MICROBIAL PSYSIOLOGY AND BIOCHEMISTRY

(DMB03)

(MSC MICROBIOLOGY)

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Lesson - 1

BACTERIAL NUTRITION

1.0 Objective

To understand the types of bacteria based on their nutritional requirements, the various nutrient elements required by them and the modes of nutritional transport into their cells form the external environment.

1.1 Introduction

Bacteria need a variety of chemical substances to obtain energy and to construct the new cellular components. The basic elements or constituents of a cell come from the natural environment and are transformed by the cell into characteristic constituents of the cell. The substances used in biosynthesis and energy production by the cell are called

nutrients and therefore required for growth. The process of building of cell constituents by the cell from the nutrients obtained from its environment is called Anabolism or Biosynthesis. Anabolism is an energy-required process and the cell acquire this energy either directly from the light or from the breakdown of organic compounds or inorganic compounds into simpler substances. This process of breakdown of organic or inorganic chemicals into simpler constituents is known Catabolism.

1.2 Nutritional Classes of bacteria

Besides the nutrient elements, bacteria also require sources of energy, hydrogen and electrons for their growth. Basing on the sources from which these requirements are available, bacteria can be grouped into different nutritional classes.

Energy sources

1) Light energy ---------- Phototrophs
2) Organic or Inorganic compounds ---------- Chemotrophs

Hydrogen or Electron sources

1) Reduced inorganic substances ----------Lithotrophs
2) Organic molecules ---------- Organotrophs

Principal Carbon source

1) CO_2 ---------- Autotrophs
2) Reduced, preformed, organic molecules ----- Heterotrophs

Bacteria exhibit a great diversity in metabolism. Basing on the primary sources of energy, hydrogen and/or electrons and carbon used, bacteria are categorized into four main nutritional classes viz., Photolithotrophic autotrophs, Photoorganotrophic heterotrophs, Chemolithotrophic autotrophs and Chemoorganotrophic heterotrophs. Of these, photolithotrophic autotrophs and chemoorganotrophic heterotrophs include large majority of the organisms that are studied well. The other two classes include fewer organisms but are ecologically very important. In response to environmental changes, a particular species belonging to a particular nutritional class may alter its nutritional or metabolic pattern.

1.2.1 Photolithotrophic autotrophs

Also called simply as Photoautotrophs. Organisms use the light as energy source and CO_2 as carbon source. Inorganic molecules like hydrogen, hydrogen sulfide and elemental sulfur are used as electron donors. Eg: Purple and green sulfur bacteria and Cyanobacteria.

1.2.2 Photoorganotrophic heterotrophs

Organisms use light as energy source and organic matter as electron donor as well as carbon source. These organisms are common inhabitants of polluted lakes and streams. Eg: Purple non-sulfur bacteria and Green non-sulfur bacteria.

1.2.3 Chemolithotrophic autotrophs

Organisms derive both energy and electrons for biosynthesis from reduced inorganic compounds such as iron, nitrogen and sulfur molecules. The carbon source is CO_2. These chemolithotrophs greatly contribute to the chemical transformations of elements that continually occur in ecosystem. The best known examples of this class are Sulfur-oxidizing bacteria, Hydrogen bacteria, Nitrifying bacteria and Iron bacteria.

1.2.4 Chemoorganotrophic heterotrophs

This class is also referred as chemoheterotrophs and sometimes even as heterotophs. They use the organic compounds as sources of energy, hydrogen, electron and carbon for biosynthesis. In most of the cases, the same organic nutrient will satisfy all these requirements. This group includes most non-photosynthetic bacteria.

1.3 Nutritional Elements

The approximate elementary composition of the bacterial cell is 50% carbon, 20% oxygen, 14% nitrogen, 8% hydrogen, 3% phosphorus, 1% sulfur, 1% potassium, 1% sodium, 0.5% calcium, 0.5% magnesium, 0.5% chlorine, 0.2% iron and others account to 0.3%. This cell composition shows that over 95% of cell dry weight is made up of a few major elements. Basing on the amounts in which required , the elements are grouped into two main categories viz., Macroelements and Microelements.

1.3.1 Macroelements

Also be called major elements or macronutrients. This category includes elements such as carbon, oxygen, nitrogen, hydrogen, sulfur, phosphorus, potassium, magnesium, calcium and iron and contribute to over 95% of the cell dry weight. The first six (C,O,H,N,S,and P) are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining four macroelements exist in the cell as cations and play a variety of roles.

Element	**Usual form in nature**	**Chemical form in culture media**	**Functions**
Carbon	CO_2; Organic compounds	Glucose, malate, acetate, pyruvate etc; complex mixtures (yeast/peptone)	Backbone for all cell organic molecules
Hydrogen	H_2O; Organic compounds	H_2O; Organic compounds	pH maintenance, Hydrogen bonds in macromolecules, prime force in oxidation-reduction reactions
Oxygen	H_2O; O_2; Organic compounds	H_2O; O_2; Organic compounds	Major component in carbohydrates, lipids, proteins
Nitrogen	NH_3, NO_3^-, N_2, Organic Nitrogen compounds	Inorganic: NH_4Cl, $(NH_4)_2SO_4$, KNO_3, N_2 Organic: Amino acids, nitrogen bases, N-containing organic compounds	Major constituents of proteins and nucleic acids; present in peptidoglycan of cell wall
Phosphorus	PO_4^{3-}(inorganic), Organic phosphates	KH_2PO_4, Na_2HPO_4	Present in nucleic acids, phospholipids, ATP, several cofactors, some

			proteins and other cell components
Sulphur	H_2S, SO_4^{2-}, Organic S compounds, Metal sulphides	Na_2SO_4, $Na_2S_2O_3$, Na_2S, Cysteine, other organic sulphur compounds	Play a structural role in cysteine and methionine amino acids; present in a number of vitamins like thiamine, biotin, lipoic acid
Potassium	K^+ solution /various K salts	KCl, KH_2PO_4	Important in enzyme action and maintain osmotic potential and electrical potential within the cell
Magnesium	Mg^{2+}solution/various Mg salts	$MgCl_2$, $MgSO_4$	For stabilization of ribosomes, cell membranes and nucleic acids; for the activity of many enzymes that involve in phosphate transfer; integral part of chlorophyll molecule
Calcium	Ca^{2+}solution/$CaSO_4$/other Ca salts	$CaCl_2$	Actually not essential for growth of many bacteria, in certain higher bacteria it forms deposits of calcium carbonate and calcium oxalate; gives stability to some extracellular enzymes and to cell

			wall; contributes to the heat-stability of bacterial endospores
Iron	Fe^{2+}/Fe^{3+}solution /FeS, $Fe(OH)_3$/other Fe salts.	$FeCl_3$, $FeSO_4$, various chelated iron solutions	Plays a major role in cellular respiration; key component of cyto-chromes, ferridoxins and iron-sulphur proteins that involves in electron transport

1.3.2. Microelements: These are also called micronutrients or trace elements. These trace elements are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure. These microelements such as manganese, zinc, cobalt, molybdenum, nickel and copper are needed by most of the cells.

Element	**Function**
Zinc	Plays a structural role in many enzymes including carbonic anhydrase, alcohol dehydrogenase, RNA and DNA polymerases, alkaline phosphatase, aldolase; in DNA binding proteins; holds the protein subunits together in proper configuration for enzyme activity
Manganese	Activator of many enzymes; component in certain superoxide dismutases; component in water-splitting enzyme of photosystem II in oxygenic phototrophs
Molybdenum	Present in certain enzymes viz., molybdoflavoproteins; in nitrogenases; in formate dehydrogenases
Copper	Play a role in certain enzymes involved in respiration; present in cytochrome oxidase and oxygenases; in some superoxide dismutases; involves in synthesis of melanin
Cobalt	Constituent of B_{12}
Nickel	Present in urease and hydrogenases; part of coenzyme F_{430} of methano-gens; required for autotrophic growth of hydrogen-oxidizing bacteria

1.3.3 Growth factors

Many photoautotrophic microorganisms often grow well and reproduce when minerals and sources of energy, carbon, nitrogen, phosphorus and sulfur are supplied. These organisms have the enzymes and pathways necessary to synthesize all cell components. But many microorganisms lack one or more essential enzymes to build up their organic cell constituents as they cannot synthesize them. Any organic compound, other than the carbon and energy source, required essentially but cannot be synthesized by organisms is called growth factor. So, these substances must be provided as nutrients.

There are three major classes of growth factors viz., (1) amino acids, (2) purines and pyrimidines, and (3) vitamins. The amino acids are required for protein synthesis whereas purines and pyrimidines are needed for nucleic acid synthesis. Vitamins are the small organic molecules that usually make up all or part of enzyme cofactors, and very small amounts sustain growth. Some microorganisms like *Enterococcus faecalis*, a lactic acid bacterium, require as many as eight different vitamins for growth. Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth response assays for a variety of substances.

Vitamin	Functions	Example
Biotin	Carboxylation (CO_2 fixation); Carboxyl transfer; Fatty acid biosynthesis	*Leuconostoc mesenteroides*
Cobalamin (B_{12})	Molecular rearrangements; reduction and transfer of single carbon fragments; synthesis of deoxyribose	*Lactobacillus* spp.
Folic acid	One-carbon metabolism; methyl group transfer	*Enterococcus faecalis*
Thiamine (B_1)	Pyruvate decarboxylation; α-keto acid oxidation	*Bacillus anthracis*
Riboflavin (B_2)	Precursor of FMN, FAD in	*Caulobacter vibrioides*

	flavoproteins involved in electron transport	
Pyridoxine (B_6)	Amino acid and keto acid transformations	*Lactobacillus* spp.
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives; pyruvate oxidation; fatty acid metabolism	*Proteus morganii*
Nicotinic acid (Niacin)	Precursor of NAD and NADP; electron transfer in oxidation-reduction reactions; and Dehydrogenations	*Brucella abortus*
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α-ketoglutarate	*Lactobacillus casei*

1.4 Nutrient transport in bacteria

Nutrients and other substances needed by the cell must penetrate the cell boundary layers before they can be metabolized. The cell wall does not present much of a barrier to small molecules and ions, but it excludes large molecules with a relative molecular mass above 600 daltons. The cell boundary component that governs the uptake of most substances into the cell is the cytoplasmic membrane. Transport of nutrients through the cytoplasmic membrane is usually specific; only those nutrients are taken up by the cell for which transport systems are available. With few exceptions, the transport mechanism is dependent on specific permeases and translocases. These are the membrane proteins, and the names indicate that they exhibit some of the properties of enzymes.

The term transport can have several quite different meanings in cell biology. Two main types of transport processes that occur in or through the cytoplasmic membrane such as primary transport and secondary transport are distinguishingly considered. Primary transport consists of those processes that lead to the transfer of ions like $H^{+,}$ Na^{+}, K^{+}, and

hence to alterations in the electrochemical potential. The term secondary transport is applied to all processes resulting in the uptake (influx) or outflow (efflux) of cellular metabolites, which are driven by electrochemical potential gradients. In view of the enormous variety of nutrients and the complexity of the task, it is not surprising that microorganisms make use of several different transport mechanisms. The most important of these are simple diffusion, facilitated diffusion, active transport, and group translocation.

1.4.1 **Simple or Passive diffusion**

The non-specific penetration of substances into the cell is called simple or passive diffusion (Fig 1.1). It is a process in which molecules move from a region of higher concentration to one of lower concentration because of random thermal agitation. The rate of passive diffusion is dependent on the size of the concentration gradient between a cell's exterior and its interior. A fairly large concentration gradient is required for adequate nutrient uptake by passive diffusion and the rate of uptake decreases as more nutrient is acquired unless it is used immediately. Uptake of sugars by passive diffusion has never been demonstrated. Apparently water, non-polar toxins, inhibitors and other substances that are not part of the normal intracellular mileu are taken up by passive diffusion. Thus passive diffusion is an inefficient process and is not employed extensively by microorganims.

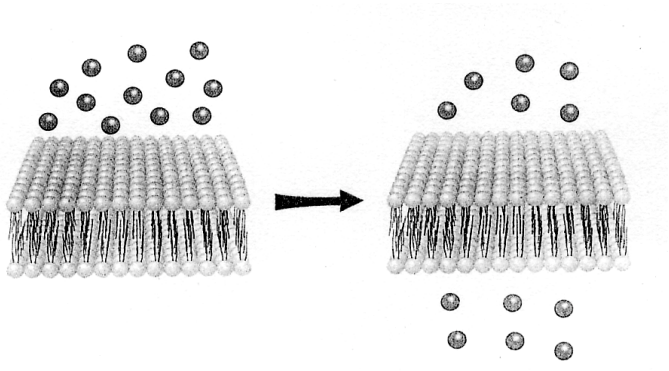

Fig. 1.1- Simple diffusion

4.2. Facilitated diffusion

The transportation of a substance into the cell along its concentration gradient, i.e. towards equilibrium between the external and internal concentrations. In most of the cases, this process is mediated by a substrate-specific permeases which are embedded in the plasma membrane. Because a carrier aids the diffusion process, it is called facilitated diffusion (Fig-1.2). The rate of this transport is governed, over a wide range, by the substrate concentration in the medium. The rate of facilitated diffusion increases with the concentration of the diffusing molecule than that of passive diffusion. Facilitated diffusion is independent of metabolic energy and the nutrient cannot accumulate inside the cell against a concentration gradient. Carrier proteins are selective and specific to transport only closely related solutes. It appears that carrier protein complex spans the membrane. After the solute molecule binds to the outside, the carrier may change conformation and release the molecule on the cell interior. Subsequently the carrier molecule change back to its original shape and be ready to pick up another molecule. This facilitated diffusion does not seem to be important in prokaryotes. Glycerol is transported by facilitated diffusion in *E. coli*, *Salmonella typhimurium*, *Pseudomonas* spp., *Bacillus* spp. and many other bacteria.

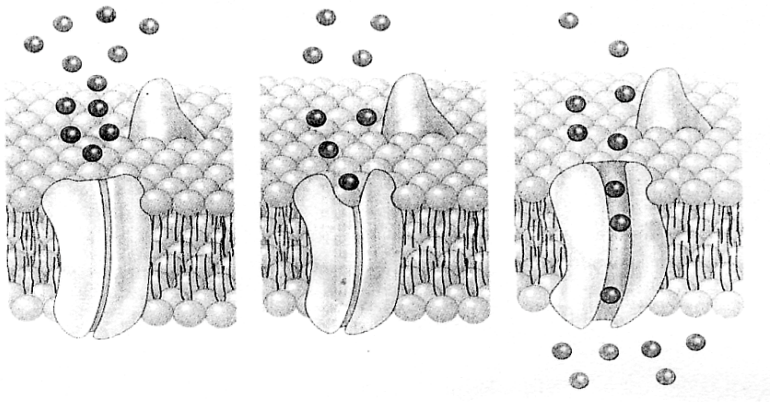

Fig-1.2 Facilitated diffusion

4.3. Active Transport

The transportation of solute molecules to higher concentrations, or against a concentration gradient, with the use of metabolic energy input is referred as active transport (Fig-1.3). Active transport resembles the facilitated diffusion in some ways as it involves the protein carrier activity. The carrier proteins bind particular solutes with great specificity for the molecules transported. Active transport is characterized by the carrier saturation effect at high solute concentrations. The active transport differs from facilitated diffusion in its use of metabolic energy and in its ability to concentrate substances. Metabolic inhibitors that block energy production will inhibit active transport but will not affect facilitated diffusion, at least for some time.

Bacteria use protonmotive force to drive active transport. The membrane transport proteins responsible for this process lack special periplasmic solute-binding proteins. The lactose permease in *E. coli* transports a lactose molecules inward as a proton simultaneously enters the cell. Such linked transport of two substances in the same direction is called symport. *E. coli* also uses proton symport to take up amino acids and organic acids like succinate and malate. Protonmotive force can power active transport indirectly, often through the formation of a sodium ion gradient. In this case, the sodium transport system pumps sodium outward in response to the inward movement of protons. Such linked transport in which the transported substances move in opposite directions is termed antiport. The sodium gradient generated by this proton antiport system then drives the uptake of sugars and amino acids.

It seems reasonable for a microorganism to have only one transport system for each nutrient. But the bacterium, *E. coli*, has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes. When there are several transport systems for the same substance, the systems differ in their energy source, in their affinity for the solute transported, and in their nature of regulation. Figure 1.4 shows the uniport, antiport and symport systems.

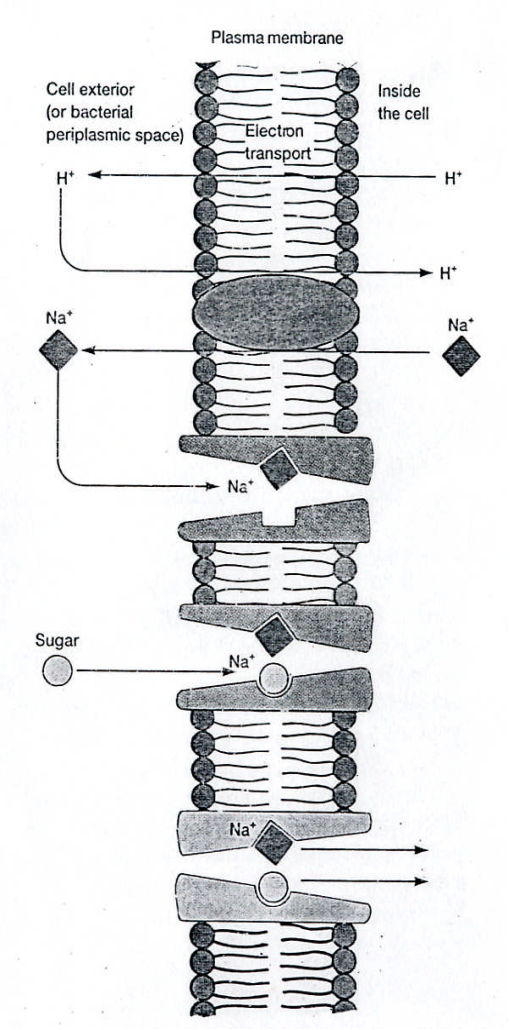

Fig.-1.3 Active transport mechanism

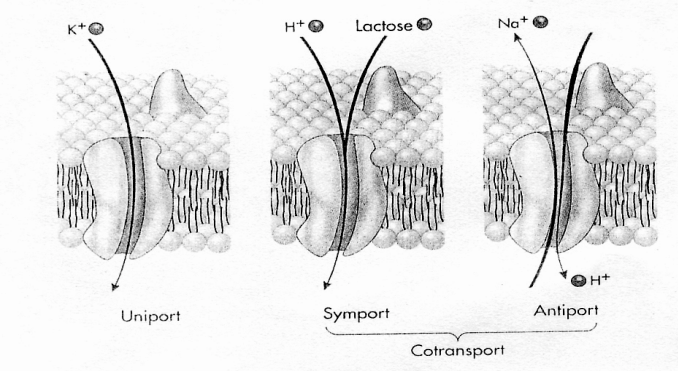

Fig.-1.4 Transport across the Cytoplasmic Membrane- Uniport, Symport, Antiport

1.4.4 Group Translocation

The process in which a molecule is transported into the cell while being chemically altered is called group translocation (Fig. 1.5). The best known group translocation system is the phosphoenolpyruvate : sugar phosphotransferase system (PTS). This system transports a variety of sugars into prokaryotic cells while phosphorylating them using phosphoenolpyruvate (PEP) as the phosphate donor. The PTS system is quite complex and consists of two enzymes and a low molecular weight heat-stable protein (HPr) in *E. coli* and *Salmonella typhimurium*. HPr and enzyme I (EI) are cytoplasmic. Enzyme II (EII) is more variable in structure and often composed of three subunits or domains. EIIA is cytoplasmic and soluble. EIIB is hydrophilic but frequently is attached to EIIC, a hydrophobic protein that is embedded in the membrane. A high-energy phosphate is transferred from PEP to enzyme II with the aid of enzyme I and HPr. Then, a sugar molecule is phosphorylated as it is carried across the membrane by enzyme II. With PTS enzyme II varies and transports only specific sugars, whereas enzyme I and HPr are common to all PTSs.

PTS are widely distributed in prokaryotes. Except for some species of *Bacillus* that have both the Embden-Meyerhof pathway and phosphotransferase systems, aerobic bacteria seem to lack PTSs. Members of the genera *Escherichia*, *Salmonella*, *Staphylococcus*, and other facultatively anaerobic bacteria have phosphotransferase systems. Some obligately anaerobic bacteria like *Clostridium* also have PTSs. *E. coli* takes up glucose, fructose, mannitol, sucrose, N-acetylglucosamine, cellobiose and other carbohydrates by group translocation.

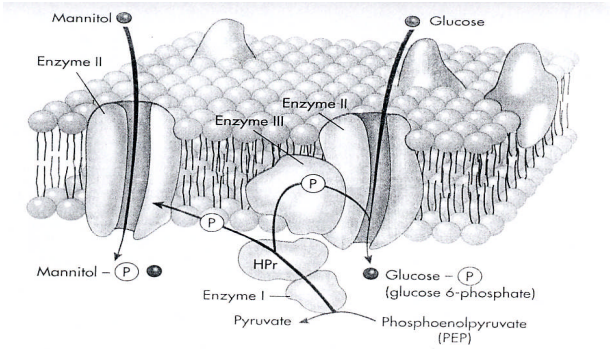

Fig.-1.5 Group translocation mechanism

1.5 Summary

Bacteria require nutrient materials that are used in biosynthesis and energy production. These materials are drawn by the bacteria from their surrounding environments. Bacteria can be categorized into nutritional classes based on the sources from which they acquire the carbon, energy and electrons. Accordingly the nutritional types of bacteria are photolithotrophic autotrophs, photoorganotrophic heterotrophs, chemolithotrophic autotrophs and chemoorganotrophic heterotrophs. Several elements require by bacteria in varying quantities as nutrients. Basing on the amounts or quantities in which they are required the elements are Macroelements (needed in larger quantities) and Microelements (needed in smaller quantities).

Various amino acids are also required by some bacteria as essential growth factors. The nutritional requirements of a bacterium are determined by the kind and number of its enzymes. So, the nutritional complexity reflects a deficiency in biosynthetic enzymes. Some nutrients can enter the bacterial cells by simple passive diffusion. But a membrane carrier protein is usually required for the uptake of some nutrients. In facilitated diffusion the transport protein carries a molecule across the plama membrane of the cell. Active transport systems use metabolic energy and membrane carrier protein for the movement of molecules into the cell. Bacteria also transport the organic molecules by modifying them in the process of group translocation.

Dr. V. Umamaheswara Rao, M.Sc., Ph.D.

Questions:

Q.1. Define nutrition and explain the types and functions of elements required by bacteria as nutrients.

Q.2. Explain the mechanisms of nutrient transport by the bacteria.

Q.3. Write a note on different nutritional classes of bacteria.

Q.4. Give short notes on growth factors.

Books for reference:

1. Prescott, L.M., Harley, J.P., and Klein, D.A. --- Microbiology
2. Brock, T.D. and Madigan, M.T. ---- Biology of Microorganisms
3. Atlas, R.M. ----- Principles of Microbiology
4. White, D. --- The Physiology and Biochemistry of Prokaryotes
5. Gottschalk, G. ----- Bacterial Metabolism

LESSON - 2

BACTERIAL GROWTH (PART – I)

2.0 Objective

To study the pattern of bacterial growth in culture medium and also the methods employed for the measurement of growth in terms of cell numbers and cell mass.

2.1 Introduction

Growth in a biological system is defined as 'an increase in mass or size accompanied by the synthesis of macromolecules, leading to the production of new organized structure'. In the case of multinucleate, coenocytic microorganisms the nuclear divisions are not accompanied by cell divisions, so growth results in an increase in cell size but not in cell number. But in the case of many unicellular microorganisms, like bacteria, which multiply or divide by binary fission, 'growth' leads to a rise in cell number. So, in microbiology the growth is defined as an increase in the number of cells. Growth is an essential component of microbial function because of the finite life span of microbes in nature. Because of their small size, it is usually not convenient to investigate the growth and reproduction of individual microorganisms. Therefore, usual practice of investigation of growth is to follow the changes in the total population number from time to time.

2.2 Bacterial Growth

2.2.1 Growth Curve

The bacterial population generally shows a characteristic growth pattern when grown in a batch culture system or closed system. In this system, cells are incubated in a closed culture vessel with a single batch of medium without the addition of fresh medium into the vessel. In this set up, nutrient concentrations decline and concentrations of wastes increase. The growth pattern of bacteria reproducing by binary fission in the culture system follows a typical curve when a graph is plotted between logarithm of cell number versus the incubation time. The resulting curve is called bacterial growth curve which consists four distinct phases namely Lag phase, Exponential phase, Stationary phase and Death phase (Fig.- 1.1).

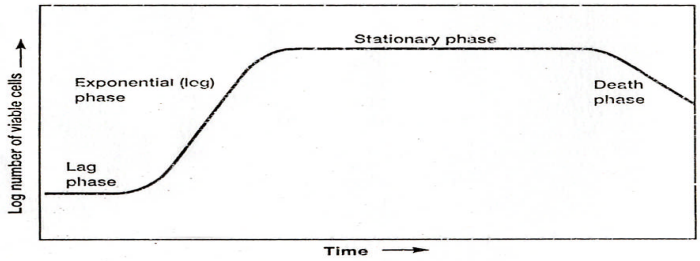

Fig.- 2.1 Bacterial growth curve

2.2.2 Lag Phase

When bacterial population is inoculated into fresh liquid culture medium, usually no immediate growth will occur. This period is called as lag phase and the increase in cell number of bacteria takes place only after this phase. During this phase, cells adjust to the new environment and undergo the synthesis of new components like essential cofactors, various enzymes which are required for the growth. This lag phase is an essential phase for a population prior to its cell division. The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase. On the other hand, when a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase will be short or absent.

2.2.3 Exponential Phase

This phase is also called Log phase. During this phase, bacteria grow and divide at the maximum possible rate and the number of cells increase exponentially in a geometrical progression. The growth rate in this phase is dependant on the genetic potential of the organism, nature and composition of the medium and conditions of culturing. The exponential growth rate of one organism differ with that of the another organism. For a given organism, the growth rate during this exponential phase is constant as the cells divide and double in number at regular intervals. The growth curve rises smoothly rather than in discrete jumps in this phase. The population is most uniform in terms of chemical

and physiological properties during this phase, therefore exponential phase cultures are usually used in biochemical and physiological studies.

Mathematical expression of growth

During the exponential phase each cell divides at constant intervals and thus population will double in number during a specific length of time called the generation time or doubling time. When culturing is started with the inoculation of one cell, increase in population occurs in a simple geometric progression of the number 2 as the cell number doubles for every generation. This increase can be expressed as a geometric progression as the following

$1 - 2^1 - 2^2 - 2^3 - 2^4 - 2^5$ -------- 2^n

the exponent 'n' is the number of generations

Let N_0 = the initial population number

N_t = the final population number at the time 't'

n = the number of generations in the time 't'

So, $N_t = N_0 \times 2^n$

Solving for the number of generations 'n' by converting the cell number into logarithms of base 10

$$\text{Log}_{10} N_t = \text{Log}_{10} N_0 + n.\log_{10}2$$

$$n = \frac{\log_{10}N_t - \log_{10}N_0}{\log_{10}2}$$

$$n = \frac{\log_{10}N_t - \log_{10}N_0}{0.301} \quad \text{… as the value of } \log_{10}2 \text{ is } 0.301$$

$$n = 3.3\,(\log_{10}N_t - \log_{10}N_0)$$

By using this formula the number of generations can be calculated if initial and final populations are known.

Generation time --- the time required for completion of one generation is called the generation time. Also be called doubling time as the cell number becomes double in one generation. It is denoted by letter 'g' and g = t/n where, t is the time of incubation and 'n' is the number of generations occurred during the incubation time of 't'.

Growth rate ---- is the change in cell number or mass per unit time and denoted by 'R'. During exponential growth phase the growth rate is reciprocal to the generation time. So,

$$R = 1/g = n/t$$

2.2.4 Stationary Phase

In a batch culture the exponential growth cannot continue for a long period or cannot occur indefinitely. At some point, the growth of population ceases and the growth curve comes horizontal . This phase is called as stationary phase where the total number of viable cells remain constant without any net increase or decrease in the cell number due

to the balance between cell division and cell death, but cells remain metabolically active. The stationary phase for bacteria is attained when the population level reaches to a level of 10^9 cells per ml of broth. Bacterial populations enter into the stationary phase for several reasons like (i) limitation and depletion of an essential nutrient in the culture medium (ii) accumulation of some toxic waste products to an inhibitory level.

2.2.5 Death Phase

This phase is also be called as decline phase. When the culturing is continued after the stationary phase, cells may die due to the occurrence of detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes. The death of a microbial, like its growth during exponential phase, is usually logarithmic.

2.3 Continuous culturing

Exponential growth of organisms occurs for only a few generations and soon reaches the stationary phase in the batch culture system. The growth of bacterial population at a particular rate in the exponential phase can be maintained through continuous culturing system in which the constant environmental conditions are maintained through continual provision of nutrients and removal of wastes. There are two major types of continuous culture systems in common use – 1) The Chemostat and 2) The Turbidostat.

2.3.1. Chemostat

A chemostat is constructed so that the sterile medium is fed into the culture vessel at the same rate as the medium containing microorganisms is removed. The culture medium for a chemostat possesses an essential nutrient like an amino acid in limiting quantities. Because of the presence of a limiting nutrient, the growth rate is determined by the rate at which new medium is fed into the growth chamber, and the final cell density depends on the concentration of the limiting nutrient. So, the concentration of the limiting nutrient in substrate of the culture vessel controls the growth rate. The concentration of the substrate is in turn controlled by the dilution rate.

$$\text{The dilution rate 'D'} = \frac{\text{flow rate (f)}}{\text{vessel volume (v)}}$$

Both the microbial population level and the generation time are related to the dilution rate. As the dilution rate increases the generation time decreases. If the dilution rate rises too high, the microorganisms can actually be washed out of the culture vessel before reproducing because the dilution rate is greater than the maximum growth rate. The limiting nutrient concentration rises at higher dilution rates because fewer microorganisms are present to use it. By adjusting the flow rate the growth rate can be controlled. The chemostat apparatus is shown in Fig. 2.2.

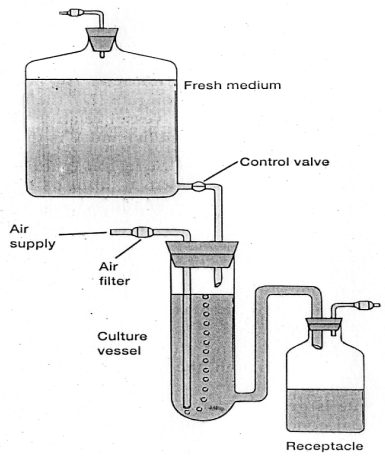

Fig.- 2.2 Chemostat apparatus

2.3.2 Turbidostat

Turbidostat is the second type of continuous culture system. This device (Fig. 2.3) has a photocell that measures the absorbance or turbidity of the culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity of cell density. The turbidostat differs from the chemostat in several ways. The dilution rate in turbidostat varies rather than remaining constant, and its culture medium lacks a limiting nutrient. The turbidostat operates best at high dilution rates whereas the chemostat is most stable and effective at lower dilution rates.

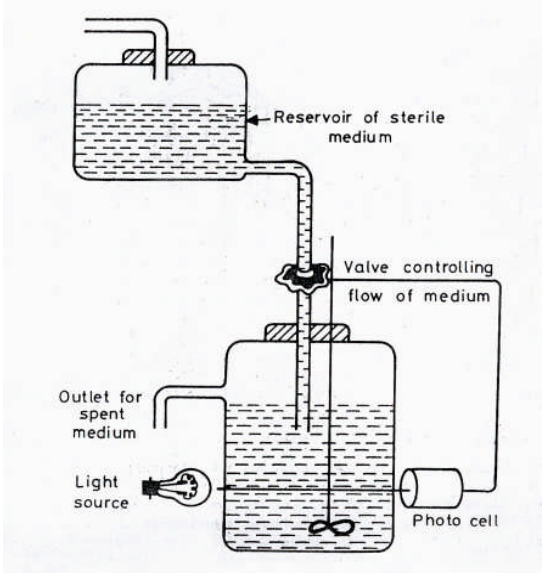

Fig. – 2.3 Turbidostat apparatus

2.4 Synchronous culturing

The bacterial population, at any given instant during log phase consists of cells in various stages of division viz., about-to-divide cells, just-divided cells, and cells in the different physiological stages preparatory to division. So, the population is said to be heterogenous in behaviour. To eliminate this heterogeneity synchronous culturing is used in which all the cells provoked to divide at the same time. In this synchronous culture since all the cells are physiologically identical, cell division occurs periodically at constant intervals (Fig. 2.4). This can be achieved by the methods that provoke the entire log-phase culture into the division process simultaneously. Such methods involve the arrest of cell division but not of cytoplasmic growth by chemical or physical means for a period followed by the sudden relief of this inhibition. With this sudden relief, a marked degree of synchronous division of an entire culture is obtained.

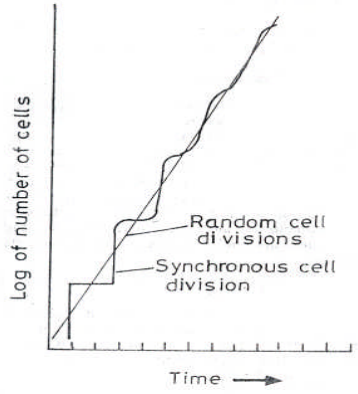

Fig.– 2.4 Synchronous growth curve

A population can be synchronized by manipulating the physical environment or the chemical composition of the medium. For example, when cells inoculated into a medium at sub-optimal temperature and maintained the same condition for some time, they will metabolize slowly but not divide. When the temperature is subsequently raised, the cells will undergo a synchronized division. Similarly, E. coli growth can be synchronized by changing the chemical nature of the medium. When a thymine requiring mutant is starved for thymine by placing it in a thymine deficient medium, it is incapable to grow. Then the addition of thymine in the culture medium causes the surviving of cells to undergo several synchronous divisions.

2.5 Biphasic Growth Curve

A biphasic growth curve reflects the preferential utilization of substrates and this is referred as diauxie phenomenon. A combination of catabolite repression and operon control mechanisms results in a biphasic growth curve (Fig.-2.5). In this diauxie phenomenon, when two carbon sources are present in the medium the organism preferentially utilizes one source completely before the use of other. For example, cultures of E. coli exhibit the biphasic growth curve when inoculated into a medium containing both glucose and lactose as substrate. While growing on glucose E. coli exhibits the normal lag, log and stationary phases of growth. Rather than exhibiting a prolonged stationary phase, E. coli enters a second lag phase when the glucose is no

longer readily available in concentrations that suppress disaccharide utilization by catabolite repression. During this second lag phase, allolactose acts as an inducer to derepress the lac operon system. The enzymes that are necessary for lactose metabolism are synthesized, and the bacteria begin to grow exponentially by using the lactose substrate. When the lactose is also utilized, the bacterium enters into the secondary stationary phase.

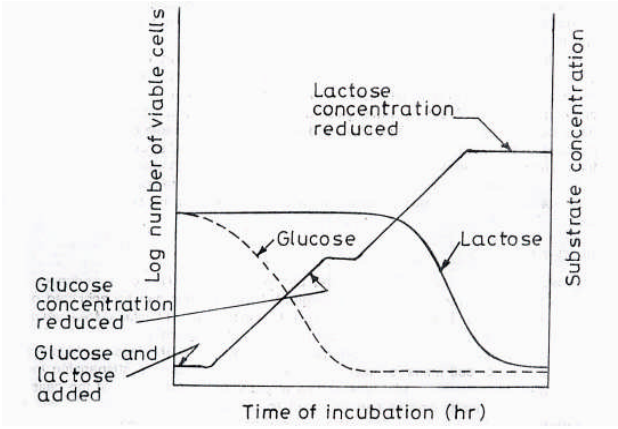

Fig. - 2.5 Biphasic growth curve

2.6 Measurement of bacterial growth

The growth of bacteria can be measured in two different ways – measurement of cell numbers and measurement of cell mass.

2.6.1 Measurement of cell numbers

2.6.1.1 Counting chamber

The most obvious way to determine bacterial numbers is through direct counting. Using a counting chamber for the purpose is easy, inexpensive and relatively quick. It also gives information about the size and morphology of bacteria. The most widely and extensively used counting chamber is Petroff-Hausser counting chamber (Fig.-2.6). This chamber is a specially designed slide with chamber of known depth with an etched grid on the chamber bottom. The grid is located in the center of the slide and it consists 25 squares covering an area of. The depth of the chamber is 0.02 mm. The number of bacteria can be calculated by taking the chamber's volume into account. The total number of bacteria in 1 mm^2 area is number of bacteria per square x 25 squares. To get the number of bacteria per mm^3

$$\text{Bacteria/mm}^3 = (\text{bacteria/square})(25\ \text{squares})(50)$$

The number of bacteria per cm^3 is 10^3 times of mm^3 value, so

$$\text{Bacteria/cm}^3 = (\text{bacteria/square})(25\ \text{squares})(50)(10^3)$$

Counting chambers yield counts of both live and dead cells.

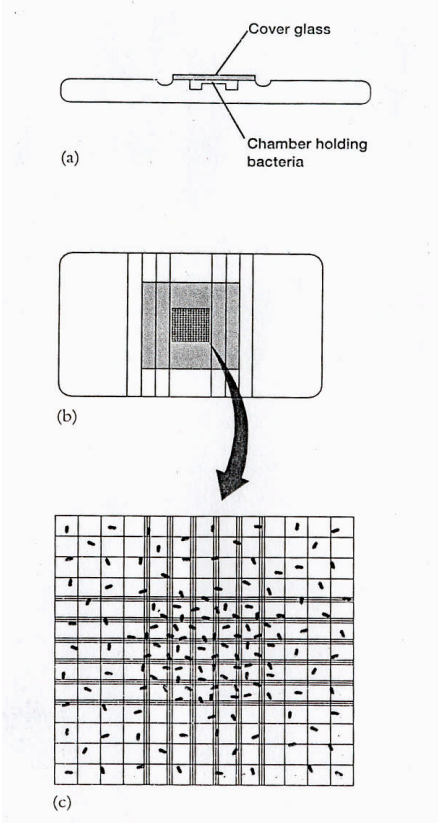

Fig.- 2.6 Petroff-Hausser counting chamber

2.6.1.2 Plating method

Plating techniques such as spread plate and pour plate methods which are simple and sensitive are also widely used to measure the viable count of bacteria in the samples of food, water and soil. In spread plate method, bacterial sample is diluted by 10-fold serial dilution technique to the required dilution. A small amount of bacterial sample from the appropriate dilution is spread or dispersed over a solid agar medium surface and incubated for the development of the colonies. In pour plate method also sample is diluted to the desired level of dilution. But here, a known volume of the sample is dispersed first into the sterilized empty petri plate and then the sterilized molten agar medium is poured. The contents of the plate are mixed well by rotation of the plate and incubated for the development of the colonies. In both the methods, the original number of viable cells in the sample can be calculated from the number of colonies formed and the sample dilution. The cell count can be measured more accurately by the use of special colony counter. For best results, the samples used should yield between 25 and 250 colonies per plate.

2.6.1.3 Membrane filter technique

This technique can be routinely used for the enumeration of bacteria from the liquid samples. Bacterial numbers are determined from counts of colonies growing on special membrane filters having pores small enough to trap bacteria. In this technique, the sample is drawn through a special membrane filter. The filter is then placed on an agar

medium or on a pad soaked with liquid media and incubated until each cell forms a separate colony. The colony count gives the number of bacteria in the filtered sample and some special media can be used to select for specific bacteria.

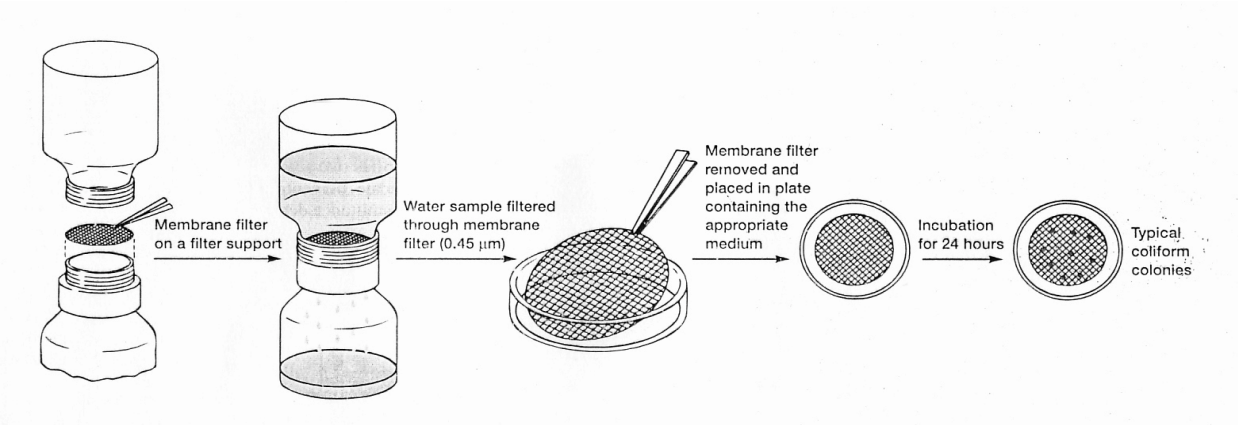

Fig.-2.7 Membrane filter technique

2.6.2 Measurement of Cell Mass

2.6.2.1 Dry weight method

The most direct approach in the measurement of cell mass of bacteria is the determination of bacterial dry weight. Cells growing in liquid medium are collected by centrifugation, washed, dried in hot-air-oven to a constant weight and then weighed. This method is time consuming and not very sensitive. Because bacteria weigh so little, it is necessary to centrifuge several hundred milliliters of culture to collect a sufficient quantity. So, this method is not so preferable for bacteria.

2.6.2.2 Turbidity measurement method

More rapid and sensitive method for the measurement of cell mass is the determination of the turbidity developed in the liquid medium due to the increased cell density during incubation. This technique depends on the fact that microbial cells scatter the light that strikes them. Because the microbial cells in a population are more or less of constant size, the amount of scattering is proportional to the concentration of cells present. When the concentration of bacteria reaches about 10^7 cells per ml, the medium appears slightly cloudy or turbid. Further increase in concentration result in greater turbidity and less light is transmitted through the medium. The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to the bacterial concentration at low absorbance levels. Thus population growth can be easily measured spectrophotometrically as long as the population is high enough to give detectable turbidity.

2.6.2.3 Estimation of cell constituents

If the amount of a substance in each cell is constant, the total quantity of that cell constituent is directly related to the total microbial cell mass. In this mode of estimation, a known volume of sample is centrifuged and washed cells are collected. This sample of

cells is analyzed for total protein or ATP. An increase in the microbial population will be reflected in higher total protein levels or ATP.

Protein is a reasonable measure of growth since it normally constitutes the majority (50-70%) of the organic cellular dry weight. The method by which cell protein is assessed depends upon the purpose and the amount of material involved. There are three methods that are commonly used for protein measurement. They are 1) the biuret method, 2) Folin reagent method, and 3) the Coomassie blue dye-binding method. Each procedure is useful under different conditions.

The biuret procedure depends upon the interaction of the cupric ammonium ion with the peptide bond. The procedure is considered by many to be the most unbiased protein measurement, since it depends only on the peptide bond and is independent of the amino acid composition. Reaction of copper with the peptide bond yields a complex that is blue-colored and absorbs broadly over a range between 500 and 650 nm. At an appropriate wavelength, a linear relationship is found between the copper-protein complex and protein concentration. Relatively large amounts of protein are required to obtain substantial absorbance readings in the visible wavelength range.

The Folin reaction method which is relatively sensitive depends upon interaction of protein with the cupric ion followed by oxidation of the complex by the Folin reagent. The oxidation of the complex is coupled with the reduction of a mixture of phosphotungstic and phosphomolybdic acids in the reagent, producing a blue color that is proportional to the amount of copper-protein complex.

Coomassie blue method is an alternate sensitive procedure for protein measurement. It takes the advantage of the fact that, in highly acidic solution, interaction of protein with Coomassie blue dye leads to a change in the dye's absorption from 465 nm to 595 nm that is proportional to the concentration of the protein-dye complex. When carefully standardized, the procedure is highly sensitive, rapid and less subject to interferences than the Folin procedure.

2.7 Summary

Growth is an increase in cellular constituents and results in an increase in cell size, cell number of both. When bacteria are grown in a closed system or batch culture, the resulting growth curve usually has four phases viz., lag phase, exponential or log phase, stationary phase and death phase. In the lag phase, the cells adapt to the new environment and prepare itself to undergo multiplication. During the exponential phase, the population number doubles at a constant interval called the doubling or generation time. The mean growth rate constant is the reciprocal of the generation time. In the stationary phase the cell numbers remain constant due to the depletion of nutrients. During death or decline phase of growth bacterial cells die due to the accumulation of toxic substances to the lethal levels.

Bacteria can be grown in an open system in which nutrients are constantly provided and wastes are removed. A continuous culture system is an open system that can maintain a

bacterial population in the log phase for desired period. There are two types of these systems namely chemostat and turbidostat.

Bacterial populations can be counted directly with counting chambers, electronic counters or fluorescence microscopy. Viable counting techniques such as spread plate technique, pour plate technique and the membrane filter method can be employed. Population changes also can be followed by determining variations in microbial mass through the measurement of dry weight, turbidity, or the amount of a cell component. The amount of microbial mass produced from a nutrient can be expressed in terms of the growth yield.

Questions

Q.1. What is the growth curve? Explain the growth pattern of bacteria in a batch culture system.
Q. 2. Describe the various methods used to measure the bacterial growth.
Q. 3. Write notes on biphasic growth curve.
Q .4. Write notes on synchronous culturing.

Books for reference:

1. Prescott, L.M., Harley, J.P., and Klein, D.A. --- Microbiology
2. Brock, T.D. and Madigan, M.T. ---- Biology of Microorganisms
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LESSON – 3

BACTERIAL GROWTH (PART – II)

3.0 Objective

To know the impact and influence of various environmental factors of a habitat on the growth of bacteria.

3.1 Introduction

Microorganisms are found in nearly every environment on earth, including environments in which no other life forms can survive. The growth of these microorganisms is greatly affected by the chemical and physical nature of their surroundings. So, the kinds of organisms found in a given environment and the rates at which they grow can be influenced by a variety of factors, both physical and biochemical. The physical factors include pH, temperature, oxygen concentration, moisture hydrostatic pressure, osmotic pressure and radiation.

3.2 Physical Factors:

3.2.1 pH :

pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration. The pH scale is invented by the Danish Chemist Soren Sorenson to describe the limits of growth of microorganisms in various media. The pH scale extends from 0.0 to 14.0 and each pH unit represents a tenfold change in hydrogen ion concentration. Bacteria, as they grow, usually cause changes in pH of their environments. So, to maintain the pH of the medium constant several buffers are used and each type of buffer work only for a narrow pH range. pH dramatically affects the microbial growth. Each species has a definite pH growth range with an optimum pH value. Most natural environments have pH values between 5.0 and 9.0 and the organisms with optima in this range are common.

Basing on the tolerance for acidity or alkalinity, the bacteria can be categorized into Acidophiles, Neutrophiles and Alkaliphiles. However, no single species can tolerate the full pH range of any of these categories, and many bacteria tolerate a pH range that overlaps two categories. Acidophiles or acid-loving bacteria have their growth optimum between pH 0.0 and 5.5. Lactobacillus, which produces lactic acid is an acidophile but it tolerates only mild acidity. Neutrophiles exist between

pH 5.5 and 8.0. Most of the bacteria that cause disease in humans are neutrophiles. The third catergory alkaliphiles or alkali-loving or base-loving bacteria prefer the pH range of 8.5 to 11.5. Vibrio cholerae, the causative agent of the disease Asiatic cholera, grows best at a pH of about 9.0. The alkaliphiles having the growth optima at pH 10.0 or higher are called as Extreme alkaliphiles. Most bacteria prefer the near neutral pH for their optimum growth.

Despite wide variations in habitat pH, the internal pH of most bacteria is close to neutrality. This may be due to the relatively impermeability of plasma membrane of bacteria to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system. Extreme alkaliphiles maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons. Although bacteria often grow over wide ranges of pH, there are limits to their tolerance. Drastic variations in pH can harm bacteria by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Bacterial death occurs if the internal pH drops much below 5.0 to 5.5. Changes in the external pH also might alter the ionization of nutrient molecules and thus reduce their availability to the organism. Bacteria often must adapt to environmental pH changes to survive. In bacteria, potassium/proton and sodium/proton antiport systems probably correct small variations in pH. If the pH becomes too acidic, other mechanisms come into play. When the pH drops below 5.5 to 6.0, bacteria like *Salmonella typhimurium* and *E. coli* synthesize an array of new proteins as part of their acidic tolerance response. This protective response is contributed by a proton-translocating ATPase either by making more ATP or by pumping protons out of the cell. In the case of a decrease in external pH to 4.5 or lower, chaperons such as acid shock proteins and heat shock proteins are synthesized that may prevent the acid denaturation of proteins.

3.2.2 Temperature:

Environmental temperature is one of the most important environmental factors influencing the growth and survival of organisms. Microorganisms are particularly susceptible because they are unicellular and '*poikilothermic*' i.e., their temperature varies with that of external temperature. A most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme-catalyzed reactions. Temperature affect the organisms in either of two opposing ways. At low temperatures, the rate of enzymatic reactions in the cell will become roughly double for every 10^{o}C rise in temperature. As a result the growth of the organism becomes faster. But above a certain point of temperature, a further increase in temperature slows down the growth and sufficiently high temperatures are lethal.

High temperatures damage the microorganisms by denaturing the enzymes, transport carriers, and other proteins. Other affects include the disruption of microbial membranes and simple melting and disintegration of lipid bilayer. Because of these opposing influences of temperature, each type of microorganism possesses three characteristic temperatures to explain their dependence on temperature. These are minimum temperature, optimum temperature and maximum temperature that are collectively called as 'cardinal temperatures'. The cardinal temperatures for a particular species are not rigidly fixed but often depend to some extent on other environmental factors such as pH and the available nutrients. And the cardinal temperatures vary greatly among the microorganisms. To define these temperatures

Minimum temperature ----- temperature below which no growth occurs

Optimum temperature ----- temperature at which growth rate is rapid
Maximum temperature ----- temperature above which growth is not possible
The microbial growth can occurs at temperatures extending from –20^{o}C to over 100^{o}C, but no single organism shows growth over this whole temperature range. Usually a given microorganism exhibit a span of about 30^{o} – 40^{o}C temperature range. Species having a small range of growth temperature are called as 'Stenothermal' (eg: *Neisseria gonorrhoeae*) and that can grow over a wide range are known as 'Eurythermal' (eg: *Enterococcus faecalis*).

Basing on their temperature ranges, microbes can be categorized into different classes (Fig.-3.1).

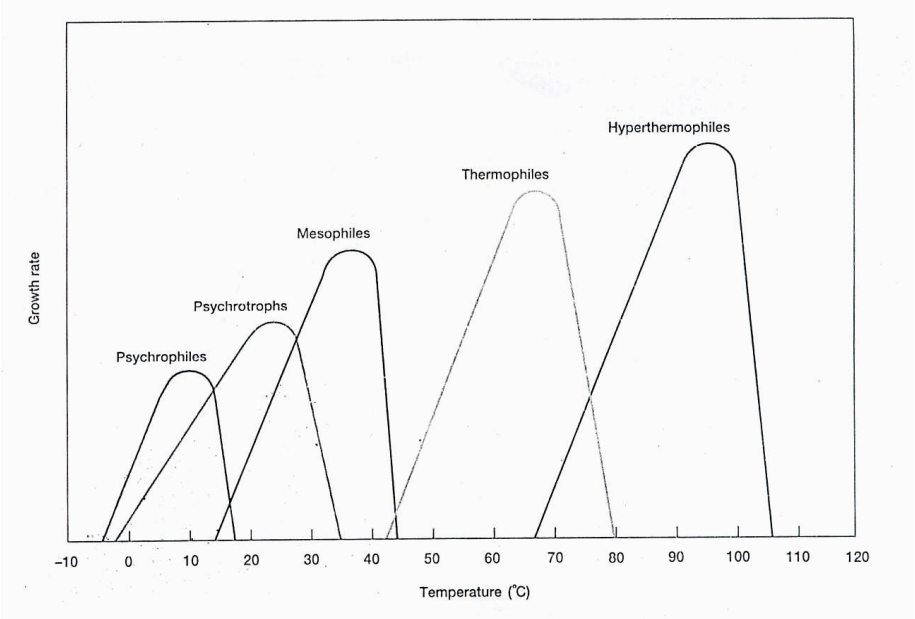

Fig.-3.1 Temperature ranges of bacteria

Psychrophiles:
These are cold-living organisms showing low temperature optima and are found in environments that are constantly cold like Arctic and Antarctic habitats. They grow well at O^{o}C with an optimum growth temperature of 15^{o}C or below and maximum temperature is around 20^{o}C. Some members of *Pseudomonas*, *Flavobacterium*, *Achromobacter* and *Alcaligenes* are best known examples of psychrophiles. The enzymes, transport systems and protein synthetic mechanisms of these psychrophiles are adapted to function well at low temperatures. The cell membranes of these organisms have high levels of unsaturated fatty acids and remain semifluid when cold. Many species can grow at 0^{o} C even though they have optima between 20^{o} and 30^{o}C, and maxima at about 35^{o}C. These are called as psychrotrophs or facultative psychrophiles or psychrotolerants and are mainly responsible for the spoilage of the refrigerated foods.

Mesophiles:
Organisms with mid-range temperature optima are referred as mesophiles. Their growth optima is around 20^{o} to 45^{o}C, minimum is of 15^{o} to 20^{o}C and the maximum temperature is about 45^{o}C. Most of the microorganisms probably fall within this category. Almost all the human pathogens are

included in this category as they grow best near human body temperature of 37^{o}C. Some mesophiles that can withstand to the exposure of higher temperatures for short periods are called as thermoduric organisms which are responsible for the canned food spoilage.

Thermophiles:
These organisms are heat loving in nature and exhibit relatively high temperature optima. Their growth minimum is around 45^{o}C and have optimum temperature between 55 and 65^{o}C. Some bacteria have growth optima between 80^{o}C and about 113^{o}C and are called hyperthermophiles (*Pyrococcus* and *Pyrodictium* spp.). Thermophilic bacteria flourish in many habitats including composts, self-heating hay stacks, hot water lines, and hot springs. The molecular adaptation of these thermophiles or thermophily is mainly due to the presence of heat-stable enzymes and protein synthesis systems that are able to function at higher temperatures. The membrane lipids of thermophiles are also more saturated than those of mesophiles and have higher melting points which enable the thermophile membranes to remain intact at higher temperatures.

3.2.3 Oxygen Concentration:
Oxygen is a peculiar environmental factor as it can be both essential and toxic. Depending on the effect and requirement of oxygen, bacteria can be divided into different groups (Fig.-3.2).

Obligate aerobes: Require the presence of oxygen for growth. Oxygen serves as the terminal electron acceptor for the electron-transport chain in aerobic respiration. Eg: *Pseudomonas* spp.
Obligate anaerobes: These organisms do not tolerate O_2 and die in the presence of oxygen. These are also termed as strict anaerobes. Examples include species of *Clostridium*, *Bacteroides*, *Fusobacterium* and *Methanococcus.*
Facultative anaerobes: Do not require O_2 for growth but grow better in its presence. These forms carry on aerobic metabolism in the presence of O_2 and shift to anaerobic metabolism in the absence of oxygen. Eg: *Escherichia coli*
Aerotolerant anaerobes: These forms simply ignore the presence of O_2 and grow equally well whether it is present or not.Eg: *Enterococcus faecalis* and *Lactobacillus* spp.
Microaerophiles: Few aerobes such as *Campylobacter* spp. require O_2 levels below the range of 2 to 10% and are damaged by the normal atmospheric level of O_2 i.e. 20%.

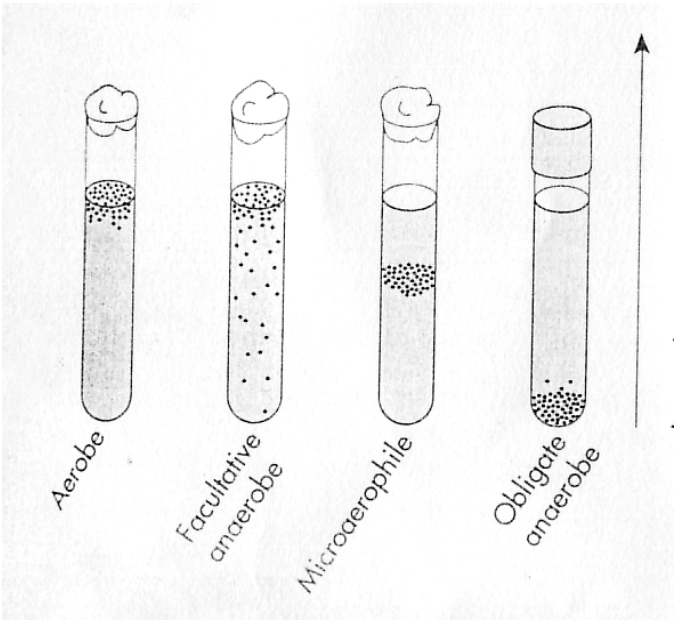

Fig.-3.2 Oxygen relationship with growth of bacteria

These different O_2 relationships are due to either inactivation of proteins or effect of toxic O_2 derivatives. During the reduction of oxygen to water, several toxic intermediates like hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and superoxide (O_2^-) are formed. This reduction reaction involves

$O_2 + e^-$ ---------- $O_2^-\cdot$ (superoxide radical)
$O_2^-\cdot + 2H^+$ ---------- H_2O_2 (hydrogen peroxide)
$H_2O_2 + e^- + H^+$ ------- $H_2O + OH\cdot$ (hydroxyl radical)

These products of oxygen reduction are extremely toxic because they are powerful oxidizing agents and rapidly destroy cellular constituents. Superoxide radical is potentially toxic and highly reactive that can cause oxidative destruction of lipids and other biochemical components. This intermediate may even pass from one cell to another cell. Hydrogen peroxide is produced in small amounts by almost all aerobic organisms. Hydroxyl radical is the most reactive of all the O_2 intermediates. It is the most potent oxidizing agent and capable of attacking any organic substance in cells. A microorganism must be able to protect itself against such oxygen products or it will be killed.

Many bacteria possess enzymes that afford protection against toxic O_2 products. The important enzymes in this regard are ‘catalase’, ‘peroxidase’ and superoxide dismutase. Both the catalase and peroxidase enzymes act on H_2O_2 , whereas superoxide dismutase acts on superoxide radical. Superoxide dismutase and catalase together can bring about the conversion of superoxide back to O_2. No enzymatic system exists to deal with hydroxyl radicals. Removal of H_2O_2 from cells, however protect the cells in part by preventing the formation of hydroxyl radicals. Obligate aerobes and facultative anaerobes usually contain superoxide dismutase and catalase enzymes. Aerotolerant

bacteria may lack catalase but always posses superoxide dismutase. Obligate anaerobes lack both catalase and superoxide dismutase or posses in very low concentrations and so cannot tolerate O_2.

3.2.4 Water Activity:

In natural environments, water availability is one of the most important factors affecting the growth of microbes as they all require water for life. The amount of water available to microorganisms can be reduced by interaction with solute molecules (the osmotic effect) or by adsorption to the surfaces of solids (the matric effect). Water availability is generally expressed in physical terms such as water activity or water potential. Water activity is denoted as a_w and expressed as a ratio of the vapor pressure of the air in equilibrium with the substance or solution divided by the vapor pressure of pure water at the same temperature.

$$a_w = \frac{P_{soln}}{P_{water}}$$

The values of a_w vary between '0' and '1'.

The movement or diffusion of water across a selectively permeable membrane from a dilute solution (higher water concentration) to a more concentrated solution (low water concentration) is called as Osmosis. Microorganisms can be affected by changes in the osmotic concentration of their surroundings as they are separated by a selectively permeable plasma membrane from their environment. If a microorganism is placed in a hypotonic solution i.e., solution with low osmotic concentration, water will enter the cell and cause it to burst unless something is done to prevent the influx. When microorganisms with rigid cell walls are placed in a hypertonic environment, water leaves the cell and plasma membrane shrinks away from the wall, a process known as plasmolysis. This condition dehydrates the cell and may damage the plasma membrane and the cell usually becomes metabolically inactive and ceases to grow.

Most of the bacteria, to maintain the shape and integrity of their cells, keep the osmotic concentration of their protoplasm above that of the habitat by the use of solutes referred to be as Compatible solutes. These compatible solutes are the substances that are compatible with metabolism and growth and always maintain the plasma membrane pressed firmly against the cell wall. Most bacteria increase their internal osmotic concentration through the synthesis or uptake of choline, betaine, proline, glutamic acid, and other amino acids. Some times elevated levels of potassium ions are also used for the purpose.

Bacteria that grow or live in environments that are high in sugar concentration are known as Osmophiles. Bacteria greatly differ in their ability to adapt to habitats with low water activity. To grow in a low a_w habitat, the bacteria should expend extra effort to maintain a high internal solute concentration. The bacteria having this capacity of growing over a fairly wide range of water activity or solute concentration are called Osmotolerants. The bacteria that require moderate to large quantities of sodium chloride are referred to as salt-loving bacteria or halophiles. The halophiles are typically found in ocean, where the salt concentration is optimum for their growth. The membrane transport systems of these halophiles actively transport sodium ions out of the cells and concentrate potassium ions inside them. This high levels of potassium in the cells of halophiles is required for the

stability and activity of various enzymes, ribosomes and transport proteins. Bacteria that are able to live in very dry environments are known as Xerophiles and they use glycerols as compatible solutes.

3.2.5 Radiation:

On the earth sunlight is the major source of radiation which includes visible light, ultraviolet light, infrared rays and radio waves. The wavelength of these radiations decreases with the increase in the energy. Many forms of electromagnetic radiation particularly ionizing radiation are very harmful to microorganisms due to their shorter wavelength and high energy that cause atoms to lose electons or ionize. The two major forms of ionizing radiations are X-rays and γ-rays. X-rays are artificially produced and the γ-rays are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations and may indirectly result in cell death. But the higher levels of ionizing radiation is directly lethal and cause death. Though microorganisms are comparatively resistant to ionizing radiation, they will be destroyed by sufficiently large doses. A variety of changes in cells occur due to ionizing radiation. Of these, breaking of hydrogen bonds, oxidization of double bonds, destruction of ring structures and polymerization of some molecules are important.

Ultraviolet radiation is the non-ionizing radiation having short wavelength and high energy. This UV radiation can kill all kinds of microorganisms. The most lethal wavelength of UV radiation that is absorbed effectively by DNA of the cell is 260 nm. The principal affect of ultraviolet radiation is the formation of thymine dimers in which the two adjacent thymines of the same DNA strand are covalently joined. This thymine dimer formation inhibits the DNA replication. This UV damage can be repaired by either photoreactivation mechanism or dark reactivation mechanism.

3.2.6 Pressure:

Most organisms that spend their lives on land or on the surface of water, always subjected to a pressure of 1 atmosphere and never affected significantly by pressure. However, the standing waters in oceans exerts a pressure called as hydrostatic pressure in proportion to its depth. Such pressure doubles with every 10 m increase in depth and it may reach to a pressure of 600-1100 atms in deep sea. Despite these extremes, some bacteria survive and adapt. These bacteria are not much affected as the others and are known as barotolerant bacteria. Some bacteria in the gut of deep-sea invertebrates grow more rapidly at high pressures and are referred as barophilic bacteria or simply barophiles. The membranes and enzymes of these barophiles require pressure to function properly. The high pressure is necessary to keep their enzyme molecules in the proper three-dimensional configuration without which the enzymes lose their shape and denature ultimately resulting in the death of organism.

3.3 Summary

The bacterial growth will be influenced by various environmental factors of their habitats either directly or indirectly. Most bacteria have rigid cell walls and are hypertonic to the habitat because of solutes such as amino acids, polyols, and potassium ions. Different bacteria exhibit their respective optimum pH for growth and can be classified as an acidophiles, neutrophiles and alkaliphiles. Bacteria can alter the pH of their surroundings, and most culture media must be buffered to stabilize the pH.

Bacteria have distinct temperature ranges for growth with minima, maxima and optima collectively referred as cardinal temperatures. These ranges are determined by the effects of temperature on the rates of catalysis, protein denaturation, and membrane disruption. Basing on their tolerance and requirement of temperature for the growth, bacteria can be classified majorily to Psychrophiles, Mesophiles and Thermophiles.

Bacteria can be placed at least into five different categories basing on their response to the presence of oxygen namely obligate aerobes, facultative anaerobes, aerotolerant anaerobes, obligate anaerobes and microaerophiles. The amount of water actually available to bacteria is expressed in terms of the water activity. Though most bacteria will not grow well at water activities below 0.98 due to plasmolysis and associated effects, osmotolerant bacteria survive and even flourish at low water activities.

High-energy or short-wavelength radiation harms organisms in several ways. Ionizing radiation which include X-rays and gamma rays ionizes molecules and destroys DNA and other cell components. Ultraviolet (UV) radiation induces the formation of thymine dimmers and strand breaks in DNA and cause the cell death. Most deep-sea bacteria are barotolerant, but some are barophilic and require high pressure for optimal growth

Q. 1. Explain the influence of different environmental factors on the growth of bacteria.
Q.2. What are the physical factors of an environment? How these factors affect the bacterial growth.
Q.3. Describe the types of bacteria basing on their relationship O_2 requirement for growth.
Q.4. Write a note on the types of bacteria in relation to the effect of temperature on their growth.
Q.5. Explain the effects of pH and water activity on the growth of bacteria.

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Lesson – 4

Oxygenic Photosynthesis (Cyanobacteria)

4.0 Objective

In this lesson pigments involved in oxygenic photosynthesis, organization of photosynthetic electron transport chain, generation of reducing power, formation of ATP by cyclic and non-cyclic photophosphorylations and mechanism of carbon dioxide fixation in cyanobacteria are discussed.

4.1 Introduction

Life on earth depends on energy derived from the Sun. Photosynthesis is the only known process of biological importance that can harvest this energy. Literally, the term photosynthesis means “synthesis using light”. Green plants, cyanobacteria and pigment containing prokaryotes carry out this process. They are called photoautotrophs. Photoautotrophs are organisms that can use light as energy source and CO_2 as carbon source for the synthesis of carbohydrates. Photoautotrophs contain a photosynthetic apparatus that enables them to absorb light energy and package it as a bond energy in the form of ATP and NADPH. The energy of these compounds is used to drive the reactions that are involved in the fixation of carbon dioxide into carbohydrates. The carbohydrates, in turn provide cellular source of energy and of starting materials for the synthesis of proteins, lipids, nucleic acids and other cellular constituents not only in the photoautotrophs that produce them but also in non photosynthetic organisms that directly or indirectly consume photosynthetic organisms. In addition, a large fraction of the earth’s energy resource such as coal, oil and gas (fossil fuels) is due to the activity of photosynthesis in ancient times. It has been estimated that photosynthesis annually fixes more than 10^{11} tons of carbon which represents the storage of

over 10^{18} kj of energy. Over half of this photosynthetic CO_2 fixation on earth is carried out by microorganisms.

Photosynthesis as a whole is divided into light reactions and dark reactions. In the light reactions light energy is trapped and converted to chemical energy. This energy is then used to fix CO_2 in the dark reactions.

4.2 Photosynthetic bacteria

There are three groups of photosynthetic bacteria. They are cyanobacteria, purple bacteria and green bacteria. In this lesson the nature of photosynthesis in cyanobacteria is discussed.

4.3 Cyanobacteria

The cyanobacteira are the largest and most diverse group of photosynthetic prokaryotes. They perform oxygenic photosynthesis using a photosynthetic apparatus similar to that of chloroplasts of algae and higher plants. They use water as an electron donor and produce oxygen as a by-product during photosynthesis. Cyanobacterial photosynthesis can be represented as follows :

$$6CO_2 + 12\ H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6O_2 + 6H_2O$$

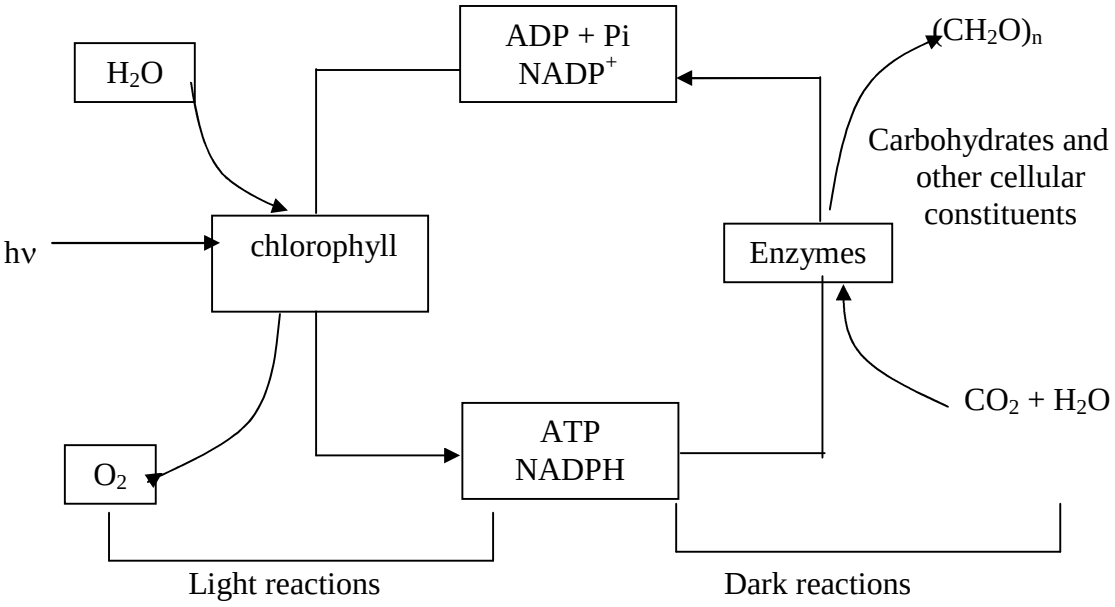


Figure 4.1 Cyanobacterial oxygenic photosynthesis. The light and dark reactions together make up photosynthesis. The energy flows from light to high energy intermediate compounds (ATP and NADPH) and then to long term energy bonds connecting carbon atoms of organic molecules.

4.3.1 Photosynthetic Apparatus

In various groups of photoautotrophs, photosynthetic apparatuses are compartmentalised in discrete highly specialized organelles called plastids. Photosynthetic cyanobacteria are devoid of typical cell organelles such as chloroplasts. However, the photosynthetic plastids of these organisms are represented by isolated and freely lying lamellae or thylakoids in the protoplasm. Schachman, Pradec and Stamier (1952) designated these membranous structures as **chromatophores**.

Cyanobacterial photosynthetic apparatus is made up of concentrically arranged circular layers of thylakoid membranes lying freely in the protoplasm without any distinction of grana and stroma regions (Figure 4.2). Thylakoids are lipoproteinaceous bilayer membranes. They contain both lipids and proteins. Phospholipids, galactolipids and sulfolipids are major lipids present in thylakoids. These thylakoid lipids contain a high proportion of highly unsaturated fatty acids, the major one being α – linolenic acid which can comprise as much as 90 percent of the total fatty acid content. They confer high degree of fluidity on membrane, which is essential for efficient photochemical functioning of the thylakoid membranes.

Figure 4.2 Cyanobacterial thylakoids and phycobilisomes. *Synechococcus lividus* with an extensive thylakoid system. The phycobilisomes lining these thylakoids are clearly visible as granules.

The proteins of thylakoid membranes in association with pigments are organized into five intrinsic and exstrinsic pigment protein complexes. They are designated as 1) PS II pigment – protein complex 2) Cytochrome b_6f pigment protein complex 3) PS I pigment-protein complex 4) Phycobilisome antenna and 5) ATP synthase complex. The first three complexes are involved in light driven electron and proton transport while fourth complex acts solely as a light harvesting antenna and has no photochemical activity. ATP synthase of thylakoids catalyses the synthesis of ATP from ADP and Pi during photosynthetic electron transport.

4.3.2 Pigments

Photosynthesis is a photobiological phenomenon that requires the participation of a molecule to absorb light energy. A molecule capable of absorbing light is known as pigment. Pigment molecules process the energy and information content of light into a form that can be used by the organism. The principal pigments found in cyanobacteria are chlorophyll a, carotenoids and phycobilins. Chlorophyll b is absent in cyanobacteria.

Chlorophyll a

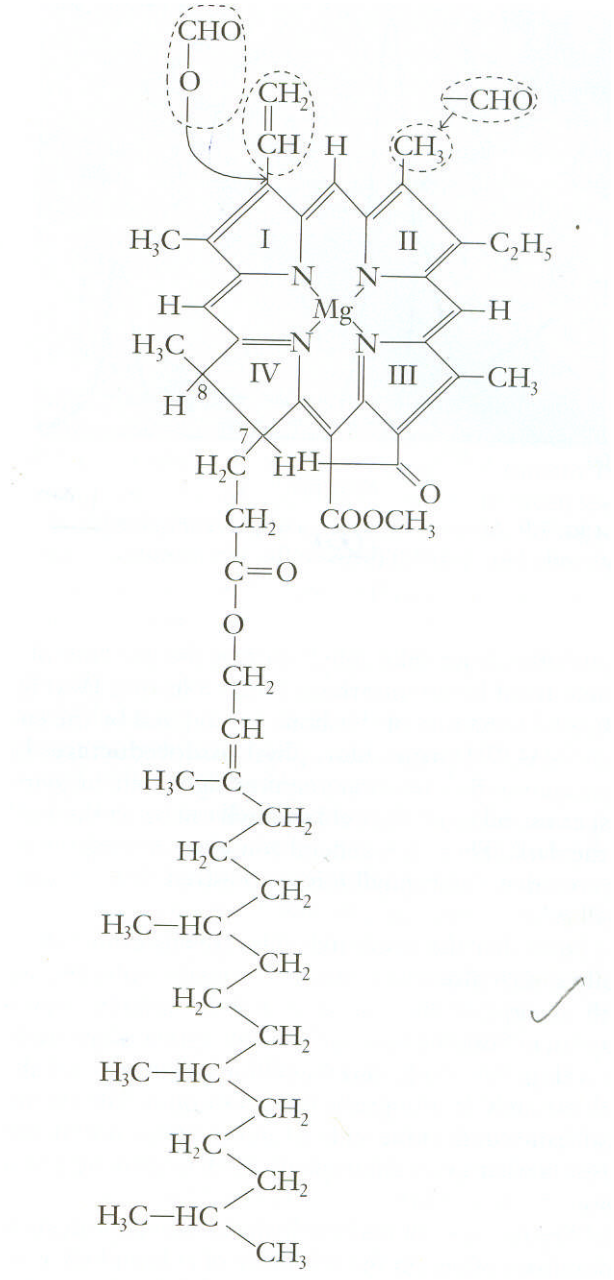


Figure 4.3 Chemical structure of chlorophyll a. Chlorophyll b found in higher plants is similar except that formyl group replaces the methyl group on ring II. Chlorophyll d present

in other algae is similar to chlorophyll a except that a-O-CHO group is substituted on ring I as shown.

Chlorophyll a is a principal light absorbing pigment. It consists of two parts, a porphyrin head and a long hydrophobic hydrocarbon tail also called phytol tail. A porphyrin is a cyclic tetrapyrrole, made up of four nitrogen containing pyrrole rings arranged in cyclic fashion. Magnesium is present at its centre. A long lipid soluble hydrocarbon phytol tail can extend from ring IV of porphyrin head (Figure 4.3)

Chlorophyll a has absorption maxima in the blue (435 nm) and red (663 nm) region of the visible spectrum (Figure 4.4). In thylakoid membranes, it is present as a water insoluble pigment protein complex.

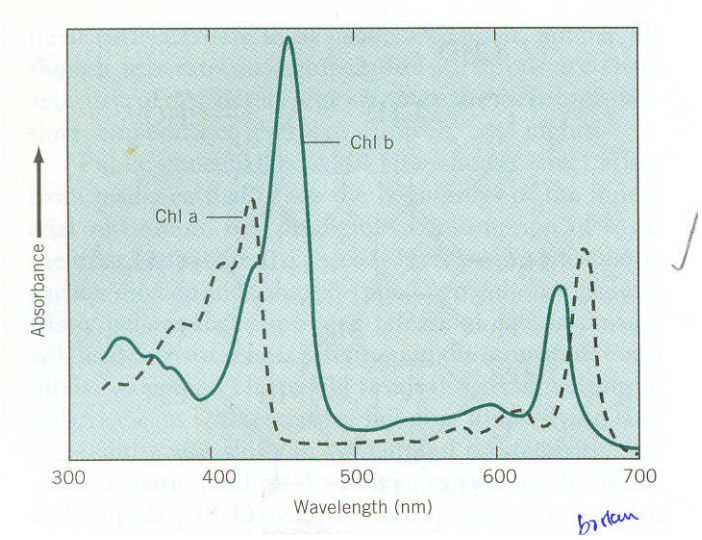


Figure 4.4 Absorption spectra of chlorophyll a (broken line) in comparision with chlorophyll b (solid line) in acetone.

Phycobilins

There are three photosynthetic phycobilins in cyanobacteria. They are phycoerythrin (also known as phycoerythrobilin), phycocyanin (phycocyanobilin) and allophycocyanin (allophycocyanobilin). These pigments differ from chlorophyll a in containing open chain tetrapyrrole with covalently linked protein molecule (Figure 4.5). A pigment that contains protein as an integral part of the molecule is known as a chromoprotein. Hence phycobilins are chromoproteins. On the surface of thylakoid membranes, the phycobiliproteins are organized into large macromolecular antenna complexes called **phycobilisomes**.

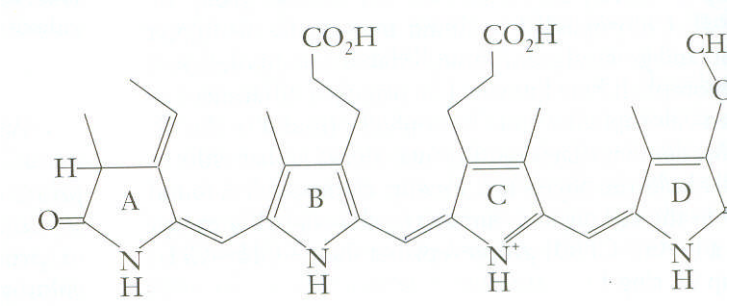


Figure 4.5 The open chain tetrapyrrole chromophore of pycocyanin.

The phycobilins are water soluble photosynthetic accessory pigments. They impart blue colour to the thallus and chlorophyll a imparts green. For this reason, the cyanobacteria are also called blue-green algae. These pigments absorb light energy in the green region where chlorophyll a does not absorb it (Figure 4.6). Phycobilims absorb light energy between 500-600 nm range and transfer that absorbed energy to chlorophyll a for its conversion into chemical energy.

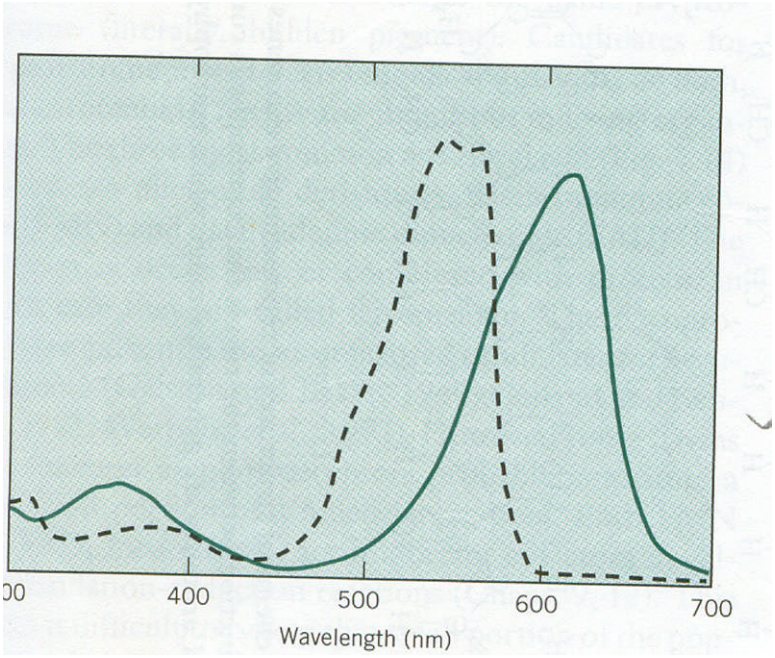


Figure 4.6 Absorption spectra of phycocyanin (solid line) and phycoerythrin (broken line) in dilute buffer.

Carotenoids

The carotenoid group of pigments includes carotenes and xanthophylls. Carotenes are carbon and hydrogen containing orange or red – orange coloured long hydrocarbon pigments. β–carotene is the major carotenoid in cyanobacteria (Figure 4.7).

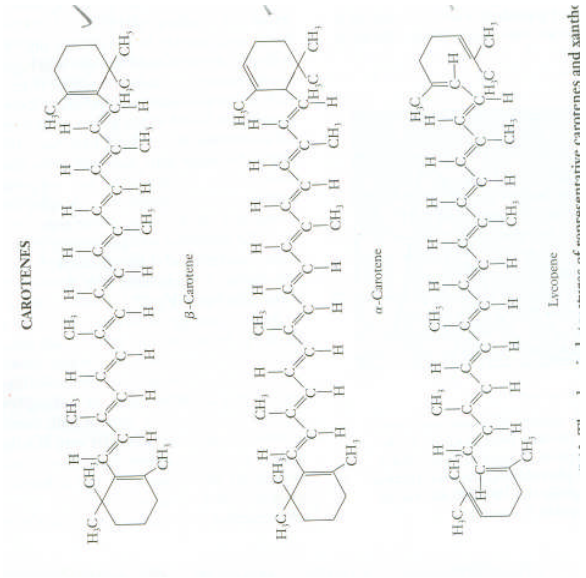


Figure 4.7 The chemical structures of β-carotene, α-carotene and lycopene.

The oxygenated, yellow carotenoids are called xanthophylls. Lutein, zeaxanthin and violaxanthin are principal xanthophylls found in cyanobacteria (Figure 4.8)

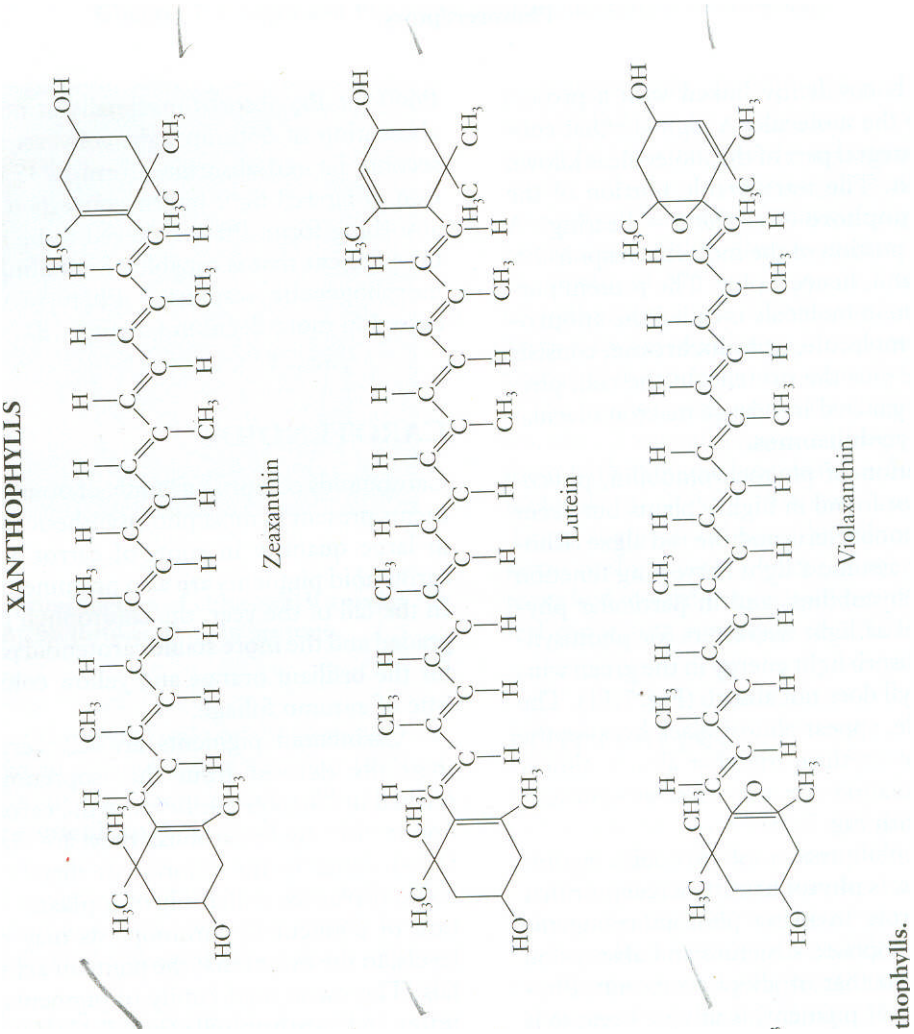


Figure 4.8 The chemical structure of representative xanthophylls. The principal distinction between the carotenes and the xanthophylls is that xanthophylls contain oxygen and carotenes do not (Figure 4.7).

Like chlorophyll a, carotenoids in the thylakoid membranes are present as water insoluble pigment protein complexes. Carotenoids may serve two principal functions during the course of photosynthesis.

1) They absorb and transfer light energy to chlorophyll a.
2) They protect the photosynthetic system from photooxidative damage.

As an accessory light harvesters, these pigments absorb light strongly between 460 nm and 490 nm and transfer that absorbed energy to chlorophyll a (Figure 4.9). β – carotene is the main pigment involved in this function. Xanthophylls on the other hand, are mainly involved in protecting the chlorophyll a against its photooxidative damage.

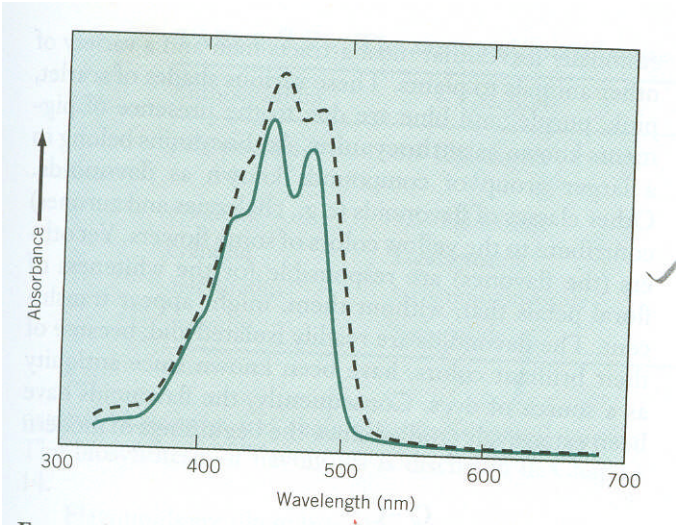


Figure 4.9 Absorption spectra of α-carotene (solid line) and β-carotene (broken line).

4.4 Mechanism of Light Reactions

Photosynthetic light reactions of cyanobacteria are carried out by four major protein complexes: The Photosystem II, cytochrome b_6f complex, Photosystem-I and the light harvesting phycobilisomes (Figure 4.10)

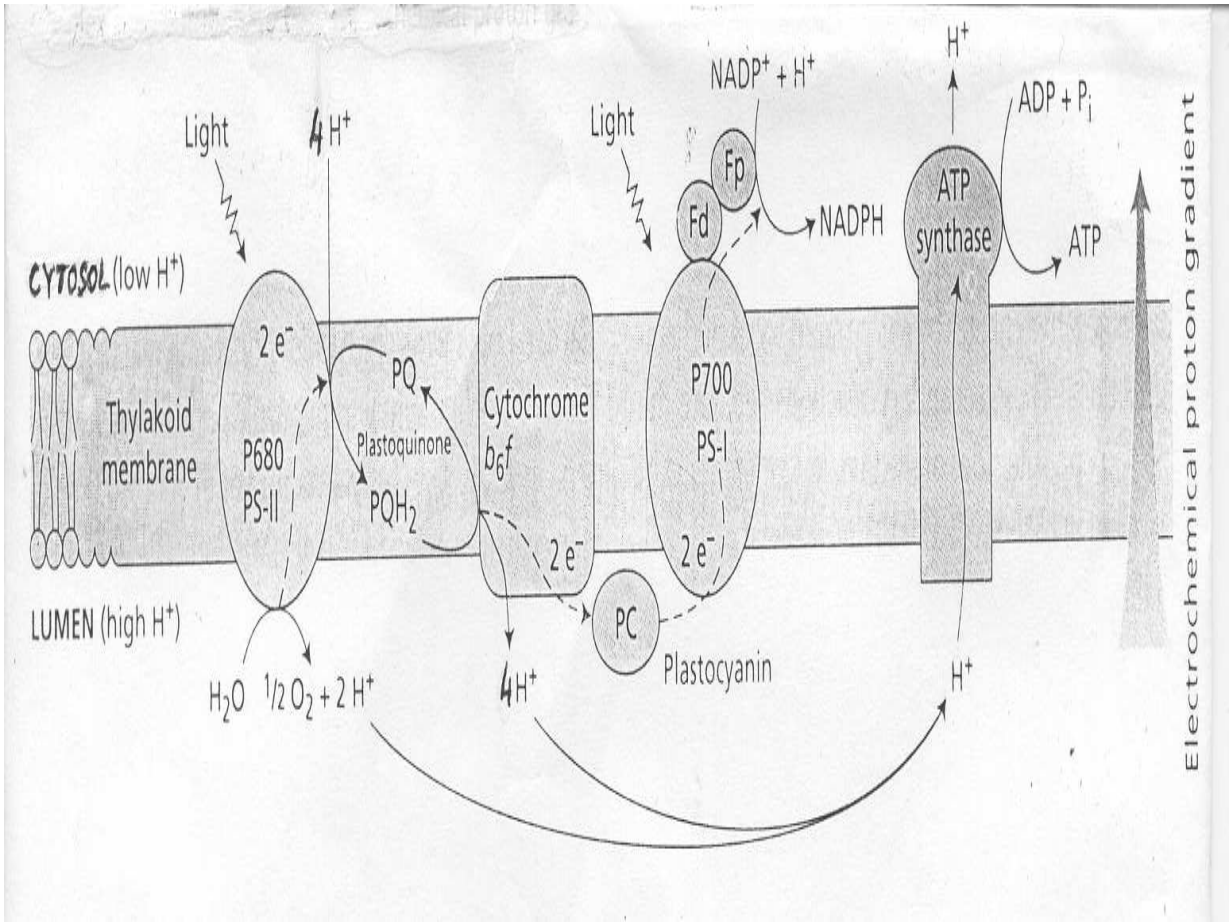


Figure 4.10 The transfer of electrons and protons in the thylakoid membrane is carried out vertically by four protein complexes. Water is oxidized and protons are released in the lumen by PS II. PS I reduces $NADP^+$ to NADPH in the cytosol, via the action of ferredoxin (Fd) and the flavoprotein ferredoxin – NADP oxidoreductase (Fp). Protons are also translocated into the lumen by the action of the cytochrome b_6f complex and contribute electrochemical proton gradient. These protons must then diffuse to the ATP synthase enzyme, where their diffusion down the electrochemical gradient is used to synthesize ATP in the cytosol.

The PS II reaction center core of cyanobacteria like higher plant chloroplasts is a heterodimeric protein with D_1 and D_2 as polypeptides. This heterodimer binds all the redox components necessary for primary photochemical reaction. These include reaction center light processing special chlorophyll a called P680, primary electron acceptor pheophytin, quinones and electron donor Z. This core in thylakoids in turn occurs along with the associated antenna components CP43 and CP47, cytochrome-b*559*, and the oxygen evolving complex (OEC) (Andersson and Styring 1991; Vermaas and Ikeuchi 1991). Phycobilisomes are present in close association with PS II.

Photosystem II traps light at shorter wavelength (≤ 680 nm) and transfer its energy to the special chlorophyll P680. The designation P680 reveals that it is a special chlorophyll-a of PS II with an absorption maxima at 680 nm. Light absorption causes this special pigment to be present in excited state. The excited form of P680 is designed as P680*. The excited

P*680** is rapidly photooxidised (within picoseconds, 10^{-12} sec) by donating its excited or high energy electron to pheophytin (Pheo). Pheophytin is a primary electron acceptor in PS II. It is a form of chlorophyll a in which the magnesium ion has been replaced by two hydrogens. This initial photochemical act results in the formation of $P680^+$ and $Pheo^-$. This reaction represents the conversion of light energy into chemical energy. Reduced pheophytin quickly passes the electron on to an other electron acceptor called Q_A and then down the electron transport chain to P700 via plastoquinone, cytochrome b_6f complex and plastocyanin.

Plastoquinone is a lipid soluble mobile electron carrier. It shuttles electrons in between PS II and cytochrome b_6f complex. Cytochrome b_6f complex is a multiprotein membrane spanning complex. It is composed of cytochrome b_6 (Cyt b_6), cytochrome f and rieske iron-sulfur protein [Fe –S]. Plastocyanin (PC) is a copper-binding small protein present on the luminal surface of thylakoid membrane. It is an electron carrier between cyt b_6f complex and P700.

Oxidised P680 is a very strong oxidant and is able to extract electrons from the oxidation of water through oxygen evolving complex (OEC). As a result P680 is reduced and is again available for next round of excitation. Oxygen evolving complex is a manganese containing multiprotein. The OEC is responsible for the oxidation of water and the consequent evolution of O_2. It is located on the lumen side of the thylakoid membrane in association with D_1 and D_2 proteins of the PS II reaction center.

In the mean time, a light driven charge formation similar to that involving P680, has also occurred in the reaction center PS I. PS I contains P700 chlorophyll a as a special pigment molecule on its polypeptides. The P700 absorbs light at a wavelength of 700 nm. In addition, the complex also contains chlorophyll a, primary electron acceptor (A) and several iron-sulfur (Fe-S) proteins. When light above 680nm is harvested and transferred to P700, the P700 is excited. Excited P700 then donates its high energy electron to primary acceptor (A) and then to iron-sulfur proteins. Finally, the electron is used to reduce ferredoxin on the cytoplasmic side of the membrane. In the presence of an enzyme ferredoxin – $NADP^+$ oxidoreductase, the electron of ferredoxin is transferred to $NADP^+$ to produce NADPH. The electron deficiency in $P700^+$ is finally fulfilled by withdrawing an electron from reduced plastocyanin. In this electron transport system the light driven electrons move from water to $NADP^+$ in a non-cyclic route through the two separate photosystems and the interveining cytochrome complex. This kind of electron transport is termed as **non-cyclic electron transport**. This is represented in the form of Z scheme (Figure 4.11)

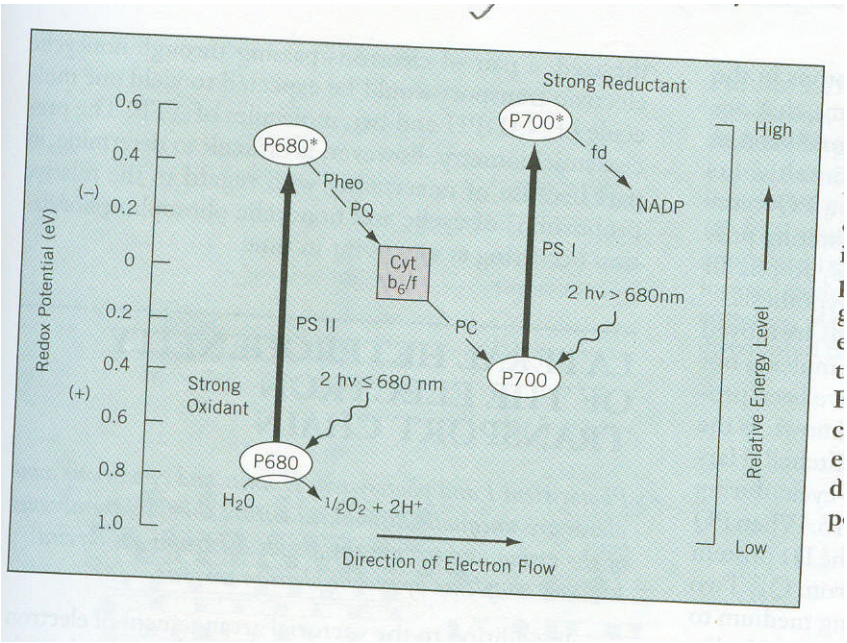


Figure 4.11 The Z scheme for photosynthetic electron transport. The redox components are placed at their approximate midpoint redox potentials. The vertical direction indicates a change in energy level. The horizontal direction indicates electron flow.

In addition to non-cyclic electron transport, the cyanobacteria like higher plants, under conditions of high ATP demand or low light intensities also employ another mode of electron transport called **cyclic electron transport**. In the cyclic pathway (Figure 4.12) the electron moves in a cyclic route through a series of electron carriers and back to the oxidized P700. In this case ferredoxin transfers the electron back to PQ rather than to $NADP^+$. The electron then returns to P700 passing through the cytochrome complex and plastocyanin.

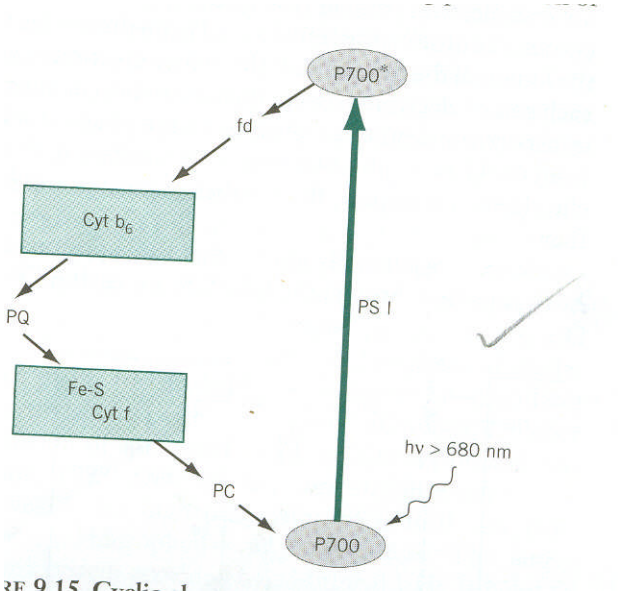


Figure 4.12 Cyclic electron transport. PS I units operating independently of PS II may return electrons from P700* to P700 through ferredoxin (fd), plastoquinone (PQ), and the cytochrome complex. No NADPH is produced by cyclic electron transport, but the energy can be used to produce ATP.

In photosynthetic non-cyclic electron transport light energy is absorbed at two points, the PS II and PS I. A portion of this absorbed energy is conserved as NADPH (218 kj mol^{-1}) while the remaining balance can be conserved as ATP by photophosphorylation. In cyclic electron transport light is absorbed only by P700 and is converted into ATP.

4.5 Photophosphorylation

Light driven synthesis of ATP by thylakoids is known as photophosphorylation. Two types of photophosphorylations are now known: the **non-cyclic photosphorylation** and **cyclic photophosphorylation**.

The formation of ATP in association with non-cyclic electron transport is known as non-cyclic photophosphorylation, while its synthesis by cyclic electron transport is referred to as cyclic photophosphoryaltion. ATP synthase complex of thylakoid membranes catalyses the synthesis of ATP from ADP and Pi in both cyclic and non-cyclic photophosphorylations.

ATP synthase is made up of two parts called F_0 and F_1. F_0 is a hydrophobic transmembrane protein that contains channel for proton translocation from the lumen to the cytoplasm. F_1 is a hydrophilic peripheral membrane protein present on the cytoplasmic side of the membrane in access with ADP and Pi. ATP synthesis is an energy requiring reaction (endergonic). ATP synthase complex then uses energy of the proton gradient which is

developed across the membrane i.e. in between the thylakoid lumen and the cytoplasm during photosynthetic electron transport.

4.5.1 Proton gradient

Proton gradient formation is a light driven process by which accumulation of protons (H^+) takes place in the lumen. In other words a part of light energy is primarily conserved in the form of proton gradient. It is developed in two ways :

1) The evolution of one molecule of O_2 from $2H_2O$ molecules releases four protons into the thylakoid lumen.

$$2H_2O \xrightarrow{\text{Light}} 4H^+ + 4e^- + O_2$$

2) The transport of the liberated 4 electrons (to produce 2 NADPH) through the cytochrome b_6f complex occurs with the translocation of nearly 8 protons from the cytoplasm to the lumen (Figure 4.10).

The mechanism of proton translocation across the membrane by the cytochrome complex was least understood, but can be explained on the basis of widely accepted chemiosmotic hypothesis proposed by Mitchell (1961). According to this hypothesis, the reduced plastoquinol (PQH_2) oxidation involves a cyclic series of reactions called **Q cycle** (Figure 4.13). In this cycle PQH_2 is oxidized to semiquinone anion (PQ^-) by passing one electron to plastocyanin and releasing two protons into the lumen. The semiquinone is fully oxidized to plastoquinone by cytochrome b_6. Reduced cytochrome b_6 ultimately passes the electron back to plastoquinone, reducing it to the semiquinone. The semiquinone form of plastoquinone then diffuses to D_1 protein of PS II and reduced to PQH_2 by taking one electron from the D_1 and two protons from the cytoplasm. Thus for each electron passing from plastoquinone to plastocyanin, two protons are translocated from the cytoplasm into the lumen of the thylakoid. According to this scheme, each molecule of evolved O_2 by non-cyclic electron transport contributes 12 protons to the gradient -8 from the Q-cycle and 4 from water oxidation. For cyclic electron transport, the number of protons would be 2 for one turn of electron.

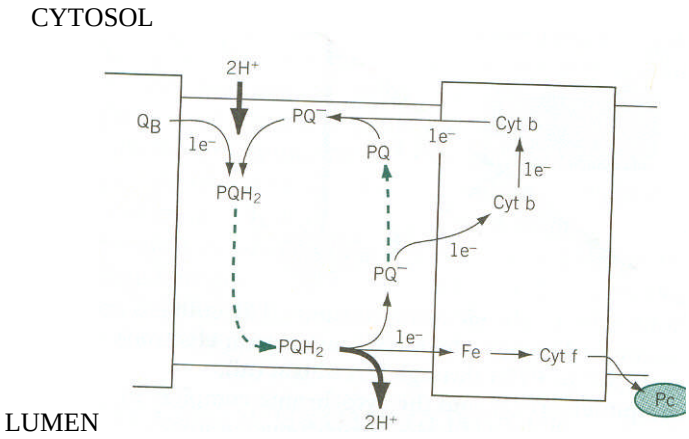


Figure 4.13 The Q-cycle, a model for coupling electron transport from platoquinol to cytochrome with the translocation of protons across the thylakoid membrane. Two protons are translocated for each electron that passes through the electron transport chain.

It is generally agreed that ATP synthase produces one ATP for every three protons it transports out of the thylakoid lumen. Non-cyclic electron transport in thylakoids, therefore results in the production of 12/3 = 4 molecules of ATP per molecule of O_2 evolved (although this quantity is subject to revision) or around one half of an ATP per photon absorbed (4/8 = ½ ATP per light quanta absorbed)

The overall light reaction can be represented as:

$$2H_2O + 2NADP^+ + 4\ ADP + 4\ P_i \xrightarrow[\text{Pigments}]{8h\nu} 2NADPH + 4\ \ ATP + 2H^+$$

4.6 Carbon fixation Mechanism

In the previous sections we saw how the light energy is harnessed to generate ATP and NADPH by the photosynthetic cyanobacteria. The important role of these products in the synthesis of carbohydrates and other substances from CO_2 will be discussed in this section.

Photosynthetic cyanobacteria when growing with CO_2 as carbon source will employ the reactions of the Calvin cycle for CO_2 fixation and is described below.

4.6.1 Calvin cycle

The metabolic pathway by which all autotrophs incorporate CO_2 into carbohydrates was elucidated between 1946 and 1953 by Melvin Calvin, James Bassham and Andrew Benson. It is called by several names : the Calvin cycle, Calvin-Benson cycle, Reductive Pentose Phosphate cycle (RPP) and more recently as Photosynthetic Carbon Reduction cycle (PCR). It is located in the cytoplasm of cyanobacteria.

4.6.2 Overview of Calvin cycle

In the Calvin cycle, CO_2 and H_2O from the environment are enzymatically combined with a five-carbon acceptor molecule to generate two molecules of a three carbon 3-phosphoglyceric acid, the first stable intermediate in the Calvin cycle. This part of the cycle is called the **carboxylation** stage. The 3-phosphoglycerate is reduced to Glyceraldehyde-3-phosphate, a three carbon carbohydrate by use of ATP and NADPH generated photochemically by a process what is known as **reduction stage**. At this point one GAP can be blend off from the cycle for use in biosynthesis. The cycle is completed by regeneration of the five carbon acceptor ribulose-1,5-bisphosphate (Figure 4.14).

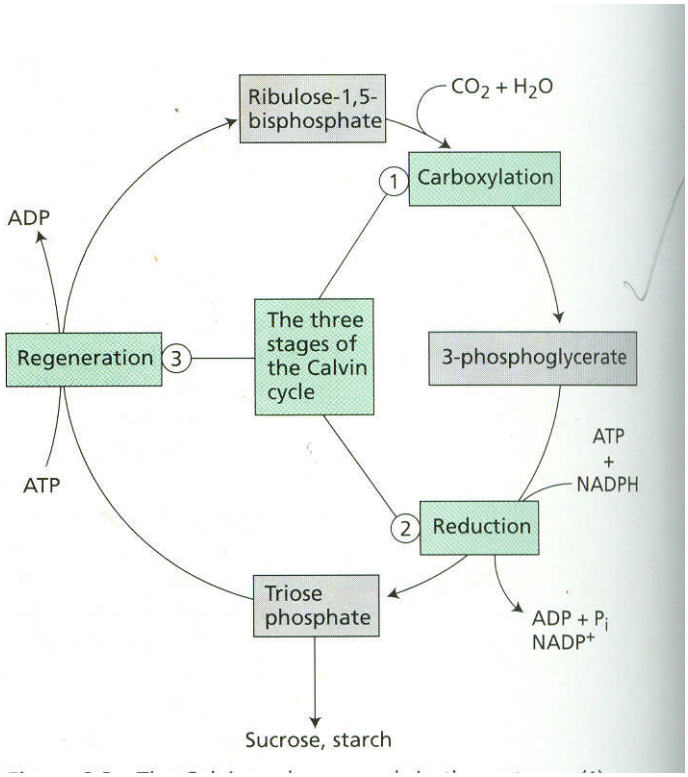
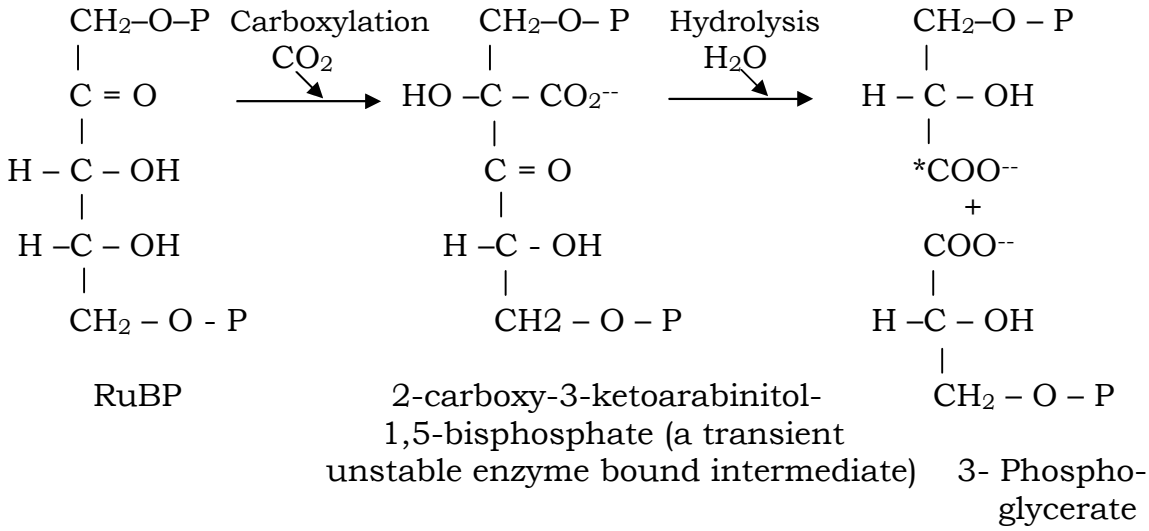


Figure 4.14. The three stages of Calvin cycle.

4.6.3 Mechanism of Calvin cycle

Calvin cycle as a whole consists of 13 enzymatic reactions which are described below.

1) Carbon dioxide is first accepted by ribulose-1, 5-bisphophate and forms an unstable enzyme bound intermediate 2-carboxy-3-ketoarabinitol-1,5-bispshosphate. This is further hydrolyzed to yield two molecules of the stable product 3-phophoglycerate. This carboxylation reaction is catalyzed by the enzyme **ribulose-1,5-bisphosphate carboxylase/oxygenase** generally called **rubisco**.



RuBP

2-carboxy-3-ketoarabinitol-1,5-bisphosphate (a transient unstable enzyme bound intermediate)

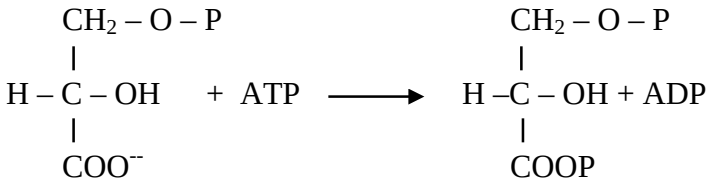
3- Phospho-glycerate

(*Symbol on C indicates incorporated carbondioxide)

Cyanobacteria and several other photosynthetic bacteria possess **Carboxysomes**. These are polyhedral inclusions consist of ribulose-1,5-bisphosphate carboxylase. Their biological function is not absolutely clear. They may be the site of CO_2 fixation or may store the carboxylase and other proteins. In some photoautotrophs, rubisco amounts to upto 20 percent of the total protein.

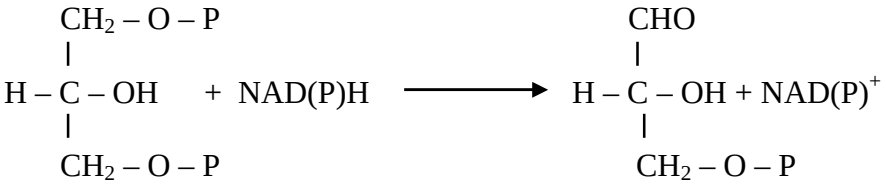
In various groups of photoautotrophs, rubisco is functionally analogous and structurally diverse. Oxygenic phototrophs (cyanobacteria) and many photosynthetic bacteria contain a form of the enzyme made up of eight large (L) catalytic subunits (about 55 kDa each) and eight small (S) subunits (about 14 kDa each). The complete protein (L_8S_8) has the molecular mass of about 560 kDa. In some photosynthetic purple non-sulphur bacteria, rubisco is composed of only two large subunits, each 50 kDa.

2) In the reduction stage of the Calvin cycle, the 3-phosphoglycerate (3-PGA) formed as a result of carboxylation is reduced to glyceraldehyde-3-phosphate (GAP). This reaction occurs in two steps. In the first step the 3-phosphoglycerate reacts with ATP and forms 1-3- bisphosphoglycerate (BPGA). This reaction represents the activation of PGA and it is the first step where light energy is used as ATP. The **phosphoglycerate kinase** catalyses this reaction.



3 - PGA BPGA

3) In the second step-1,3-bisphosphoglycerate is reduced to glyceraldehyde-3- phosphate by using the NAD(P) H generated by the light reactions. This reaction is regulated by the enzyme **glyceraldehyde-3-phosphate dehydrogenase**.



BPGA GAP

4) The phosphoglyceraldehyde molecule then converted into dihydroxy acetone phosphate (DHAP) by the enzyme **triose phosphate isomerase**. This triose is available for the biosynthesis of sugars and other cellular components. If this triose is completely used for biosynthesis, CO_2 fixation would be stopped due to the shortage of acceptor RuBP. Thus a third process must follow i.e. the regeneration of the CO_2 acceptor. This is accomplished by a series of reactions involving 4-,5-,6-, and 7-carbon sugars (Fig.4.14).

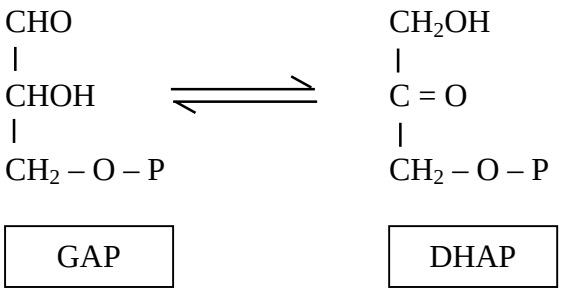
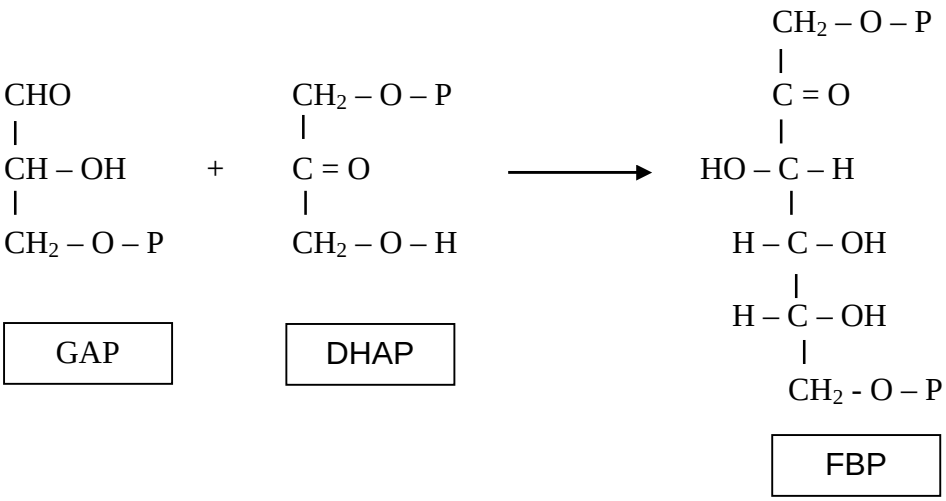
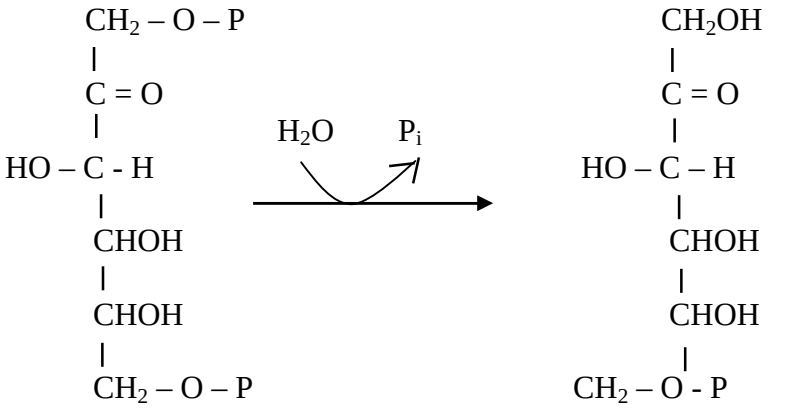


Fig.4.14 shows that for every three turns of the cycle i.e. the uptake of three CO_2, there is sufficient carbon to regenerate the required number of acceptor molecules as well as one additional triose phosphate available for sugar formation.

5) In the presence of enzyme **aldolase**, the 3-phosphoglyceraldehyde and dihydroxyacetone phosphate undergo condensation to yield fructose-1,6-bisphosphate (FBP).

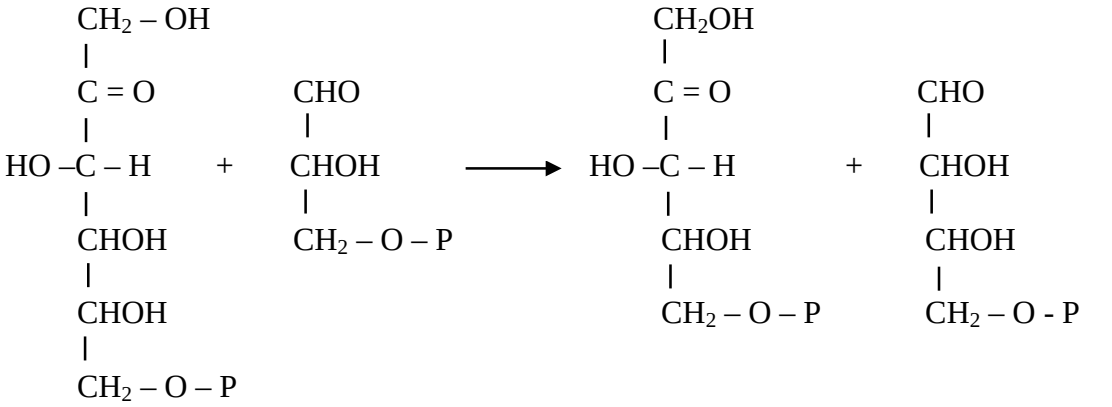


6) **Fructose-1,6-bisphosphate phosphatase** catalyses the dephosphorylation of fructose-1,6-bisphosphate to form fructose 6-phosphate (F6P).



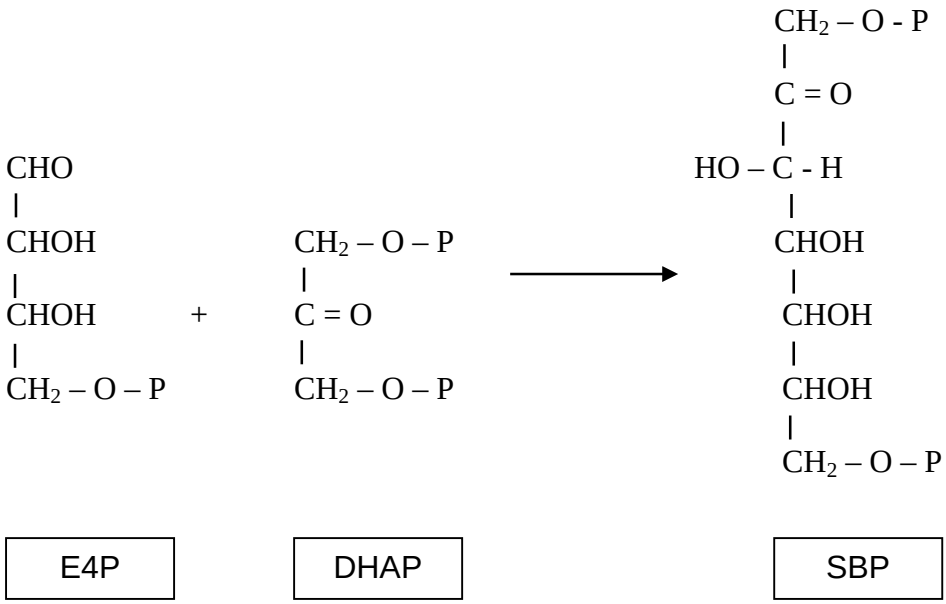
FBP F6P

7) Fructose-6-phosphate then reacts with 3-phosphoglyceraldehyde and form one molecule each of xylulose-5-phosphate (Xu5P) and erythrose-4-phosphate (E4P) in the presence of an enzyme **transketolase**.

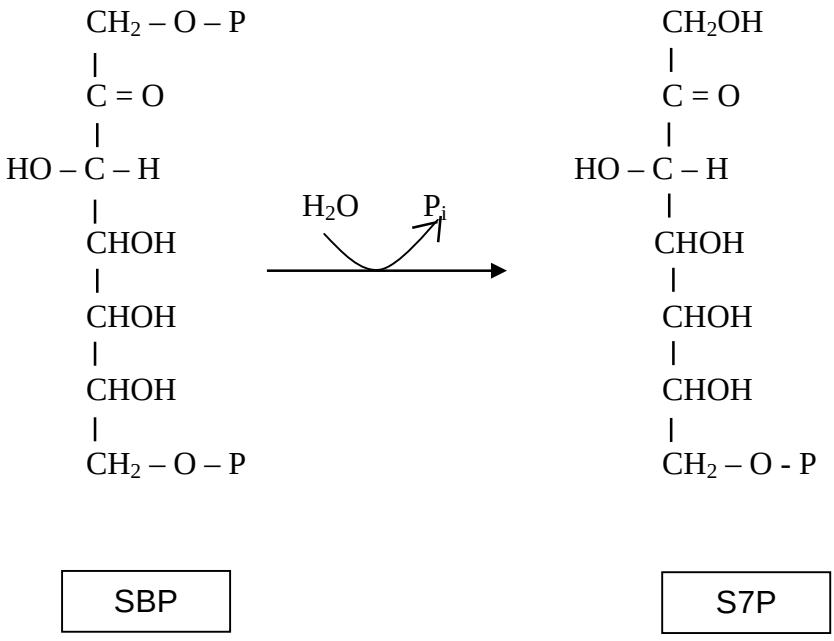


F6P GAP Xu5P E4P

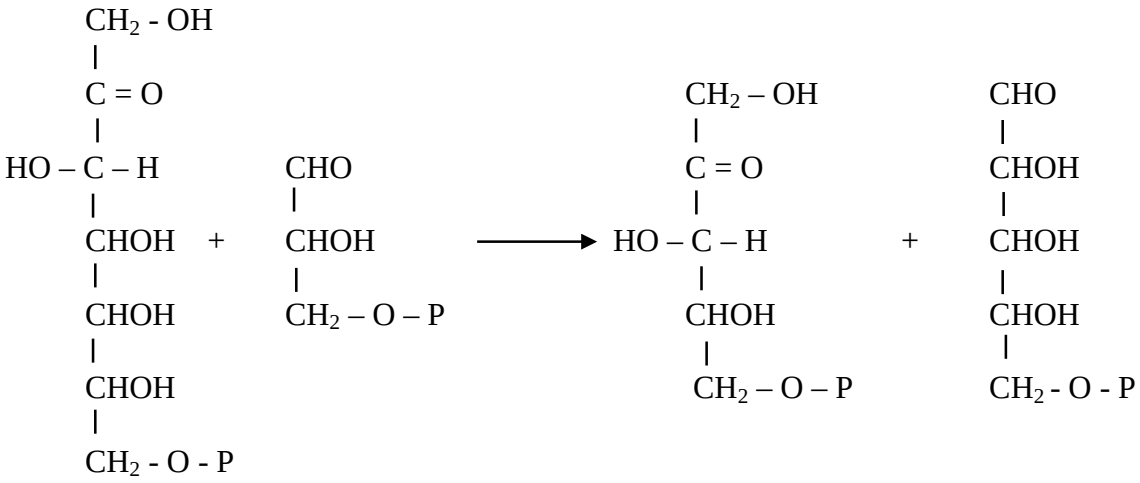
8) Erythrose-4-phosphate combines with a molecule of dihydroxyacetone phosphate to form sedoheptulose-1,7-bisphosphate (SBP) in the presence of **aldolase**.



9) Sedoheptulose-1,7-bisphosphate is further hydrolysed by way of a specific **phosphatase** to give sedoheptulose-7-phosphate (S7P).

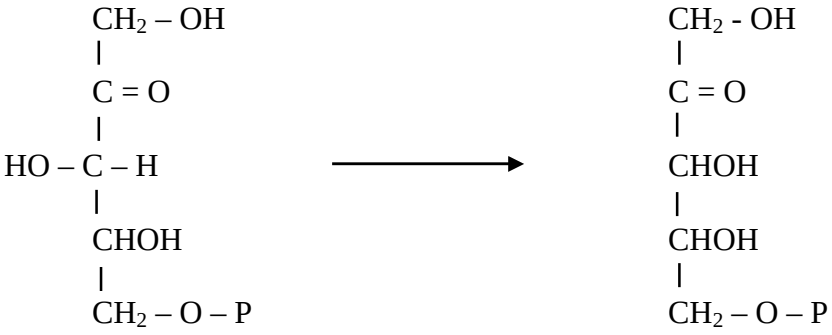


10) Sedoheptulose-7-phosphate donates a two carbon unit to the molecule of glyceraldehyde-3-phosphate via **transketolase** and produces ribose-5-phosphate (R5P) and xylulose-5- phosphate.



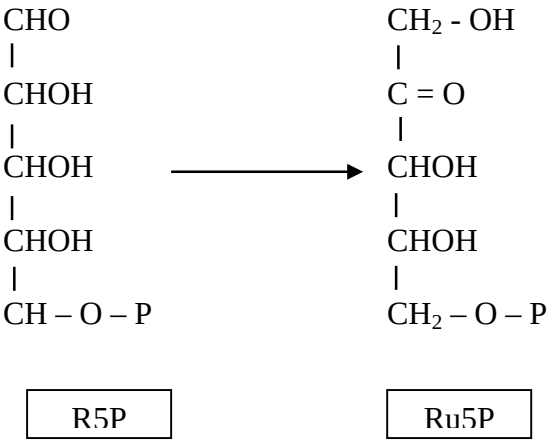
S7P GAP Xu5P R5P

11) The two molecules of xylulose-5-phosphate formed in reactions 7 and 10 are converted to two ribulose-5-phosphate (Ru5P) sugars by an **epimerase**.



Xu5P Ru5P

12) By the action of **isomerase**, the ribose-5-phosphate (formed in reaction 10) is also converted to ribulose-5-phsophate.



13) Finally, in the presence of **ribulose-5-phosphate kinase** (phosphoribulo kinase), ribulose-5-phosphate is phosphorylated with ATP and regenerating the three molecules of initial CO_2 acceptor ribulose-1,5-bisphosphate.

Sugar formed in the Calvin cycle can then be used to synthesize other essential molecules.

The carbon balance of the whole cycle can be described schematically as follows :

$3\ C_5 + \boxed{3\ CO_2} \longrightarrow 5\ C_3 + \boxed{C_3} \longrightarrow$ Sugar formation

fixation and reduction

$$5\ C_3 \longrightarrow 3\ C_5$$

regeneration

A total of 9 ATP and 6 NADPH are required for the formation of one glyceraldehyde-3-phosphate (GAP) from CO_2. In otherwords incorporation of one CO_2 into an organic material requires three ATP and two NADPH.

$$3\ CO_2 + 9\ ATP + 6\ NADPH.H^+ \longrightarrow GAP + 9\ ADP + 8\ P_i + 6NADP^+$$

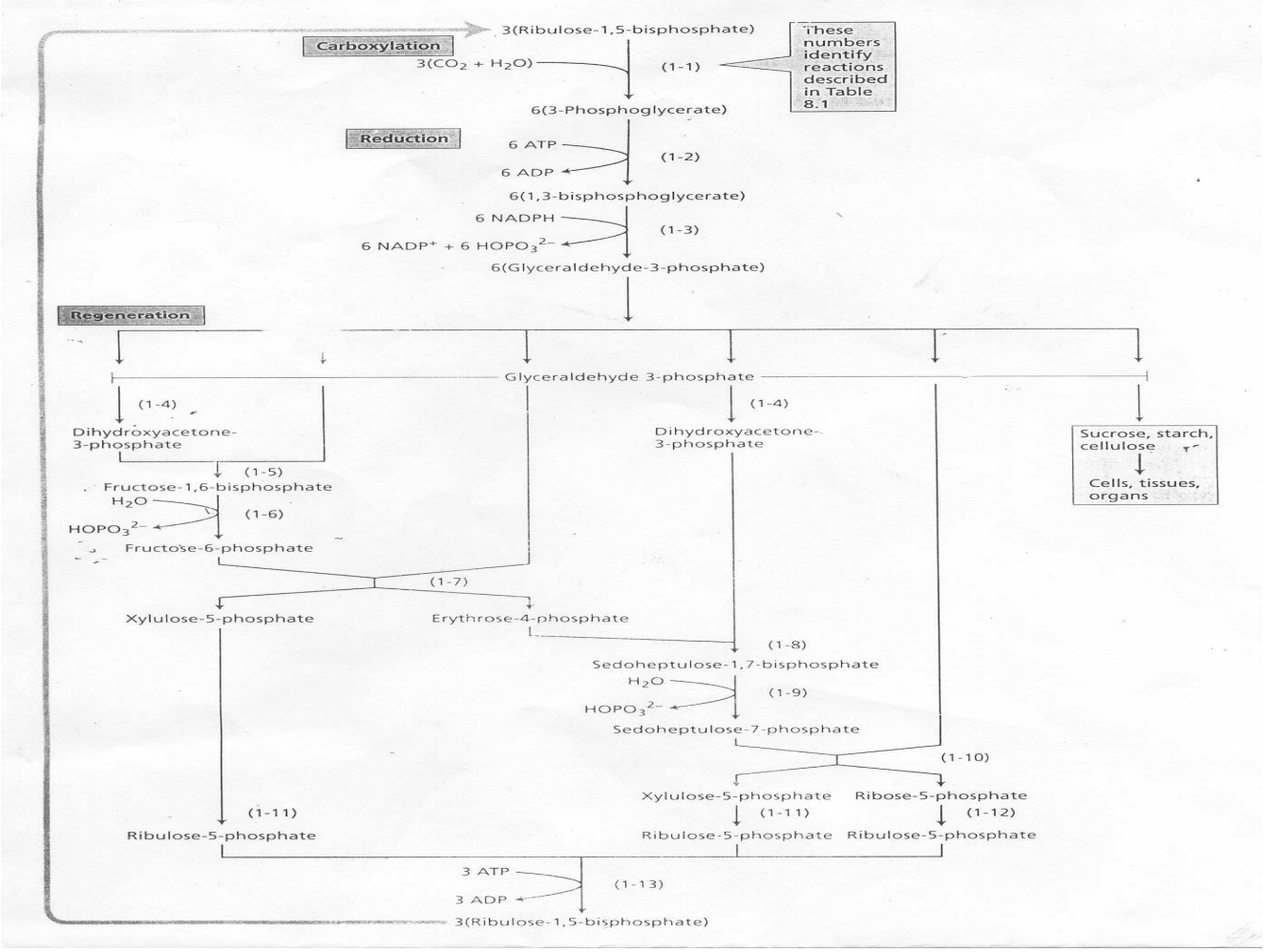


Figure 4.14 Reactions of Calvin cycle 1, RuBP carboxylase/oxygenase; 2, Phsophoglycerate kinase; 3, Glyceraldehyde-3-phosphate dehydrogenase; 4, Triosephosphate isomerase; 5, Aldolase; 6, Fructose-1, 6-bisphosphate phosphatase; 7, Transketolase; 8, Aldolase; 9, Sedoheptulose-1,7-bisphosphate phosphatase; 10, Transketolase; 11, Phosphoribulo epimerase; 12, Ribose-5-phosphate isomerase; and 13, Phosphoribulo kinase.

4.7 Differences between cyanobacteria and eukaryotic photosynthesizers

The cyanobacterial photosynthetic system differs from that of higher plant chloroplasts in atleast four key ways.

1) Cyanobacteria have phycobilins as the major light harvesting pigments, organized in phycobilisomes on the surface of the thylakoid membranes (Bryant 1991). In contrast, the chlorophyll a/b binding light harvesting chlorophyll protein complexes of higher plants are integral components of the thylakoid membranes, surrounding the PS-II core.

2) Cyanobacterial thylakoids are arranged in concentric layers at the periphery of the cell, while in chloroplasts of higher plants the thylakoids are differentiated into stacked grana regions enriched in PS II and unstacked stromal membranes (Andersson and Anderson 1988).

3) In cyanobacteria, the photosynthetic and respiratory electron transport chains share the same plastoquinone pool and the cytochrome b_6f complex, while in higher plants photosynthesis and respiration are confined to separate organelles (Peschek 1987).

4) Cyanobacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) has a much lower affinity for CO_2 than the higher plant counter part (Raven 1984) and cyanobacteria have compensatory mechanisms to accumulate inorganic carbon (Miller et al 1991).

4.8 Summary

Photosynthesis is the storage of solar energy carried out by photoautotrophs. Light is absorbed mainly in the antenna complexes, which in cyanobacteria comprise chlorophyll a, carotenoids, phycobilins and proteins. They are located in the concentrically arranged thylakoid membranes.

Photosynthetic antenna pigments transfer the light energy to a reaction center. The reaction center contains multiple subunit protein complexes and closely associated chlorophyll a containing proteins. Reaction center processes the radiant energy of light into chemical energy by initiating a series of photochemical events.

Cyanobacteria possess two reaction centers designated as PS I and PS II and carry out an oxygenic photosynthesis like the green plants. The reaction center chlorophylls of PS I absorb maximally at 700 nm and those from photosystem II at 680 nm. PS II and PS I together carry out non-cyclic electron transport, oxidize water to O_2 and reduce $NADP^+$ to NADPH. Photosystem I alone is involved in cyclic electron transport.

A portion of the light energy (photons) is initially stored as chemical potential energy, largely in the form of a pH difference across the thylakoid membranes. This energy is quickly converted into chemical energy during ATP formation by the action of an enzyme ATP synthase. ATP synthesis takes place either by non-cyclic photophosphorylation or cyclic photophosphorylation. NADPH and ATP generated in the light driven electron transport serve as energy source for carbon reduction.

The cyanobacteria reduce CO_2 by Calvin cycle in the cytoplasm. Here, CO_2 and H_2O are combined with acceptor ribulose-1,5-bisphosphate to form two molecules of 3-phosphoglycerate, which are reduced and converted to carbohydrate. The continued operation of the cycle is ensured by the regeneration of ribulose-1,5-bisphosphate. The Calvin cycle consumes two molecules of NADPH and three molecules of ATP for every molecule of CO_2 fixed.

4.9 Model Questions

1. What is oxygenic photosynthesis? Describe the light dependent phase of photosynthesis.
2. Describe dark reactions of photosynthesis.
3. Write short notes on :
   (1) Photophosphorylation
   (2) Pigments
   (3) Organization of the photosynthetic electron transport chain components
   (4) Rubisco

4.10 Reference Books and Journals

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Lesson - 5

Anoxygenic photosynthesis (Purple and Green bacteria)

5.0. Objective

In this lesson photosynthetic bacteria that carry out anoxygenic photosynthesis, organization of their photosynthetic apparatus, pigments, electron transport chain and its organization and carbon dioxide fixation mechanisms are discussed.

5.1. Introduction

Microorganisms that carry out anoxygenic photosynthesis are fall into two categeories the purple bacteria and the green bacteria. Unlike cyanobacteria, these bacteria carry out anoxygenic photosynthesis, in that they usually employ reduced compounds such as hydrogen sulfide, sulfur, hydrogen and organic molecules as their electron source and generate sulfur or other matter as a by-product. These organisms are unable to use water as an electron source consequently do not produce O_2 during photosynthesis. For this reason their photosynthesis is called anoxygenic.

Schematically anoxygenic photosynthesis can be represented as follows :

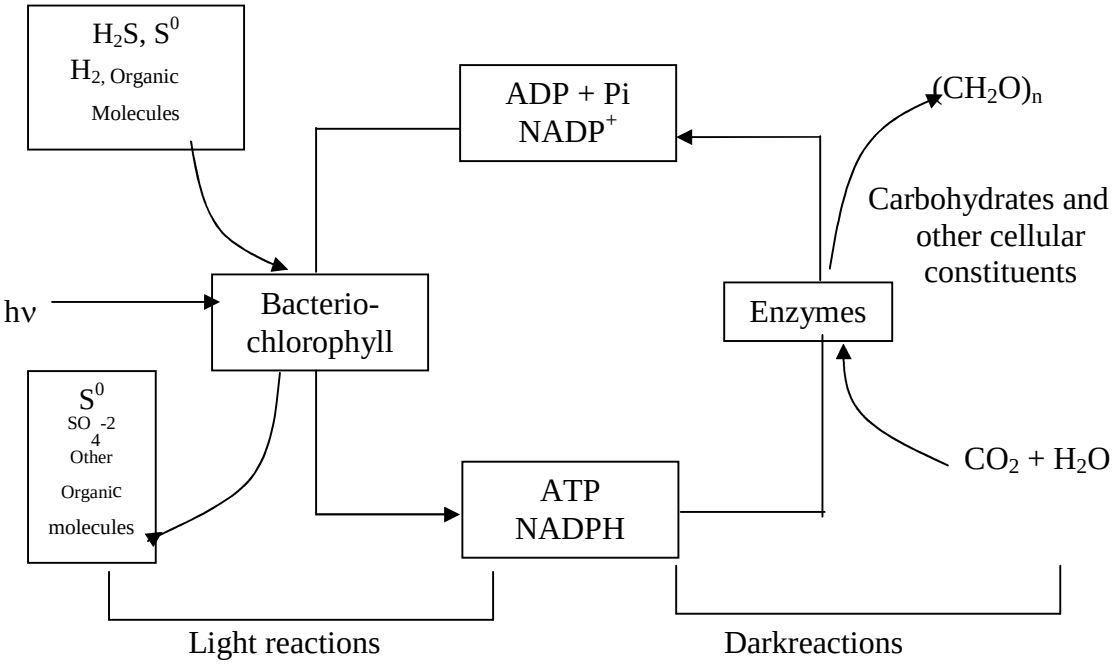


Figure 5.1 Anoxygenic photosynthesis in purple and green bacteria.

5.2. Purple bacteria

Purple bacteria are of two types namely purple sulfur bacteria and purple non-sulfur bacteria. *Thiospririllum, Thiocapsa* and *Chromatium* are typical purple sulfur bacteria located in the family Chromatiaceae. They are strict anaerobic photolithoautotrophs. Purple sulfur bacteria generally use hydrogen sulfide and oxidize it to sulfur.

$$2\,H_2S + CO_2 \xrightarrow[\text{Bacteriochlorophyll}]{\text{Light}} (CH_2O)_n + H_2O + 2S$$

Sulfur produced in this reaction is deposited internally within invaginated pockets of the plasmamembrane. Some times this sulfur and hydrogen may also serve as electron donors. Purple sulfur bacteria are found in anaerobic sulfide rich zones of lakes and ponds.

The purple non-sulfur bacteria are grouped in the family Rhodospirillaceae. They trap light energy and use organic molecules as electron source. *Rhodospirillum, Rhodopseudomonas* and *Rhodomicrobium* are some of the purple non-sulfur bacteria. They fix CO_2 into carbohydrate according to the following general equation.

$$2HOOC - CH_2 - CH_2 - COOH + CO_2 \xrightarrow[\text{Bacteriochlorophyll}]{\text{Light}} (CH_2O)_n + 2HOOC - CH = CH - COOH + H_2O$$

5.2.1 Photosynthetic apparatus in purple bacteria

The photosynthetic apparatus of purple bacteria is located in the vesicular invaginations of the cytoplasmic membrane. These structures maintain continuity with the plasma membrane. The vesicular invaginations consist of bacteriochlorophyll a and b, carotenoids and several proteins.

Bacteriochlorophyll a and b like chlorophyll a contain a tetrapyrrolic porphyrin head and a phytol tail. But they differ from chlorophyll a in having different chemical substituents in the positions R_1 to R_7 as shown in Fig. 5.2 and accompanying table 5.1.

Table 5.2 Different chemical substituents of Bacteriochlorophyll

Pigment	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Infrared absorption maxima (nm) *In vivo*	Infrared absorption maxima (nm) Extract (methanol)
Bacteriochlorophyll *a* (purple bacteria)	$-\overset{\overset{O}{\|}}{C}-CH_3$	$-CH_3$[b]	$-CH_2-CH_3$	$-CH_3$	$-\overset{\overset{O}{\|}}{C}-O-CH_3$	P/Gg[a]	—H	805 830–890	771
Bacteriochlorophyll *b* (purple bacteria)	$-\overset{\overset{O}{\|}}{C}-CH_3$	$-CH_3$[c]	$=\overset{\overset{H}{\|}}{C}-CH_3$	$-CH_3$	$-\overset{\overset{O}{\|}}{C}-O-CH_3$	P	—H	835–850 1020–1040	794
Bacteriochlorophyll *c* (green sulfur bacteria)	$-\overset{\overset{H}{\|}}{\underset{\underset{OH}{\|}}{C}}-CH_3$	$-CH_3$	$-C_2H_5$ $-C_3H_7$[d] $-C_4H_9$	$-C_2H_5$ $-CH_3$	—H	F	$-CH_3$	745–755	660–669
Bacteriochlorophyll c_s (green nonsulfur bacteria)	$-\overset{\overset{H}{\|}}{\underset{\underset{OH}{\|}}{C}}-CH_3$	$-CH_3$	$-C_2H_5$	$-CH_3$	—H	S	$-CH_3$	740	667
Bacteriochlorophyll *d* (green sulfur bacteria)	$-\overset{\overset{H}{\|}}{\underset{\underset{OH}{\|}}{C}}-CH_3$	$-CH_3$	$-C_2H_5$ $-C_3H_7$ $-C_4H_9$	$-C_2H_5$ $-CH_3$	—H	F	—H	705–740	654
Bacteriochlorophyll *e* (green sulfur bacteria)	$-\overset{\overset{H}{\|}}{\underset{\underset{OH}{\|}}{C}}-CH_3$	$-\overset{\overset{O}{\|}}{C}-H$	$-C_2H_5$ $-C_3H_7$ $-C_4H_9$	$-C_2H_5$	—H	F	$-CH_3$	719–726	646
Bacteriochlorophyll *g* (heliobacteria)	$-\overset{\overset{H}{\|}}{C}=CH_2$	$-CH_3$[b]	$-C_2H_5$	$-CH_3$	$-\overset{\overset{O}{\|}}{C}-O-CH_3$	F	—H	670, 788	765

[a] P, Phytyl ester ($C_{20}H_{39}O-$); F, farnesyl ester ($C_{15}H_{25}O-$); Gg, geranylgeraniol ester ($C_{10}H_{17}O-$); S, stearyl alcohol ($C_{18}H_{37}O-$).
[b] No double bond between C_3 and C_4; additional H atoms are in positions C_3 and C_4.
[c] No double bond between C_3 and C_4; an additional H atom is in position C_3.
[d] Bacteriochlorophylls *c*, *d* and *e* consist of isomeric mixtures with the different substituents on R_3 as shown.

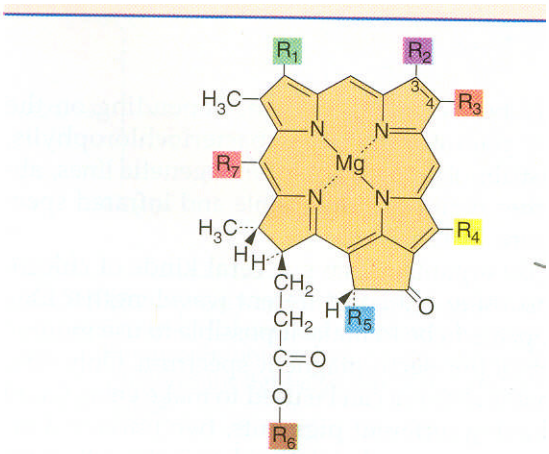


Figure 5.2 Structure of all known bacteriochlorophylls. The different substituents present in the positions R_1 to R_7 are given in the accompanying table.

Methanol extracts of bacteriochlorophyll a and b show absorption maxima at 771 and 794 nm, respectively. However, their in vivo maxima are about 830 to 890 nm for bacteriochlorophyll a, and 1,020 to 1,040 nm for bacteriochlorophyll b. This long wave length absorption maxima of bacteriochlorophyll permits these organisms to grow best in deeper anaerobic zones of aquatic habitats. These pigments also show second light absorption peak between 400 and 500 nm.

Carotenoids are accessory pigments. They are responsible for red-purple and brownish colour of the organisms. A typical carotenoid of purple bacteria is spirilloxanthin. It absorbs light in between 400 and 500 nm (Figure 5.3) and transfers that energy to the reaction center where it may be used in photophosphorylation in the same way as light energy captured directly by chlorophyll.

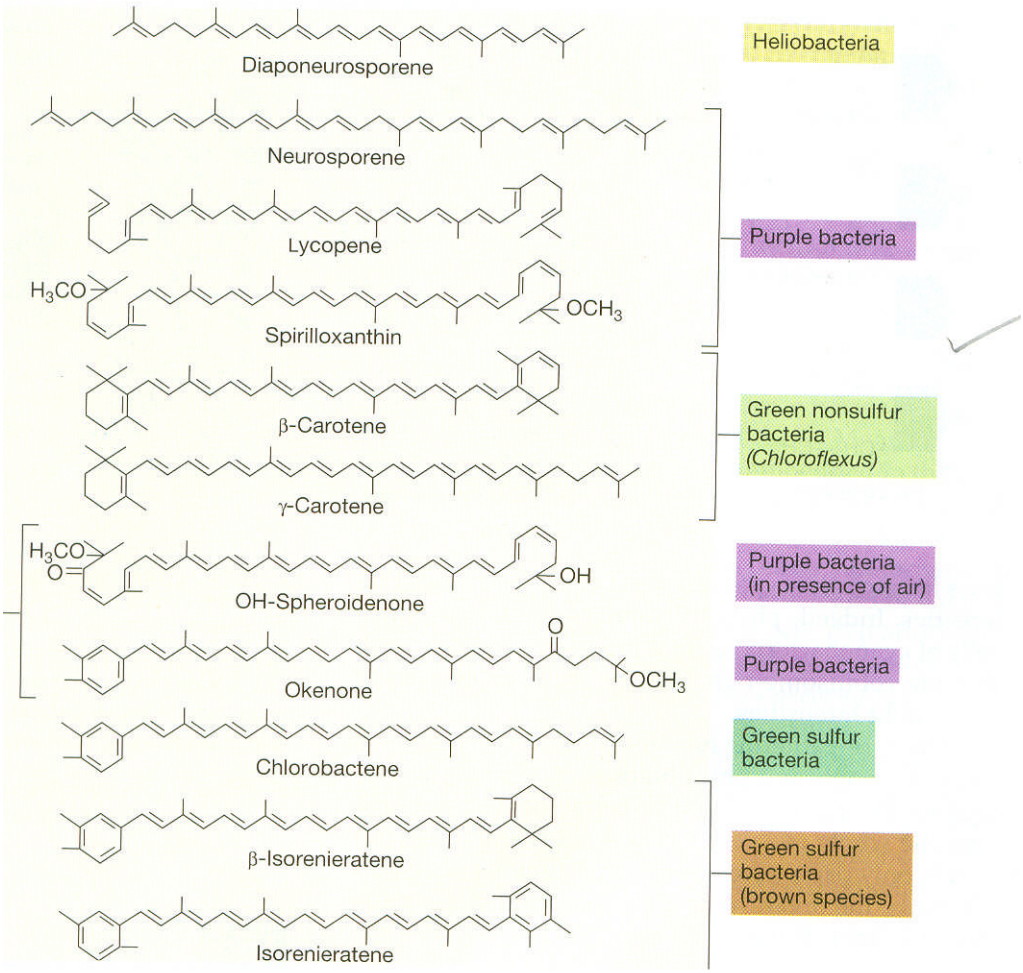


Figure 5.3 Structures of some common corotenoids found in anoxygenic phototrophs. For simplicity, in the structures shown here, methyl (CH_3) groups are designated by their bonds only.

5.2.3 Light reactions in purple bacteria

Purple bacteria do not use water as an electron source or produce O_2 photosynthetically, that is they are anoxygenic. Further they do not produce NADPH and NADH directly in their photosynthetic light reactions due to lack of photosystem II. The purple bacteria, however can generate ATP by having cyclic electron transport. ATP synthesized through cyclic photophosphorylation may then be used to generate NADPH and NADH by using electron donors like H_2, H_2S, S^0 and organic compounds by the mechanism of reverse electron flow.

The photosynthetic electron transport chain of purple bacteria is contained in intra-cytoplasmic membrane invaginations. It consists of a multisubunit membrane spanning reaction center and cytochrome bc_1 complex. Reaction center is surrounded by several light

harvesting complexes (LH). Two types of light harvesting centers known as LH I or B_{870} and LH 2 or $B_{800\text{-}850}$ are found in most purple bacteria. The LH I can present adjacent to the reaction center whereas LH2 are peripheral proteins present on the outer surface of the membrane. Both LH I and LH 2 are oligomers of two short peptides, α and ß, which specifically bind either Bacteriochlorophyll a or b depending on bacterial species and a carotenoid in stochiometric amounts. From these antenna pigments the energy is transferred to the reaction centers. About 20-40 light harvesting complexes are linked to one reaction center.

Reaction center particles from several species of purple bacteria have similar compositions. In 1984, Hartmut Michel, Johann Deisenhofer, Robert Huber and coworkers in Munich solved the three dimentional structure of the reaction centre from the purple photosynthetic bacterium *Rhodospirillum viridis.* It consists of three polypeptides, designated H (heavy mass), M (medium) and L (light) subunits. The L and M subunits of this membrane spanning center collectively bind four molecules of Bacteriochlorophyll a (or Bacteriochlorphyll b in others), which maximally absorb light at 960 nm (B chl a at 870 nm), two molecules of bacteriopheophytin b (B pheo a in others), one non-haeme iron-sulfur cluster (Fe-S), one molecule of ubiquinone or menaquinone, and two molecules of a carotenoid pigment. Two of the bacteriochlorophyll molecules are photochemically active. They are referred to as the "special pair".

A second membrane integrated complex of photosynthetic electron transport chain is cytochrome bc_1 Complex. This complex consists of one b type and c_1 type cytochrome plus an iron-sulfur protein. Other components of photosynthetic electron transport chain are ubiquinone and cytochrome c_2 both being mobile carriers within the membrane. Several molecules of these carriers are present per reaction center (Figure 5.4). The major function of the cytochrome bc_1 complex is to transfer electrons from ubiquinone to cytochrome C_2.

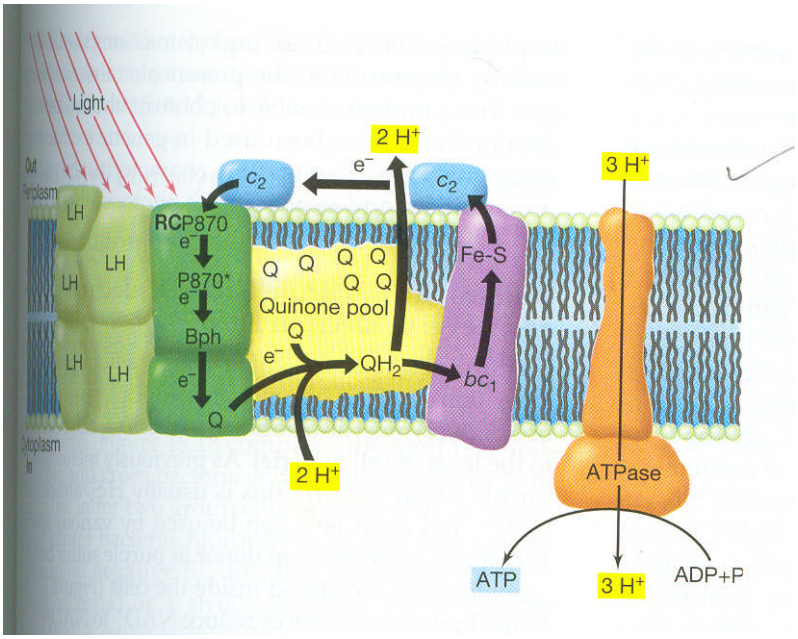


Figure 5.4 Arrangement of protein complexes in the photosynthetic membrane of a purple phototrophic bacterium. Light-generated proton gradient is used in the synthesis of ATP by the ATP synthase (ATPase). LH, light harvesting bacteriochlorophyll complexes; RC, reaction center; Bchl, bacteriochlorophyll; Bph, bacterio pheophytin; Q, Quinone; Fe-s, Iron-sulfur protein; bc_1, cytochrome bc_1 complex; C_2 cytochrome C_2.

The sequence of photochemical events which happens when the light energy is transferred to the reaction center is schematically shown in Figure 5.5. Light energy is transferred from the antenna to the reaction center in packets called *excitons.* Photosynthesis begins when exciton energy strikes the special pair present in the reaction center. The reaction center of purple bacteria containing bacteriochlorophyll a is referred to as P870 and P960 for the reaction center containing bacteriochlorophyll b (in the figure 5.5 reaction center containing bacteriochlorophyll a, P870, is given).

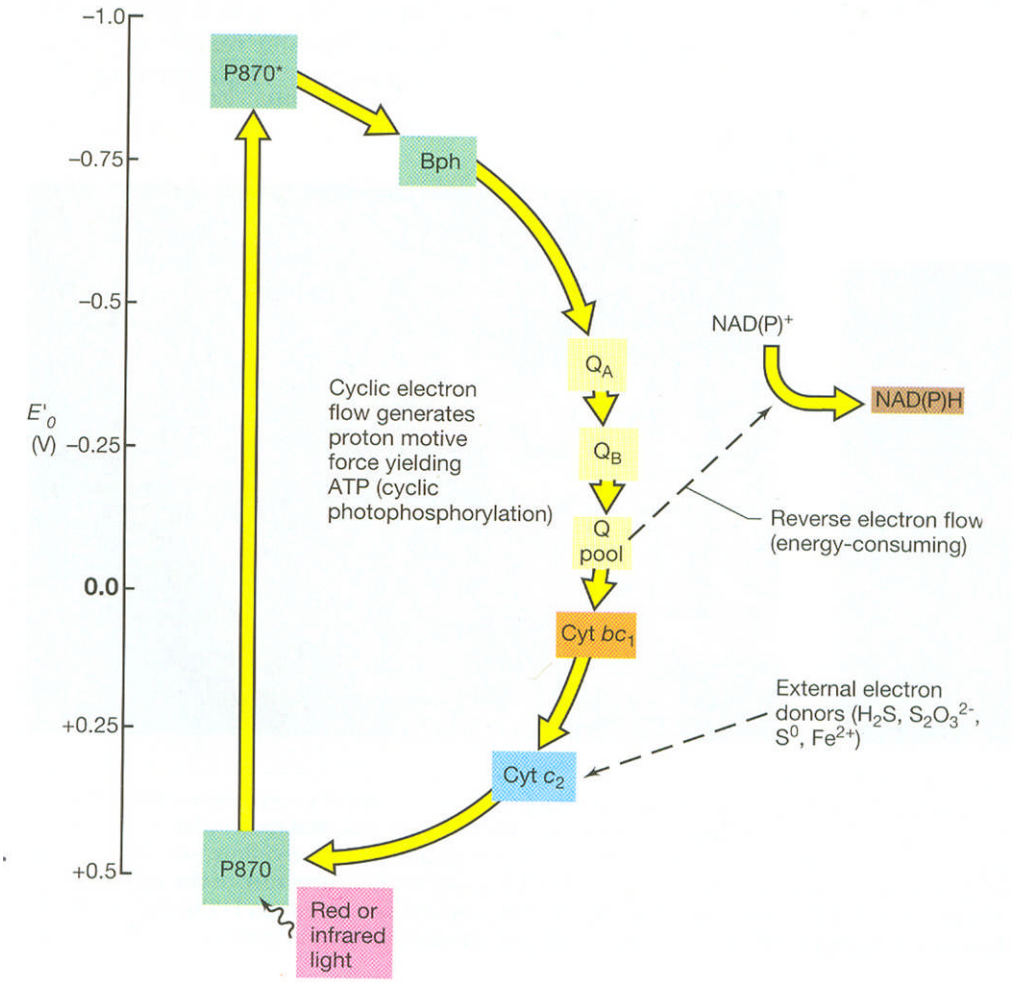


Figure 5.5 General scheme of electron flow in anoxygenic photosynthesis in a purple bacterium. Only a single light reaction occurs. P870, reaction center photochemically active bacteriochlorophyll a; Bph, bacteriopheophytin; Q_A, Q_B, intermediate quinones; Q pool, mobile ubiquinone pool in membrane; Cyt, chtochrome.

Light absorption by the reaction center P870 causes it to be present in P870*. Photoexcited P 870 then quickly transfers its electron ($3x10^{-12}$ sec) to bacteriopheophytin to yield $P870^+$ and B pheo a^-. This primary photochemical event represents the conversion of absorbed radiant energy into the chemical energy. The electron has further migrated to the intermediate iron-ubiquinone complexes (or in some species the menaquinane) designated as Q_A and Q_B. Reduced quinones then transfers excited electron to mobile ubiquinone pool Q, to form semiquinone anion, Q^-.

When the reaction center once again becomes excited, it transfers a second electron to Q^- to form the fully reduced anionic quinol, Q^{2-}. This anionic quinol takes up two protons from the cytoplasm to form QH_2. The electrons taken up by QH_2 are eventually returned to $P870^*$ via a cytochrome bc_1-Fe-S complex and mobile cytochrome c_2. Cytochrome c_2 is a periplasmic cytochrome and serves as an electron shuttle between the membrane-bound bc_1

complex and the reaction center. The electron return journey is accompanied by the release of protons to outside of the cell by the bc_1 complex catalyzed Q cycle as in cyanobacteria. The reaction series is completed when cytochrome c_2 donates an electron to the special pair bacteriochlorophylls, returning these molecules to their original grond state potential (E_o' = +0.5v). The reaction center is then capable of absorbing new energy and repeating the process.

The importance of this cyclic electron transport for the organism performing it is that for each electron transported, two protons are translocated from the inside to the outside of the cell. The energy of the resulting proton gradient is then used in the synthesis of ATP by the ATP synthase complex through cyclic photophosphorylation.

In addition to ATP, carbon fixation process requires reducing equivalents. Purple bacteria can synthesize NADH or NADPH with either organic substrates (Rhodospirilaceae) or H_2S (Chromatiaceae) as electron donors. Two mechanisms have been proposed for NAD(P)H formation in purple bacteria.

1) The organic substrates are oxidized by NAD^+-depending reactions, for example, malate by malate dehydrogenase.

$$\text{Malate} + NAD^+ \xrightarrow{\text{Malate dehydrogenase}} \text{Oxaloacetate} + NADH$$

2) **Reversed electron flow**. This mechanism involves use of light generated ATP or pH gradient to reverse the flow of electrons from an electron transport chain probably from "quinone pool" to $NAD(P)^+$ to form NAD(P)H. This is because that the oxidation – reduction potential of quinone is insufficiently negative (E_0' = O volts) to reduce $NAD(P)^+$ (-0.32 volts) directly, such that electrons from quinone pool must be forced *backward,* against the thermodynamic gradient, to reduce $NAD(P)^+$ to NAD(P)H. This energy requiring process is called **reversed electron flow** and is driven by energy inherent in the proton motive force or by ATP. The electron hole which is created as a result of reverse electron flow at cytochrome c_2 probably be fulfilled by oxidation of inorganic or organic electron donors (Figigure 5.6).

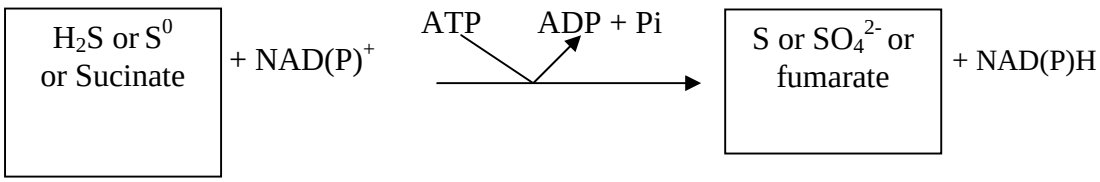


Figure 5.6. $NAD(P)^+$ reduction in purple bacteria. The arrow in this diagram represents an electron transport chain that is being driven in reverse by light generated pH gradient or ATP. This process requires energy because electrons are moving from donors with more positive reduction potentials to an acceptor ($NAD(P)^+$) with a negative potential.

Hydrogen and H_2S may feed their electrons to the cytochrome c_2 and succinate at the level of ubiquinone in the electron transport chain through their respective enzyme systems.

5.3 Green bacteria

The photosynthetic green bacteria are divided into green sulfur bacteria (Chlorobiaceae) and green non-sulfur bacteria (Chloroflexaceae). The green sulfur bacteria are a small group of obligately anaerobic photolithoautotrophs. Like purple sulfur bacteria they also use H_2S, S^0 and H_2 as an electron source. In contrast to purple sulfur bacteria the elemental sulfur produced by sulfide oxidation in them is deposited outside the cell. They also differ from purple sulfur bacteria in having the light harvesting centers in specialized photosynthetic structures known as **chlorosomes**. These bacteria flourish well in the anaerobic sulfide rich zones of lakes or muds at the bottom of the lakes and ponds. Representative genera are *Chlorobium*, *Prosthecochloris* and *Pelodictyon*.

The green non-sulfur bacteria resemble the green bacteria in their ultrastructure and photosynthetic pigments but differ from them in having purple non-sulfur bacterial kind of anoxygenic photosynthesis. Example, **Chloroflexus.**

5.3.1 Photosynthetic Apparatus

Green bacteria are charecterised by the presence of specialized membrane bound photosynthetic apparatus called **chlorosomes** or **chlorobium vesicles**. Chlorosomes are ellipsoidal vesicles. They are attached to the plasma membrane but are not continuous with it. The chlorosome membrane is not a normal lipid bilayer. It is a non-unit membrane made up of galactolipids. Chlorosomes contain bacteriochlorophyll c, d or e in addition to bacteriochlorophyll a, carotenoids and proteins (Figure 5.7).

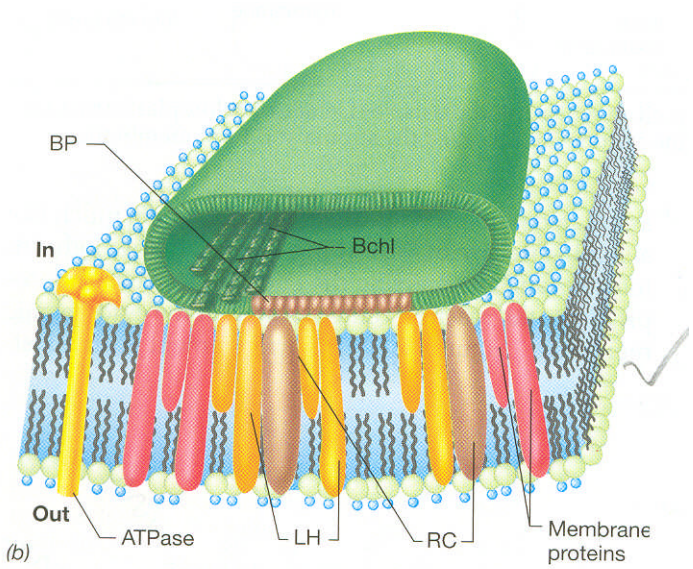


Figure 5.7 The chlorosome of green sulfur and green nonsulfur bacteria. Antenna bacterio chlorophyll molecules (Bchl c, d or e) are arranged in tube like arrays inside the chlorosome. Base plate (BP) proteins function as connectors between the chlorosome and cytoplasmic membrane.

The absorption maxima of these pigments are observed to be 850 to 910 nm for Bacteriochlorophyll a, 745 to 760 nm for Bacteriochlorophll c, 705 to 740 nm for Bacteriochlorophyll d and 715 to 725 nm for Bacteriochlorophyll e (Figure 5.2).

Green sulfur and green non-sulfur bacteria contain predominantly carotenoids bearing aromatic rings such as β-carotene, α-carotene, β-isorenieratene and isorenieratene. A typical carotenoid of the green bacteria is isorenieratene (Figure 5.3).

5.3.2 Light Reactions

Like purple bacteria, the green bacteria are anoxygenic photosynthesizers and use inorganic and organic compounds as electron donors in the synthesis of NAD(P)H and ATP. Unlike purple photosynthetic bacteria, these photoautotrophs contain a simple form of non-cyclic photosynthetic electron flow to form NAD(P)H in addition to cyclic electron flow (Figure 5.8). The photosynthetic electron transport in green bacteria involves two multisubunit membrane integrated complexes known as reaction center and cytochrome b-Fe-S complex.

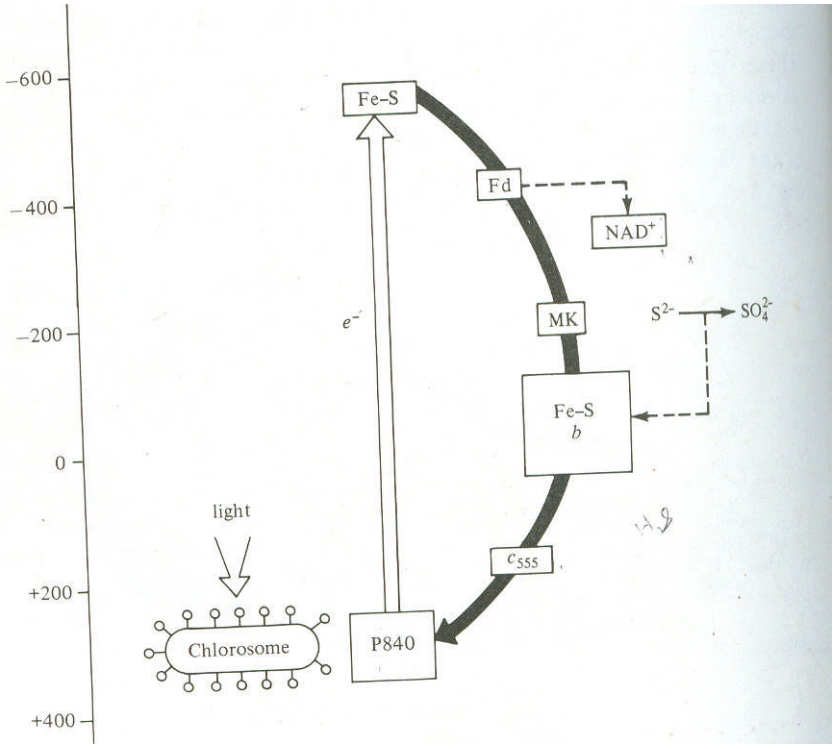


Figure 5.8 Cyclic electron transport in green bacteria and light dependent reduction of NAD^+. MK, Menaquinone; Fe-S, iron-sulfur protein; b, Cytochrome b; C_{555} , cytochrome C_{555}.

The reaction center of a green bacterium is called P840. It is situated in the cytoplasmic membrane. It contains photochemically active pair of bacteriochlorophyll a and an iron-sulphur protein as the primary electron acceptor. Each reaction center may be surrounded by more than thousand light harvesting centers (chlorosomes) and this organization is much higher than that found in purple bacteria. In this way the chlorosomes function as some sort of super antenna and permits the green bacteria to grow well even at low light intensities. Chlorosomes that contain bacteriochlorophyll c, d or e and carotenoids can absorb blue and infra red portion of the electromagnetic spectrum and transfer it to the reaction center for its further processing.

Reaction center P840 is connected to the second membrane integrated complex which is formed from one b type cytochrome and an Fe-S protein. Menaquinone(MK), the structural analogue of the ubiquinone acts as a mobile electron carrier between the reaction center and the cytochrome b – Fe-S complex. Cytochrome c_{555} is the another mobile peripheral membrane protein that shuttle electrons in between cyt b- Fe-S complex and P840 during cyclic electron transport.

Light absorbed by pigmented chlorosomes is transferred to reaction center. As a result that the photochemically active pair of bacteriochlorophyll a in the reaction center are excited and oxidized. Here, the reductant is probably not a bacteriopheophytin but an iron sulfur protein. The electron from reduced Fe-S protein is transferred to ferredoxin. From ferredoxin an electron can move in two directions. In the cyclic path way, the electron moves back to the oxidized P840 through menaquinone, cytochrome complex and cytochrome C_{555}.

In the non-cyclic route, the electron is transferred to $NAD(P)^+$ via ferredoxin. In this case " the electron hole" at cyt C_{555} of electron transport chain be filled by the oxidation of an external inorganic and organic electron donors.

The mechanism of proton extraction is analogous to the one described in purple bacteria. NAD(P)H and ATP formed by the light reactions then provide the energy for carbon fixation.

Purple and green bacteria differ from cyanobacteria and eukaryotic photosynthesizers -

(1) in performing anoxygenic photosynthesis
(2) in the absence of PS II
(3) in the mode of reducing equivalents synthesis [NAD(P)H] and
(4) in having bacteriochlorophylls as light processing units (Table 5.2)

Table 5.2 Properties of microbial photosynthetic systems

S.No	Property	Higher Plants	Cyanobactera	Purple and green bacteria
1	Photosynthetic pigment	Chlorophyll a	Chlorophyll a	Bacterio chlorophyll
2	PS II	Present	Present	Absent
3	Photosynthetic electron donors	H_2O	H_2O	H_2, H_2S, $S^0$and organic molecules
4	O_2 production pattern	Oxygenic	Oxygenic	Anoxygenic
5	Primary products of energy conservation	ATP + NADPH	ATP + NADPH	ATP
6.	Carbon source	CO_2	CO_2	CO_2 and/or organic

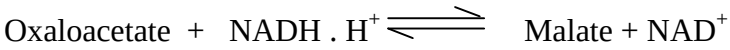
5.4 Carbon fixation in purple and green bacteria

Photosynthetic bacteria such as rhodospirillaceae, chromatiaceae and chloroflexaceae, when growing with CO_2 as carbon source, will have the carbon fixation mechanism similar to the one found in higher plants and cyanobacteria i.e. fix CO_2 through Calvin cycle. The members of chlorobiaceae which do not possess key enzymes of Calvin cycle, such as ribulose-5-phosphate kinase and ribulose-1-5-bisphosphate carboxylase can employ different type of carbon fixation in that CO_2 is incorporated into acetyl CoA via carboxylic acids of TCA cycle. This cycle was first formulated by Evans, Buchanan and Arnon (1966) and confirmed with isotope experiments by Fuchs. They named it as a **reductive tricarboxylic acid** cycle. This cycle involves most of the tricarboxylic acid cycle reactions that are reversible (Figure 5.9).

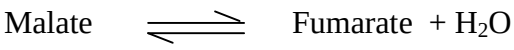
5.4.1 Reductive tricarboxylic acid cycle

The tricarboxylic acid cycle is actually meant for the oxidation of acetyl CoA and most of its reactions are reversible except succinate dehydrogenase and α – oxoglutarate dehydrogenase and citrate synthase. These enzymes are irreversible in aerobically respiring cells and are replaced by necessary enzymes for incorporation of CO_2 into acetyl CoA and then to carbohydrates and other cellular constituents. The sequential events of the cycle are described below.

1) Reductive tricarboxylic acid cycle begins with the reduction of four-carbon oxaloacetate to malate by **malate dehydrogenase**. NADH is used as a reducing power.

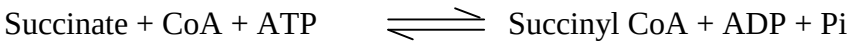


2) The next step in the reductive tricarboxylic acid cycle is the conversion of malate to furmarate. The reaction is catalyzed by **fumarase** with elimination of water from malate.

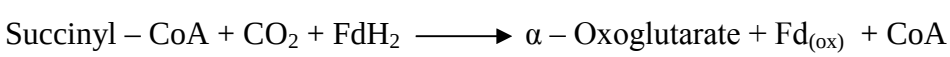


3) Fumaric acid, the product of reaction 2, is reduced to succinic acid by **fumarate reductase**. This reaction of TCA cycle is irreversible and occurs in the presence of succinate dehydrogenase. Succinate dehydrogenase is not favourable for fumarate reduction in reductive tricarboxylic acid cycle and is replaced by fumarate reductase which probably uses NADH as H donor.

4) In the next step catalyzed by enzyme **succinyl CoA synthetase** one molecule of coenzyme A is added to succinate to yield succinyl CoA. The energy of ATP is used to drive the reaction.



5) This is the most important reaction of the reductive tricarboxylic acid cycle, where a molecule of CO_2 is incorporated into the succinyl CoA to form α – oxoglutarate by the enzyme **α – oxoglutarate: ferredoxin oxidoreductase** also called **α – oxoglutarate synthase**. It is an another irreversible reaction in the catabolic TCA cycle, where it is catalyzed by α – oxoglutarate dehydrogenase and here it is replaced by α – oxoglutarate synthase. FdH_2 is the reducing agent.



6. The α – oxoglutarate is then reduced and carboxylated to isocitric acid. The enzyme involved is isocitrate dehydrogenase. This is another step in the cycle to incorporate CO_2 into isocitrate.

$$\alpha - \text{oxoglutarate} + CO_2 + NADH.H^+ \rightleftharpoons \text{Isocitrate} + NAD^+$$

7. Next, with the removal and addition of molecule of water in the presence of cis-aconitase, isocitrate is converted into citrate via cis-aconitate.

$$\text{Isocitrate} \rightleftharpoons \text{Cis-aconitate} + H_2O$$

$$\text{Cis-aconitate} + H_2O \rightleftharpoons \text{Citric acid}$$

8. This is another important reaction in the reductive tricarboxylic acid cycle where its enzyme ATP-citrate lyase takes the place of citrate synthase of the normal TCA cycle. This enzyme catalyzes the conversion of citric acid into acetyl CoA, the product of the cycle and oxalocetate from which the cycle is started. The chlorobiaceae are the only prokaryotes known to possess ATP - citrate lyase enzyme.

$$\text{Citrate} + \text{ATP} + \text{CoA} \longrightarrow \text{Acetyl CoA} + \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

The entire cycle is represented in Figure 5.9. The net result of this cycle is that 2 CO_2 molecules are reduced to acetyl CoA at the expense of four reducing equivalents and two ATP molecules.

Acetyl CoA can further be used to synthesize the carbohydrates and other organic substances needed for cell growth and function as shown in figure 5.9.

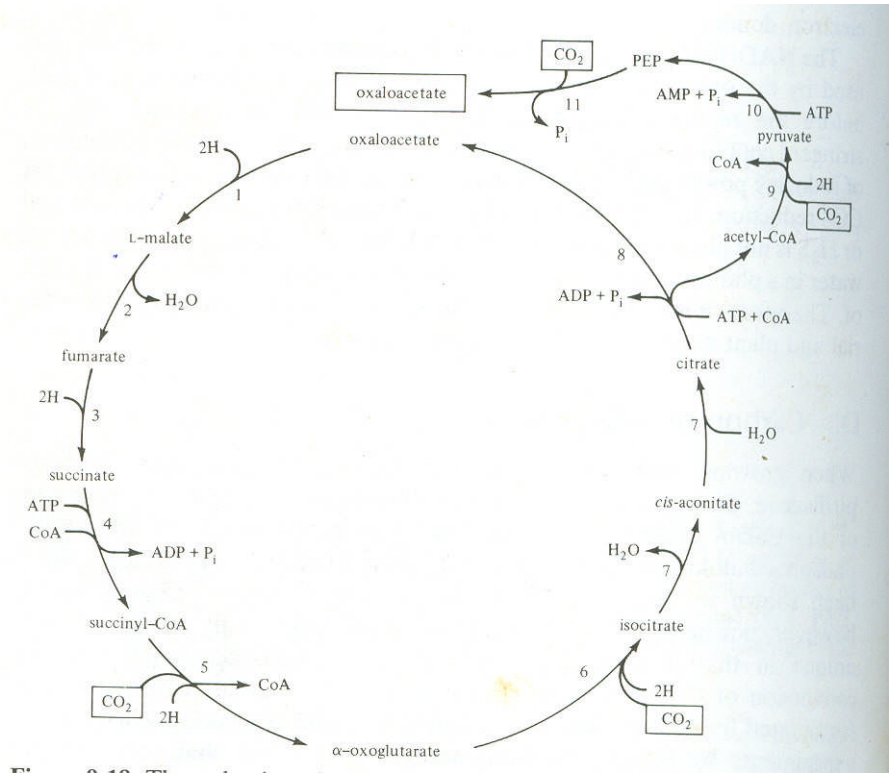


Figure 5.9 The reductive tricarboxylic acid cycle of the chlorobiaceae. 1, malate dehydrogenase; 2, fumarase; 3, fumarate reductase; 4, succinyl CoA synthetase; 5, α – oxo glutarate synthase; 6, isocitrate dehydrogenase; 7, cis-aconitase; 8, ATP: citrate lyase; 9, pyruvate synthase; 10, PEP synthetase; 11, PEP carboxylase.

5.5 Summary

The purple and green bacteria carry out anoxygenic photosynthesis. The photosynthetic apparatus of this group, made up of bacteriochlorophyll, carotenoids and proteins is located in plasmamembrane invaginations (purple bacteria) or chlorosomes (green bacteria).

The photosynthetic apparatus consists of membrane-bound pigment-protein complexes plus an ATP ase complex that drives ATP synthesis at the expense of a proton motive force.

The reaction center is a multiple subunit pigment protein complex. It is surrounded by 20-40 light harvestening centers (purple bacteria) or 1000 light harvesting clorosomes (green bacteria).

Purple bacteria contain only one pigment system called P 870 (or P 960). The light energy transferred to this center from antenna gives rise to a cyclic electron transfer. The cyclic electron transport around the reaction center causes the cytochrome bc_1 complex in the

extrustion of H^+ from the cytosolic side of the membrane to the medium side of the membrane. The resulting proton gradient is used either for ATP synthesis through photophosphorylation or for reverse electron flow to form NAD(P)H from $NAD(P)^+$ with inorganic or organic substrates as electron donors.

P 840 is the reaction center found in green bacteria. Primary acceptor of the electron from P 840 is an iron sulfur protein, from which an electron can be fed into a cyclic flow or can be used for NAD^+ reduction.

Purple bacteria and green bacteria of chloroflexaceae employ the Calvin cycle for CO_2 fixation. Members of chlorobiaceae, however, involve the reductive tricarboxylic acid cycle for CO_2 incorporation. This cycle contains three important enzymes, the fumarate reductase, the α – oxoglutarate : Fd oxidoreductase, and the ATP : citrate lyase in addition to TCA cycle enzymes to incorporate two molecules of CO_2 into acetyl CoA at the expense of two ATP and four reducing equivalents.

5.6 Model Questions

1. Describe anoxygenic photosynthesis as carried out by purple and green bacteria.
2. Discrbe the organization of photosynthetic apparatus in anoxygenic photosynthesizers.
3. Write short notes on :
    (a) Reversed electron flow
    (b) Reaction center in purple bacteria
    (c) Chlorosomes
    (d) Reductive tricarboxylic acid cycle.

5.7 Reference Books

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Dr.G.ROSAIAH

6. CHEMOAUTOTROPHY

6.0. Objective:

Different groups of chemoautotrophic bacteria such as hydrogen oxidizing bacteria, carbon monoxide oxidizing bacteria, sulfur oxidizers, iron oxidizers, ammonia oxidizers and nitrite oxidizers are discussed in this chapter.

6.1. Introduction

Carbon is required for the skeleton of all organic molecules. **Autotrophs** are the organisms that use CO_2 as their sole or principal source of carbon. The reduction of CO_2 is a very energy expensive process. Thus many microorganisms cannot use CO_2 as their sole carbon source, but must rely on the presence of more reduced, complex molecules for the supply of carbon. Organisms that use reduced, preformed organic molecules as their principal carbon source are called **Heterotrophs**.

Microorganisms can be grouped into two classes basing on the source of energy they can utilize. **Phototrophs** use light as an energy source, while **chemotrophs** use inorganic/organic chemicals as their energy source. Organisms can be classified into four categories if both carbon and energy sources are taken into consideration. They include **photoautotrophs**, **chemoautotrophs**, **photoheterotrophs** and **chemoheterotrophs**. Chemoautotrophs (chemolithotrophs) obtain their energy from the oxidation of inorganic compounds while chemoheterotrophs (chemoorganotrophs) obtain their energy from the oxidation of organic compounds.

Chemolithotrophic bacteria are able to obtain all their carbon from CO_2. Two components are needed for growth on CO_2 as sole carbon source: energy in the form of ATP and reducing power. In chemolithotrophs, ATP generation is in principle similar to that in chemoorganotrophs, except that the electorn donor is inorganic rather than organic. Thus ATP synthesis is coupled to oxidation of the electron donor. Reducing power in chemolithotrophy is obtained from the inorganic compounds.

Aerobic chemolithotrophs are categorized into six groups depending on the nature of the inorganic compound oxidized. They include (a) Hydrogen oxidizing bacteria, (b) Carbon monoxide oxidizing bacteria, (c) Sulfur oxidizers, (d) Fe^{2+} oxidizers, (e) Ammonia oxidizers and (e) nitrite oxidizers.

6.2. Hydrogen Oxidizing Bacteria

These bacteria use molecular hydrogen as energy source. They are highly diverse in their physiological and morphological features. The hydrogen oxidizing bacteria include *Pseudomonas saccharophila*, *P. facilis*, *Alcaligenes eutrophus* and *Nocardia autotrophica*. Generation of ATP during H_2 oxidation comes from the oxidation of H_2 by O_2 leading to the formation of a proton motive force.

$$H_2 + ½\ O_2 \rightarrow H_2O \qquad \Delta G^{0'} = -237\ kJ$$

The reaction is highly exergonic and can support the synthesis of at least one ATP. The reaction is catalyzed by the enzyme **hydrogenase**, the electrons from H_2 initially being transferred to a quinone acceptor. From here electrons pass through a series of cytochromes to eventually reduce O_2 to water (Fig.6.1). Some hydrogen bacteria have two hydrogenases, one soluble and one membrane-bound. The **soluble hydrogenase** takes up H_2 and reduces NAD^+ to NADH directly while the **membrane bound enzyme** is involved in energetics. These organisms fix CO_2 by the calvin cycle. The stoichiometry observed here is

$$6H_2 + 2O_2 + CO_2 \rightarrow (CH_2O) + 5H_2O$$

where (CH_2O) represents cell material.

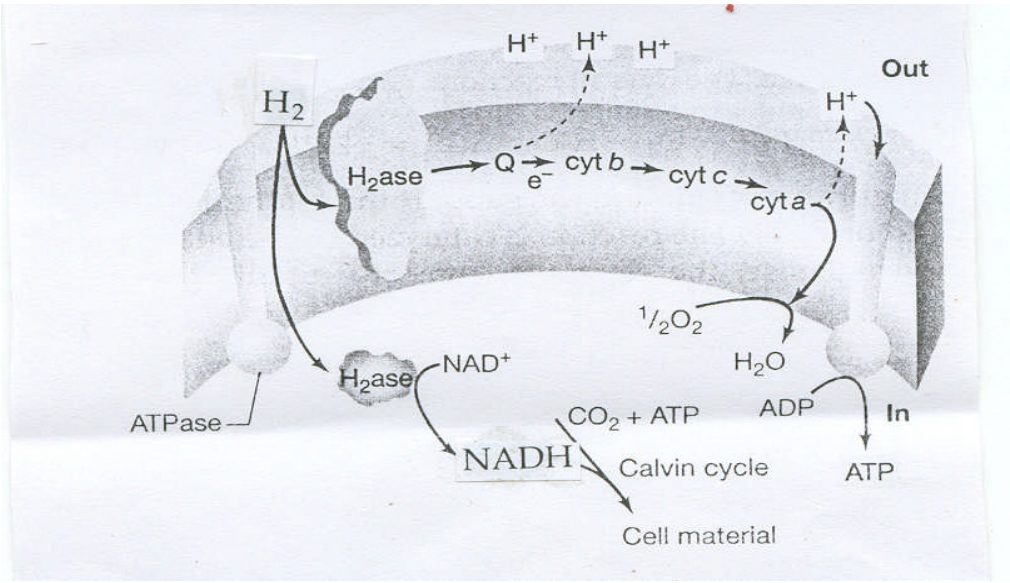

Fig.6.1. : Bioenergetics and function of the two hydrogenases of aerobic H_2 bacteria. In *Ralstonia eutropha*, where two hydrogenases are present, the membrane-bound hydrogenase is involved in energetics while the cytoplasmic hydrogenase makes

NADH for the Calvin cycle. Some H_2 bacteria have only the membrane-bound hydrogenase, and in these organisms reducing power synthesis occurs from reverse electron flow. H_2ase, hydrogenase; cyt, cytochrome; Q, quinone.

Physiology and ecology of hydrogen bacteria

Most hydrogen bacteria grow best under microaerobic conditions because hydrogenases are oxygen sensitive enzymes. Oxygen levels of about 5-10% support best growth. Nickel must be present in the medium for chemolithotrophic growth of hydrogen bacteria because virtually all hydrogenases contain Ni^{2+} as a metal cofactor.

6.3. Carbon Monoxide Oxidizing Bacteria

Some hydrogen bacteria can grow on carbon monoxide as energy source, with electrons from the oxidation of CO to CO_2 entering the electron transport chain to drive ATP synthesis. All CO-oxidizing bacteria are H_2 oxidizers (not vice versa). They include *Pseudomonas carboxydovorans, Alcaligenes carboxydus* and the thermophilic *Bacillus schlegelii*.

CO–oxidizing bacteria (carboxydotrophic bacteria) grow autotrophically using calvin cycle reactions to fix the CO_2 generated from the oxidation of CO. The enzyme **carbon monoxide dehydrogenase** oxidizes CO to CO_2. The molybdenum in CO dehydrogenase is bound to a small cofactor consisting of a multiringed structure called pterin.

CO consumption by carboxydotrophic bacteria is a significant ecological process. Although much CO is generated from various human and other sources, CO levels in air have not risen significantly over many years. This may be attributed to CO consumption by microbes. Carboxydotrophic bacteria in the upper layers of soil represent the most significant sink for CO in nature.

6.4. Sulfur Oxidizers

The concept of chemolithotrophy emerged from studies of the sulfur bacteria, as the great Russian Microbiologist Winorgradsky first proposed the idea of chemolithotrophy from studies of these organisms. Many reduced sulfur compounds can be used as electron donors by a variety of sulfur bacteria. The ability to grow chemolithotropically on reduced sulfur compounds is a property of a diverse group of proteobacteria such as *Thiobacillus thioparus, T.denitrificans, T.novellus, Beggiatoa, Thiothrix, Thiomicrospira, Thiosphaera* and *Thermothrix*.

The most common sulfur compounds used as electron donors are hydrogen sulfide, elemental sulfur and thiosulfate. The final product of sulfur oxidation in most cases is sulfate and the total number of electrons involved between H_2S (oxidation state, -2) and sulfate (oxidation state, +6) is eight.

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \qquad \Delta G^{0'} = -798.2 \text{ kJ/reaction}$$

The oxidation of H_2S occurs in stages, and the first oxidation step results in the formation of elemenal sulfur, S^0. Some H_2S- oxidizing bacteria deposit this elemental sulfur inside the cell eg. *Beggiatoa*. The sulfur deposited as a result of the initial oxidation is an energy reserve, and when the supply of H_2S has been depleted, additional energy can be obtained from the oxidation of sulfur to sulfate.

When elemental sulfur is provided externally as an electron donor, the organism must grow attached to the sulfur particle because of the extreme insolubility of elemental sulfur. By adhering to the particle, the organism can efficiently obtain the atoms of sulfur needed eg. *Sulfolobus acidocaldarius.*

Biochemistry and energetics of sulfur oxidation

The biochemical steps in the oxidation of reduced sulfur compounds are summarized in fig 6.2. If sulfide is the starting material, sulfite is produced. When S^0 is the starting substrate, sulfite is also produced, although the S^0 must first be reduced to sulfide. Sulfite can be oxidized to sulfate in two ways. In one type, **sulfite oxidase** transfers electrons from sulfite directly to cytochrome C, and ATP is made from this during electron transport and proton motive force formation. In addition to sulfite oxidase, a few sulfur chemolithotrophs oxidize sulfite to sulfate via a reversal of the activity of **adenosine phosphosulfate** (APS) **reductase**. When thiosulfate is the electron donor for sulfur chemolithotrophs, it is split into S^0 and SO_3^{2-}, both of which are eventually oxidized to SO_4^{2-}.

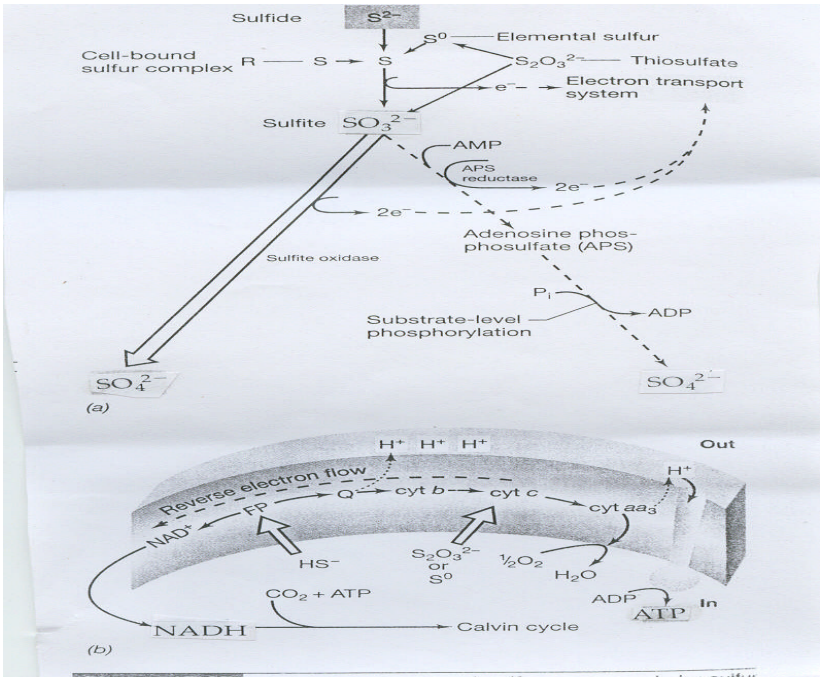

Fig.6.2. Oxidation of reduced sulfur compounds by sulfur chemolithotrophs. (a) Steps in the oxidation of different compounds. The sulfite oxidase pathway accounts for the majority of sulfite oxidized. (b) Electrons from sulfur compounds feed into the electron transport chain to drive a proton motive force; electrons from thiosulfate and elemental sulfur enter at the level of cytochrome c. NADH must be made by energy-consuming reactions of reverse electron flow since the electron donors have a

more electropositive E_0' than does NAD^+/NADH. Cyt, cytochrome; FP, flavoprotein; Q, quinone.

6.5. Iron Oxidizers

Iron is one of the most abundant elements in Earth's crust. On the surface of the Earth, iron exists naturally in two oxidation states, ferrous (Fe^{2+}) and ferric (Fe^{3+}). Fe^0 is a major product of human activities in the smelting of ferrous or ferric iron ores to form cast iron. In nature, iron cycles primarily between the ferrous and ferric forms, the reduction of Fe^{3+} occurring both chemically and as a form of anaerobic respiration, and the oxidation of Fe^{2+} occurring both chemically and as a form of chemolithotrophic metabolism.

The aerobic oxidation of iron from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state is an energy-yielding reaction for a few bacteria. Only a small amount of energy is available from this oxidation. Hence iron bacteria must oxidize large amounts of iron in order to grow. The ferric iron produced forms insoluble ferric hydroxide {Fe (OH_3)} precipitates in water.

The best-known iron-oxidizing bacteria (*Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) are able to grow autotrophically using ferrous iron as electron donor. These bacteria are very common in acid-polluted environment such as coal-mining dumps. *Gallionella* is active under neutral pH conditions and *Sulfolobus* functions under acidic, thermophilic conditions.

One of the most common forms of iron in nature is pyrite (FeS_2). Pyrite is formed from the reaction of sulfur with ferrous sulfide (FeS) to form a highly insoluble crystalline structure which is very common in bituminous coals and in many ore bodies. The bacterial oxidation of pyrite is of great significance in the development of acidic conditions in mining operations. Oxidation of pyrite by bacteria is of considerable importance in the process called **microbial leaching** of ores. The oxidation of pyrite is a combination of chemically and bacterially catalyzed reactions.

Energy from ferrous iron oxidation

The bioenergetics of iron oxidation by *Thiobacillus ferrooxidans* is of interest because of the very electropositive reduction potential of the Fe^{3+}/Fe^{2+} couple (+ 0.77 V at pH 2). The respiratory chain of *T. ferrooxidans* contains cytochromes of the c and a types and a periplasmic copper containing protein called rusticyanin. Ferrous iron oxidation begins in the periplasm where rusticyanin oxidizes Fe^{2+} to Fe^{3+}, a one electron transition. This protein then reduces cytochrome c, and this subsequently reduces cytochrome a. The latter interacts directly with O_2 to form H_2O (Fig. 6.3). ATP is then synthesized from proton-translocating ATPases in the membrane and ATP yields are relatively low because of the high potential of the electron donor.

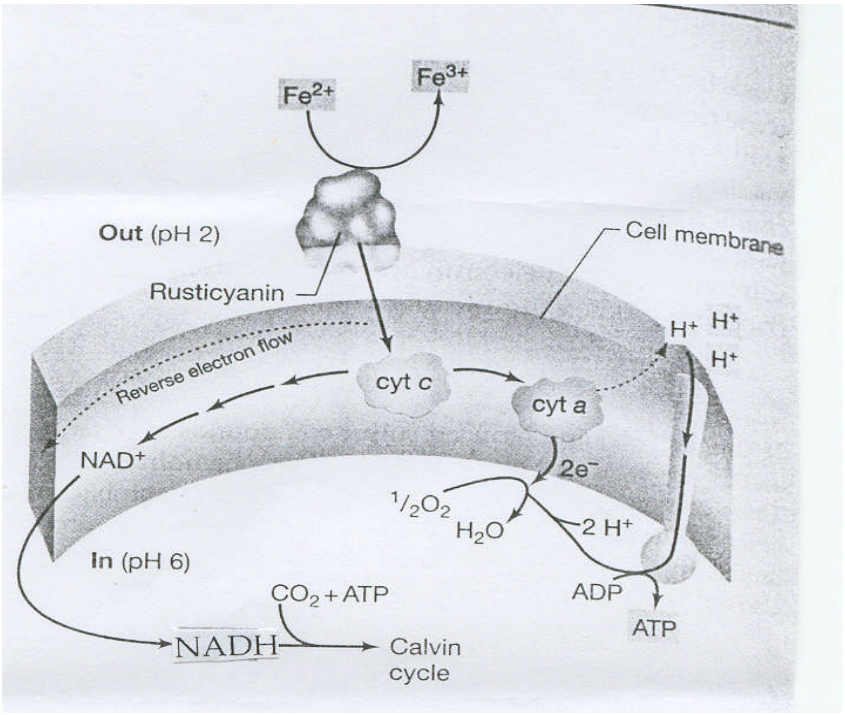

Fig.6.3. Electron flow during Fe^{2+} oxidation by the acidophile *Thiobacillus ferrooxidans*. The periplasmic copper-containing protein rusticyanin is the immediate acceptor of electrons from Fe^{2+}. From here, electrons travel a short electron transport chain resulting in the reduction of O_2 to H_2O. Reducing power to drive the Calvin cycle comes from reactions of reverse electron flow.

Autotrophy in *T. ferrooxidans* is driven by the Calvin cycle. Because of the high potential of the electron donor, Fe^{2+}, much energy is consumed in reverse electron flow reactions to obtain the reducing power necessary to drive CO_2 fixation. A relatively poor energetic picture coupled with large energetic demands means that *T. ferrooxidans* must oxidize large amounts of Fe^{2+} in order to produce even a very small amount of cell material.

Ferrous iron can be oxidized under anoxic conditions by certain anoxygenic phototrophic bacteria. The ferrous iron is used in this case not as an electron donor in energy metabolism, but as an electron donor for CO_2 reduction.

6.6. Ammonia Oxidizers and Nitrite Oxidizers

Bacteria able to grow chemolithotrophically at the expense of reduced inorganic nitrogen compounds (NH_3 and NO_2^-) are called **nitrifying bacteria**. Nitrification in nature results from the sequential action of two separate groups of organisms, the **ammonia oxidizing bacteria** (Nitrosofyers) and the **nitrite oxidizing bacteria** (the true nitrifying bacteria). Nitrosofyers typically have genus names beginning in "Nitroso" (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus,*

Nitrosovibrio) while true nitrifyers usually begin with "Nitro" (*Nitrobacter*, *Nitrospina*, *Nitrococcus* and *Nitrospira*). *Nitrosomonas* and *Nitrobacter* are major genera of nitrifying bacteria. The nitrifying bacteria were the first organisms to be shown to grow chemolithotrophically.

The nitrifying bacteria are widespread in soil and water. They are present in highest numbers in habitats where considerable amounts of ammonia are present, such as sites where extensive protein decomposition occurs and in sewage treatment facilities.

Enrichment cultures of nitrifying bacteria are readily established by using mineral salts media containing ammonia or nitrite as electron donor and bicarbonate as sole carbon source. An easy means of monitoring their growth is to assay for the production of nitrite (with ammonia as electron donor) or the disappearance of nitrite or production of nitrate (with nitrite as electron donor).

Several key enzymes are involved in oxidizing reduced nitrogen compounds. In ammonia – oxidizing bacteria, NH_3 is oxidized by **ammonia monooxygenase** that produces NH_2OH and H_2O. **Hydroxylamine oxidoreductase** then oxidizes NH_2OH to NO^-_2 removing four electrons in the process. **Ammonia monooxygenase** is an integral membrane protein where as **hydroxylamine oxidoreductase** is periplasmic (fig.6.4).

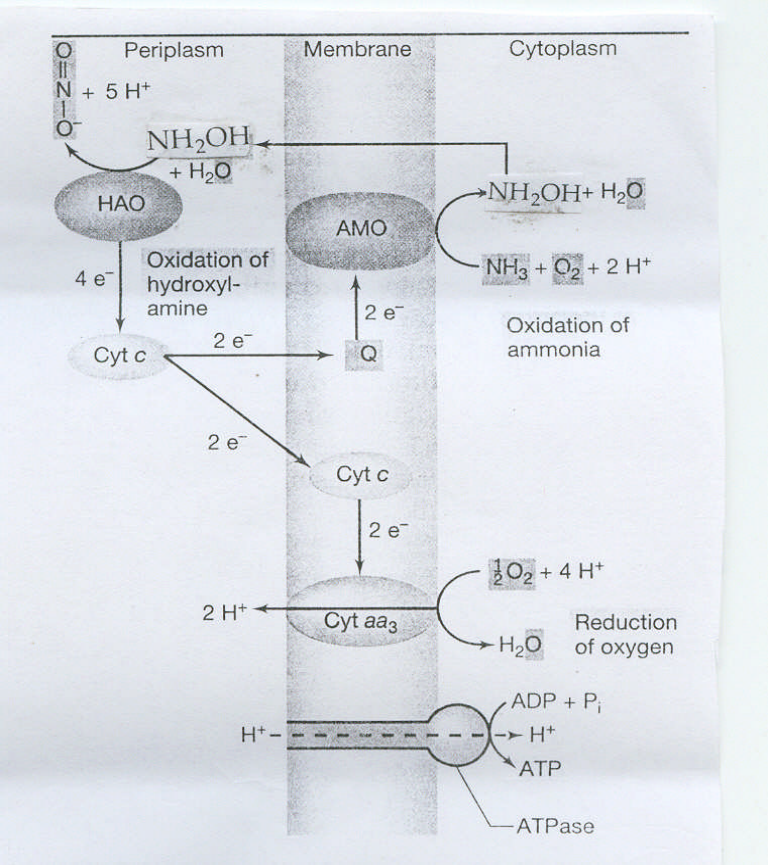

Fig.6.4. Oxidation of ammonia and electron flow in ammonia-oxidizing bacteria. The reactants and the products of this reaction series are highlighted. The cytochrome c

(cyt c) in the periplasm is a different form of cyt c than that in the membrane. AMO, Ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; Q, ubiquinone.

In the reaction carried out by **ammonia monooxygenase**,

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$$

there is need for two exogenously supplied electrons plus two protons to reduce one atom of dioxygen to water. These electrons originate from the oxidation of hydroxylamine and are supplied to **ammonia monooxygenase** from **hydroxylamine oxidoredectase** via cytochrome c and ubiquinone. Thus, for every four electrons generated from the oxidation of NH_3 to NO^-_2, only two actually reach the terminal oxidase (cytochrome aa_3).

Nitrite-oxidizing bacteria employ **nitrite oxidoreductase** to oxidize nitrite to nitrate, with electrons traveling a very short electron transport chain to the terminal oxidase (fig.6.5). Cytochromes of the a and c types are present in the electron transport chain of nitrite oxidizers, and generation of a proton motive force (which ultimately drives ATP synthesis) occurs through the action of cytochrome aa_3. Only small amounts of energy are available as is the case with iron oxidation. Thus, growth yields of nitrifying bacteria are relatively low.

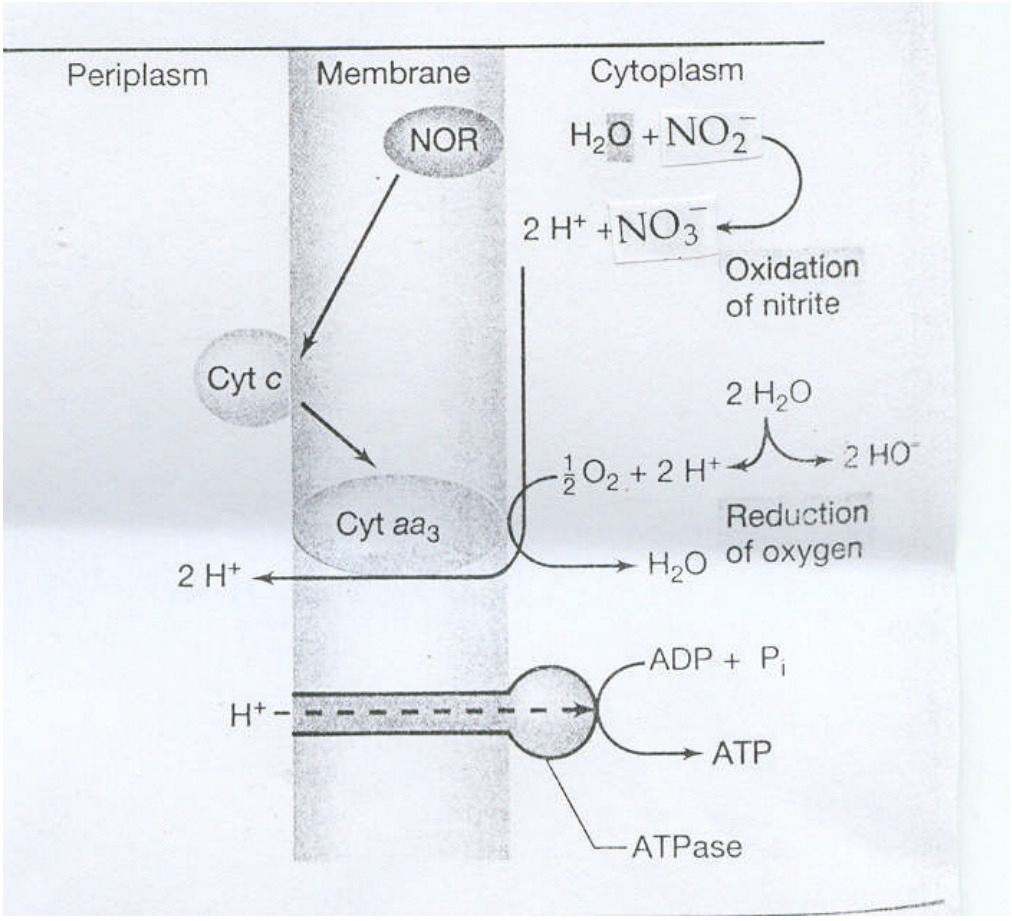

Fig.6.5. Oxidation of nitrite to nitrate by nitrifying bacteria. The reactants and products of this reaction series are highlighted. NOR, Nitrite oxidoreductase.

Assimilation of CO_2

Chemolithotrophs use the ATP and reducing power produced by oxidation of inorganic substrates to reduce CO_2 and to convert it to cell material. Since CO_2 functions as sole source of carbon in these organisms, they are often called **C-autotrophs**. The mechanism of CO_2 fixation employed by chemolithotrophs is the calvin cycle, the same mechanism that occurs in cyanobacteria and green plants.

Anoxic ammonia oxidation : anammox

Although classical nitrifying bacteria are strict aerobes, ammonia can also be oxidized under anoxic conditions. This process, known as **anammox** (anoxic ammonia oxidation), is highly exergonic and linked to energy metabolism of the organisms involved. Anammox involves the oxidation of ammonia with nitrite as the electron accepter to yield gaseous nitrogen.

$$NH_4 + NO^-_2 \rightarrow N_2 + 2H_2O \quad \Delta G^{0'} = -357 \text{ kJ}$$

The organism that catalyzes anammox, *Brocadia anammoxidans*, is a phylogenetically distinct member of the planctomycetes. Anammoxosome found in *B. anammoxidans* is the location of the anammox reaction. The source of NO^-_2 in the anammox reaction is the product of ammonia oxidation by aerobic nitrifying bacteria. The two groups of nitrifyers, aerobic (*Nitrosomonas*) and anaerobic (*Brocadia*) live together in ammonia-rich habitats such as sewage and other wastewaters. In these environments suspended particles are present that contain both oxic and anoxic zones where the two groups of ammonia oxidizers can coexist. Like classic nitrifying bacteria, *B. anammoxidans* is also an autotroph. It can grow with CO_2 as sole carbon source and uses nitrite as electron donor to produce cell material:

$$CO_2 + 2NO^-_2 + H_2O \rightarrow CH_2O + 2NO^-_3$$

6.7. Methylotrophs

Methane, CH_4, is found extensively in nature. It is produced in anoxic environments by methanogenic Archaea and is a major gas of anoxic muds, marshes, anoxic zones of lakes, the rumen and the mammalian intestinal tract. It is a relatively stable molecule, but a variety of bacteria, the **methanotrophs**, oxidize it readily, utilizing methane and a few other one-carbon compounds as electron donors for energy generation and as sole sources of carbon.

In addition to methane, a number of other one – carbon compounds such as methanol, methylamine and dimethylamine can be utilized by microorganisms. Organisms that can grow using only one carbon organic compounds are called **methylotrophs**. Many, but not all, methylotrophs are also methanotrophs.

Methanotrophs possess a specific enzyme, **methane monooxygenase**, for the introduction of an oxygen atom in to the methane molecule, leading to the formation of methanol. All methanotrophs are obligate aerobes. They also appear to be obligate C_1 utilizers, unable to utilize compounds containing carbon-carbon bonds. By contrast, nonmethanotrophic methylotrophs are able to utilize organic acids, ethanol and sugars. The individual steps in methane oxidation to CO_2 can be summarized as :

$$CH_4 \rightarrow CH_3OH \rightarrow CH_2O \rightarrow HCOO^- \rightarrow CO_2$$

The enzyme **methane monooxygenase** is a key enzyme in the catabolism of methane. C_1 units get assimilated into cell material at the level of formaldehyde in methanotrophs by either the ribulose monophosphate pathway or the serine pathway.

6.8. Summary

Chemolithotrophic microorganisms gain ATP by oxidation of inorganic compounds with oxygen. The inorganic compounds oxidized are : molecular hydrogen (hydrogen – oxidizing bacteria); CO (CO- oxidizing bacteria); hydrogen sulfide, elemental sulfur and thiosulfate (sulfur-oxidizing bacteria); ferrous iron (iron-oxidizing bacteria); ammonia (ammonia oxidizers) and nitrite (nitrite oxidizers). Cell material is formed from CO_2. With the exception of some hydrogen-oxidizing bacteria, chemolithotrophs use reverse electron transfer in order to reduce NAD^+ with their hydrogen donors (nitrite, ammonia, elemental sulfur, H_2 etc.). Chemolithotrophs use the calvin cycle for CO_2 fixation.

6.9. Model Questions

1. Explain different kinds of chemoautotrophic bacteria.
2. Write short notes on

    (a) Ammonia oxidizers
    (b) Methylotrophs
    (c) Sulfur oxidizers
    (d) Ironoxidizers

6.10. Reference Books

1. Microbial Physiology – A.G. Moat and J.W. Foster, John Wiley, New York.
2. Bacterial Metabolism – G.Gottschalk, Springer – Verlag, New York.
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11.FERMENTATIONS

11.0. Objective

Nature and Kinds of Fermentations are discussed in this chapter.

11.1. Introduction

The term “fermentation” is derived from the Latin verb *fervere* which means to boil. It describes the appearance of the action of yeast on fruit extracts. The boiling appearance is due to the production of carbon dioxide bubbles produced by the anaerobic catabolism of sugars present in the extract. The term fermentation was first defined by Pasteur as life in the absence of oxygen. In a strict biochemical sense, the term fermentation has been used to mean an energy generation process in which organic compounds act as both electron donors and terminal electron acceptors. In many fermentations, ATP is formed only by substrate – level phosphorylation. However, in a number of fermentations electron transport phosphorylation is also involved in ATP synthesis.

The bacteria carrying out fermentations are either facultative or obligate anaerobes. Facultative anaerobes such as the enterobacteria grow as aerobic heterotrophs in the presence of oxygen; under anaerobic conditions they carry out a fermentative metabolism. In contrast, obligate anaerobes are not able to synthesize the components of an oxygen linked respiratory chain. Consequently, they can not grow as aerobes. Moreover, many of the obligate anaerobes do not even tolerate oxygen and are killed in air.

When reduced flavoproteins or reduced iron-sulfur proteins come together with oxygen, two toxic compounds are produced: hydrogen peroxide and the super oxide radical. Aerobes contain catalase and super oxide dismutase for destruction of these compounds. Most aerotolerant anaerobes are devoid of catalase but contain super oxide dismutase. Strict anaerobes lack both enzymes. Aero intolerant species

die in the presence of oxygen because of the deleterious effects of the super oxide radical.

Fermentative anaerobes carry out a variety of oxidation-reduction reactions involving organic compounds. These reactions are coupled to substrate level and/or electron transport phosphorylation. The ATP yield (mol ATP per mol substrate consumed), however, is very low. Fermentations are usually classified according to the main fermentation end products. For example alcohol is the end product in alcohol fermentations.

11.2. Alcohol fermentation by yeasts

Alcohol fermentation is the domain of yeasts notably of *Saccharomyces* species. Most of the ethanol produced by the fermentation industry comes from the anaerobic breakdown of glucose and other hexoses by these organisms. Gay –Lussac (1815) had established that hexoses are converted into ethanol and CO_2 according to the following equation.

Glucose $\longrightarrow$ 2 ethanol + 2 CO_2.

Figure 11.1 Summarizes the alcohol fermentation as carried out by yeasts. For glucose degradation, yeasts employ the Embden-Meyerh of-Parnas pathway resulting in the production of 2 mol of pyruvate from 1 mol of glucose. In aerobic metabolism pyruvate is converted to acetyl Co-A. But in fermentations pyruvate is decarboxylated to acetaldehyde. The enzyme catalyzing this reaction is **pyruvate decarboxylase** which is regarded as the key enzyme of alcohol fermentation. Acetaldehyde is then reduced to ethanol by **alcohol dehydrogenase**. During the formation of two molecules of 1,3-bisphosphoglyceric acid in glycolysis, two molecules of NAD^+ are reduced to NADH. These two molecules of NADH are utilized for the reduction of acetaldehyde.

In any energy yielding process, oxidation must balance reduction and there must be an electron acceptor for each electron removed. In this case, the reduction of NAD^+ at one enzymatic step in glycolysis is balanced with its oxidation at another.

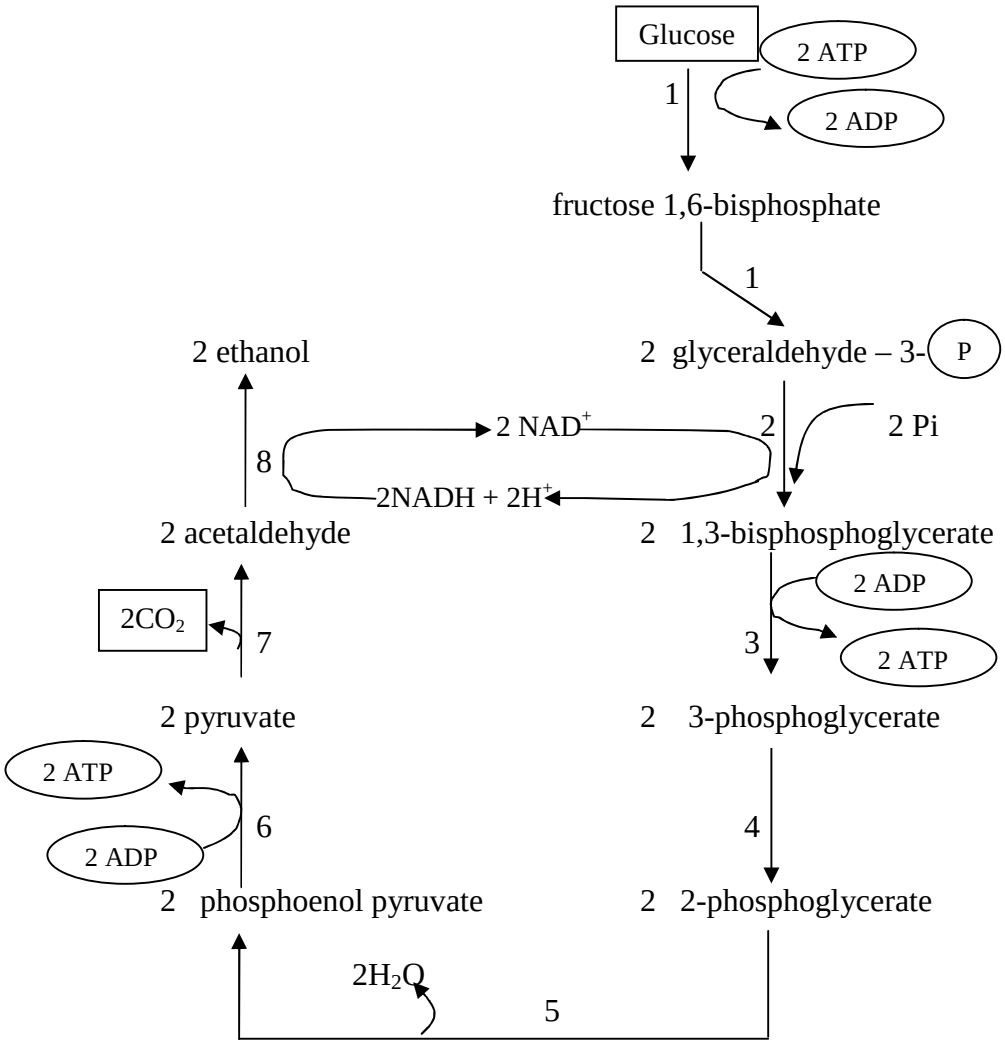

Fig 11.1: Fermentation of glucose to ethanol and CO_2 by yeasts.

1. Initial enzymes of the Embden-Meyerhof-paranas pathway.
2. glyceraldehyde-3-phosphate dehydrogenase
3. 3-phosphoglycerate kinase
4. phosphoglycerate mutase
5. enolase
6. pyruvate kinse
7. pyruvate decarboxylase
8. alcohol dehydrogenase

The net ATP yield of the alcohol fermentation is 2 mol ATP/mol glucose – much lower than the ATP yield of aerobic metabolism.

11.2.1. Alcohol fermentation by bacteria

Some bacterial species such as *Zymomonas mobilis* and *Z. anaerobica* carry out alcohol fermentation and grow well under anaerobic conditions. They degrade glucose to pyruvate via the Entner-Doudoroff pathway. They contain **pyruvate decarboxylase** and form nearly 2 mol each of ethanol and carbon dioxide from 1 mol glucose. *Sarcina ventriculi*, a strict anaerobe capable of growth under extremely acidic conditions and *Erwinia amylovora*, a facultatively anaerobic enterobacterium ferment glucose to ethanol and CO_2 via the EMP pathway and the **pyruvate decarboxylase** and **alcohol dehydrogenase** reactions.

In general, pyruvate decarboxylase is rare in bacteria. Many enterobacteria, lactic bacteria and clostridia form considerable amounts of ethanol but do not employ pyruvate decarboxylase for acetaldehyde synthesis. In these organisms acetyl-coA functions as ultimate precursor of acetaldehyde.

Ethanol is now produced on a large scale by fermentation for industrial purposes. Yeasts and to a small extent *Zymomonas mobilis* have been employed for the production of ethanol on a commercial scale.

11.3. Lactate Fermentation

Lactate is a very common end product of bacterial fermentations. Some genera often referred to as lactic acid bacteria produce large amounts of lactate. The lactic acid bacteria are grampositive rods and cocci. Members of this group do not carry out electron transport phosphorylation as they lack porphyrins and cytochromes. They obtain energy only by substrate level phosphorylation. They are highly saccharolytic and exhibit very complex nutritional requirements. Most lactic acid bacteria are strictly fermentative but are aerotolerant. The genera of lactic acid bacteria such as *Streptococcus, Lactobacillus, Lactococcus, Leuconostoc* and *Pediococcus* have been defined on the basis of cell morphology, DNA base composition and type of fermentative metabolism.

For the fermentation of carbohydrates to lactate these bacteria may employ either of three pathways viz., homofermentative pathway, heterofermentative pathway and bifidum pathway.

The homofermentative pathway yields 2 mol of lactate per mol of glucose:

Glucose $\longrightarrow$ 2 Lactate

The heterofermentative pathway yields 1 mol each of lactate, ethanol and CO_2 per mol of glucose:

Glucose ⟶ Lactate + ethanol + CO_2

The bifidum pathway yields 3 mol of acetate and 2 mol of lactate per 2 mol of glucose:

2 Glucose ⟶ 3 Acetate + 2 lactate.

11.3.1. Homofermentative Pathway

Fig. 11.2 summarizes the homofermentative pathway. Glucose is degraded via the Embden-Meyerhof-Parnas pathway to pyruvate. Lactate is produced by the reduction of pyruvate by **lactate dehydrogenase**. The net ATP yield of the lactate fermentation is 2 mol ATP/glucose as in alcohol fermentation.

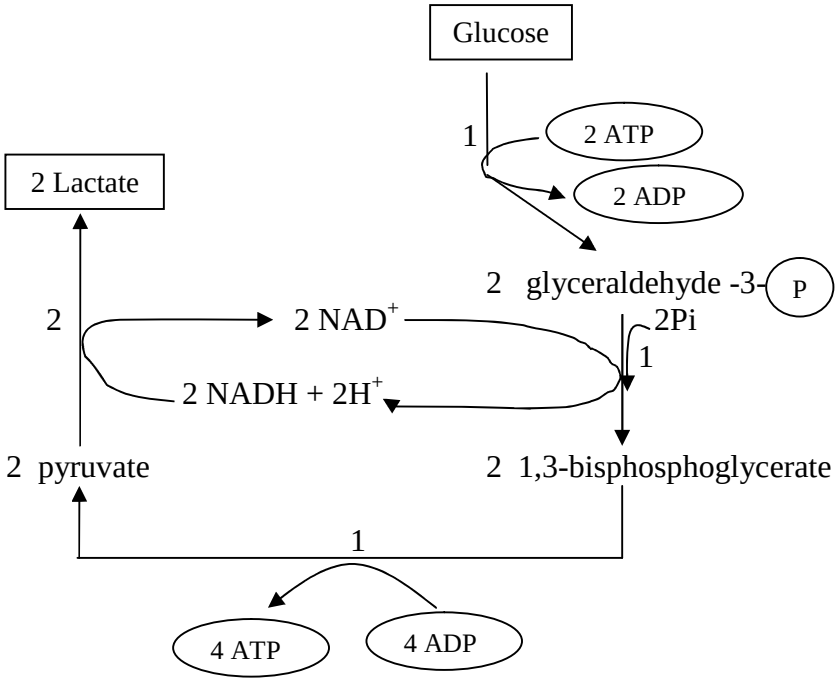

Fig 11.2 Formation of lactate from glucose by the homofermentative pathway.

1- Enzymes of Embden-Meyerhof-Parnas pathway
2- Lactate dehydrogenase

11.3.2. Hetero fermentative pathway

The heterofermentative pathway is illustrated in Fig.11.3. Ribulose-5-phosphate is formed via 6-phosphogluconate as in the oxidative pentose phosphate cycle. Epimerization of ribulose-5-phosphate yields xylulose-5-phosphate, which is cleaved into glyceraldehydes-3-phosphate and acetyl phosphate by an enzyme **phosphoketolase**.

Acetyl phosphate is converted into acetyl-coA by **phospotransacetylase**. Acetaldehyde is formed by the reduction of acetyl coA by **acetaldehyde dehydrogenase**. Subsequent reduction of acetaldehyde by **alcohol dehydrogenase** yields ethanol. The glyceraldehyde-3-phosphate formed in the phosphoketolase reaction is converted to lactate as in the homofermentative pathway.

In the fermentation process 2 NADH are formed and consumed; the ATP yield is 1 per mol of glucose i.e. half of that of the homofermentative pathway.

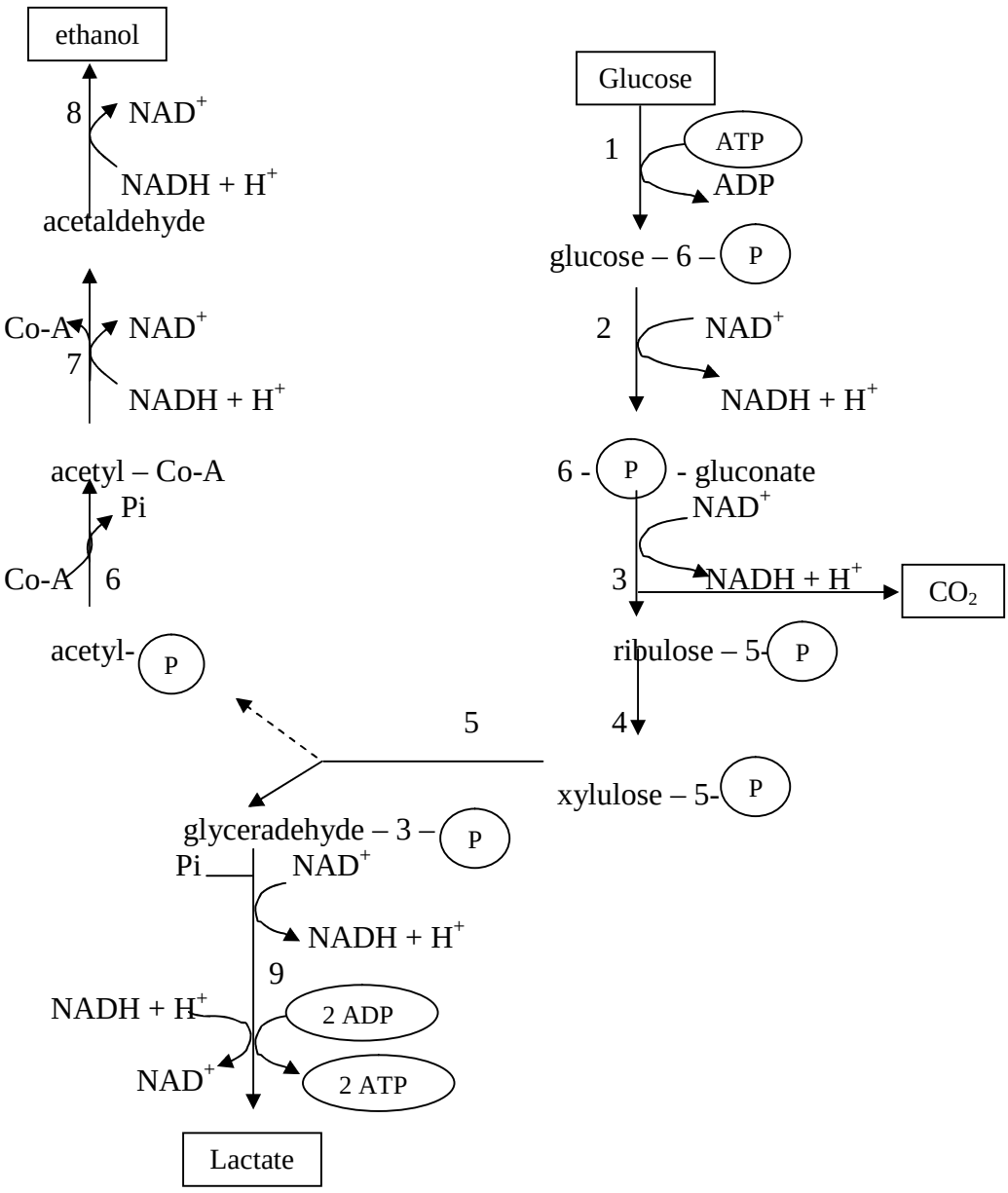

Fig. 11.3. : Formation of CO_2, lactate and ethanol from glucose by the heterofermetnative pathway.

1- Hexokinase; 2- glucose-6-phospahte dehydrogenase; 3- 6-phosphogluconate dehydrogenase; 4- ribulose-5-phosphate 3-epimerase; 5-phosphoketolase (the cleavage reaction yields glyceraldehyde 3-phosphate and enzyme, bound α,β-dihydroxy-ethyl thiamine pyrophosphate. This is converted to acetyl – TPP-E via the α-hydroxyvinyl derivative; phosphorylytic cleavage results in acetyl phosphate formation); 6- phosphotransacetylase; 7-acetaldehyde dehydrogenase; 8-alcohol dehydrogenase; 9-enzymes in homofermentative pathway.

11.3.3. Bifidum pathway

Two **phosphoketolases** are involved in the break down of glucose by *Bifidobacterium bifidum*: one specific for fructose-6-phosphate and one specific for xylulose-5-phosphate. The bifidum pathway is presented in Fig. 11.4. It exhibits a very interesting sequence of reactions. In this pathway, 2 mol of glucose are converted into 3 mol of acetate and 2 mol of glyceraldehyde-3-phosphate without the participation of hydrogenation and dehydrogenation reactions. The 2 mol of glyceradehyde-3-phosphate are converted to 2 mol of lactate as in the homofermentative pathway.

The formation of acetate from acetyl phosphate is coupled to the formation of ATP from ADP. This reaction catalysed by **acetate kinase** is of great importance for all anaerobes that form acetate because it effects ATP synthesis by substrate level phosphorylation. The ATP yield of the bifidum pathway is 2.5 mol of ATP per mol of glucose. Hence the ATP yield of the bifidum pathway is higher than that of the homo - and heterofermentative pathway.

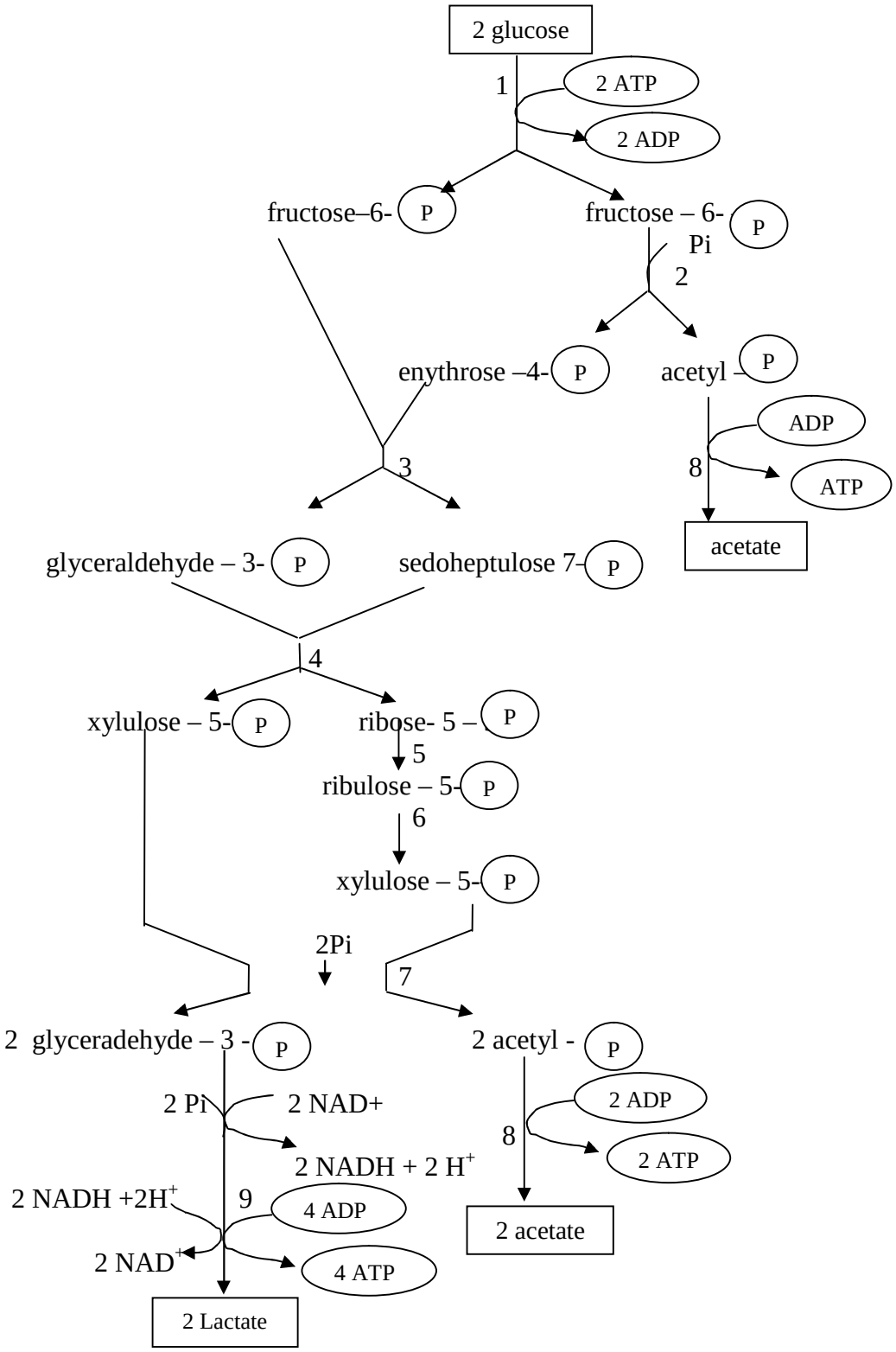

Fig. 11.4: Formation of acetate and lactate from glucose by the bifidum pathway
1. hexokinase and glucose-6-phosphate isomerase; 2-fructose-6-phsophate phosphoketolase; 3-transaldolase; 4-transketolase; 5-ribose-5-phosphate isomerase; 6-

ribulose-5-phosphate 3- epimerase; 7-xylulose-5-phosphate phosphoketolase; 8-acetate kinase; 9-enzymes as in homofermentative pathway.

11.4. Mixed acid fermentation

Enterobacteria such as *Escherichia, Salmonella* and *Shigella* carry out mixed acid fermentation. One of the key taxonomic characteristics separating the various genera of enteric bacteria is the type and proportion of fermentation products produced by fermentation of glucose. Two broad patterns are recognized, the mixed acid fermentation and the 2,3-butanediol fermentation.

In mixed acid fermentation, three acids are formed in significant amounts--acetic, lactic and succinic; ethanol, CO_2 and H_2 are also formed, but not butanediol. In butanediol fermentation, smaller amounts of acids are formed, and butanediol, ethanol, CO_2 and H_2 are the main products. As a result of mixed acid fermentation equal amounts of CO_2 and H_2 are produced, whereas with a butanediol fermentation considerely more CO_2 than H_2 is produced. Mixed acid fermenters produce CO_2 only from formic acid by means of the enzyme system **formate hydrogen lyase**:

$$HCOOH \longrightarrow CO_2 + H_2$$

This reaction results in equal amounts of CO_2 and H_2. The butanediol fermentations also produce CO_2 and H_2 from formic acid, but they produce two additional molecules of CO_2 during the formation of each molecule of butanediol.

Fig 11.5 summarizes the mixed acid fermentation Enterobacteria employ the Embden-Meyerhof-Parnas pathway for hexose breakdown. The pathway leading to the production of succinate branches off at phosphoenol pyruvate; all other end products are derived from pyruvate. Three enzymes systems act on pyruvate and the amounts in which the fermentation products are formed depend on the activity of these enzyme systems. **Lactate dehydrogenase** catalyzes the reduction of pyruvate to lactate. The enterobacteria are able to synthesize two enzyme systems for pyruvate breakdown to acetyl-coA. In aerobic metabolism, the pyruvate dehydrogenase multienzyme complex is involved. This enzyme is not synthesized under anaerobic conditions. Instead, the synthesis of **pyruvate formate lyase** is induced under anaerobic conditions. The reaction mediated by this enzyme proceeds in two steps with an acetyl-enzyme as intermediate and formate and acetyl coA as products:

$$CH_3\text{-}CO\text{-}COOH + \text{enzyme} \longrightarrow CH_3\text{-}CO\text{-enzyme} + HCOOH$$

$$CH_3\text{-}CO\text{-enzyme} + \text{Co ASH} \longrightarrow \text{enzyme} + CH_3\text{-Co-S CoA}$$

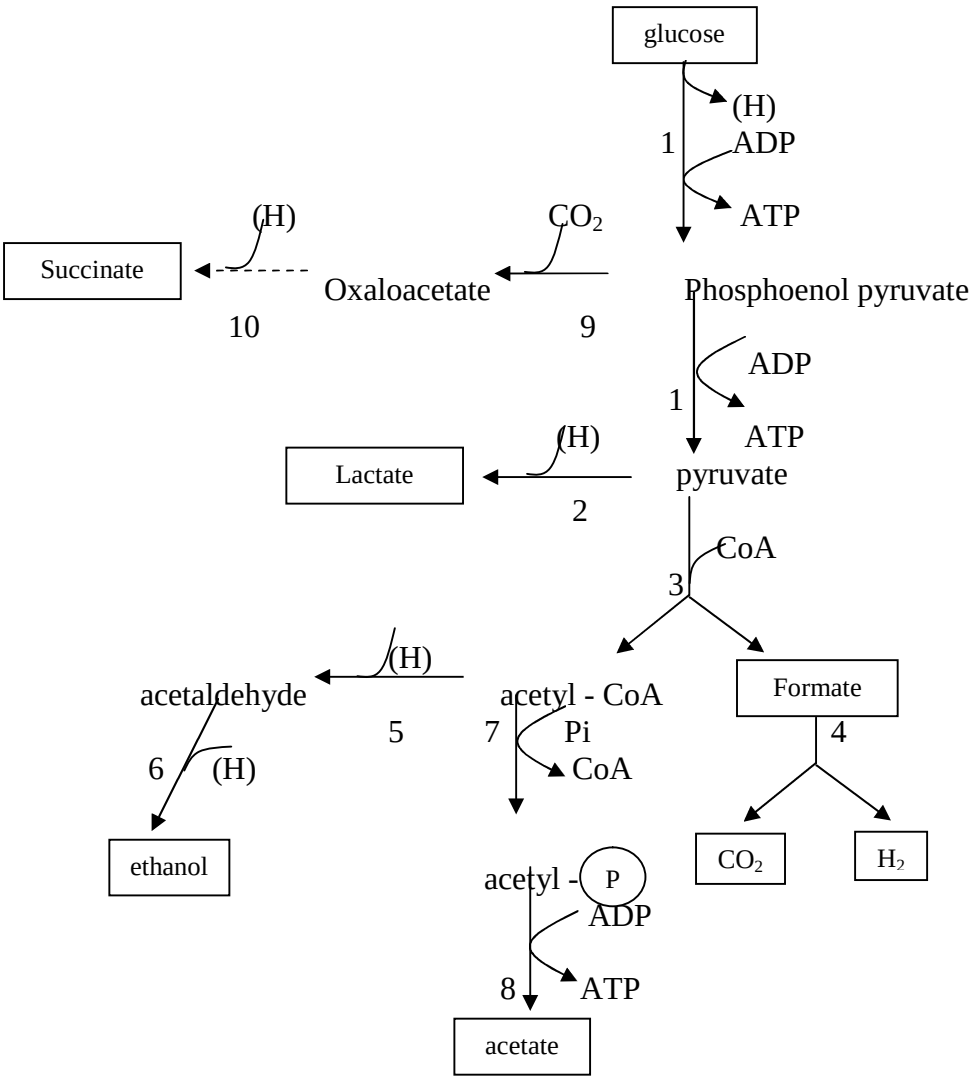

Fig. 11.5: Mixed acid fermentation
1. Enzymes of the Embden – Meyerhof-- Parnas pathway;
2. Lactate dehydrogenase; 3. Pyruvate-formate lyase; 4. Formate-hydrogen lyase; 5. Acetaldehyde dehydrogenase; 6. Alcohol dehydrogenase; 7. Phosphotranscetylase; 8. Acetate kinase; 9. PEP carboxylase; 10. Malate dehydrogenase, Fumarase and Fumarate reductase.

Pyruvate formate lyase is irreversibly inactivated in air so that it functions only in fermentative metabolism of the enterobacteria. Genera such as *Erwinia* and *Shigella* produce considerable amounts of formate. They do not contain formate-hydrogen lyase which cleaves formate into CO_2 and H_2. *Escherichia coli* and *Enterobacter aerogenes* contain formate-hydrogen lyase when grown on sugars

under anaerobic conditions and formate is cleaved into CO_2 and H_2. Formate-hydrogen lyase is not a single enzyme entity. The formation of H_2 and CO_2 from formic acid is the result of the combined activity of a special formate dehyderogenase and a hydrogenase. Acetyl Co-A produced from pyruvate is reduced to acetaldehyde which is further converted to ethanol by alcohol dehydrogenase.

11.5. Summary

Fermentations are anaerobic, energy generating processes in which organic compounds act as both electron donors and terminal electron acceptors. ATP is formed mostly by substrate – level phosphorylation. In alcohol fermentation, yeasts ferment glucose to ethanol and CO_2. The key enzyme of this fermentation is **pyruvate decarboxylase**. Two mol of ATP are formed per mol of glucose fermented. *Zymomonas* species, *Sarcina ventriculi* and *Erwinia amylovora* also carry out alcohol fermentations.

Lactic acid bacteria employ the homofermentative, the heterofermentative or the bifidum pathway for the fermentation of hexoses. The homofermentative pathway yields 2 lactate/glucose, the heterofermentative pathway yields lactate, ethanol and CO_2, whereas acetate and lactate are formed in the ratio of 3:2/2 mol glucose by the bifidum pathway. The key enzyme of the latter two pathways is phosphoketolase.

Microorganisms belonging to the genera *Escherichia*, *Salmonella* and *Shigella* carry out a mixed acid fermentation and produce lactate, acetate, succinate, formate, CO_2 and H_2. Characteristic enzymes of this fermentation are pyruvate – formate lyase, which cleaves pyruvate in to acetyl CoA and formate, and formate – hydrogen lyase, which splits formate into H_2 and CO_2. Microorganisms belonging to the genera *Enterobacter*, *Serratia* and *Erwinia* produce less acids than the above mentioned enterobacteria but more CO_2, ethanol and 2,3-butanediol.

11.6. Model Questions

1. Discuss different types lactic acid fermentations.
2. Write short notes on
   (a) Alcohol fermentation
   (b) Mixed acid fermentation.

11.7. Reference Books

1. Microbial Physiology – A.G. Moat and J.W. Foster, John Wiley, New York.
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LESSON-7

B I O E N E R G E T I C S

7.0 Objective : Bioenergetics, the energy transformations in living organisms, are discussed in this chapter.

7.1 Introduction

Bioenergetics is the application of thermodynamic laws to the study of energy transformations in biological systems. The energetics of cellular processes can be related to chemical equilibrium and oxidation and reduction potentials of chemical reactions. Whether at the level of molecules, cells or ecosystems, the flow of energy is central to the course of biology. Energy flow, in biological systems, is dictated by certain fundamental thermodynamic laws. Most of thermodynamic properties are extensive properties. These include energy (E), enthalpy (H), entropy (S), and free energy (G). E, H, S and G are all properties of the state of a substance. A general understanding of thermodynamic principles is necessary because these principles provide the quantitative framework for understanding energy transformation in biology. In addition to energy transformations, thermodynamics also helps to describe the capacity of a system to do work. In nature biological energy transductions are based on two thermodynamic laws.

7.2 Thermodynamic laws

The first law, commonly known as the **Law of conservation of energy** says that the total amount of energy in the universe is constant. Energy can undergo transformations from one form to

another. For example, the chemical energy of a molecule can be transformed into thermal, electrical or mechanical energy. It means, energy is never "lost" in reaction – an apparent decrease in one form of energy will be balanced by an increase in some other form of energy. To apply the first law to a chemical reaction, we must consider all the energy changes that occur. Including changes in the surroundings, as well as in the system of interest. The system might be a reaction occurring in a test tube or a living cell. The surroundings are all the rest of the universe. According to the first law, the over all energy remains constant even though energy in some form may flow from the system to its surroundings or from the surroundings to the system.

According to the first law of thermodynamics, the change in the energy (Δ E) of system is the difference between the heat absorbed by the system (q) and the work done by the system (w):

$$\Delta E = q - w \qquad \rightarrow (1)$$

Both q and w depend on the path of the reaction, but their difference, Δ E, is independent of the path and therefore defines a state function.

The second law of thermodynamics

The second law of thermodynamics states that the universe inevitably proceeds from states that are more ordered to states that are less ordered. This phenomenon is measured by a thermodynamic function called entropy, which is denoted by symbol, S. Entropy(s) is the measure of the degree of randomness or disorder of a system. The entropy of system increases (Δ S is positive) when it becomes more disordered. In spontaneously occurring reaction the total entropy change must be greater than zero.

$$(\Delta \text{ System} + \Delta \text{ S surroundings} > 0 \text{ for a spontaneous process} \qquad \rightarrow (2)$$

(A process can occur spontaneously only if the sum of the entropies of the system and its surroundings increases). In general, the entropy of a system can decrease during a spontaneous process, provided that the entropy of surroundings increases so that their sum is positive. For example, the formation of a highly ordered biomolecule is thermodynamically feasible because the decrease in the entropy of such system is more than offset by an increase in the entropy of its surroundings.

Free energy concept

The difficulty in using entropy as criterion of whether a biochemical process can occur spontaneously is that the entropy changes of a chemical reactions are not readily measured. In addition, both the entropy change of the surroundings and that of the system of interest be known. These difficulties are obviated by using a different thermodynamic function called the **free energy**, which is denoted by the symbol G (or F, in the older literature). In 1878, Josaiah Willard Gibbs created the free-energy function by combining the first and second laws of thermodynamics. The amount of energy actually available to do work, called the free energy, G (or Gibbs free energy expresses the amount of energy capable of doing work during a reaction at constant temperature and

pressure) will always be somewhat less than the theoretical amount of energy released, because some energy is dissipated as the heat.

7.3.1 Free energy change (Δ G)

Gibbs developed the theory of free energy changes during a chemical reaction. The energy change as the system moves from its initial state to equilibrium, with no changes in temperature or pressure, is given by the **free energy change**. Free energy change denoted by Δ G and it is expressed in the following way:

$$\Delta G = \Delta H - T \Delta S$$

Where Δ H = change in Enthalpy of this system and 'T' is the temperature
Δ S = change in entropy of system

Enthalpy, H is the heat content of systems. It reflects the number and kinds of chemical bonds in the reactants and products.

Δ H, reflecting the kinds and numbers of chemical bonds and non-covalent interactions broken and formed.

Entropy, S, is a quantitative expression for randomness or disorderness in a system.

Δ S = Any change in the randomness of system is expressed as entropy change.

- Free energy change of a reaction depends both on the change in internal energy and on the change in entropy of the system.
- A reaction occurs spontaneously, the products have less free energy than the reactants. The reactions proceed with the release of the energy are exergonic. The free energy change of exergonic reactions is negative (-ΔG).
- A reaction cannot occur spontaneously if ΔG is positive. An input of energy is required to drive such a reaction, called endergonic (i.e. the products have more free energy than the reactants).
- A system is at equilibrium and no net change in the concentration of reactants and products can take place if free energy change (ΔG) is zero.
- The free energy change of the reaction depends only on the free energy of products (the final state) minus that of the reactants.
- The ΔG of reaction independent of the path of the transformation (molecular mechanism).

- The ΔG provides no information about the rate of the reaction.
- ΔG of a reaction depends on the nature of their reactants and on their concentrations.
- ΔG is a function of reactant and product concentrations and temperature prevailing during the reaction.

Units of ΔG and ΔH are Joules / mol. or calories / mol (1 cal = 4.184 J)

Units of Entropy are Joules / mol. Kelvin (J/mol.K).

Standard free-energy change of a reaction and its relation to the equilibrium constant

At the equilibrium of the system, the rates of the forward and reverse reactions are exactly equal and no further net change occurs in the system. The ratio of the reactants and products at equilibrium define the equilibrium constant, K'eq is expressed by

$$K'eq = \frac{[C]^c\ [D]^d}{[A]^a\ [B]^b}$$

Where a, b, c and d are the number of molecules of A, B, C and D; and [A], [B], [C] and [D] are the molar concentrations of the reaction components at the point of equilibrium.

When a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force, the magnitude of which can be expressed as the free energy change for the reaction, Δ G (free energy change at cell conditions).

Under standard conditions – that is temperature is 298 K, the concentrations of the each reactant is 1 M (or for gas 1 atm concentration) and pH about 7, the force driving the system toward equilibrium is defined as **the standard free-energy change**, $\Delta G^{o\prime}$. Just as K'eq is a physical constant characteristic for each reaction, $\Delta G'^{0}$ is also a constant. The standard free-energy change of a chemical reaction is simply an alternative mathematical way of expressing its equilibrium constant. Table 7.1 shows the relationship between $\Delta G^{o\prime}$ and K'eq.

Table 7.1 Relation between $\Delta G^{o\prime}$ and K'eq (at 25°C)

K'eq	**$\Delta G^{o\prime}$**	
	kJ/mole	**k cal/mol**
10^{-5}	28.53	6.82
10^{-4}	22.84	5.46
10^{-3}	17.11	4.09

10^{-2}	11.42	2.73
10^{-1}	5.69	1.36
1	0	0
10	-5.69	-1.36
10^{2}	-11.42	-2.73
10^{3}	-17.11	-4.09
10^{4}	-22.84	-5.46
10^{5}	-28.53	-6.82

If the K'eq for a given chemical reaction is 1.0, the standard free energy change of that reaction is zero. If K'eq of a reaction is greater than 1.0, its $\Delta G^{o\prime}$ is negative. If K'eq is less than 1.0, $\Delta G^{o\prime}$ is positive. Because the relationship between $\Delta G^{o\prime}$ and K'eq is exponential relatively small changes in $\Delta G^{o\prime}$ correspond to large changes in K'eq. The other way of expression for $\Delta G^{o\prime}$ is the difference between the free energy content of the products and the free-energy content of the reactants under standard conditions. Each chemical reaction has a characteristic standard free energy change. The standard free energy change tells us in which direction and how far a given reaction must go to reach equilibrium when the initial concentration of each component is 1.0 M, the pH is 7.0, the temperature is $25^{o}C$, and a pressure is 1 atm. Thus $\Delta G^{o\prime}$ is a constant; it has a characteristic, unchanging value for a given **reaction**.

Δ G and $\Delta G^{o\prime}$ for any reaction A + B $\leftrightarrow$ C + D are related by the equation

$$\Delta G = \Delta G^{o\prime} + RT \ln \frac{[C]\,[D]}{[A]\,[B]} \qquad \rightarrow (3)$$

Where Δ G = actual free energy change

R = gas constant (8.315 J/mol. K or 1.987 cal /mol. K)

T = absolute temp. $25^{o}C$ = 298 K

[A], [B], [C] and [D] = actual molar concentrations of reacting components

When the reaction attains equilibrium the ΔG (actual free energy change) become zero.

Equation 3 then becomes

$$O = \Delta G^{o\prime} + RT \ln \frac{[C]\,[D]}{[A]\,[B]} \qquad \rightarrow 4$$

$$\text{and so } \Delta G^{o\prime} = -RT \ln \frac{[C]\,[D]}{[A]\,[B]} \quad \rightarrow 5$$

K'eq under standard conditions, is defined as

$$K'eq = \frac{[C]\,[D]}{[A]\,[B]} \quad \rightarrow 6$$

Substituting equation 6 into equation 5 gives
the equation 7 which relating the standard free-energy change and equilibrium constant

$$\Delta G^{o\prime} = -RT \ln K'eq \quad \rightarrow 7$$

$$\Delta G^{o\prime} = -RT \log K'eq \quad \rightarrow 8$$

$$\Delta G^{o\prime} = -2.303\, RT \log_{10} K'eq \quad \rightarrow 9$$

The criterion for spontaneity of a reaction is the value of ΔG, not $\Delta G^{o\prime}$. A reaction with a positive $\Delta G^{o\prime}$ can go in the forward direction if ΔG is negative. **Standard free energy changes are additive** i.e., the $\Delta G^{o\prime}$ values of sequential chemical reactions are additive. For example, the overall reaction, $A \leftrightarrow C$, $\Delta G^{o\prime}_{total}$ is the algebriaic sum of the individual standard free energy changes, $\Delta G^{o\prime}_1$ and $\Delta G^{o\prime}_2$, of the two separate reactions.

$A \leftrightarrow C$ $\Delta G^{o\prime}$ total

(1) $A \rightarrow B$ $\Delta G^{o\prime}_1$

(2) $B \rightarrow C$ $\Delta G^{o\prime}_2$

$A \rightarrow C$ $\Delta G^{o\prime}_1 + \Delta G^{o\prime}_2$

For example, the synthesis of glucose 6-phosphate from glucose and ATP.

(1) Glucose + Pi $\rightarrow$ Glucose 6-Phosphate + H_2O $\Delta G^{o\prime} = +13.8$ kJ/mol

under standard conditions, the reaction is not spontaneous in the direction written because $+\Delta G^{o\prime}$ value. But this reaction become spontaneous when it is coupled to the another exergonic reaction (the reaction with, $-\Delta G^{o\prime}$ values)

(2) ATP + H_2O $\rightarrow$ ADP + Pi $\Delta G^{o\prime}_2 = -30.5$ kJ/mol

Because of these two reactions share the common intermediates Pi and H_2O, they may be expressed as sequential reactions

	Glucose + Pi $\rightarrow$ Glucose – 6-Phosphate + H_2O	$\Delta G^{o\prime}_1$ 13.8 kJ/mol
	ATP + H_2O $\rightarrow$ ADP + Pi	$\Delta G^{o\prime}_2$ -30.5 kJ/mol
Sum	ATP + Glucose $\rightarrow$ Glucose 6-phosphate + ADP	

The overall free energy change is obtained by adding the $\Delta G^{o\prime}$ values of individual reactions.

$$\Delta G^{o\prime}_{total} = \Delta G^{o\prime}_1 + \Delta G^{o\prime}_2$$

$$= 13.8 \text{ kJ/mol} + (-30.5) \text{ kJ/mol} = -16.7 \text{ kJ/mol}$$

The overall reaction is exergonic.

Standard Free energy change ($\Delta G^{o\prime}$) of a reaction and its relation to the reduction potential

The reduction potential of reducing agent is a measure of its thermodynamic reactivity (E). As in the definition of $\Delta G^{o\prime}$, biochemists define the state for oxidation reduction reactions as pH 7 and express the reduction potential as $E^{o\prime}$. Electrons tend to flow to the electron acceptor with more positive E, and the strength of that tendency is proportional to the difference in reduction potentials, ΔE. The energy made available by this spontaneous electron flow (the free energy change for the oxidation – reduction reaction) is proportional to ΔE:

$$\mathbf{\Delta G^{o\prime} = - nF \quad \Delta E^{o\prime}} \qquad \rightarrow 10$$

In the above equation 'n' represents the number of electrons transferred from donor to acceptor (in the reaction), and **F** is Faraday's constant (96.48 kJV^{-1} mol^{-1}).

$\Delta G^{o\prime}$ is defined as the difference in volts between the standard reduction potential of the electron-acceptor and that of the electron-donor system.

$$\Delta E^{o\prime} = E^{o\prime}_{\text{electron acceptor}} \text{ --- } E^{o\prime}_{\text{electron donor}} \qquad \rightarrow 11$$

Standard free-energy change calculation is based on Oxidation. Reduction potential. When $NADH_2$ is oxidised by aerobic electron transport chain, (ETC), the amount of free energy released is calculated by using the following formulae:

$\Delta G^{o\prime} = - n\mathcal{F} \quad \Delta E^{o\prime}$

Net reaction is : $NADH^- + H^+ + ½\ O2 \rightarrow NAD^+ + H_2O$

This reaction is highly exergonic, in which 2 electrons are transfer from NADH to molecular oxygen through ETC.

(i) $E^{o\prime}$ for the redox pair $NAD^+ / NADH_2$ -0.320 V
(ii) $E^{o\prime}$ for the redox pair O_2 / H_2O +0.816 V

Standard reduction potential change $\Delta E^{o\prime} = 0.816 – (-0.320) = 1.14$ V
Standard free energy change $\Delta G^{o\prime}$ = -2(96.5 kJ/v.mol) (1.14 V)
= -220 kJ/mol (of NADH)

Free energy change for ATP hydrolysis

Although the hydrolysis of ATP is highly exergonic ($\Delta G^{o\prime}$ = -30.5 kJ/mol), the molecule is kinetically stable at pH 7 because the activation energy for ATP hydrolysis is relatively high. Rapid cleavage of the phosphoanhydride bonds occurs only when catalyzed by an enzyme. The free energy change for ATP hydrolysis is –30.5 kJ/mol under standard conditions, but the actual free energy of hydrolysis (ΔG) of ATP in living cells is very different; the cellular concentrations of ATP, ADP and Pi are not identical and are much lower than the 1.0 M of standard conditions. Furthermore, in most of the reactions catalysed by enzymes that involve ATP as phosphoryl group donor, the true substrate is $Mg\ ATP^{2-}$. The relevant $\Delta G^{o\prime}$ is therefore for $Mg\ ATP^{2-}$ hydrolysis. In intact cells, ΔG for ATP hydrolysis, usually designated ΔG_P, is much more negative than $\Delta G^{o\prime}$, ranging from –50 to –65 kJ/mol. ΔG_P is often called the **phosphoryl potential**.

$$\Delta G_P = \Delta G^{o\prime} + RT\ In \frac{[ADP]\ [Pi]}{[ATP]} \qquad \rightarrow 15$$

Equation 15 indicates the calculation of actual free energy of hydrolysis of ATP under intra cellular conditions.

7.4 ATP (Adenosine triphosphate)

ATP occurs in all known life forms, is the “high-energy” intermediate that constitutes the most common cellular energy currency. ATP was first discovered in muscle extracts by C. Fiske and Y. Subbarow in the United States and independently by K. Lohmann in Germany in 1929. In the 1930’s Ottowarburg and Otto Meyerhof observed the formation of ATP from ADP in coupled enzymatic reactions during the anaerobic break down of glucose to lactic acid in muscle. Later H. Kalckar in Denmark and V. Belister in the Soviet Union identified the generation of ATP from ADP during aerobic oxidations in animal tissues, in the process of oxidative phosphorylation.

Engelhardt and M.N. Lyubimova discovered that ATP is hydrolysed to ADP and Pi in energy requiring functions.

7.4.1 Structure

The structure of ATP was first deduced by in Lohman in 1930 and ultimately confirmed by Alexander Todd and his colleagues in 1948.

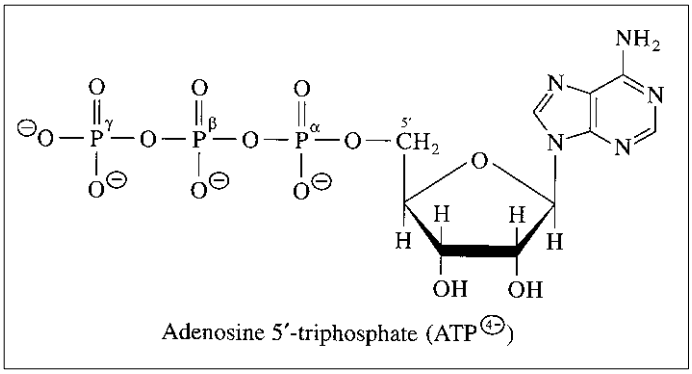

Fig. 7.1 ATP structure

ATP consists of an adenosine moiety to which three phosphoryl groups (α, β and γ) are sequentially linked via a phosphoester bond followed by two phosphoanhydride bonds. The phosphoester bond formed by linkage of α-phosphoryl group to the 5'-oxygen of ribose and two phosphoanhydride bonds formed by the α, β and β, γ linkages between phosphoryl groups (Fig. 7.1). Bonds whose hydrolysis proceeds with large negative values are often referred to as "high-energy" bonds or "energy-rich" bonds and are frequently symbolized by the squiggle (~). Thus may ATP may be represented as AR-P~P~P where A, R, and P symbolize adenyl, ribosyl and phosphoryl groups, respectively.

7.4.2 Rate of turnover of ATP

The cellular role of ATP is that of free energy transmitter rather than a free energy reservoir. The amount of ATP in a cell is typically only enough to supply its free energy needs to for a minute or two. Hence, ATP is continually being hydrolysed and regenerated. ^{32}P- labelling experiments indicate that the metabolic half-life of an ATP molecule varies from seconds to minutes depend on the cell type and its metabolic activity. For instance, brain cells have only a few seconds supply of ATP. An average person at rest consumes and regenerates ATP at a rate of ~3 mol (1.5 kg) h^{-1} and as much as an order of magnitude faster during strenuous activity.

7.4.3 The role of ATP

The role of ATP in energy metabolism was first recognised in 1941 by Fritz Lipmann and Herman Kalckar. The ATP serve as an energy conduit between "high-energy" phosphate donors and "low-energy" phosphate acceptors (Fig. 7.2).

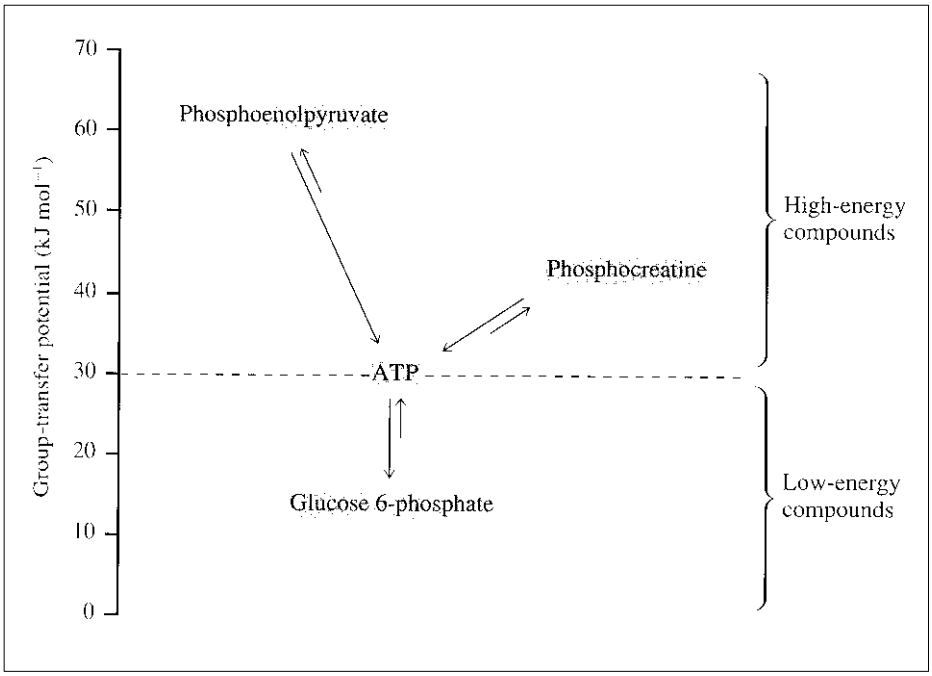

Fig. 7.2 Relative phosphoryl-group-transfer potentials. A compound with a high group-transfer potential (i.e., a large negative value of $\Delta G_{hydrolysis}$) can donate its phosphoryl group to a compound that is less energy rich. The reaction arrows indicate the predominant direction of phosphoryl-group transfer under standard conditions.

(1) Early stages of nutrient breakdown

The exergonic hydrolysis of ATP to ADP may be enzymatically coupled to an endergonic phosphorylation reaction to form "low-energy" phosphate compounds. For example, Formation of Glucose 6-phosphate from glucose and ATP by the activity of phosphofrutokinase.

Endergonic half reaction-1	Pi + Glucose $\rightarrow$ glucose 6-phosphate + H_2O	$\Delta G^{o\prime}$ + 13.8 kJ/mol
Exergonic half reaction-2	ATP + H_2O $\rightarrow$ ADP + Pi	$\Delta G^{o\prime}$ -30.5 kJ/mol
Overall coupled reaction	ATP + glucose $\rightarrow$ ADP + glucose 6-phosphate	-16.7 kJ/mol

(2) Interconversion of nucleoside triphosphates

Many biosynthetic processes, such as synthesis of proteins and nucleic acids, require nucleoside triphosphates other than ATP. These include the ribonucleoside triphosphates CTP, GTP and UTP which together with ATP, are utilized in the biosynthesis of RNA and the deoxyribonucleoside triphosphates (DNA precursors dATP, dCTP, dGTP and dTTP). All these nucleoside triphosphates (NTPS) are synthesized from ATP and corresponding nucleoside diphosphate (NDP) in reactions catalyzed by the non-specific enzyme nucleoside diphosphate kinase:

$$ATP + NDP \xleftrightarrow{\text{nucleoside diphosphate kinase}} ADP + NTP$$

(3) Physiological processes

The hydrolysis of ATP to ADP and Pi energizes many essential endergonic physiological processes such as muscle contraction and the transport of molecules and ions against concentration gradients. In general, these processes result from conformational changes in proteins that occur in response to their binding ATP. This is followed by the exergonic hydrolysis of ATP and release of ADP and Pi, thereby causing these processes to be unidirectional.

(4) Additional phosphoanhydride cleavage in highly endergonic reactions

Although many reactions involving ATP yield ADP and Pi (orthophosphate cleavage), others yield AMP and PPi (Pyrophosphate cleavage). In these latter cases, the PPi is rapidly hydrolyzed to 2 Pi by inorganic pyrophosphatase ($\Delta G^{o\prime} = -33.5$ kJ/mol^{-1}) so that the pyrophosphate cleavage of ATP ultimately results in the hydrolysis of two "high-energy" phorphoanhydride bonds. The first step in the oxidation of fatty acids provides an example for this process. Pyrophosphate cleavage alone is insufficiently exergonic to drive the fatty acid-activation reaction to completion. This reaction is made irreversible by the hydrolysis of Ppi.

7.4.4 Different types of Phosphorylations

In living organisms, formation of ATP occurs through five different types of processes. They are: (a) Substrate level phosphorylation, (b) Oxidative phosphorylation, (c) Photophosphorylation, (d) Adenylate kinase reaction, and (e) from Phosphocreatine.

(1) Substrate-level phosphorylation

ATP may be formed from 1,3 bisphosphoglycerate by direct transfer of a phosphoryl group from a "high-energy" compound to ADP. Such reactions, which are referred to as substrate-level phosphorylations, most commonly occur in the early stages of carbohydrate metabolism.

$$1,3\text{-Bisphosphoglycerate} + ADP + Mg^{2+} \xrightarrow{\text{phosphoglycerate kinase}} ATP + 3 - \text{Phosphoglycerate}$$

(2) Oxidative Phosphorylation and (3) Photophosphorylation

Both oxidative metabolism and photosynthesis generate a proton (H^+) concentration gradient across a membrane. Dissipation of this gradient is enzymatically coupled to formation of ATP from ADP and Pi. In oxidative metabolism, this process is called **oxidative phosphorylation**. In this process, oxidation of fuel molecules involved in the generation of proton concentration gradient whereas in photosynthesis, the generation of proton concentration gradient is driven by the sunlight. Formation of ATP in the photosynthesis is termed as **photophosphorylation**. Most of the ATP produced by respiring and photosynthesizing organisms is generated in this manner.

(4) Adenylate Kinase reaction

The AMP formed from pyrophosphate cleavage reactions of ATP is converted to ADP in a reaction catalyzed by the enzyme adenylate kinase:

$$AMP + ATP \xleftrightarrow{\text{adenylate kinase}} 2\ ADP$$

The ADP is subsequently converted to ATP through substrate-level phosphorylation, oxidative phosphorylation or photophosphorylation.

(5) ATP formation from Phosphocreatine

Phosphocreatine also called creatine phosphate serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP. The enzyme creatine kinase catalyzes the reversible reaction.

$$ATP + \text{Creatine} \leftrightarrow \text{Phosphocreatine} + ADP \qquad \Delta G^{o\prime} = -12.5\ \text{kj/mol.}$$

Phosphocreatine acts as an ATP "buffer" in cells that contain creatine kinase.

7.5 Oxidation-Reduction Reactions

Oxidation-reduction (also called redox) reactions are involved in the energy yielding reactions of cells. Chemically, an oxidation is defined as the removal of an electron or electrons from a substance. A reduction is defined as the addition of electron (or electrons) to a substance. In biochemistry, oxidation and reductions frequently involve the transfer of not just electrons but whole hydrogen atoms also. A substance that loses electrons and is oxidized is **reducing agent**. A molecules that accepts electrons and is reduced is an **oxidizing agent**. The net oxidation – reduction reaction is

$$A_{red} + B_{ox} \leftrightarrow A_{ox} + B_{red}$$

For example, hydrogen gas, H_2, can release electrons and hydrogen ions (protons) and become oxidized:

First half reaction: $H_2 \rightarrow 2e^- + 2\ H^+$ $\rightarrow$ (1)

Electrons cannot exist alone in solution; they must be part of atoms or molecules. The above reaction is only a half reaction, or term that implies the need for a second half reaction. This is because of an oxidation of one molecule is necessarily coupled with the reduction of another molecule. For example, the oxidation of H_2 could be coupled to the reduction of many different substances including O_2 in a second reaction

Second half reaction: $\frac{1}{2}\ O_2 + 2e^- + 2\ H^+ \rightarrow H_2O$ $\rightarrow$ (2)

The second half reaction is reduction. The two half reactions are summarised in the following way.

$H_2 + \frac{1}{2}\ O_2 \rightarrow H_2O$

In reactions of this type, the substance oxidized, in this case H_2, is called as electron donor, and the substance reduced in this case O_2, is called as electron acceptor (Fig. 7.3).

$H_2 \rightarrow 2e^- + 2\ H^+$

Electron donating half reaction

$\frac{1}{2}\ O_2 + 2e^- \rightarrow O^{2-}{}_2$

Electron accepting half reaction

$2H^+ + O_2^- \rightarrow H_2O$

Formation of water

$H_2 + \frac{1}{2}\ O_2 \rightarrow H_2O$: H_2 = electron donor

Net reaction : $\frac{1}{2}\ O_2$ = electron acceptor

Fig. 7.3 Example of an oxidation-reduction reaction: The formation of H_2O from H_2 and O_2.

7.6 Reduction potentials (or Oxidation-reduction potential)

Compounds vary in their tendency to give up electrons or accept electrons. This tendency is expressed quantitatively as the **reduction potential** ($E^{o\prime}$) of the compound. The reduction potential of a reducing agent is a measure of its thermodynamic reactivity. This potential is measured electrically in reference to a standard substance, H_2. By convention, reduction potentials are

expressed for half reactions written as **reductions**. Thus, oxidized form + e^- $\rightarrow$ reduced form. The O/R potential some extent influenced by the pH when the protons involved in the reaction. By convention in biology, reduction potentials are given for neutrality (pH 7) because the cytoplasm of the cell is neutral or nearly so. Reduction potentials of substances measured in volts (V).

For example : The reduction potential ($E^{o\prime}$) of

$$\frac{1}{2} O_2 + 2H^+ + 2e^- \rightarrow H_2O$$

is +0.816 volts (V), and that of

$$2H^+ + 2e^- \rightarrow H_2 = -0.412 \text{ V}$$

The reduction potential of half-reaction depends not only on the chemical species present but also on their activities, approximated by their concentrations. Walther Nernste derived an equation that relates standard reduction potential (E^o) to the reduction potential at concentration of oxidized and reduced species in the cell:

$$E = E^o + \frac{RT}{nF} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \rightarrow 1$$

R = gas constant

T = temperature 298 K (25^oC)

n = number of electrons transferred per molecule, and

F = Faraday constant. Above equation simplified to

$$E = E^{o\prime} + \frac{0.026 \text{ V}}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

Because of protons involved in all the biological reactions, biochemists expressed the reduction potentials of reactions as standard reduction potential ($E^{o\prime}$). As in the definition of $\Delta G^{o\prime}$, biochemists define the standard state for oxidation-reduction reactions as pH 7.

7.6.1 Oxidation-reduction couples

Most of the molecules are either electron donors or electron acceptors under different circumstances, depending on what other substances they react with. The same atom on each side of

the arrow in the half reactions can be thought of as representing an oxidation-reduction (O-R) couple, such as $2H^+/H_2$ or ½ O_2/H_2O.

In constructing complete oxidation-reduction reactions from their constituent half reactions, it is simplest to remember that the reduced substance of an O-R couple whose reduction potential is more negative donates electrons to the oxidized substance of an O-R couple whose potential is more positive. Thus, in the couple $2H^+/H_2$, which has a potential of –0.42 V, H_2 has a great tendency to donate electrons. On the other hand, in the couple ½ O_2 / H_2O, which has a potential of +0.82 V, H_2O has a very slight tendency to donate electrons, but O_2 has a great tendency to accept electrons. It follows then that in a reaction of H_2 and O_2, H_2 serves as the electron donor and becomes oxidized, and O_2 serves as the electron acceptor and becomes reduced (Fig. 7.3). Even though by chemical convention both half reactions are written as reductions, in an actual O-R reaction one of the two half reactions must be written as an oxidation and therefore proceeds in the reverse direction. In the reaction shown in Fig. 7.3, the oxidation of H_2 to $2 H^+ + 2 e^-$ is reversed from the formal half reaction, written as a reduction.

7.6.2 The electron tower

A convenient way of viewing electron transfer in biological systems is to imagine a vertical tower (Fig. 7.4). The tower represents the range of reduction potentials for O-R couples from the most negative at the top to the most positive at the bottom. The reduced substance in the pair at the top of the tower has the greatest amount of potential energy whereas the oxidized substance in the couple at the bottom of the tower has the greatest tendency to accept electrons.

As electrons from the electron donor at the top of the tower fall, they can be "caught" by acceptors at various levels. The difference in electric potential between two substances is expressed as ΔE_o'. The farther the electrons drop from a donor before they are caught by an acceptor, the greater the amount of energy released, that is, $E^{o\prime}$ is proportional to $\Delta G^{o\prime}$ (Fig.7.4). O_2, at the bottom of the tower, is the most favourable electron acceptor used by organisms. In the middle of the tower, O-R couples can act as either electron donors or acceptors. For instance, the $2H^+ / H_2$ couple has a reduction potential of –0.42 V. The fumarate-succinate couple has a potential of +0.02 V. Hence, the oxidation of hydrogen (the electron donor) can be coupled to the reduction of fumarate (the electron acceptor):

$$H_2 + \text{fumarate}^{2-} \rightarrow \text{succinate}^{2-}$$

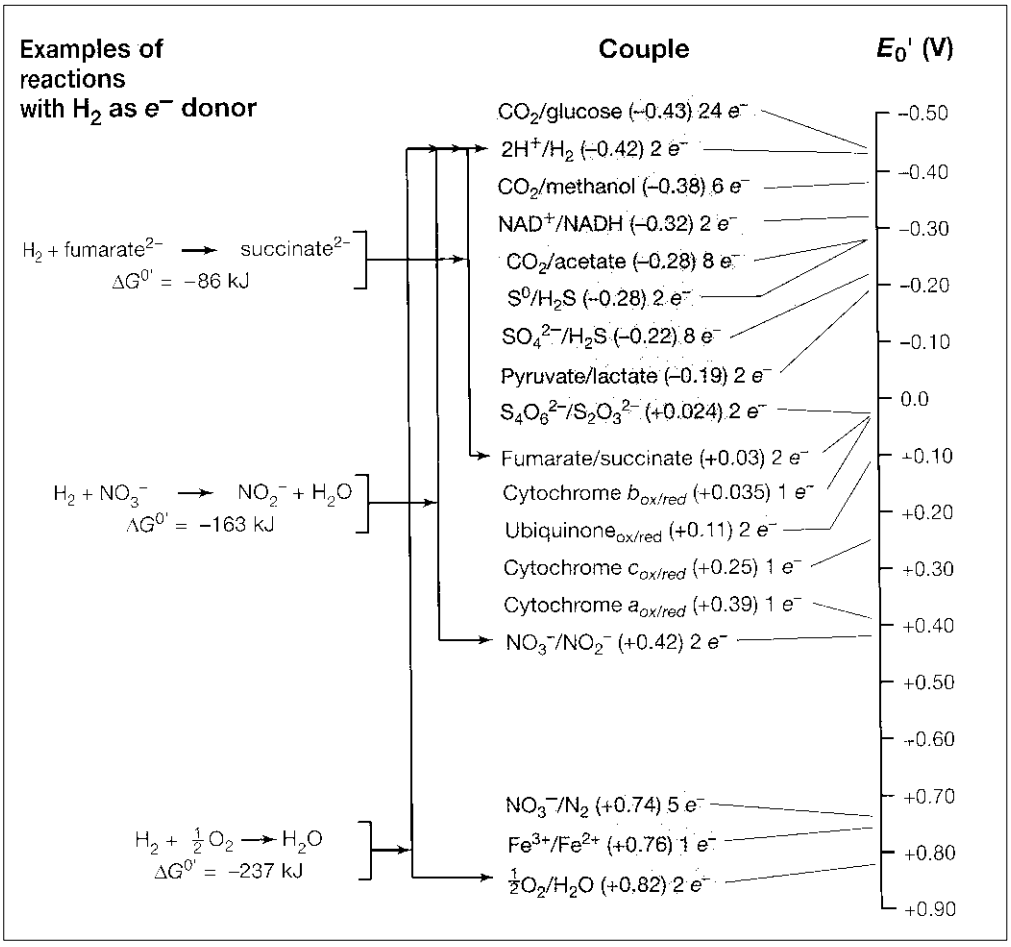

Fig. 7.4 The electron tower

7.7 Summary

Bioenergetics is the quantitative study of energy relationships and energy conversions in biological systems. Biological energy transformations obey the laws of thermodynamics. All biological reactions are influenced by two forces - enthalpy (H) and entropy (S). The net driving force in a reaction is ΔG, the free-energy changes which represents the net effect of these two factors: $\Delta G = \Delta H - T\Delta S$. Cells require sources of free energy to perform work. The standard free energy-change, $\Delta G^{o\prime}$ is a physical constant characteristic for a given reaction and can be calculated from the equilibrium constant for the reaction: ΔG, is a variable, which depends on $\Delta G^{o\prime}$ and on the concentrations of reactants and products. $\Delta G = \Delta G^{o\prime}$ + RT In ([Products] / [reactants]). When it is large and negative, the reaction tends to go in the forward direction; when it is large and positive, the reaction tends to go in the reverse direction; and when $\Delta G = 0$, the system is at equilibrium. The free energy-change for a reaction is independent of the pathway by which the reaction occurs. Free energy changes are additive.

ATP is the chemical link between catabolism and anabolism. ATP is the energy currency of the living cell. Its exergonic conversion to ADP and Pi, or to AMP and PPi, is coupled to a large number of endergonic reactions and processes. By the group (phosphoryl or pyrophosphoryl) transfer reactions, ATP provides the energy for anabolic reactions, physiological as well was mechanical processes. ATP is formed by the five different types of processes. Oxidation-reduction reaction involve the transfer of electrons from one substance to another. The tendency of a compound to accept or donate electrons is expressed quantitatively by its reduction potential. The standard free energy change for an oxidation-reduction reaction is directly proportional to the difference in standard reduction potentials of electron acceptor and electron donor.

7.8 Model Questions

1) Define the enthalpy and entropy
2) Discuss the free energy concept.
3) Describe the structure of ATP and its role in living cells.
4) Write about the different types of phosphorylations.
5) Define the reduction potential.
6) Describe the electron tower.

7.9 Reference Books

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Y.R.K.V. TIRUPATI RAO

LESSON-8

AEROBIC RESPIRATION-I

8.0 Objective: This lesson describes the some important carbohydrate catabolic pathways and their importance. They are:

⇒ Glycolysis
⇒ ED pathway
⇒ HMP pathway

8.1 Introduction

Metabolism is a highly coordinated cellular activity in which many multienzyme systems (metabolic path ways) cooperate to accomplish four functions: (1) obtain chemical energy by capturing solar energy or degrading energy-rich nutrients from the environment; (2) convert nutrient molecules into the cell's own characteristic molecules, including precursors of macro-molecules; (3) polymerize monomeric precursors into macromolecules: proteins, nucleic acids, and polysaccharides; and (4) synthesize and degrade biomolecules required in specialized cellular functions, such as membrane lipids, intracellular messengers, and pigments.

Metabolism, the sum of all the chemical transformations taking place in a living cell, occurs through a series of enzyme catalyzed reactions that constitute **metabolic pathways**. Each of the consecutive steps in a metabolic pathway brings about a specific, small chemical change, usually the removal, transfer or addition of a particular atom or functional group. **Metabolites** are the small molecules that are intermediates in the degradation or biosynthesis of biopolymers. 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 (generally Mr <1,000). It is convenient to consider the metabolism into two separate processes namely anabolism and catabolism. Catabolism is the degradative phase of metabolism in which organic nutrient molecules (proteins, fats, carbohydrates) degraded into simpler 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 ($NHDH_2$, $NADPH_2$ and $FADH_2$); the rest is lost as heat. Catabolism is considered as convergent process. In anabolism, also called biosynthesis, small, simple precursors

are built up into larger and complex molecules like proteins, lipids, carbohydrates and nucleic acids. Anabolic reactions requires energy in the form of ATP and the reducing power of $NADPH_2$, $NADH_2$ and $FADH_2$. In anabolism from few simple precursors large number of diversified compounds are formed hence it considered as divergent process.

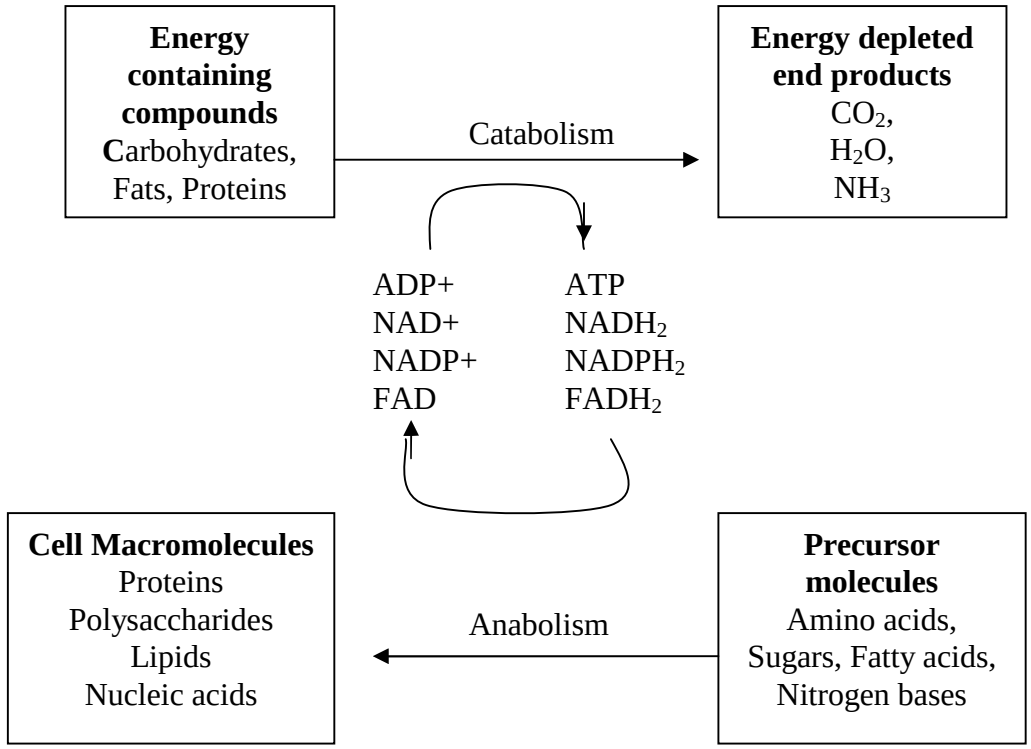


Fig. 8.1 Energy relationships between catabolic and anabolic pathways

Individual metabolic pathways can take different forms. They are: (a) linear metabolic pathway, (b) cyclic metabolic pathway, and (c) spiral metabolic pathway.

(a) **Linear pathway:** It is a series of enzyme-catalysed reactions in which the product of one reaction is the substrate for the next reaction (Fig. 8.2a). For example biosynthesis of serine.

(b) **Cyclic pathway:** It is also a sequence of enzyme catalyzed steps, but the sequence forms a closed loop, so the intermediates are regenerated with every turn of the cycle (Fig. 8.2b). For example: citric acid cycle.

(c) **Spiral pathway:** In spiral metabolic pathway, such as the biosynthesis of fatty acids, the same set of enzymes is used repeatedly for lengthening or shortening a given molecule (Fig. 8.2c). Each type of pathway may have branch points where metabolites enter or leave.

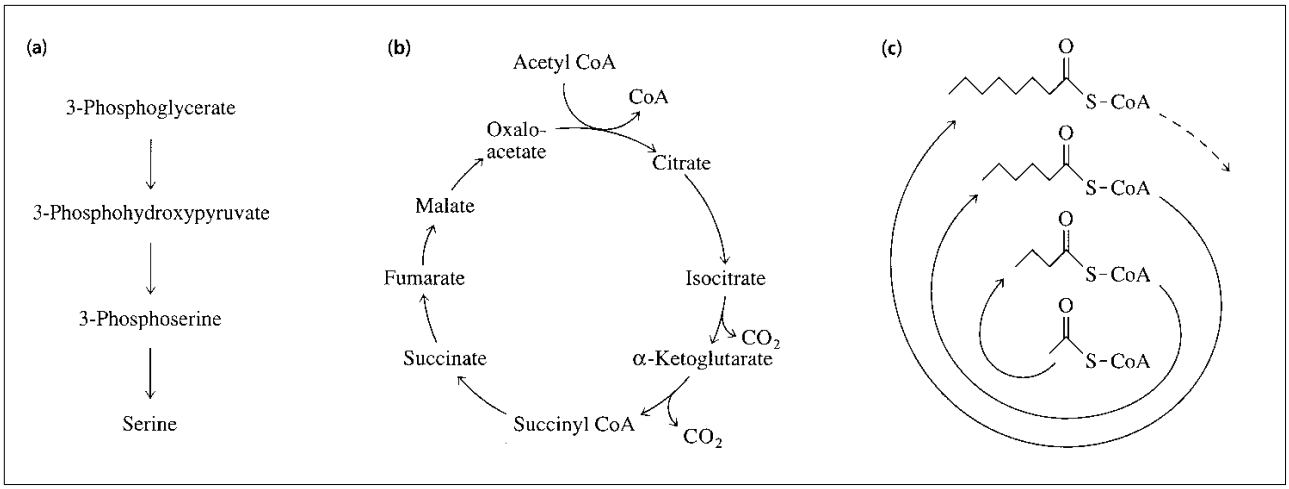


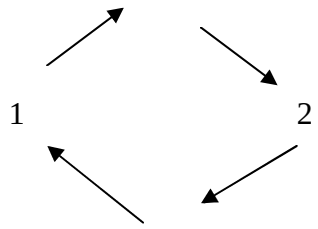
Fig. 8.2 Forms of metabolic pathways, (a) The biosynthesis of serine is an example of a linear metabolic pathway. The product of each step is the substrate for the next step. (b) The sequence of reactions in a cyclic pathway forms a closed loop. In the citric acid cycle, an acetyl group is metabolized via reactions that regenerate the intermediates of the cycle. (c) in fatty acid biosynthesis, a spiral pathway, the same set of enzymes catalyzes the progressive lengthening of the acyl chain.

Four principal characteristics of metabolic pathways stem from their function of producing products for use by the cell.

1. Metabolic pathways are irreversible

They are highly exergonic (have large negative free energy changes) so that their reactions go to completion. This characteristic provides the pathway with direction. Consequently, if two metabolites are metabolically interconvertible, the pathway from the first to the second must differ from the second back to the first.

A



B

The existence of inpendent interconversion routes is an important property of metabolic pathways because it allows independent control of the rates of the two processes. If metabolite 2 is required by the cell, it is necessary to ‘turn off’ the pathway from 2 to 1 while “turning on” the pathway from 1 to 2. Such independent control would be impossible without different pathways.

(2) Every metabolic pathway has a first committed step

Although metabolic pathways are irreversible, most of their component reactions function close to equilibrium. Early in each pathway, however, there is generally an irreversible (exergonic) reaction that "commits" the intermediate it produces to continue down the pathway.

(3) All metabolic pathways are regulated

In order to exert control on the flux of metabolites through a metabolic pathway, it is necessary to regulate its rate-limiting step. The first committed step, being irreversible; functions too slowly to permit its substrates and products to equilibrate. Since most of the other reactions in a pathway function close to equilibrium, the first, the first committed step is often its rate limiting step. Most metabolic pathways are therefore controlled by regulating the enzymes that catalyze their first committed steps. This is the most efficient way to exert control because it prevents the unnecessary synthesis of metabolites further along the pathway when they are not required.

(4) Metabolic pathways in eukaryotic cells occur in specific cellular locations

The spatial separation of pathways result in effective regulation and processing. However, in procaryotes there is no spatial separation in metabolic pathways.

Aerobic respiration is an energy generating process in which oxygen serves as the ultimate electron acceptor. If the terminal electron acceptor is other than the oxygen (nitrogen, SO_4, CO_2 etc.), the respiration is called **anaerobic respiration**. The electron donor can be either an organic compound or an inorganic one. According to biochemists and cell biologists, the respiration is a molecular processes by which cells consume O_2 and produce CO_2. Respiration occurs in three major stages. In the first stage, organic fuel molecules, glucose, fatty acids and some amino acids – are oxidized to yield two carbon fragment is known as acetyl-coenzyme A (acetyl-CoA). In the second stage, the acetyl-CoA oxidized to CO_2 through citric acid cycle. The energy released by oxidation conserved in the form of reduced electron carriers $NADH_2$ and $FADH_2$ and in the energy currency ATP. In the third stage of the respiration, these reduced coenzymes themselves oxidized by electron transport chain. In the course of electron transfer, the large amount of energy released is conserved in the form of ATP by a process called oxidative phosphorylation.

8.2 Glycolysis

The term glycolysis is derived from the Greek stem glyk-, "sweet", and the word lysis dissolution. It is a universal pathway in biological systems. Glycolysis is the sequence of enzyme-catalyzed reactions that converts glucose into two molecules of three-carbon compound pyruvate. During the sequential reactions of glycolysis relatively small amount of energy released from glucose is conserved in the form of ATP and NADH. Glycolysis was the first metabolic pathway to be elucidated and is probably the best understood. By the 1940s, the complete glycolytic pathway – including its enzymes, intermediates and coenzymes – was known. Glycolysis, also named the

Embden, Meyerhof and Paranas pathway for its major discoverers. Glycolysis is a sequence of 10 enzyme-catalyzed reactions. Most of the enzymes of this pathway are found in all living organisms and are located in the cytosol. Glycolysis can be divided into two stages.

Stage I (Preparatory phase): In this stage six-carbon glucose molecule break down to two molecules of glyceraldehyde 3-phosphate (C_3). This stage consists of five enzyme-catalyzed steps. In which two ATP's are used per glucose metabolized.

Stage-II ("Pay off" phase): This stage catalyzes the oxidation of glyceradehyde 3-phosphate to pyruvate. It consists of five enzyme-catalyzed reactions. In this stage, 4 ATPs are generated. The net yield is two molecules of ATP, per molecule of glucose metabolized, because of two molecules of ATP were invested in the preparatory phase. Energy is also conserved in the pay off phase in the formation of two molecules of NADH per molecule of glucose. Glycolytic pathway shown in Fig. 8.3.

Stage-I

(1) Phosphorylation of Glucose

The first step of the glycolysis is the posphorylation of glucose at C-6 to form glucose 6-phosphate. The phosphoryl donor is ATP. The reaction is irreversible and catalyzed by **Hexokinase**. Kinases are enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor. Hexokinase, then, catalyzes the transfer of a phosphoryl group from ATP to a variety of hexoses, such as glucose, D-fructose and D-mannose. Hexokinase, like all other kinases, requires Mg^{2+} (or Mn^{2+}), for activity, because the true substrate of the enzyme is not ATP^{4-} but the Mg ATP^{2-} complex.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase, } Mg^{2+}} \text{Glucose 6-phosphate + ADP}$$

Some bacteria (*E. coli*) phosphorylate glucose during transport into the cell via the phosphotransferase (PTS) system, in which case the phosphoryl donor is phosphoenol pyruvate (PEP)

$$\text{Glucose + PEP} \xrightarrow{\text{PTS}} \text{Glucose 6 – phosphate + puruvate}$$

(2) Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate

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

$$\text{Glucose 6-phosphate} \xrightleftharpoons{\text{Phosphohexo isomerase}} \text{Fructose 6-phosphate}$$

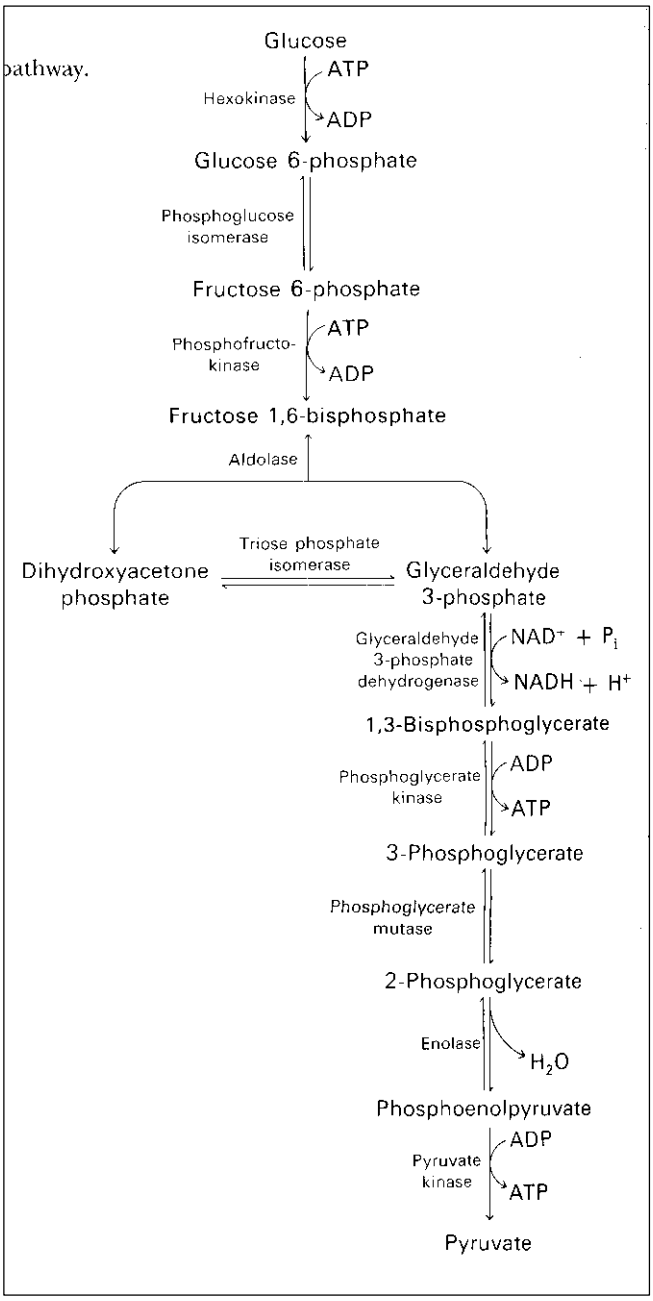


Fig. 8.3 The Glycolytic pathway

3) Phosphorylation of Fructose 6-Phosphate to Fructose 1,6 bisphosphate

Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-bisphosphate. This reaction is catalyzed by phosphofructokinase-I (PFK-I). The reaction is essentially irreversible. It is regulatory enzyme. It is major point of regulation in glycolysis.

$$\text{Fructose 6-phosphate} + \text{ATP} \xleftarrow{\text{Phosphofructokinase-I}} \text{Fructose 1,6-bisphosphate} + \text{ADP}$$

(4) Cleavage of Fructose 1,6-bisphosphate

The enzyme fructose 1, 6-bisphosphate aldolase, often simply, called aldolase, catalyzes a reversible aldol condensation. Fructose 1,6-bisphosphate is cleaved to yield two different triosephosphates, a glyceraldehyde 3-phosphate and dihydroxy acetone phosphate.

$$\text{Fructose 1,6-bisphosphate} \xleftrightarrow{\text{Aldolase}} \text{Glyceraldehyde 3-phosphate} + \text{Dihydroxyacetone phosphate}$$

(5) Interconversion of triose phosphates: Only one of the two triose phosphates formed by aldolase – glyceraldehyde 3-phosphate is a substrate for next reaction in the glycolytic pathway. Dihydroxyacetone phosphate is not, but it can be readily converted into glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase.

$$\text{Dihydroxyacetone phosphate} \xleftrightarrow{\text{Isomerase}} \text{Glyceraldehyde 3-phosphate}$$

Stage-II

(6) Oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate: This reaction is catalyzed by the enzyme glyceraldehyde 3-phosphate dehydrogenase. In this step glyceraldehyde 3-phosphate oxidized and phosphorylated to produce 1,3-bisphosphoglycerate. In the process, a molecule of NAD^+ is redued to NADH. This reaction generates a compound, 1,3-bisphosphoglycerate has a higher phosphoryl-group potential than ATP. The oxidation of the aldehyde group of glyceraldehyde 3-phosphate proceeds with a large negative standard free energy-change, and some of that energy is conserved in the acid-anhydride linkage of 1,3-bisphosphoglycerate.

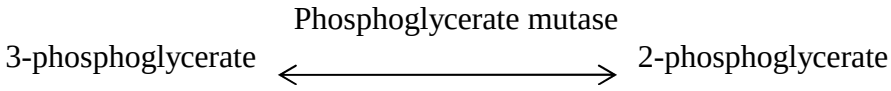
$$\text{Gyceraldehyde 3-phosphate} + NAD^+ + \text{Pi} \xleftrightarrow{\text{Glyceraldehyde 3-phosphate dehydrogenase}} \text{1,3-bisphosphoglycerate} + NADH_2$$

(7) Formation of ATP from 1,3-bisphosphoglycerate: The enzyme phosphoglycerate kinase catalyzes the transfer of phosphoryl group from 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate.

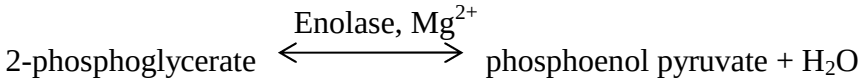
$$\text{1,3 –bisphosphoglycerate} + \text{ADP} \xleftrightarrow{\text{Phosphoglycerate kinase}} \text{3-phosphoglycerate} + \text{ATP}$$

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**.

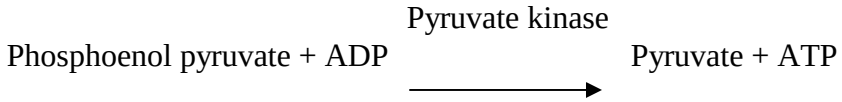
(8) Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate: This step is catalyzed by the enzyme phosphoglycerate mutase. In which phosphate group transferred from 3-carbon to 2-carbon of triose sugar. It is reversible process and it requires Mg^{2+}.



(9) Dehydration of 2-Phosphoglycerate to Phosphoenol pyruvate: Enol is formed by the dehydration of 2-phosphoglycerate. Enolase catalyzes the formation of phosphoenolpyruvate. Enolphosphate has a high phosphoryl group transfer potential.



(10) Formation of Pyruvate and the generation of a second ATP: The enzyme pyruvate kinase 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.



Pyruvate kinase is regulated both by allosteric modulators and by covalent modification.

Balance Sheet of Energy of Glycolysis: The net reaction in the transformation of glucose into two molecules of pyruvate is

$$\text{Glucose} + 2\ Pi + 2\ ADP + 2NADP^+ \rightarrow 2\ \text{pyruvate} + 2H_2O + 2NADH + 2H^+ + 2ATP$$

In the stage-I of 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.

ATP consumed per glucose : 2 (Stage-I)
ATP produced per glucose : 4 (Stage-II)
Net ATP production per glucose : 2

In glycolysis, the glucose is partially oxidized. Glycolytic degradation of glucose to two molecules of pyruvate yields only ($\Delta G^{o\prime}$ = -146 kJ/mol) 5.2% of the total energy that can be released from glucose by complete oxidation.

Regulation of Glycolysis: Glycolytic pathway is regulated at three steps. These steps are called as regulatory steps. These three steps catalyzed by the enzymes hexokinase, phosphofructokinase-I

and pyruvate kinase. These enzymes are called as regulatory enzymes. The required adjustment in the glycolysis is achieved by the reversible allosteric regulation of hexokinase, PFK-I and pyruvate kinase enzymes..

Glycolysis – amphibolic pathway: The glycolytic pathway serves not only to oxidize carbohydrate to pyruvate and to generate ATP, but also provides building blocks for other anabolic reactions. Glycolytic intermediate metabolites acts as precursors for the formation of aromatic amino acids, aminosugars, pentose sugars, and phospholipids. Glycolytic pathway perform the both catabolic and anabolic roles. Hence, it is considered as **amphibolic pathway**.

8.3 Entner-Doudoroff pathway

It is the another important pathway for the degradation of carbohydrates, which is widely distributed among aerobic gram negative bacteria. It was first discovered by Entner and Doudoroff in Pseudomonas saccharophila. It is usually not found among anaerobic bacteria, perhaps because of the low ATP yields. It is also present in many other micro-organisms including archae-bacteria. The ED pathway is also important when compounds such as gluconate, mannonate, or hexouronates serve as substrates. If *E. coli* transferred from a glucose medium to a gluconate medium it uses the E.D. pathway for the degradation of gluconate. Distribution of the EMP and ED pathways in bacteria shown in Table 8.1.

Table 8.1 Distribution of the Embden-Meyerhoff-Parnas (EMP) and Entner-Doudoroff (ED) pathways in certain bacteria

Bacterium	**EMP**	**ED**
Arthrobacter species	+	-
Azotobacter chroococcum	+	-
Alcaligenes eutrophus	-	+
Bacillus species	+	-
Escherichia coli and other enteric bacteria	+	-
Pseudomonas species	-	+
Rhizobium species	-	+
Thiobacillus species	-	+
Xanthomonas species	-	+

Reactions of Entner-Doudoroff pathway (Fig. 8.4)

(1) Phosphorylation of glucose: The first step of ED pathway is the phosphorylation of glucose at C-6 to form glucose 6-phosphate. The phosphoryl group donor is ATP. The reaction is irreversible and catalyzed by the enzyme *Hexokinase*.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose 6-phosphate + ADP}$$

(2) Dehydrogenation of Glucose 6-phosphate: Glucose 6-phosphate is converted to 6-phosphogluconolactone an intramolecular ester by the enzyme glucose 6-phosphate dehydrogenase. Oxidation of glucose 6-phosphate yield the reducing equivalent $NADPH_2$. The lactone is then hydrolyzed to 6-phosphogluconate by gluconolactonase.

$$\text{Glucose 6-phosphate + NADP+} \xrightarrow[\text{dehydrogenase}]{\text{glucose 6-phosphate}} \text{6-phosphogluconolactone + } NADPH_2$$

$$\text{6-phosphogluconolactone + } H_2O \xrightarrow{\text{Lactonase}} \text{6-phosphogluconate}$$

(3) Formation of KDPG: 6-P-gluconate is dehydrated to 2-keto-3-deoxy 6-phospho-gluconate (KDPG). This step is catalyzed by the enzyme 6-phospho-gluconate dehydratase.

$$\text{6-phosphogluconate} \xrightarrow[\text{dehydratase}]{\text{6-phosphogluconate}} \text{2-keto-3-deoxy-6-phosphogluconate (KDPG) + } H_2O$$

At this step ED pathway diverges from pentose phosphate pathway

(4) Cleavage of KDPG: The KDPG is cleaved by KDPG aldolase to two-three carbon molecules pyruvate and glyceraldehyde 3-phosphate.

$$\text{2-keto-3-deoxy-6-phosphogluconate} \xrightarrow{\text{Aldolase}} \text{Pyruvate + Glycoraldehyde 3-phosphate}$$

The glyceraldehyde-3-phosphate is oxidized to pyruvate in a sequence of reactions identical to those in the stage two of the EMP pathway.

(5) Formation of 1,3-Bisphosphoglycerate: Glyceraldehyde 3-phosphate oxidized to 1,3-bisphospho-glycerate by the activity of glyceraldehyde-3-phosphate dehydrogenase enzyme. This step is utilized the inorganic phosphate as the source of phosphate and NAD^+ as the electron acceptor.

$$\text{Glyceraldehyde 3-phosphate} \xrightarrow[\text{dehydrogenase}]{\text{Glyceraldehyde 3-phosphate}} \text{1,3-bisphosphoglycerate}$$

(6) Formation of 3-phosphoglycerate: The bisphosphoglycerate serves as the phosphoryl group donor for a substrate level phosphorylation catalyzed by the enzyme phosphoglycerate kinase. The product of phosphoglycerate kinase reaction is 3-phosphoglycerate (3-PGA).

$$\text{1,3-bisphosphoglycerate + ADP} \xleftrightarrow{\text{Phosphoglycerate kinase}} \text{3-phosphoglycerate + ATP}$$

(7) Epimerization of 3-phosphoglycerate to 2-phosphoglycerate: The 3-phosphoglycerate epimerised to 2-phosphoglycerate by the activity of enzyme phosphoglycerate mutase (PGM). In this reaction intramolecular rearrangement of phosphoryl group takes place.

$$\text{3-phosphoglycerate} \xleftrightarrow{\text{Phosphoglycerate mutase}} \text{2-phosphoglycerate}$$

(8) Formation of Phosphoenolpyruvate (PEP): In this reaction, 2-phosphoglycerate dehydrated to phosphoenolpyruvate. This step is catalyzed by the enzyme *enolase*. PEP attains the high phosphoryl group transfer potential because of the presence of ene bond.

$$\text{2-phosphoglycerate} \xleftrightarrow{\text{Enolase, } Mg^{2+}} \text{Phosphoenol pyruvate + } H_2O$$

(9) Conversion of PEP to Pyruvate: In this reaction, conversion of PEP to pyruvate is coupled with the formation of ATP by the substrate level phosphorylation. PEP seres as a phosphoryl group donor. This reaction is catalyzed by the enzyme pyruvate kinase.

$$\text{Phosphoenol pyruvate + ADP} \xrightarrow{\text{Pyruvate kinase}} \text{Pyruvate}$$

The overall reaction for the Entner-Doudoroff pathway is:

$$\text{Glucose} + NADP^+ + NAD^+ + ADP + Pi \rightarrow 2 \text{ Pyruvic acid} + NADPH + 2H^+ + NADH + ATP$$

ED pathway catalyzes the same overall reaction as the Embden-Meyerhoff-Paranas pathway (i.e., one molecule of glucose oxidized to two molecules of pyruvic acid) except that only one ATP is made, and one NADPH and one NADH are made instead of two $NADH_2$. In ED pathway, only one ATP is formed due to only one glyceraldehyde-3-phosphate is made from glucose.

Several procaryotes (e.g., Pseudomonads) completely devoid of phosfofructokinase and fructose 1,6-bisphosphate aldolase enzymes. Hence, they cannot carry out complete EMP pathway instead, they use the Entner-Dardoroff pathway for the oxidation of glucose.

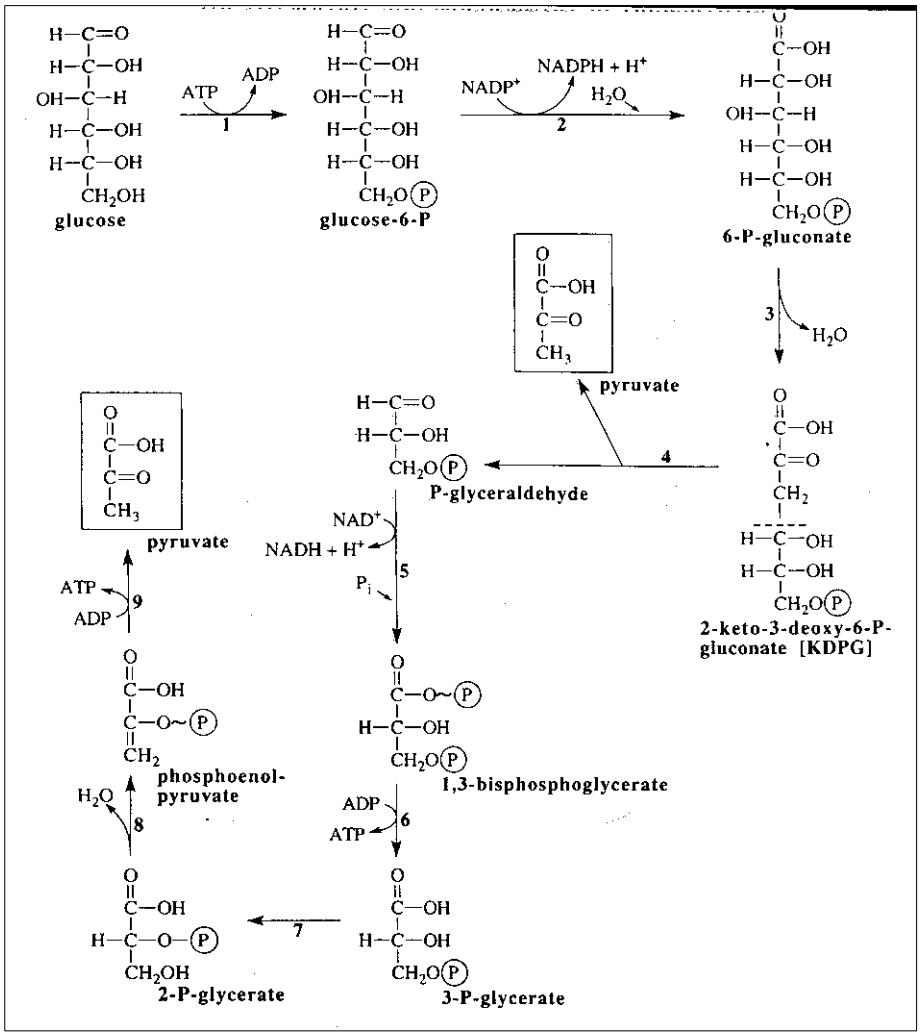


Fig. 8.4 The Entner-Doudoroff pathway. Because there is only one PGALD formed, there is only one ATP made. The enzymes unique to this pathway are the 6-phosphogluconate dehydratase (reaction 3) and the KDPG aldolase (reaction 4). The other enzymes are present in the pentose phosphate pathway and the glycolytic pathway. Enzymes: (1) hexokinase; (2) glucose-6-phosphate dehydrogenase; (3) 6-phosphogluconate dehydratase; (4) KDPG aldolase; (5) triose phosphate dehydrogenase; (6) PGA kinase; (7) mutase; (8) enolase; (9) pyruvate kinase.

Similarities with other pathways:

(1) ED pathway showed similarity with EMP pathway through oxidation stage (Stage-II) steps.
(2) ED pathway also showed resemblance with HMP pathway at first two i.e., Hexokinase and Glucose 6-phosphate dehydrogenase catalysing steps.

8.4 Pentose phosphate Pathway or HMP pathway:

Warburg and Christian provided the first evidence for the existence of an alternative pathway for the utilization of hexose sugars. It is common to both procaryotes and eucaryotes. In eucaryotes, it is active in tissues that synthesize fatty acids or steroids. Since large amounts of NADPH are consumed in these biosynthetic reactions. In other cells, such as muscle and brain, the pentose phosphate pathway accounts for little of the overall consumption of glucose. In both procaryotes and eucaryotes all the enzymes that catalyze the reactions of this pathway are present in the cytosol.

The pentose phosphate pathway (Fig. 8.5) is complex and can be best learned by dividing the reactions into three stages. Stage one consists of oxidation – decarboxylation reactions. The CO_2 and NADH are produced in stage one. Stage two consists of isomerization reactions that make the precursors for stage three. Stage three consisting of rearrangement reactions.

Stage-I: Oxidative phase: This phase comprises three enzyme catalyzed reactions. In which two reducing equivalents ($NADPH_2$) and one molecule of CO_2 are formed.

1. **Glucose Phosphorylation:** Glucose is phosporylated at C-6 carbon, by the enzyme hexokinase, to glucose-6-phosphate. In this reaction, ATP serve as phosphoryl group donor.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase, } Mg^{2+}} \text{Glucose 6-phosphate + ADP}$$

2. **Dehydrogenation of Glucose 6-Phosphate:** Glucose-6-phosphate is dehydrogenated, by the enzyme glucose 6-phosphate dehydrogenase, to 6-phospho-gluconolactone. In which NADP act as electron acceptor. Subsequently, 6-phosphogluconolactone converted to 6-P-gluconate by utilizing one water molecule. This step is catalyzed by the enzyme gluconolactonase.

$$\text{Glucose 6-phosphate + } NADP^+ \xrightarrow[\text{dehydrogenase}]{\text{Glucose 6-phosphate}} \text{Phosphogluconolactone + } NADPH_2$$

$$\text{6-phosphoglyconolactone + } H_2O \xrightarrow{\text{Lactonase}} \text{6-phosphogluconate}$$

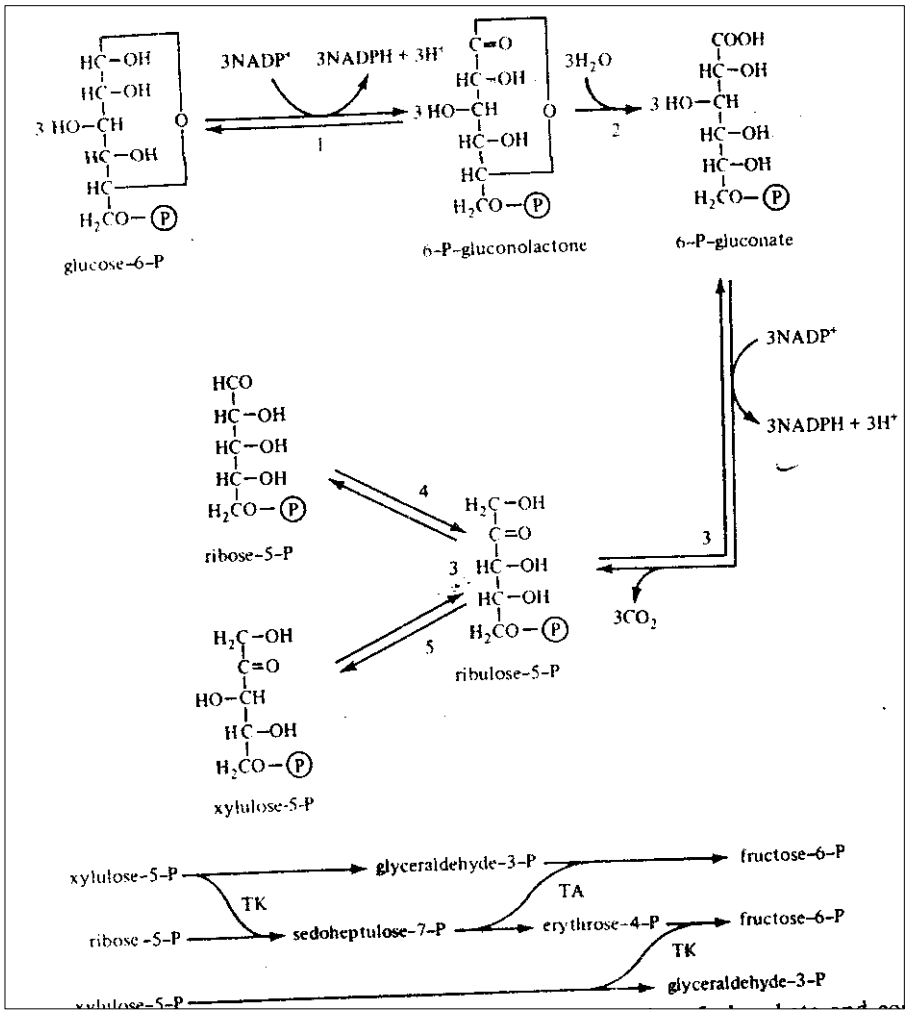


Fig. 8.5 Oxidation of glucose 6-phosphate to ribulose 5-phosphate and conversion of pentose phosphate into hexose phosphate. 1. Glucose 6-phosphate dehydrogenase; 2. lactonase; 3, 6-phosphogluconate dehydrogenase; 4, ribose-5-phosphate isomerase; 5, ribulose-5-phosphate-3-epimerase. The formed ribulose-5-phosphate is in equilibrium with ribose-5-phosphate and xylulose-5-phosphate if enzymes 4 and 5 are present. TK, transketolase, TA, transaldolase.

3. **Oxidative-decarboxylation of 6-Phosphogluconate:** In this reaction, 6-phosphogluconate is transformed to five carbon compound ribulose 5-phosphate by oxidation and decarboxylation. This reaction is catalyzed by the enzyme 6-phosphogluconate dehydrogenase. $NADP^+$ acts as electron acceptor and CO_2 is formed from the first carbon of the 6-phosphogluconate.

$$\text{6-phosphogluconate} + NADP^+ \xrightarrow[\text{dehydrogenase}]{\text{6-phosphogluconate}} \text{Ribulose 5-phosphate} + CO_2 + NADPH_2$$

The overall reaction of the first stage is

$$\text{Glucose} + \text{ATP} + 2\text{NADP}^+ + H_2O \rightarrow \text{Ribulose 5-phosphate} + \text{ADP} + 2\text{NADPH} + 2H^+ + CO_2$$

Stage-II

Isomerization reactions: During the second stage, some of the ribulose 5-phosphate is isomerized to ribose-5-phosphate and to xylulose-5-phosphate. The isomerization reactions generate the substrates for the sequential actions of transketolase and transaldolase.

(4) Formation of Ribose-5-Phosphate: The enzyme isomerase converts ribulose 5-phosphate to ribose 5-phosphate.

$$\text{Ribulose 5-phosphate} \xleftarrow[\text{isomerase}]{\text{Ribose 5-phosphate}} \text{Ribose 5-phosphate}$$

(5) Xylulose-5-Phosphate formation: Ribulose 5-phosphate is epimerized to xyluloe 5-phosphate by the enzyme ribulose 5-phosphate epimerase. In this reaction, the enzyme epimerase catalyzes a movement of the hydroxyl group from one side of the C_3 in the ribulose-5-phosphate to the other.

$$\text{Ribulose 5-phosphate} \xrightarrow[\text{epimerase}]{\text{Ribulose 5-phosphate}} \text{Xylulose 5-phosphate}$$

Stage-III

Rearrangement reactions: In this stage, there are two basic types of reactions are involved. One kind transfers a "two-carbon" fragment from a ketose to an aldose. The enzyme that catalyzes the transfer of the two carbon fragment is called a '**transketolase'** (TK). A second kind of reaction involves transfers a three carbon fragment from a ketose to an aldose. The enzyme that catalyzes the transfer of a three carbon fragment is called a **transaldolase** (TA).

(6) Formation of Glyceraldehyde-3-Phosphate and Sedoheptulose-7-Phosphate: The reversible transfer of a two carbon fragment (C-1 and C-2) from xylulose-5-phosphate to ribose 5-phosphate generates glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate. In which ketose phosphate is shortened by two carbon atoms while the aldose-phosphate is lengthened by two carbon atoms (5C + 5C $\leftrightarrow$ 3C + 7C). This reaction is catalyzed by the enzyme transketolase.

$$\text{Xylulose 5-phosphate} + \text{Ribose 5-phosphate} \xleftrightarrow{\text{Transketolase}} \text{Glyceraldehyde 3-phosphate} + \text{Sedoheptulose 7-phosphate}$$

(7) Formation of Fructose-6-Phosphate and Erythrose 4-Phosphate: The reversible transfer of a three-carbon unit (dihydroxyacetone phospahte) from sedoheptulose 7-phosphate to C-1 of

glyceraldehyde-3-phosphate generates a new ketose phosphate, fructose-6-phosphate, and releases a new aldose phosphate, erythrose 4-phosphate. The enzyme transaldolase catalyzes this reaction.

Glyceraldehyde 3-phosphate + Sedoheptulose 7-phosphate $\xleftrightarrow{\text{Transaldolase}}$ Erythrose 4-phosphate + Fructose 6-phosphate

(8) Formation of Fructose 6-Phosphate and Glyceraldehyde 3-Phosphate: This reaction is catalyzed by the enzyme transketolase. In which xylulose 5-phosphate tranfers a two carbon fragment to the erythrose 4-phosphate forming glyceraldehyde 3-phosphate and fructose 6-phosphate.

Xylulose 5-phosphate + Erythrose 4-phosphate $\xleftrightarrow{\text{Transketolase}}$ Fructose 6-phosphate + Glyceraldehyde 3-phosphate

Three moles of glucose 6-phosphate are converted to two moles of fructose 6-phosphate and one mole of phosphoglyceraldehyde. The two moles of fructose 6-phosphate become glucose 6-phosphate by isomerization and the net result is the conversion of one mole of glucose 6-phosphate to one mole of phosphoglyceraldehyde, three moles of carbondioxide and six moles of $NADPH_2$. The net result of this process is

3 Glucose 6-phosphate + 6 $NADP^+$ + $3H_2O \rightarrow$ 2 Fructose 6-phosphate + Glyceraldehyde 3-phosphate + 6 NADPH + 3 CO_2 + $6H^+$

Carbon balance for the pentosephosphate pathway

$3 \times C_6 \rightarrow 3 \times C_5 + 3 \times CO_2$
$3 \times C_5 \rightarrow 2 \times C_5 + 1 \times C_5$
$C_5 + C_5 \rightarrow C_7 + C_3$
$C_7 + C_3 \rightarrow C_6 + C_4$
$C_5 + C_4 \rightarrow C_6 + C_3$

Sum = $C_6 \rightarrow C_3 + 3 \times C_1$

Rapidly dividing cells, which require both ribose 5-phosphate and NADPH, generally have high pentose phosphate pathway activity

Similarities with other pathways:

(1) The first two reactions of the HMP pathway are the same as the reactions in the ED pathway.
(2) Rearrangement reactions in the HMP pathway are similar to reactions in the calvin cycle.
(3) Oxidation of 3 carbon molecule (glyceraldehyde-3-phosphate) of the end product of HMP shunt is similar to oxidation phase of EMP pathway.

Importance of HMP pathway:

(1) This pathway produces pentose phosphates which are the precursors to the ribose and deoxyribose nucleic acids.
(2) Pentose phosphate pathway produce erythrose 4-phosphate, which is the precursor to the aromatic amino acids.
(3) $NADPH_2$ formed in this pathway acts as reducing power for several anabolic pathways.
(4) Pentose phosphates formed in this pathway are used as CO_2 acceptors in the calvin cycle.

The remaining part of the aerobic respiration includes pyruvate oxidation, citric acid cycle, elecron transport chain, proton motive force and glyoxylate cycle are discussed in the aerobic respiration Part-II.

8.5 Summary

The nearly ubiquitous pathway for glucose degradation is the EMP pathway, also called the glycolytic pathway. Glycolysis is the set of reactions that converts glucose into pyruvate. In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain. The 10 reactions of the glycolysis occur in the cytoplasm. In the first stage, glucose is converted into fructose 1,6-bisphosphate by a phosphorylation, an isomerization, and a second phosphorylation reaction. Two molecules of ATP are consumed per molecule of glucose in these reactions which are prelude to the net synthesis of ATP. In the second stage, fructose 1,6-bisphosphate is cleaved by aldolase into dihydroky acetone phosphate and glyceraldehyde 3-phosphate, which are readily interconvertible. Glyceraldehyde 3-phosphate is then oxidized and phosphorylated to 1,3-bisphosphoglycerate. 1,3-bisphosphoglycerate generates ATP by its conversion to 3-phosphoglycerate. Another ATP is generated as phosphoenol pyruvate is converted into pyruvate. There is a net gain of two molecules of ATP in the formation of two molecules of pyruvate from one molecule of glucose. In the second stage of glycolysis two moles of NADH are formed. These are oxidised by ETC. The glycolytic pathway has a dual role: it generates ATP, and it provides the building blocks for the synthesis of cellular components.

In bacteria, a second sugar catabolizing pathway is the Entner-Doudoroff pathway. It is widespread among prokaryotes where it can be used for growth on gluconate. An intermediate of this pathway is phosphoglyceraldehyde which is oxidized to pyruvate using the same reactions that occur in stage 2 of glycolysis. Only one molecule of ATP is formed for conversion of glucose to pyruvate. Several bacteria lack key enzymes in the first of the EMP pathway (i.e. phosphofractokinase and 1,6-bisphosphate aldolase) and rely on the ED pathway for growth on glucose.

The pentose phosphate pathway generates NADPH and ribose 5-phosphate in the cytosol. NADPH is used in reductive biosyntheses, whereas ribose 5-phosphate is used in the synthesis nucleic acids and nucleotide coenzymes. The pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate to form a lactone, which is hydrolyzed to give 6-

phosphogluconate and then oxidatively decarboxylated to yield ribulose 5-phosphate. $NADP^+$ is theelectron acceptor in both of these oxidations. The last stage is the isomerization of ribulose 5-phosphate to ribose 5-phosphate and xylulose 5-phosphate. By pentose phosphate pathway, three moles of glucose 6-phosphate are converted to two moles of fructose 6-phosphate and one mole of phosphoglyceraldehyde. The two moles of fructose 6-phosphate become glucose 6-phosphate by isomerization and the net result is the conversion of one mole of glucose 6-phosphate to one mole of phosphoglyceraldehyde, three moles of carbondioxide and six moles of $NADPH_2$.

8.6 Model Questions

1. Describe the glycolysis and its importance.
2. ED pathway.
3. Write about the pentose phosphate pathway and its importance.
4. Compare the glycolysis and ED pathway.

8.7 Reference Books

1) Michael T. Madigan, John M. Martinko and Jack Parker (2000). **Brock Biology of Micoorganisms**, 9th Edition, Prentice-Hall International, Inc.
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Y.R.K.V. TIRUPATI RAO

LESSON-10

ANAEROBIC RESPIRATION

10.0 Objective: In this chapter anaerobic respiration and nitrate reduction, sulfate reduction and methanogenesis are discussed.

10.1 Introduction

Metabolism is the total of all chemical reactions occurring in the cell. The flow of energy and the participation of enzymes make metabolism possible. Metabolism may be divided into two major parts: Catabolism and Anabolism. In catabolism, larger and more complex molecules are broken down into smaller, simpler molecules with the release of energy. Some of the energy is trapped and made available for work; the remainder is released as heat. The trapped energy can then be used in anabolism which involves the synthesis of complex molecules from simpler ones with the input of energy. Microorganisms employ several metabolic pathways to catabolize glucose and other sugars. Respiration includes the catabolic reactions producing ATP in which either organic or inorganic compounds are primary electron donors and organic or inorganic compounds are ultimate electron acceptors. Although oxygen is the most widely used electron acceptor in energy – yielding metabolism (aerobic respiration), a number of other compounds can also be used as electron acceptors.

The anaerobic energy-yielding process in which the electron transport chain acceptor is an oxidized inorganic molecule other than O_2 is called **anaerobic respiration**. The major electron acceptors are nitrate, sulfate and CO_2 (Table 10.1). Anaerobic metabolism is a hallmark of procaryotes. In eucaryotes, anaerobic growth is rare. In procaryotes, by contrast, anaerobic growth is common, and the mechanisms of anaerobic metabolism highly diverse. This process of anaerobic respiration is less energy efficient but makes it possible for respiration to occur in environments where O_2 is absent.

Table 10.1. Some electron acceptors used in respiration

	Electron acceptor	Reduced products	Examples of microorganisms
Aerobic	O_2	H_2O	All aerobic bacteria, fungi, algae and protozoa
Anaerobic	NO_3^-	NO_2^-	Enteric bacteria
	NO_3^-	NO_2^-, N_2O, N_2	*Pseudomonas* and *Bacillus*
	SO_4^{2-}	H_2S	*Desulfovibrio* and *Desulfotamaculum*
	CO_2	CH_4	All methanogens
	S^o	H_2S	*Desulfuromonas* and *Thermoproteus*
	Fe^{3+}	Fe^{2+}	*Pseudomonas* and *Bacillus*

The bacteria carrying out anaerobic respiration generally possess electron transport systems containing cytochromes, quinones, iron-sulfur proteins and other typical electron transport proteins. Their respiratory systems are analogous to those of conventional aerobes. In denitrifying bacteria, the anaerobic respiration process competes in the same organism with an aerobic one. In such cases, if O_2 is present, aerobic respiration is usually favoured, and when O_2 is depleted from the environment, the alternate electron acceptor is reduced. Other organisms carrying out anaerobic respiration are *Obligate anaerobes* and are unable to use O_2.

10.2 Nitrate reduction

Inorganic nitrogen compounds are some of the most common electron acceptors in anaerobic respiration. The oxidation states of various nitrogen compounds are summarised in Table 10.2. The most widespread inorganic nitrogen compounds in nature are ammonia and nitrate.

Table 10.2. Oxidation states of key nitrogen compounds

Compound	Oxidation state
Organic N ($R - NH_2$)	-3
Ammonia (NH_3)	-3
Nitrogen gas (N_2)	0
Nitrous oxide (N_2O)	+1
Nitrogen oxide (NO)	+2
Nitrite (NO_2^-)	+3
Nitrogen dioxide (NO_2)	+4
Nitrate (NO_3^-)	+5

One of the most common alternative electron acceptor is nitrate (NO_3^-) which is reduced to N_2O, NO and N_2. All these products of nitrate reduction are gaseous and can easily be lost from the

environment. Hence, this process is called **denitrification** (Fig. 10.1). The process is the main means by which gaseous N_2 is formed biologically.

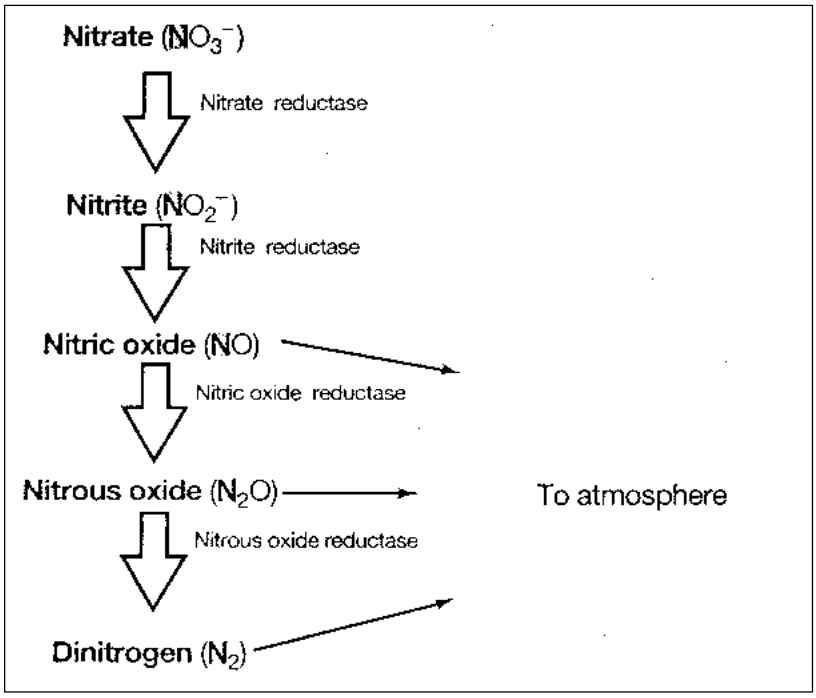

Fig. 10.1. Steps in the dissimilative reduction of nitrate. Some organisms, for example *Escherichia coli*, can carry out only the first step. All enzymes involved are derepressed by anoxic conditions.

10.2.1 Biochemistry of dissimilative nitrate reduction

The enzyme involved in the first step of nitrate reduction, nitrate reductase, is a molybdenum – containing membrane – integrated enzyme. Its synthesis is repressed by molecular oxygen. The first product of nitrate reduction is nitrite (NO_2^-). The enzyme **nitrite reductase** reduces it to nitric oxide (NO). Nitrous oxide (N_2O) is produced by the reduction of nitric oxide mediated by **nitric oxide reductase**. Finally, **nitrous oxide reductase** reduces nitrous oxide to N_2.

The biochemistry of dissimilative nitrate reduction has been studied in several organisms. In *Escherichia coli* nitrate is reduced only to nitrite by nitrate reductase. This enzyme accepts electrons from a b-type cytochrome. A comparison of the electron transport chains in aerobic versus anaerobic respiration in *E. coli* is shown in Fig. 10.2. In *Paracoccus denitrificans* and *Pseudomonas stutzeri*, nitrate is reduced to nitrogen (Fig. 10.2). In these bacteria series of

enzymes including nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase are involved in nitrate reduction.

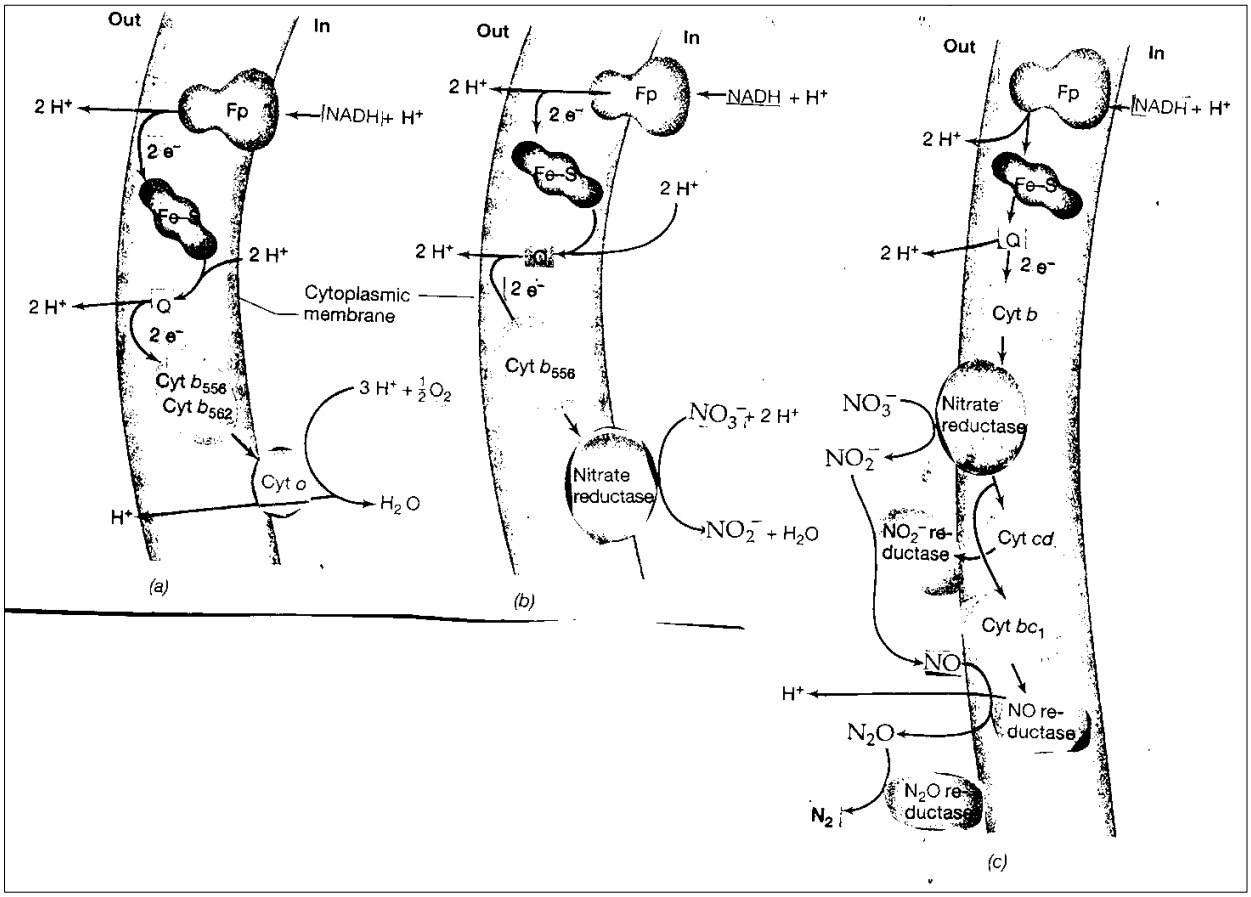

Fig. 10.2 Electron transport processes in *Escherichia coli* when (a) O_2 or (b) NO_3^- is used as an electron acceptor and NADH is the electron donor. Fp, Flavoprotein; Q, ubiquinone. Under high [O_2] conditions, the sequence of carriers is cyt b_{562} → cyt o → O_2. (c) Possible scheme for electron transport in *Pseudomonas stutzeri* during denitrification. Nitrate and nitric oxide reductases are located in the cytoplasmic membrane whereas nitrite and nitrous oxide reductases are periplasmic.

During electron transport, a proton motive force is established and ATP is produced by ATPase in the usual fashion. Additional ATP is available when NO_3^- is reduced to N_2 because the NO reductase is linked to proton extrusion.

10.3 Sulfate reduction

Several inorganic sulfur compounds serve as electron acceptors in anaerobic respiration. A summary of the various sulfur compounds with their oxidation states is given in Table 10.3.

Sulfate, the most oxidized form of sulfur, is used by **sulfate-reducing bacteria**. The end product of sulfate reduction is H_2S. In assimilative sulfate reduction, the H_2S formed is immediately converted into organic sulfur in the form of amino acids and so on, but in dissimilative sulfate reduction, the H_2S is excreted.

Table 10.3. Oxidation states of sulfur compounds

Compound	Oxidation state
Organic S (R – SH)	-2
Sulfide (H_2S)	-2
Elemental sulfur (S^o)	0
Thiosulfate ($S_2O_3^{2--}$)	+2
Sulfur dioxide (SO_2)	+4
Sulfite (SO_3^{2--})	+4
Sulfate (SO_4^{2--})	+6

10.3.1 Biochemistry of sulfate reduction

The reduction of SO_4^{2-} to H_2S, an eight-electron reduction, proceeds through a number of intermediate stages. The sulfate ion is stable and cannot be reduced without being activated. ATP sulfurylase catalyzes the attachment of the sulfate ion to a phosphate of ATP, leading to the formation of adenosine phosphosulfate (APS). The sulfate moiety of APS is reduced directly to sulfite (SO_3^{2-}) by the enzyme **APS reductase** with the release of AMP in dissimilative sulfate reduction. Sulfide is formed from sulfite by the enzyme **sulfite reductase** (Fig. 10.3).

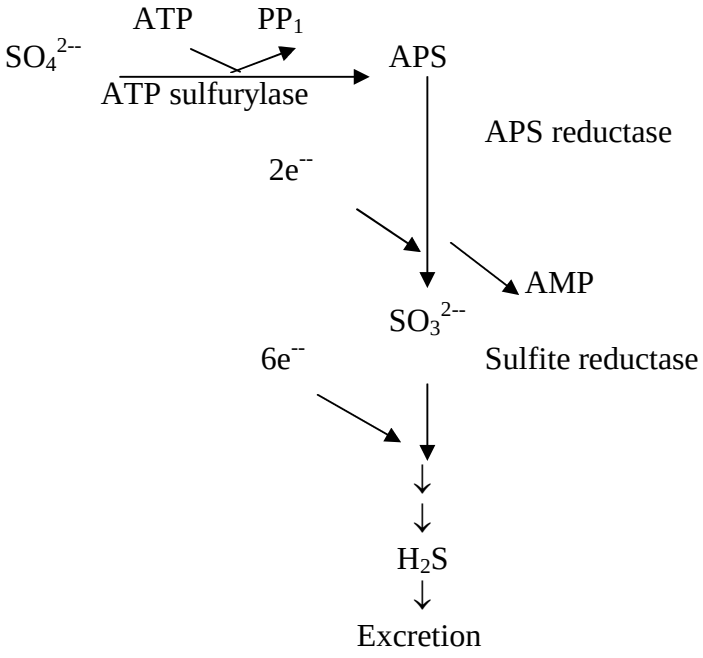

Fig. 10.3. Scheme of dissimilative sulfate reduction

In the process of disssimilative sulfate reduction, electron transport reactions occur leading to proton motive force formation, and this drives ATP synthesis by ATPase. A major electron carrier cytochrome C_3 accepts electrons from a periplasmically located hydrogenase and transfers these electrons to a membrane – associated protein complex called Hmc. It carries them across the cytoplasmic membrane, thus making them available to APS reductase and sulfite reductase, which are cytoplasmic enzymes (Fig. 10.4).

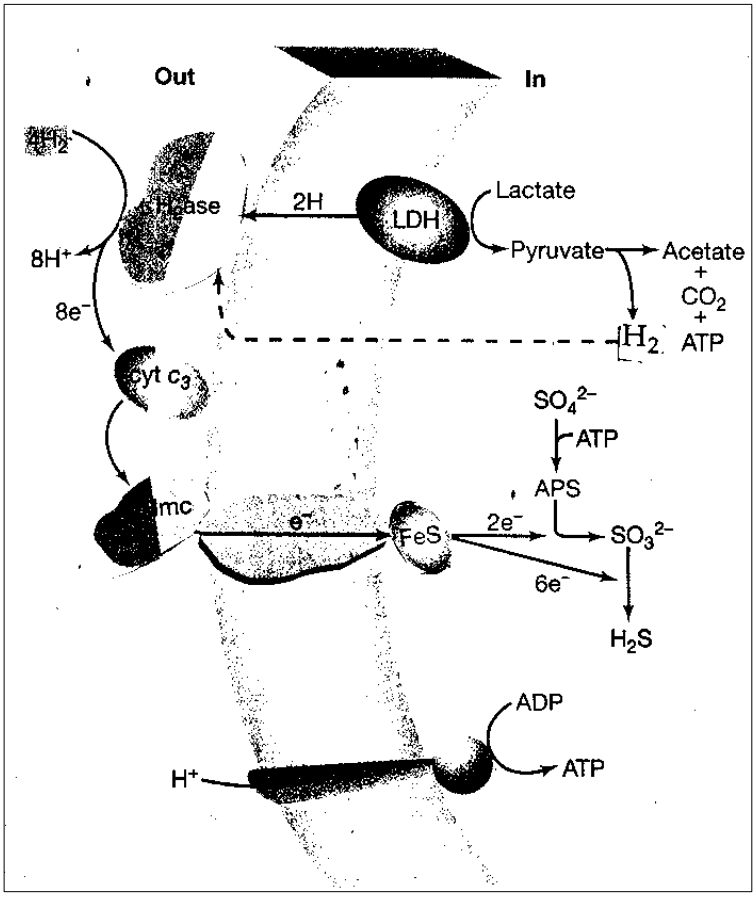

Fig. 10.4. Electron transport and energy conservation in sulfate-reducing bacteria. In addition to external hydrogen (H_2), H_2 originating from the catabolism of organic compounds such as lactate and pyruvate can fuel hydrogenase. The enzymes hydrogenase, cytochrome C_3, and a cytochrome complex (Hmc) are periplasmic proteins. A separate protein functions to shuttle electrons across the cytoplasmic membrane from Hmc to a cytoplasmic iron-sulfur protein that supplies electrons to APS reductase (forming SO_3^{2-}) and sulfite reductase (forming H_2S).

The enzyme hydrogenase appears to play a key role in sulfate reduction whether the organism grows on H_2 or on an organic compound like lactate. Evidence indicates that lactate is

converted through pyruvate to acetate with the production of H_2. The H_2 produced crosses the cytoplasmic membrane and is oxidized by the periplasmic hydrogenase to initiate a proton motive force. Growth yields of sulfate-reducing bacteria suggest that one ATP is produced for each SO_4^{2--} reduced to HS^{--}.

10.4 Methanogenesis

The biological production of methane is carried out by a group of strictly anaerobic Archaea called the **Methanogens**. The process of methane formation is called **Methanogenesis**. Methanogens obtain energy by converting CO_2, H_2, formate, methanol, acetate and other compounds to either methane or methane and CO_2. They are autotrophic when growing on H_2 and CO_2.

Methanogens thrive in anaerobic environments rich in organic matter; the rumen and intestinal system of animals, fresh water and marine sediments, swamps and marshes, hot springs, anaerobic sludge digesters and even within anaerobic protozoa. A wide variety of morphological types of methanogens have been described (Table 10.4). Their taxonomy is based on both phenotypic as well as phylogenetic (comparative 16S rRNA sequencing) analyses. Structurally methanogens are procaryotic cells that show a diversity of cell wall chemistry. The latter includes the pseudopeptidoglycan walls of *Methanobacterium* species and relatives, the methano chondroitin walls of *Methanosarcina* and relatives, the protein or glyco-protein walls of *Methanocaldococcus* and *Methanoplanus* species respectively and the S-layer walls of *Methano-spirillum*. Most known methanogens are mesophilic, although 'extremophilic' species growing optimally at very high or very low temperature, or at very high salt concentration, have also been described. The substrates utilized by various methanogenic Archaea include: i) CO_2 – type substrates, ii) methyl substrates, and iii) acetotrophic substrates (Table 10.5). The first class includes the important substrate CO_2, which is reduced to methane using H_2 as electron donor:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^{0\prime} = -131 \text{ kJ}$$

The second class of methanogenic substrates are methyl group substances. For example, methanol can be reduced to methane using an external electron donor such as H_2 :

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \qquad \Delta G^{0\prime} = -113 \text{ kJ}$$

The final methanogenic process is the cleavage of acetate to CO_2 and CH_4, called the acetotrophic reaction:

$$CH_3COO^{--} + H_2O \rightarrow CH_4 + HCO_3^{--} \qquad \Delta G^{0\prime} = -31 \text{ kJ}$$

A variety of unique coenzymes are involved in methanogenesis. The key coenzymes can be divided into two classes, those involved in carrying the C_1 unit from the initial substrate CO_2 to the final product CH_4 and those that function in redox reactions to supply the electrons necessary for the reduction of CO_2 to CH_4.

Table 10.4 Characteristics of methanogenic Archaea*

Genus	Morphology	Number of species	Substrates for methanogenesis	DNA (mol % G)
Methanobacteriates				
Methanobacterium	Long rods	8	$H_2 + CO_2$, formate	30-55
Methanobrevibacter	Short rods	7	$H_2 + CO_2$, formate	27-31
Methanosphaera	Cocel	2	Methanol + H_2 (both needed)	23-26
Methanathermus	Rods	2	$H_2 + CO_2$, can also reduce S^o; hyperthermophile	33
Methanothermobacter	Rods	6	$H_2 + CO_2$, formate, thermophiles	32-61
Methanococcales				
Methanococcus	Irregular cocci	3	$H_2 + CO_2$, pyruvate + CO_2, formate	29-35
Methanothermococcus	Cocci	1	$H_2 + CO_2$, formate	31-34
Methanocaldococcus	Cocci	4	$H_2 + CO_2$	31-33
Methanotorris	Cocci	1	$H_2 + CO_2$	31
Methanomicrobiales				
Methanomicrobium	Short rods	1	$H_2 + CO_2$, formate	49
Methanogenium	Irregular cocci	4	$H_2 + CO_2$, formate	47-52
Methanospirillum	Spirilla	1	$H_2 + CO_2$, formate	45-50
Methanoplanus	Plate-shaped cells—occurring as thin plates with sharp edges	3	$H_2 + CO_2$, formate	39-50
Methanocorpusculum	Irregular cocci	4	$H_2 + CO_2$, formate, alcohols	48-52
Methanoculleus	Irregular cocci	6	$H_2 + CO_2$, alcohols, formate	49-61
Methanofollis	Irregular cocci	2	$H_2 + CO_2$, formate	54-60
Methanolacinia	Irregular cocci	1	$H_2 + CO_2$, alcohols	38-45
Methanosarcinales				
Methanosarcina	Large irregular cocci in packets	5	$H_2 + CO_2$, methanol, methylamines, acetate	36-43
Methanolobus	Irregular cocci in aggregates	5	Methanol, methylamines	39-46
Methanohalobium	Irregular cocci	1	Methanol, methylamines, halophilic	37
Methanococcoides	Irregular cocci	2	Methanol, methylamines	42
Methanohalophilus	Irregular cocci	3	Methanol, methylamines methyl sulfides, halophile	39-41
Methanosacta	Long rods to filaments	2	Acelate	52-61
Methanosalsum	Irregular cocci	1	Methanol, methylamines, dimethylsulfide	38-40
Methanopyrales				
Methanopyrus	Rods in chains	1	$H_2 + CO_2$, hyperthermophile, growth	60

			at 110^{o}C	

*Taxonomic orders are listed in bold.

Table 10.5 Substrates converted to methane by various methanogenic *Archaea*

I.	**CO_2-type substrates** Carbon dioxide, CO_2 (with electrons derived from H_2 certain alcohols, or pyruvate) Formate, $HCOO^-$ Carbon monoxide, CO
II	**Methyl substrates** Methanol, CH_3OH Methylamine, $CH_3NH_3^+$ Dimethylamine, $(CH_3)_2NH_2^+$ Trimethylamine $(CH_3)_3NH^+$ Methylmercaptan, CH_3SH Dimethylsulfide, $(CH_3)_2S$
III	**Acetotrophic substrates** Acetate, CH_3COO^- Pyruvate, CH_3COCOO^-

The coenzyme *methanofuran* is involved in the first step of methanogenesis. Methanofuran contains the five-membered furan ring and an amino nitrogen atom that binds CO_2. *Methanopterin* is a methanogenic coenzyme that resembles the vitamin folic acid and is the C_1 carrier in the intermediate steps of CO_2 reduction to CH_4. Coenzyme M (CoM) is a small molecule that is involved in the terminal step of methanogenesis, the conversion of a methyl group to CH_4.

Although not a C_1 carrier, the nickel containing tetrapyrrole coenzyme F430 is also involved in the terminal step of methanogenesis as a part of **methyl reductase** enzyme complex. The pathway for methane synthesis is thought to function as shown in Fig. 10.5. It appears that ATP synthesis is linked with methanogenesis by electron transport, proton pumping and a chemiosmotic mechanism.

Methanogemic bacteria are of great practical importance since methane is a clean – burning fuel and an excellent energy source. Sewage treatment plants have been using the methane they produce as a source of energy for heat and electricity. Anaerobic digester microbes degrade particulate wastes like sewage sludge to H_2, CO_2 and acetate. CO_2 – reducing

methanogens form CH_4 from CO_2 and H_2, while aceticlastic methanogens cleave acetate to CO_2 and CH_4. A kilogram of organic matter can yield upto 600 litres of methane.

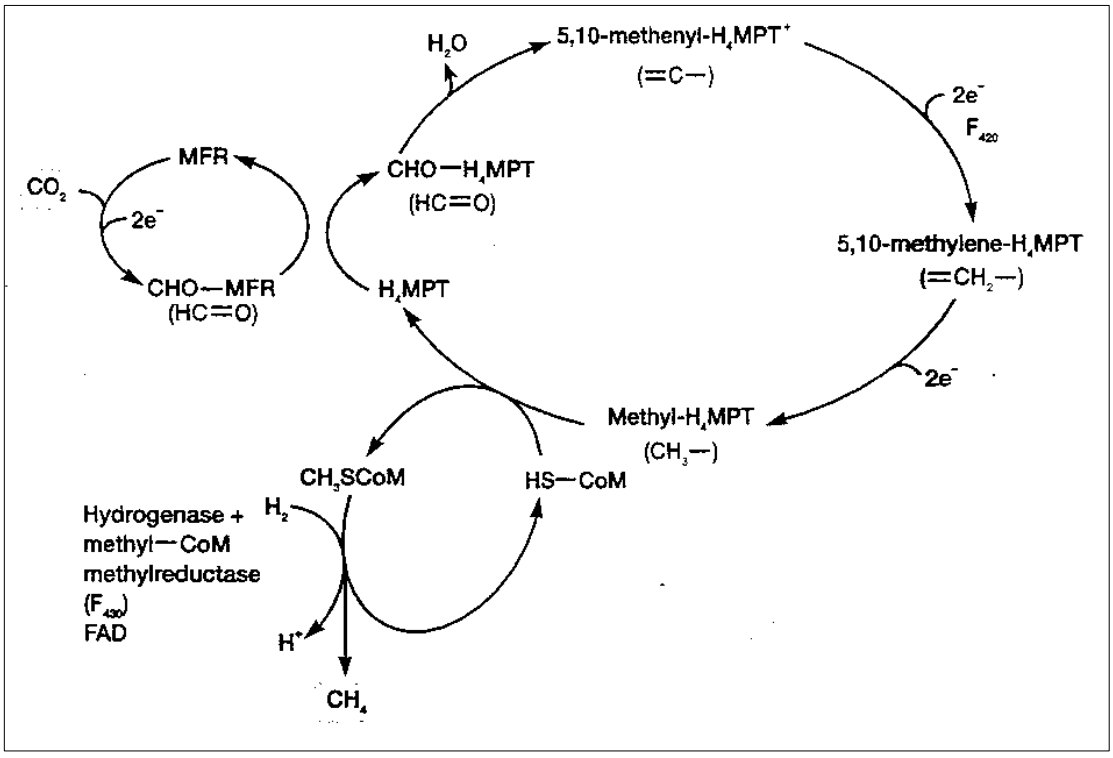

Fig. 10.5. Methane synthesis. Pathway for CH_4 synthesis from CO_2 in Methanobacterium thermoautotrophicum. Cofactor abbreviations: methanopterin (MPT), methanofuran (MFR), and 2-mercaptoethanesulfonic acid or coenzyme M (CoM). The nature of the carbon-containing intermediates leading from CO_2 to CH_4 are indicated in parentheses.

10.5 Summary

Anaerobic respiration is the process of ATP production by electron transport in which the terminal electron acceptor is an oxidized inorganic molecule other than O_2. The most common acceptors are nitrate, sulfate and CO_2. This process of anaerobic respiration is less energy efficient but makes it possible for respiration to occur in environments where O_2 is absent.

The enzyme nitrate reductase reduces nitrate to nitrite. Many bacteria that use nitrate in anaerobic respiration eventually produce nitrogen gas (N_2), a process called **denitrification**. The

sulfate reducing bacteria reduce sulfate to hydrogen sulfide. The reduction of sulfate first requires activation by a reaction with ATP to form the compound adenosine phosphosulfate (APS). Electron donors for sulfate reduction include hydrogen and organic compounds. Methanogenesis is the biological production of CH_4 from either CO_2 reduction with H_2 or from methylated compounds. A variety of unique coenzymes are involved in methanogenesis and the process is strictly anaerobic.

10.6 Model Questions

1. What is anaerobic respiration? Describe the process of denitrification?
2. How sulfate reducing bacteria can reduce sulfate to hydrogen sulfide?
3. Describe the process of methanogenesis.

10.7 Reference Books

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Dr. M. VIJAYALAKSHMI

LESSON 12

NATURE AND CLASSIFICATION OF ENZYMES

12.0. OBJECTIVES

After completion of this lesson, you will know

* Historical aspects of enzymes
* Properties of enzymes and
* Classification of enzymes.

12.1. INTRODUCTION

Enzymes are wonderful substrates – they consist of wonderful molecules.

L. Pauling (1956)

Enzymes have extraordinary catalytic power, which is greater than synthetic catalysts. These are proteinaceous molecules. They are able to accelerate specific chemical reactions in a living cell. They have high degree of specificity for their substrates. Like catalysts, they are not used up in the reaction, but unlike catalysts they are produced by living cells only. They function in dilute aqueous solutions under mild conditions of temperature and pH. They catalyze the reactions by which nutrient molecules are degraded. In this process, chemical energy is conserved and transformed, and macromolecules are made from simple precursors. There are special classes of enzymes called regulatory enzymes, which can sense metabolic signals and change their catalytic rates.

Enzyme catalyzed reactions differ from ordinary chemical reactions in four important ways:

1) High specificity: - Enzymes are capable of recognizing subtle and highly specific differences in substrate and product molecules, to the extent of discrimination between mirror images of the same molecules, called stereoisomers or enantiomers in the same way you do not fit your right hand into your left glove.
2) High reaction rates: - The rates of enzyme-catalyzed reactions are typically 10^6 to 10^{12} greater than rates of uncatalyzed reactions. Many enzymes are capable of converting thousands of substrate molecules every second.
3) Mild reaction conditions: - Enzyme reactions typically occur at atmospheric pressure, relatively low temperature and within a narrow range of pH near neutrality. There are exceptions, such as certain protein-degrading enzymes that operate in vacuoles with a pH near 4.0 or enzymes of thermophilic bacteria where their surrounding temperatures are close to 100^o C. Most enzymes in reactions are under far milder conditions than those required for most chemical reactions.
4) Opportunity for regulation: - The presence and amount of a particular enzyme is regulated by controlled gene expression and protein turnover. Enzyme activity is subject to regulatory control by a variety of activators and inhibitors.

Biological catalysis was first recognized and described in the early 1800s, in studies of the digestion of meat by secretions of the stomach and the conversion of starch into sugar by saliva and various plant extracts. In 1850 Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments". These ferments were named as **"enzymes"** that are in yeasts. The name enzyme (Greek en = in; zyme = yeast) was coined in 1878 by Kuhne to describe something present in yeast that catalyzes the reactions of fermentation. In 1897 Edward Buchner extracted a set of enzymes that catalyses the fermentation of sugar to alcohol. He prepared a cell free juice by grinding yeast cells. This juice promoted the fermentation of sugar like that by original intact yeast cells. His experiment is considered as starting of modern enzyme chemistry.

In twentieth century Emil Fischer studied about the enzyme specificity. In 1926 James Sumner at Cornell University isolated enzymes from Jackbean and crystallized it. This was named as Urease. He found that the Urease consisted of only protein. He postulated that, all enzymes are proteins. Protein nature of enzymes was confirmed further by Northrop and Kunitz (1930) through their studies on proteolytic enzymes such as pepsin, trypsin and chymotrypsin. Today some 2000 different enzymes have been identified and hundreds have been obtained in pure crystalline form. All pure enzymes examined to date are proteins except ribozymes, which are RNA molecules having the catalytic properties of enzymes.

12.2. PHYSICO-CHEMICAL NATURE

Enzyme catalytic activity depends on the integrity of their structures as proteins. Enzymes have molecular weight ranging from 12,000 to over one million. Some enzymes consist only of polypeptides and contain no chemical groups other than amino acid residues. Other enzymes require an additional chemical component for activity called a cofactor. It may be either inorganic such as Fe^{+2}, Mn^{+2} or Zn^{+2} ions (Table 12.1) or it may be a complex organic molecule called a coenzyme (Table 12.2).

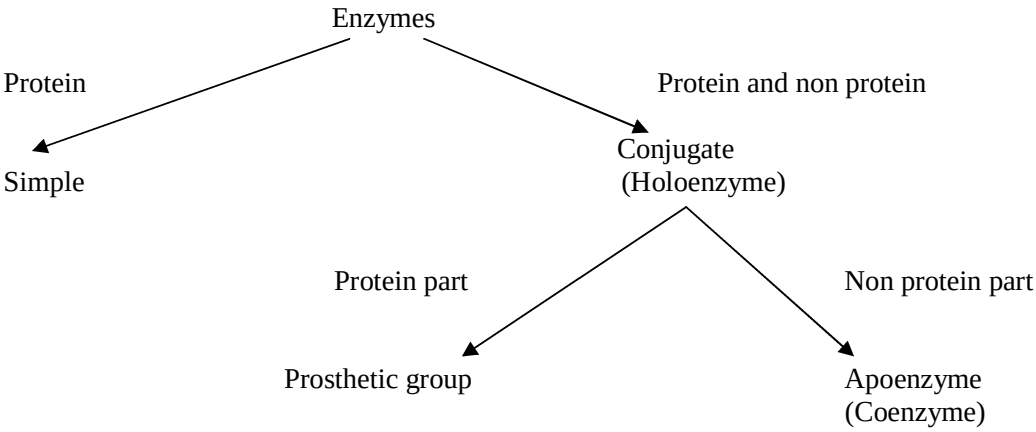
Table 12.1: Some enzymes containing or requiring essential inorganic elements as cofactors.

Element	**Cofactor**
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, Catalase, Peroxidase
Cu^{2+}	Cytochrome oxidase
Zn^{2+}	DNA polymerase, carbonic anhydrase, alcohol dehydrogenase
Mg^{2+}	Hexokinase, Glucose 6-phosphate
Mn^{2+}	Arginase
K^{+}	Pyruvate kinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase

Table 12.2: Coenzymes serving as Transient carriers of specific atoms or functional groups.

Coenzyme	**Entity transferred**
Thiamine pyrophosphate	Aldehydes
Flavin adenine dinucleotide	Hydrogen atoms
Nicotinamide adenine dinucleotide	Hydrogen ions (H^{-})
Coenzyme A	Acyl groups
Pyridoxal phosphate	Amino groups
5'-Deoxyadenosylcobalamine (Coenzyme B_{12})	H atoms and alkyl groups
Biocytin	CO_2
Tetrahydrofolate	other one-carbon groups

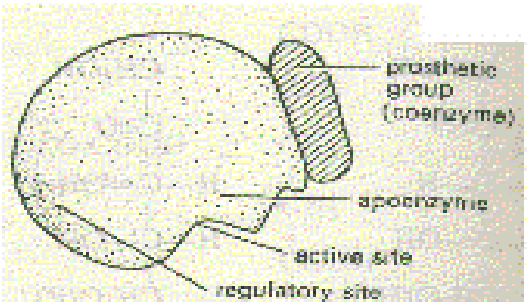
Some enzymes require both a coenzyme and one or more metal ions for activity. In some enzymes, the coenzyme or metal ion is loosely bound or in others it is tightly and permanently bound by covalent bonds, in which case it is called a prosthetic group. The catalytically active enzymes and metal ions are stable on heating, whereas the protein part of an enzyme, called the apoenzyme is denatured on heating.



Both portions of the enzymes are inactive when separated from each other. Enzymes show specificity. This is due to its apoenzyme portion. The coenzymes are non-specific. Apoenzyme is a protein with α-amino acid units. They show colour reactions and UV spectrum, a characteristic of proteins.

Some enzymes show absolute specificity for binding the substrates in catalyzing their reactions. This stereospecificity arises because enzymes form asymmetric active sites. For example, trypsin hydrolyzes polypeptide composed of L-amino acids but not those containing D-amino acids. Enzymes are absolutely stereospecific in the reactions they catalyze. For example, alcohol dehydrogenase catalyzes the interconversion of ethanol and acetaldehyde.

$$\text{Ethanol} + NAD^+ \xrightleftharpoons[\text{Alcohol Dehydrogenase}]{\text{Yeast}} \text{Acetaldehyde} + NADH + H^+$$



In addition to their stereospecificity, most enzymes are quite selective about the identities of the chemical groups on their substrates. This is known as geometric specificity. A substrate of the wrong chirality will not fit into an enzymatic binding site like you can not fit your right hand into your left glove. Some have broader specificity and may accept several specific substrates. The specificity is due to its active site (Fig.12.1)

Fig. 12.1. Morphology of an enzymes

The active site is made up of a few amino acids. This site shows flexibility in its structural organization so that it can accommodate substrate molecule. Enzymes exist either as a single unit (monomeric) or aggregate of several units (oligomeric). Apoenzyme behaves as a true catalyst during enzymatic reactions. After the reaction, it comes back to its original physico-chemical configuration.

Enzymes are colloidal in nature. So they provide large surface area for reaction to take place. Being colloidal, enzymes are hydrophilic in nature. Enzymes react with both acidic and basic substances as major parts of enzymes are proteins. Enzymes inactivate by the factors and substances, which can precipitate or denature the protein. Denaturation depends on degree of hydration. They are heat sensitive (thermolabile). Activity of enzymes depends on the activators, like inorganic salts, ions (Mn,Ni,Cl and Mg) which are required in low concentrations. Enzyme activity is also influenced by pH.

12.3. CLASSIFICATION

Enzymes are classified on the basis of the reactions they catalyze. Many enzymes have been named by adding the suffix "-ase" to the name of their substrates. Urease catalyzes hydrolysis of urea and arginase catalyzes hydrolysis of arginine. Lactase catalyzes hydrolysis of lactose. Many enzymes have been given names that do not denote their substrates for example pepsin, trypsin and transcarboxylase. Some names do indicate the substrate of the reaction, for example catalase. The enzyme is known by two or more names or two different enzymes have been given the same name. To overcome these ambiguities, because of lack of consistency in the nomenclature as well as the ever increasing number of newly discovered enzymes, a systematic basis for naming and classifying enzymes have been adopted by international agreement. The International Union of Biochemistry (IUB) for naming and classifying enzymes appointed a commission.

The commission assigned to each enzyme a systematic name in addition to its existing trivial name. This systematic name includes the name of the substrate or substrates in full and a word ending in '-ase' indicating the nature of the process catalysed. This word is either one of the six main classes of enzymes or a subdivision of one of them. When a reaction involves two types of overall change, e.g. oxidation and decarboxylation, the second function is indicated in brackets, e.g. oxidoreductase (decarboxylating). Examples are given below.

The systematic name and the Enzyme Commission (E.C.) classification number unambiguously describe the reaction catalysed by an enzyme and should always be included in a report of an investigation of an enzyme, together with the source of enzyme, e.g. rat liver mitochondria.

However, these names are likely to be long and unwiedly. Trivial names may, therefore, be used in a communication, once they have been introduced and defined in terms of the systematic name and E.C. number. Trivial names are also inevitably used in everyday situations in the laboratory. The Enzyme Commission made recommendations as to which trivial names were acceptable, altering those which were considered vague or misleading. Thus, 'fumarase' mentioned above, was considered unsatisfactory and was replaced by 'fumarate hydratase'. This commission places all enzymes in six major classes, each with subclasses, based on the type of reaction catalyzed (Table 12.3).

Table 12.3: - International Classification of Enzymes, based on the reactions they catalyze.

No.	Class	Type of reaction catalyzed
1.	Oxidoreductases	Transfer of electrons
2.	Transferases	Group transfer reactions
3.	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4.	Lyases	Addition of groups to double bonds or the reverse
5.	Isomerases	Transfer of groups within molecules to yield isomeric forms
6.	Ligases	Formation of C-C, C-S, C-O and C-N bonds by condensation reactions coupled to ATP cleavage.

Each enzyme was assigned a code number, consisting of four elements separated by dots. The first digit shows to which of the main classes the enzyme belongs:

First digit	Enzyme class	Type of reaction catalyse
1	Oxidoreductases	Transfer of electrons (H^+ or OH)
2	Transferases	Transfer of functional groups
3	Hydrolases	Hydrolysis reactions
4	Lyases	Addition of groups to double bonds,or formation of double bonds by removal of groups
5	Isomerases	Isomerization by transfer of groups within molecules
6	Ligases	Bond formation coupled with ATP hydrolysis

The second and third digits in the code describe the kind of reaction being catalyzed. The fourth digit distinguishes between them by defining the actual substrate.

The commission assigned to each enzyme a systematic name. This name includes the name of the substrate or substrates in full and a word ending in '-ase' indicating the nature of the process catalyzed. The systematic name and the Enzyme Commission (E.C) classification number describe the reaction catalyzed by an enzyme.

E.g.: - The formal systematic name of the enzyme is ATP:glucose phosphotransferase.

ATP + D-glucose ⟶ ADP + D-glucose-6-phosphate

This enzyme catalyzes the transfer of a phosphate group from ATP to glucose. Its classification number is 2.7.1.1. the first digit (2) stands for the class name (transferase), the second digit (7) for the subclass (phosphotransferases), the third digit (1) for the sub-subclass (phosphotransferases with a hydroxyl group as acceptor) and the fourth digit (1) for D-glucose as the phosphate-group acceptor. When the systematic name is long, a trivial name may be used. In this case the trivial name is hexokinase.

1. Oxidoreductases: - These enzymes catalyze oxidation – reduction reactions, that is, they transfer H atoms, O atoms or electrons from one substrate to another. For example, alcohol: NAD^+ oxidoreductase (alcohol dehydrogenase) catalyzes the oxidation of an alcohol to an aldehyde. It removes two electrons and two hydrogen atoms from the alcohol to yield an aldehyde. The two electrons originally in the carbon – hydrogen bond of the alcohol are transferred to the NAD^+, which is reduced. NAD^+ is a cofactor that mediates many biological oxidation-reduction reactions. In addition to the alcohol and aldehyde groups, dehydrogenases also act on the $–CH_2-CH_2$, $-CH_2-NH_2-$ and –CH = NH as well as the cofactors NADH and NADPH.

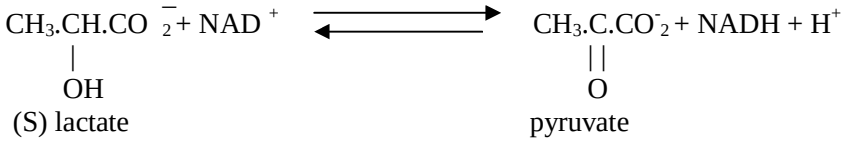
The second digit in the code number of oxidoreductases indicates the donor of the reducing equivalents (Hydrogen or electrons) involved in the reaction.

Second digit	Hydrogen/electron donor
1	Alcohol (>CHOH)
2	Aldehyde or ketone (>C=O)
3	–CH.CH-
4	Primary amine ($-CHNH_2$ or $–CHNH_3$)
5	Secondary amine (>CHNH-)
6	NADH or NADPH

The third digit refers to the hydrogen or electron acceptor

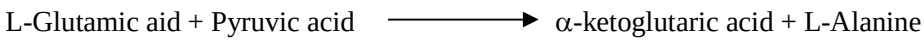
Third digit	Hydrogen or electron acceptor
1	NAD+ or NADP+
2	Fe+3
3	O_2
99	Unclassified acceptor

E.g.: - (S)-lactate:NAD+ oxidoreducatse (E.C.1.1.1.27)(Trivial name is lactate dehydrogenase) catalyses:



The alcohol group of lactate is involved in the reaction

2. Transferases: - These enzymes transfer functional groups between donors and acceptors. The amino, acyl, phosphate, one-carbon and glycosyl groups are the major moieties that are transferred. Aminotransferases (transaminases) transfer an amino group from one amino acid to an α-keto acid acceptor. This results in the formation of a new amino acid and a new keto acid.

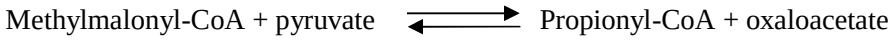


Kinases are the phosphorylating enzymes that catalyze the transfer of the γ phosphoryl group from ATP or another nucleoside triphosphate to alcohol or amino group acceptor.

Second digit	Group transfer
1	1-carbon group
2	aldehyde or ketone group (>C=O)
3	acyl group (-C-R) ‖ O
4	glycosyl (carbohydrate) group
7	phosphate group

E.g.: - E.C.2.1.1 enzymes are methyltransferases (transfer of $-CH_3$)

(S)-2-methyl-3-oxopropanoyl-CoA: Pyruvate carboxyltransferase (E.C. 2.1.3.1) (trivial name: methylmalonyl-CoA carboxyltranferase, formerly transcarboxylase) which catalyses the transfer of a carboxyl group from methylmalonyl-CoA to pyruvate:



The third digit describes the group transferred

Third digit	Group transferred
1	methyltransferease
2	hydroxymethyltransferase
3	carboxyl or carbamoyl transferases

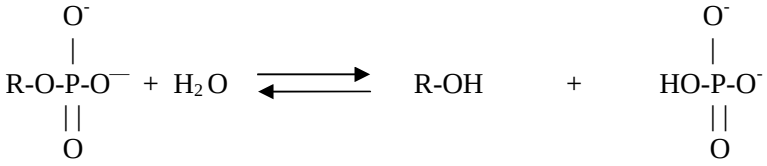
3. Hydrolases: - These enzymes catalyze hydrolytic reactions and can be considered as a special class of the transferases in which the donor group is transferred to water. These are classified according to the type of bond hydrolyzed. The generalyzed reaction involves the hydrolytic cleavage of C-O, C-N, O-P and C-S bonds. Proteolytic enzymes are a special class of hydrolases called peptidases.

Second digit	**Bond hydrolyzed**
1	ester
2	glycosidic
4	peptide (-C-N-)
5	C-N bonds other than peptides

The third digit describes the type of bond hydrolyzed.

Third digit	**Bond hydrolysed**
1	Carboxylic ester (-C-O-)
2	Thiol ester (-C-S-)
3	Phosphoric monoester ($-O-PO_3^{-2}$)
4	Phosphoric diester (-O-P-O-)

E.g.: - Orthophosphoric monoester phosphohydrolase (E.C.3.1.3.1) (Trivial name: alkaline phosphatase) catalyses



Organic phosphate inorganic phosphate

Alkaline phosphatases are relatively non-specific, and act on a variety of substrates at alkaline pH. The trivial names of hydrolases are recommended to be the only ones to consist simply of the name of substrate plus '-ase'

4. Lyases: - These enzymes catalyze the non-hydrolytic removal of groups from substrates, leaving double bonds. Lyases add or remove the element of water, ammonia or carbondioxide. Decarboxylases remove the element of CO_2 from α- or β- keto acids or amino acids. Dehydratases remove H_2O in a dehydration reaction. Fumarase vonverts fumarate to malate. The second digit indicates the bond broken.

Second digit	Bond broken
1	C-C
2	C-O
3	C-N
4	C-S

The third digit refers to the type of group removed.

Third digit	Group removed
1	carboxyl group
2	aldehyde group
3	ketoacid group

E.g.: - L-histidine carboxyl-transferase (E.C.4.1.1.22) (Trivial name is histidine decarboxylase) catalyses:

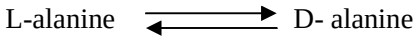
5. Isomerases: - These enzymes catalyze isomerization reactions. These are heterogenous group of enzymes catalyze isomerization of several types. These include cis-trans and aldose/ ketose interconversions. Isomerases that catalyze inversion at asymmetric carbon atoms are either epimerases or racemoses. Mutases involve intramolecular transfer of a group such as a phosphoryl. The transfer may be direct but can involve a phosphorylated enzyme as an intermediate. Phosphoglycerate mutase catalyzes conversion of 2-phosphoglycerate to 3-phosphoglycerate.

Second digit	Type of reaction
1	epimerization or recemization
2	cis-trans isomerization
3	intramolecular transfer reaction
4	intramolecular transfer reaction

The third digit describes the type of molecule undergoing isomerization

Third digit	Substrate
1	amino acids
2	hydroxy acids
3	carbohydrates

E.g.: - Alanine racemase (E.C.5.1.1.1) catalyses



6. Ligases: - These enzymes catalyze the synthesis of new bonds along with the breakdown of ATP or other nucleoside triphosphates. Ligation means, “to bind”. These enzymes are involved in synthetic reactions, when two molecules are joined at the expense of a “ high-energy phosphate bond” of ATP. The term synthetase is reserved for this particular group of enzymes. The reactions catalyzed by ligases are the formation of aminoacyl tRNAs, acyl coenzyme A and glutamine and the addition of CO_2 to pyruvate. Pyruvate carboxylase is a good example of a ligase enzyme.The substrate bicarbonates and pyruvate are ligated to form a four-carbon (C_4) α-keto acid.The second digit indicates the type of bond synthesized.

Second digit	Bond synthesized
1	C-O
2	C-S
3	C-N
4	C-C

The third digit describes the bond being formed.

Third digit	Bond formed	
1	amide synthases	$-\overset{\overset{O}{\|\|}}{C}-NH_2$
2	peptide synthases	$-\overset{\overset{O}{\|\|}}{C}-\underset{\underset{H}{\|}}{N}-$

Ex:- L-glutamate: ammonia ligase (E.C.6.3.1.2) (Trivial name: glutamate ammonia ligase) catalyses:

$$\text{L-glutamate} + \text{ATP} + NH_3 \rightleftharpoons \text{L-glutamine} + \text{ADP} + \text{Pi}$$

12.4. SUMMARY

- Enzymes have catalytic power greater than synthetic catalysts. They are proteinaceous molecules.
- Louis Pasteur concluded that sugar is converted to alcohol by fermentation in the presence of a ferment, which is named as " Enzymes".
- Enzymes have been used for many centuries, although their true nature has only become known relatively recently, and they are still of great importance in scientific research, clinical diagnostics and industry.
- Each enzyme has a protein and non-protein part. Proteinaceous are called simple enzymes and enzymes with protein and non-protein parts are called holoenzymes/ conjugate enzymes. Protein part of holoenzyme is called a prosthetic group and non-protein part is called apoenzyme.
- Enzymes show specificity toward substrate is due to their active sites. The active site is made up of a few amino acids.
- Because of the lack of consistency and occasional lack of clarity in the names of enzymes, an Enzyme Commission appointed by the International Union of Biochemistry has given all known enzymes a synthetic name and a four-figure classification. These, together with the source of the enzyme concerned, should be quoted in any report.
- There are six classes, oxidoreductases, transferases, hydrases,lyases, isomerases and ligases.

12.5. MODEL QUESTIONS

1. What are enzymes? Write the physico-chemical properties of enzymes?
2. Write an essay on nature and classification of enzymes?
3. Write briefly about the enzyme classification given by IUB?

12.6. REFERENCE BOOKS

1. ENZYMES: Biochemistry, Biotechnology and clinical chemistry- Trevor palmer, Harwood Publishing Limited, Chichester, 2001.
2. Introduction to Plant Physiology 2nd ed., - William G. Hopkins, Joh Wiley & Sons, Inc. 1999.
3. Principles of Biochemistry- Albert Lehninger, CBS Publishers and Distibutors.

Dr. P. KIRANMAYEE

LESSON 12

REGULATION OF ENZYMES

12.0. OBJECTIVES

After completion of this lesson you will know

- How enzymes are regulated by induction and repression
- Regulation of enzyme activity by feedback inhibition, allosteric control and covalent modification of enzymes.

12.1. INTRODUCTION

Enzyme catalyzed reactions must proceed with the demands of the cell for energy and for cellular constituents. So a cell must contain mechanisms to slow down or to speed up the synthesis of a particular amino acid or the formation of ATP. This may either increase or decrease the activity of certain key enzymes of the metabolic pathways.

Under certain growth conditions microorganisms need a number of catabolic and anaplerotic enzymes. β-galactosidase enzyme is required when ***E.coli*** is growing on lactose and this enzyme is not required when glucose acts as substrate. Likewise, ***Pseudomonas*** strain requires several specific enzymes to utilize phenol. These enzymes are not needed if organic acids like malate or succinate were the substrates. So it is reasonable and economical that organisms do not synthesize all the enzymes, but synthesize whenever they are necessary for metabolism under physiological conditions. This is called regulation and regulation of enzyme synthesis is accomplished by induction and repression.

12.2. REGULATION BY INDUCTION AND REPRESSION

The substrate mediated enzyme synthesis is called an induction and the compound responsible for the synthesis of enzyme is called an inducer.

12.2.1. Enzyme induction

There is an increase in the specific activity of β-galactosidase in ***E.coli*** following the addition of lactose to a cell suspension (Fig.12.1).

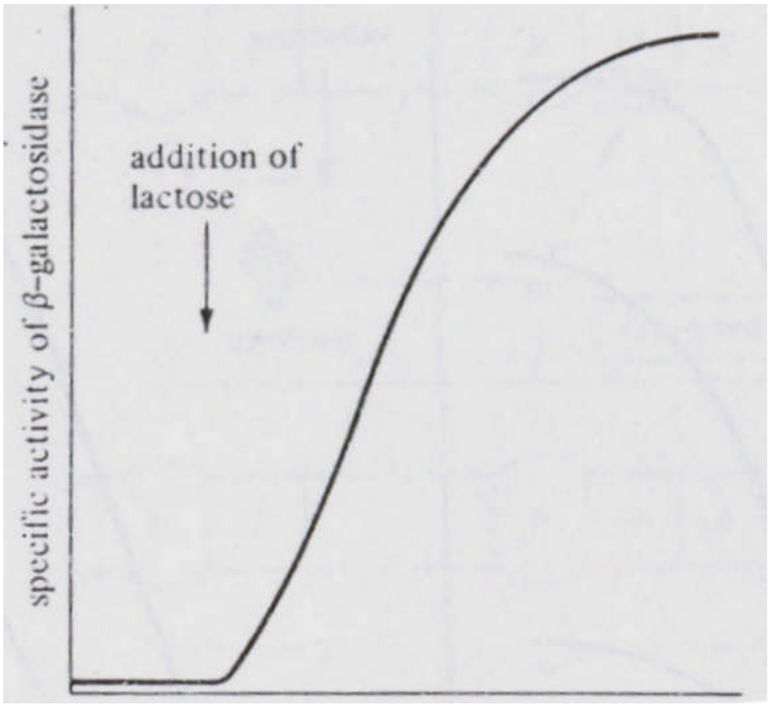


Fig. 12.1. Increase of the specific activity of β- galactosidase in ***E. coli*** following the addition of lactose to a cell suspension.

Enzyme level is low when there is absence of lactose. A measurable increase in enzyme activity as early as 1.4 minutes after the addition of lactose. Depending on the growth conditions, enzyme activity reaches a plateau after 15-180 minutes.

Pseudomonas putida has to synthesize 10 enzymes when growing on aromatic compounds. More than one enzyme is required to convert a substrate into the simple compound involved in metabolism. There are two kinds of mechanisms, coordinate and sequential induction.

Coordinate induction

The growth substrate switches on the synthesis of all enzymes required for its degradation. For example, the enzymes of the Enter-Doudoroff pathway are formed by both coordinate induction and the enzymes for the metabolism of arabinose. In presence of lactose permease, β-galactosidase and a transacetylase are induced. Galactose formed in the β-galactosidase reaction induces the enzymes for its conversion into glucose-1-phosphate.

Sequential induction

It is found for long catabolic pathways that serve for the degradation of several substrates. One of the best examples is the formation of the enzymes for the degradation of aromatic compounds (Fig. 12.2).

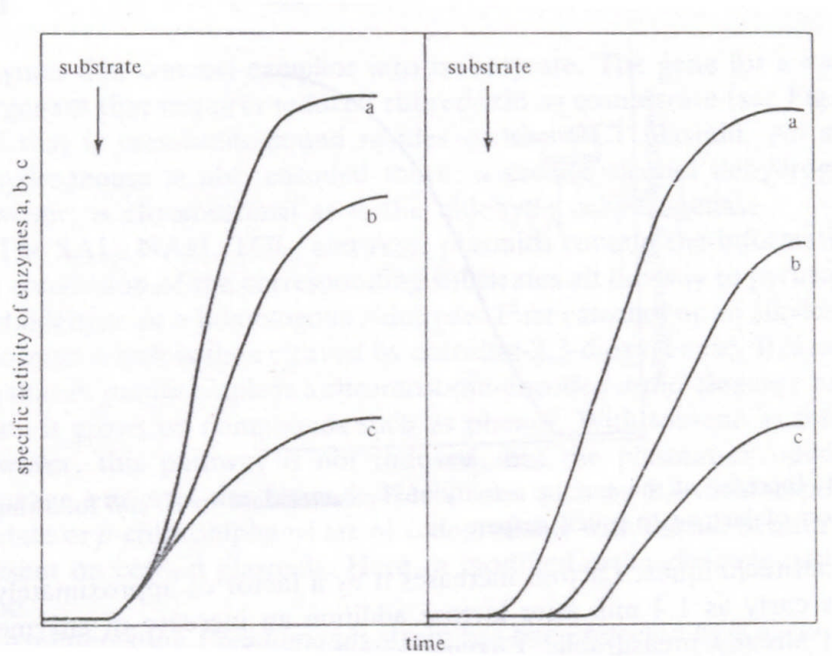


Fig. 12.2. Coordinate and sequential induction of enzymes a, b and c.

In this figure, the two branches are regulated in a different manner. Protocatechuate is the inducer of protocatechuate oxygenase. The four subsequent enzymes are coordinatedly induced by one of the terminal intermediates, 3-oxoadipate. In the catechol branch, *cis, cis*-muconate acts as product-inducer of catechol 1,2-oxygenase and as substrate-inducer of muconate-lactonizing enzyme and muconolactone isomerese. Catechol 1,2-oxygenase and the enzymes of this pathway are product-induced, requires that catechol be converted to *cis, cis*-muconate and muconolactone to 3-oxodipate before the induction of the enzymes required if catechol is the substrate.

In ***Acinetobacter calcoaceticus***, all enzymes for the degradation of protocatechuate are induced by *cis-cis* muconate, induces all enzymes required if catechol is the sunbstrate.

The glucose-grown ***E. coli*** cells do not contain the enzymes for the catabolism of lactose. It means that the entire bacterial genome is not blindly transcribed into RNA sequences and translated into proteins. Many genes always express in growing cells and produce a number of enzymes that are present in all conditions, which are called constitutive enzymes.

The molecular and genetic relationships between enzyme induction and enzyme repression have been clarified by the genetic research of Francois Jacob and Jacques Monod at Pasteur Institute in Paris. They worked on induction of β-galactosidase activity in ***E.coli*** cells and proposed the operon hypothesis. The type of regulation of protein synthesis provided by the operon concept is spoken of as transcriptional control. The control is exerted primarily on the rate of transcription of genes into their corresponding mRNAs. There is another regulation, the translational control, i.e., control of the rate of synthesis of the polypeptide chain from its mRNA template. Transcriptional control is the primary mechanism and traslational control is the secondary mechanism in bacteria but very important in eukaryotes.

Jacob and Monod proposed the three structural genes *z*, *y* and *a* coding for the synthesis of β-galactosidase, β-galactosidase permease and A protein. All of these can be induced by lactose and are located adjacent to each other in the ***E.coli*** chromosome (Fig.12.3).

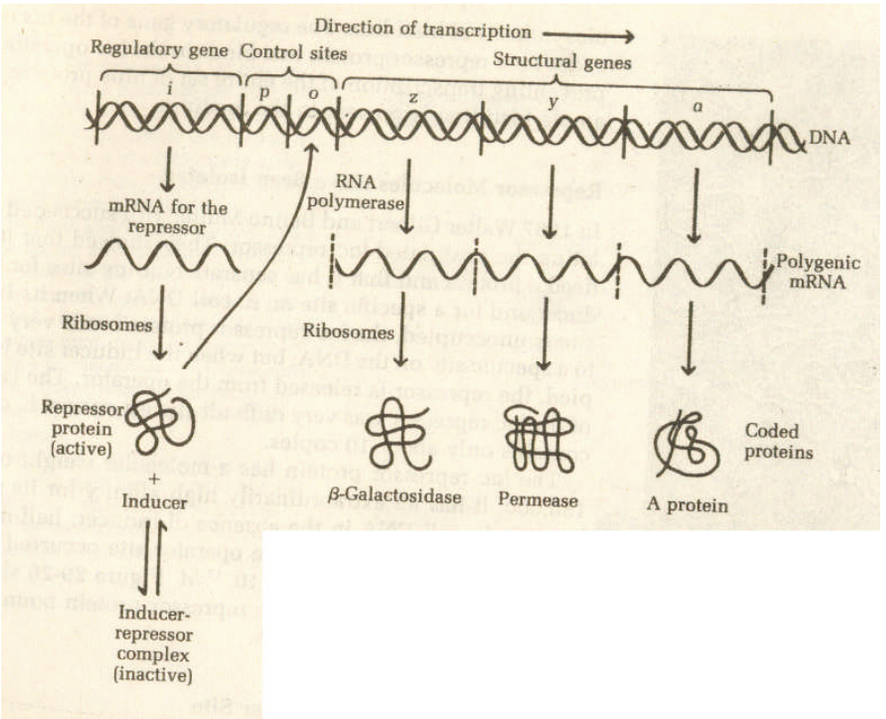


Fig. 12.3. Schematic diagram of the lac operon .

The three lac structural genes *z*, *y* and *a* are adjacent to each other. They are preceded by two control sites, *p* for promoter and *o* for operator. The *p* and *o* regions are very short compared with the other genes. A regulatory gene *i* codes for the repressor protein. The repressor protein has two binding sites, one for the operator and the other for the inducer. The active form of the repressor protein can bind to the operator thus preventing the binding of RNA polymerase and subsequent transcription of the structural genes *z, y* and *a*. Under these conditions, β-galactosidase and the other two proteins are not made by the cells. When lactose is available and glucose is lacking, the inducer binds to the repressor, converting into an inactive form that will not bind to the operator. In this case RNA polymerase can bind to the *p* site, move through the *o* site and begin to transcribe the three structural genes into polygenic mRNA, which codes for the synthesis of the three lac proteins on the ribosome. Lactose itself is not an inducer of the lac operon, but is converted into its isomer allolactose, the actual inducer.

The *lac* operon ia an example of a negative control mechanism. The operon itself is ready to be transcribed, but transcription is prevented by a specific repressor. This repressor has to be removed by the inducer. The operon has to combine with an activator and the inducer to transcribe. This is called positive control.

In another example, the metabolism of L-arabinose involves the expression of six genes. Genes *ara A, B, C* and *D* are located on the bacterial chromosome, genes *ara E* and *F* are located on DNA at far off from *A* to *D*. *Ara E* and *ara F* code for proteins that are involved in active transport of L-arabinose into the cell and genes *ara A, B* and *D* enzymes catalyze the conversion of L-arabinose into D-glucose-5-phosphate. All these five genes are called *regulon*. The *ara C* gene product is the regulator protein (Fig.12.4).

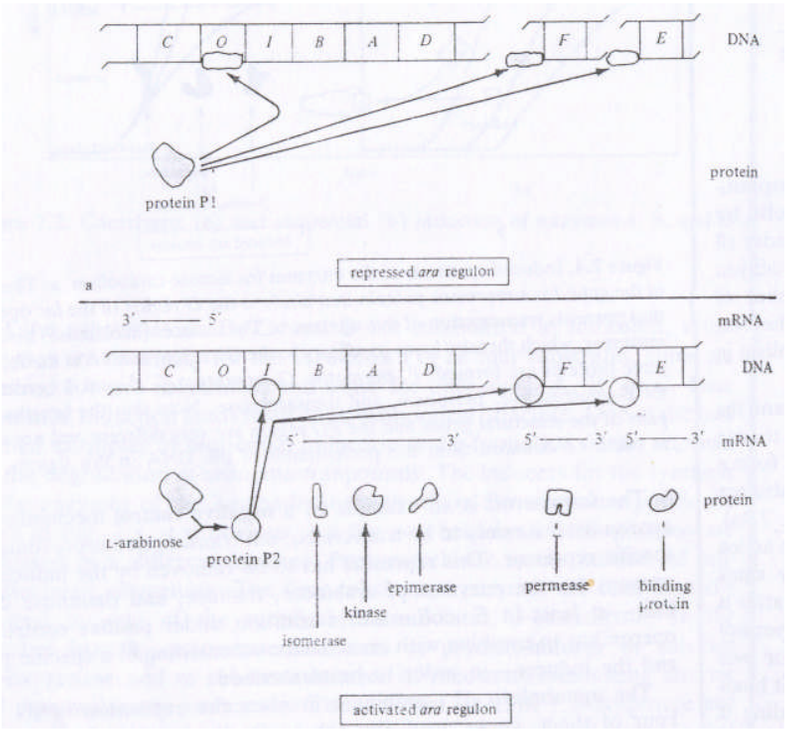


Fig. 12.4. Induced synthesis of the enzymes for L-arabinose catabolism

The gene *ara C* is separated from the *ara A B D* operon. In absence of L-arabisone, *ara C* is transcribed and P1 protein is formed. This protein binds at the *ara o* region and the transcription of *ara A B D E F* is prevented. Protein P1 here functions as repressor like the *lac* repressor. Engelsberg concluded that a modified gene product protein P2, functions as an activator for the transcription of the *ara B A D* and *E F* genes. So the *ara* genes are under positive control of the protein P2.

12.2.2. Catabolite repression

In 1942 Monod discovered the phenomenon of *diauxie*. A culture of ***Bacillus subtilis*** supplemented with glucose and arabinoise as carbon and energy sources, utilize glucose first and then arabinose. This resulted a biphasic growth curve (Fig. 12.5).

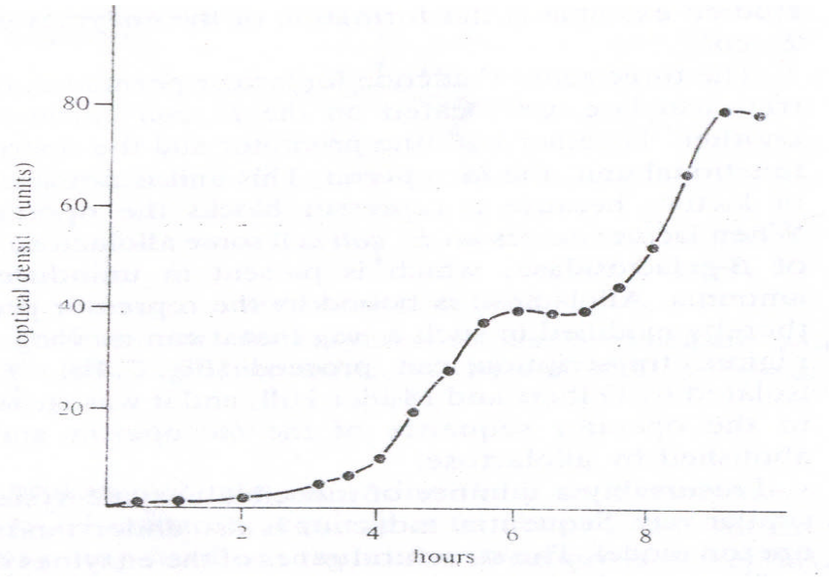


Fig. 12.5. Diauxic growth of ***B. subtilis*** on glucose and arabinose.

E. coli confronted with a mixture of glucose and lactose in its growth medium, will grow first with glucose. The three structural genes of the *lac* operon are not expressed during this period. Expression begins when the glucose concentration becomes low.

When glucose is absent and lactose is present in the medium, the inducer binds to the repressor, releasing the repressor from the operator. This allows transcription of the *lac* genes and subsequent synthesis of the *lac* proteins. Suppose both glucose and lactose are present in the medium, ***E. coli*** uses only glucose and ignore lactose. Cells no longer make the *lac* proteins. The repression of the *lac* proteins by glucose is called catabolite repression.

In addition to the gene *i* and *o* site there is another control site, the promoter or *p* site. This lies between the *i* and *o* sites. The promoter contains two specific regions. Next to the operator is the RNA polymerase entry site to which RNA polymerase first bound. The other portion is the specific binding site for another regulatory protein, the catabolite activator protein (CAP) (Fig. 12.6).

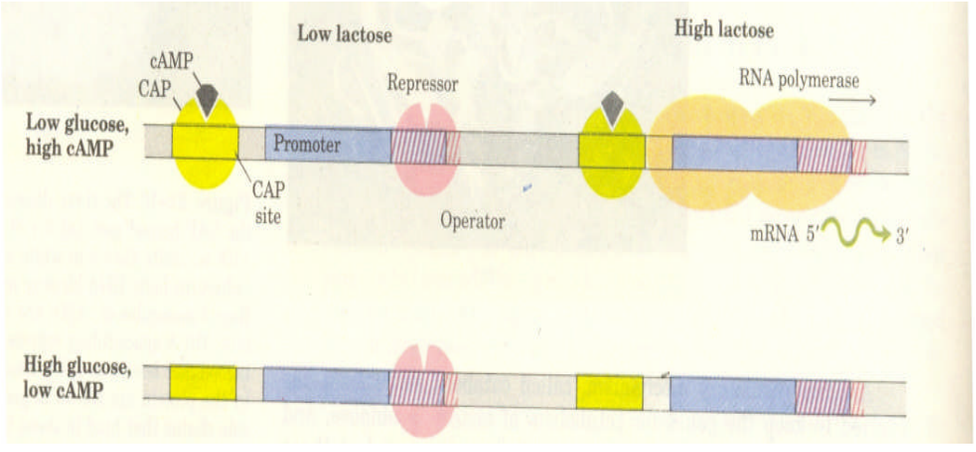


Fig. 12.6. Catabolic repression of ***E.coli.***

The CAP site controls the polymerase site. When glucose is lacking CAP complexes with cyclic AMP (cAMP). It is bound to CAP site and allows the entry site to bind RNA polymerase moves from its entry site through the operator and begins to transcribe the three *lac* genes. When ample glucose is available the concentration of cAMP is very low and the CAP-cyclic AMP complex can not form. Since the CAP site can make the entry site available to RNA polymerase only if it binds the CAP-cyclic AMP complex, RNA polymerase can not enter and the *lac* genes can not be transcribed. When glucose is unavailable, transcrtiption of the three *lac* genes is possible.

The CAP has two binding sites. One for the CAP site in the promoter DNA and the other for cyclic AMP. In ***E. coli***, cyclic AMP serves as messenger. It signals whether glucose is available as fuel or not. Cells contain adenylate cyclase, which can make cyclic AMP from ATP. They also contain a phosphodieserase. This enzyme hydrolyzes cyclic AMP and inactivate it. When glucose concentration is high, the concentration of cyclic AMP in the cell is very low. When glucose concentration is low, the cyclic AMP level increases, due to increased adenylate cyclase activity and decreased phosphodiesterase activity. The formed cyclic AMP binds to the CAP protein and the CAP-cyclic AMP complex in turn binds to the imitation site and starts transcribing the *lac* genes into mRNA. Cyclic AMP in bacteria thus called as “hunger signal”.

12.2.3. End product repression and attenuation

When ***E. coli*** grows in a minimal medium with glucose as carbon and energy source, all the monomers for the formation of macromolecules have to be synthesized. Amino acids and nucleotides are needed in correct amounts for polymer synthesis. This stops the overproduction of monomers. If histidine or tryptophane can be taken up, the biosynthesis of these compounds is superfluous. This way organisms adjust the level of anabolic enzymes. This adjustment is achieved by end product repression and/or attenuation.

For an understanding of this kind of regulation, the nine genes responsible for the enzymes of histidine biosynthesis in ***Salmonella typhimurium*** represent on operon. The five genes coding for the enzymes that catalyze the synthesis of tryptophan also form one operon. When the product of a pathway accumulates in cells, it combines with a repressor protein to give an active repressor. The repressor binds to the operator and prevents transcrtiption of operon. (Fig. 12.7).

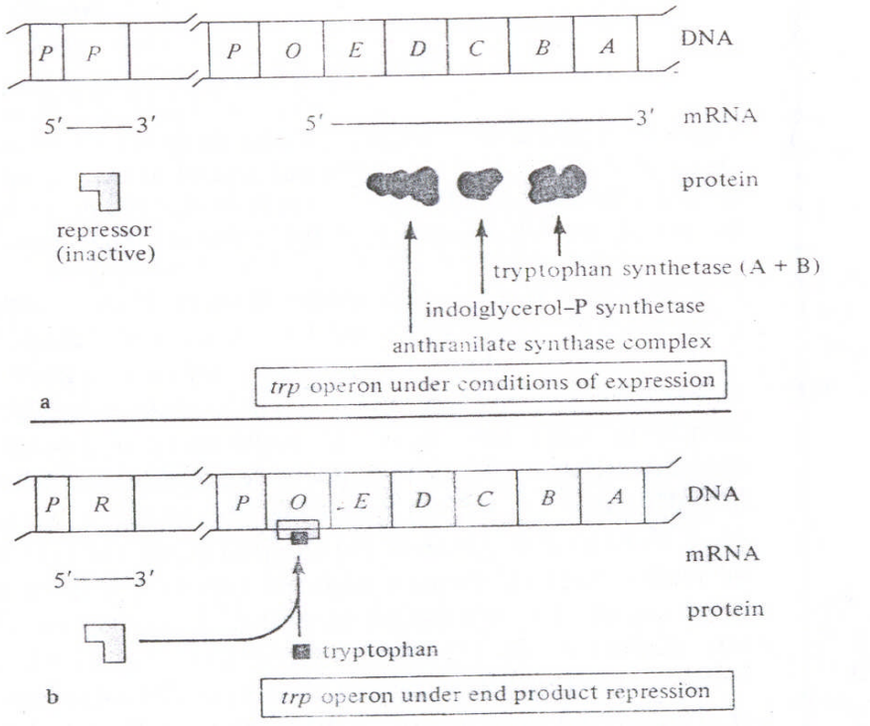


Fig. 12.7. The tryptophan operon and the mechanism of end product repression.

End product repression or attenuation stops enzyme synthesis with in a short time because of the short half-life of mRNA. In ***E. coli*** the genes for the enzymes involved in arginine biosynthsis are located at different positions on the chromosome. All of them are switched off by the same repressor-arginine complex.

In branched anabolic pathways, enzyme regulation is more difficult. If one end product would completely repress the formation of enzymes, that also serves in the synthesis of other monomers. This could result in a shortage of these compounds and a stoppage of growth. Two principles are applied to avoid the difficulties:

1) Isoenzymes are formed for common reactions, so that each end product has its enzyme whose synthesis can be regulated.

2) Repression of enzyme synthesis requires that all products synthesized by these enzymes are present in excess. This kind of regulation is called as multivalent attenuation.

Another branched pathway is the synthesis of L-isoleusine, L-valine and L-leucine. The four steps involved in L-valine and L-isoleucine synthesis is catalyzed by the same enzyme. The starting material for L-leucine and the precursor of L-valine is α-oxo-β-methylbutyrate. A trivalent attneuation mechanism is present in order to prevent a shortage of one of the three amino acids. L-leucine alone attenuates the synthesis of the enzymes leading from α-oxo-β-methylbutyrate to L-leucine (Fig. 12.8).

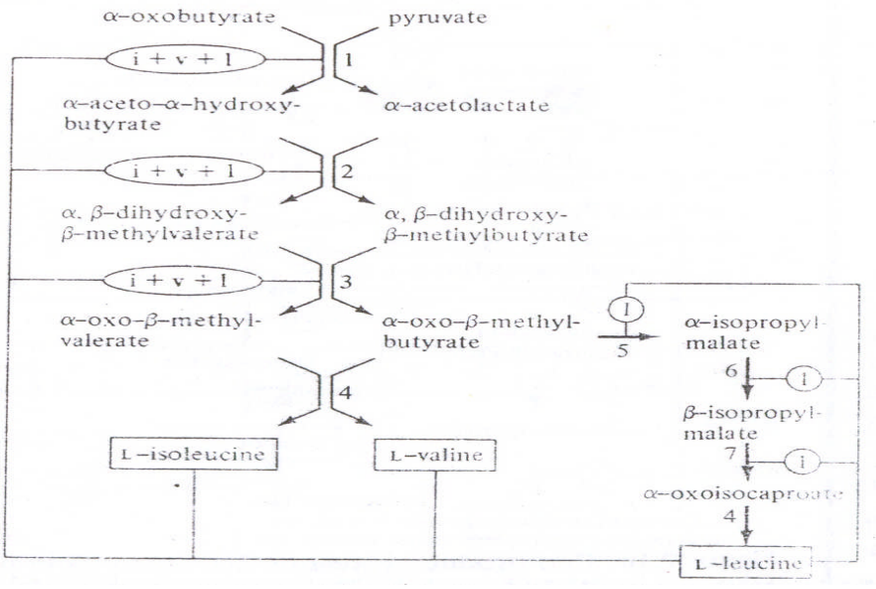
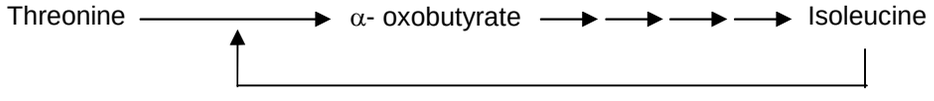


Fig. 12.8. Regulation of synthesis of the enzymes involved in L-isoleucine, L-valine and L-leucine formation in ***Salmonella typhimurium***.

12.3. REGULATION OF ENZYME ACTIVITY

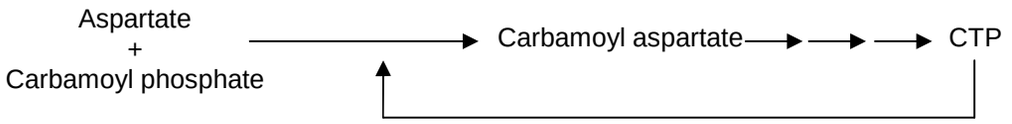
12.3.1. Feedback inhibition

If a monomer is synthesized in larger amounts than is needed for polymer synthesis, it is necessary to stop the synthesis of the enzymes involved and to reduce the synthesis of that monomer. This is known as feedback inhibition. If the end product accumulates in the cell, it inhibits the activity of the first enzyme involved in its formation. For example:



If isoleucine is synthesized in excess, it inhibits the activity of the first enzyme threonine deaminase, of its biosynthetic pathway.

If pyramidine nucleotides accumulate in the cell, CTP inhibits the activity of aspartate transcarbamoylase of their biosynthetic pathway.



When threonine deaminase and aspartate transcarbamoylase are inhibited, the enzymes of these pathways run out of substrates and monomer synthesis stops. This shows that the substrate flows through most biosynthetic pathways is regulated by feedback inhibition.

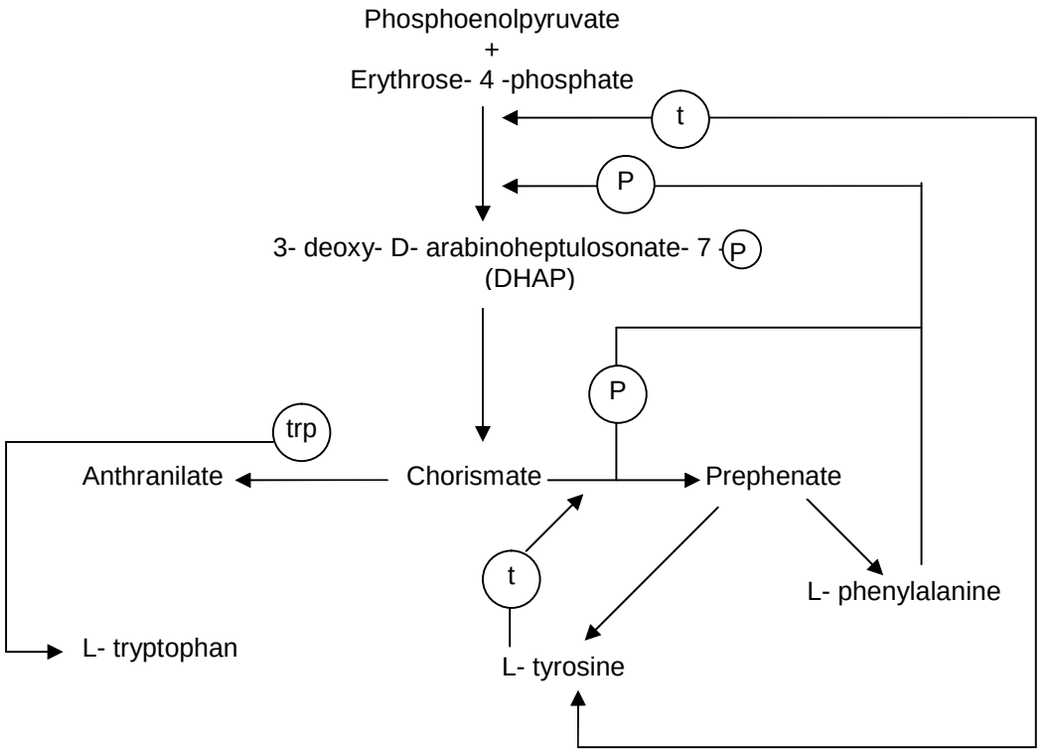


Fig. 12.9. Feedback inhibition in the aromatic amino acid pathway of ***E. coli***. L-Phenylalanine inhibits DAHP synthase I and chorismate mutase I; L-tyrosine inhibits DAHP synthase II and chorismate mutase II; L-tryptophan inhibits anthranilate synthase.

This figure shows the control of enzyme activity in the aromatic amino acid pathway in ***E. coli*** (Fig. 12.9). One DAHP synthase is inhibited by L-phenylalanine and the second one by L-tyrosine. Tryptophan inhibits anthranilate synthase. The two chorismate mutases are also under the control of corresponding end products. This type of inhibition is seen in enterobacteria where the three DAHP synthases are present, where as in ***Bacillus*** species, there is only one synthsae present. It is inhibited by the intermediates chorismate or prephenate. This type of control of activity is called sequential feedback inhibition.

Another type of inhibition called cumulative inhibition in ***Alcaligenes eutrophus***, which is hydrogen-oxidizing bacteria. The DAHP synthase is inhibited by phenylalanine and tyrosine (Table 12.1.)

Table 12.1:- Regulation of DAHP synthase activity of ***Alcaligenes eutrophus*** by cumulative inhibition.

Aminoacid present in the DAHP synthase assay mixture	Inhibition(%)	Residual activity (100%= 1)	
		Observed	Calculated
Phenylalanine Tyrosine Mixture	25 47.5 58.2	0.75 0.525 0.418	0.75 × 0.525 0.394

The above table gives the percent inhibition observed with phenylalanine, tyrosine and with the mixture of these amino acids. The residual activity observed in presence of the mixture is equal to the product of the residual activities observed with phenylalanine and tyrosine. This is characteristic for cumulative inhibition.

From the above examples, it is evident that the inhibition differs in its chemical structure from the substrates of the enzyme it acts upon. Carbamoyl phosphate and aspartate are the substrates of aspartate transcarbamoylase. These are different from the inhibitor CTP. Since the inhibitors are analogues of the substrates, enzymes must contain special binding sites for them. Monod, Changeux and Jacob coined the term allosteric sites for these areas on the enzymes. Compounds that are bound at these sites and that alter the activity of the enzymes are called allosteric effectors and the enzymes are called allosteric enzymes.

12.3.2. Allosteric enzymes

Enzymes have cleft or crevices called active sites in which the substrate molecules are bound. In the active site substrate fits in like a key into a lock. This is not true for all enzymes. These enzymes are oligomers and contain 2,4,6 or more subunits which may be identical or not.

Allosteric control of central pathways

The main objective of catabolic and central pathways are to provide energy to the cell and starting material for biosynthesis. So it is reasonable that the regulatory signals used here for control are ultimate products of the energy metabolism and central precursors of the biosynthetic metabolism (Table 12.2.)

Table 12.2:- Allosteric enzymes involved in central pathways of ***E. coli***

Enzyme	Inhibitor	Activity
ADP- glucose pyrophosphorylase	AMP	Pyruvate, F-6-P, F-P_2
Fructose bisphosphotase	AMP	
Phosphofructokinase	PEP	ADP, GDP
Pyruvate kinase		F-P_2
Pyruvate dehydrogenase	NADH, acetyl- CoA	PEP, AMP, GDP
PEP carboxylase	Aspartate, malate	acetyl- CoA, F-P_2, GTP, CDP
Citrate synthase	NADH, α-oxoglutarate	
Malate dehydrogenase	NADH	

An increase of the NADH concentration in the cell signals that the respiratory chain is saturated with NADH and TCA cycle may slow down. Citrate synthase, malate dehydrogenase and pyruvate dehydrogenase complex are inhibited by NADH. Citrate synthase is inhibited by α-oxoglutarate and the pyruvate dehydrogenase complex by acetyl-CoA.

In all bacteria citrate synthases are not inhibited by NADH. NADH inhibitable type of enzyme is formed in Gram-negative bacteria, the synthase enzyme is inhibited by ATP in Gram-positive bacteria like eukaryotic organisms. In eubacteria, α-oxoglutarate functions as inhibitor.

PEP carboxylase is inhibited by aspartate and malate. High levels of aspartate and malate signals that C_4-dicarboxylic acids need to be synthesized. Increased acetyl-CoA concentration indicates a shortage of C_4-dicarboxylic acids. Thus acetyl-CoA is an activator of PEP carboxylase. In most cases, PEP carboxylase is replaced by pyruvate carboxylase, which is also activated by acetyl CoA (Fig. 12.10).

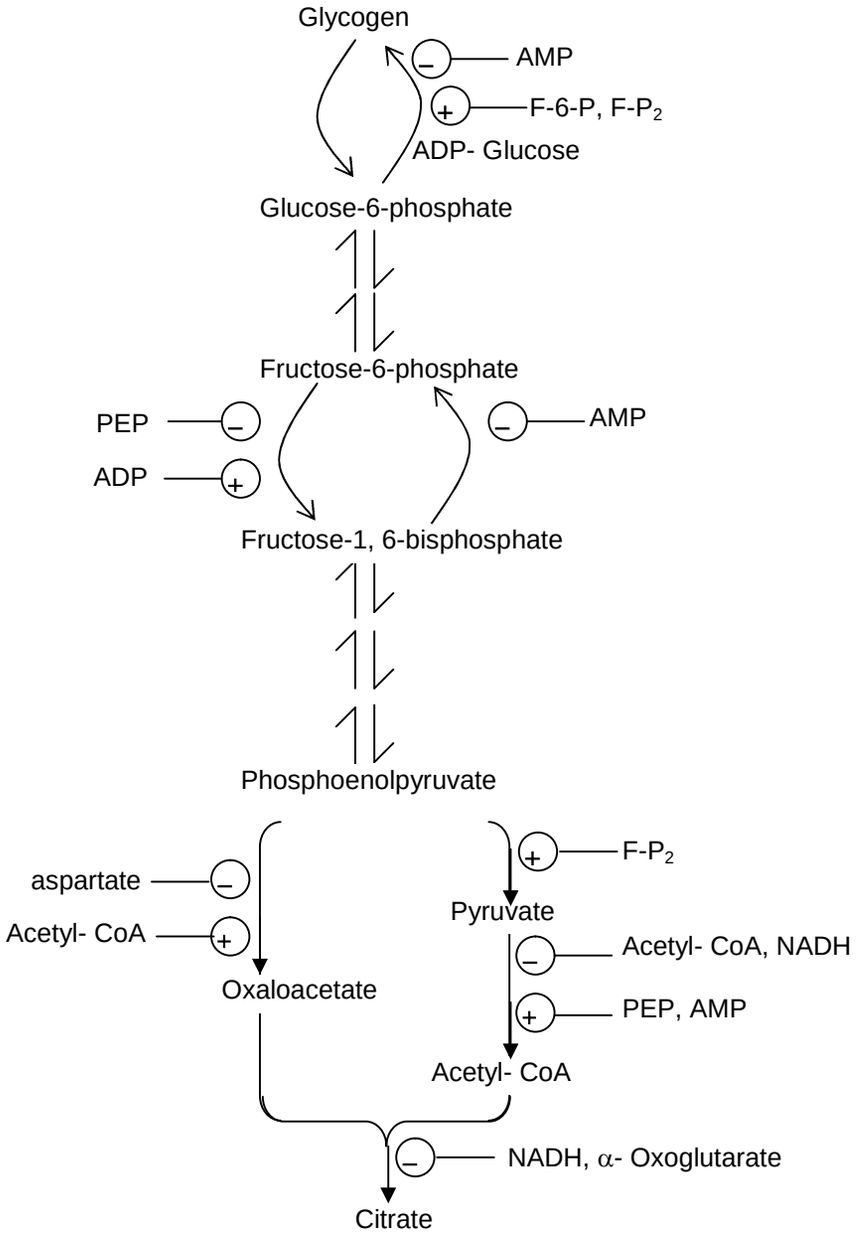


Fig.12.10.: - Schematic representation of glycolytic and glycogenic reactions in ***E.coli*** and their control. $F\text{-}P_2$ – Fructose 1,6- bisphosphate, ⊖=inhibitor, ⊕ =activater.

Fructose-1,6-bisphosphate is a stratagic branch point of glycolysis and of glycogen formation and its intracellular level is under regulatory control. An increased concentration signals ATP deficiency. This results in an inhibition of glycogen formation, because ADP-glucose pyrophosphorylase and fructose-bisphosphatase are inhibited by AMP. Carbohydrates in excess lead to an increase of the fructose-1,6-bisphophate level. This has a positive effect on glycolysis, as both pyruvate kinase and PEP carboxylase are activated by fructose-1,6-bisphosphate. A sufficient supply of ATP in the cell is singalled by an elevated PEP level. As a result phosphofructokinase is inhibited and glycogen formation is favoured by activation of ADP-glucose pyrophosphorylase.

In the above figure it can be seen that the target enzymes for control of reaction sequences catalyze reactions that are irreversible under physiological conditions. Phosphofructokinase, pyruvate kinase function as pacemakers and regulation is very efficient at these points. Antagonistic enzymes occur in the cells simultaneously, for example, phosphofructokinase and fructose-1,6-bisphosphate. These enzymes have to be under stringent control. Otherwise futile cycles would be established. This causes the hydrolysis of ATP as net reaction. Fructose-6-phosphate is phosphorylated by phosphofructokinase and the bisphosphate is hydrolyzed again by the phosphatase. A futile cycle could also be established with glucose-1-phosphate, ADP-glucose and glycogen as participating metabolites.

For maintaining constant energy status, organisms employ another regulation. AMP is formed in many biosynthetic reactions from ATP and so is ADP. Any increase in the concentration of these nucleotides leads to a stimulation of ATP-yielding reactions. By regulation of ATP producing and consuming reactions, organisms try to maintain a constant energy status. According to Atkinson this energy status can be described by the

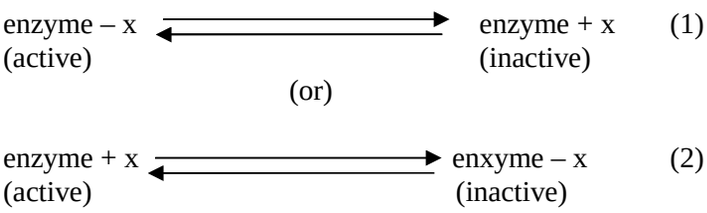
energy charge (ec),

$$ec = \frac{[ATP] - 1/2\,[ADP]}{[ATP] + [ADP] + [AMP]}$$

Systems containing only ATP have an energy charge of 1 and for those containing only AMP the ec is zero. The energy charge of growing organisms is about 0.8. ***E.coli*** cell dies at ec values below 0.5.

12.3.3. Covalent modification of enzymes

In this type of regulation, the enzyme is covalently modified in an enzyme-catalyzed reaction.



Equation one describes enzyme systems that are active only when substituted with x. When x is removed the enzyme systems are inactive. In second equation, enzymes are also known that are inactivated by substitution.

The entry of glucose residues into the glycolytic sequence is regulated. The entry of glucose residues into glycolysis is from glycogen through the action of glycogen phosphorylase. This is a regulatory enzyme, which occurs in two forms. Phophorylase a, catalytically active, phosphorylated form and phosphorylase b, less active and dephosphorylated form. The interconversion of active and inactive forms of glycogen phosphorylase is brought about by specific enzymes.

Phosphorylase a is converted into less active phosphorylase b by an enzyme called phosphorylase a phosphatase, which removes the phosphate group from phosphorylase a by hydrolysis (Fig. 12.11).

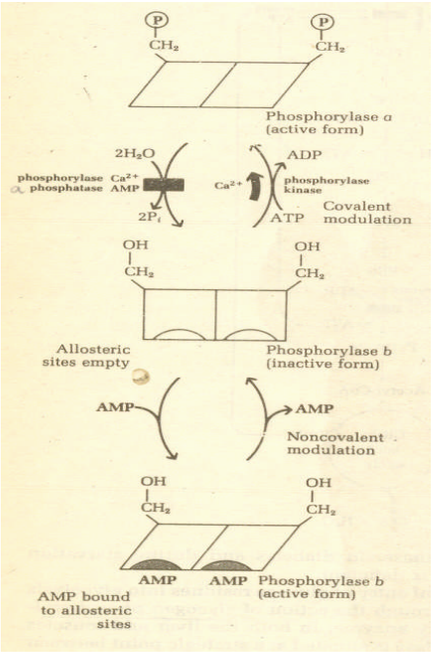


Fig. 12.11. Regulation of glycogen phosphorylase.

Phosphorylase b can be converted back into active phosphorylase a by the enzyme phosphorylase b kinase, which promotes a reaction in which ATP phosphorylates the serine residues of phosphorylase b to yield phosphorylase a.

Glycogen phosphorylase is regulated in a second way. Phosphorylase b, an inactive form, can be stimulated by non covalent binding of its allosteric modulator AMP (Fig.12.11). The stimulation of phosphorylase b by AMP is prevented by ATP, a negative modulator, but phosphorylase a is not stimulated by AMP. Phosphorylase a is sometimes referred to as the AMP-independent form and phosphorylase b as the AMP-dependent form. Glycogen phosphorylase is regulated by two different mechanisms:

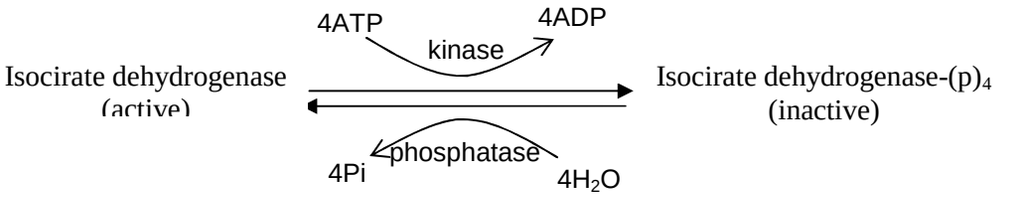
(a) Covalent modification through phosphorylation and dephosphorylation of its serine hydroxyl groups

(b) Allosteric regulation of phosphorylase b by the non covalent binding of AMP or ATP.

It is important to note that glycogen phosphorylase of prokaryotes is not regulated by covalent modification. These enzyme systems modified by phosphorylation/dephosphorylation are generally more common among eukaryotes.

A bacterial enzyme system regulated by phosphorylation/dephosphorylation are generally more common among eukaryotes. A bacterial enzyme system regulated by phosphorylation/dephosphorylation is the $NADP^+$ - isocitrate dehydrogenase of ***E. coli*** and ***Salmonella typhimurium***.

It was observed that addition of acetate to a culture containing limited glucose resulted in rapid inactivation of isocitrate dehydrogenase. This inactivation is due to the phosphorylation of the enzyme. One serine residue per subunit is phosphorylated, this brings about the inactivation of the enzyme:



The isocitrate dehydrogenase kinase and the isocitrate dehydrogenase phosphatase are activities that are associated with the same protein. Isocitrate is a branch point between the tricarboxylic acid cycle and the glyoxylate cycle. Inactivation of isocitrate dehydrogenase brings the glyoxylate cycle more into play and favours oxaloacetate and PEP formation.

Two other interesting systems of covalent modification have been observed in bacteria are glutamine synthetase of ***E.coli*** and in other organisms regulated by adenylation/deadenylation and citrate lyase of ***Rhodopseudomonas gelatinosa*** is regulated by acetylation/deacetylation.

Allosteric inhibition of glutamine synthetase is well studied in ***E. coli***. Glutamine is the amino-group donor in the biosynthesis of many metabolic products (Fig. 12.12).

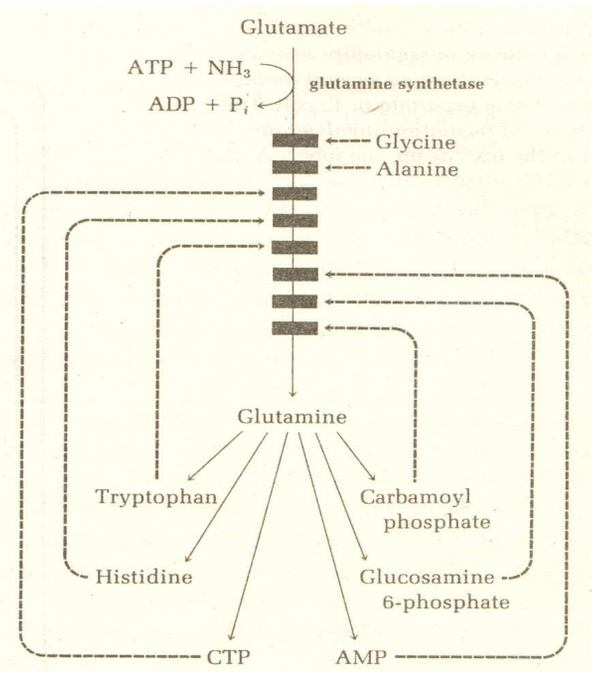


Fig. 12.12. Allosteric inhibition of glutamine synthatase in ***E.coli.***

In this organism glutamine is a precursor of the eight products shown. All of these can act as feedback inhibitors or negative feedback modulators of the activity of glutamine synthetase, one of the complex enzymes known. Such inhibition by several negative modulators is called concerted inhibition. Glutamine synthetase is also strongly inhibited by excess ATP, which converts it into an inactive form by a covalent modification of essential tyrosine groups in its subunits.

Citrate lyase in a phototrophic bacterium, ***Rhopodopseudomonas gelatinosa*** is regulated by acetylation/deacetylation (Fig.12.13).

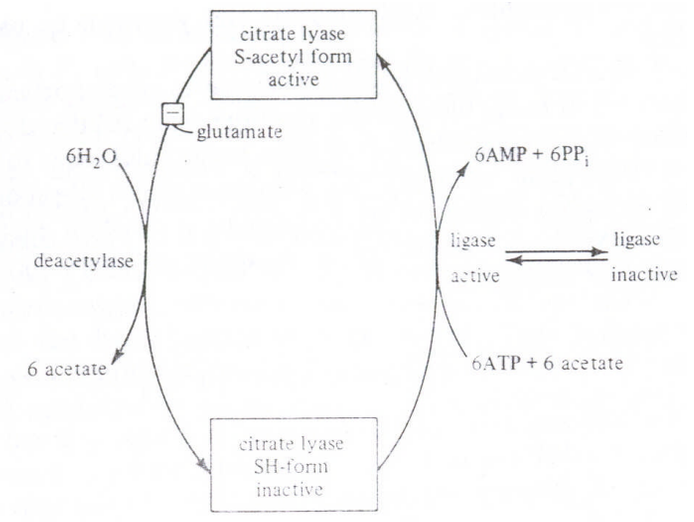


Fig. 12.13. Regulation of citrate lyase of ***Rhodopseudomonas gelatinosa*** by acetylation and deacetylation.

Citrate lyase cleaves citrate to oxaloacetate and acetate. Active enzyme is present if citrate is available to the cells as substrate. At this time, the deacetylating enzyme is inhibited by L-glutamate for which citrate serves as precursor. In absence of citrate, the glutamate concentration decreases and citrate lyase is inactivated by deacetylase. If citrate is available, the active enzyme is formed by acetylation. In citrate lyase system, the acetyl group participates in the enzymatic reaction.

Acetyl-s-enzyme + citrate $\rightleftharpoons$ citryl-s-enzyme + acetate

Citryl-s-enzyme $\rightleftharpoons$ acetyl-s-enzyme + oxaloacetate

The acetyl group participates in the initial transferase reaction where, HS-enzyme must be inactive. In lactic acid bacteria and clostridia, enzyme activity is regulated by dissocation/association. Lactate dehydrogenase is inactive as a dimer. In presence of fructose-1,6-bisphosphate, the dimers associate to enzymatically acive tetramers. Lactate is produced only if there is a build-up of fructose-1,6-bisphosphate in the organisms.

Enzyme regulation in chloroplast was described by Buchanan and coworkers. Certain enzymes are activated in the light and inactivated in the dark by oxidation. The light-driven electron transport to ferridoxin leads to an increase of the reduced ferredoxin: oxidized ferredoxin ratio. Under catalysis of ferridoxin-thioredoxin reductase this affects the level of reduced thioredoxin. This is required for the activation of certain enzymes like fructose-1,6-bisphosphate of chloroplasts.

12.4. SUMMARY

- The process of substrate-mediated enzyme synthesis is called enzyme induction. Depending on the involvement of inducers, ther are coordinate induction, where all enzymes are under the control of one inducer and sequential induction where several inducers are involved.
- The genes of inducible enzymes are located on the bacterial genome adjacent to one another. When operator and promotor are there, they form a regulatory unit, the operon. The transcription of operons under negative control is repressed by a repressor protein and the inducer prevents binding of the repressor (eg *lac* operon). Transcription of operons under positive control requires binding of activator protein at the DNA.
- Some readily utilizable substrates prevent the induction of enzymes of other catabolic pathways is called catabolite repression. When these substrates are transported to the cells, there is a decrease in the intracellular level of cyclic AMP. Cyclic AMPs are required for the initiation of RNA synthesis at operons that code for inducible pathways.
- The level of enzymes of anabolic pathways is regulated by end product repression or attenuation. An end product combines with a specific aporepressor to yield anactive repressor.
- In feedback inhibition, the end product of an anabolic pathway decreases the activity of the first enzyme that involved in its formation. This allows the adjustment of the rate of product synthesis to the cellular demands.
- The enzyme for feedback inhibition are allosteric enzymes. Besides their active sites, allosteric enzymes contain specific binding sites for their inhibitors or activators.
- Regulatory signals used for the control of the activity of central pathways are products of energy metabolism (AMP, ADP, ATP, NADH) and important precursors of biosynthetic metabolism (PEP, acetyl-CoA, aspartate). The enzymes under allosteric control function as pacemakers in central pathways.
- The energy status is explained by energy charge.
  ec = [ATP] + ½ [ADP]/[ATP] + [ADP] + [AMP]
- Some key enzymes are regulated by covalent modification. These enzymes may exist as an active or inactive forms. Interconversion is achieved by phosphorylation/dephosphorylation, adenylation/deadenylation and acetylation/deacetylation. Other regulatory mechanisms are association/dissociation of proteins and oxidation/reduction by thioredoxin.

12.5. MODEL QUESTIONS

1. Write an essay on Enzyme regulation by induction and repression
2. Write short notes on feedback inhibition , allosteric enzymes.
3. Discuss about regulation of enzyme activity by covalent modification of enzymes.
4. Write briefly about feedback inhibition, allosteric enzymes and covalent modification of enzymes.

12.6. REFERENCE BOOKS

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2. Bacterial metabolism 2nd ed., Gerhard Gottschalk Springer-Verlag.

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