single enzyme, whereas the carboxyl group of nearly all other aminoacids arises from the carboxyl group of a corresponding α-keto acid.

The first step in the reaction sequence leading to histidine is catalyzed by an allosteric enzyme, ATP phosphoribosyl transferase, which is inhibited by histidine, the end product of the sequence.

2.2.3.4 Biosynthesis of the Aromatic Aminoacids

(Phenyl alanine and tryptophan : and tyrosine)

The aromatic aminoacids phenylalanine, tyrosine and tryptophan are all formed by means of the shikimate pathway to build the benzene ring. The branch point compound for all these diverse is chorismate.

In the pathway to shikimic acid, a four carbon sugar phosphate. D-crytheose 4-phosphate reacts with phosphoenol pyruvate to yield a

phosphorylated seven – carbon keto sugar acid, it in turn cyclizes to 5-dehydroguinic acid, which has a six – carbon aliphatic ring. This intermediate is then converted into shikimic acid, which leads via phosphorylated intermediates to chorismic acid at which there is an important metabolic branch point. One branch leads to antheanilic acid and hence to tryptophan whereas the other leads to the quinonoid compound prephenic acid, the last nonaromatic compound in the sequence prephenic acid can be aromatized in two ways (1) by dehydration and simultaneous decarboxylation to yield phenylpyruvic acid, the precursor of phenyl alanine, and (2) by dehydrogenation and decarboxylation to yield P-hydroxy phenyl pyruvic acid, the precursor of tyrosine.

1) Tryptophan is synthesized in Five steps from chorismate

The pathway leading from chorismate to L-tryptophan is the most thoroughly studied of any biosynthetic pathway.

The first specific step in tryptophan biosynthesis is glutamine – dependent conversion of chorismate to the simple aromatic compound anthranilate like most other glutamine dependent reactions the reaction can also occur with ammonia as the source of the amino group. However high concentrations of ammonia are required thus far, almost all the anthranilate synthesis examined have the glutamine amido transferase activity (component II) and the chorismate. Co-antheanilate activity (component I) on separate proteins. Component I actually catalyzes two reactions with 2-amino – 4 deoxy chorismate being an enzyme bound intermediate.

Anthranilate is transferred to a ribose phosphate chain in a phosphoribosyl – pyrophosphate – dependant reaction catalyzed by anthranilate phosphoribosyl transferase. An isomerase catalyzes an Another rearrangement in which the ribosyl moiety of phosphoribosyl antheanilate becomes a ribulosyl moiety. The product, 1-)O-carboxy phenyl amino)-1-deoxyribuylose – 5^1-phosphate is cyclized to indole glycerol phosphate by the removal of water and loss of the ring carboxyl by indole glycerol phosphate synthase. The final step in tryptophan biosynthesis is a replacement reaction, catalyzed by tryptophan synthase in which glyceraldehydes 3-phosphate is removed from indole glycerol phosphate and the enzyme bound indole so formed condensed with serine.

Tryptophan synthase is a pyridoxal phosphate enzyme having a molecular weight of about 135,000; it has been isolated in crystalline form. It catalyses the overall reaction.

Indole-3-glyceeophosphate + serine → tryptophan + glyceraldehyde 3-phosphate which takes place in two steps, with the intermediate indole remaining bound to the active site.

Indole – 3-glycerolphosphate → (Indole) + glyceraldhyde – 3-Phosphate - (1)

(Indole) + Serine → tryptophan + H_2O - (2)

The enzyme of E.Coli contains four polypeptide chains made up of two α chains and two β chains which can be separated the α subunit also can catalyze reaction (1) but at a low rate, and the β chains are associated as the dimes β_2 can catalyse reaction (2) also at low rate when the α and β chains are mixed both reaction rates are greatly increased

2) Carbon flow in the Biosynthesis of aromatic aminoacids is regulated at barnchpoints

Metabolite flow to tryptophan is controlled by inhibition of anthranilate synthase by tryptophan. Regulation of metabolite flow in phenyl alanine and tyrosine biosynthesis varies from organism to organism, owing to a variety of enzyme patterns in the conversion of chorismate to the two aminoacids. In E.Coli and other related organisms, phenyl alanine inhibits both activities of chorismate mutase P-prophenate dehydratase, whereas tyrosine inhibits only the mutase activity of chorismate mutase T-prophanate dehydrogenase.

There are tow general patterns of control over the common aromatic pathway one is found in *E.coli* and related organisms. In this, there are three isozymic deoxyarabino – heptulosonate 7-phosphate synthases. Each is inhibited by one of the three aromatic amino acids (there is, in addition a tryptophan – specific repression of the tryptophan – sensitive enzyme, a tyrosine – specific repression of the tyrosine – sensitive enzyme and a tryptophan plus phenyl alanine – specific multivalent repression of the phenyl alanine sensitive enzyme. The three enzymes contribute to a common pool of deoxy – arabino – heptulosenate – 7-phosphate that is drawn upon for all the compounds formed from chorismate. Indeed in some strains, the phenyl alanine – sensitive enzyme is predominant, where as in other the tyrosine sensitive enzyme is predominant.

Another pattern is found in Bacillus subtilis. This single deoxy – arabino – heptulosonate – 7-phosphate synthase is carried on the same protein that exhibits chorismate mutase activity. The protein is complexed with another protein the exhibits shikimate kinase activity. Both the deoxyarabino – heptulosonate – 7-phosphate synthase activity and the shikinmate kinase acivitiy are inhibited by chorismate and prephenate, which way inhibit by binding to the substrate and product sites of the chorismate mutase. The chorismate mutase activity is inhibited by prephanate. Prephanate dehydratase is inhibited by phenyl alamine, whereas prephanate dehydrogenase is inhibited by tyrosine.

2.2.3.5 Regulation of Amino acid Biosynthesis

Two general mechanisms for regulation of amino acid biosynthesis have been noted.

(1) allosteric or feed back inhibition.

(2) Repression of the biosynthesis of one or more of the enzymes of the pathway, thus lowering their concentration in the cell.

Feed back inhibition of the first reaction in the biosynthetic sequence by its end product yields fine control over biosynthesis, since it is capable of second – by – second adjustment of the rate of biosynthesis of the amino acid to the steady state level of the biosynthetic end product allosteric control of amino acid biosynthesis is especially conspicuous in bacteria because of their basic need to conscene nitrogen sources. In mammalian tissues allosteric regulation of the biosynthesis of aminoacids does not appear to be quite so conspicuous.

The second type of control mechanism, repression of enzyme synthesis. Such control is afforded by changes in the rate of DNA transcription or of messenger RNA translation, thus changing the rate of synthesis of enzymes. Repression and depression of enzyme synthesis are slows to respond to changing metabolic conditions than feedback inhibition of regulatory enzymes.

2.2.3.6 Summary

The albino rat and man can synthesize 10 of the 20 aminoacids required as building blocks for protein biosynthesis. The remainder of the aminoacids are nutritionally indispensable and must be obtained from other sources. In the nonessential or nutritionally dispensable group, glutamic acid is formed by reductive amination of α-ketoglutarate and is the direct precussor of glutamine and proline. Alanine and aspartic acid are formed by transamination to pyruvate and oxaloacetate respectively. Tyrosine is formed by hydroxylation of phenylalanine cystine is formed from methionine by a complex series of reactions in which S-adenosyl methionine, S-adenosyl homocysteine, and cystathoinine are the most significant intermediates. Serine is synthesized from 3-phosphoglycerate. Serine is also a precussor of glycine, its β carbon atom is transferred to tetrahydrofolate.

The pathways of biosynthesis of the essential aminoacids have been established largely from studies on bacteria. The carbon skeletons of methionine and threonine arise from aspartic acid lysine is made by two routes, the amino adipic (bacteria) and the aminopimelic acid (fungi) pathways Isolucine, valine and leucine synthesis begin with α-keto acids. Arginine is formed from ornithine, which in turn is derived from glutamic acid the precussors of the aromatic

aminoacids are aliphatic compounds which are cyclized to shikimic acid, an essential precussor of many aromatic biomolecules. Skikimic acid yields phenylalanine and tyrosine via prephenic acid and tryptophan via anthenilic acid. The pathway of histidine is most complex and unusual and involves the carbon chain of a pentase and the fragmentation of the purine ring of ATP.

Most of the biosynthetic pathways leading to the aminoacids are subject to allosteric or end product, inhibition, the regulatory enzyme is usually the first in the sequence.

2.2.3.6 Model Questions

1. Write about chorismate pathway.
2. Synthesis of Histidine.
3. Write about biosynthesis of glycine, serine and alanine.

2.2.3.7 Reference Books

1. Biochemistry – Lehninger.
2. Test Book of Biochemistry – Voet & Voet.
3. Zubey.

Lesson 2.3.2

THE METABOLIC FATE OF AMINO ACIDS

(AMINOACID – DEGRADATION)

objective

Objective

This chapter deals with the various degradative pathways of amino acids and degradation of individual amino acids were discussed

2.3.2.1 Introduction

Although amino acids sence as important components or precursors of many biological compounds, they all ultimately undergo degradation reactions. Each amino aid is degraded by a specific pathway. In complex eukaryotes the main purpose of degradation is sometimes merely to remove excess amino acids which can be toxic to the organism. Excess dietary amino acids are neither stored for future use nor excreted. Rather they are converted to common metabolic intermediates such as pyruvate, oxaloacetate and α ketoglutarate. Consequently amino acids are also precursors of glucose, fatty acids and ketone bodies and are therefore metabolic fuels.

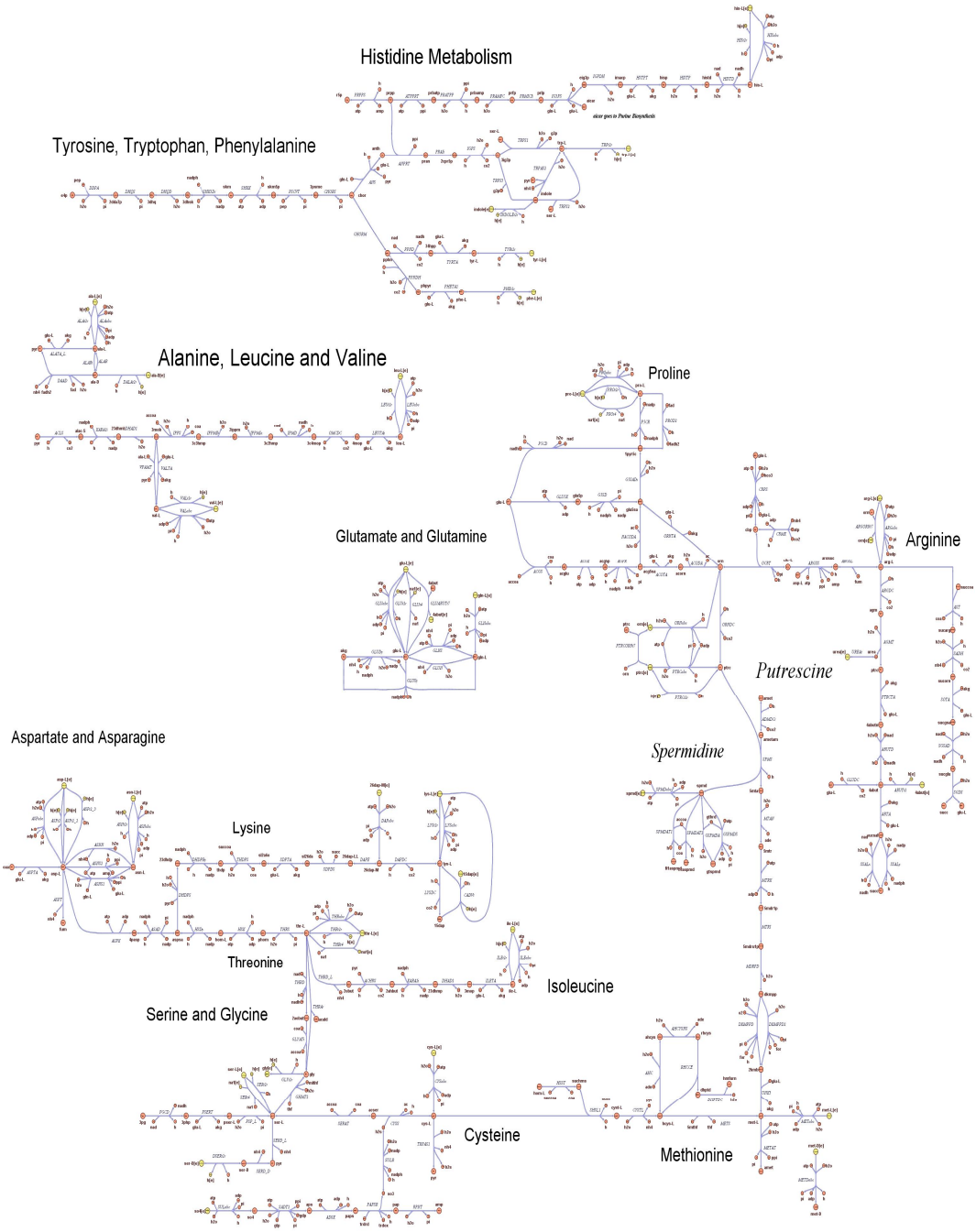
In this chapter, we consider the pathways of amino acid breakdown. We begin by examining the three common stages of amino acid breakdown. Degradative pathways for most amino acids begin by removal of the α-amino nitrogen the three stages are :

1. Deamination (amino group removal), where by amino groups are converted either to ammonia or to the amino group of aspartate.

2. Incorporation of ammonia and aspartate nitrogen into uses for excretion.

3. Conversion of aminoacid carbon skeleton (the α ketoacids produced by deamination) to common metabolic intermediates.

AMINO ACID METABOLISM



2.3.2.2. Amino acid deamination

There are two major lanes of deamination: transamination and oxidative deamination.

Both these processes are of major importance. The first reaction in the breakdown of an amino acid is almost always removal of its alfa amino group with the object of excreting excess nitrogen and degrading the remaining carbon skeleton. Urea the predominant nitrogen excretion product in terrestrial mammals, is synthesized from ammonia and aspartate. Both of these latter substances are derived mainly from glutamate, a product of most deamination reactions.

Most amino acids are deaminated by trans amination, the transfer of their amino group to an α-ketoacid to yield the α-keto acid of the original amino acid and a new amino acid, in reactions catalysed by amino transferases (or transaminases). The predominant amino group acceptor is α-ketoglutarate, producing glutamate as the new amino acid.

Amino acid + α ketoglutarate → α ketoacid + glutamate

Glutamate is amino group, in turn, is transferred to oxaloacetate in a second transamination reaction, yielding aspartate.

Glutamate + oxaloacetate → α ketoglutarate + aspartate

Transamination, of course does not result in any net deamination. Deamination occurs largely through the oxidative deamination of glutamate by glutamate dehydrogenase, yielding ammonia. The reaction requires NAD^+ or $NADP^+$ as an oxidizing agent and regenerates α-ketoglutarate for use in additional transamination reactions.

Glutamate + $NAD(P)^+$ H_2O → α ketoglutarate + NH_4^+ + NAD(P)H

a. Transamination

Aminotransferase reactions occur in two stages.

1. The amino group of an aminoacid is transferred to the enzyme, producing the corresponding ketoacid and the aminated enzyme.

Aminoacid + enzyme → α ketoacid + enzyme – NH_2

2. The amino group is transferred to the ketoacid acceptor (e.g., α-ketoglutarate), forming the aminoacid product (e.g., glutamate and regenerating the enzyme).

α ketoglutarate + enzyme – NH_2 → enzyme + glutamate

To carry the aminogroup, aminotransferases require participation of an aldehyde – containing coenzyme. Pyridoxal – 5 – phosphate (PLP), a derivative of pyridoxine (vitamin B_6) (Figure). The aminogroup is accommodated by conversion of the co-enzyme to pyridoxamine – 5^1 – phosphate (Figure). PLP is covalently attached to the enzyme via a schiff base (imine) linkage formed by the condensation of its aldehyde group with the E amino group of an enzymatic Lys residue. A proton is removed from the α carbon of the aminoacid and donated to carbon 4^1 of pyridoxal – 5^1 – phosphate (an inteemdeculae transaldimination). Upon hydrolysis of the isomerised addcut, the corresponding α-ketoacid is released, and the enzyme is in its pyridoxamine – 5^1 – phosphate form.

Aminotransferases differ in their specificity for amino acid substrates in the first stage of the transamination reaction, thereby producing the correspondingly different α-ketoacid products. Most aminotransferases however accept only α-ketoglutarate or (to a larses extent) oxaloacetate as the α-keto acid substrate in the second stage of reaction, thereby yielding glutamate or aspartate as their only amino acid products. The amino groups of most aminoacids are consequently funneled into the formation of glutamate or aspartate, which are themselves interconverted by glutamate – aspartate aminotransferase.

Amino acid + oxaloacetate → α ketoglutarate 4 aspartate

Amino acid 1 + α ketoacid 2 → α ketoacid 1 + amino acid 2

Oxidative deamination of glutamate yields ammonia and regenerates α-ketoglutarate for another round of transamination reactions. Ammonia and aspartate are the two amino group donors in the synthesis of urea.

The Glucose – Alamine cycle transports nitrogen to the lives

An important exception to the foregoing is a group of muscle amino transferases that accept pyruvate as their α-ketoacid substrate. The product amino acid, alanine is released into the blood stream and transported to the lives,

where it undergoes transamination to yield pyruvate for use in gluconeogenesis the resulting glucose is returned to muscles, where it is glycolytically degraded to pyruvate. This is glucose – alanine cycle. Evidently, the glucose – alanine cycle functions to transport nitrogen from muscle to lives.

b) Oxidative Deamination

Glutamate Dehydegenase transamination does not result in any net deamination, since one amino acid is replaced by another amino acid. The main function of transamination is to funnel the amino nitrogen into one or a few amino acids. For glutamate to play a role in the net conversion of amino groups to ammonia, a mechanism for glutamate deamination is need, so that α-ketoglutarate can be regenerated for further transamination, the regeneration is accomplished by the oxidative deamination of glutamate, a reaction catalysed by an NAD^4 – linked enzyme, glutamate dehydegenase. This enzyme is located in the mitochondria of eukaryotic cells. It catalyses release of the α-amino group of glutamate leading to the regeneration of α-ketoglutarate.

Glutamate + NAD^+ +H_2O → α ketoglutaate + NH_4^+ + NADH

The catalysis by glutamate dehydrogenase involves covalent bond formation between an intermediate and the enzyme. In the first step of the reaction there is a hydride transfer from the α carbon of the amino acid to $NAD^{+.}$ The resulting electron deficient imino carbon is attacked by a lysyl side chain of the enzyme, leading to the displacement of ammonia and formation of an imino linkage with the enzyme. In the last step a hydrolysis restores the enzyme to its original state and α-ketoglutarate is released. Like glutamate some amino acids that can undergo transamination can be deaminated more directly by oxidative reactions, either by a flavoprotein or by an NAD^+ - linked enzyme.

Whether transamination or direct deamination is more important as an initial step in amino acid breakdown depends on the organism or tissue under investigation. However, where two mechanisms are available for one amino acid in a given cell type, it may will be that both mechanisms are employed.

The Fate of Nitrogen Derived from Amino acid Breakdown

Living organism excrete the excess nitrogen resulting from the metabolic breakdown of amino acids in one of these ways. Many aquatic animals simple excrete ammonia. Where water is less plentiful, however, processes have evolved that convert ammonia to less toxic waste products that therefore require less water for excretion. One such product is urea, which is excreted by most terrestrial vertebrates; another is uric acid, which is excreted by birds and terrestrial reptiles.

Accordingly living organisms are classified as being either ammonotelic (ammonia excreting) ureotelic (urea excreting), or uricotelic (Uricacid excreting). Some animals can shift from ammonotelism to ureotelism or uricotelism if their water supply becomes restricted. Here we focus our attention on urea formation.

2.3.2.3 Urea Formation in the Animal lives Detoxifies NH_3 : (The urea cycle)

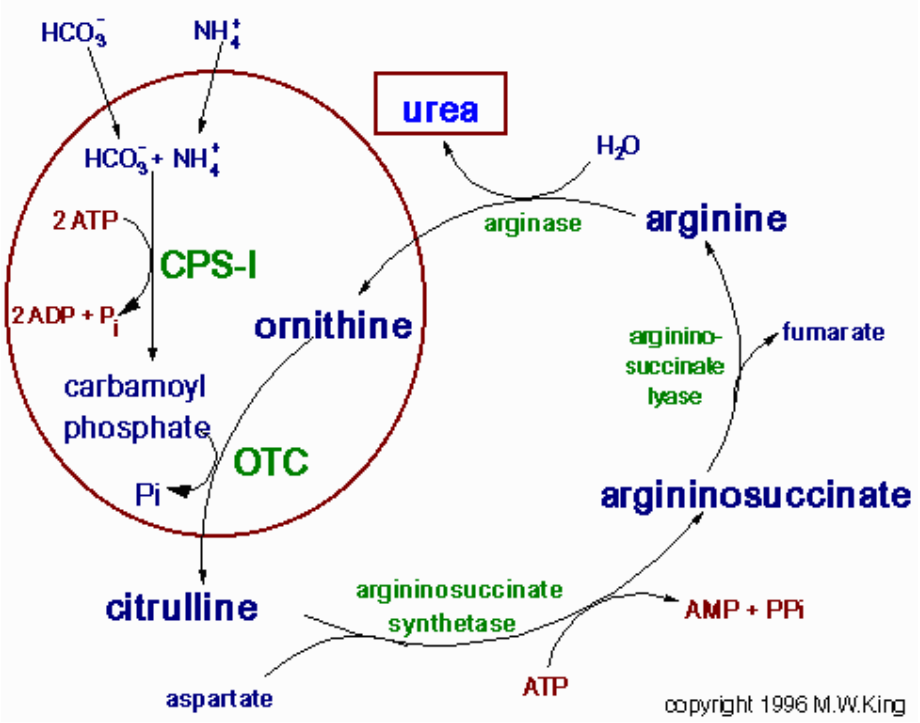
Urea is synthesized in the lives by the enzymes of urea cycle. It is then secreted into the blood steam and sequestered by the kidneys for excretion in the urine the urea cycle was elucidated in 1932 by Hans Krebs and Henseleit in 1932.

The complete urea cycle involves five enzymes, orginosuccinate synthase, arginase and arginosuccinate lyase which function in the cytosol and ornithine transcarbamylase and carbamoyl phosphate synthase which function in the mitochondria. Additional specific transport proteins are required for the mitochondrial uptake of L-ornithine, NH_4^+ and HCO_3^- and for the release of L-citrulline.

The free ammonia formed by oxidative deamination of glutamate is converted into carbomoyl phosphate in a reaction requiring two ATP molecules.

$NH_4^+ + HCO_3^- + H_2O$ 2ATP → Carbamoyl phosphate + HPO_4^{2-} + 2ADP

This reaction involves three steps, all of which takes place by the same enzyme.



1. Carbamoyl phosphate synthetase

Acquisition of the first urea nitrogen atom

Carbamoyl phosphate synthetase (LPS) is technically not a member of the urea cycle. It catalyses the condensation and activation of NH_4^+ and HCO_3^- to form carbamoyl phosphate, the first of the cycles two nitrogen – containing substrates, with the concomitant hydrolysis of two ATPS. Eukaryotes have two forms of CPS.

1. Mitochondrial CPS I uses ammonia as its nitrogen donor and participates in urea biosynthesis.

2. Cytosolic CPS II uses glutamine as its nitrogen donor and is involved in pyrimidine biosynthesis.

The reaction catalyzed by CPS I is thought to involve three steps.

1. Actuation of HCO_3^- by ATP to form carbonyl phosphate and ADP.

2. Attack of Ammonia on carboxyl phosphate displacing the phosphate to form carbonate and Pi.

3. Phosphorylation of carbomate by the second ATP to form carbamoyl phosphate and ADP.

   The reaction is essentially irreversible and is the rate limiting step of the urea cycle.

b. Ornithine transcarbamoylase

Ornithine transcarbomoylase transfers the carbamoyl group of carbamoyl phosphate to ornithine, gielding liteullive. The reaction occurs in the mitochondrian so that ornithine, which is produced in the cytosol, must enter the mitochondrion via a specific transport system like wise, since the remaining urea cycle reactions occur in the cytosol, citrulline must be exported from the mitochondrion.

c. Arginino succinate synthetase

Acquisition of the second urea Nitrogen Atom.

Urea's second nitrogen atom is introduced in the urea cycles third reaction by the condensation of citrulline's ureido group with an asparatate amino group by arginino succinate synthetase. The ureido oxygen atom is activated as a leaving group through formation of a citenllyl – AMP intermediate, which is subsequently displaced by the aspartate amino group. Support for the existance of the citenllyl AMP intermediate comes from experiments using ^{18}O – labeled citrulline (Figure).

d. Argino succinase

With the formation of argino succinate, all of the urea molecule components have been assembled. However the amino group donated by aspartate is still attached to the aspartate carbon skeleton. This situation is remedited by the arginino succinase catalysed elimination of argnine from the aspartate carbon skeleton forming fumarate (Reaction 4, Fig). Arginine is urea as immediate precursor. The fumarate produced in the arginino succinase reaction can be reconverted to aspartate for use in the arginino succinate synthetase reaction. This occurs via the fumarase and malate dehydrogenase reactions to form oxaloacetate followed by transamination. These are the same reactions that occur in the citric acid cycle, although they take place in the cytosol rather than in the mitochondrion.

e. Arginase

The urea cycles fifth and final reaction is the arginase – catalysed hydrolysis of arginine to yield urea and regenerate ornithine. Ornithine is then returned to the mitochondrion for another round of the cycle. The urea cycle thereby converts two amino groups. One from ammonia and one from aspartate, and a carbon atom from HCO_3^- to the relatively non toxic excretion product urea, at the cost of four "high – energy" phosphate bonds (three ATP hydrolyzed to two ADP, two Pi, AMD and Ppi followed by rapid Ppi hydrolysis). This energetic cost is more than recovered, however by the energy released upon the formation of urea cycle substrates. The ammonia released by the glutamate dehydrogenase reaction is accompanied by NADH formation, as is the reconversion of numarate through oxaloacetate to aspartate. Mitochonrial reoxidation of this NADH yields six ATPS.

f. Regulation of the urea cycle

Carbomoyl phophate synthetase I, the mitochondrial enzyme that catalyses the first committed step of the urea cycle, is allosterically activated by N-acetyl glutamate.This metabolite is synthesized from glutamate and acetyl coA N-acetyl glutamate synthase and hydrolysed by a specific hydrolase. The rate of urea production by the lives is, in fact, correlated with the N-acetyl glutamate concentration. Increased urea synthesis is required when amino acid breakdown rate increase, generating excess nitrogen that must be excreted increase in glutamate concentration through transamination reactions. This situation is then, causes are increase in N-acetyl glutamate synthesis, stimulating cabamoyl phosphate synthetase and thus the entire urea cycle.

The remaining enzymes of the urea cycle are controlled by the concentrations of their substrates. Thus, inherited deficiencies in urea cycle enzymes other than arginase do not result in significant decrease in urea production (the total lack of any urea cycle enzyme results in death shortly after birth).

The urea cycle and the TCA cycle are linked

In the urea cycle the carbon skeleton of the aspartate is released as fumarate this product links the urea cycle with the TCA cycle. Fumarate is hydrated to malate, which is oxidized to oxalo-acetate. The carbons of oxaloacetate can stay in the TCA cycle by condensation with acetyl coA to form citrate, or they can levae the TCA cycle either by gluconeogenesis to form glucose or by transamination to regenerate the aspartate .

2.3.2.4 Metabolic breakdown of individual aminoacids

The degradation of amino acids converts them to citric acid cycle intermediates or their precursors so that they can be metabolized to CO_2 and H_2O or used in gluconeogenesis. The 20 "standard" amino acids (the amino acids of proteins) have widely differing carbon skeletons, so their conversions to citric acid cycle intermediates follow correspondingly diverse pathways.

a. Aminoacids can be glucogenic, ketogenic, or both

"Standard" amino acids are degraded to one of seven metabolic intermediates: Pyruvate, α-ketoglutarate, succinyl coA, fumarate oxaloacetate, acetyl coA or aceto acetate (Figure). The aminoacids may therefore be divided into two groups based on their catabolic pathways.

1. Glucogenic aminoacids

Their carbon skeletons are degraded to pyruvate. α Ketoglutarate succinyl – coA, fumarate or oxaloacetate and are therefore glucose precursors.

2. Ketogenic aminoacids

Whose carbon skeletons are broken down to acetyl coA or aceto acetate and thus can be converted to fatty acids or ketone bodies.

For example, alanine is glucogenic because its transamination product, pyruvate can be converted to glucose via gluconeogenesis encine, on the other hand, is ketogenic; its carbon skeleton is convertedto acetyl coA and acetoacetate.

Isoleucine, phenyl alanine, threonine, tryptophane and tyrosine, however are both glucogenic and ketogenic; isoleucine for example, is broken down to succinyl coA and acetyl coA and hence is a percussor of both carbohydrates and ketone bodies. The remaining 13 amino acids are purely glucogenic.

In studying the specific pathways of amino acid breakdown, we shall organize the amino acids into groups that are degraded into each of the seven metabolic intermediates: Pyruvate, oxaloacetate, α-ketoglutarate, succinyl coA, fumarate, acetyl coA and acetoacetate. When acetoacetyl – coA is a product in amino acid degradation, it can of cause be directly converted to acetyl – coA.

b. Alanine, cystine, glycine, serine and threnine are degraded to pyruvate

Five amino acids, alanine, cystine, glycine serine and threonine, are broken down to yield pyruvate .

Tryptophan should also be included in this group since are of its breakdown products is alanine, which is transaminated to pyruvate.

Alanine

Alanine undergoes a reversible transamination directly to pyruvate. Recall that this reaction is part of the glucose alanine cycle.

Threonine

Threonine is degraded in more than one way. In the pathway shown in the Figure, threonine is converted to glycine and acety coA via threonine dehydrogenase and α-amino - β-ketobutyrate lyase. The glycine so formed can give rise to a second acetyl coA via pyruvate. Threonine is both glucogenic and ketogenic. Since it produces both pyruvate and acetyl coA.

Glycine

It is converted to serine by the enzyme serine hydroxy methyl transferase a PLP containing enzyme (Figure) this enzyme utilizes N^5, N^{10} methylene – tetrahydrofolate (N^5,N^{10} – methylene THF) as a cofactor to provide the C_1 unit necessary for this conversion.

The methylene group of the N^5, N^{10} methylene THF utilized in the conversion of glycine to serine is obtained through a second glycine degradation (Figure reaction 3) catalysed by the glycine cleavage system (also called glycine synthase when acting in the reverse direction. The glycine cleavage system, a multi enzyme complex resembles pyruvatedehydrogenase.

Serine

Serine is converted to pyruvate through dehydration by serine dehydratase. This PLP – enzyme, like the amino transferases, functions by forming a PLP amino acid schiff base so as to facilitate the removal of the aminoacids α-hydrogen atom. In the scrine dehydratase reaction, however, the Cα carbanion breaks down with the elimination of the amino acids C_β-OH, so that the substrate undergoes α,β elimination of H_2O rather than deamination. The product of the dehydration the examine aminoaceylate, tautomerizes non-enzymatically to the corresponding imino which spontaneously hydrolyzes to pyruvate and ammonia.

Serine hydroxy methyl trnasferase catalyzes PLP – Dependent Cα - C_β bond cleavage

Threonine may also be converted directly to glycine and acetaldehyde (that latter being subsequently oxidized to acetyl – coA) (Reaction 5 Figure). This reaction is catalyzed by serine hydroxy methyl transferase. Degradation of threonine to glycine and acetaldehyde by serine hydroxy methyl transferase demonstrates that PLP also facilitates cleavage of an amino acids Cα - C_β bond by delocalyzing the electrons of the resulting carbanion into the conjugated PLP ring.

Cysteine may be converted to pyruvate via several routes in which the sulfhydryl group is released as H_2S, SO_3^{2-}, or SCN.

C. Asparagine and aspartate are degraded to oxaloacetate

Transamination of aspartate leads directly to oxaloacetate.

Asparagine is also converted to oxaloacetate in this manner after its hydrolysis to aspartate by L-asparaginase.

d. Arginine, glutamate, glutamine, histidine and proline are degraded to α-ketoglutarate

α-ketoglutarate is the end point for degradation of these aminoacids. It is also the starting point for the synthesis of these five aminoacids. Hence these are reversible conversions.

Glutamine and Glutamate

Conversion of glutamine to glutamate involves only one reaction: hydrolysis by glutaminase the reactions were discussed earlier in this chapter when we were considering deamination.

Proline

Proline is converted into Δ^1 – pyrroline 5-carboxylate in a reaction catalysed by proline oxidase. It then converts into glutamate 50semialdehyde and to glutamate and finally to α-ketoglutarate.

Arginine

Arginine is converted to ornithine by the arginase of the urea cycle is broken down in most organisms by a transaminase to yield glutamate – V – semialdehyde

and to glutamate which in then is oxidized to α-ketoglutarate by glutamate dehydrogenase.

Histidine

The major route for histidine catabolism in mammals involves the conversion of histidine to glutamate. In the first step histidase catalyzes the removal of NH_3 with the formation of urocanate. This step is followed by an internal oxidation and reduction involving addition of the elements of water in a reaction catalysed by urocanase. The resulting intermediate, 4-imidazolone 3-propianate contains an imidazolone ring which is opened by a hydrolytic reaction to form N-formimino glutamate. Cleavage of N-formimino L-glutamate leads to form imino group transfer to the N^5 position of tetrahydrofolate and free glutamate.

Degradation pathways of arginine, glutamate, glutamine, histidine and proline to α ketoglutarate. The enzymes catalyzing the reactions are (1) glutamate dehydrogenase, (2) glutaminase, (3) arginase, (4) Ornithine – f-aminotransferase, (5) glutamate semialdehyde dehydrogenase, (6) Proline oxidase, (7) spontaneous, (8) histidine ammonia lyase, (9) urocanate hydratase, (10) imidazolone propionase and (11) glutamate forminino transferase.

e. Leucine and Lysine are degraded to acetoacetate and/or acetyl – coA

Leucine is oxidized by a combination of reactions used in β-oxidation and ketone body synthesis.

The four carbon atoms of leucine are converted into aceto acetyl coA leucine is thus ketogenic. The other two carbon atoms of leucine are converted into acetyl – coA. Following transamination of the α-amino group of leucine and oxidative decarboxylation of the corresponding α keto acid, isolvalaryl coA is formed. After its dehydrogenation, addition of a carboxyl group and hydration the resulting six carbon β-hydroxy - βmethyl glutaryl coA is cleaved to yield one molecule of acetyl coA and one of acetoacetate which is inturn converted into aceto acetyl coA by reaction with succinyl coA.

Lysine, another ketogenic amino acid degrades in a rather complex pathways by which four of the six carbon atoms of lysine are converted into aceto acetyl coA. The other two carbon atoms are lost in decarboxylation reactions lysine does not undergo transamination. In one pathway, lysine first condenses with α-ketoglutarate to yield saccharopine, which is ultimately converted into aceto – acetyl coA. In the other pathway the α-amino group of lysine is oxidized

presumabely by 2-amino acid oxidase. Both pathways converse into formation of α-amino adipic semialdehyde.

The saccharopine pathway is thought to predominate in mammals because a genetic defect in the enzyme that catalyzes reaction 1 results in hyperlysinemia and hyperlysinuria (elevated levels of lysine in blood and mine respectively along with mental and physical retardation).

f. Phenyl alanine and tyrosine are degraded to fumarate and aceto acetate

The first reaction in phenyl alanine degradation is its hydroxylation to tyrosine by the action of phenylalamine – 4 – monooxygenase. The enzyme requires tetrahydrobiopterin, a tolic – acid like compound, as a cosubsteatetetra hydrobiopterin is an infrequently used electron carrier coenzyme. Dihydrobiopterin is its oxidized form. The biopterin is kept in the reduced form by NADPH, the ultimate hydrogen donor in the hydroxylation reaction.

Minor pathways for phenyl alamine breakdown in animals involve transamination to yield phenyl pyruvate. Although phenyl pyruvate can be reduced to lactate and metabolized to other phenyl derivatives, the disposal of dietary phenyl alanine by these routes is insufficient so that in the inherited absence of the hydroxylation to tyrosine, high blood levels of phenyl alamine and phenyl pyruvate result. The condition is known as phenyl ketonuria.

Tyrosine

The P oxidation of tyrosine (and phenyl alanine) by the lives proceeds by way of acetoacetate and the dicarboxylic acid fumarate. Thus tyrosine and phenylalanine are both ketogenic and glycogenic.

The first step in tyrosine catabolism involves its conversion to 4-hydroxy phenyl pyruvate by a tyrosine – glutamate transaminase the next step is catalyzed by 4-hydroxy phenyl pyruvate dioxygenase, a copper containing enzyme that is stimulated by ascorbate. The enzyme is called a dioxygenase because both atoms of the oxygene become incorporated into the product. The product homogentisate, results from oxidation of the aromatic ling and an oxidative decarboxylation and migration of the side chain the aromatic ring is further oxidized and cleaved by nonhomogentisate 1,2-dioxygenase to 4-maleyl acetoacetate. Nearly all cleavages of aromatic rings in biological systems are catalysed by dioxygenases the enzyme requires ferrous ion and is also stimulated by ascorbate. An isomerase, maleyl aceto acetate isomerase, yields the teans compound 4-fumaeyl acetoacetate, which is hydrolytically cleaved to fumarate and acetoacetate by fumarylacetoacetate hydrolase.

g. Tryptophan Degradation

The major pathway for tryptophan catabolism in the mammalian lives and for many micro-organisms proceed by way of kynmenine kynurenine itself canbe metabolized in liver by way of α-ketoadipate, which is also an intermediate in lysine degradation. The first step in the breakdown of tryptophan is catalyzed by tryptophan oxygenase which yields N-formyl kynurenine (Figure) the enzyme is a dioxygenase and cleaves the indole ring by incorporating an oxygen atomon both C-2 and C-3 of the indole ring kynurenine itself is formed by the liberation of formate by kynuenine formamidase.

Kynurenine is converted to 3-hydroxy kynurenine by the NADPH – dependent kynurenine 3-monooxygenase. Kynureninase a pyridoxal phosphate enzyme, Catalyzes a hydrolytic cleavage of the alanine side chain to yield 3-hydroxy antheanilate. The aromatic ring is cleaved to2-amino 3-carboxy muconate 6-semialdehyde (ACS) by 3-hydroxy anthranilate oxygenase. Again this enzyme is a dioxygenase, and oxygen atoms are incorporated on both the carbons at the site of ring cleavage. Ferrousions are required by the enzyme ACS can be spontaneously cyclized to quinolinate with the liberation of a molecule of H_2O quinolinate is an intermediate in the biosynthesis of nicotinamide, the ACS is decarboxylated by a specific decarboxylase to yield 2-aminomuconate 6-semialdehyde. 2-aminomuconate 6-semi aldehyde is oxidized by an NAD – dependent amino muconate semialdehyde dehydrogenase the resulting 2-aminomuconate is reduced to α-ketoadipate by an NAD(P) H dependant reducase.

The further catabolism of α-ketoadipate results in the liberation of two molecules of CO_2 and two of acetyl coA. The first step is a coenzyme-A dependent oxidative decarboxylation by an enzyme probably identical to α-ketoglutarate dehydrogenase. The product, glutaryl – coA is oxidized by a fluvin – linked dehydrogenase to an intermediate common to the oxidation of fatty acids, geotonyl coA. Finally, two molecules of acetyl – coA are formed by the action of the fatty acid oxidising enzymes.

h. Methionine catabolism leads to succinyl coA

The catabolism of methionine involves nine steps leading to succinyl coA.

In the first step methionine is adenylated (reacts with ATP) to form S-adenosyl methionine (SAM). SAM is probably the most used transmethylating agent in the cell. Transfer of the methyl group SAM to an appropriate receptor leads to S-adenosyl homocysteine. This is hydrolyzed to adenosine and homocysteine. The homocysteine is condensed with serine to yield cystathionine which in one more step is converted to cysteine and α-ketobutyrate. The α-ketobutyrate is converted in one step into propionyl coA and then to succinyl coA.

i. Isolencine and valine are degraded to succinyl coA

Degradation of branched chain aminoacids isoleucine, leucine and valine beings with three reactions that employ common enzymes (1) transamination to the corresponding α-ketoacid (2) oxidative decarboxylation to the corresponding acyl – coA and (3) dehydrogenation by FAD to form a double bond.

The remainder of the isoleucine degradation pathway (Figure) is identical to that of fatty acid oxidation (4) double bond hydration (5) dehydrogenation by NAD^+ and (6) thiolytic clevage yielding acetyl coA and peopionyl coA which is subsequently converted to succinyl coA.

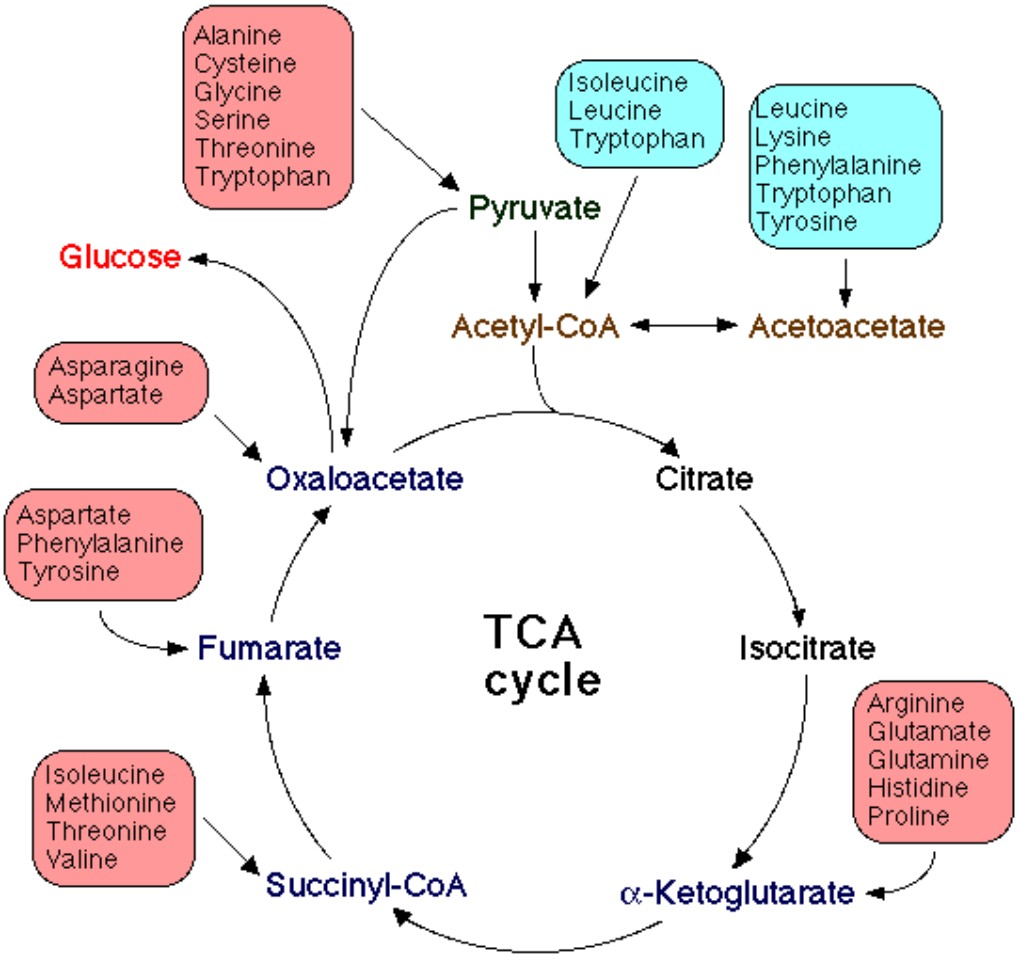
Valine degradation is a variation following (7) double bond hydration (8) the coA thioester bond is hydrolyzed before (9) the second dehydrogenation reaction. The thioester bond is then regenerated as peopienyl coA in the sequencest last reaction (10) an oxidative decarboxylation rather than a thiolytic cleavage.

Maple syrup urine disease results from a defect in branched – chain amino acid.

Degradation

Branched chain α-keto acid dehydrogenase (BCKDH; also known as α-ketoisovalerate dehydrogenase), which catalysis reaction 2 of branched – chain amino acid degradation is a multi enzyme complex that closely resembles the pyruvate dehydrogenase and α-ketoglutarate dehydeogenase multienzyme complexes. Indeed, all three of these multienzyme complexes share a common protein component, E_3 (dihydrolipoamide dehydrogenase) and employ the coenzymes TPP, lipeamide and FAD in addition to their terminal oxidizing agent, NAD^+.

A genetic deficiency in BCKDH causes maple syrup urine disease, so named because the consequent buildup of branched chain α-keto acid imparts the urine with the characteristic odour of maple syrup. Unless promptly treated by a diet low in branched chain amino acids, maple syrup urine disease is rapidly fatal.



2.3.2.5 Summary

In the digestive teact of vertebrates, the peotedytic enzymes pepsin, trypsin, chymotrypsin, carboxypeptidase and lencine amino peptidase function to carry out complete hydrolysis of ingested proteins yielding free aminoacids, which are then absorbed into the blood for transport to the lives, where the major portion of amino acid catabolism takes place.

The carbon skeletons of amino acids undergo oxidative degradation to compounds that can enter the tricarboxylic acid cycle for oxidation. The amino groups of most of the L-amino acids are removed by transamination to α-ketoglutarate, yielding glutamate, which undergoes oxidative deamination by glutamate dehydrogenase. The amino groups of most of the L-amino acids are

removed by transamination to α-ketoglutarate, yielding glutamate which undergoes oxidative deamination by glutamate dehydrogenase. There are five pathways by which carbon atoms of L-amino acids enter the tricarboxylate cycle: via (1) actyl coA, (2) α-ketoglutarate (3) succinate (4) fumarate and (5) oxaloetate. The aminoacids entering via acetyl coA are divided into two groups. The first, which includes alanine, theronine, glycine, serine and cystrine yields pyruvate (and is thus glycogenic) and finally to acetyl coA.

The second group (phenyl alanine, tyrosine, leucine, lysine and tryptophan) yields acetoacetyl coA and finally to acetyl coA. The amino acids arginine, histidine, glutamine, glutamic acid and proline enter via α-ketoglutarate.

Methionine, isoleucine and valine enter via succinate, four carbon atoms of phenyl alanine and tyrosine enter via femarate; and asparagines and aspartic acid enter via oxaloacetate. In ureotelic animals (terrestrial mammals and adult amphibia), urea formed by the urea cycle, is the final excretion product of amino nitrogen urea results from the action of arginase or arginine the other cleavage product being ornithine the urea cycle takes place in the lives.

2.3.2.6 Model Questions

1. Discuss urea cycle?
2. Write about the degradation of aromatic amino acids.
3. Degradation of branched chain amino acids.

2.3.2.7 Reference Books

1. Text book of biochemistry – Voet & Voet.
2. Biochemistry – Zubey.
3. Biochemistry – Lehninger.

Lesson 2.3.3

BIOSYNTHESIS OF PURINES & PYRIMIDINES

Objective

2.3.3.1. Biosynthesis of Purine Ribonucleotides

A. Denovo synthesis
   i. Synthesis of IMP – 11 steps
   ii. Synthesis of Adenine & Guanine
      1. Synthesis of AMP
      2. Synthesis of GMP
      3. ATP & GTP synthesis
B. Salvage pathway
   i. APRT
   ii. HGPRT
C. Regulation
D. Disorders

2.3.3.2. Biosynthesis of Pyrimidine Nucleotides

A. Denovo synthesis – 8 steps
B. Salvage pathway
C. Regulation
D. Disorders

2.3.3.3. Biosynthesis of Deoxyribonucleotides

A. Ribonucleotide reduction
B. d NTP's production
C. Thymine synthesis

2.3.3.4 Summary
2.3.3.5 Model Questions
2.3.2.6 Reference Books

OBJECTIVE

The present lesson deals with the biosynthetic path ways of nucleotides.The pathways includes both de nova and salvage path ways

Introduction

An ample supply of nucleotides is essential for many life processes.

1. They are the precursors of DNA and RNA. As such, they are necessary for replication and transcription.

2. They are essential carriers of chemical energy – a role primarily of ATP and to some extent GTP.

3. They are components of cofactors NAD, FAD, S-adenosyl methionine, and coenzyme A

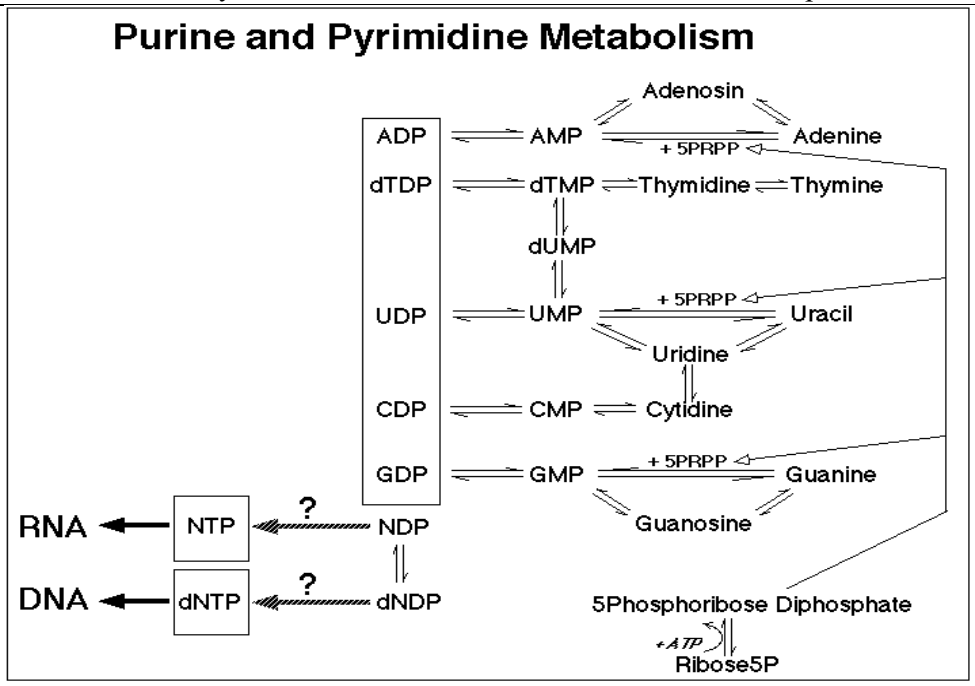
4. Nucleotides are essential components of signal – transduction pathways – many hormonal signals are mediated intracellularly by CAMP and CGMP.

Two types of pathways lead to nucleotides: de novo pathways and salvage pathways. De novo synthesis of nucleotides beings with their metabolic precursors: amino acids, ribose – 5-phosphate, CO_2, NH_3, etc., salvage pathways recycle the free bases and nucleosides released from nucleic acid breakdown. Both types of pathways are important in cellular metabolism. Both de novo and salvage pathways lead to the synthesis of ribonucleotides. However, DNA is built from deoxyribonucleotides.

2.3.3.1. Biosynthesis of Purine Ribonucleotides

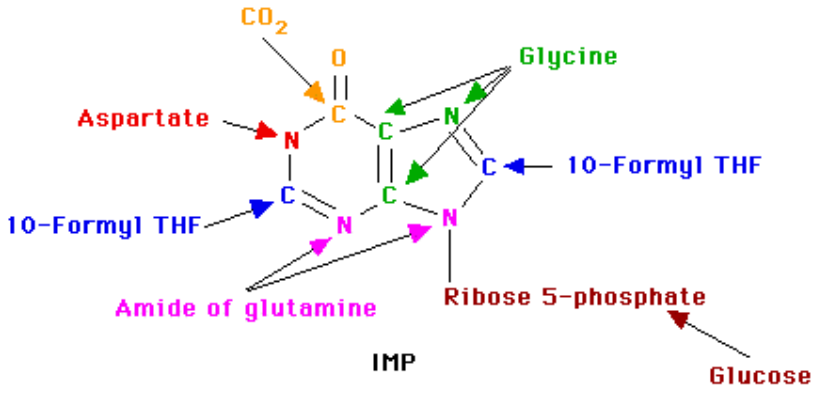
In 1948, John Buchanan obtained the first clues as to how this process occurs de novo by feeding a variety of isotopically labeled compounds to pigeons and chemically determining the positions of the labeled atoms in their excreted uric acid. He used birds in these experiments because they excrete waste nitrogen almost entirely as uric acid, a water soluble, and therefore easily isolated substance.

Most cells have an active turnover of many of their nucleic acids which result in the release of adenine, guanine and hypoxanthine. These free purines are reconverted to their corresponding nucleotides through salvage pathways.



A. De Novo synthesis

The actual pathway of precursors incorporation into the purine ring was elucidated in subsequent investigations of Buchanan and G. Robert Greenberg. They demonstrated that N_1 of purines is from amine group of Aspartate; C_2 and C_8 are from formate; N_3 and N_9 are from amide group of glutamine; C_4, C_5 and N_7 are derived from glycine; and C_6 is from HCO_3^-.



Buchanan and Greenberg investigations showed that:

i. Initially synthesized nucleotide is Inosine Mono Phosphate (IMP)

ii. Subsequently synthesized nucleotides are AMP and GMP.

i. Synthesis of IMP

IMP is synthesized in a pathway comprised of 11 reactions.

1. Activation of ribose – 5-phosphate

2. Acquisition of purine atom N_9

3. Acquisition of purine atoms C_4, C_5 and N_7

4. Acquisition of purine atom C_8

5. Acquisition of purine atom N_3

6. Formation of purine imidazole ring

7. Acquisition of C_6

8. Acquisition of N_1

9. Elimination of Fumarate

10. Acquisition of C_2

11. Cyclization to form IMP

1. Activation of ribose – 5-phosphate

The starting material for purine biosynthesis is α- D-ribose-5-phosphate. In this step 5-phosphoribosyl - 1- pyrophosphate (PRPP) will be formed from reaction of ATP with ribose – 5-phosphate on catalysis by ribose phosphate pyrophosphokinase. For this enzyme Ppi and 2,3-bisphosphoglycerate are the activators and, ADP and GDP are the inhibitors.

2. Acquisition of purine atom N_9

The PRPP's pyrophosphate group is displaced with glutamine by the catalyst amido phosphoribosyl transferase. Then inversion of configuration at C_1 occurs forming β-5-Phosphoribosylamine. The amido phosphoribosyl transferase is subjected to feed back inhibition by purine nucleotides.

3. Acquisition of purine atoms C_4, C_5 and N_7

The enzyme glycinamide ribonucleotide synthetase facilitates the formation of an amide linkage between Glycine's carboxyl group and amino group of phospho-ribosylamine. This is the only step of purine biosynthesis pathway in which more than one purine ring atom is acquired.

4. Acquisition of purine atom C_8

Glycinamide ribotide's free β-amino group is formylated to yield formylglycinamide ribotide. The formyl donor in this reaction is N^{10} – formyl tetrahydrofolate.

5. Acquisition of purine atom N_3

The amide amino group of a second glutamine is transferred to the growing purine ring to form formylglycinamidine ribotide with the enzyme Formylglycinamidine ribotide synthetase. This reaction involves the coupled hydrolysis of ATP.

6. Formation of purine imidazole ring

The closing of imidazole ring is facilitated by the tautomeric shift of the reactant from its imine to its enamine form giving out the product 5-aminoimidozole ribotide.

7. Acquisition of C_6

In a reaction catalyzed by aminoimidazole ribotide carboxylase purine C_6 is introduced as HCO_3^-.

8. Acquisition of N_1

Purine atom N_1 is contributed by aspartate in an amide forming condensation reaction yielding 5-aminoimidazole –4- (N-succinyl carboxamide) ribotide (SACAIR). The reaction needs the hydrolysis of ATP.

9. Elimination of fumarate

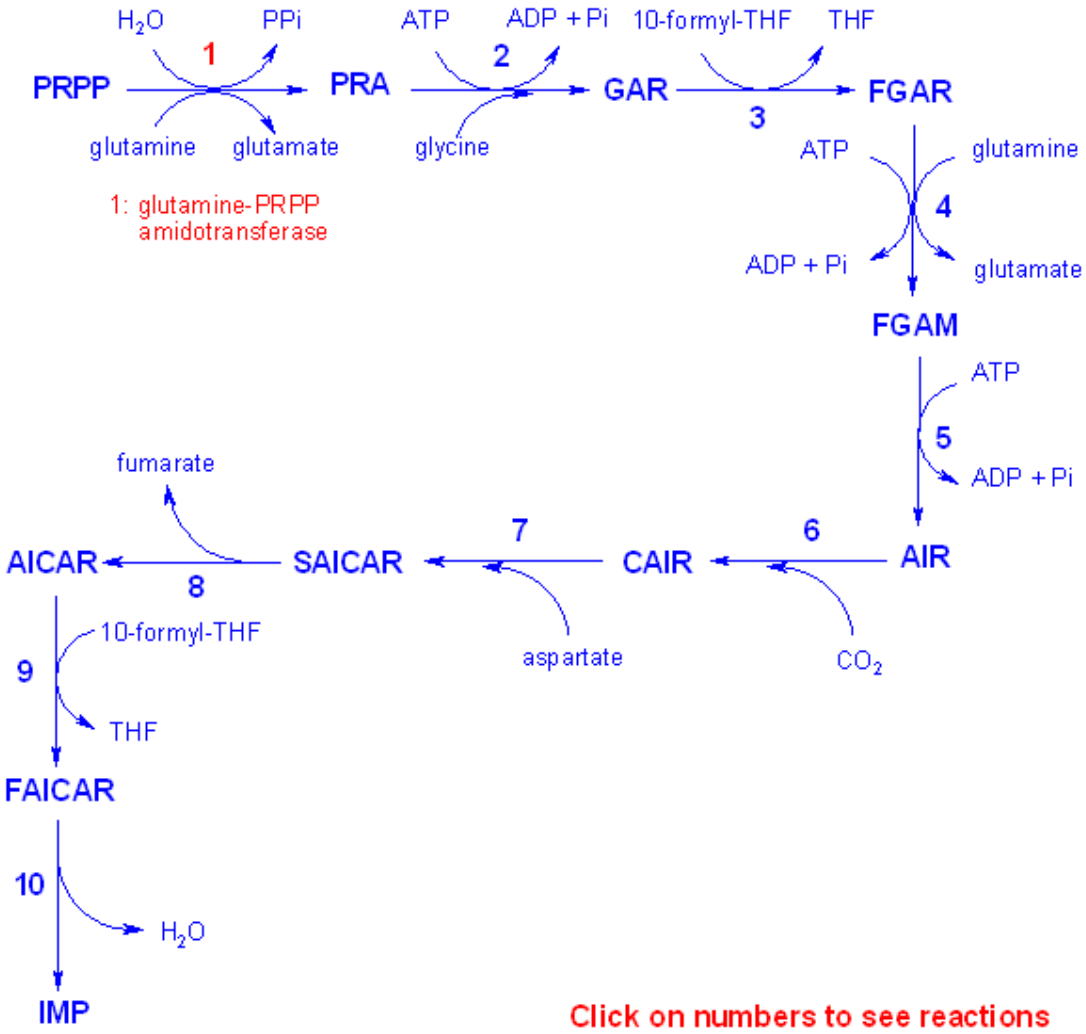
SACAIR is cleaved with the release of fumarate, yielding 5-amino imidazole – 4-carboxamide ribotide (AICAR).

10. Acquisition of C_2

The last atom of C_2 of purine ring is from N^{10} – formyl THF, yielding 5-Formamino imidazole – 4- carboxamide ribotide (FAICAR). The reactions 4 and 10 are inhibited by sulfonamides in bacteria.

11. Cyclization to form IMP

The final reaction of the pathway is ring closure to form 1 MP that occurs through the elimination of water.



ii. Synthesis of Adenine and Guanine Ribonucleotides

AMP and GMP will be synthesized from IMP.

1. Synthesis of AMP
2. Synthesis of GMP
3. Synthesis of ATP and GTP

1. Synthesis of AMP

AMP will be synthesized from IMP only by the replacement of 6-keto group by an amino group. This is a two reaction pathway. In the first reaction aspartates aminogroup is linked to IMP to yield adenylosuccinate. In the second reaction, adenylo succinate lyase forms AMP by removing fumarate from adenylosuccinate.

2. Synthesis of GMP

GMP also will be synthesized from IMP in a two step pathway. In the first step IMP is dehydrogenated to form xanthosine monophosphate (XMP). The GMP will be synthesized from XMP using glutamine amide nitrogen.

3. ATP and GTP synthesis

In order to participate in nucleic acid synthesis, the nucleotide monophophates must be converted to the corresponding nucleoside triphosphates.

From the corresponding nucleoside monophosphates nucleoside diphosphates are synthesized with the catalytic reaction of nucleoside monophosphate kinases.

AMP + ATP → 2 ADP

GMP + ATP → GDP + ADP

The nucleoside diphosphates are converted to the corresponding triphosphates by nucleoside diphosphate kinase.

GDP + ATP → ADP + GTP

B. Salvage Synthesis

The free purines are reconverted to the corresponding nucleotides through salvage pathways. The de novo pathway is common in all the cells. But salvage

pathway differs in different cells. The salvage pathway is by two important enzymes role.

i. Adenine phosphoribosyl transferase (APRT)

ii. Hypoxanthine – Guanine Phosphoribosyl Transferase (HGPRT)

i. APRT

APRT mediates AMP formation through the transfer of adenine to PRPP.

Adenine + PRPP → AMP + Ppi

ii. HGPRT

HGPRT mediates synthesis of 1MP and GMP of both hypoxanthine and guanine.

Hypoxanthine + PRPP → 1 MP + Ppi

Guanine + PRPP → GMP + Ppi

C. Regulation of Purine Biosynthesis

The synthesis of AMP and GMP will be coordinately controlled along their individual concentrations regulation.

The synthesis of IMP will be regulated in the first two steps itself i.e., at PRPP synthetase and glutamine – PRPP amidotransferase. Both ADP and GDP inhibit the PRPP synthetase activity. The second enzyme glutamine – PRPP amidotransferase inhibited by all ATP, ADP, AMP, GTP, GDP and GMP. This indicating that IMP synthesis is under the control of adenine and guanine nucleotides.

A part from regulation at the level of synthesis of IMP at the level of AMP and GMP also maintained. GTP powers the synthesis of AMP and ATP powers the synthesis of GMP. This will balance the production of AMP and GMP.

D. Disorders

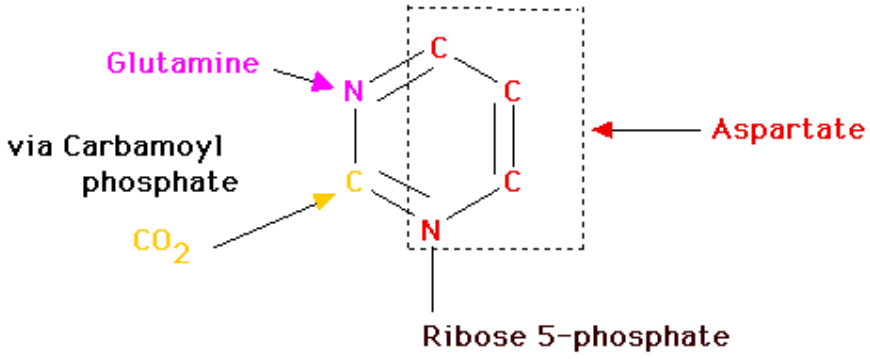
If the enzymes participating in the purine nucleotide biosynthesis were not functioning properly or if the concentrations of precursors is not at satisfactory levels that leads to disorders of the pathway and also automatically disorder of organism.

One of the very important disorder related to this is Lesch-Nyhan syndrome. This results from HGPRT deficiency. Excessive uric acid production causes it. This disorder affects mostly males.

The neurological symptoms arising with the enzyme deficiency are spasticity, mental retardation, and highly aggressive and destructive behavior.

2.3.3.2. Biosynthesis of Pyrimidine Nucleotides

The common pyrimidine ribonucleotides are cytidine – 5^1- monophosphate and uridine 5^1-monophosphate, which contain. The pyrimidines cytosine and uracil. The De novo pyrimidine nucleotide biosynthesis proceeds in a some what different manner from purine nucleotide synthesis. The six membered pyrimidine ring is made first and then attached to ribose – 5-phosphate. Isotopic labeling experiments have shown that N_1, C_4, C_5 and C_6 of the pyrimidine ring are all derived from aspartic acid. C_2 arises from HCO_3^- and N_3 is contributed by glutamine amide.



Generic pyrimidine base

A. De novo synthesis

The studies of de novo synthesis of pyrimidine ribonucleotides was with the observation of mutants of the bread mold, neurospora crassa.

It involves the steps :

1. Synthesis of carbamoyl phosphate
2. Synthesis of carbamoyl aspartate
3. Synthesis of dihydro orotate

4. Synthesis of orotate
5. Synthesis of OMP
6. Synthesis of UMP
7. Synthesis of UTP
8. Synthesis of CTP

1. Synthesis of Carbamoyl Phosphate

HCO_3^- and amide nitrogen of glutamine reacts in the presence of the enzyme cytosolic carbamoyl phosphate synthetase II to form carbamoyl phosphate. However in animals carbamoyl phosphate required in urea synthesis is made by mitochondrial carbamoyl phosphate synthetase I. In bacteria, a single enzyme supplied carbamoyl phosphate for the synthesis of arginine and pyrimidines.

2. Synthesis of Carbamoyl Aspartate

Carbamoyl phosphate reacts with aspartate to yield N-carbamoyl aspartate in the first committed step of pyrimidine biosynthesis. This reaction is catalyzed by aspartate transcarbamoylase. In bacteria this step is highly regulated, and bacterial aspartate transcarbamoylase is one of the most thoroughly studied allosteric enzymes.

3. Synthesis of Dihydro Orotate

By removal of water from N-Carbamoyl aspartate the pyrimidine ring is closed to form L-dihydroorotate. This reaction is catalysed by the enzyme dihydroorotase.

4. Synthesis of Orotate

The L-dihydroorotate is oxidized to the pyrimidine derivative orotate using NAD^+ as electron acceptor.

5. Synthesis of OMP

Orotate reacts with PRPP to yield orotidine – 5- monophosphate (OMP) in a reaction catalyzed by orotate phosphoribosyl transferase.

6. Synthesis of UMP

The OMP is decarboxylated by OMP decarboxylase to form UMP.

7. Synthesis of UTP

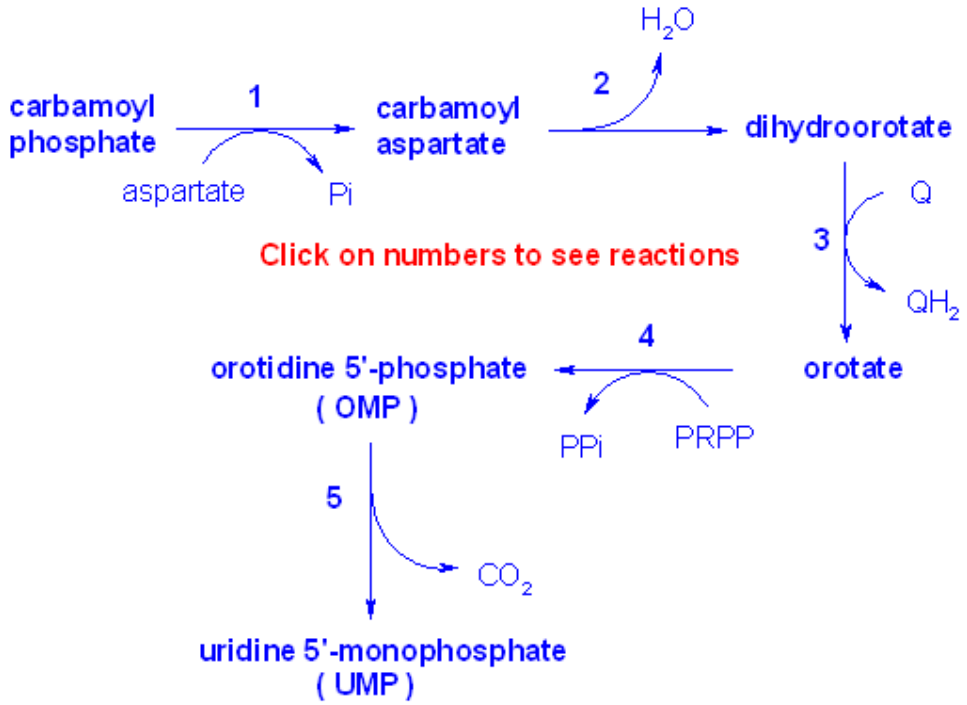
The UMP is phosphorylated to UTP by consuming ATP with the catalysis of nucleoside monophosphate kinase and nucleoside diphosphate kinase.

UMP + ATP → UDP + ADP

UDP + ATP → UTP + ADP

8. Synthesis of CTP

CTP is synthesized from UTP by animation with the help of glutamine. The enzyme catalyzing this reaction is CTP synthetase. In animals the amino group is donated by glutamine, but in bacteria it is directly supplied by ammonia.



B. Salvage pathway

The enzyme orotate phosphoribosyl transferase, which is catalyzing the synthesis of OMP from orotate, also catalyzes the salvage synthesis of UMP and CMP from uracil and cytosine.

Uracil + PRPP → UMP

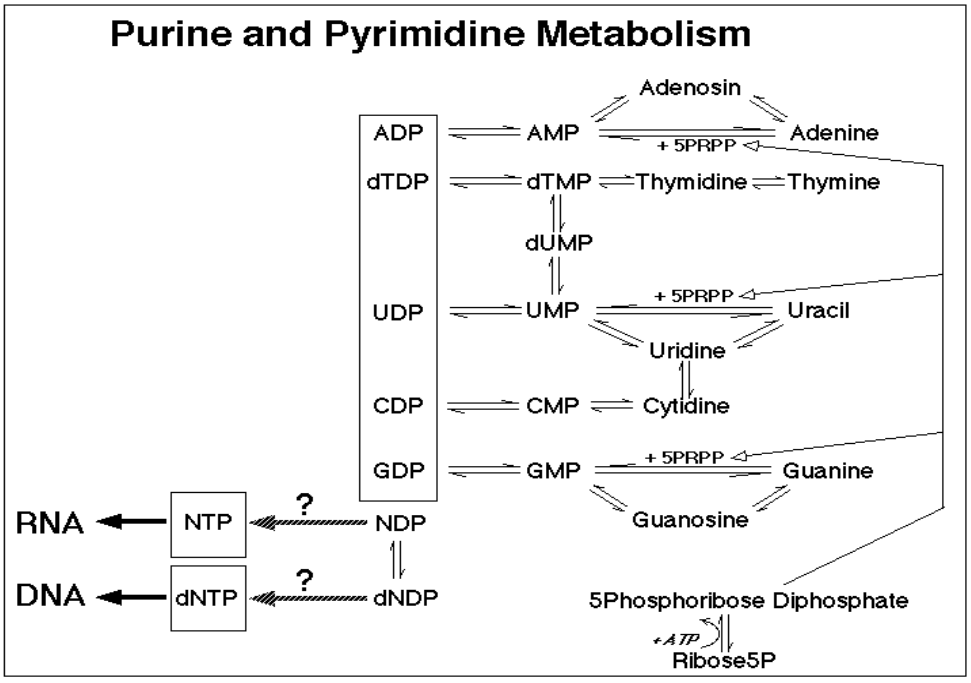
Cytosine + PRPP → CMP

C. Regulation

In bacteria the pyrimidine biosynthesis is primarily regulated at the ATcase reaction point. ATP acts as the stimulator of Atcase and UTP acts as inhibitor of AT case in many bacteria, but in E.Coli CTP is the inhibitor.

In animals ATC ase is not a regulatory enzyme. Rather is controlled at carbamoyl phosphate synthetase II. This enzyme is inhibited by UDP and UTP; and activated by ATP and PRPP. In mammals at OMP decarboxylase activity step also pathway regulation is maintained as CMP is inhibitor of the enzyme.

One of the important precursor PRPP concentration will be effected by the ADP and GDP, as these are inhibitors of ribose phosphate pyrophosphokinase.



D. Disorders

The inherited disease orotic aciduria is a consequence of deficiency of orotate phosphoribosyl transferase and OMP decarboxylase. The disease is characterized by the excretion of large amounts of urine, retarded growth and severe anaemia.

2.3.3.3. Biosynthesis of Deoxyribonucleotides

The concentrations of Deoxyribonucleotides are extremely low in nonproliferating cells. Only at the time of DNA replication the deoxyribonucleotides concentration increases to support the required DNA synthesis.

A. Ribonucleotides reducation

By the catalytic action of ribonucleotide reductase the 2' – deoxynucleotides are synthesized from corresponding ribonucleotide 5'-disphosphate.

The ribonucleotide reductase is regulated by a complex Feedback network. The maintenance of the proper intracellular ratios of dNTP s is essential for normal growth, as a deficiency of any dNTP is lethal, where as an excess is mutagenic.

In the presence of a mixture of NDPs, ribonucleotide reductase commences dNDP production by the ATP stimulated reduction of CDP and UDP. The resulting dUDP is converted to dTTP, which inhibits further CDP and UDP reduction but stimulates dGDP production.

B. dNTP's production

dNTP's are produced by the phosphorylation of dNDP's. The enzyme catalyzing reaction is nucleoside diphosphate kinase.

dNDP + ATP → ADP + dNTP

C. Thymine synthesis

dTMP component of DNA is synthesized bymethylation of dUMP. The enzyme thymidylate synthetase with N^5, N^{10} – methylene tetrahydro folate methyl donor synthesizes dTMP from dUMP.

The medical significance of steps with tetrahydrofolate role is very high. This is because THF will be regenerated from dihydrofolate (DHF) with the enzyme dihydrofolate reductase (DHFR). The inhibition of DHFR therefore not only prevents dTMP synthesis, but also blocks all other THF dependent biological

reactions such as the synthesis of purines, histidine and methionine. DHFR therefore offers an attractive target for chemotherapy.

2.3.3.4 Summary

*The nucleotides significant need in the biological process indicates the necessary to maintain their concentrations in cells.

*Purine biosynthesis with de novo pathway is from precursors like amino acid, CO_2, formate, etc.

- The free purines released during cell turn over are reconverted to corresponding nucleotides through salvage pathway.
- The pyrimidine biosynthesis is mainly regulated at ATCase in bacteria and carbamoyl phosphate synthetase II in animals.
- Ribonucleotides will be reduced to deoxyribonucleotides only in NDP forms.
- Abnormalities in biosynthesis of nucleotides causes diseases like Lesch-Nyhan syndrome.

2.3.3.5 Model Questions

1. Describe in detail about the ATP de novo synthesis
2. How can you synthesize dTTP
3. Write about the disorders related to nucleotide biosynthesis.

2.3.3.6 Reference Books

1. Biochemistry – Donald Voet and Judith G. Voet.
2. Lehningers Principles of Biochemistry – Nelson & Cox

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Lesson 2.3.4

CATABOLISM OF NUCLEOTIDES

Objective

Objective:

The main objective of the present lesson is to bring the degradative pathways of purine and pyrimidinne nucleotides

Introduction

The nucleotides are synthesized in cells by the synthetic pathway. These inturn participates in the synthesis of nucleic acids and other functions. The cellular nucleotides will be in continuous turnover. In this chapter we will see about the pathway and biological significance of nucleotide catabolism.

Apart from the cellular nucleotide most of the food stuffs also contain nucleic acids as they are also of cellular origin. Dietary nucleic acids survive the acid medium of the stomach, they are degraded to their component nucleic acids, mainly in the duodenum, by pancreatic nucleases and intestinal phosphodiesterases. They are then hydrolyzed to nucleosides by a variety of group specific nucleotidases and nonspecific phosphatases. The nucleosides may be

absorbed or digested to free bases and ribose or ribose – 1-phosphate by the action of nucleosidases and nucleoside phosphorylases.

Nucleic acids + H_2O $\xrightarrow{\text{nucleases}}$ nucleotides

Nucleotides + H_2O $\xrightarrow{\text{nucleotidases}}$ nucleoside + Pi

Nucleoside + H_2O $\xrightarrow{\text{nucleosidases}}$ Base + ribose

Nucleoside + Pi $\xrightarrow{\text{nucleoside phosphorylase}}$ Base + ribose – 1-phosphate.

2.3.4.1. Catabolism of Purines

The degradation of purine nucleotides, nucleosides, and bases is through a common pathway leading to the formation of uric acid. The enzymes involved in degradation of nucleic acids, nucleotides and nucleosides vary in specificity. Nucleases show specificity towards either RNA or DNA and also toward the bases and also position of cleavage site. Nucleotidases shows broad specificity, which will hydrolyse any of 3' or 5' nucleotides.

A. Purine Catabolism

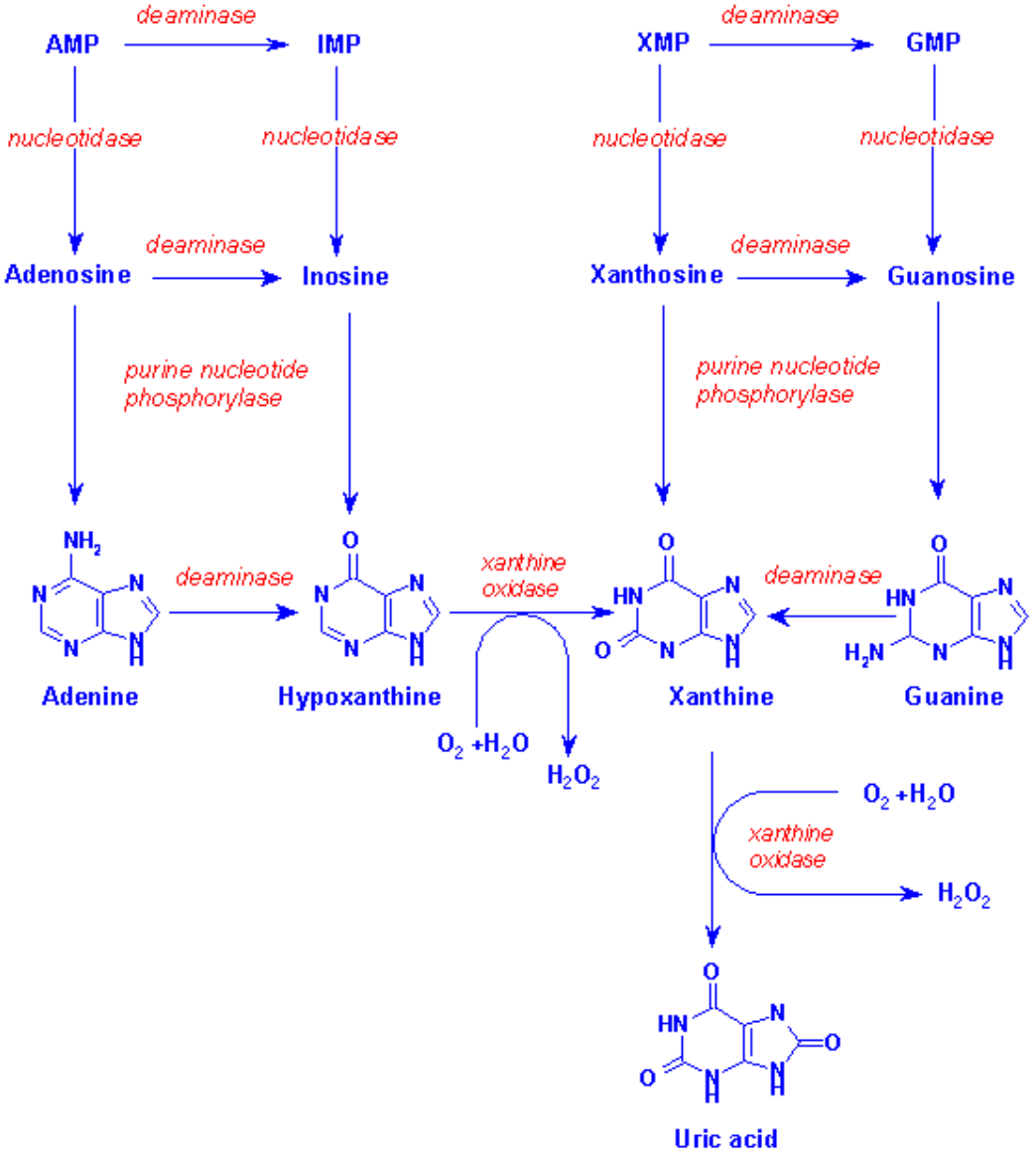
1. The first step of the degradation of all the nucleotides is the removal of phosphate from the nucleotide by the nucleotidase to its corresponding nucleoside.

2. All the nucleosides except the adenosine and deoxyade nosine will be deribosylated to its corresponding base by the enzyme purine nucleoside phosphorylase.

3. Adenosine and deoxy adenosine are not degraded by mammalian purine nucleoside phosphorylase. The AMP or dAMP will be deaminated to IMP and; adenosine or deoxyadenosine will be deaminated to inosine successively by the action of AMP deaminase and adenosine deamminase.

4. The products of the catalysis of purine nucleoside phosphorylase hypoxanthine and guanine will also be converted to xanthine. The xanthosine will also be catalysed to xanthine.

5. Xanthine will be finally catabolized to uric acid, the end product of purine catabolism.



B. Fate of Uric Acid

The final product of purine metabolism in humans and other primates, birds, reptiles and insects is uric acid. This will be excreted as such in urine. Mammals other than primates oxidize it to their excretory product, allantoin. The enzyme catabolyzing this reaction is urate oxidase.

The allantoin further degraded to allantoic acid, is excretory form in teleost fishes. Cartilaginous fish and amphibia further degrade allantoic acid to urea. Finally, marine invertebrates decomposes urea to Ammonia.

C. Disorders

Gout is a disease characterized by elevated levels of uric acid in body fluids. Symptoms of this disease are painful joint inflammation of sudden onset caused by deposition of insoluble crystals of sodium urate. These crystals also precipitate in kidneys and ureters as stones, resulting in renal damage and urinary tract obstruction.

2.3.4.2. Catabolism of Pyrimidines

Animals cells degrade pyrimidine nucleotides to their component bases. The resulting uracil and thymine are then broken down in the liver through reduction, rather than oxidation as occurs in purine catabolism. The end products of pyrimidine catabolism are β-alanine and β-aminoisobutyrate. These are converted to malonyl COA and methyl malonyl – COA for further utilization.

1. The pyrimidines CMP, UMP and dTMP will be dephosphorylated to their nucleosides form in the presence of the enzyme nucleotidase.

2. The cytidine will also be converted to uridine form in the presence of cytidine deaminase by a deamination reaction.

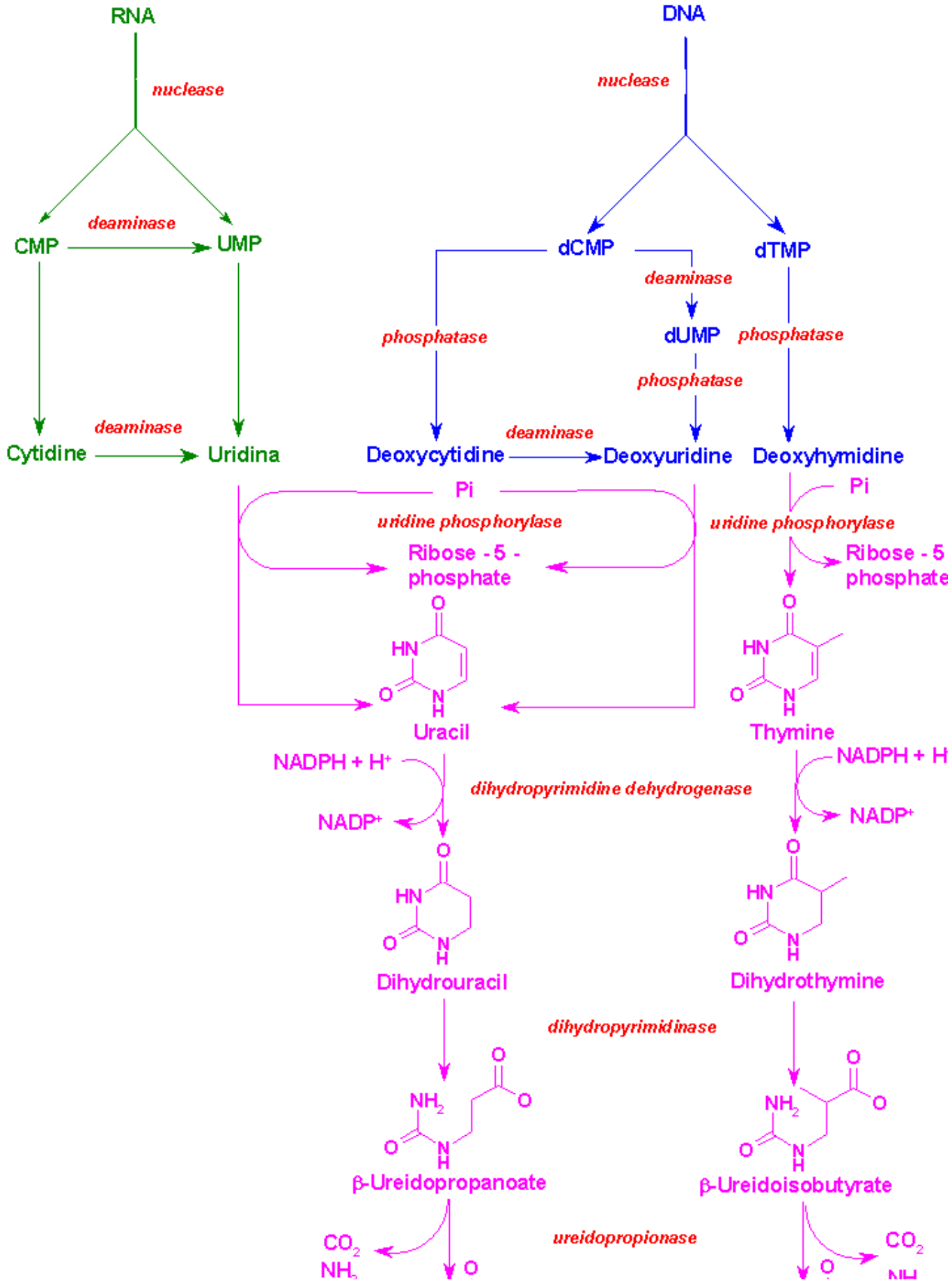
3. The uridine and deoxythymidine will be deribosylated to corresponding nitrogen bases in presence of the enzyme phosphorylase.

4. Uracil and thymine will then be reduced to dihydrouracil and dihydrothymine respectively in presence of NADPH + H^+ by the catalytic reduction of dihydro uracil dehydrogenase.

5. The dihydrouracil and dihydrothymine will then be hydrated to β-ureidopropionate and β-ureidoisobutyrate opening the base ring. The reaction is catalyzed by hydropyrimidine hydratase.

6. β-ureidopropionate by deamination and decarboxylation reaction forms amino acids, β-alanine and β-aminoisobutyrate will be converted tomalonyl COA and methyl malonyl COA, the useful forms for utilizations like fatty acid biosynthesis.

7. β–alanine and β-aminoisobutyrate will be converted to malonyl COA and methylmalonyl COA, the useful forms for utilizations like fatty acid biosynthesis.


RNA
nuclease
deaminase
CMP
UMP
Cytidine
deaminase
Uridina
DNA
nuclease
dCMP
dTMP
deaminase
dUMP
phosphatase
phosphatase
phosphatase
Deoxycytidine
deaminase
Deoxyuridine
Deoxyhymidine
Pi
uridine phosphorylase
Ribose - 5 - phosphate
Pi
uridine phosphorylase
Ribose - 5 phosphate
Uracil
Thymine
NADPH + H+
NADP+
dihydropyrimidine dehydrogenase
NADPH + H
NADP+
Dihydrouracil
Dihydrothymine
dihydropyrimidinase
β-Ureidopropanoate
β-Ureidoisobutyrate
CO2
NH3
ureidopropionase
CO2
NH

2.3.4.3 SUMMARY

- The nucleotides present in the body may be cellular origin or may be as food stuff component.
- The end product of purine catabolism is uric acid, which will be excreted in different forms in different organisms.
- Gout is a very important disease related to the purine catabolism abnormalities and other consequences.
- Pyrimidines will be degraded to amino acids β-alanine and β-amino isobutyrate.

2.3.4.4 Model Questions

1. The degradative pathway of uracil results in amino acid β-alanine – explain.
2. What is Gout.
3. What is the fate of uric acid

2.3.4.5 Reference Books

1. Lehningers principles of biochemistry – Cox and others.
2. Harpens review of biochemistry.

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Lesson 2.4.1

DNA AS HERIDITARY MOLECULE AND ITS STRUCTURE

Objective

Objective

This chapter explains the different experiments that proves DNA as hereditary material and its structure is also clearly explained.

2.4.1.1 Introduction

In 1865, Mendel showed that genes transmitted genetic information. The classical genetics of early twentieth century showed that the genetic material must perform three essential functions.

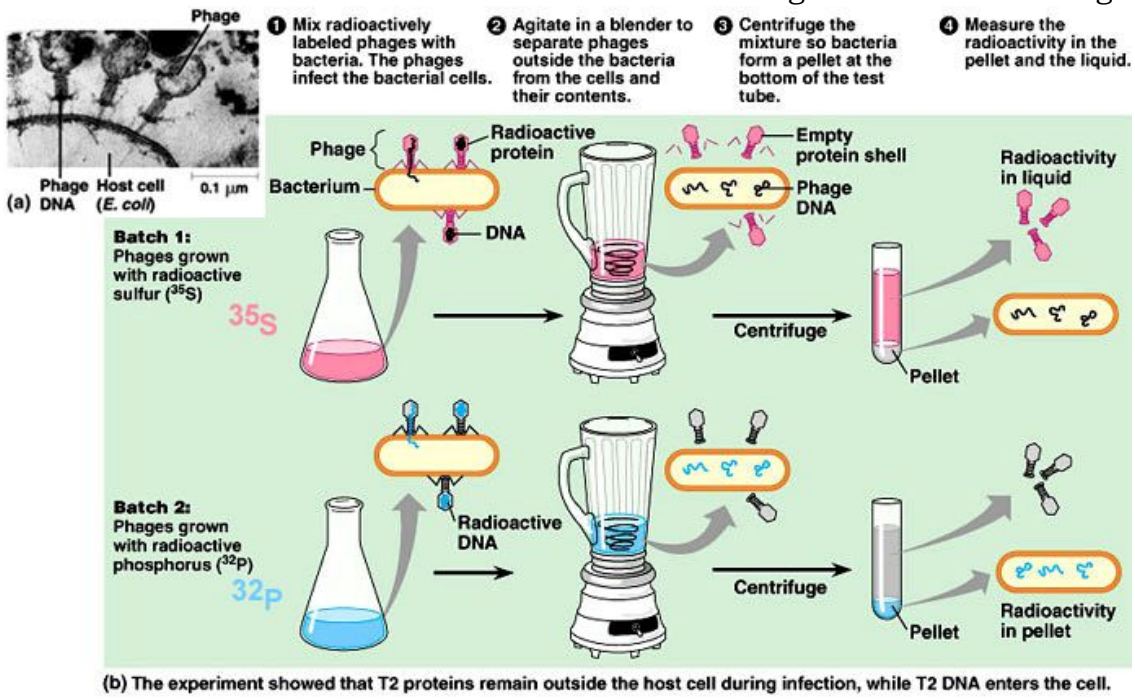
1. Replication – genotypic function
2. Gene expression – phenotypic function
3. Mutation – evolutionary function

Other early genetic studies established a precise correlation between the patterns of transmission of genes and the behaviour of chromosomes during sexual reproduction, providing strong evidence that genes are usually located on chromosomes.

Chromosomes are composed of two types of large organic molecules (macromolecules) called proteins and nucleic acids. The nucleic acids are of two types : Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). During the 1940s and early 1950s, the results of elegant experiments clearly established that the genetic information is stored in nucleic acids, not in proteins. In most organisms, the genetic information is encoded in the structure of DNA. However, in many small viruses, the genetic information is encoded in RNA.

2.4.1.2 Proof that genetic information is stored in DNA

Several lines of indirect evidences suggested that DNA harbors the genetic information of living organisms.



(b) The experiment showed that T2 proteins remain outside the host cell during infection, while T2 DNA enters the cell.

- Most of the Cell's DNA is located in the chromosomes, whereas RNA and proteins are also abundant in cytoplasm.
- A precise correlation exists between the amount of DNA per cell and the no of sets of chromosomes per cell.
- Most somatic cells of diploid organisms contain twice the amount of DNA as the haploid germ cells (gametes) of the same species.

- The molecular composition of the DNA is the same (with rare exceptions) in all the cells of an organism, where as the composition of RNA and proteins is highly variable from one cell type to another.

- DNA is more stable than RNA or proteins.

1) Transformation

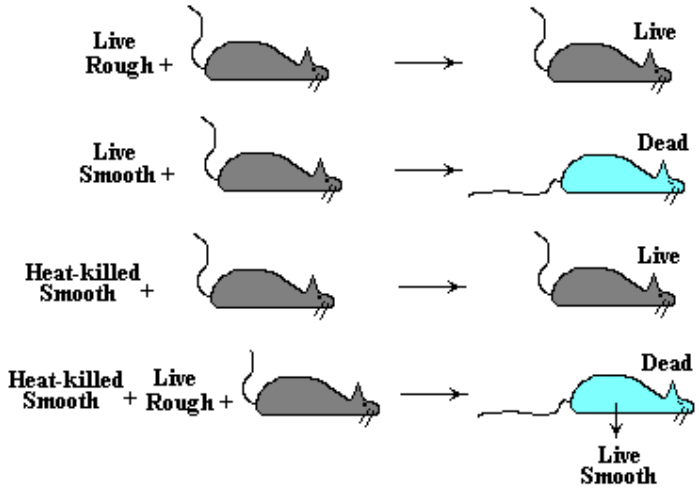
It involves the uptake of naked DNA molecules from one bacterium (the donor) by other bacterium (the recipient). It was discovered by Frederick Griffith in 1928 which laid foundation for the identification of DNA as the genetic material in the bacterium streptococcus pneumoneae.

Griffith's experiment

The wild-type organism is a spherical cell surrounded by mucous coat called capsule. They form large, glistening, smooth 9s) colonies. These cells are virulent, capable of causing lethal infections upon injection into mice.

A certain mutant strain of S.pneumoniae has lost the ability to form a capsule. As a result, it forms small, rough ® colonies and is avirulent.

When virulent strains were injected into mice, they died. The mice was live when both avirulent and treat killed virulent strains were injected independently. However, the mice was died when treat killed virulent strain+avirulent strain is injected. Griffith called this conversion of avirulent strains to virulent strains as trasformation. This transformation was not transient, the ability to make capsule and therefore to kill host cells, once conferred upon the avirulent bacteria, was passed to their descendants as a heritable trait. In otherwords, the gene for virulence, missing in avirulent cells, was somehow restored during transformation. It means that the transforming substance in the heat killed bacteria was probably the gene for virulence itself.



2)DNA as the transforming principle

In 1944, Avery Mc Leod and Madyn Mc Carty showed that the transforming principle was DNA.

- First, they removed the protein from the extract with organic solvents and found that it still transformed.
- Trypsin, chymotrypsin which destroy protein had no effect on transformation. Neither did Ribonuclease which destroy RNA.
- On the otherhand they found that the enzyme Dnase which breaksdown DNA, destroyed the transforming ability of the virulent cell extract.

Finally, direct physico-chemical analysis showed the purified transforming substances to be DNA.

Ultracentrifugation

The material with transforming activity sedimented rapidly suggesting a very high molecular weight, characteristic of DNA.

Electrophoresis

Transforming activity had a relatively high mobility, also characteristic of DNA.

UV-absorption spectrophotometry

Its absorption spectrum matched that of DNA, i.e, maximum absorption at 260nm.

Elementary chemical analysis

This yielded an average Nitrogen / Phosphorous ratio of 1.67, equal to that of DNA which is rich in both elements.

Genetic transformation has been demonstrated in several other genera including Haemophilus,

Bacillus

Salmonella

Steptococeus

Rhizobium

Neisseria and in Higher organisms

Drosophila

Σ phestia insects

Bombyx

Mice and humans cells cultured invitro. Even in these species, all cells in a given population are not capable of active uptake of DNA.

Only competent cells, which possess a so called competence factor, are capable of serving as recipients in transformation. Competance of bacteria is not a permanent feature but occurs only at certain times in life cycle. Competence is commonly observed towards the ends of the `log' phase of growth just before the stationary phase.

There are two theories to explain development of competence.

1. Structure of cell wall is critical. It permits uptake of DNA only during the restricted competence phase and its permeability to macromolecules may change with growth conditions.

2. Competence results from the synthesis of specific receptor sites on the surface of the cell. This view is supported by the fact that synthesis of new proteins is necessary for development of competence. Inhibition of proteins or RNA synthesis inhibits the transformation.

Stages in transformation

1. DNA comes into contact with the bacterial cell surface as a result of random collision. The binding becomes irreversible after a very short period (5-6 seconds).

2. Permanently bound DNA penetrates the bacterium ds DNA is converted into ss DNA by the action of exonuclease. Penetrating DNA must have a minimum length of about 750 base pairs.

3. ssDNA is stabilized by a competence-specific protein. ssDNA migrates from the periphery of the cell to the chromosome DNA.

4. The homologous portion synapses with the recipient chromosome. The unsynapsed DNA is out by means of nuclease action.

5. The transformation heteroduplex undergoes replication to form transformation homoduplexes. One of these is a normal duplex, while the other is transformed duplex. The clone produced from the transformed duplex is the transformed. The normal duplex will give rise to a non-transformed duplex.

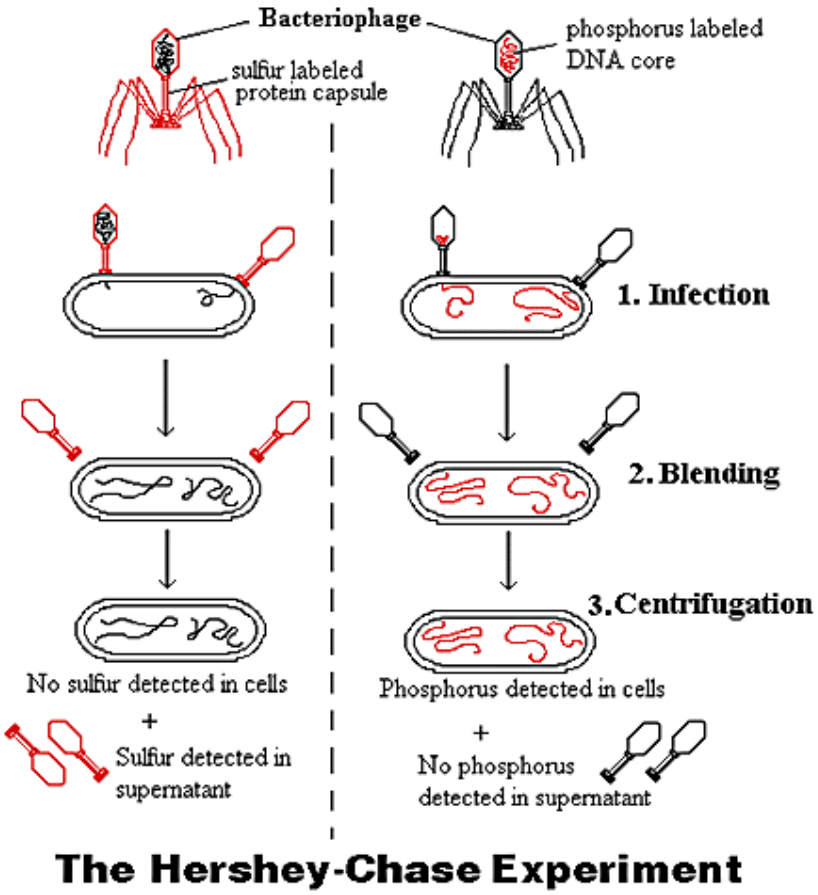
2.4.1.3 Proof that DNA is the Genetic Material in T_2 Bacteriophage

Finally in 1952, A.D. Hershey and Martha chase performed an experiment to prove that DNA was the genetic material. Their experiment involved a bacteriophage, called T_2 that infects the bacterium *E.coli*. During infection, the phage genes enter the host cell and direct the synthesis of new phage particles.

Their experiment depended on radioactive labels on the DNA and protein, a different label for each. ^{32}p for DNA, ^{35}s for protein, DNA is rich in phosphorous while phage protein has none, and protein contains sulfur but DNA does not.

Hershey and chase allowed the labeled phages to attach by their tails to bacteria and inject their genes into their hosts. Then they removed the empty phage coats by homogenizing in a blender.

Since the phage genes must enter the cell, they reasoned that the type f label found in the infected cell would indicate the nature of the genes. From the above experiment the conclusion was that the genes are made of DNA



2.4.1.4 Deoxyribonucleic acid

Friedrich Miescher, in 1869 had isolated a previously unidentified macromolecular substance, to which he gave the name nuclein. Nuclein was later renamed nucleic acid. Development of DNA-specific staining techniques by Feulgen and Rossenbeck in 1924 enabled Feulgen to demonstrate in 1937 that most of the DNA content of a cell is located in the nucleus.

Nucleic acids are macromolecules present in all living cells, either in free state or in combination with proteins. Nucleic acids are polymers consisting of units called nucleotides. They are hence called polynucleotides.

Nucleotides

These are the compounds consititued by purine or pyrimidine bases, deoxyribose sugars and phosphoricacid.

Importance

1. Purine nucleotides act as the high energy sources: ATP, GTP.
2. Serve as monomeric precurosors of RNA & DNA.
3. Play an important role in carbohydrate, fat, protein metabolism.
4. They also serve as chemical signals Ex: camp, cGMP.
5. Function as components of coenzymes FAD, NAD^+ etc. and an important methyl donor, SAM.
6. also act as high energy intermediates such as UDP-glc & UDP-gal in carbohydrate metabolism and CDP-acyl glycerol in lipid synthesis.

Nitrogenous bases

The bases are derivatives of two parent compounds: purines & pyrimidines. These are weakly basic.

Pyrimidine bases

Pyrimidine bases found in Nucleic acids are mainly three.

- Cytosine – found in both DNA and RNA
- Thymine – found in only DNA
- Uracil - found in only RNA

All the pyrimidine bases can exist in lactam form and lactim form. If the group is –NH-CO- it is called Lactam (keto) type, while the same if isomerizes to –N=C-OH, it is called lactim (enol) type. At the physiological pH, the lactam forms are predominant.

chemically 2-oxy-4-amino pyrimidine

- is found in all nucleicacids except DNA of certain viruses.

Thymine

Chemically, if is 2,4-dioxy – 5- methyl pyrimidine

- Also called as 5-methyl uracil
- Occurs only in DNA, however, minor amounts have recently been found in tRNA.

Uracil

chemically it is 2,4-dioxy pyrimidine

- is confined to RNA only, not found in DNA

Purines

- Purine ring is more complex than pyrimidine
- It can be considered as the product of fusion of a pyrimidine ring with an imidazole ring.
- Adenine & Guanine are the two principal purines
- Found in both DNA & RNA.

Adenine

- chemically it is 6-amino purine

Guanine

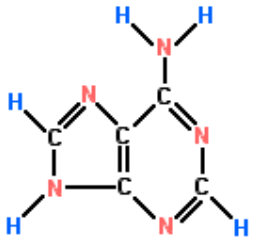
- chemically it is 2-amino- 6-oxy purine

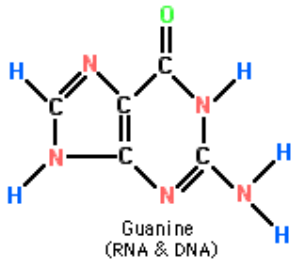
Minor bases in DNA

- 5-methyl cytosine occur in plants
- N^6-methyl adenine in bacterial DNA
- 5-hydroxy methyl cytosine in bacteria infected with certain bacteriophages.

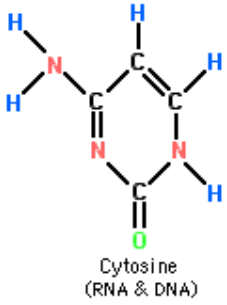
The Nitrogen Bases Occurring in Nucleic Acids:

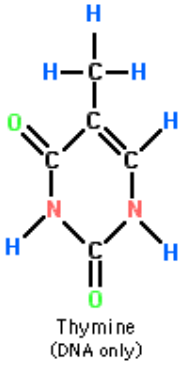
Purines:

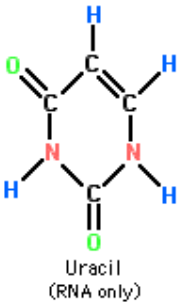




Pyrimidines:







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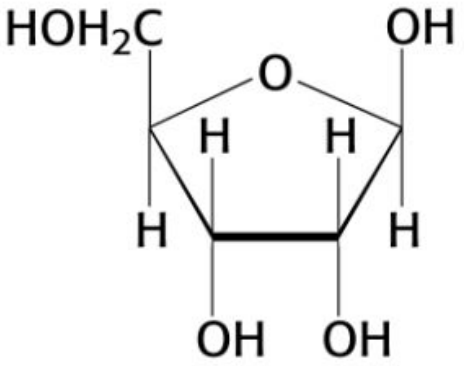
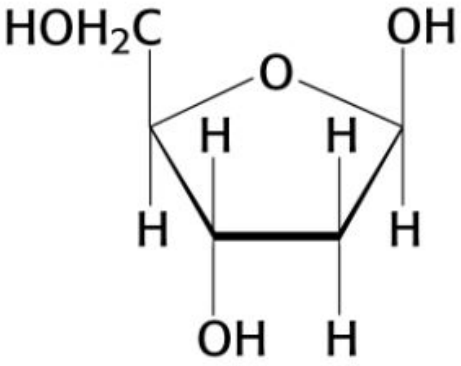
Sugar

2-deoxy B-D-ribose. The sugar is in its furanose form in nucleic acids. An important property of the pentoser is their capacity to form esters with phosphoricacid. The –OH group of the pentose, especially those at C_3 & C_5 are involved forming a 3', 5' – phosphodiester bond.



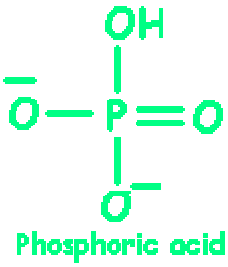
D-Ribose



2-Deoxy-D-ribose

Phosphoric acid

The molecular formula of phosphoric acid is H_3PO_4. It contains 3 monovalent –OH groups and a divalent oxygen atom, all linked to the pentavalent phosphorous atom.



Phosphoric acid

Nucleotides are the phosphoric acid esters of nucleosides. These occur either in the free form or as subunits in Nucleicacids.

Deoxyribonucleotides

Deoxy adenylic acid

Deoxy cytidylic acid

Deoxy thymidylic acid

Deoxy guanylic acid

Nucleosides are composed of a purine or pyrimidine base and a deoxyribose sugar.

The base is joined covalently, at N-1 of pyrimidine and N-9 of purines in an N-glycosyl linkage to the 1 carbon of the pentose.

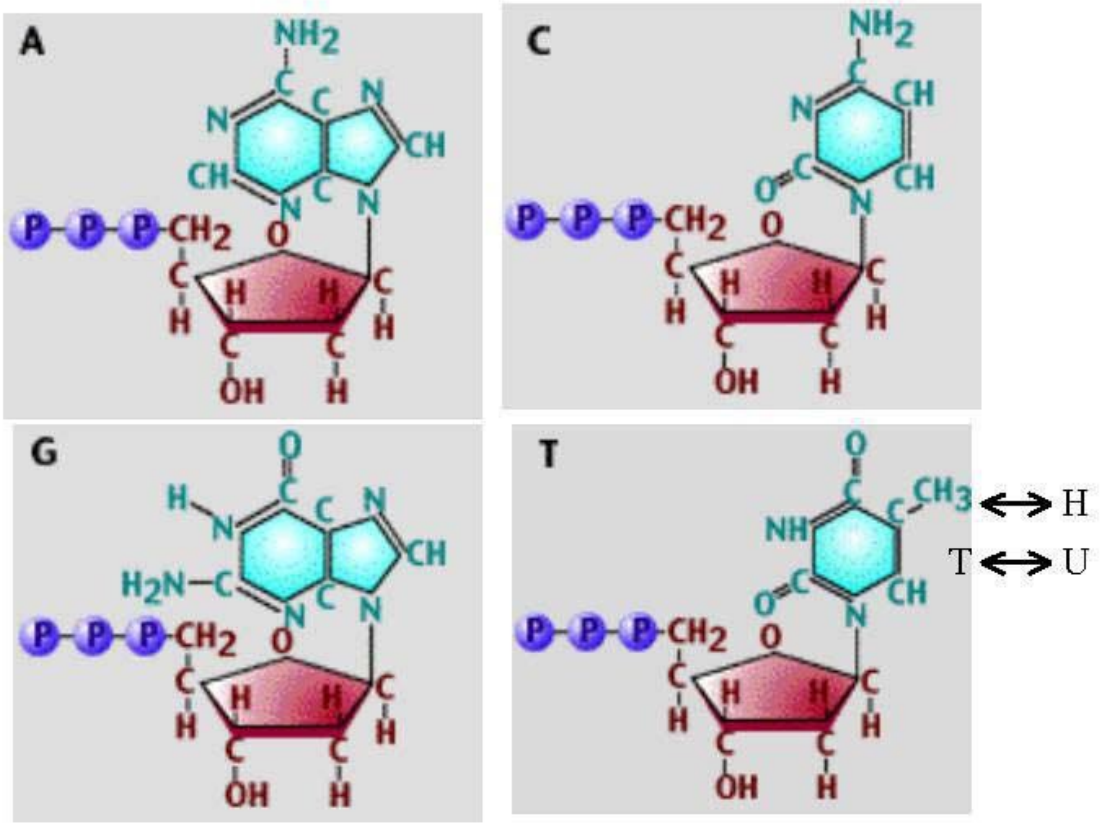
The antiform is necessary for the proper positioning of the complementary purine an dpyrimidine bases in the dsDNA.

Deoxyribonucleosides : Deoxy adenosine

Deoxy Guanosine

Deoxy Cytidine

Deoxy Thymidine



Synthetic derivatives

Synthetic nucleobases, nucleosides, nucleotides are widely used in the medical science & clinical medicine. Changes in heterocyclic ring structure and sugar moiety, induces toxic effects when incorporated into cells and inhibits the activities of enzymes.

- 6-thioguanine
- 6-mercaptopurine
- 4- hydroxy pyrazole pyrimidine

- also called as allopurinol
- inhibitor of xanthine oxidase.

- cytarabine (arabinosyl cytosine) – used in the chemotherapy of cancer & viral infections
- Vidarabine (arabinosyl adenine)
- Azathioprine – useful in organ transplantation

Nucleic acid

A nucleic acid is a polymer of a nucleotide monomer and can be considered as a polynucleotide.

The successive nucleotides in DNA are covalently linked through phosphate groups bridges, specifically the 5'-OH group of one nucleotide unit is joined to the 3'-OH group of the next nucleotide by a phosphodiester bond. Thus the back bone of nucleic acids consist of alternating phosphate and pentose residues, and the characteristic bases may be regarded as side groups joined to the backbone at regular intervals.

Each linear nucleicacid has a specific polarity and distinct 5' and 3' ends. The 5^1 end lacks a nucleotide at 5^1 position and the 3^1 end lacks a nucleotide at 3^1 position.

The back bone of phosphate and sugar is hydrophilic, where as the bases is hydrophobic.

Structure

DNA structure contains hierarchial levels of complexity.

1. Primary structure → covalent structure of nucleotides forming a linear chain.
2. Secondary structure → any regular, stable structure taken up by some or all of the nucleotides.
3. Tertiary structure → The complex folding of large chromosomes with in the bacterial nucleoid & eukaryotic chromatin.

A most important due to the structure of DNA came from the work of Erwin Chargraff and his colleagues in the late 1940s. They concluded that:

- the base composition of DNA generally varies from one species to another.
- DNA specimens isolated from different tissues of the same species have the same base composition.

- The base composition of DNA in a given species does not change with the organism's age, nutritional state or changing environment.

- In all DNAs, regardless, of species, the no. of A residues is equal to the no. of T residues and the no. of G is equal to the no. of C residues, i.e., the no. of purines = the no. of pyrimidines. (A+G = T+C). This is sometimes referred as Chargraff's rule and is the key for establishing 3-dimensional structure of DNA.

Secondary structure / double helical structure

Evolution

- W.T. Astbury was the first person to propose the 3-D-structure of DNA. By his X-ray crystallography study on DNA, he concluded that, because DNA has a high density, its polynucleotide was a stack of flat nucleotides, each of which was oriented perpendicularly to the long axis of the molecule & was placed 3-4 A" apart from each other.

- Continued crystallography studies by Wilkins & R. Franklin confirmed Astbury's 3-4 A^o internucleotide distance and suggested a helical configuration for DNA molecule. They also suggest that, the helix is folded into many turns and each turn causes a vertical rise of 34 A^o.

- Analytical studies also suggests that the polynucleotide chains were held together by H-bonding between the base residues.

Watson and Crick model of DNA

- In 1953, Watson & Crick postulated a three-dimensional model of DNA structure from the all available data.

- It consists of two helical DNA chains coiled around the same axis to form a right handed helix.

- They hydrophilic backbones of alternating deoxyribose & -vely charged phosphates are on the outside of the double helix, facing the surrounding water.

- The purine & pyrimidine bases of both strands are stacked inside the double helix with their hydrophobic & nearly planar ring structure very close together and as perpendicular to the long axis.

- The spatial relationship between these two strands creates a major groove and a minor groove between the two strands.

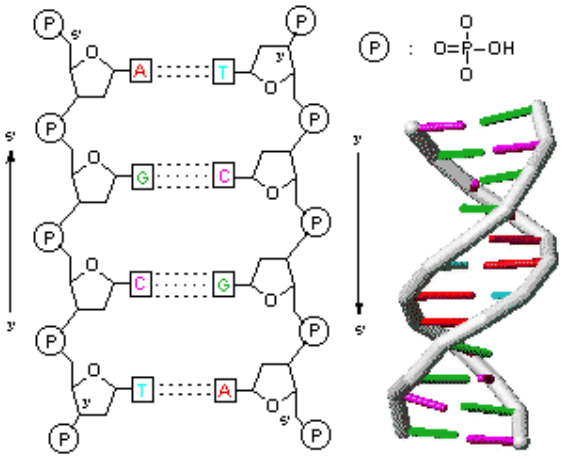
- The diameter of the helix is 20 A^o, the bases are 3.4 A^o apart along the helix axis. Each turn of the helix contains 10 nucleotides residues. Therefore the helical structure repeats at intervals of 34 A^o.

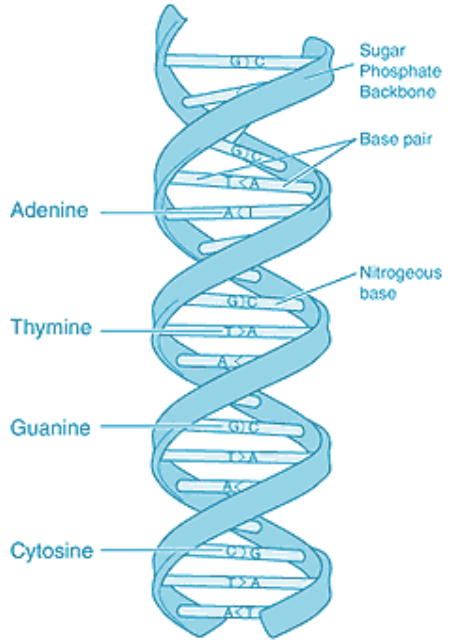
- The two chains are held together by H-bonds between pairs of bases. Adenine always pairs with thymine by two H-bonds and guanine always pairs with cytosine by three

- The two chains or strands of the helix are antiparallel, Their 5^1, 3^1-phosphodiester bonds run in opposite directions.

- The two strands are complementary to each other. Whereever adenine appears in one strand, T is found in the other; Similarly wherever G is found in one chain, C is found in the other.

-





Different structural forms of DNA

DNA is remarkably flexible molecule. The Watson-Crick structure is also referred as B-form; which is the more stable, right handed DNA molecule. Many significant derivations from the DNA structure are found in cellular DNA, and some (or) all of these may play an important role in DNA metabolism.

A-form

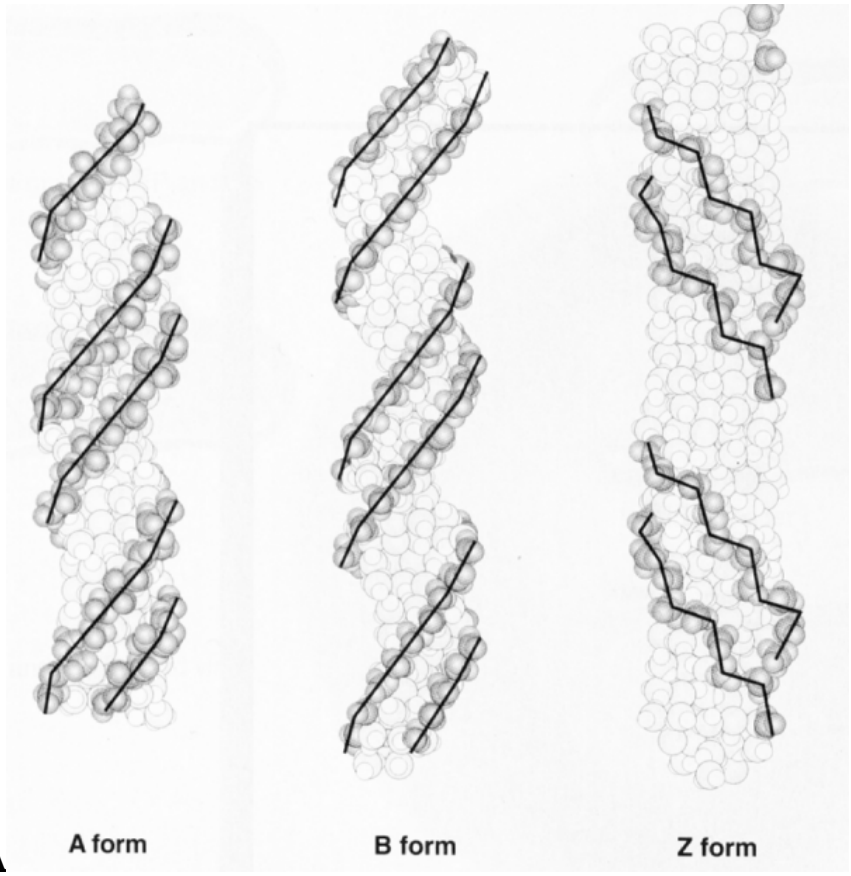
- it is a right handed helix
- it is favoured in many solutions that are relatively devoid of water.
- No. of base pairs per helical turn is 11.
- Rise per basepair is 2.3 A^o
- Is shorter and have a greater diameter
- The reagents used to promote crystallization of DNA tend to dehydrate it, and this leads to a tendancy for many DNAs to crystallize in the A-form.

Z-form

- left-handed double helix

- 12-bp per helical turn
- rise per basepair is 3.8 A^o
- DNA back bone takes zig-zag appearance.
- Sequences in which pyrimidine alternating with parine will give Z-forms.
- There is evidence for short stretches of Z-DNA both in prokaryotes & eu-karyotes.
- These Z-DNA tracts may play an undefined role in the regulation of gene expression or genetic recombination.

-



H-DNA

- triple helical DNA
- unusual structure
- occur in polypyrimidine / polypurine tracts
- pairing and interwinding of three strands.

- They form spontaneously only within long sequences containing only pyrimidines or only purines in one strand.

- Two of the 3 strands inn the H-DNA triple helix contain pyrimidines and the third contains purines.

- Found within regions involved in the regulation of expression of a number genes in eukaryotes.

Tertiary structure

The DNA double helix can undergo coiling about its own axes to produce a supercoiled tertiary structure. Thus DNA can exist in forms other than a linear molecule. In bacterial, viral replicative forms, plasmids, mitochondrial, and chloroplast DNA, the ends of the DNA molecules are covalently joined to form a closed, circular duplex molecule. In the much larger eukaryote chromosomes, supercoiling arises when the DNA coils around histones.

The terms supercoiling, superhelicity and supertwisting are employed for the twisting of DNA duplex upon itself. This property of DNA is an integral feature of all chromosomes, whether circular or linear. It has been shown to be essential for the stages of replication, transcription and recombination.

2.4.1.5 Summary

Chromosomes are composed of two types of large organic molecules (macromolecules) called proteins and nucleicacids. In 1940,s there are several experiments were conducted by different groups of scientists that proves the genetic information is stored in DNA not in proteins.the DNA contains deoxyribose sugar,phosphate and nitrogenbases such as adenine ,Guanine ,thymine and Cytosine.The structure of DNA was proposed by Watson and crick i.e double helical model.the DNA helix can undergo coiling about its own axes to produce a supercoiled tertiary structure

2.4.1.6 Model Questions

1)write in detail about s the components of nucleic acids

2)Prov the DNA as genetic material

2.4.1.7 Reference books

Freifelder, David., Physical Biochemistry, W.H.freeman & company

Griffiths, Anthony JF. , Wessler, Susan R. , Lewontin, Richard C. , Gelbart William M., Suzuki, David T. , Miller, Jeffrey H. *An Introduction to Genetic Analysis* 8/e, W.H. Freeman

Lewin B., Genes, Oxford University Press, Newyork

Author :

Dr.N.Srinivasa Reddy
Centre for Biotechnology
Acharya Nagarjuna University
Guntur

Lesson 2.4.2

REPLICATION

Objective

2.4.2.1 Introduction

2.4.2.2 Replication models

1) Semi-conservative model
2) Conservative model
3) Dispersive model
4) Meselson & Stahl experiment
5) Replication Origin
6)Replication fork
7)Replication Direction

2.4.4.3 Prokaryotic Replication

1) Enzymology of Replication

2**) Replication of E.Coli Chromosome**

1) Initiation
2) Elongation
3). Termination

2.4.4.4 Replication Mechanism of Bacteriophage M 13

1)Replication Mechanism of 17 A

2.4.4.5 Fidelity of Replication

2.4.4.6 Eukaryotic Replication

1) Enzymology of Eukaryotic Replication
2) Mechanism of Replication
3) Eukaryotic Chromosome Origin
4) Mitochondrial DNA Replication
5) Termination

2.4.4.7 Inhibitors of DNA Replication

2.4.4.8 DNA Repair

1) Direct Reversal of the Damage
2) Excision Repair

2.4.4.9 Summary

2.4.4.10 Model Questions

2.4.4.11 Reference books

RNA : Ribonucleic acid
DNA : Deoxyribonucleic acid
dsDNA : double stranded DNA
Ss DNA : single stranded DNA
Nt : nucleotide
Bp : base pair
Ori : origin

OBJECTIVE

During the synthetic phase of cell division the content of DNA increases by a process known as replication. In this chapter the process of replication ,types and inhibitors were clearly explained. Different DNA repair mechanisms were also discussed.

2.4.4.1 INTRODUCTION

The ability to reproduce is one of the most fundamental properties of all living organisms. This duplication is observed at various levels:

Organisms duplicate by sexual / asexual methods

Cells duplicate by cellular division

Genetic material duplicates by Replication.

The capacity of duplication is thought to be the first critical properties to have appeared on the path toward evolution.

2.4.4.2 Replication Models

The formulation of DNA structure by WATSON & CRICK in 1953 accompanied the proposal for its self duplication. (Watson and Crick envisioned that gradual separation of the helix by the successive breakage of H-bonds is possible and as the two strands are complementary and follow strict base pairing

rules: each strand contains information for the synthesis of other. Thus once strands are separated, each can act as template to direct the assembly if nucleotides).

1. Semi-conservative Model

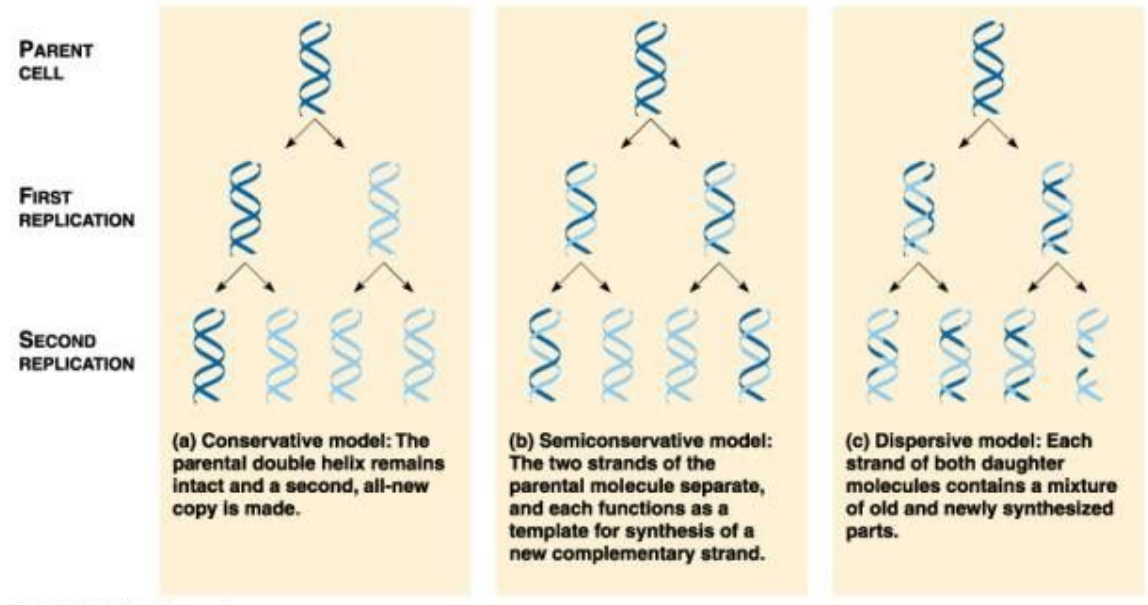
Watson and Crick proposed several predictions concerning the behavior of DNA and, most important is the physical separation of the two strands. According to Watson and Crick, each of the daughter duplexes should be composed by one parent strand and one new strand.

2. Conservative Model

The two original strands would remain together and two newly synthesized strands remain together. One daughter cell contains fully conserved duplex, while the other cell contains newly synthesized duplex.

3. Dispersive Model

The integrity of the each of parental strands would become disrupted, as a result, the daughter cell contains duplexes in which each strand is composite of old and new DNA i.e., neither the strands nor the duplex itself is conserved.



To decide the possibilities among the three models, it is necessary to distinguish newly synthesized DNA from the original DNA that served as template. This was first accomplished in studies on bacteria in 1958, by Matthew Meselson, Franklin Stahl of California Institute of Technology.

4. Melson and Stahl's Experiment

-Meselson and Stahl grew bacteria (E.Coli) for many generations in media containing N^{15} – NH_4Cl as sole source of nitrogen until all the cellular DNA contained the isotope.

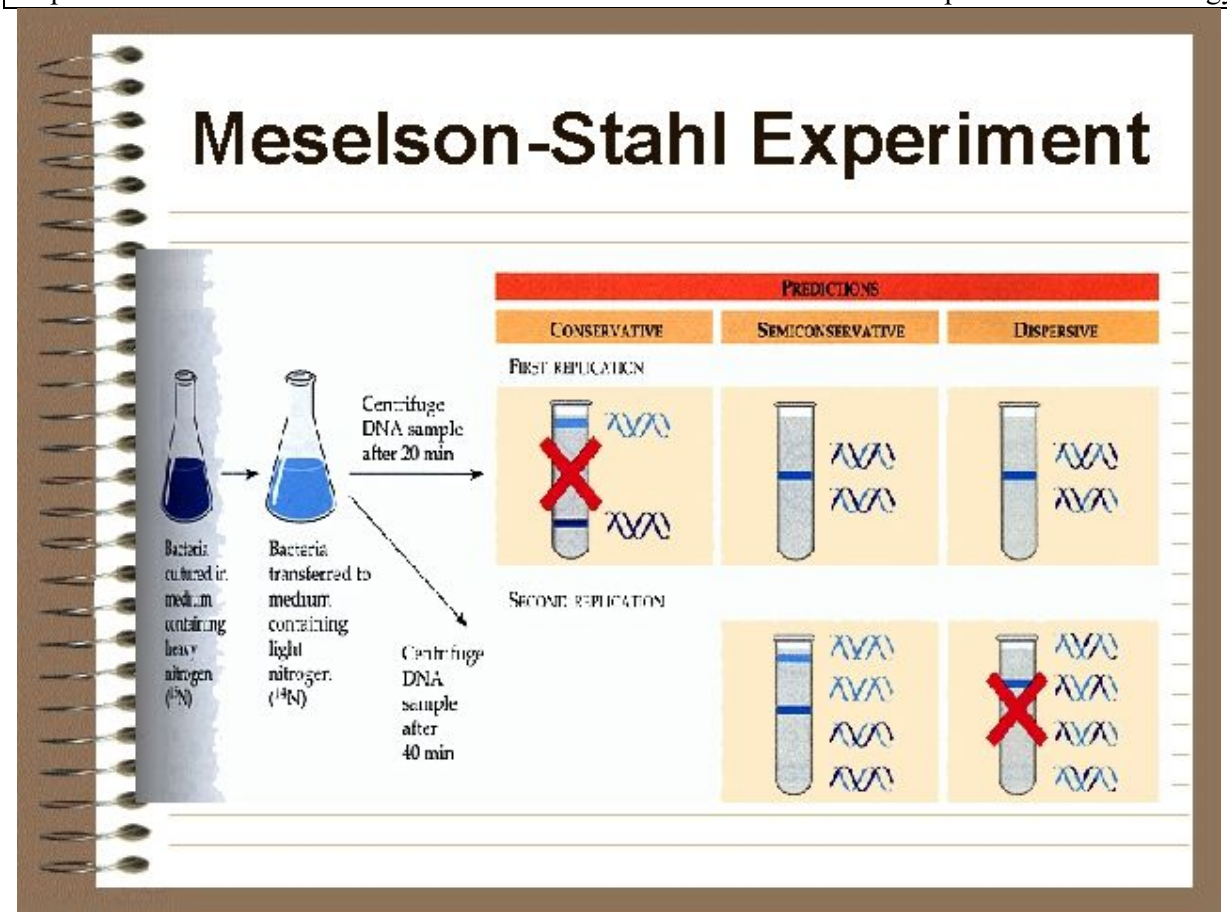
- The cells were then transferred to a medium containing the normal `light' isotope N^{14} and samples were removed periodically from the cultures.

- The DNA in each sample was analysed by density-gradient equilibrium centrifugation which can separate heavy-heavy, (N^{15}-N^{15}) , light-light (N^{14}-N^{14}), heavy-light (N^{15}-N^{14}) duplexes into distinct bands.

- The results showed that replication occurs by semi-conservative model.

DNA extracted & centrifuged

To equilibrium inn Cscl Density gradient



Semi-conservative replication of chromosomes can also be visualized through an examination of chromosomes that are allowed two rounds of replication in a medium containing Bromodeoxy uridine. These duplicated chromosomes are then stained with a fluorescent dye and Giemsa stain to produce harlequin chromosomes. The newly synthesized DNA stains differently than the parental DNA.

5. Replication Origin

Replication begins at a specific site on the chromosomes called ORIGIN. A replication origin is analogous to a promoter of transcription in that – both regulatory sequences acts as biding sites for sequence specific DNA-binding proteins that initiate DNA or RNA synthesis at a specific site along the template.

John Cairns, in the early 1960s, developed an autoradiographic technique, to visualize the semi-conservative model. In this process, bacteria are lysed very

gently and their chromosomes spread out on a surface without any further manipulation. If the bacteria had been growing on the H^3 thymidine prior to the preparation of the chromosomes, then the light microscopic autoradiogrphy could reveal the outline picture of the labeled DNA.

Autoradiographs of this type confirmed circular nature of the bacterial chromosome and revealed the overall process of replication. In many cases the outlines of the silver grains formed a structure resembled θ - which is expected to be caught during replication of a circular duplex chromosome.

Each θ structure is composed of three distinct lengths of DNA – two newly synthesizing daughter strands and one unreplicated portion of the parent duplex.

6. Replication Fork

The point at which the pair of replicated segments come together is called the replication fork. Each replication fork corresponds to a site where the parental double helix is undergoing strand separation, and the nucleotides are being added into the new strands. The replication fork marks the advance of DNA synthesis within a replication intermediate a duplex chromosome.

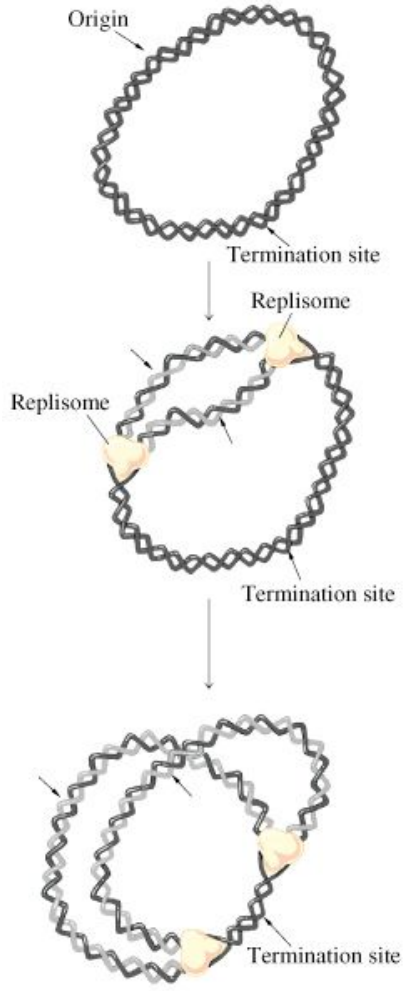
Common intermediates include:

θ Structures

Resulting in replication in

A specific region of a circular

Chromosome.



D-loop Structures

Resulting from the synthesis of

One daughter strand in a circular

Or linear chromosome with synthesis

Initially in one direction on one

Template strand.

Bubble or Y Structures

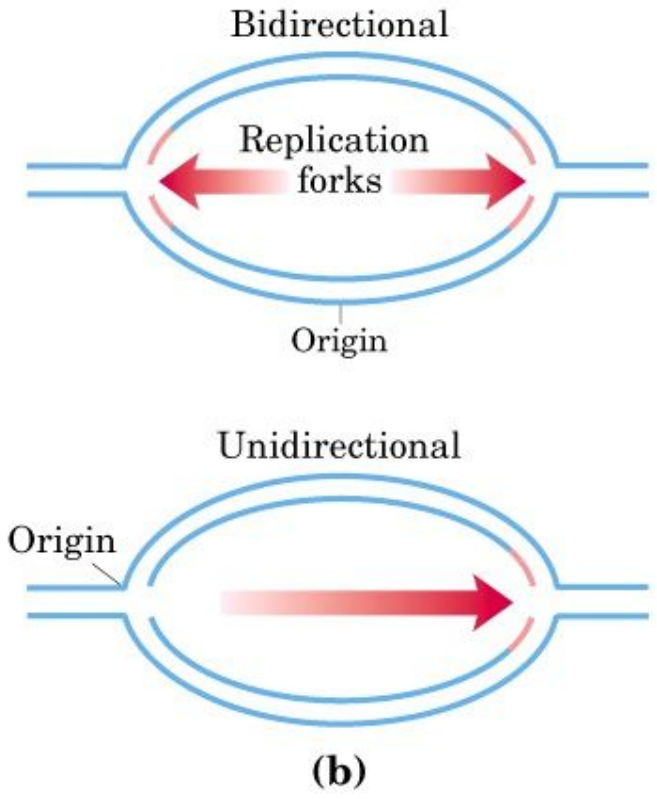
Resulting from initiation of on a linear chromosome.

Lariat forms

Resulting from covalent extension of a parental strand in a circular chromosome.

7. Replication Direction

DNA replication can be unidirectional or bi-directional, depending upon whether the replication from the point of origin proceeds only in one direction or proceeds in both the directions. In unidirectional replication, one of the two replication forks will be stationary and the other moves with replication. In bi-directional replication, none of the two replication forks will be stationary and both will be moving.



Autoradiographic experiment

If cultured mammalian cells undergoing replication are exposed first to high concentration and then to low concentration of 3_H thymidine, the resulting DNA

will be heavily labeled (hot) near replication origins (ORI) and lightly labeled farther away. When such labeled DNA is dried on a microscopic slide as long fibers and their exposure to radiation – sensitive enulsion, autoradiographs should be produced corresponding to hot-warm regions. Such autoradiographs from cultured mammalian cells show the replication to be a bi-directional.

2.4.4.3 Prokaryotic Replication

1. Enzymology of Replication

DNA Polymerase I

- It was the first polymerase recognized
- It was discovered by Arthur Kornberg
- It was the most extensively studied DNA polymerase
- A cell contains 400 polymerase I molecules.
- The enzyme is a single chain of 928 aminoacid residues
- Molecular weight is 103 Kda.
- It is a globular protein, with a diameter nearly of $65^{o}A$
- The enzyme is readily cleaved by proteases to
- A small 35Kda C-terminal fragment with 5^{1} -> 3^{1} exonuclease activity
- A large 68Kda C-terminal fragment with polymerase and 3'->5^{1} exonuclease activity. This fragment is called as klenow fragment.
- The active site of the enzyme contains binding sites for the template chain, growing chain (the primer), dNTPs, and divalent metal ion.

Binding of Mg^{2+} - dNTP, in the dNTP binding pocket of polymerase I and nucleophilic attack by the 3'-OH group of the growing chain or primer on the α phosphorous of the incoming dNTP.

- The enzyme is inactive on complete ds DNA, ss DNA which are covalently linked and nicks with 5'-OH and 3'-P, but are active at nicks with 3'-OH and

5'-P. The enzyme is able to bind at nicks with 5'-OH & 3'-P but is unable to polymerize.

- 3^1->5^1 exonuclease activity: In polymer, chains with correctly paired 3'-OH ends and in the absence of dNTPs required for polymerization, the primer terminus will be exposed to repeated nuclease actions, which can be inhibited by a correct dNTP. This activity is called proof reading.

- 5^1 ->3^1 exonuclease activity :

    - Cleaves a diester bond only at a base paired region.
    - Can excise oligonucleotides upto 10 residues long from the 5' end.
    - It has endonucleolytic activity, that it cleaves diester bonds as distant as 8 from the 5' end or even further.
    - Or the excision enzyme and polymerizing enzyme are present together, the gap formed by excision is filled immediately without giving a chance for endonucleases.
    - Removes RNA primer from 5' and of a DNA chain.

- Polymerase I turnover is 600 nucleotides / min/enzyme at 37^oc.

DNA Polymerase II

- The identification of mutants lacking polymerase I by Paula De Lucia and John Cairns in 1969 lead to the discovery of polymerase II and polymerase III enzymes.

- Pol II was isolated by T.Kornberg and Gefter in 1970.

- The presence of Pol II & Pol III was masked by the high level of activity of Pol I.

- Molecular Weight : 90Kda

- Unlike Pol I unable to use a template-primer that is simply nicked.

- Laks the 5'->3' exonuclease activity

- Strains lacking the gene for this enzyme show no replicational or growth defects.

DNA Polymerase III

- isolated by T.Kornberg & Gefter
- it is the most complex of the DNA polymerases
- pol III holoenzyme is the principal replicative enzyme of E.Coli
- only 10 molecules / cell are present
- it has a collective mass of 900KD, with 20 or more polypeptides.
- It dissociates readily upon dilution into a variety of subassemblies.
- The core pol III, while effective in filling gaps in DNA, incapable of rapidly replicating ss viral circles that are several thousand residues.
- However, a novel activity was observed in E.Coli extracts which was highly efficient in replicating long stretches of template.
- This proved upon purification to be pol III complexed with many auxillary subunits that clamp the core to the template and endow it with high processivity.
- The β - subunit is the most important processivity factor for subassemblies of pol III holoenzyme. Although, it is not demonstrably a DNA- binding protein, β can become tightly attached to a template – primer to form a preinitiation complex.
- Pol III holoenzyme lacks 5'->3' exonuclease activity.

Primase

- One of the basic rules of replication is that a DNA pol cannot start a chain and must rely on a priming device.
- So, every initiation event requires a primer.
- The opposite polarities of the two strands of DNA duplex and the exceusively 5^1->3^1 polymerization by DNA pol necessitates a semi-discontinuous mechanism of replication.

- Two different modes of priming operates for continuous (leading) and discontinuous (lagging) strands.
- The continuous strand needs to be primed only ones, usually at or near a chromosomal origin.
- The discontinuous strand must be primed repeatedly to generate the short nascent strand (1000-2000 nt long)
- The enzyme is a single polypeptide of 60Kda.
- About 50-100 copies / cell
- E. Coli primase acts alone, most commonly it teams up with the multifunctional Dna B protein in the synthesis of primers to start DNA chains.
- Primase – Dna B complex on a template requires additional prepriming proteins Pri A, Pri B, Pri C.
- The resulting mobile protein complexes track progressively along the DNA template and are called primosomes.

Pri A

Pri B + Dna B ⟶ Preprimosome ⟶ Primosome

Pri C

Helicase

- It couples the energy of NTP hydrolysis for melting the hydrogen bonds and dissipating the other forces that hold together the strand of the DNA duplex.
- The helicase activity of DNA B protein is central to replication in E.Coli.
- Dna B, a hexamer of 50Kda subunits, activate priming by primase and melts the DNA strands at replication fork.
- As a fuel for DNA melting, ATP is preferred.
- E.Coli SSB & primase together stimulate the helicase approximately 6 folds.
- Helicase binds templates very poorely and for proper binding requires a large number of proteins. This is overcome by the origin – in initiator proteins.

- At origin, the Dna A and Dna C proteins efficiently load Dna B protein at a ratio of 2-4 molecules per template.

- The Dna B and Dna C proteins fro a 6:6 complex that activates Dna B protein for entry into the Ori C-Dna A protein comples.

- The distance between polymerase and helicase on DNA appears small, indicating that melting is what limits the rate of DNA synthesis.

SSBPs

- Single stranded DNA binding proteins

- They impart regular structure to DNA single strands, a structure required for the action of a variety of enzyme of replication.

- Had strong preference for DNA than RNA and for ssDNA than for dsDNA.

- Binds tightly and cooperatively

- A cell contains 300 copies

- The protein is a tetramer of 18.9 Kda subunits.

- A polynucleotide, atleast 4-6 residues long is required for binding.

- This protein contributes to opening and unwinding of a duplex at an origin of replication in a supercoiled DNA.

- They direct priming of DNA synthesis to specific origins by covering single strand regions.

- Sustains unwinding of duplex DNA by helicase actions at replication forks and loci of repairs.

- Inhibits both exo and endonucleolytic activities of nucleases.

Ligase

- By 1967, models for recombination, repair & replication predicted an enzyme, that could reseal the breaks of DNA backbone.

- DNA ligases – phosphodiester bond in DNA polymers

- less effective in joining ssDNA

- more active with DNA than with RNA

- it is a 75 Kda polypeptide

- 300 molecules per cell, a value close to that for DNA pol I, inview of their closely related functions in filling gaps and resealing segments of DNA.

- Ligases from E. Coli & B. Subtilis use NAD^+ as coenzyme and as the energy source for the synthesis of the phosphodiester bond. In eukaryotes coenzyme is ATP.

- Ligases utilize the group transfer potential of the phospho-anhydride bonds of NAD^+ or ATP to form a phosphodiester bond between nucleic acid chains.

The reaction occurs in 3 steps.

- The formation of an enzyme – nucleotide intermediate by transfer of the adenylyl group of NAD / ATP to the E-NH_2 of a Lysine residue in the enzyme. Product is NMN or Ppi.

- Adenylyl activation of the 5'-P terminus of DNA by transfer of the adenylyl group from the enzyme.

- Phosphodiester bond formation by attack of the 3'-OH terminus of the DNA on the activated 5'-P group with release of AMP.

- The joining reaction is inhibited by the addition of coenzymes NAD^+ or ATP.

- The enzyme cannot link ss nicks and blunt ends. It joins only nicks with 3'-OH and 5'-P of DNA and 5'-P of DNA to 3'-OH of RNA, but not the reverse.

- **Topoisomerases**

- Topoisomerases catalyse the interconversion of topological isomers (topoisomers) of a DNA molecule.

- By introducing a transient break in the phosphodiester backbone, through formation of a covalent protein – DNA intermediate, the enzyme allows the DNA strands to pass through one another there by changing the topological state of the DNA molecule.

- Other DNA characteristics are not affected.
- The linking number may be changed by the introduction or relaxation of superhelical turns.

Topoisomerase I

- is a single polypeptide of about 100 Kda.
- The relaxing activity of topoisomerase I has several features.
  - ➔ negative supercoil turns are removed but no other change in the DNA occurs.
  - ➔ Relaxation of the DNA occurs gradually
  - ➔ A covalent protein – DNA intermediate conserves energy of the nicked phosphodiester bond for concerted resealing.
- The enzyme reacts with ss circles to introduce topological knots.
- It is also involved in the formation of covalently closed duplex circles from complementary single stranded ones.
- It also catenates and decatenates duplex DNA circles, provided that atleast one of the molecule contain a nick or gap in one strand.

Topoisomerase II or DNA Gyrase

- Gyrase converts relaxed, closed circular duplex DNA to the negatively superhelical form.
- ATP is required and is hydrolysed in the reaction.
- It is a tetramer (dimer of dimers) A_2B_2 and has a molecular weight of 400 Kda.
- The enzyme catalyses.
  - negative supercoiling of circular DNA.
  - Relaxation of negatively supercoiled DNA in the absence of ATP.

- Double strand breakage of DNA.
- ATP hydrolysis to ADP + Pi in the presence of duplex DNA.
- Positive wraping of DNA around Gyrase.
- Catenation and decatenation of duplex DNA.

Topoisomerase II is necessary to maintain a negatively supercoiled template for the assembly of the initiation complex and opening of the duplex at the origin, to provide swivel for the progress of replication fork and to decatenate and superc oil the daughter molecules during the terminal stages.

2) Replication of E. Coli Chromosome

The synthesis of a DNA molecule can be divided into 3 stages.

1. Initiation
2. Elongation
3. Termination.

1. Initiation

E.Coli replication origin, ori C consists of ---- 245 basepairs, many of which are highly conserved among bacteria. The key sequence include 2 series of short repeats.

- 3 repeats of a 13 bp sequence.
- 4 repeats of a 9 bp sequence.

The key enzyme in the initiation process is the Dna A protein. Dna A protein binds to the four 9 mers in oric, forming an initial complex that contains 10-20 protein subunits.

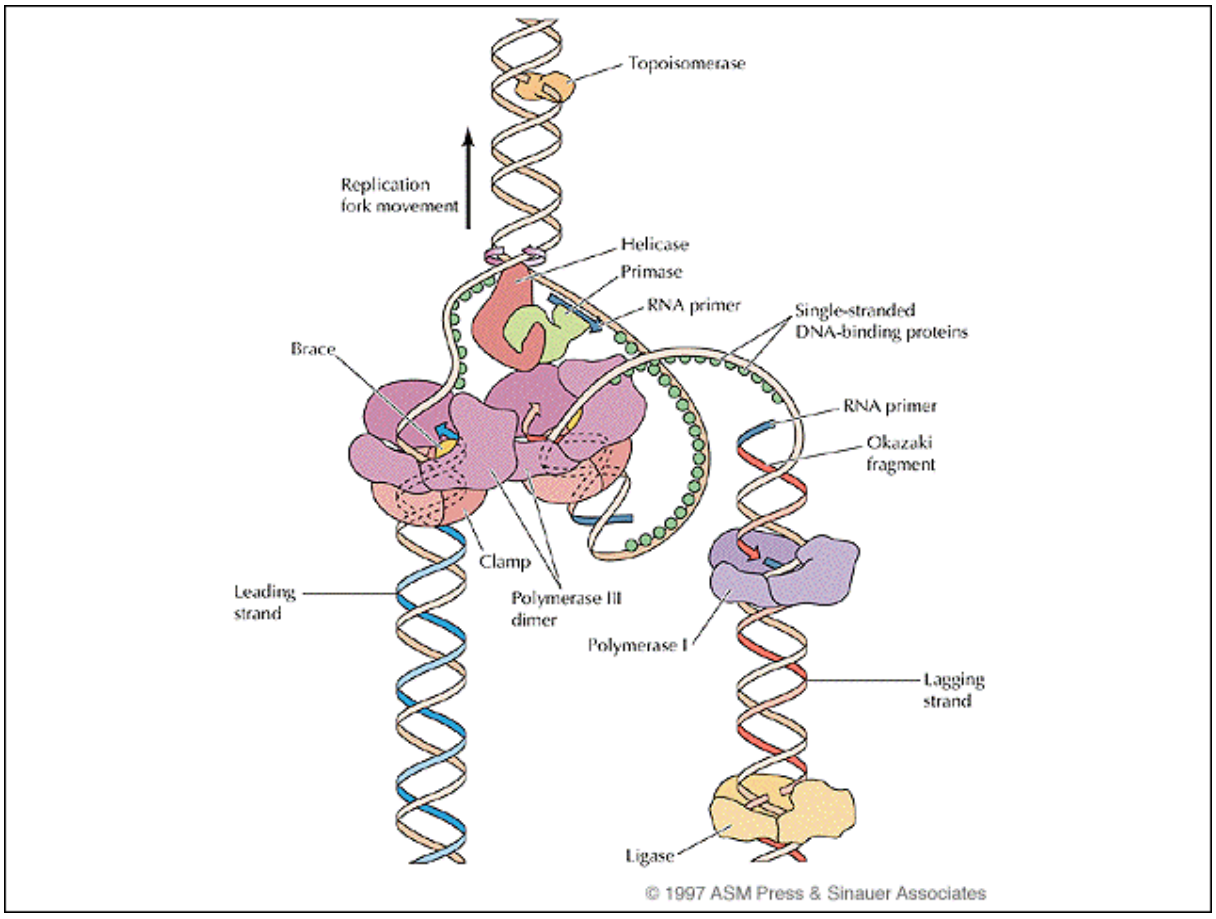
Although Dna A can bind to E. Coli Ori DNA in the relaxed state, it can initiate replication only in the negatively supercoiled DNA. This specificity is that, DNA molecules with negative supercoils are tightly wound and are easily melted, thus providing single strand templates.

Binding of Dna A protein to the Oric 9-mers facilitates the initial strand separation or melting of duplex DNA, which occurs at the Ori C 13-mers. This process requires ATP and yields, the socalled `open complex'.

The Dna B protein then binds to this region and unwinds the DNA bidirectionally and requires the presence of Dna C proteins and creates two replication forks.

Multiple molecules of SSBP bind cooperatively to ssDNA and stabilizes the separated DNA strands and prevents renaturation.

Gyrase relieves the topological stress created by the Dna B helicase action.



Regulation

DNA replication is precisely regulated so that it occurs only ones in each cell cycle. The Dna A protein hydrolyses its tightly bound ATP slowly (about 1 hr) to form an inactive Dna A –ADP complex. Reactivating this complex (replacing ADP with ATP) is facilitated by interaction with acidic phospholipids in the bacterial plasma membrane.

Initiation at inappropriate time is prevented by the presence of the inactive Dna A – ADP complex, by the binding of a protein called Ici A (Inhibitor of Chromosomal Initiation) to the 13-mer repeats.

Elongation

The elongation phase of replication consists of two seemingly similar operations that are mechanistically quiet different i.e, leading & lagging strand synthesis.

Several enzymes at the replication fork are important for the synthesis of both strands.

- DNA helicases unwind the parental duplex.
- DNA topoisomerases relieve the topological stress induced by helicase.
- SSBPs stabilize the separated strands.
- In other respects, synthesis of DNA is two strands is sharply different.

Continuous / Leading strand synthesis

- Begins with synthesis of short RNA primers (10-60nt) at the replication ori.
- Deoxy NTPs are then added to this primer by DNA pol III holoenzyme.
- Ones began leading strand synthesis proceeds continuously keeping pace with replication fork.

Discontinuous / Lagging strand synthesis

Synthesis must be accomplished in short fragments, called okazaki fragments after their discoverer Reiji Okazaki. Synthesis occurs in direction opposite to the fork movement. Each fragment must have its own RNA primer, synthesized by primase and its positioning must be controlled.

- The regulatory apparatus for lagging strand synthesis is called primosome.

- Primosome moves along the lagging strand template in 5'->3' direction.

- Primosome at intervals compels primase to synthesize a short RNA primer to which DNA is then added by the DNA pol III holoenzyme.

- When the new okazaki fragment reaches the old primers, then DNA pol I removes that primer by its 5'-3' exounceclease activity and is replaced with dNTPs by the same pol V.

- The nick left was sealed by Ligase.

In E. Coli synthesis of the leading and lagging strand synthesis may be actually coupled. This can be accomplished by looping of the lagging strand, so that synthesis can be carried concurrently on both strands by a single pol III dimeric enzyme. As the okazaki fragment synthesis proceeds, the loop between pol III and replication fork increases.

Discoverty of okazaki fragments

- The most recently synthesized or nascent / DNA molecular in preparations of T_4 phage are short pieces.

- They sediment at about 85-105s in a gradient of alkaline sucrose, representing chain lengths of 1000-2000 nt.

- To capture, T_4 replication pieces before they join the main body of growing chains, it is necessary to

    - reduce the replication rate by lowering the growth temperature (Ex: to 8^oc).

- Then use a brief pulse of a DNA precursor of high radio-activity that enters DNA rapidly and directly. Quench the pulse efficiently. Under these conditions, much of the precursor label is captured as small pieces.

- After a prolonged pulse or after subsequent exposure to large concentration of unlabeled precursors (chase), the radioactive precursor is found exclusively in high molecular weight DNA.

- Mutant E. Coli deficient in ligase or in pol I or in both accumulate large amounts of E. Coli replication fragments.

- It seems likely that, in cell systems, initiations are limited to one of the two strands at the fork and that a "semi-discontinuous" model best accounts for kinetic observation of the DNA.

Termination

- The E. Coli replication terminus is a large region flanked by six nearly identical, non-palindromic --- 23 bp terminator sites.

  ➔ Ter E, Ter D, Ter A → counter clockwise

  ➔ Ter F, Ter C & Ter B → clockwise

- A replication fork traveling counter clockwise passes through Ter E, D, A.

- A clockwise traveling replication fork transits Ter E, D, A but halts at Ter C or failing that Ter B or Ter F.

- Thus they act as one way valves that allow replication forks to enter the terminal region but not to leave it.

- The arrest of replication fork motion at Ter sites requires the action of Ter protein (monomer of 309 aminoacid residues).

  (Terminator utilization substance)

- Thus protein specifically bids to a Ter site, where it prevents strand displacement by helicase, and there by arresting the replication fork.

- However, this termination system is not essential when the replication terminus is deleted, replication simply stops. Through the collision of opposing replication forks.

The last few helical turns in the parental DNA could be removed by changing the topology of the nearby completed replicated regions, leaving the two daughter helices linked together as catenanes. Replication then could be completed before

or after decatenation to yield two separated complete daughter helices. This decatenation is catalysed by DNA gyrase and topoisomerase IV. Topo IV is responsible for separating newly replicated molecules in vivo. DNA gyrase cannot fully substitute for Topo IV, although if decatenates in vitro.

2.4.4.4. Replication Mechanism of Bacteriophage M_{13}

- Bacteriophage M_{13} carries a 6408 –nt single straded circular DNA, known as Viral or (+) strand.
- When it infects a cell (bacterium), this strand directs the synthesis of its complementary or (-) strand and thus forms the circular duplex, replicative form (RF).
- The RF may be nicked (RF II) or supercoiled (RF II).
- It gives a good paradigm for leading strand synthesis in duplex DNA.
- As the M13 (+) strand enters the *E. Coli* cell, it becomes coated with SSBP except at a palindromic 57-nt segment. That forms a hairpin.
- RNA polymerase commences the synthesis of primer, 6nt before the start of the hairpin and extends the RNA 20-30 residues to form a segment of RNA-DNA hybrid duplex.
- The DNA that is displaced from the hairpin becomes coated with SSB so that when RNA polymerase reaches it, primer synthesis stops.
- Pol III holoenzyme extends the RNA primer around the circle to form the (-) strand.
- The primer is removed by Pol I there by forming RF II, which is converted to RF I by the sequential actions of DNA ligase and DNA gyrase.

1) Replication mechanism of bacteriophage ϕ x 174

- Bacteriophage ϕ x 174 carries a small (5386 nt) single stranded circular DNA.
- The conversion of its DNA to replicative form is a much more complex process than that for M_{13} phage DNA.

- ϕ x 174 replication requires the participation of a nearly 600 Kda protein assembly called primosome.

a) (-) strand synthesis

- It acts as a good model for lagging strand synthesis.
- The (+) strand is coated with SSBP except for a 44-nt hairpin near position 2300.
- A 70nt sequence containing this hairpin known as pas (primosome assembly site), is then recognized and bound by the Pri A, Pri B and Pri C proteins.
- Dna B and Dna C proteins in the form of a $DnaB_6$. $DnaC_6$ complex add to the DNA with the help of Dna T protein. This process requires an ATP molecule.
- Dna C is then released yielding the preprimosome. The preprimosome, inturn, binds primase yielding primosome.
- The primosome moves in 2' -> 3' direction along the (+) strand by Pri A and Dna B catalysed ATP hydrolysis. This motion, displaces SSBPs in its path.
- At randomly selected sites, the primosome reverses its migration while pimase synthesizes an RNA primer.
- The initiation of primer synthesis requires the participation of Dna B protein, which through concomitant ATP hydrolysis is thought to alter DNA template conformation in a manner required by primase.
- Pol III holoenzyme extends the primers to form okazaki fragments.
- Pol I excises the primers and replaces them by DNA.
- The fragments are then joined by DNA ligase and supercoiled by DNA gyrase to form the ϕ x 174 RFI.

b) (+) strand synthesis

- The ϕ x 174 (+) strand is synthesized on an RF-I template by a variation of rolling circle or σ - replication mode called the looped rolling circle mode.
- (+) strand synthesis begins with primosome-aided binding of the phage-encoded enzyme gene A protein (60 Kda) to its ---- 30-bp recognition site. There gene A protein specifically cleaves the phosphodiester bond

preceeding (+) strand nucleotide 4306, by forming a covalent bond between a Tyr residue and the DNA's 5'-phosphoryl group.

- Rec protein subsequently attaches to the (-) strand at the gene A protein and, with the help of the primosome still associated with the (+) strand, commences unwinding the duplex DNA from the (+) stand's 5' end.

- The displaced (+) strand is coated with SSBP, which prevents it from reannealing to the (-) strand. Pol III holoenzyme extends the (+) strand from its free 3'-OH group.

- The extension process generates a looped rolling circle structure in which the 5' end of the old (+) strand remains linked to the gene A protein at the replication fork.

- When it has come full circle around the (-) strand, the gene A protein again makes a specific cut at the replication origin so as to form a covalent linkage with the new (+) strand's 5' end. Simultaneously, the newly formed 3'-OH group of the old, looped out (+) strand attacks its 5'-P attachment to the gene A protein, there by liberating a covalently closed (+) strand.

- The replication fork continues its progress about the duplex circle, producing new (+) strands in a manner reminiscent of linked sausages being pulled off a reel.

In the intermediate stages of a ϕ x 174 infection each newly synthesized (+) strand directs the synthesis of the (-) strand to form RF –I as described in 1.7.4.1. In the latter stages of infection, however, the newly formed (+) strands are packaged into phage particles.

2.4.4.5 Fidelity of Replication

Preservation of the genome of a species is entrusted to a replication process with an error frequency, in E. Coli cells, of 10^{-10} or less per bp. These rare replication errors are, principal source of the so-called spontaneous mutation. High repplication accuracy comes from 5 mechanisms.

1. Maintenance of batenced levels of d NTPs

- These are provided by fine regulation of key biosynthesis steps.

- Aberrantly high levels of a dNTP favor its misincorporation.

- Low levels of a dNTP invites the incorporation of miscorrect base.

Ex. Incorporation of Uracil inplace of dTTP is minimized by the action of UTPase and maintenance of dTTP levels.

2. Watson-crick basepairing

Of a dNTP to the template acts as a checkpoint. The error frequency at their step is estimated to be 10^{-3} to 10^{-4} bp.

3. Induced fits of polymerase and DNA

The polymerase's active site adapts to the size and shape of a correct base pair and conformational features of the DNA template are adjusted by bending and base stacking. At this sage the error frequency is reduced to $10^{-5} – 10^{-6}$ per bp.

4. Proof reading

The 3'-5' exonuclease functions of pol I and pol III detect and eliminate the occasional errors made by their polymerase functions.

5. Mismatch correction / Repair

A remarkable battery of enzyme systems, contained in all cells, function to repair residual errors in the newly synthesized DNA, as well as any damage that it may incur after its synthesis through chemical and / or physical insults.

2.4.4.6 Eukaryotic Replication

1)Enzymology

Polymerase α

- It is essential for the replication
- The enzyme contains – a catalytic core subunit 170Kda.
  - two associated primase subunits 50 & 60 Kda
  - an additional polypeptide of uncertain function.
- an essential role for pol α in chromosomal replication is based on many lines of evidence.
  - Mouse cells with temperature sensitive mutant pol α fail to replicate DNA at the restrictive temperature.

- Antibodies against pol α inhibit DNA replication when introduced into permeabilised nuclei or cultured mammalian cells.
- The levels of Pol α are high in rapidly growing mammalian cells in culture and in proliferating lymphocytes.
- Pol α mutants are not diminished in repair of DNA damage. Pol α is not responsible for DNA repair.

- it has no 3^1 ->5^1 exonuclease activity even then, the fidelity is high, one in 10^6 nts.
- It is involved in discontinuous replication.

Polymerase δ

- it has unique intrinsic 3^1 ->5^1 exonuclease activity.
- Pol δ requires PCNA (proliferating cell nuclear antigen), which is a processivity factor and needed for initiation of replication.
- Involved in continuous replication.

Polymerase ε

- It has similarities to pol δ
- It is distinguished from pol δ, by its high processivity independent of PCNA.

Polymerase β

- It is the smallest of eukaryotic polymerases
- It shows no correlation with chromosome replication.
- In regenerating rat liver and hepatomas pol α levels increase enoromously while those of pol β remain the same.
- Levels of the pol β transcript respond to doses of DNA-damaging agents.
- Pol β fills the single nucleotide gap in-short patch repair.
-

Polymerase γ

- It is the replicative polymerase of mitochondrial DNA.
- Has potent 3^1 ->5^1 exonuclease activity.
- Low error rate, nearly one per 10^6 nts.
- Although pol γ represents only about 2% as much activity as α, and 10-25% as much as β.

Primase

- Primase activity is commonly a component of DNA pol α, a nuclear enzyme responsible for chromosomal replication.
- Primer length is 12-14 nts.
- The relatively low processivity of pol α, in addition to its associated primase makes it possible candidate for the synthesis of the discontinuous strand.
- Purified pol α contains
    - 180 Kda core
    - 50 & 60 Kda primase
    - 70 Kda no known function
- The two primase units are active in pol-primase complex and also when they are separated. The two subunits function as a unit and not been separated from each other under primase activity conditions are maintained.
- Low the primer is moved from the active site on the small primase subunits to the DNA pol site on the 180 Kda polypeptide is not known.

Helicase

- Proteins from many sources have the capacity to unwind DNA duplexes.
- For lack of adequate genetic foundation and purified systems for replication, recombination and repair, the functions of most of these helicases are still unknown.
- The exceptions are certain viral systems in which the invitro replication is advanced and genetic analysis is available.

SV40 large T antigen

- The only viral protein required for SV40 replication.
- The large T – antigen binds specifically to the origin sequence, unwinds it during initiation and has NTP-dependent DNA & RNA helicase activities.
- By genetic analysis, T-antigen is essential for replication.
- Translocates in 3'->5' direction and depends NTP hydrolysis with ATP preferred.
- The rate of duplex melting is only 75-100bp per min compared to that of 300-800 bp per second achieved by the prokaryotic replicative helicases.

SSBPs

- Discoveries of such SSBPs have been limited, perhaps due to the abundance and prominence of histones in the distinctive nucleosomal organization of eukaryotic genomes.
- The most convincing examples of SSBPs are those disclosed by viral infections.
- RF-A (human SSBP; also called RP-A & protein A)

It is made up of three tightly associated polypeptides of 70-76 Kda, 32-34 Kda, and 11-14 Kda. The purified protein binds tightly to singlestrande DNA and is required for the helicase action of the virus –encoded T-antigen in opening the SV 40 origin

Ligases

- Coenzyme is ATP
- Product is Ppi

Topoisomerases

Topo I : Type I topoisomerase

- Eukaryotic topo I is a monomeric protein of --- 95 Kda.

- Although the reactions catalysed are generally the same as those of E. Coli topo I; +vely and –vely supercoiled DNAs are relaxed equally well.

- Relaxation is independent of ATP.

- DNA – covalent protein intermediate is formed with a specific tyrosine residue via a 3'-phosphate group.

  Rather than the 5'-p used by the E. Coli type I enzyme.

Topo II : Type II Topoisomerase

- Homodimers of 150-180 Kda subunits

- Relaxes negative and +ve supercoils at equal rates. Doesnot introduce negative supercoils like E.Coli Gyrase.

- Requires ATP hydrolysis.

- U-terminal region is related to the B subunit of E.Coli Gyrase and it is the ATPase domain.

- The central portion is similar in sequence to A subunit which is involved in the nicking and closing activity.

- Reactions catalysed by eukaryotic topo II and E.Coli Gyrase are different.

2) Mechanism of Replication

In Drosophila, the DNA molecules are replicated in bi-directional fashion. Instead of beginning from a single point of origin, replication of eukaryotic DNA begins at multiple points of origins. This forms a number of eyes, as the parental strands of DNA are separated and replicated until they meet.

Multiple origins are necessary because of the great length of eukaryotic DNA and the relatively slow movement of replication fork, i.e, about 2600 bp per min compared to 16,000 bp per min in E.Coli.

If there were only a single replicating fork in eukaryotes, complete replication of the nuclear DNA would require atleast two weeks. However, a Drosophila egg cell completes replication in about 3 min and is believed to employ upto 6000 replication forks simultaneously.

Eukaryotic chromosome origin

Finding of specific initiation sequence has been difficult, however, due to the complexity of eukaryotic genomes and the limitations of assays to detect the intiation sites. The clearest evidence for specific replication origin is found in yeast.

Yeast ARS

Autonomous Replication Sequences isolated from chromosomes and naturally occurring plasmids in yeast. About 400 ARS elements are estimated to be present in the yeast genome. ARS elements are mode of two functional domains.

- Domain A, always present, consists of a II-bp sequence that has the consensus sequence (A/T) TTTAT (A/G) TTT (A/T). Mutations that alter this core sequence destroy ARS function. Analogous to the binding site of an initiator protein.

- Domain B is AT rich and extends 50-100 bp to the 3' side of the core. At rich character is thought to provide a region of DNA melting.

It is thought that initiation of replication is triggered by the binding of another component to the multiprotein ORC (origin recognition complex) that is already residing at the origin. This binding event is thought to stimulate the local unwinding of the DNA at the adjacent sequences.

The origin ones utilized becomes inactivated, by the formation of new histones and methylation of newly synthesized DNA by methyl transferases.

3) Mitochondrial DNA Replication

Mitochondrial DNA is replicated by a process in which leading strand synthesis preceeds lagging strand synthesis. The leading strand therefore displaces the lagging strand template to form a displacement or D loop.

During replication the D loop is extended. When it has reached a point ---- 2/3 of the way around the chromosome, the lagging strand origin is exposed and its synthesis proceeds in the opposite direction around the chromosome. Logging strand synthesis is therefore only --- 1/3 complete when leading strand synthesis terminates.

4) Termination

No polymerase is known that can extent a chain from the 5' end and thus fill the gap created at the end after the RNA primer has been excised on the lagging strand. How, then, are the DNA sequences at the ends of eukaryotic chromosomes, the telomeres, are replicated:

Telomeric DNA has an unusual sequence. It consists of upto 1000 or more tandem repeats of a simple, species – specific depedent, G-rich sequence concluding the 3' ending strand of each chromosomal terminus.

For example, the ciliated protozoan Tetrahymena has the repeating telomeric sequence TTGGGG, where as in humans it is TTAGGG. More over, this strand ends with a 12 to 16-bp overhang.

The synthesis of telomeric sequences follow the instructions of a 3'-AACCCCAAC (ribonucleotide) sequence contained within an essential RNA (about 160 nt) in the enzyme Telomerase.

The proposed mechanism entails hybridization of a long protruding 3' end of the DNA chain to the RNA sequence to permit elongation by TTG, further by GGGTTG by translocation back on this RNA template. Thus, growth of the 3' end of the telomere is by 6nt lengths of GGGTTG. So, a 3' end terminating at any nucleotide within the TTTGGGG sequence can be elongated to yield the perfect tandem repeats.

Synthesis of the $(AACCCC)_n$complementary chain to form the duplex telomere may be primed by a primase. The problem of filling gaps at the 5' end created by removal of the RNA primer is solved by the enoromous redundancy of the telomeric tandem repeats.

2.4.4.7 Inhibitors of DNA Replication

- Inhibitors of DNA replication continue to serve as prime drugs for suppressing proliferative viral, bacterial and autoimmune diseases.
- To provide the laboratory investigator with the means to analyse biochemical pathways *Invivo & Invitro.*
- Inhibitors are especially attractive for studies of eukaryotic system in which mutant selection and genetic analysis are different.

Inhibitors of Topoisomerases

DNA replication at many stages, as well as other DNA transactions (Recombination, transcription) depends on the topological state of the DNA. Type I

and II topoisomerases, found in all cells relax –ve supercoils, except E.Coli type II topoisomerase, which is unique in inducing –ve supercoils.

Inhibitors of topoisomerases have proved to be outstanding antibacterial drugs & highly promising as antitumor agents.

Coumarins

- Novobiocin, coumermycin A & chlorobioan.
- Related streptomyces – derives antibiotics containing comermycin & sugar moieties.
- Inhibits bacterial Gyrase (B subunit), eukaryotic topoisomerase II, vaccinia type I topoisomerase.

Alkaloids

Camptothecin – inhibits eukaryotic topo I.

Quinolones

Nalidixic acid, oxolinic acid, Norfloxacin – inhibits bacterial Gyrase (A subunit).

- Some replication systems are inhibited because RNAP provides the primer for DNA replication.
- Blocks the formation of first two phosphodiester bonds.

Inhibitors of DNA polymerases

- a tetracyclic diterpenoid antibiotic, inhibits replicative eukaryotic DNA polymerases.
- α and δ pols of yeast and animal, viral encoded pols, α-like pols of plants can be inhibited by aphidicolin.
- It doesnot affect the β and α - pols.

Phosphnoacetic acid and phosphonoformic acid

- Despite their simple structures, are effective antiviral drugs.

- They selectively inhibits the DNA pol encoded by herpes simplex, Vaccinia viruses.

- The viral pols are generally over 100 times more sensitive than the host cell enzymes.

Inhibitors of postreplicational modifications

Except in some phage systems, which synthesize alternative precursors, modifications of DNA and RNA are made after the chain has assembled. As the functional significance of these modifications become clearer, there is greater interest in the development of agents that affect these processes.

For example: The methylation of certain cytosine residues in mammalian DNA is likely to have an important influence on gene activation and cell differentiation.

The methyl donor is invariably 5-Adenosyl Methionine compounds that affect – methyl transferases, hydrolytic enzymes that remove inhibitory products, enzymes of Ado Met regeneration can have major sequences.

Example : 5-azacytidine → inhibits the methyl transferase –7-Deaza – S-adenosyl homocysteine → potent inhibitor of Adomet-dependent methylases and decreases the level of methylation.

2.4.4.8 DNA Repair

DNA is the only molecule which, when altered or damaged is repaired by the cell. A bacterial gene has a 50% chance of remaining unaltered even after having been duplicated 100 million times. This remarkable stability of DNA in all cells is in considerable measure due to variety of devices, for preserving its integrity and repairing any lesion it may sustain.

The repair systems are extraordinarly diverse and effective. Perhaps, 100 loci in E.Coli are involved in DNA repair and related functions. The molecular mechanism for repair can be divided into –

- Those that reverse the damage

  i.e., photoreactivation, dealliylation

- Those that excise and replace the damaged unit by replication, recombination or the mis-match repair pathways.

When repair fails, continuity of the genome may be preserved through error-prone replication, in which bypass of the lesion permits replication to proceed.

1)Direct reversal of the damage

Photoreactivation by photolyases

The energy of visible light is used by photolyases to break the cyclobutyl pyrimidine dimer rings, restoring the bases to their monomeric form. Photoreactivation, as an alternate of excision, repairs any UV-induced cyclobutyl dimers.

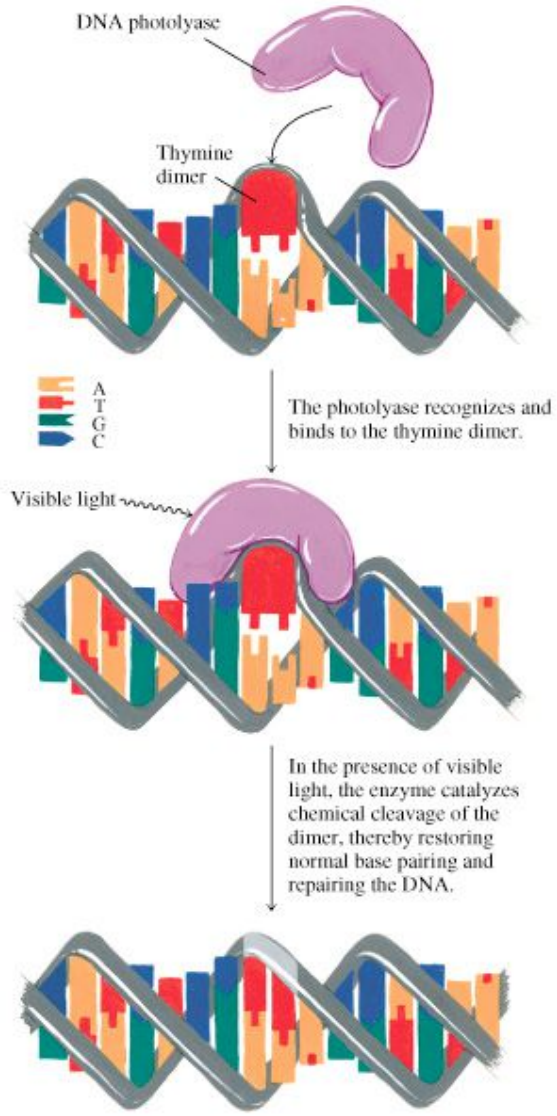
All photolyases contain two chromophores.

1. $FADH_2$
2. Pterin)folate coenzyme in yeast and E.Coli)

Or

Deaza flavin (other classes)

- In the first stage of photoreactivation, the enzyme recognizes and binds specifically to the dimer in the dark.
- When the lesion absorbs light (of a wavelength characteristic to the chromophore), the energy is used by the stable enzyme-DNA complex to convert cyclobutyl dimer to pyrimidine monomers.
- The enzyme then dissociates from the DNA.



Dealkylation

Another example of direct reversal of damage is the transfer of a methyl group from the precarcinogenic O^6-methyl guanine to a cysteire residue of an O^6-methyl guanine – DNA methyl transferase (Mtase).

The enzyme also removes an alkyl group from a phosphotriester by alkylation of another cysteine residue.

* Mtases are present in E.Coli, Yeast, and Mammalian cells.

2) Excision Repair

Repair of great variety of damaged and modified bases is achieved by excisions, that remove

- the damaged base, creating an AP site
- a fragment containing an AP site
- the nucleotide lesion and neighboring region of DNA or
- an inserted crosslink.

The gap generated by excision is filled by DNA pol and then covalently joined by a ligase.

1. Base exicision

- Removes the lesion, there by creating an AP site.
- It is carried out by one of several N-glycosylases that recognize a deaminated or altered base or a helical deformation caused by the lesion and then hydrolyze the bond linking the base to the sugar.

2. Excision of an AP region

This is achieved by either of two ways.

- By one pathway, a class II endonuclease makes the initial incision next to the AP site.

  - after which the fragment is removed by an exonuclease.

- By the second route, a class I AP endonuclease, which possesses both N-glycosylase and AP endonuclease domains, uses the latter for an incision of -O-P-O bond to the 3'-side of AP site.

- removal of the AP gragment is then carriedout by a class II endonuclease (incises the backbone to the 5' side of the AP site).

AP II endonuclease → 3'→5' exonuclease activity

AP I endonuclease → the –O-P-O-bond is broken on either side of the AP site to initiate the removal of the abasic deoxyribose for its eventual replacement by the proper nucleotide.

DNA Glycosylase → hydrolyses N-glycosyl bond that links the base to the

Deoxyribose of the DNA backbone.

3. Oligonucleotide excision

Includes the region containing a UV dimer, bulky adduct, or interstrand cross link. Their excision is performed by an excinuclease, such as Uvr ABC complex of E. Coli.

- Uvr ABC, cut's out a 12-13 nt fragment from one strand.
- The resulting gap is filled by pol I and is sealed by ligase.
- The hydrolytic incisions are made at the 8^{th} –o-p-o bond 5' to the lesion and 4^{th} or 3^{rd} bond 3' to it.
- Crosslinks are produced by many carcinogenic and chemotherapeutic agents.
- Excision requires repair of both strands.
- In E. Coli and mammalian cells, crosslinks are repaired by a combination of nucleotide exceision and recombinational repair, which requires Rec A recombinase and the assistance of Rec BCD nuclease, helicase.

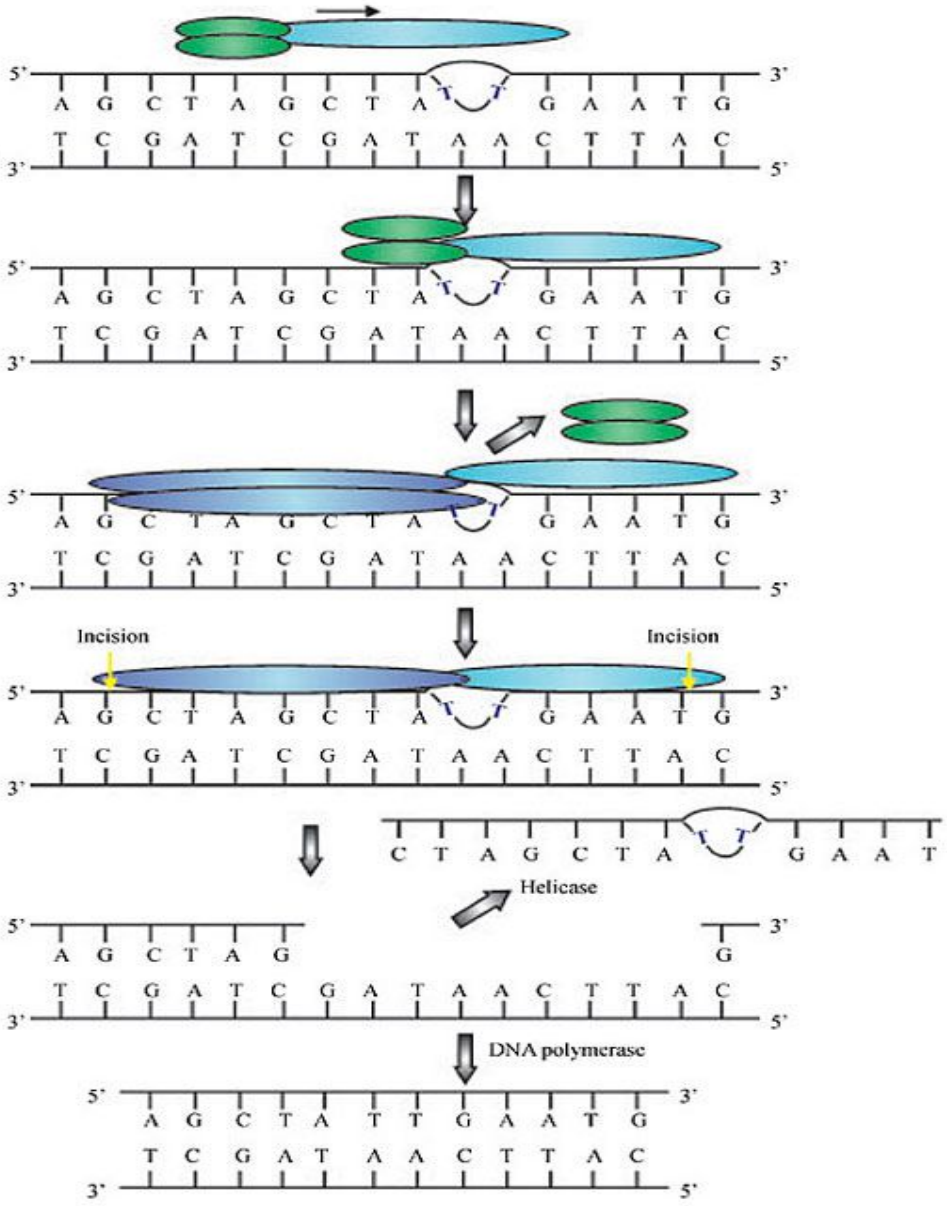


Figure 2. Schematic representation of nucleotide excision repair. The UvrAB heterodimer scans the DNA searching for large distortions in the helix such as the ones caused by pyrimidine dimers. Once a damaged site is found, UvrA proteins (dark green) dissociate, and a stable UvrB-DNA (light green) complex is formed. UvrC (blue) associates to bound UvrB and enables UvrB protein to nick the DNA at the fourth nucleotide 3' to the site of damage. Following the 3' incision, UvrC protein catalyzes nicking of the DNA at the seventh nucleotide, 5' to the damage. The potential oligonucleotide fragment that is generated is removed by a helicase. The remaining gap is filled up by polymerase synthesis and repair is completed by ligase.

4. Excision – Repair Patches

- Is produced by pol I in E. Coli and is --- 20 nt long.

- These short-patches are longer in pol A mutants deficient in 5'→3' exonuclease function. These long patches may be several thousand nucleotides long.

- In mammalian cells. The short patch is only 3-4 nt long

  The long patch is about 35 nt.

- Short patches are produced by the damage of DNA from ionizing radiation and alkylation.

- Long patches are produced from DNA distorsion caused by UV and strand linkages.

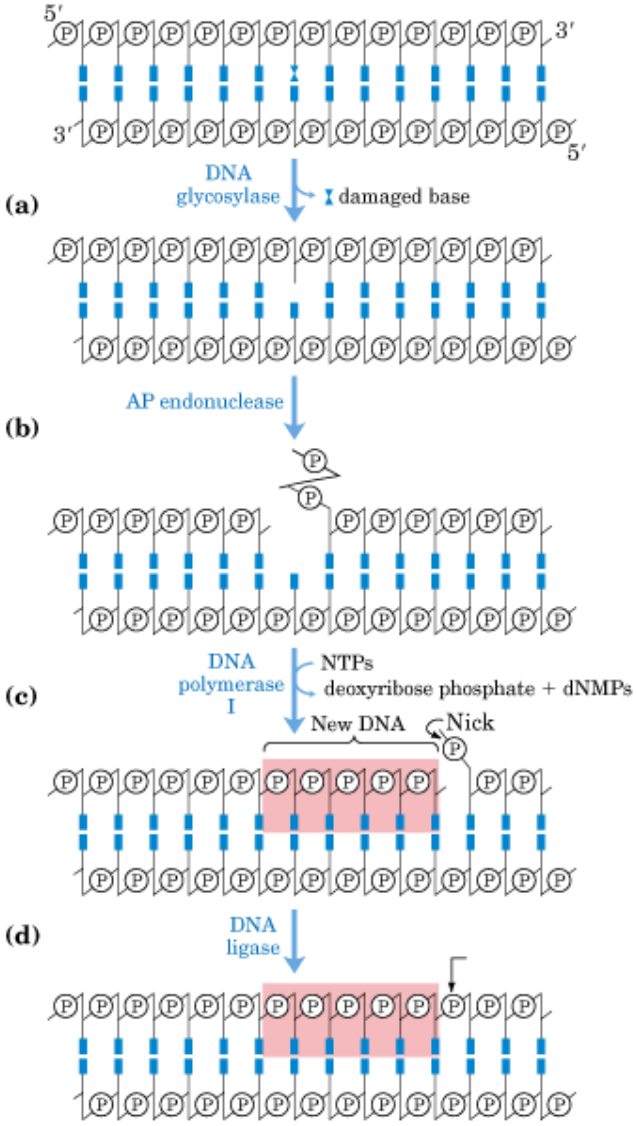
5. Translesion replication and recombinational repair

When a DNA polymerase encounters a pyrimidine dimer or certain other lesion in the template, either one of two things happens.

- The polymerase, as part of the SOS response, fills that spot by non template – directed (error-prone) replication across the lesion, employing the Uma C and Uma D proteins and pol III holoenzyme.

- The polymerase stops and then resumes 1000 nt or so down stream; the discontinuity or post replication gap may be filled with a complementary strand from the sister-duplex by Rec A mediated.

By either pathway, the lesion remains and must be removed subsequently by one or another of the direct-repair or excision-repair systems.



6. Mis Match Repairs

This repair system corrects mismatches within recombination intermediates. Ex: Thymine of a GT bp produced by spontaneous deamination of 5-methylcytosine.

Methyl-directed mis-match repair

- The newly synthesized DNA strand of a duplex can be identified because its GATC sites have not been methylated.

- Any of the possible base-base mispairs be discovered by this repair system, then that section of the unmethylated strand containing the mispaired base is removed.

- Mis Match repair depends on seven proteins.

  Mut S, Mut L, Mut H, Mut U (helicase II, Uvr Dhelicase)

  Exonuclease I, SSBP and pol IV holo enzyme.

- The sequence of events is as follows.

  - Mut S forms a complex with the heteroduplex and is then joined by Mut L, Mut H.

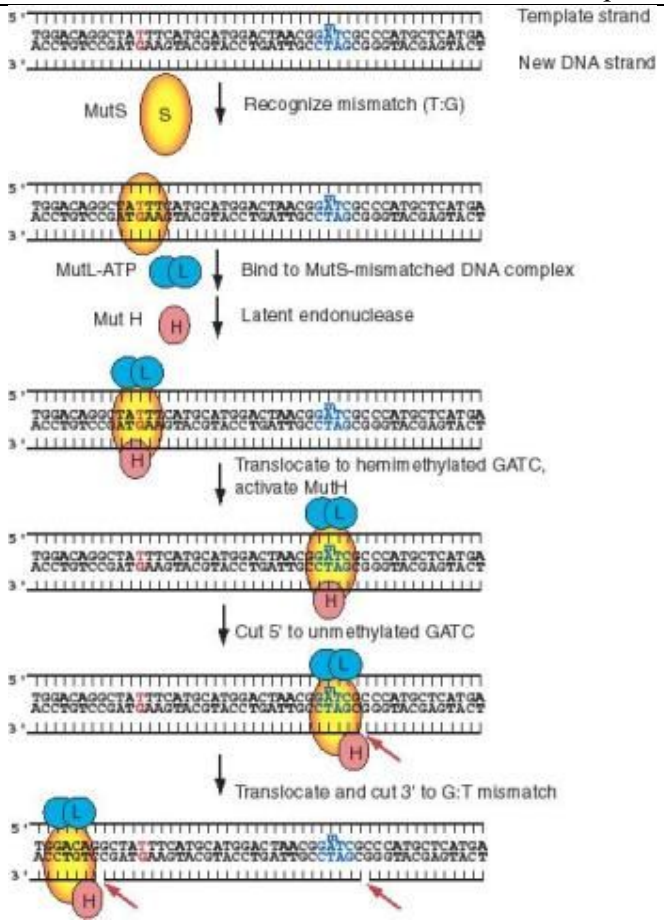
  - The S.L.H complex is then translocated along the DNA, in either direction, for several thousand bps of necessary until it encounters an unmethylated GATC sequence in one strand of the duplx.

  - The endonuclease function of Mut H in the presence of ATP, incises that strand at the GATC sequence.

  - Excision of the incised strand, from the break upto and including the mismatch, depends on exonuclease I, helicase II and SSBP.

  - Concerted replacement of the DNA by pol III holoenzyme synthesis.

Mut S can recognize the slight helical distortion of an incorrect bp and the DNA can be tracked for great distances to identify which of the strand is newly synthesized in order to remove and replace the entire section with high efficiency.



2.4.4.9 **Summary**

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The duplication of DNA is known as replication.there are different models were proposed for DNA replication.they are Semiconservative replication in which the daughter duplexes should be composed by one parent strand and one new strand Conservative Model in which the two original strands would remain together and two newly synthesized strands remain together.and Dispersive model in which the daughter cell contains duplexes in which each strand is composite of old and new DNA i.e., neither the strands nor the duplex itself is conserved.In these three models the semiconservative model is the correct model proved by Messelson and Sthal experiment.the DNA polymerase is the enzyme that synthesizes the DNA.IN addition to DNA pol number of proteins like ligase ,topoisomerase,ssb,s helicase

etc are involved in replication.there are different models of DNA replication like rolling circle replication.looped circle mechanism etc.there different agents that damage the DNA by causing mutations that should be repaired otherwise they affects the normal cellular functions sometimes they causes cell death.the cell contains different repair mechanisms to repair the DNA damage.They are Methyl-directed mis-match repair recombinational repairPhotoreactivation by photolyases,sos repair

2.4.4.8 Model questions

1)Compare the process of replication of projkaryotes with eukaryotes

2)explain the different repair mechanismss

2.4.4.9 Reference books

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Author :

Dr.N.Srinivasa Reddy
Centre for Biotechnology
Acharya Nagarjuna University
Guntur

Lesson 2.4.3

TRANSCRIPTION

Objective

Objective

The process of synthesizing RNA from DNA is known as transcription .In this chapter we have explained the process of transcription and the inhibitors of transcription

2.4.3.1 Introduction

The information in DNA, ecoded in the sequence of the four bases, is used to direct the assembly of 20 aminoacids in the correct sequence so as to produce the protein for which a given gene is responsible. A gene doesnot participate directly in the protein synthesis; in eukaryotes the DNA is enclosed inside the nuclear membrane while the protein-syntheizinng machinery is outside in the cytoplasm and the two never meet.

The protein synthesis is done by sendingout copies of its coded information to the cytoplasm. (In E.coli, the copy is in immediate contact with the cytoplasm). Since, the information is in a sequence of bases, the copy must also be a nucleic acid, but this time it is RNA and not DNA, called mRNA or messenges RNA.

- mRNA is single stranded, not a duplex, i.e., mRNA is a copy of only one of the two strands of the DNA of a gene.

- Its 4 bases are A, C, G and U. There is no T.

The flow of information in gene expression is DNA Transcription mRNA Translation protein.

In copying DNA to RNA there is transcription of the information. Hence mRNA production is called gene transcription or simply TRANSCRIPTION and the DNA is said to be transcribed. The RNA molecules produced are called transcripts.

The DNA strand that acts as the template for mRNA synthesis is called the template and the other one is called the non-template strand. But the other terms coding and non-coding, sense & non-sense strands are used more commonly.

5' CGATGCAT 3' Non-template (coding / snese) strand

DNA

3' GCTACGTA 5' template (non-coding / non-sense) strand

5' CGAUGCAU 3' mRNA strand.

Fig: Relationship of transcribed mRNA to template and non-template strands of DNA terminologies.

The base sequence of mRNA is same that of the non-template strand, this has the information for the sequence of amino acids in protein. Hence this non-template strand is called coding or sense strand or (+1) strand in viruses. The template strand is called the non-coding or non-sense strand or (-) strand inn viruses.

2.4.3.2 RNA Polymerase (RNAP)

The major enzyme of transcription is RNA polymerase, specifically DNA-dependent RNA polymerase, simply called RNAP.

Similarities:

RNA & DNA pols have basically identical catalytic properties in the growth of polynucleotide chains. RNAP require all 4 dNTPs for complementary base-pairing with a DNA template, just as DNA pols require all 4 dNTPs. The newly synthesized chain propagates in 5'→ 3' direction.

Distinctions

RNAP	DNAPOLS
1. It can both start and stop a new chain when copying a duplex.	- Cannot start chain and generally rely on RNPs for primers.
2. Terminates chain at the end of a gene or an operon.	- Normally copy a template until it is exhausted. Specific replication termination signals have been identified; but they exert their effect by impeding helicase action on a duplex, rather than by affecting a DNA pol directly.
	- Inert on duplex DNAs, require nicked or frayed regions with auxillary proteins to melt the helical duplex.
3. The true template for RNAP is a duplex that undergoes localized and transient melting during transcription.	- Had ultrahigh fidelity in copying any template.
4. Fidelity in copying is uncertain.	

2.4.3.3 RNA Polymerase

- E. coli has a single DNA-derected RNA pol that synthesizes all types of RNA.

- It is the most extensively studied and is representative of the enzymes isolated from other bacterial genera-salmonella, Bacillus, serratia, proteus, Aerobacter etc..

- It is a large and complex enzyme containing totally six subunits with a composite mass of 448 Kda.

- The core enzyme contains 5 subunits, $\alpha_2\beta\beta^1$ w.

- Association of a σ subunit with the core constitutes the holoenzyme.

- all the RNAPs of bacterial genera are complex in organization and similar in properties except for those of phages T_7 and N_4.

- The structure of the enzyme contains a channel similar to the active site cleft on DNA pol I. This channel is about 25 A^o in diameter and 55 A^o in length.

- The structural similarity between polymerase I & RNA Polymerase is supported by a small amount of amino acid sequence homology between pol I and the β-subunit of RNA Polymerase.

The σ subunit

- present only about 1/3rd the abundance of the core.

- Required only during initiation of transcription at a specific site.

- **σ** dissociates from the RNA P-template complex shortly thereafter and cycles to a new core.

- Both *E.Coli* and *B.subtilis* have multiple forms of **σ,** each responsible for recognizing a particular class of promoters.

- The predominant σ is 70Kda (σ 70) in E.coli and 43 Kda (σ 43) in β. Subtilis.

β' subunit

- antibiotics that bind RNA P and inhibit the action have provided important insig hts into the structure and functions.

- Rifampicin, for example, which binds firmly to the β subunit, completely blocks productive initiation of RNA chains by the enzyme in vivo and vitro.

- The polymerase – rifampicin complex apparently fails in performing the translocation step that follows the formation of the 1st or 2nd phosphodiester bond.

- Cells gain resistance to rifampicin by changing its β-subunit that fails to bind the drug.

- In addition to functioning in rifampicin binding, in transcription termination and interacting with the σ subunit, β also binds rNTPs and possesses an atom of Zn^{++}.

The β' subunit

- compared to β, little is known about the functions of other core subunits.

- The β' polypeptide also has a tightly bound Zn^{++}, is involved in DNA binding and is the site of action of polyanionic inhibitors such as heparin.

The α subunit

- No specific function is ascribed to α subunit.

The W subunit

- it can be dissociated from the intact enzyme (holoenzyme) without apparent loss of activity and is not required in the reconstitution of an active enzyme.

- Its presence is required, however for transcription to be inhibited by Guanosine tetraphosphate (PPGPP) in vitro.

- PPGPP is thought to be a signaling molecule involved in the stringent control.

- Thus the `w' subunit is implicated in the regulation of transcription ratherthan directly in RNA synthesis.

Reconstitution of Holoenzyme

- Separate polypeptides, individually inert, become active when reassembled in the following order:

- $\alpha + \alpha \rightarrow \alpha_2 \rightarrow \alpha_2\beta \rightarrow \alpha_2\beta\beta' \rightarrow \alpha_2\beta\beta'\sigma$

- The requirement for all the subunits in reconstituting enzyme activity indicates that each is an essential component of RNA P.

- Assembly of the enzyme is required for subunit stability: overproduced normal polypeptides are also rapidly degraded invivo for lack of subunits with which to assemble.

General reaction

The fundamental chemistry of RNA synthes is has much in common with DNA synthesis. The overall reaction is

$(NMP)_n + NTP \longrightarrow (NMP)_{n+1} + Ppi$

RNA Lengthened RNA

RNA P both intiates and terminates the chain. Formation of phosphodiester bond for chain growth is thus only one stage in a complicated sequence of reactions – template binding, site selection, initiation, elongation and termination.

2.4.3.4 Promoters

RNA synthesis is normally initiated only at specific sites on the DNA template called `promoters' that are recognized by the corresponding σ factor.

The existence of promoters was first recognized through mutations, that enhance or diminish the transcription rates of certain genes. Promoters lie on the upstream side of the RNA's starting nucleotide (+1), towards 5' end of the transcribing gene.

Analysis and comparison of sequences in many different bacterial promoters have revealed similarities in two short sequences located about to & 35 bp away from transcription start site and are represented as –10 & -35 consensus sequences. For most promoter in E.coli and related bacteria, the consensus sequen for the –10 region (also called pribnow box) is 5' TATAAT 3' and for –35 region is 5' TTGACA 3'.

Trp TTGACA N_{17} TATAAT N_8 A

lac TTTACA N_{17} TATGTT N_8 A

rec A TTGATA N_{16} TATAAT N_7 A

ara BAD CTGACG N18 TACTGT N_6 A

a)_Promoter recognition

The σ subunit, as part of the holoenzyme recognizes both the –35 and –10 sequences in the promoter and probably contribute to the melting of the duplex to form open complex.

The multiple σ forms that are normally present in bacteria, those induced during spore formation in β subtilis and those appearing during infection by certain phages have provided insights into their structure and functions. At least 17σ factors have been identified, along with the promoter sequences which they recognize.

The major σ forms from E.coli (σ 70) and β subtilis (σ43) recognize the same sequences and direct the initiation of most genes.

The minor σ factors, commonly around 30Kda redirect the core polymerase to different classes of promoters, such as those for the heat shock genes in E.coli and the sporulation genes in β subtiles.

b)Chain initiation

- The σ subunit allows holoenzyme to move rapidly along a DNA strand in search of the σ subunit's corresponding promoter.
- The holoenzyme then migrates to the –35 region, forming the closed complex.
- The DNA is then unwound for about 17 basepairs beginning at the –10 region, exposing the template strand at the initiation site.
- The RNAP binds more tightly to this unwound region, forming an open complex.
- The RNA synthesis begins.
- The binding of RNAP to promoters is facilitated by the supercoiling of the DNA.
- The initiating reaction of transcription is the coupling of two nucleoside triphosphate in the reaction:

PPPA + PPPN ⟶ PPPApN + Ppi

- bacterial RNAs therefore have 5'-triphosphate groups as was demonstrated by the incorporation of radioactive label into RNA when it was synthesized with (- P) ATP.

- Only the 5' terminus of the RNA can retain the label because the internal phosphodiester groups of RNA are derived from the α-phosphates of NTPs.

c) Chain elongation

- the σ subunit is required only to ensure the specific recognition of the promoter by the RNAP.

- Once a few phosphodiester bonds re formed the σ subunit dissociates, leaving the care polymerase to complete the syntheses of the RNA molecule.

- The σ is recycled to intiate another roand of transcription.

- The chain lengthens in 5'→ 3' direction (50nt/see), as was detected by using the antibiotic cordycepin.

- The topological problems caused by transcription are relieved through the action of topoisoerases.

- Moving of RNAP, produces +ve supercoils ahead of and –ve supercoils behind the point at which transcription is occurring.

d) Chain termination

- RNA synthesis proceeds until the RNAP encounters a sequence that triggers its dissociation.

- In E.coli there are atleast 2 classes of such fermination signals or terminators.

- One class relies on a protein factor called `p' (rho) and the other is p-independent.

- The P-independent class has two distinguishing features. The first is a region that is transcribed into self-complementary sequences, resulting in the formation of a hairpin structure, 15-20 nt before the end of the RNA.

- The 2nd is, a run of adenylates in the template strand that are transcribed into uridylates at the end of the RNA.

- It is thought the formation of hairpin disrupts part of the RNA-DNA hybrid in the transcription complex. The remaining hybrid duplex (oligo u-dA)

contains a particularly unstable combination of bases and the entire complex simply dissociates.

P-dependent

- RNAP needs no help to terminate transcription at a hairpin followed by several u residues. At othersites, however, termination requires the participation of an additional factor.

- Some RNA molecular synthesized in vitro by RNAP alone are longer than those synthesized in vivo. The missing factor, a protein, was isolated and named `rho' `p'.

- The p factor is active as hexamer and specifically binds ssRNA, a stretch of 72nt, 12 per subunit. It also has an associated RNA-dependent NTPase activity (specially ATP)

- The ATPase activity of p enables it more unidirectionally along nascent RNA toward the transcription bubble. It then breaks stops the transcription process.

Evidences for P function

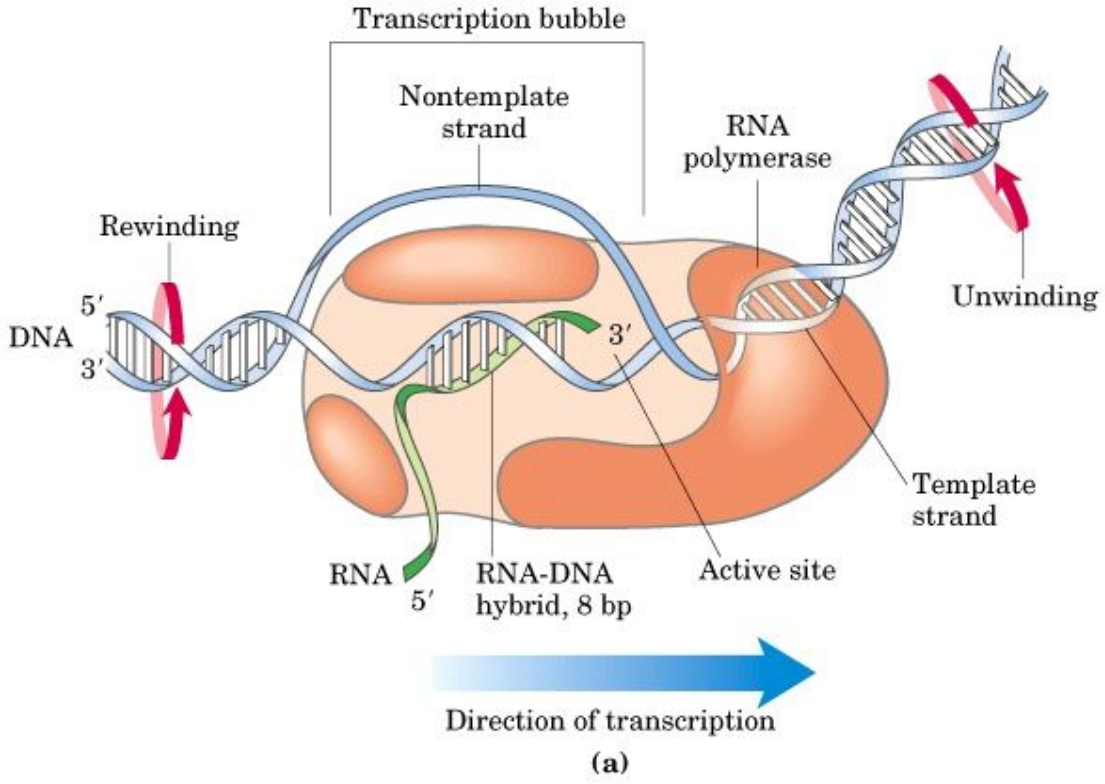
Additional information about the action of P was obtained by adding this termination factor to an incubation mixture at various timer after the initiation of RNA synthesis. RNAs with sedementation coefficients of 13S, 17S and 23S were obtained, when P was added a few seconds, 2 min, and 10 min respectively after initiation.

It is evident that the template contains atleast three termination sites that respond to P (yielding 10S, 13S & 17S) and one termination site that doesnot require --- (23s RNA)

However, P detects additional termination signals that are not recognized by RNAP alone.

2.4.3.5 Eukaryotic RNAP

In contrast to pro karyotes, eukaryotes have three different RNAPs, each transcribing a particular class of genes. The three RNAPs are designated as RNA pol I, RNA pol II, and RNA pol III, were first resolved by RoEDER and RUTTER in 1969, as three distinct proteins electing at different salt concentrations during ion-exchange chromatography.



1)RNA pol I

- Present in nucleus
- Synthesizes only one type of RNA, called pre-rRNA, which consists the precursors for the 18s, 5,8s, 28s rRNAS.
- Very insensitive to α-amanitin (upto 10μg/ml)

2)RNA pol II

- Present in nucleoplasm
- Transcribes all protein-coding genes, ie, it functions in the production of mRNAs.
- It also produces four small nuclear RNAs that participates in RNA splicing.
- Very sensitive to α-amanitin (0.1 μg/ml)

3)RNA Pol III

- present in nucleoplasm and cytoplasm
- transcribes the genes encoding tRNAs, 5S rRNA and also small, stable RNAs.

Ex : U_6 → involved in RNA splicing

7S RNA of SRP (signal recognition particle) involved in transport of protein into the Emdoplasmic reticulum.

- moderately sensitive to α-amanitin (1-10 μg/ml)

Each of the eukaryotic RNA polymerase is more complex than E.coli RNA polymerase. All 3 contain two large subunits and 12-15 smaller, some of which are present in two or all three polymerases.

The best characterized eukaryotic RNAPs are from the yeast *S.cerevisiae*.

- the largest subunit (160-220Kda) and 2nd largest subunit (128-150Kda) of each eukaryotic RNAPs are related to the E.coli β' & β subunits respectively.
- Both yeast RNAP I and III contain α subunits (19 7 40Kda), that have sequence homology with E.Coli α-subunit.
- Yeast RNAP II contains 2 copies of a different subunit (44 Kda) that exhibit most distant similarity with E.Coli α-subunit.
- In addition to their core subunits related to the E.coli polymerase subunits, all 3 yeast RNAPs contain 5 small common subunits (10-27 Kda).
- In addition, each RNAP has 4-7 enzyme specific subunits that are not present in the other two RNAPs.

The functions of these multiple polymerase subunits are not understood, but gene-knockout experiments in yeast indicate that most of them are essential for cell viability.

4)Promoters

- As prokaryotic genes, eukaryotic genes also contain promoters to the 5'side of start site.

- The start site itself has a recognizable short sequence between nucleotides – 3 and +5, called the initiator region.

- About 80% genes have a TATA box centered at about ---- 25 to 35 base pairs upstream of the start site. This highly conserved sequence is also called Goldberg – Hogness box

- Further upstream, within about 100-200 or so base pairs of the start site, are elements or boxes – short specific DNA sequences that are recognized by specific proteins called. Transcriptional factors. Three common upstream elements are the CAAT box, the GC box and an 8 bp octamer box. These are located at sites within –100 to –200 region in different genes.

- These control elements, together with the TATA box and/or initiator often are referred to as the promoter of the gene they regulate. However, we prefer to reserve the term promoter for the TATA box or initiator sequences and the term promoter-proximal elements for control regions lying within 100 – 200 bps upstream of the start site.

- 20% of genes, including many house keeping genes, lack TATA boxes, some lack other elements, some have multiple copies of an element.

5) Enhancers

The activities of many promoters in higher eukaryotes are greatly increased by sequences called `enhancers'.

- They are relatively large elements, often including several 100 bps and sometimes contains repeated sequences that are independently functional.

- They can act over considerable distances, upto several thousand bps.

- They can be upstream, downstream or even in the middle of a transcribed gene.

- The enhances element and the gene must always be present on the same DNA molecule.

- A particular, the Ig enhances functions in β-lymphocytes but not else where.

- Enhancers act only in cells that contain cognate stimulatory proteins.

Ex: The steroids interact with soluble receptors. These hormone – Receptor complexes bind to the glucocorticoid enhanurs and then lead to stimulation of transcription of a distinctive set of genes.

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Enhancers

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- The enhancer element and the gene must always be present on the same DNA molecule.

- A particular enhance is effective only in certain cells, for example, the Ig enhancer functions in β-lymphocytes but not elsewhere.

- Enhancers act only in cells that contain cognate stimulatory proteins.

Ex: The steroids interact with soluble receptors. These hormone- Receptor complexes bind to the glucocorticoid enhancers and then lead to stimulation of transcription of a distinctive set of genes.

Enhances are not the only DNA elements that can act at a distance to control transcription of a gene. There are certain elements which prohibit transcription, called Silencers. It is clear that silencers depend on silencer proteins, that bind to the silencer DNA and some how cause repression of surrounding genes. The mechanism of this repression is still unknown.

2.4.3.6 Transcriptional factors

In prokaryotes , RNAP recognizes the correct binding site on a promoter and binds directly to the DNA helped in somecases by for example, CAP. This does not occur in eukaryotes. All the genes in chromation are basically in `shutdown' or repression state because, a nucleosome blocks the initiating region of each gene promoter. A gene cannot be transcribed until the nucleosome is displaced, thus allowing the basal initial complex formation. This formation involves several proteins, collectively called as Transcriptional factors.

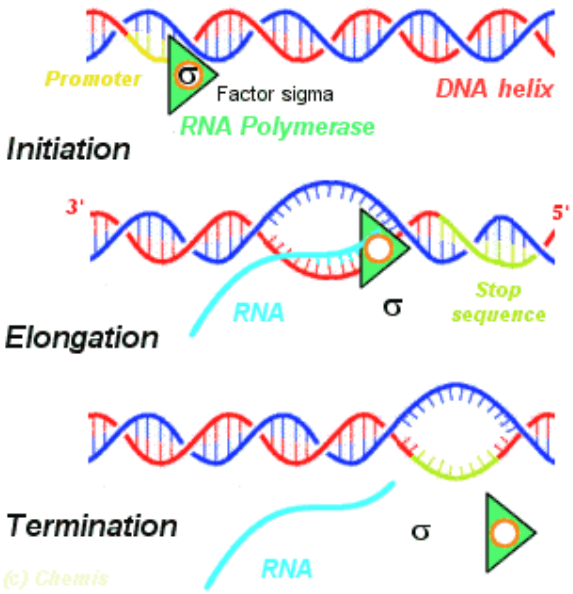
2.4.3.7 RNA synthesis and termination

Transcription by eukaryotic RNAP leads to synthesis of primary transcripts which, are longer than the mature, functional RNAs consequently, the primary transcripts require and undergo one kind of processing or the other before they finish up as mature RNAs.

Electron microscopic pictures of transcription of rRNA genes show that definite start and termination sites exist for RNAP I. Termination occur a few 100 bases past the 3' end of the 28S RNA gene.

Transcription of 5S RNA genes by eukaryotic RNAP III involves precise termination to produce 5S RNA straight away. Transcription terminates in a run of U s.

RNAP II termination is closely linked to processing. However, one general feature is that termination occurs anywhere between a few 100 to a few 1000 bases downstream of the 3' end of the coding sequence. It has been suggested that loss of proussivity of pol II ratherthan a precise signal may determine termination.



2.4.3.8 Inhibitors of transcription

A variety of antibiotics and other inhibitors affect transcription at different stages.

Actionmycin D

- it is a streptomyces antibiotic
- acts as antitumor agent but is highly toxic.
- It is a planar, tricyclic compound with a phenoxazone ring containing two –COOH groups, each of which carries a cyclic peptide chain.
- Binds to ds DNA at GC rich regions.
- Inhibits replication as well as transcription.
- At low concentrations (10^{-6} M), specifically inhibits DNA – dependent RNA polymerases.

Rifampicin, Rifamycin

- inhibitors of bacterial RNA polymerase.
- Have long aliphatic sidechains attached at both ends to planar aromatic ring systems.

- Rifampicin specifically inhibits the β subunit.
- Elongation is not affected.

α - amanitin

- an extremely toxic octapeptide from mushrooms
- inhibits eukaryotic RNAPs.
- Has one residue each of L-hydroxy proline, L-Aspargine, L-hydroxy isoleucine and 2 glycine residues linked to a central tryptophan, itself attached to an oxidized cysteine moiety.
- Obtained from poisonous mushroom Amanita phalloides.
- Forms a tight 1:1 complex with RNAP II and a looser with RNAP III so as to specifically block their elongation steps.
- RNAP I, as well as mitochondrial, chloroplast and prokaryotic RNAPs are insensitive.

Cordycepin

- naturally produced by cordyceps militaris
- inhibits transcription by getting incorporated at 3' end of the growing transcript.
- Results in chain termination, since chain elongation is not possible due to the absence of free 3'-OH group.

2.4.3.9.Summary

The synthesis of RNA from DNA is known as Transcription carried out by the enzyme RNA polymerase.In case of prokaryotes a single RNA polymerase synthesizes all the RNA's.But in the case of Eukaryotes three RNA polymerases are present RNA polymerase 1 synthesises 5.8s, 18s, 28s rRNA's .RNA polymerase synthesizes mRNA, snRNA's. RNA polymerase III synthesizes 5s rRNA,tRNA,U_6s$_n$RNA. RNA synthesis is normally initiated only at specific sites on the DNA template called `promoters'.the eukaryotes contain specific DNA sequences that increase the rate of transcription known as Enhancers.The prokaryotic RNA polymerase doesn't require any proteins for the transcription ,it can initiate ,elongate and terminate the RNA synthesis. But eukaryotic RNA polymerases need proteins known as transcriptionalfactors.The transcription in prokaryotes is inhibited by

Rifampicin, Rifamycin,Actinomycin wher as eukaryotic RNA polymerase are inhibited by amanitin,cordycepin

2.4.3.10 Model questions

1)Explain the procees of transcription

2)Write a note on inhibitors of transcriptionssss

2.4.3.11 Reference books

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Dr.N.Srinivasa Reddy

Lesson 2.4.4

POST – TRANSCRIPTIONAL MODIFICATIONS

Objective

Objective

As soon as RNA is produced from the DNA by transcription, the RNA undergoes some modifications for its protection, which are called post transcriptional modifications. All these modifications were clearlyu discussed in this chapter**.**

2.4.4.1 Introductin

The immediate products of transcription, the primary transcripts, are not necessarily functional entities. In order, to acquire biological activity, many of them must be specifically altered in several ways.

- by the endo and exo nucleolytic removal of polynucleotide segments.
- By appending nucleotide sequences to their 3' and 5' end
- By 1 modification of specific nucleosides.

2.4.4.2 M RNA processing

In prokaryotes, most primary mRNA transcripts function in translation without further modification. Ribosomes in prokaryotes usually commence translation on nascent mRNAs. In eukaryotes, however mRNAs are synthesized in the cell nucleus where as translation occurs in cytosol. Eukaryotic mRNA transcripts can therefore undergo extensive post transcriptional processing while still in the nucleus.

MRNA processing includes three major processes

1. 5' capping
2. 3' cleavage or polyadenylation
3. RNA splicing

HnRNA

Nascent RNA transcripts from protein coding genes and mRNA processing intermediates are collectively referred to as pre-mRNA. These pre-mRNAs donot exist as free RNA molecules in the nuclei of eukaryotic cells.

From the time of their first emergence as nascent transcripts until mature mRNAs are transported into the cytoplasm, they are associated with an abundant set of proteins called heterogenous ribonucleoprotein particles (hnRNP) which contain hnRNA.

A collective term for pre-mRNAs and other nuclear RNAs of various size.

hnRNPs range in size from 34-120 Kda. Binding studies with purified hnRNPs suggest that different hnRNP protein associate with different regions of a newly made pre-mRNA.

For example : hnRNP proteins A_1, C & D bind preferentially to the pyrimidine rich sequences at the 3' ends of introns.

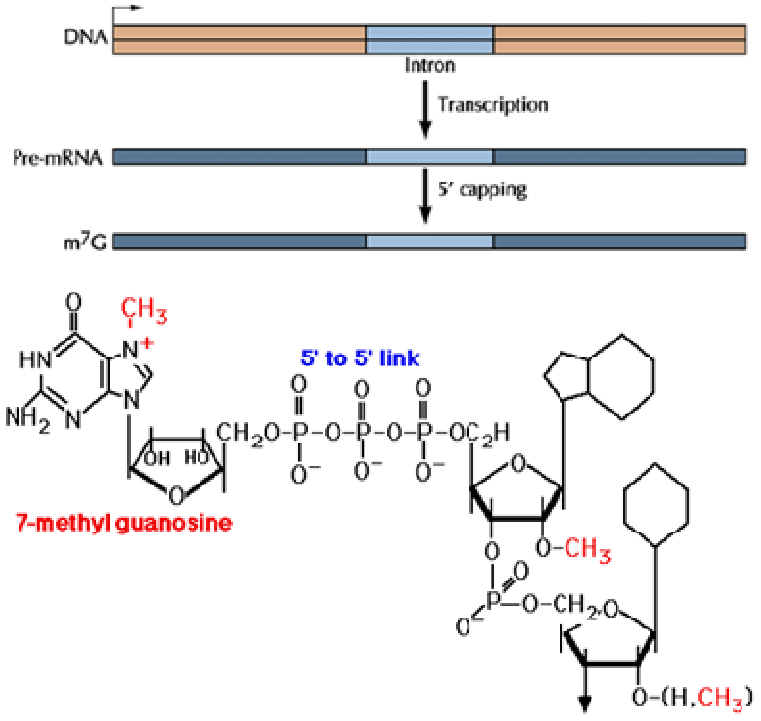
Pre-mRNAs associated with hnRNP proteins present a more uniform substrate for further processing steps, than the free, unbound pre-mRNAs, hnRNP proteins may interact with the RNA sequences that specify RNA splicing or cleavage, or polyadenylation and contribute to the structure recognized by RNA-processing factors.

2.4.4.3 5' Capping

After nascent RNA molecule produced by RNAP II reach a length of 25-30 nts, 7-methyl guanosine is added to their 5' end. This initial step in RNA processing is catalysed by a dimeric enzyme, which associates with the phosphorylated carboxyl-terminal domain (CTD) of RNAP II. Because the capping enzyme doesnot associate with pol I or III, capping is specific for transcripts produced by RNAP II.

One subunit of the capping enzyme removes the r- phosphate from the 5' end of the nascent RNA. The other subunit transfers the GMP moiety from GTP to the 5'-diphosphate of the nascent transcript, creating the guanosine 5'-5'-triphosphate structure.

In the final steps, separate enzymes transfer $-CH_3$ group from S-adenosyl methionine to the N_7 position of the guanine and the 2'-oxygens of riboses at the 5' end of the nascent RNA.



2.4.4.4 3^1 Celavage / Polyadenylation

In animal cells, all mRNAs, except histone mRNAs, have a 3^1 poly (A) tail. Early sequencing of CDNA clones from animals showed that nearly all mRNAs contain the sequence AAUAAA 10-35nts upstream from poly (A) tail.

Mutation in the sequence AAUAAA except to AUUAAA will undoubtedly terminates polyadenylatio. Further mutagenesis in a sequence downstream from the cleavage site is required for efficient cleavage and adenylation of most pre mRNAs. This downstream poly (A) signal is not a specific sequence but rather a Gu-rich or simply a U-rich region within ----50nt of cleavage site.

Identification and purification of the proteins required for cleavage and polyadenylation of pre mRNA has lead to a model. According to this model.

- a cleavage and polyadenylation specificity factor (CPSF) 360 Kda, a heterotetramer, first forms an unstable complex with the upstream AU-rich poly (A) signal.
- Then at least 3 additional proteins binds to CPSF-RNA complex.
    - a 200 Kda heterotrimer called cleavage stimulating factor (CS_tF)
    - a 150 Kda heterotrimer called cleavage factor 1 (CF1)
    - a second cleavage factor – CF II
- interaction between CS_tF and the GU or U-rich downstream poly (A) signal stabilizes the multiprotein complex
- Finally a poly (A) polymerase (PAP) binds to the complex before cleavage can occur.
- This requirement for PAP binding links cleavage and polyadenylation, so that the free 3^1 ends generated are rapidly polyadenylated.
- Assembly of this, large cleavage – polyadenylation complex resembles to assembly of transcription factors at TATA box, in both cases multiprotein complexes assemble through a network of protein-nucleicacid and protein – protein interactions cooperatively.
- Following cleavage at the poly (A) site, polyadenylation proceeds in two phases.

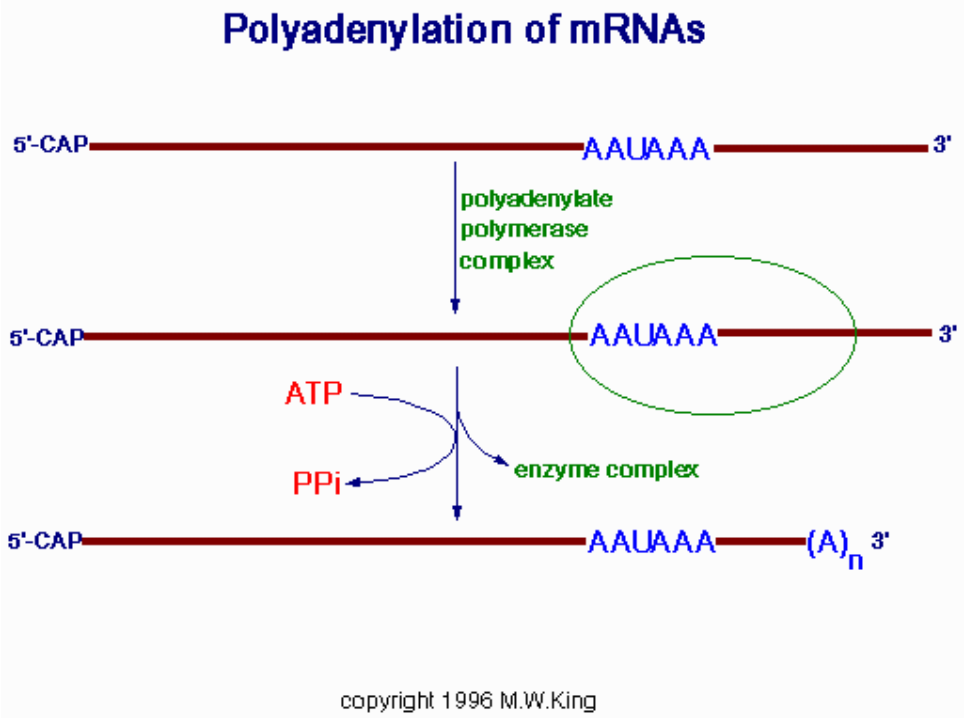
1. addition of the first 12 or so A residues occur slowly.

2. followed by rapid addition of uptp 200-250 more A residues.

- the rapid phase requires the binding of multiple copies of a poly (A) binding protein containing the RNA motif. This protein is designated as PAB II to distinguish it from poly (A) binding protein that binds to poly (A) tail of cytoplasmic mRNAs.

- PAB II binds to the short A tail initially added by PAP, stimulating polymerization of additional A residues by PAP.

- PAB II is also responsible for signaling PAP to terminate polymerization when the poly (A) tail reaches a length of 200-250 residues (the mechanism for measuring this length is not yet understood).



Split genes

mRNA in both pro and eukaryotes is proportionate in length to the size of the protein it codes for. In bacteria it is true of the primary RNA transcript, but that from most eukaryote genes is verymuch longer (even 10 times longer than would be expected). This is because the RNA coding for protein in the mRNA is split up into several parts, linked together by intervening stretches of RNA that donot code for aminoacid sequences.

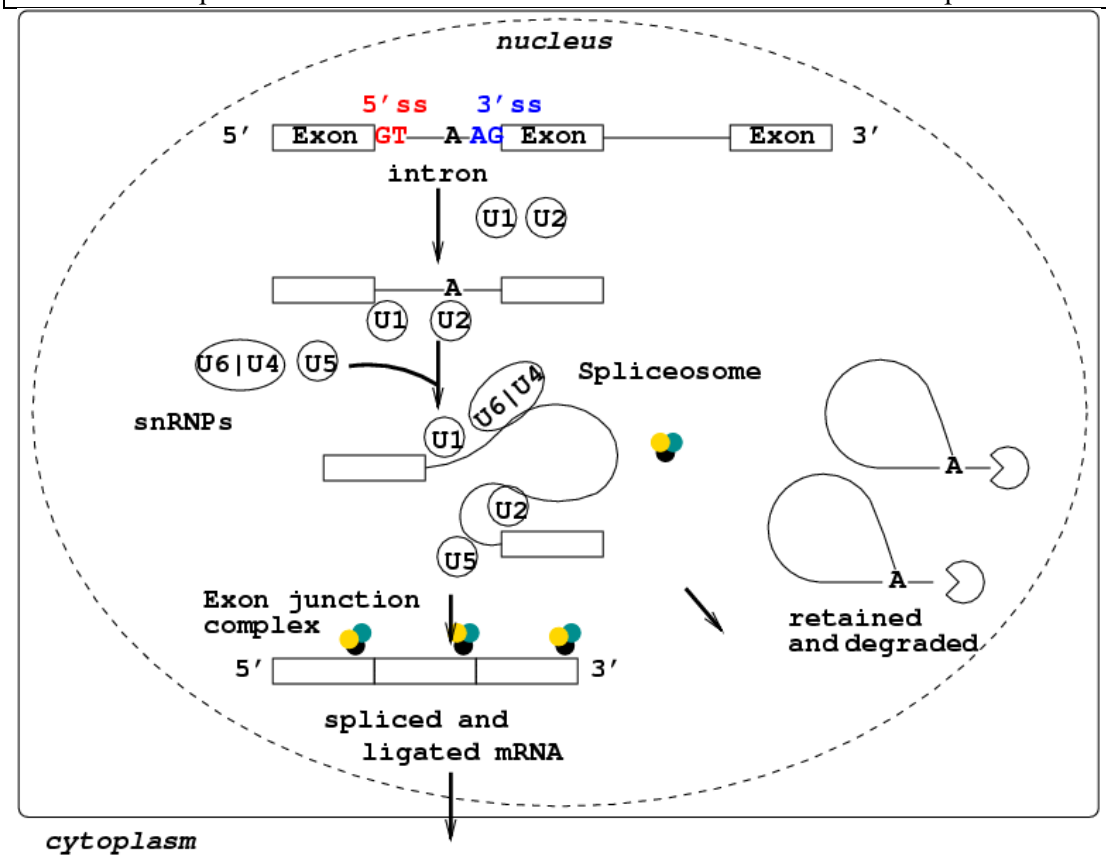
In the DNA from which such RNA molecules are transcribed, the sections coding for the interveining sequences, with no protein-coding content are called introns and the coding stretches are called exons. There can be two –50 or so introns in a human gene and introns can vary in length 50 bp to 20,000 bps. Exons are usually less than 1000bps.

An important biological aspect of the existence of introns is that they may have facilitated evolution i.e., evolution of new proteins by "domain shuffling" – the concept is that same domain is used repeatedly for a partial function of an enzyme for ex: combined with a variety of other domains, so that a new family of proteins is assembled from pre-existing domain. Domain shuffling, provides a more rapid means of producing novel proteins by recombination events ratherthan by point mutation in DNA.

2.4.4.5 Splicing

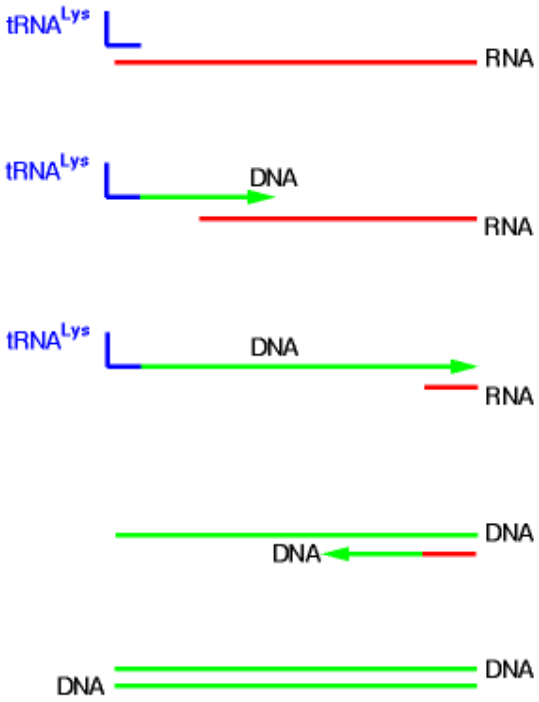
MRNA splicing

The primary transcript is processed into an mRNA molecule, known as mRNA splicing. The intron is removed as a lariat structure in which the 5^1G of the intron is joined in an unusual 2^1, 5^1 phosphodiester bond to an adenosine near the 3^1 end of the intron. This Adenosine residue is called the branch point because it forms an RNA branch in the lariat structure. The splicing proceeds via two sequential transesterification reactions. Since the number of phosphate – ester bonds in the molecule is not changed in either reaction, no energy is consumed. The net result is that two exons are ligated and the intervening intron is released as a branched lariat structure.



2.4.4.6 Reverse transcription

The retroviruses life cycle involves transcription like events. The retroviruses have genomes of ssRNA that are replicated through a double stranded DNA intermediate. The crucial steps are :



The enzyme responsible for generating the initial DNA copy of the RNA is Reverse transcriptase. This enzyme converts the RNA into a linear duplex of DNA in the cytosol of the infected cell. The linear DNA makes its way to the nucleus. One or more DNA copies become integrated into the host genome. A single enzyme called integrase is responsible for integration. The integrated proviral DNA is transcribed by the host machinery to produce viral RNAs, which serve both as mRNA and as genome for packaging into virions.

Retroviruses are called + strand viruses, because the viral RNA itself codes for the protein products. Reverse transcriptase is responsible for converting the genome into a complementary DNA strand, (-) strand DNA.

The enzymes, Reverse Transcriptase (Rtase) and integrase are carried with the genome in the viral particle.

Rtase has a polymerase activity and an Rnase H activity. All retroviral Rtases share considerable similarities of aminoacid sequence.

Viral RNA structure

- The viral RNA has direct repeats at its end. These `R' segments vary in different strains of viruses from 10-80 nt.

- Following R segment at the 5^1 end of the virus is the U_5 region (80-100 nt). The name indicates that it is unique for 5^1 end.

- Preceeding the R segment at the 3^1 terminus is the U_3 segment (170-1350 nts) unique for 3^1 end.

- The R segments are used during the conversion from the RNA → DNA form to generate more extensive direct repeats, that are found in linear DNA.

- Like other DNA pols, Rtase also requires a primer.

- The native primer is tRNA (uncharged)

- A sequence of 18 bases at the 3^1 end of the tRNA is basepaired to a site 100-200 bases from the 5^1 end of the viral RNA molecule. Synthesis proceeds to the end, generating a short DNA sequence called minus strand stop DNA.

- In this reaction, the R region at 5^1 end of the RNA template is degraded by the Rnase H activity of Rtase. This allows the R region at 3^1 end to basepair with the newly synthesized DNA.

- Now occurs the first jump on to the 3^1 end of the RNA. Then Rtase continues through the U_3 region into the body of the RNA. The result of the switch and extension is to add a U_3 segment to the 5^1 end.

- The stretch of sequence U_3-R-U_5 is called the LTR, because a similar series of events add U_5 to the 3^1 end giving it the same structure U_5-R-U_3.

- Primer is excised.

- Rtase primes the synthesis of + strand DNA from a fragment of RNA that is left after degrading the original RNA molecule. A strong stop plus strand is generated when the enzyme reaches the end of the template.

- This DNA is then transferred to the other end of the minus strand DNA. This ds DNA then requires completion of both strands to generate a duplex LTR at each end.

- This duplex DNA is then integrated into the host genome, by the action of another viral encoded enzyme, integrase.

2.4.4.7 Summary

The immediate product of transcription is known as primary transcript or pre RNA which undergo number of modifications to produce functional RNA's collectively termed as post transcriptional modifications. MRNA processing includes three major processes i.e.5' capping ,3' cleavage or polyadenylation RNA splicing From the RNA also the DNA is synthesized known as reverse transcription carried out by enzyme reversetranscriptase

2.4.4.8 Model questions

1)Discuss in detail the various post transcriptional modifications

2)Write an account on reverse transcription

2.4.4.9 Reference books

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Griffiths, Anthony JF. , Wessler, Susan R. , Lewontin, Richard C. , Gelbart William M., Suzuki, David T. , Miller, Jeffrey H. *An Introduction to Genetic Analysis* 8/e, W.H. Freeman

Lewin B., Genes, Oxford University Press, Newyork.

Dr.N.Srinivasa Reddy

Lesson 2.5.1

Prokaryotic Gene Regulation

Objective

Objective

The main objective of this chapter is to describre,how and when . the prokaryotic genes are expressed and repressed . the expression and repression depends on the demand for the product of the gene.

2.5.1.1 Introduction

Gene regulation is one of the most active areas of genetic research. Developmental biology, the biology of aging, genetic diseases research and cancer research all look at how genes are expressed and controlled. Some of the answers to how genes are regulated are coming from work on recombinant DNA research, some from genetics including the effect of mutations on gene expression, and some from research on disease. Much is coming from our increasing knowledge of cancers and the failure of the body to control cell division in cancer formation. Another current research interest is stem cells – the cell lines that lead to the development of precise tissue types, such as skin, immune system or blood cells. At some point in development, stem cells are "programmed", do their job, and, as a part of their programming, may even lead to programmed cell death, or apoptosis. Such programming relies on genetic controls. It is important for cells to be able to control gene activity. We have genetic information for thousands of proteins. We do not want to synthesize enzymes that are not needed, nor do we want to synthesize molecules in greater quantity than needed. The bacteria of our oral cavity for example, secrete a slime sheath when sucrose is in their diet. When no sucrose is present, they do not secrete the slime, nor do they synthesize the enzymes that would process the sucrose. Prokaryotes, in general, control genes for rapid

response to their environment. By selectively activating or inhibiting gene activity, bacterial cells can take advantage of changing conditions.

For eukaryotes, gene regulation is tied to maintaining homeostasis – a consistent internal environment in the face of ever-changing external conditions. Multicellular organisms require different genes at different times of growth and development in different tissues. We have more complex controls of gene expression to ensure that

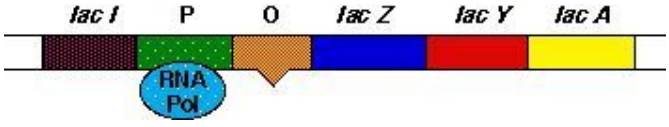
Gene control is exerted chemically in two general ways: affecting molecules that interact with DNA, RNA and/or the polypeptide chains, or controlling the synthesis of an enzyme or the activity of an enzyme in the cell. Gene controls can be positive – inducing gene activity, or negative – repressing gene activity.

2.5.1.2 The Need for Gene Regulation

Our consideration of gene expression has until now focused on the actual mechanism of RNA synthesis, without regard to whether or not the mRNA (and the protein it encodes) is actually **required**. This is an important consideration. Think of it this way: in the winter, do you run your furnace nonstop, 24 hours a day? Of course not. Your house would get way too hot and your heating bill would probably force you into bankruptcy (in other words, there would unnecessary heat produced at great expense). The same thing is true of bacteria. Why should they go to the expense of producing the enzymes that metabolize a particular nutrient if that nutrient is not present in the environment? To put this another way, bacteria need to be able to respond to their environment by changing their patterns of gene expression. They accomplish this using strategies of gene regulation.

2.5.1.3 Operons

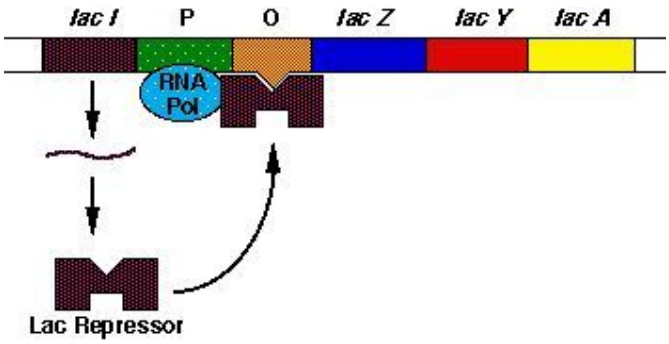
Genes that affect the same biochemical pathway in bacteria (for example, genes encoding proteins involved in metabolizing the sugar lactose) will all be expressed under the same conditions (such as when lactose is present). Therefore, it is economical to have all of these genes grouped together under the control of the same regulatory system. Such a grouping of similarly-regulated genes in bacteria is called an operon. Some operons are inducible, others are repressible. We will discuss the differences between these two types of operons later. An example of an inducible operon is the **lactose operon** (lac operon for short), which contains genes that encode enzymes responsible for lactose metabolism. The lac operon looks like this:



The three structural (protein-encoding) genes of the lac operon are *lac Z*, which encodes the enzyme beta-galactosidase (which breaks down lactose into glucose and galactose), *lac Y*, which encodes a permease (that transports lactose into the cell), and *lac A*, which encodes a transacetylase (whose function we won't consider here). These three genes are under the control of the same promoter, designated P in the figure. As discussed elsewhere, the promoter is where RNA polymerase binds to the DNA and prepares to initiate transcription. The other regulatory element in an operon is the operator (designated O). As we'll see, this is the element that determines whether or not the genes of the operon are transcribed. In addition to all of the above, there is another gene, which is technically not part of the operon (it is controlled by a separate promoter, and is expressed all the time, or constitutively), but plays an important role in operon function. This is the *lac I* gene, which encodes a protein called the lac repressor. The lac repressor has two functional domains or regions: one that binds to the DNA of the operator region, and one that binds to lactose. When the repressor binds to the operator, it prevents RNA polymerase advancing along the operon, and transcription does not occur. The regulation of the operon depends on regulating whether or not the repressor binds to the operator. To understand how this works, we need to consider what happens when lactose is present, and what happens when lactose is absent.

When Lactose is Absent

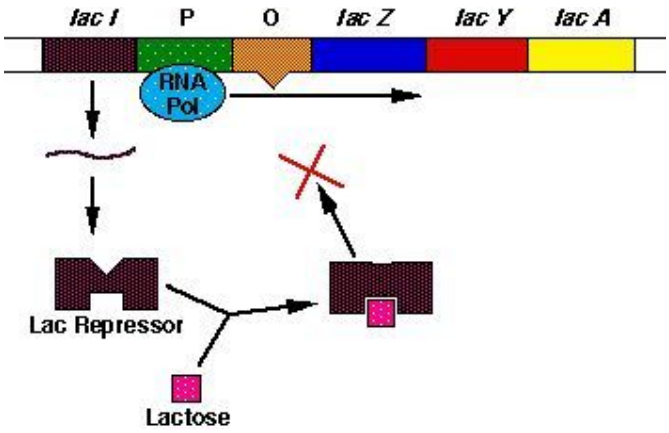
If there is no lactose present in the environment, the following series of events occurs:



The *lac I* gene is transcribed (remember, it is expressed constitutively), and the mRNA is translated, producing the lac repressor. The ,,. With RNA polymerase blocked, there is no transcription, and the enzymes for lactose metabolism are not synthesized. This is good, because there is no lactose to metabolize.

When Lactose is Present

If there is lactose in the environment, the events unfold differently. A small amount of the lactose gets into the cell, and affects regulation of the operon:



The lac repressor is still synthesized. As mentioned previously, the repressor can bind to lactose. When it does so, the repressor undergoes a **conformational change** (change of shape). Molecules that change shape when they bind to another molecule are called allosteric molecules. When it undergoes the conformational change, the lac repressor is unable to bind to the operator region. RNA polymerase is therefore not blocked, and is able to transcribe the genes of the operon. The enzymes encoded by those genes will be produced, the lac permease will transport more lactose into the cell, and beta-galactosidase will cleave the lactose into glucose and galactose, which can then be further metabolized by other enzymes, producing energy for the cell. Lactose, therefore, is able to **induce** the synthesis of the enzymes necessary for its metabolism (by preventing the action of the repressor). As such, lactose is the inducer of the lac operon

The bottom line for the lac operon, then, is that when lactose is absent, lactose-metabolizing enzymes are not produced, and when lactose is present, those enzymes are produced.

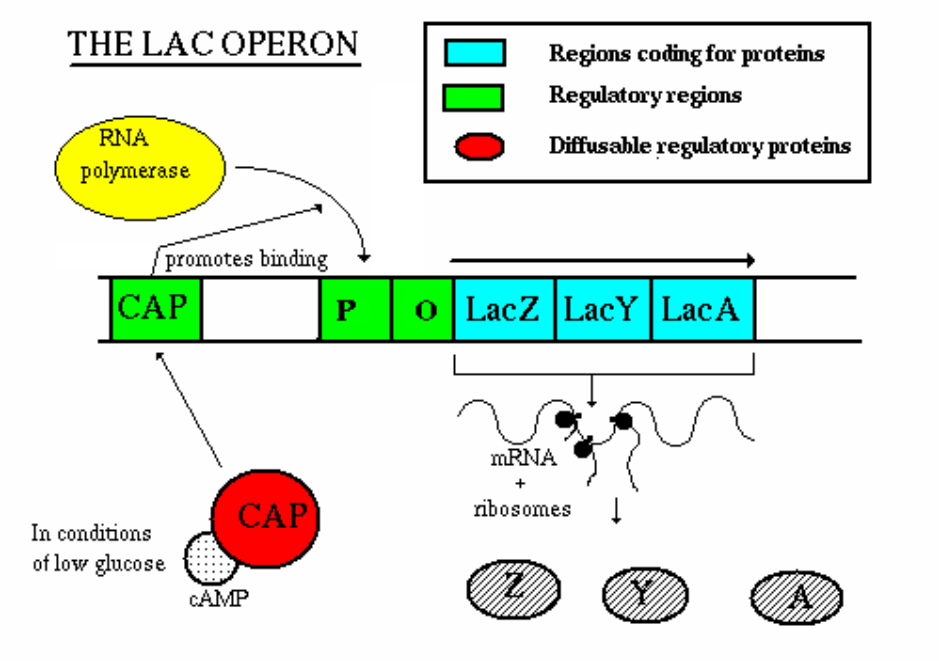
Catabolite Repression

Expression of the *lac* operon can be regulated another way. Glucose is preferable to lactose as an energy source, so if glucose is present in the environment, the *lac* operon is down-regulated (transcription is reduced). Here is how it works:

Although it wasn't mentioned previously, transcription of the lac operon requires another protein, called catabolite activator protein (CAP for short). This CAP protein binds to the *lac* promoter and promotes transcription, but only after it has

bound to a small molecule called cyclic AMP (cAMP). Without cAMP, CAP will not bind to the promoter, and no transcription will occur. In the previous examples involving the *lac* operon, we can assume that cAMP was present, and the CAP-cAMP complex was bound to the promoter.

cAMP is produced by an enzyme called adenylcyclase. When bacteria encounter glucose in the environment, adenylcyclase is inhibited, and cAMP production drops. There is no cAMP to bind to CAP, so CAP will not bind to the *lac* promoter, and no lac transcription takes place. In this way, the bacterium does not produce enzymes for lactose metabolism when they are not necessary because of the presence of glucose. This also offers a form of feedback inhibition. Beta-galactosidase (the *lac Z* gene product) breaks lactose down to glucose and galactose, so when enough lactose has been metabolized, glucose (one of the products) accumulates and causes repression of the *lac* operon



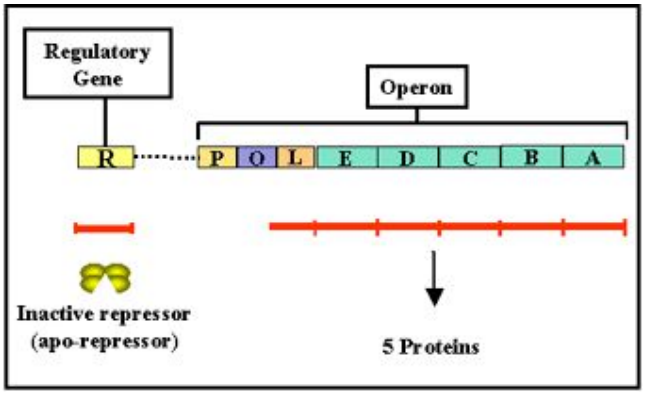
The Operon Model

1. Definition - Repressible genes are those in which the presence of a substance (a co-repressor) in the environment turns off the expression of one or more genes (structural genes) involved in the metabolism of that substance.

e.g., Tryptophan represses the expression of the trp genes.

Repression is common in metabolic pathways that result in the biosynthesis of a substance and the co-repressor is normally the end product of the pathway being regulated

2. Tryptophan operon -The tryptophan operon is illustrated in Figure .

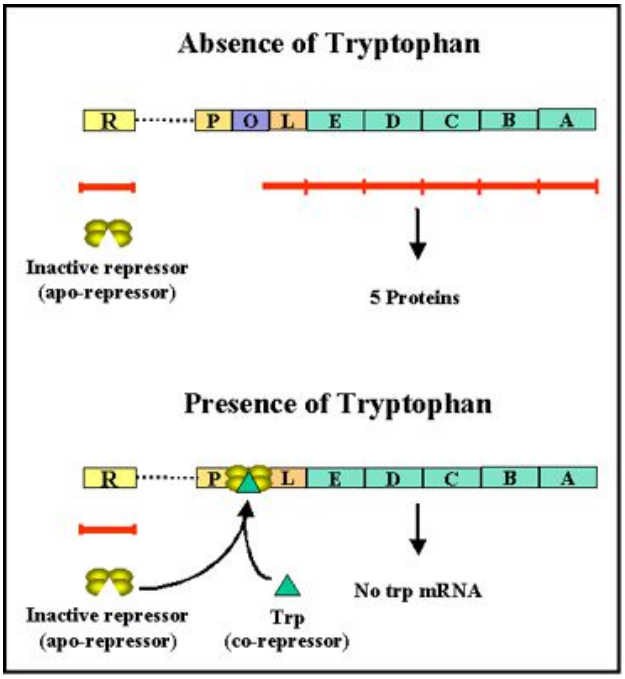


a. **Structural genes** - The tryptophan operon contains five structural genes that code for enzymes involved in the synthesis of tryptophan. These genes are transcribed from a common promoter into a polycistronic mRNA, which is translated to yield the five enzymes.

b. **Regulatory gene** - The expression of the structural genes is not only influenced by the presence or absence of the co-repressor, it is also controlled by a specific regulatory gene. The regulatory gene may be next to or far from the genes that are being regulated. The regulatory gene codes for a specific protein product called an REPRESSOR (sometimes called an apo-repressor). When the repressor is synthesized it is inactive. However, it can be activated by complexing with the co-repressor (*i.e.* tryptophan).

c. **Operator** - The active repressor/co-repressor complex acts by binding to a specific region of the DNA called the operator which is adjacent to the structural genes being regulated. The structural genes together with the operator region and the promoter is called an OPERON. Thus, in the presence of the co-repressor the repressor is active and binds to the operator, resulting in repression of transcription of the structural genes. In contrast, in the absence of co-repressor the repressor is inactive and does not bind to the operator, resulting in transcription of the structural genes. This kind of control is referred to a NEGATIVE CONTROL since the function of the regulatory gene product (repressor) is to turn off transcription of the structural genes.

d. **Co-repressor** - Transcription of the tryptophan genes is influenced by the presence or absence of a co-repressor (tryptophan) (Figure 7).



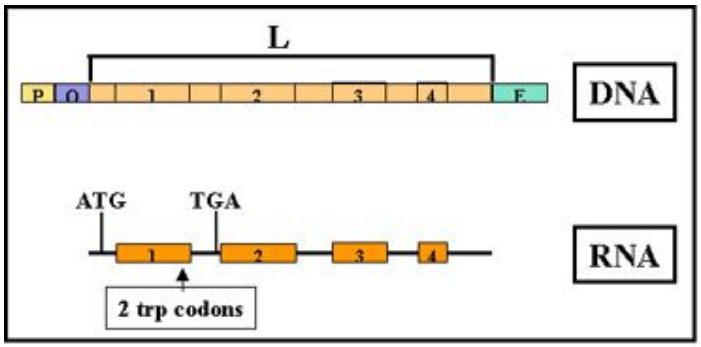
e.g

co-repressor	no expression
co-repressor	expression

3. Attenuation

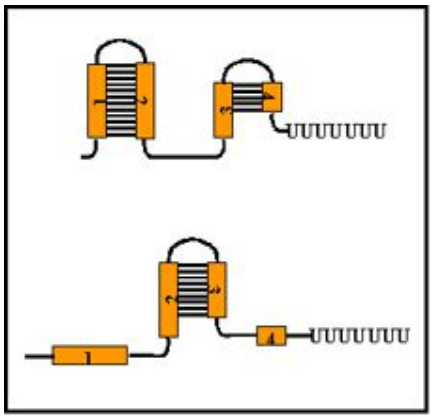
In many repressible operons transcriptions that initiate at the promoter can terminate prematurely in a leader region that precedes the first structural gene. (*i.e.* the polymerase terminates transcription before it gets to the first gene in the operon. This phenomenon is called ATTENUATION; the premature termination of transcription. Although attenuation is seen in a number of operons, the mechanism is best understood in those repressible operons involved in amino acid biosynthesis. In these instances attenuation is regulated by the availability of the cognate aminoacylated t-RNA.

a. Mechanism (See Figure)



When transcription is initiated at the promoter, it actually starts before the first structural gene and a leader transcript is made. This leader region contains a start and a stop signal for protein synthesis. Since bacteria do not have a nuclear membrane, transcription and translation can occur simultaneously. Thus, a short peptide can be made while the RNA polymerase is transcribing the leader region. The test peptide contains several tryptophan residues in the middle of the peptide. Thus, if there is a sufficient amount of tryptophanyl-t-RNA to translate that test peptide, the entire peptide will be made and the ribosome will reach the stop signal. If, on the other hand, there is not enough tryptophanyl-t-RNA to translate the peptide, the ribosome will be arrested at the two tryptophan codons before it gets to the stop signal.

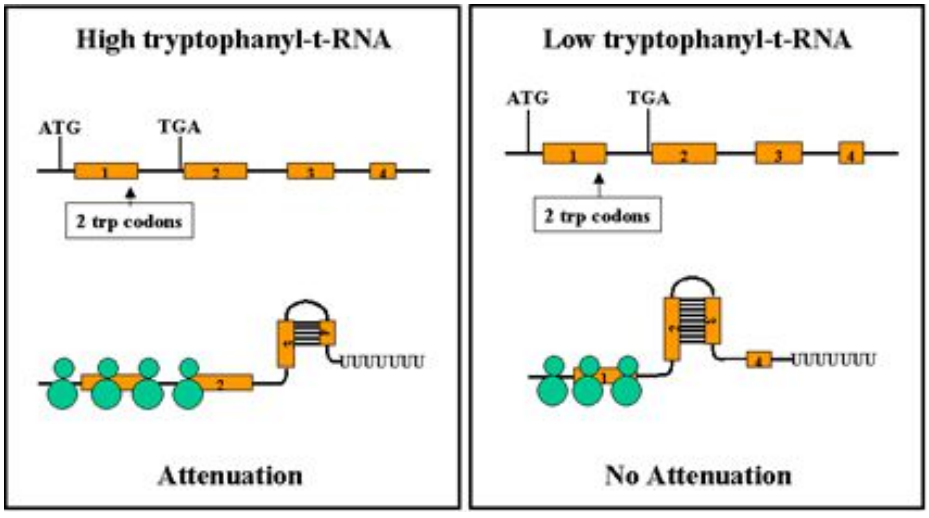
The sequence in the leader m-RNA contains four regions, which have complementary sequences (Figure 9). Thus, several different secondary stem and loop structures can be formed. Region 1 can only form base pairs with region 2; region 2 can form base pairs with either region 1 or 3; region 3 can form base pairs with region 2 or 4; and region 4 can only form base pairs with region 3. Thus three possible stem/loop structures can be formed in the RNA.



region 1:region 2
region 2:region 3
region 3:region 4

One of the possible structures (region 3 base pairing with region 4) generates a signal for RNA polymerase to terminate transcription (*i.e.* to attenuate transcription). However, the formation of one stem and loop structure can preclude the formation of others. If region 2 forms base pairs with region 1 it is not available to base pair with region 3. Similarly if region 3 forms base pairs with region 2 it is not available to base pair with region 4.

The ability of the ribosomes to translate the test peptide will affect the formation of the various stem and loop structures Figure 10. If the ribosome reaches the stop signal for translation it will be covering up region 2 and thus region 2 will not available for forming base pairs with other regions. This allows the generation of the transcription termination signal because region 3 will be available to pair with region 4. Thus, when there is enough tryptophanyl-t-RNA to translate the test peptide attenuation will occur and the structural genes will not be transcribed. In contrast, when there is an insufficient amount of tryptophanyl-t-RNA to translate the test peptide no attenuation will occur. This is because the ribosome will stop at the two tryptophan codons in region 1, thereby allowing region 2 to base pair with region 3 and preventing the formation of the attenuation signal (*i.e.* region 3 base paired with region 4). Thus, the structural genes will be transcribed.



summary

Gene regulation is one of the most active areas of genetic research. . the prokaryotic genes are expressed and repressed . the expression and repression depends on the demand for the product of the gene.

Genes that affect the same biochemical pathway in bacteria under the control of the same regulatory system. Such a grouping of similarly-regulated genes in bacteria is called an operon.there are different operons. Some operons are inducible, others are repressible. An example of an inducible operon is the **lactose operon** (lac operon for short), which contains genes that encode enzymes responsible for lactose metabolism. If there is no lactose,the repressor binds to the operator, and prevents the traccription of lactose metabolizing genes.

If there is lactose, the repressor can bind to lactose undergo some conformational chages unable to bind to the operator region. RNA polymerase is therefore not blocked, and is able to transcribe the genes of the operon.the Tryptophan pperon is a repressible operon in which the presence of tryptophan inhibits the expression of operon.inaddition to this a leader region that precedes the first structural gene terminates transcription before it gets to the first gene in the operon. This phenomenon is called ATTENUATION; the premature termination of transcription.

2.5.1.4 Model Questions.

1) Write in detail abot the gene expressin with lac operon as an exmple

2.5.1.5 Reference books

Freifelder, David., Physical Biochemistry, W.H.freeman & company

Griffiths, Anthony JF. , Wessler, Susan R. , Lewontin, Richard C. , Gelbart William M., Suzuki, David T. , Miller, Jeffrey H. *An Introduction to Genetic Analysis* 8/e, W.H. Freeman

Lewin B., Genes, Oxford University Press, Newyork.

Dr.N.Srinivasa Reddy

Lesson 2.5.2

GENETIC CODE

Objective

2.5.2.1Introduction

2.5.2.2 Genetic code

2.5.2.3 Wobble hypothesis

2.5.2.4 Summary

2.5.2.5 Model questions

2.5.2.6 Reference books

Objective

This chapter gives detailed explanation of genetic code

2.5.2.1 Introduction

Three major advances set the stage for our present knowledge of protein bio-synthesis.

1. In the early 1950s Paul Zamecnik and his colleagues designed a set of experiments to investigate where in the cell proteins are synthesized.

They injected radioactive amino acids into rats and, at different time intervals after injection, the liver was removed, homogenized and fractionated by centrifugation. The sub cellular fractions were then examined for the presence of radioactive protein.

When hours or days were allowed to elapse after injection of the labeled amino acids, all the sub cellular fractions contained labeled proteins. However, when only minutes had elapsed, labeled protein was found only in a fraction containing small ribonucleo protein particles

These particles visible in animal tissues by electron microscopy, were therefore identified as the site of protein synthesis from amino acids, and later were named as ribosomes.

2. The second key advance was made by Mahlon Hogland and Zamecnik, when they found that amino acids were activated when incubated with ATP and the Cytosolic fraction of liver cells. The amino acids became attached to a heatstable RNA later called tRNA forming aminoayl –tRNA.

3. The third major advance occurred with the rise of a question, how genetic information encoded in the four-letter sequence codes for 20 amino acids of proteins.

Crick reasoned that a small nucleic acid could serve the role as an adaptor, one part of adaptar molecule binding a specific amino acid and another part recognizing the nucleotide sequence encoding that amino acid in mRNA. This idea was soon verified. The tRNA adaptar translates the nucleotide sequence of an mRNA into the amino acid sequence of a polypeptide.

These three developments soon lead to recognition of the major stages of protein syntheis and ultimately to the elucidation of the genetic code that specifies each amino acid.

2.5.2.2 Genetic Code:

The sequence of nucleotides in the mRNA molecule is read consecutively in groups of three. Since RNA is a linear polymer of 4 different bases, there are 4^3=64 possible combinations. Each group of three consecutive nucleotides in RNA is called a CODON, and each specifies.

The mRNA codons cannot directly recognize amino acids, Rather they specifically bind molecules of tRNA that each carry a corresponding amino acid. Each tRNA contains a trinucleotide sequence anticodon, which is complementary to an mRNA codon specifying the tRNS's amino acid.

In 1961, Marshall Nirenberg and Himrich Matthaei reported an observation, that solved the genetic code. They used *invitro* translation systems. Basically such systems generally include the following:

- Ribosomes, mRNA, and Mg++ - the polysome component
- Amino acids, activating enzymes, 20 different tRNAs, ATP and mg++ to provide activated amino acids.
- Protein factoros (initiation, elongation and termination factors) – auxillary requirements.
- Phosphoenol pyruvate and pyruvate kinase – ATP generation system.
- GTP, Thiol compounds – auxillary requirements.

They incubated the synthetic polyribonucleotides polyuridylate(polycul) with an E.coli extract, GTP and a mixture of 20 amino acids in 20 different tubes. In each tube a different amino acid is radiolabelled. A radio active polypeptide was formed in only one of the 20 tubes, that containing radioactive phenylalnine. Nirenberg and Matthai therefore concluded that the triplet UUU codes for phenyl alanine. The same approach revealed that synthetic poly © codes for proline and poly(A) codes for poly Lysine.

The synthetic polynucleotides used in such experiments were made by the action of polynucleotide phosphonylase. If it is presented with a mixture of 5 parts of ADP and I part of CDP, it will make a polymer in which about $5/6^{th}$, if the residues are adenylates and $1/6^{th}$ cytidylates. Such a random polymer is likely to have many triplets of the sequence AAA, lesser numbers of AAc, CAA, relatively few ACC, CCA, CAC and very few CCC triplets.

AAC A_2C : Asn

AAC A_2C : Gln

ACC AC_2 : His

AAA : Lys

ACC AC_2, CCC : Pro

CCA C_2A : Thr

With the use of different artificial mRNAs made by polynucleotide phosphorylase, from different starting mixtures of ADP, GDP, UDP and CDP, the base compositions of the triplets were soon identified. However, these experiments could not reveal the sequence of the bases in each coding triplet.

a) Triplet binding assay

In 1964, Nirenberg and Philip Leder found that isolated *E.Coli* ribosomes will bound a specific aminoacyl tRNA, if the corresponding synthetic polynucleotide messenger is present.

For example, robosomes incubated with poly(U) and phenylalanyl –$tRNA^{phe}$ binds both RNAs, but if the ribosomes are incubated with poly (U) and some other aminoacyl tRNA, the aminoacyl-tRNA is not bond because it does not recognise the UUU triplets in poly(U)

Trinucleotide	C^{14} – labeled aminoacyl –tRNA bound to ribosomes		
	Phe-$tRNA^{phe}$	Lys-$tRNA^{lys}$	Pro-$tRNA^{pro}$
UUU	4.6	0	0
AAA	0	7.7	0
CCC	0	0	3.1

Each number represents the factor by which the amount of bound ^{14}C-increased when the indicated trinucleotide was present, relative to a control in which no trinuicleotide was added.

Even trinucleotides could promote specific binding of appropriate tRNAs, allowing the use of chemically synthesized oligonucleotides. Researchers identified, aminoacyl –tRNAs bound to 50 of the 64 possible triplet codons. For some codons, either no aminocyl-tRNAs or more than one would bind.

Chemical Methods

Haragobind Khorana, developed chemical methods to synthesize polyribonucleotides with defined, repeating sequences of 2-4 bases. The polypeptide produced by these mRNAs had one or few animoacids in repeating patterns. These patterns, when combines with information from the random polymers used by Nirenberg and colleagues permitted unambiguous codon assignments.

Example

The copolymer$(AC)_n$, has alternating ACA and CAC codons: ACACAC ACA CAC. The polypeptide synthesized from this messenger contained equal amounts of Threonine and histidine. Given that Histidine codon has 1A & 2_{cs}, CAC must code for his tidine and ACA for threonine.

An RNA with three bases in a repeating pattern should give three different types of polypeptide, each derived from a different reading frame and containing a single type of amino acid.

Example : $(GUA)_n$

GUA GUA GUA GUA

An RNA with 4 bases in a repeating pattern should yield a single type of polypeptide with a repeating pattern of four aminoacids. Consolidation of the results

from all such experiments permitted the assignment of 61 of the 64 codons. The other three were identified as termination cudons, because they disrupted aminoacid coding patterns, when they occurred in a synthetic RNA polymer.

Example: $(GUAA)_n$

Reading frame1:5[1] .....GUA AGU AAG UAA GUA AGU AA .....3[1]

Reading frame2:5[1].....GUAA GUA AGU AAG UAA GUA A.....3[1]

Reading frame3:5[1].....GU AAG UAA GUA AGU AAG UAA.....3[1]

Dipeptides an tripeptides are synthesized depending on where the ribosome initially binds. Termination codons encountered every fourth codon in all three reading frames.

Meanings for all the triplet codons were established by 1966 and have been verified in many different ways.

b)START and STOP codons

Codons are the key to translation of genetic information, allowing the synthesis of specific protens. Several codons serve special functions. The initiation codon, AUG signals the beginning o a polypeptide in all cells, in addition to coding for Methionine residues in internal positions of polypeptides. Three of the 64 possible codons do not code for

The Genetic Code

	U	C	A	G	
U	UUU Phenyl alanine UUC UUG Leucine UUA	UCU UCC UCA Serine UCG	UAU Tyrosine UAC UAA Stop UAG	UGU Cysteine UGC UGA Stop UGG Tryptophan	U C A G
C	CUU CUC CUA Leucine CUG	CCU CCC CCA Proline CCG	CAU Histidine CAC CAA Glutamine CAG	CGU CGC CGA Arginine CGG	U C A G
A	AUU AUC Isoleucine AUA AUG Methionine	ACU ACC ACA Threonine ACG	AAU Asparagine AAC AAA Lysine AAG	AGU Serine AGC AGA Arginine AGG	U C A G
G	GUU GUC GUA Valine GUG	GCU GCC GCA Alanine GCG	GAU Aspartic acid GAC GAA Glutamic acid GAG	GGU GGC GGA Glycine GGG	U C A G

Table: Dictionary of aminoacid code words

Any known aminoacids. These termination codons (stop/non sense codons) normally signal the end of polypeptide synthesis.

In a random sequence of nucleotides, one in every 20 codons in each reading frame, or on average a termination codon. In general, a reading frame without a termination codon among 50 or more codons is called an open reading frame(URF). Long ORFs usually correspond for a typical protein with a molecular weight of 60,000 would require an ORF with 500/more codons.

Codon Degeneracy

The code is highly degenerate. Three aminoacids-arginine, Leucine and serve are each specified by six codons and most of the rest are specified by either 3 or 2 codons. Only Methionine and Tryptophan, two of the least common aminoacids in proteins, are represented by a single codon. Codons that specify the same aminoacids are termed synonyms

When an amino acid has multiple codons. The difference between the codons usually lies in the third base (at the 3'end) for example, Alanine is coded by the triplets GCU,GCC, GCA & GCG

-XYC and XYU always specify the same aminoacid

-XYG and XYA do so in all but two cases

AUA Ile UAA

AUG Met UAG stop codons

UGG Trp UGA

- codons with a second position pyrimidine encode mostly hydrophobic amino acids.
- codons with second position purins encode mostly polar amino acids.

As a consequence of the genetic codes' degeneracy, many point mutations at a 3rd codon position are phenotypically silent, i.e. the mutated codon specifies the same aminoacid as the wild type.

2.5.2.3 Wobble hypothesis

In protein synthesis, the proper tRNA is selected only through codon-anticodon interactions; the aminoacyl groups doesnot participate in this process. Many tRNAs bind to 2nd or 3rd of the codons specifying their cognate amino acids.

For example:

- yeast $tRNA^{phe}$, which has the anticodon GAA, recognizes the codon UUU and UUC

anticodon : 3` – A-A-Gm-5` 3`-A-A-Gm-5`

codon: 5`-U-U-C-3` 5`-U U-U-3`

-yeast $tRNA^{Ala}$, which has the anticodon IGC recognizes the codons GCU, GCC, GCA

anticodon: 3`-C-G-3-5` 3`-D G I – 5` 3`-C G I – 5`

codon : 5`-G-C-U-3` 5`-G C C –3` 5`-G C A-3`

The 3rd base of the codons form weak H-bonds with Inosinate residue at the 1st position of anticodon. Examination of such codon-anticodon pairings led crick to conclude that the 3rd base of most codons paire rather loosely with the corresponding base of its anticodon. The third bases of such codons were called `wobble' crick proposed a set of relationship called the wobble hypothesis

1. The first two bases of codon is mRNA always form strong Watson-crick basepairs with the corresponding bases of the anticodon and confer most of the coding specificity.

2. The first base of some anticodons (in 5`-3` direction) determines the number of codons read by a given tRNA.

5`-anticodon base	3`-codon base
C	G
A	U
U	A or G
G	U or C
I	U, C or A

3. When an amino acid is specified by several different codons, those codons that differ in either of the first two bases require different t RNAs.

4. A minimum of 32 tRNAs are required to translate all 61 codons

The wobble base of the codon contributes to specificity, but because it pairs only loosely with its corresponding base in the anticodon, it permits rapid dissociation of the t RNA from its codon during protein synthesis. In all three forms strong Watson-crick base pairs. t RNAS would dissociate too slowly and severly limit the rate of protein synthesis. This codon-anticodon interactions optimize both accuracy and speed.

Universality

For many years it was thought that the standard genetic code was universal. This assumption was based on the observation that one kind of organism (ex:E.coli) can accurately translate the genes from quite different organisms (ex:humans). DNA sequencing studies in 1981 reveled that the genetic codes of certain mitochondria are variants of the standard genetic code.

For example, in mammalian mitochondria AUA as well as the standard AUG, is a methionine or initiation codon.

*UGA specifies Trp rather than stop

*AGA and AGG are `stop' rather than Arg

*more recent studies, however revealed that in ciliated protozoa, the codons UAA and UGA specify Glu rather than stop.

Normalcode Assigned :	UGA Stop	AUA Ile	AG^{A}G Arg	CUN Leu	CCG Arg
Vertebrates :	Trp	Met	Stop	+	+
Drosophila :	Trp	Met	Ser	+	+
S.cerevisiae :	Trp	Met	+	Thr	+
Filamentous fungi :	Trp	+	+	+	+
Trypanosomes :	Trp	+	+	+	+
Higher plants :	+	+	+	+	Trp

+ - the codon has same meaning as in 1 normal cod

** in E.coli UGA some times codes for selenocysteine, in addition to termination

At any rate, the standard genetic code is widely used, but not universal. The limited scope of code variants strengthens the principle that all life on this planet evolved on the basis of a single (very slightly flexible) genetic code.

2.5.2.4. Summary

The sequence of nucleotides in the mRNA molecule is read consecutively in groups of three nucleotides known as codons .The mRNA codons cannot directly recognize amino acids but Each tRNA contains a trinucleotide sequence anticodon, which is complementary to an mRNA codon specifying the tRNS's amino acid.In 1961, Marshall nirenberg and Himrich Matthaei reported an observation, that solved the genetic code..They decipher the genetic code by using homopolymers(poly-U,polyC,poly-A),tripletbindingassay,and polymers.the genetic code is universal and degenerate.In somecodons even though the third base is varid is recognized by same t-RNA which is known as wobble hypothesis

2.5.2.5 Model Questions

1) Explain the properties of genetic code

2.5.2.6 Reference books

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Dr.N.Srinivasa Reddy

Lesson 2.5.3

PROTEIN SYNTHESIS

Objective

This chapter gives an idea about protein synthesis from m-RNA with the help of t-RNA, ribosomes, and inhibitors of translation

2.5.3.1 Introduction

Proteins are the end products of most information pathways A typical cell requires thousands of different proteins at any given moment. These must be synthesized in response to the cell's current needs, transported to their appropriate cellular locations, and degraded when no longer needed.

-It is the most complex biosynthetic process

-Overall, almost 300 different macromolecules cooperate to synthesize polypeptides

- To different ribosomal proteins
- 20 or more enzymes to activate the amino acid precursors
- a dozen or more auxillary enzymes
- other protein factors for the initiation, elongtion, termination of polypeptide synthesis
- 100 additional enzymes for the final processing of different proteins.

- It accounts for upto 90% of the chemical energy used by a cell for all biosynthetic reactions.

-The 20,000 ribosomes, 100,000 related protein factors and enzymes and 200,000 tRNAs in a typical bacterial cell can account for more than 35% of the cell's dryweight.

-Despite the great complexity proteins are made at exceedingly high rates.

Example: a polypeptide of 100 residues is synthesized in *E.coli* cell in about 5 sec.

2.5.3.2 Transfer of RNA or tRNA

In 1955, crick proposed "adaptor hypothesis", according to which translation occurs through the mediation of adaptor molecules. Each adaptor was postulated to carry a specific enzymatically appended amino acids and to recognize the corresponding codon.

Crick suggested that these adaptors contain RNA because codon recognition would then occurs by complementary base pairing. At about this time, Zamecnik & Hogland discovered that, in the course of protein synthesis, c^{14}-labeled amino acids became transiently bound to a low molecular weight fraction of RNA. Further investigations indicated that these are tRNAs which were first called as sRNAs. (Soluble RNA's)

-tRNAs are relatively small and consists of a single stand of RNA folded into 3-dimensional structure.

-In bacteria & in cyosol of eukaryotes, tRNAs have between 73 and 93nt, corresponding to molecular weight 24-31Kda.

-There is at least one kind of tRNA for each amino acid, for some amino acids there are two or more tRNAs.

Structure

- Many tRNAs have been isolated in homogenous form.

- In 1965, Robert W.Holley & his colleagues reported the first known base sequence of yeast Alanine tRNA ($tRNA^{Ala}$)

- This is the very first nucleic acid to be sequenced.

- It has 76nt residues, 10 of which have modified bases.

- Since then, the base sequences of many other tRNAs from various species have been solved and revealed many common features.

- 8 or more of the nucleotide residues of all tRNAs have unusual modified bases, many of which are methyl derivatives of the bases.

- Most tRNAs have a guanylate residue at 5`end and all have the Tri nucleotide sequence CCA(3') at the 3'end.

- All the tRNAs have a common secondary structures, called cloverleaf structure and have the following common features.

- a 5`-terminal phosphate group
- a 7bp stem including the 5` terminal nucleotide and that may contain non-Watson-Crick base pairs such as G.U. This is known as acceptor arm or amino acid stem because the amino acid residue carried by the tRNA is appended to its 3`-terminal OH.
- A 3- or 4- base pair stem ending in a loop that frequently contains the modified base DHU (dihydrouracil). The stem and loop are therefore collectively termed the D arm.
- A 5 base pair stem ending in a loop that usually contains the anticodon, the triplet of bases complementary to the codon. This is called anticodon arm.
- A 5 base pair stem ending in a loop that usually contains the sequence TψC, and called TψC arm.

- The longer tRNAs have a short variable extra arm. It has 3-21 nucleotides and may have a stem consisting of upto 7 base pairs.

Tertiary Structure

- In 1974, Alexander Rich in collaboration with, Sung Hou Kin elucidated

the X-ray crystal structure of Yeast $tRNA^{Phe}$.

- The molecule assumes an L-shaped conformation in which one leg of L is formed by the acceptor and T-stems folded into a continuous double helical like structure and the other leg is similarly composed of the D and anticodon stems.

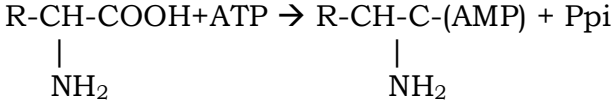
- Each leg of the L is ~60 A^o long and the antocodon and amino acid acceptor sites are at opposite ends of the molecules, some 76 A^0 apart. The narrow 20-25 A^0 width of native tRNA molecule is essential to its biological function.

tRNAs complex tertiary structure is maintained by H-bonding as well as stocking interactions. The structure also contains 9 base pairing interactions that cross link its tertiary structure. Remarkably all but one of these tertiary interactions are non-Watson-Crick interactions, and they are all located near the corner of the L structure. The structure is also stabilized by several unusual H-bonds between bases and either phosphate groups on 2`-OH group of ribose residues.

Aminoacyl –tRNA synthetases

Accurate translation requires the covalent attachment of the correct amino acid to a tRNA, which is catalysed by amino acid specific enzymes known as aminoacyl-tRNA synthetases (aa RSs). These enzymes append an amino acid to the 3`-terminal ribose residues of corresponding tRNA to form an aminoacyl-tRNA. This unfavourable process is driven by the hydrolysis of ATP in two sequential reactions that are catalyzed by a single enzyme.

1. The amino acid is first "activated" by reaction with ATP to form an aminoacyl – adenylate



This intermediate may be isolated although if normally remains lightly bound to the enzyme.

2. This anhydride then reacts with tRNA to form the aminoacyl-tRNA

Aminoacyl – AMP +tRNA → aminoacyl – tRNA + AMP

Some Aminoacyl – tRNA synthetases exclusively append an amino acid to the terminal 2=OH group of their corresponding tRNAs, and other do so at the 3`-OH group. This selectivity was established with the use of chemically modified tRNAs that lack either the 2-or 3-OH group of their 3`-terminal ribose residues.

The overall amino acylation reaction is:

Amino acid +tRNA +ATP→amino acyl+tRNA+AMP+Ppi

These reaction steps are radily reversible because the free energies of hydrolysis of the bonds formed in both amino acyl – AMP and amino acyl – tRNA are comparable to that of ATP hydrolysis. The overall reaction is driven to completion by the pyrophosphatase catalysed hydrolysis of the pyrophosphate generated in the first reaction step.

As elucidated by Paul Berg, tRNA is the acyl acceptor is amino acid activation, where as CoA performs this function in fattyacid activation.

There are two unrelated families of aminoacyl – tRNA synthetases, termed class I and class II aminoacyl RNA synthetases. Each have the same 10 member in all organisms. Detailed sequence and structural comparison of these two families is given by Diro Moras.

Many class I aaRSs require anticodon recognition to aminoacylate their cognate tRNAs. Incontrast, several class II enzymes do not interact with their bound tRNAs anticodon. All class I enzymes aminoacylate their bound tRNAs 3`-terminal 2`-OH group. Where as class II enzymes charge the 3`-OH group.

Prokaryotic aaRSs occur as individual proteins. In many higher eukaryotes aa RSs associate to form a multienzyme complex. The advantages of this system are unknown.

Non-sense Suppression

Non-sense mutations are usually lethal when they prematurely terminate the synthesis of an essential protein. Restoring the gene to its normal function requires a 2nd mutation that either converts the termination codon to a codon specifying an amino acid or alternatively suppresses the effects of the termination codon. The 2nd class of restorative mutations are called non-sense

suppresors – generally involve mutations in tRNA genes that produce altered tRNAs that can recognizes the termination codon and insert an amino acid at that position. Most suppressor tRNAs are produced by single base substitutions in the anticodons of minor tRNA species.

Non sense suppression does not completely disrupt information transfer in the cell. This is because, usually there are several copies of the genes for some tRNAs in any cell; some of these duplicate genes are weakly expressed and account for only a minor part of cellular pool of a particular tRNA. Supressor mutations usually involve these minor tRNA species, leaving the major tRNA to read its codon normally.

For example

There are three identical genes for $tRNA^{Tyr}$ in E.coli, each producing a tRNA with the anticodon 5` GUA 3`. One of these is expressed at relatively high levels-major $tRNA^{Tyr}$; the other two are transcribed in only small amounts. A change in the anticodon from 5` GUA to 5` CUA, produces a minor $tRNA^{Tyr}$ species that will insert tyrosine at UAG stop codons. This insertion of Tyr at UAG is inefficient, but can permit production of enough useful full-length protein from a gene with a non-sense mutation to allow the cell to live.

Some E.coli Nonsesnse suppressors

	Codn suppressed	aminoacid iserted
SU1	UAG	Ser
SU3	UAG	Tyr
SU6	UAA	Leu
UGA1	UGA	Trp
UGA2	UGA	Trp

2.5.3.3 Ribosomes

-1st observed by albert claude (1930s) and referred them to as Microsomes – by darkfield microscopy.

-In mid 1950s, George palade observed them in cells by electron microscopy – ribosomes.

Contain 2/3rd of RNAS & 1/3rd of protein.

Bacterial ribosomes

-There are 15,000 ribosomes or more in E.Coli cell and make up 1/4th of the cell's dry weight.

-Contain 65% RNA and 35% protein

-Have a diameter of about 18nm and sedementation coeffieicnt of 70s

-Can be disassociated into two unequal subunits as given by lalatson

-The larger one having a S of 50s and

-The smaller 30s subunit

-The 50s submit contains one molecule of 5s rRNA, one molecule of 23S rRNA and 34 proteins.

-The 30s submit contains one molecule of 16s rRNa and 21 proteins.

-The proteins are designated by numbers. Those in the large 50s subunit are numbered L1-L34 and those in smaller subunit S1 to S21.

-Each of the 55 proteins in *E.coli* ribosome is believed to play a role in the synthesis of polypeptides, either as enzymes or as a structural component in the overall process.

-The two oddly shaped subunits fit together in such a way that a cleft is formed through which the mRNA, passes as the ribosome moves along it during the translation.

Eukaryotic ribosomes

-larger and more complex than bacterial ribosomes

-have a diameter of about 23nm and as of about 80s

-they also have subunits which vary in size between species but on average are 60s and 40s

-the small subunit contains 18s rRNA and ~ 33 proteins.

-The large subunit contains 5s rRNA, 5.8s rRNA, 28s rRNA and 49 proteins

-in contrast, the ribosomes of mitochondria and chloroplasts are some what smaller and simpler than bacterial ribosomes.

Structure and functionalities of ribosome subunits

-The 3`end of the rRNA participate in mRNA binding – located on the small subunit's platform

-The anticodon binding sites occur in the small subunit's cleft region

-Large subunit's stalk participate in the ribosome's various GTP^{ase} reactions.

-The peptidyl transferase function (p) occupies the valley, between the large subunits other two protuberances

Thus the large subunit appears to be mainly involved in mediating biochemical tasks such as catalyzing the reactions of polypeptide elongation, where as the small subunit is the major anchor in ribosomal recognition process, such as mRNA and tRNA binding processes.

2.5.3.4 Polypeptide synthesis

Polypeptide synthesis begins at the Amino-terminal end

The direction of ribosomal polypeptide synthesis was established in 1961, by Howard Dintzis. Reticulocytes, that were actively synthesizing hemoglobin were incubated withradioactive leucine. (Leu occurs frequently along both the α-and β-globin chains) samples of completed β-chains were isolated from the reticulocytes at various times after addition of radioactive leu. The distribution of radioactivity along the β-chain was determined, with the expectation that it would be concentrated at the end that was synthesized last.

Isolated after 60min of incubtion, rearly all the Leucine residues were radioactive in β-chains. However, in completed globin chains that were isolated only a few min after *leu was added, * Leu were concentrated at the c-terminal end.

Fig. Proof of polypeptide chains grow in N→C terminal

From these observations it was concluded that polypeptide chains are begun at the N-terminal end and are elongated by sequential addition of residues to C-terminal end. This applies to all proteins in all cells.

Ribosomes read mRNA in 5`→ 3` direction

The direction that the ribosome reads mRNAs was determined through the use of a cell-free protein synthethesis system in which the mRNA was poly(A) with the 3-terminal cytosine.

5` A-A-A----------A-A-A-C-8`

Such a system synthesizes poly(Lys) that has C-terminal Aspargine.

H_3N^+-Lys-Lys-Lys……Asn – coo-.

This together with the knowledge that AAA and AAC code for Lys and Asn and the polarity of polypeptide synthesis, indicates that the ribosome reads mRNA in the 5`→ 3` direction. Since, mRNA is synthesized in the 5`→ 3` direction, this accounts for the observation that, in prokaryotes, ri9bosomes initiate translation on nascent mRNA.

2.5.3.5 Prokaryotic Translation

The complex process of translating mRNA into protein can be divided into three stages

- initiation
- Elongation
- Termination

Initiation

The initiation of polypeptide synthesis in bacteria requires

- the 30s ribosomal subunit which contain 16s rRNA
- the mRNA coding for the polypeptide to be synthesized
- initiating fMet - $tRNA^{tmet}$
- a set of three proteins called Ifs
- GTP
- 50^s ribosomal subunit

A specific amino acid initiates protein synthesis

Although there is only one codon for Met(AUG), there are two tRNAs for Met in all organisms. One tRNA is used exclusively when AUG represents the initiation codon for protein synthesis. The 2nd is used when Met is added at an internal position in a polypeptide.

In bacteria, the two tRNAs specific for Met are designated as $tRNA^{Met}$ and $tRNA^{iMet.}$ The starting aminoacid at N-terminal end is N-formyl Methionine. It enters the ribosome as N-formyl Methionyl-$tRNA^{fMet}$, which is formed in two successive reactions.

1. Methionine is attached to $tRNA^{tMet}$ by the Met=tRNA synthetase. There is only one of these enzymes in E.Coli and aminoacylates both $tRNA^{Met}$ and $tRNA^{tMet}$.

Met +$tRNA^{tMet}$+ATP→ Met-$tRNA^{tMet}$+AMP+ppi

2.A fomayl group is transferred to the $–NH_2$ group of the Met residue from N^{10} – formyl tetrahydrotolate by a trans-formylase enzyme.

N^{10}-formyl FM_4+Met-$tRNA^{tMet}$→fMet-$TRNA^{fMet}$+FM_4

This transformylase is more slective, it is specific for Met residues attached to $tRNA^{tMet}$. Presumably recognizing some unique structural feature of that tRNA.

Initiation Complex

To begin the assembly of a bacterial translation complex, sequential interactions occur between specific proteins initiation factors and the small 30s ribosomal subunit. The resulting preintiation complex, together with fMet-$tRNA^{fMet}$ then binds the mRNA at specific sequence near the initiating AUG and thus forms the 30s initiation complex. The formation of initiation complex takes place in three steps.

1.- The 30s ribosomal submit binds IF-3 which prevents the 30s and 50s subunits from combining prematurely.

-binding of mRNA to the 30s subunit then takes place in such a way that the initiation codon binds to precise location on 30s Ribosomal subunit.

-the initiating AUG is guided to the correct position on the 30s ribosomal subunit by an initiating signal called the shine-dalgarno sequence in the mRNA,centered 8-13 bases from the 5`end of AUG.

-The sequence generally contains 4-9 purine residues and base pairs with the complementary pyrimidine rich sequence at the 3`end of 16s rRNA of 30s ribosomal subunit.

-This mRNA –rRNA interaction fixes the mRNA so that the AUG is correctly positioned for initiation of translation.

-AUG is positioned in the P or peptidyl site, which is the only site to which fMet-$tRNA^{fMet}$ can bind.

2. Complex consisting of the 30s subunit, IF-3, mRNA now forms a still larger complex by binding IF-2, which already is bound to GTP and the initiating fMEt-$tRNA^{fMet}$. The anticodon of this tRNA pairs correctly with initiation codon in this step.

3. This large complex combines with the 50s ribosomal subunit. Simultaneously, the GTP molecule bound to IF-2 is hydrolyzed to $GDP^{+}Pi$. IF-3 and IF-2 also depart from the ribosome.

- The correct binding of the fMet – $tRNA^{tMet}$ to the P site in the complete 70s initiation complex is assured by Codon-anticodon interactions

- Binding interactions between the P site and f Met-$tRNA^{fMet.}$

Elongation

The stepwise addition of aminoacids to the polypeptide chain requires – the initiation complex

-the next aminoacyl-tRNA sepecified by the next codon in mRNA

-cytosolic protein factors – Elongation factors

-GTP

Three steps takes place in the addition of each amino acid residue,and this cycle is repeated as many times as there are residues to be added.

1.The next aminoacyl-tRNA is first bound to a complex of EF- Tu containing a molecule of bound GTP.

-The resulting aa-tRNA –EF-Tu.GTP complex is then bound to the a site of the 70s initiation complex.

-The GTP is hydrolyzed, an EF-Tu. GDP complex is released from the 70s ribosome and an EF-Tu. GTP complex is regenerated.

2. A new peptide bond is formed between the amino acids bound their tRNAs to the A & P sites on the ribosome.

-This occurs by the transfer of the initiating N-formyl methionyl group from its tRNA to the – NH_2 group of the second amino acid now in the A-site.

-NH_2 group of the aminoacid in A site, acts as a nucleophite, displacing the tRNA in the P site to form the peptide bond. This reaction produces a dipeptidyl – tRNA in the A site and the now deacylated tRNa remains bound to the P site.

-The enzyme activity that catalyzed peptide bond formation has been referred to as peptidyl transferase and was widely assumed to be intrinsic for 1 or more proteins in the 50s ribosomal subunit. In 1992, Harry Noller and colleagues discovered that this activity was catalyzed not by a protein, but by the 235 rRNA, another critical biological function for ribozymes.

3. In the final step of elongation, called translocation, the ribosome moves by the distance of one codon toward the 3`end of the mRNA.

-because the dipeptidyl – tRNA is still attached to the 2^{nd} codon of mRNA, the movement of the ribosome shifts the dipeptidyl – tRNA from the A site to P site and the deacylated tRNA is released from the P site back into the cytosol.

-the 3^{rd} codon of the mRNA is now in the A site and the 2^{nd} codon in Psite.

-This shift of the ribosome along the mRNA requires EF-G(translocase) and the energy provided by the hydrolysis of another molecule of GTP.

-the ribosome, with its attached dipeptidyl – tRNA and mRNA is now ready for another elongation cycle to attach the 3^{rd} amino acid residue.

-the ribosome moves from codon to codon along the mRNA toward the 3`end, adding one amino acid residue at a time to the growing chain.

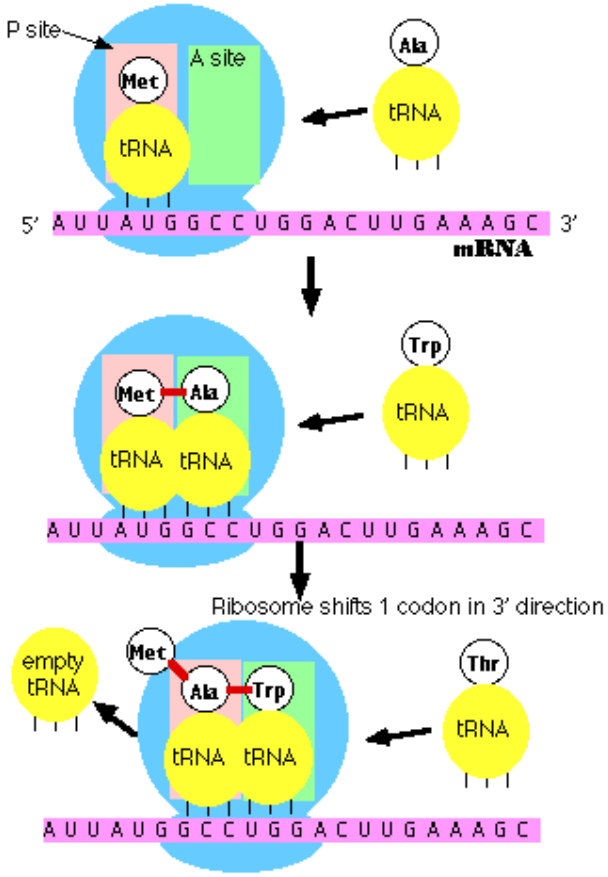
Termination

Elongation continues until the ribosome adds the last amino acid, eompleting the polypeptide coded by the mRNA. Termination, is signaled by one of the three termination codons in the mRNA (UAA, UAG,UGA), immediately following the last amino acid codon.

In bacteria, once a termination codon occupies the ribosomal A site three termination factors/releasing factors, RF_1, RF_2, and RF_3 contribute to the

hydrolysis of the terminal peptidyl – tRNa bond release of the free poly peptide and the last tRNA, now unchanged from P site the dissociation of the 70s ribosome into 30s & 50s subunits, ready to start a new cycle of polypeptide synthesis.

RF 1 recognizes termination codons UAA & UAG and RF2 recognises termination codons UAA & UGA. Either RF1 & RF2 bind at termination codon and induces peptidyl transferase to a water molecule rather than to another aminoacid. The specific function of RF3 has not yet been firmly established.



2.5.3.6 Eukaryotic translation

Initiation

Two eukaryotic factors eIF_3 (a large multimeric protein with about 8 subunits) and eIF_6 keep the ribosomal subunits apart. Eukaryotic preinitiation complex will be formed from an active ternary complex of eIF_2 bound to a GTP

molecule & Met-tRNAi^{Met} associated with a small (40s) ribosomal subunit complexed with two other factors eIF_3 and eIF 1A.

Cells can regulate protein synthesis by phosphorylating a ser residue on eIF_2 bound to GDP, this complex is then unable to bind Met- tRNA^{Met}, thus inhibiting protein synthesis.

Most eukaryotic mRNAs have a single start site near the 5` capped end of the mRNA. A cap binding protein, $eIF_4$ recognises the 5`-cap structure present on all eukaryotic mRNA. After recognition any secondary structure at the 5`end is removed by an associated helicase activity.

The bound preinitiation complex then probably slides along the mRNA, most often stopping at the first AUG, However, selection of the initiation AUG is facilitated by specific surrounding sequences called KOZAK sequences.

mRNA 5` - ACCAUGG---------

The A preceeding the AUG seems to be most important nucleotide affecting initiation efficiency.

Scanning of the mRNA by preinitiation complex yields 40s initiation complex, in which Met-tRNA^{Met} is correctly positioned at the translation start site. Once,40s initiation complex is formed with its correctly positioned Met-tRNA^{Met} at the start codon, the 60s ribosomal submit binds completing the formation of 80s initiation complex.

Elongation

-same as in prokaryotes

-Elongation factors only differ

EF1 &-GTP ~ EF – Tu = GTP

EF2 – GTP ~ EF – G-GTP

Termination

- same as in prokaryotes

- Contains a single releasing factor CRF 1

-

Proof Reading

The GTPase activity of EF-Tu makes an important contribution to the rate and fidelity of the overall biosynthetic process. The EF-Tu. GTP complex exists for a few milliseconds and the EF-Tu. GDP complex also exist for the same period, before it dissociates. Both of these intervals provide an opportunity for the codon – anticodon interactions to be verified(i.e. proof red). Incorrect, aa-tRNAs normally dissociate during one of these periods.

If the GTP analog, GTPs us used in place of GTP, hydrolysis is slowed, improving the fidelity but reducing the rate of protein synthesis.

This proof reading mechanism establishes only that the proper codon-anticodon pairing has taken place. The identity of amino acids attached to tRNAs is not at all checked on the ribosome. This was demonstrated experimentally by two research groups led by Fritz Lipmann and Seymour Benzer in 1962.

They isolated enzymatically formed cys-$tRNA^{cys}$ and then chemically converted it into Ala-$tRNA^{cys}$. This hybrid aa-tRNA, whichcarries Ala but contains the anticodon for cys, was then incubaked with a cell free system capable of protein synthesis. The newly synthesized polypeptide was found to contain Ala residues in positions that should have been occupied by cys. So, on ribosomes only the codon-anticodon interactions will be verified and the fidelity of this proteinsynthesis process depends on the central role of aminoacyl t-RNA synthetases.

Proof reading by aminoacyl tRNA synthetases

The potential for any enzymes to descriminte between two different substates is limited by the available binding energy that can be derived from Enzyme-substate interactions.

-The first filler is the initial amino acid binding and activation to amino acid – AMP

-The second filler is the separate active site, which catalyses the deacylation of incorrect amino acid – AMPs

-in addition to proof reading after formation of the amino acid – AMP, most amino acid tRNA synthetases are also capable of hydrolyzing the ester linkage between amino acid and tRNA in aa-tRNA. This hydrolysis is greatly accelerated for incorrectly charges tRNAs – 3rd filter.

-in a few aminoacyl –tRNA synthetases that activate amino acids, that have no close structural relatives little or no proof-reading occurs, in these cases the active site can sufficiently discriminte between the proper substrate amino acid and the incorrect amino acids.

2.5.3.7 Inhibitors of Protein Synthesis

Protein synthesis is the primary target of a wide variety of naturally accruing antibiotics and toxins. This is presumably a consequence of translational machinery's enoromous complexity which makes it vulnerable to disruption in many ways. Antibiotics have become vulnerable tools in the study of protein synthesis, nearly every step in protein synthesis can be specifically inhibited by one antibiotic or another.

PUROMYCIN

-made by the mold streptomyces alboniger

-has structural similarity to the 3`end of aa-tRNA

-it binds to the A site and participates in all elongation steps including peptide bond formation, producing a peptidyl puromycin.

-However, puromycin will not bind to the P site.

-it dissociates from the ribosome shortly after it is linked to the – coo terminus of the peptide, prematurely terminating synthesis of the polypeptide.

Streptomycin

- Medically important number of a family of antibiotics known as aminoglycosides, that inhibit prokaryotic ribosome's in a variety of ways.

-a low concentrations, it induces the ribosome to characteristically misread the mRNA – one pyrimidine may be mistaken for the other in 1st and 2nd codon positions and either pyrimidine may be mistaken for A in 1st codon position.

-At higher concentrations, however streptomycin prevents proper chain initiation and there by causes cell death.

-certain streptomycin – resistant mutants(str^{R}) have ribosomes with an altered protein S12 compared with streptomycin sensitive bacteria.

Chloroamphenicol

-inhibts the peptidyl transferase activity on the large subunit of prokaryotic ribosomes.

-protein L 16 is necessary for chloramophenicol binding

-chloramphenicol`s binding site must lie near the A site since it competes for binding with the 3`end of aa-tRNAs and puromycin but not with peptidyl-tRNAs.

Tetracyclins

-Tetracyclin and its derivatives are broad spectrum antibiotics

-binds to the small subunit of prokaryotic ribosome

-inhibts aa-tRNA binding

-Tetrocyclin resistant bacterialstrains have become quite common. Resistence is conferred by a decrease in bacterial cell membrane permeability to the drug rather than any alteration of ribosomal components.

2.5.3.8 Post-translational Modifications

Newly synthesized polypeptides in the membrane and lumen of the ER undergo 5 principal modifications before they reach their final destinations.

*formation of disulfide bonds

*proper folding

*addition and processing of carbohydrates

*specific proteolytic cleavages

*assembly into multimeric proteins

Only properly folded and assembled proteins are transported from the rough ER to the Golgi complex and ultimately to the cell surface or other final destination. Unfolded, misfolded, or partly folded proteins are retained in the rough ER or are retrieved from the cis-Golgi network and returned to the ER. Misfolded proteins and unassembled subunits of multimeric proteins often move from ER lumen back through the translocation into the cytosol where they are degraded.

1. Formation of disulfide bonds

Intermolecular & intramolecular disultide bonds help stablize the 3^0 and 4^0 structure of many proteins. These covalent bonds formed by the oxidative linkage of $^{-SH}$ groups, on two cysteine residues in the same or different polypeptide chains. In eukaryotic cells, disulfide bonds are formed in the lumen of the rough ER but not in cytosol. Thus disulfide bonds are found only in secretory proteins and in the exoplasmic domains of membrane proteins synthesis on the rough ER.

Because of higher amounts of reduced glutathione (GSH) cytosolic proteins synthesized on free ribosomes lack –s-s-bonds and depend on other interactions to stabilize their structures. (GSH=GSSG ratio 50:1 in cytosol)

In proteins that contain more than one –s-s-bond, the proper pairing of cys residues is essential for normal structure and activity. Disulfide bonds sometimes are formed sequentially while a polypeptide is still growing on the ribosome.

For example: During synthesis of the Ig light chain, which contains two –s-s-bonds, the first and second cysteins closest to the N-terminus from a-s-bond before the 3rd cys has even been added to the nascent chain, automatically ensuring the correct pairing of cys. Similarly, the 3rd pairs with the 4th to create the second –s-s-bond.

The –s-s-bonds in some proteins however, do not link cys that occur sequentially in the amino acid sequence.

For example: Proinsulin has three –s-s-bonds that link cystine 1-4,2-6, and 3-5. In this case the 1st-s-s-bonds that form spontaneously by oxidation of –SH groups may have to undergo rearrangements.

In cells, the rearrangement of –s-s-is accelerated by the enzyme protein –s-s-isomerase(PDI) which is found in abundance in the Er of secretory tissues in such organs as the liver & pancreas. In catalyzing rearrangement, PDI forms a disulfide bonded substate – enzyme intermediate. –s-s-bonds generally form in a specific order, first stabilizing small domains of a polypeptide, then stabilizing the interactions of more distant segments.

2. Proper folding

The ER contains several proteins that accelerate the folding of newly synthesized proteins with in the ER lumen. Protein disulfide isomerase(PDI) is one such folding catalyst; the chaperone HSP 70 is another. Like cytosolic HSP 70, this ER chaperone transiently binds to proteins and prevents them from misfolding or forming aggregates, there by enhancing their ability to fold into proper conformation. Two other ER proteins, the homologous lectins – calnexin

and calreticulin bind to certain carbohydrates attached to newly made proteins and aid in protein folding.

Other important protein folding catalysts are peptidyl-prolyl isomerase, a family of enzymes that accelerate the rotation about peptidyl-prolyl bonds in unfolded segments of a polypeptide.

For example: In drosophila an ER peptidyl –prolyl isomerase called Nina A is required for the folding of opsin, the membrane protein that absorbs light and triggers the visual response.

3.**Glycosylation**

Most plasma membrane and secretory proteins contain one or more carbohydrate chains. The addition and subsequent processing of carbohydrates is the principal chemical modification to most proteins some glycosylation reaction occur in the lumen of ER, others in lumina of Cis-medial – or trans golgi. Thus the presence of certain carbohydrates provide useful markers for their movement from the ER and through the Golgi cisternae.

The oligosacharide bound to either Asn or Ser/Thr are referred as N-linked and 0-linked oligo saccharides respectively. O-linked oligosaccharides are generally short, often containing only 1 to 4 sugar residues. Typical N-linked oligosaccharide always contain mannose as well as N-acetyl glucosamine and usually have several branches each terminating with a negatively charged sialic and residues.

NANA→ N-acetyl Neuraminic acid or sialic acid

Gal→ Galactose

Glc→ Glucose

GalNAc→ N-acetyl galactosamine

Glc Nac→ N-acetyl glucosamine

Fuc→ fucose

Man→ Mannose

O-linked sugars are added one at a time, and each sugar transfer is catalyzed by a different glycosyl transferees enzyme. In contrast, biosynthesis of N-linked oligosaccharide begins with the addition of a large preformed

oligosaccharide containing 14 sugar residues; subsequently certain sugars are removed and others are added, one at a time in a defined order with each reaction catalyzed by a different enzyme.

4. Assembly into multimeric proteins

Many important secretory & membrane proteins are built of two or more polypeptides. In all cases, these multimeric proteins are assembled in the ER. One important example is provided by the Igs which contain two heavy and two light chains, all linked by –s-s-bonds.

Haemagglutinin(HA) –the trimeric protein that forms the spikes protruding from the surface of the influenza virus particle.

-each spike is formed within the ER of an infected host cell from three copies of a precursor protein termed HAO, which has a single membrane spanning &-helix.

-In the Golgi complex, each of the 3 HA_o proteins is cleaved to form two polypeptides, HA_1 and HA_22; thus each spike in the virus particle contains 3 copies of HA_1 and HA_2.

-The timer is stabilized by interactions between the exoplasmic domains of the constituent polypeptides as well as by interactions between the 3 cytosolic and membrane spanning domains.

5. Proteolytic cleavage

Mutant misfolded secretary & Membrane proteins, as well as, the unassembled subunits of multimeric proteins, often are degraded within an hour or two after this synthesis in the RER. For many years researchers thought that the proteolytic enzymes in the ER catalyzed degradation of misfolded or unassembled polypeptides, but such proteases were never found. Recent studies have shown that misfolded membrane and secretory proteins are transported from ER lumen “backwards” through translocation into the cytosol where they are degraded by the ubiquitin-mediated proteolytic pathway.

2.5.3.9 summary

Proteins must be synthesized in response to the cell’s current needs, transported to their appropriate cellular locations, and degraded when no longer needed protein synthesis can be divided into three stages. Initiation, Elongation, Termination.The formation of peptide bond between amino acids is thermodynamically un favourable and the amino acids are unable to reconise the nucleotides on the m-RNA these two problems are solved by the activation of amino acids carried out by aminoacyl t-RNA synthetases. The activated amino

acids bind to the ribosome m-RNA complex with the help of translation factors. After binding to the ribosomes the peptide bond is formed by the peptidyl transeferase.

Then the de acylated t RNA's are removed by translocation step .This process is repeated for number of cycles until it reaches the termination codons UAA,UAG & UGA.These terminating codons are recognized by releasing factors which changes the specificity of peptidyl transferase leads to theformation bond between water and amino group of amino acid resulting in the release of nascent polypeptide chain.the polypeptide chin undergo number of post translational modifications such as *formation of disulfide bonds *proper folding *addition and processing of carbohydrates *specific proteolytic cleavages *assembly into multimeric proteins

2.5.3.10. Model questions

1) Compare the process of translation in prokaryotes with eukaryotes

2) Write a note on post translational modifications

2.5.3.11 Reference books

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Dr.N.Srinivasa Reddy

Lesson 2.5.4

MUTATIONS

Objective

2.5.4.1 Introduction

2.5.4.2 Mutations at the level of DNA sequence

2.5.4.3 Mutations at the level of organism

2.5.4.4 Detection of mutations

2.5.4.5.summary

2.5.4.6. Model questions

2.5.4.7. Reference books

Objective

Mutation is nothing but a sudden change. This chapter explain different types of mutations both at sequence of DNA level and at the level of chromosomes.

2.5.4.1 Introduction

The term mutation refers to all the heritable changes in the genome, excluding those resulting from incorporation of genetic material from other organisms. A mutation is an abrupt qualitative or quantitative change in the genetic material of an organism.

Hugo de Vries was the first to use the term `mutation' to describe phenotypic changes in oenothera lamarkiana, which were heritable.

First, the changes occur in the DNA sequence itself. These changes in the nucleotide sequence may occur in a gene. Finally different kinds of phenotypic changes can arise in an organism as a result of changes in a gene.

Changes in nucleotide sequence may result in changes in gene may lead to altered phenotypes in organisms

2.5.4.2 Mutations at the level of DNA sequence

A point mutation is the replacement of one nucleotide or few nucleotide pair(s) by another. A point mutation can be classified as

- Substitution of one nitrogen base by the other

  Transition : of purine substituted by another purine or if pyrimidine is replaced by another pyrimidine.

  Transversion : if purine is replaced by pyrimidine or vice versa.

- an insertion or deletion is the addition or removal of anything from one basepair upto quite extensive pieces of DNA.

- Inversion : excision of portion of the double helix followed by its reinsertion at the same position but in a reverse orientation.

1 2 3 4 5 6 7

-A-T-G-T-T-C-A

-7-A-C-A-A-G-T

Fig : Various types of sequence changes in the given DNA sequence.

1)Mutations at the level of Gene

1. Silent mutation

Occur of a point change takes place at the third nucleotide position of a codon and changes the codon, but owing to the degeneracy of the genetic code, not the amino acid sequence of the gene product and does not give rise to a mutant phenotype.

Missence mutation

This is also a point mutation, but in this case it does change the amino acid. Most point changes at the first or second nucleotide positions of a codon result in missence mutation. A few third position nucleotides also.

Changing nucleotide 4, from G to A produces an Arg codon instead of a Gly codon.

Similarly changing nucleotide 15, from A to T specifies phe ratherthan Leu.

A missence mutation gives rise to a polypeptide with a single amino acid change. Whether or not it causes phenotypic change depends on its precise location in the protein. Many proteins can tolerate some changes in their amino acid sequence, although a missence mutation that alters an amino acid essential for structure or function will inactivate the protein, that lead to a mutant phenotype.

Non-sense mutations

This is also a point mutation that changes a codon specifying an amino acid into a termination codon. The result is a truncated gene which codes for a polypeptide that has lost a segment at its carboxy terminees. In many cases, although not always, thus segment will include amino acids essential for the proteins activity and a mutant phenotype results.

Ex:

5' – ATG GGA GCT CTA TTA ACC TAA – 3'

Met Gly Ala Leu Leu Thr Stop

5' – ATG GGA GCT CTA TGA ACC TAA – 3'

Met Gly Ala Leu Stop

Frameshift mutations

This is the usual consequence of an insertion or delection event because the addition or removal of any number of basepairs that is not a multiple of three causes the ribosome to read a completely new set of codon down stream the mutation. It usually produces mutant phenotypes.

ATG GGA GCT CTA TTA ACC TAA TTT GA

Met Gly Ala Leu Leu Thr stop

ATG GGG AGC TCT ATT AAC CTA ATT TGA

Met Gly Ser Ser Ile Asn Leu/ Ile Stop

Deletion

ATG GGG CTC TAT TAA CCT AAT TT GA

Met Gly Leu Tyr Stop

2.5.4.3 Mutations at the level of organism

In order to produce a mutant phenotype, the nucleotide sequence alteration must produce a mutated gene product, that is unable to fulfill its function in the cell. In many cases the cell will be unable to tolerate the loss of the function and will die. Such mutations are called lethal mutations.

Some mutations inactivate proteins that are not essential to the cell and others result in proteins with reduced or modified activities. This is true for both pro-and Eukaryotes.

Auxotrophic mutants

This type of mutant lacks a gene product involved in synthesis of an essential metabolite such as an amino acid. However, these mutants can be kept alive if, the metabolite is supplied as a nutrient in the culture medium.

Example : A Trp auxotroph to *E.coli* that lacks one of the enzyme involved in Trp biosynthesis.

Conditional – Lethal mutants

This type of mutations can survive, but only if cultured under a particular set of conditions. The most common example are temperature, sensitive mutants which are able to survive at one temperature range (<30^{o}c, say) but die if the temperature is raised above this permissive threshold.

The mutations carried by these temperature sensitive organism is often one that affects an amino acid product. The mutated protein is able to retain its correct structure at a low temperature, but is essentially denatured and inactivated by heat.

Antibiotic resistant mutants

Antibiotics kill wild-type bacteria but have no effect on resistant mutants. Resistance to an Antibiotic can arise in several ways, but the commonest is when the biomolecule that is target for the antibiotic becomes altered.

Streptomycin interferes with protein synthesis by binding to ribosomal protein S_{12}, one of the small subunit component of E.coli ribosome.

When streptomycin is bound to the small subunit, the initiator tRNA cannot enter the p site and mRNA is not translated and the bacterium dies.

In streptomycin resistant mutant, the gene for the ribosomal protein S_{12} is mutated. This leads to an altered S_{12} protein, which is still able to fulfill its role as a ribosomal protein, but can no longer bound to streptomycin. Has no effect on the mutated bacterium.

Regulatory mutants

These have lost the ability to control expression of a gene or operon normally subject to regulation. For instance, it is possible to obtain E.coli mutants that express the genes of the lac operon even in the absence of lactose. These are called constitutive mutants.

Constitutive mutants arise through a mutation in the gene for the lac repressor, so that the repressor is either not produced or has an altered structure.

Reverse mutations

A point mutation can be reversed by a 2nd point mutation, an insertion event by a subsequent deletion and so on. These events are called back mutations and occur rarely.

Many mutations can also be corrected by second site reversions → a 2nd mutation that restores the original phenotype but does not return the DNA sequence to its precise un mutated form.

ATG GGA GCT CTA TTA ACC TAA

Met Gly Ala Leu Leu Thr stop

Missence mutation

ATG GGA GCT CTA TTT ACC TAA

Met Gly Ala Leu Phe Thr Stop

2nd site reversion

ATG GGA GCT CTA CTT ACC TAA

Met Gly Ala Leu Leu Thr Stop

Mutations can occur at any stage during development.

- if mutations occur in a germinal cell, before differentiation of gametes, it would influence several gametes and will thus affect all the individuals derived from these affected gametes.

- If mutation occurs in a gamete / zygote, a single individual will carry the mutation.

- If a mutation occurs in a cell after the zygote has undergone one or more divisions, only a part of the body will show the mutant character.

The first two are called germinal mutations and the last is called the somatic mutation.

Spontaneous and induced mutations

The background or spontaneous mutations occur suddenly in the nature. They have been reported in oenothera, maize, bacteria, bread molds, viruses, Drosophila, mice, man etc.

Besides naturally occurring mutations, the mutations can be induced artificially in the living organisms. Those agents which cause mutations artificially are called mutagens and the mutations are called as induced mutations.

Chemical Mutagens that effect replicating DNA

Base analoguer

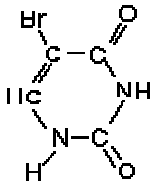
5-Bromo Uracil (5-bU) is derived from thymine by replacement of the methyl group with Bromine. It is sufficiently similar to thymine to be incorporated into a polynucleotide chain, in place of normal nucleotide.

5-bu, as tymine analog, base pairs with A and also pairs with G after a tautomeric shift.

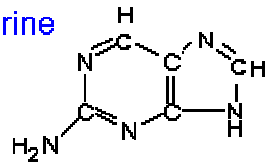
If the tautomeric shift happens during DNA replication, then one of the daughter molecules will have a 5bu-G bp instead of the original A-T bp. A further round of replication of the mutant molecule produces a G-C pair in one double helix, in which the mutation is now established and 5-bu-G/5-bu-A in the other daughter cell. This is an example how a point mutation can be brought about.

I. Base analogs

5-Bromouracil

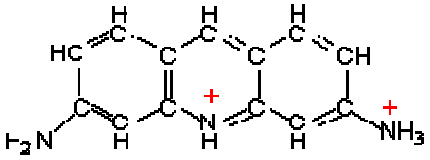


2-Aminopurine



II Acridines

2,8, Diamino acridine
(Proflavin)



Intercalating agents

Ex: Acridine dyes (Ethidium bromide, EtBr). Et Br is a A ringed molecule whose dimensions are similar to those of a purine – pyrimidine basepair, so that the compound can intercalate into a double helix, moving adjucent basepairs slightly apart. An insertion of a single nucleotide is likely to occur at the intercalation position and cause a frameshift mutation if the position lies within a gene.

Chemical mutagens that effect non replicating DNA

These are the substrates that can alter a base that is already incorporated in DNA and there by change its Hydrogen bonding specificity. Three commonly used mutagens are

Nitrous acid(HNO_2)

Primarly converts – NH_2 groups to keto groups by oxidative deamination. Thus Cytosine, Adenine and Guanine are converted to Uracil Hypoxanthine (H) and xanthine (X) respectively. These bases can form base pairs :

U – A

H – C

X – C

Conversion of G→ X is not directly mutagenic, since both G and X pairs with C. But in some single stranded DNA phages, x undergoes an undiscovered tautomeric change and is able to pair with thymine.

Hydroxylamine (HA)(NH_2OH)

HA probably causes hydroxylation of cytosine at amino group giving rise to hydroxyl cytosine, which then subsequently pairs with Adenine. So, a G-C pair can ultimately become an A-T pair.

EMS(CH_3-CH_2-O-SO_2-CH_3)

It is an alkylating agent and is a potent mutagen extensively with eukaryotes. Many sites in DNA are alkylated by these agents.

Of prime importance is the addition of an alkyl group to the H-bonding oxygen of Guanine and Thymine. These alkylations impair the normal H-bonding of these bases and cause mis pairing of Guanine and Thymine leading to the transitions.

Another phenomenon resulting from alkylation of Guanine is depurination. Depurination is not always mutagenic, since the gap left by loss of purine can be repaired by AP endonuclease.

However, sometimes the replication fork may reach the apurinic site before repair has occurred, when this happens replication stops just before the AP site (or) if the cell contains a functional SOS system, replication restarts after a brief pause.

However, with high probability an adenine is put in the daughter strand opposite to AP site. It gives a mismatch Purine-Adenine.

Physical mutagens

Heat

It is probably the most important environmental mutagen. Its effect on DNA molecule is to cause cleave the bonds between purine bases and their sugars, resulting in apurinic sites in polynucleotides. Pyrimidines are also removed but at a much slower rate.

Upto 10,000 apurinic sites are created everyday in every human cell, equivalent to one per chromosome every min or so. Those that escapes repair can cause point or deletion mutations when DNA is replicated.

Radiation

Several types of radiation are mutagenic. UV radiation of about 260nm is absorbed by purine and pyrimidine and can cause structural damages, particularly result in the formation of cyclobutyl dimers between adjacent pyrimidine in a polynucleotide. Dimerization causes the bases to stack closer together and can give rise to deletions during DNA replication.

2.5.4.4 Detection of mutants

Induced mutations and visible mutations. Both these types could be located either on sex chromosomes or on autosomes.

The procedures used to identify and isolate the mutants are referred to as Genetic screens and they depend on whether the experimental organism is haploid or diploid. If it is diploid whether dominant or recessive.

Detection of lethal mutants

In Haploids

In haploid organims all mutations are in effect dominant, so that the mutant phenotype is exhibited immediately in the progeny of the mutagenized population. For instance, mutations that disrupt Arginine synthesis lead to cells that require Arginine for growth. Such mutations are easily detected by growing them in the presence and absence of Arginine.

In prokaryotes and haploid eukaryotes viz, yeast, essential genes can be studied through the use of conditional mutations.

Example: Temperature sensitive mutants.

L.H. Hartwell & colleagues studied a particulary important temp. sensitive screen in the yeast *Saccharomyces cerevisiae* in lak 1960s and early 1970s. They setout to identify genes important in regulation of the cell cycle.

Screening

- Yeast cells were grown in a large liquid culture, treated with a chemical mutagen and then subcultured into a – small aliquots.

- After a – 5 he growth period at 23^{o}c, aliquots from each tube were separately plated onto a medium and incubated at 23^{o}C.

- Then, the colonies developed were replica-plated onto two plates → incubated at permissive temp 23^{o}C

  → other at non-permissive temp. 36^{o}c

- the temp sensitive colonies grow at 23^{o}c but not at 36^{o}c were assessed to determine whether they were blocked at specific stages in the cell cycle.

- The cell cycle stage at which cell growth was arrested at the non-permissive temp. indicated when the protein encoded by the mutated gene was required.

In diploids

In diploid organism (ex. Drosophila), phenotypes resulting from recessive mutations can be observed only in individuals homozygous for the mutant allele.

Autosomal mutations

Mutation on chromosome 3.

This approach requires. Three sequential crosses. Many males are treated with a mutagen (EMS), producing flies carring various mutations (M_1, M_2 etc..) in their germline cells. The level of mutagen used is sufficiently to induce atleast one mutation on each chromosome. These males carry a non-lethal recessive mutation that gives rise to a visible phenotype in homozygotes, the marker in this example is rosy (ry) eye color.

1st cross: In the first cross ($P_{1)}$, mutagenized males are mass-mated to a large no.of females.

The females carry dominant visible markers (D_1 and D_2) on chromosome 3. These are non-lethal in heterozygous condition, but are lethal in homozygoles.

2nd cross: In the second cross (P_2), individual heterozygous F_1 ----- carrying mutagenised chromosome 3 are mated individually to non – mutagenized organisms.

The F_2 progeny homozygous for either dominant marker will die, those heterozygous for both markers are easily identified and excluded. The F_2 heterozygotes includes O and O that have the identical mutagenized chromosome carrying `ry' marker and one non-mutagenized chromosome carrying a single dominant visible marker.

3rd cross :

F_2 generation, heterozygous brothers and sisters are mated individually in the third cross (P_s).

The absence of flies with rasy colored eyes in F_3 progeny indicates the presence of an induced lethal mutation (M_1 for ex). Although flies homozygous for M_1 donot survive, heterozygotes carrying M_1 on one chromosome and one of the dominant marker on the other will survive.

The mutation can be maintained in heterozygous flies.

Sex-linked lethals

Muller-5 method: This method makes use of a muller-5 Drosophila stock, which carrier two marker genes, dominant `Bar' (barred eye) and recessive `apricot' but doesn't contain a lethal gene.

In F_2 generation 50% -- are muller-5 in phenotype and remaining 50% are wild type. If a lethal mutation is induced in x-chromosome of irradiated ---, no wild type ---would appear in F_2 generation. Therefore, the absence of wild type ---- in F_2 is an indication of an induced lethal mutation.

Sexlinked visible mutations

For detection of sex-linked visibles, muller-5 and attached x-chromosome were used. The attached x ---- (xxy) have a special advantage when these --- are crossed to an irradiated ---, x-chromosome of irradiated --- goes either to super female daughter or to the sons. Since in sons there is only a single – x chromosome, any visible induced mutation will immediately express itself and can be easily scored.

Isolation of mutants

There are a number of selective techniques to isolate mutants, when they arise in a parent organism.

Enrichment

It is possible to selectively pickout strains of microorganisms with specific capabilities. For example, we may wish to isolate a rare bacterial cell, present in

the culture of a prototroph with the ability to utilize a particular, non-utilizable growth substance.

For this purpose, the bacterial cell culture is switched to a minimal medium, containing x as the only energy source. Although most bacterial cells cann't grow, a few cells utilize x and grow slowly. By repeated sub-culturing on medium having x, we could enrich the culture and exclude, large population which lack the ability to utilize x.

Filteration enrichment (fungal spores)

Concentration of auxotrophs can also be increased by filtration of a culture grown in suspension culture with minimal medium on which only protorophs grow, and yield mycelia, Auxotrophic spores cannot do so. Suring filtration, only prototrophs will be filtered out due to filamentous mycelia and non-growing auxotrophs will be in the filtrate. This filtrate can be used for culturing of auxotrophs on supplemented media and thus they can be concentrated. This method was derived by Woodward, 1954 and is known as Woodward's filtration technique.

Penicillin enrichment

Bacteria and fungi (Neurospora) in proliferative stage are sensitive to penicillin as it kills growing cells by specifically blocking the synthesis of cell wall precursors and is not lethal to non-proligerating cells. Therefore in a suspension culture with minimal medium, if penicillin is added, the prototrophs will be killed. The auxotrophs survive due to lack of growth. After killing prototrophs, the penicillin can be removed by washing the cells on a filter. If the culture is now plated on a supplemented medium, auxotrophs will grow. Multiple auxotrophs can be selected by this method.

Replica plating

It is a simple and powerful technique deviced by `Lederberg' in 1952. This tecynique is conveniently utilized for microorganisms forming colonies.

The material is first grown on complete medium. For ex: if streptomycin resistant mutants are to be isolated, material should be allowed to grow on medium lacking streptomycin, so that both mutant and wild types may grow. These colonies are imprinted on velveteen by inverting the potriplate. As a result, all colonies are imprinted and leave cells at corresponding position on velveteen. Other plates having streptomycin can then be pressed on velvet to get an impression. On this plate now, only resistant cells will form colonies. Knowing the position of mutants in the original plate, they could be isolated and multiplied.

Utility of mutants

Mutations are normally deleterious and recessive and therefore majority are of no practical value. A gustafsson estimated that < 1 in 1000 mutants produced may be useful in plant breeding. .

- Mutant microorganisms are useful in industry.
- Mutation breeding helps to generate varieties of plants with characters like resistance to drought, pests and pesticides.
- One of high yielding varieties of Rice, Reimei was developed through mutations isolated after irradiation.
- Protein and Lysine content was increased through mutations in wheat.
- Crop harvesting time is decreased in Aruna variety castor from 270 days to 140 days.
- Increased yield of penicillin was obtained from mutant penicillium fungus.
- Many of our fruit varieties resulted from spontaneous somatic mutations.
- Mutants are important tools for unraveling metabolic pathways.

2.5.4.5.summary

The term mutation refers to all the heritable changes in the genome . A point mutation or gene mutation is the replacement of one nucleotide or few nucleotide pair(s) by another. A point mutation can be classified asSubstitution, ransition,Transversion or Inversion and these mutations may be natutal or induced by some chemical and physical agents.the mutant organisms can be isolated by replicaplating method. Mutations are normally deleterious and recessive and therefore majority are of no practical value However, mutants had some uses. in industry,or for getting good yielding varities etc

- **.5.4.6 Model questions**

1) Explain different types of mutations in detail

2) Write in detail about the detection of mutations

2.5.4.7. Reference books

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Dr.N.Srinivasa Reddy