Uttar Pradesh Rajarshi Tandon
Open University

UGBCH-103

Intermediary Metabolism

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BLOCK

1

BIOENERGETICS AND THERMODYNAMICS

Course Design Committee

Prof. K.N. Singh — **Vice Chancellor**
Uttar Pradesh Rajarshi Tandon Open University

Dr. (Prof.) Ashutosh Gupta, — **Chairman**
School of Science, UPRTOU, Prayagraj

Prof. Prof. Umesh Nath Tripathi — **Member**
Department of chemistry
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. S.I. Rizvi — **Member**
Department of Biochemistry, University of Allahabad, Prayagraj

Prof. Denish Yadav — **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. Sharad Kumar Mishra — **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Dr. Ravindra Pratap Singh — **Member**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh — **Course Coordinator**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Course Preparation Committee

Dr. Ajay Yadav — **Writer** — (Unit: 1,2 & 3)
Department of Zoology, Dr. B.R. Ambedkar
Gov. Girls P.G. College. Fatehpur. U.P.

Dr. Sushma Chauhan — **Author** — Unit: 4 & 9
Academic Consultant (Botany)
School of Sciences, UPRTOU, Prayagraj

Dr. Ravindra Pratap Singh — **Author** — Unit: 5
Academic Consultant (Biochemistry)
School of Sciences, UPRTOU, Prayagraj

Dr. Dharmveer Singh — **Author** — Unit: 6,7 &8
Academic Consultant (Biochemistry)

Dr. Ayodhya Prasad Verma — **Editor** — (All blocks and units)
Associate Professor,
Department of Botany
Bappa Sri Narain Vocational Post Graduate College, Lucknow

Dr. Dharmveer Singh — **(Course Coordinator)**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

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Introduction

This the first block of intermediary metabolism. It consists of following three units:

Unit-1 : Bioenergetics is a field in biochemistry and cell biology that concerns energy flow through living systems. This is an active area of biological research that includes the study of the transformation of energy in living organisms and the study of thousands of different cellular processes such as cellular respiration, Other metabolic and enzymatic processes that lead to production and utilization of energy in forms such as adenosine triphosphate (ATP) molecules. The goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics.

Unit-2 : In thermodynamics, interactions between large ensembles of objects are studied and categorized. Central to this are the concepts of the thermodynamic system and its surroundings. A system is composed of particles, whose average motions define its properties, and those properties are in turn related to one another through equations of state. Properties can be combined to express internal energy and thermodynamic potentials, Which are useful for determining conditions for equilibrium and spontaneous processes.

Unit-3 : Adenosine triphosphate (ATP) is a complex organic chemical that provides energy to drive many processes in living cells, e.g. muscle contraction, nerve impulse propagation, and chemical synthesis. Found in all forms of life, ATP is often referred to as the "molecular unit of currency" of intracellular energy transfer. When consumed in metabolic processes, it converts either to adenosine diphosphate (ADP) or to adenosine monophosphate (AMP). Other processes regenerate ATP so that the human body recycles its own body weight equivalent in ATP each day. It is also a precursor to DNA and RNA, used as a coenzyme.

UNIT : 1

BIOENERGETICS

Structure

1.1. Introduction

Living cells and organisms must perform work to stay alive, to grow, and to reproduce. The ability to harness energy and to channel it into biological work is a fundamental property of all living organisms; it must have been acquired very early in cellular evolution. Modern organisms carry out a remarkable variety of energy transductions, conversions of one form of energy to another. They use the chemical energy in fuels to bring about the synthesis of complex, highly ordered macromolecules from simple precursors. They also convert the chemical energy of fuels into concentration gradients, electrical gradients, into motion, heat and in a few organisms such as fireflies and deep-sea fish, into light. Photosynthetic organism transducer light energy into all these other forms of energy. Biological energy transductions obey the same chemical and physical laws that govern all other natural processes. It is therefore essential for a student of biochemistry to understand these laws and how they apply to the flow of energy in the biosphere.

Objectives

- To explain what is meant by the terms free energy, entropy, enthalpy, exergonic, and endergonic.
- To explain about activation energy and transition state

- To know about photochemical reaction in photosynthesis
- To know about chemical energy of organic substance

1.2. Bioenergetics overview

Bioenergetics is the quantitative study of energy transductions—changes of one form of energy into another—that occur in living cells, and of the nature and function of the chemical processes underlying these transductions. Although many of the principles of thermodynamics have been introduced in earlier chapters and may be familiar to you, a review of the quantitative aspects of these principles is useful here.

Thermodynamics : Knowledge of thermodynamics, which is the description of the relationships among the various forms of energy and how energy affects matter, enables one to determine whether a physical process is possible. The first and second laws of thermodynamics are combined in the thermodynamic function, free energy (*G*). If the change in free energy (ΔG) of a reaction is negative, that reaction can occur spontaneously. If ΔG is positive, an input of energy is required to drive the reaction. The unit of energy is the Joule (J) or the calorie (cal).

Free energy change : The difference in energy level between the substrates and products is termed the change in Gibbs free energy (ΔG). A negative ΔG indicates that the reaction is thermodynamically favourable in the direction indicated, whereas a positive ΔG indicates that the reaction is not thermodynamically favourable and requires an input of energy to proceed in the direction indicated. An energetically unfavourable reaction is often driven by linking it to an energetically favourable reaction, such as the hydrolysis of ATP.

Chemical Equilibrium : A chemical reaction often exists in a state of dynamic equilibrium. The equilibrium constant (*K*) defines the ratio of the concentrations of substrates and products at equilibrium. Enzymes do not alter the equilibrium position, but do accelerate the attainment of the equilibrium position by speeding up the forward and reverse reactions.

1.3. Photosynthesis:

Harvesting light energy

Photosynthesis is the reaction sequence in which the flow of electrons is coupled to the synthesis of ATP: light-driven phosphorylation. The capture of solar energy by photosynthetic organisms and it's conversion to the chemical energy of reduced organic compounds is the ultimate source of nearly all biological energy on Earth. Photosynthetic and heterotrophic organisms live in a balanced steady state in the biosphere. Photosynthetic organisms trap solar energy and form ATP and NADPH, which they use as energy sources to make carbohydrates and

other organic compounds from CO_2 and H_2O; simultaneously, they release O_2 into the atmosphere. Aerobic heterotrophs (humans, for example, as well as plants during dark periods) use the O_2 so formed to degrade the energy-rich organic products of photosynthesis to CO_2 and H_2O, generating ATP. The CO_2 returns to the atmosphere, to be used again by photosynthetic organisms. Solar energy thus provides the driving force for the continuous cycling of CO_2 and O_2 through the biosphere and provides the reduced substrates—fuels, such as glucose—on which nonphotosynthetic organisms depend. Photosynthesis occurs in a variety of bacteria and in unicellular eukaryotes (algae) as well as in plants. Although the process in these organisms differs in detail, the underlying mechanisms are remarkably similar, and much of our understanding of photosynthesis in vascular plants is derived from studies of simpler organisms.

The overall equation for photosynthesis in plants describes an oxidation-reduction reaction in which H_2O donates electrons (as hydrogen) for the reduction of CO_2 to carbohydrate (CH_2O):

$$CO_2 + H_2O \quad \text{light} \rightarrow \quad O_2 + (CH_2O)$$

General Features of Photophosphorylation

Unlike NADH (the major electron donor in oxidative phosphorylation), H_2O is a poor donor of electrons; its standard reduction potential is 0.816 V, compared with 20.320 V for NADH. Photophosphorylation differs from oxidative phosphorylation in requiring the input of energy in the form of light to *create* a good electron donor and a good electron acceptor. In photophosphorylation, electrons flow through a series of membrane-bound carriers including cytochromes, quinones, and iron-sulfur proteins, while protons are pumped across a membrane to create an electrochemical potential. Electron transfer and proton pumping are catalyzed by membrane complexes homologous in structure and function to Complex III of mitochondria. The electrochemical potential they produce is the driving force for ATP synthesis from ADP and Pi, catalyzed by a membrane-bound ATP synthase complex closely similar to that of mitochondria and bacteria. Photosynthesis in plants encompasses two processes: the **light-dependent reactions**, or **light reactions**, which occurs only when plants are illuminated, and the **carbon-assimilation reactions** (or **carbon fixation reactions**), sometimes misleadingly called the dark reactions, which are driven by products of the light reactions. In the light reactions, chlorophyll and other pigments of photosynthetic cells absorb light energy and conserve it as ATP and NADPH; simultaneously, O_2 is evolved. In the carbon-assimilation reactions, ATP and NADPH are

used to reduce CO_2 to form triose phosphates, starch, and sucrose, and other products derived from them.

Photosynthesis in Plants Takes Place in Chloroplasts

In photosynthetic eukaryotic cells, both the light dependent and the carbon-assimilation reactions take place in the chloroplasts, intracellular organelles that are variable in shape and generally a few micrometers in diameter. Like mitochondria, they are surrounded by two membranes, an outer membrane that is permeable to small molecules and ions, and an inner membrane that encloses the internal compartment. This compartment contains many flattened, membrane- surrounded vesicles or sacs, the **thylakoids**, usually arranged in stacks called **grana**. Embedded in the thylakoid membranes (commonly called **lamellae**) are the photosynthetic pigments and the enzyme complexes that carry out the light reactions and ATP synthesis. The **stroma** (the aqueous phase enclosed by the inner membrane) contains most of the enzymes required for the carbon-assimilation reactions.

Light Drives Electron Flow in Chloroplasts

In 1937 Robert Hill found that when leaf extracts containing chloroplasts were illuminated, they (1) evolved O_2 and (2) reduced a non-biological electron acceptor added to the medium, according to the **Hill reaction**:

$$2H_2O + 2A \quad \text{light} \rightarrow \quad 2AH_2 + O_2$$

where A is the artificial electron acceptor, or **Hill reagent**. One Hill reagent, the dye 2,6-dichlorophenolindophenol, is blue when oxidized (A) and colourless when reduced (AH_2), making the reaction easy to follow. When a leaf extract supplemented with the dye was illuminated, the blue dye became colourless and O_2 was evolved. In the dark, neither O_2 evolution nor dye reduction took place. This was the first evidence that absorbed light energy causes electrons to flow from H_2O to an electron acceptor. Moreover, Hill found that CO_2 was neither required nor reduced to a stable form under these conditions; O_2 evolution could be dissociated from CO_2 reduction. Several years later Severo Ochoa showed that $NADP^-$ is the biological electron acceptor in chloroplasts, according to the equation

$$2H_2O + 2NADP^+ \quad \text{light} \rightarrow \quad 2NADPH + 2H^+ + O_2$$

1.4. Photochemical reaction in plants

Photochemical Reaction Definitions:

"The photochemical reaction is none other than a chemical reaction that starts with light being absorbed as a form of

energy". Temporary peak states would be triggered while the molecules absorb light and there would be physical and chemical property differences to a large extent from the real molecules.

The resultant chemical structures could be separated, modified, mixed among the similar or different molecules along with the transfer of hydrogen atoms, electronic charge to separate molecules, protons and electrons. The peak states in comparison to the real ground states are stronger reductants and acids that are stronger.

1.5. Photochemical Reaction in Photosynthesis:

- *Photosynthesis is a photochemical process* by which green plants, seaweeds, algae and certain bacteria absorb solar energy and utilize it to convert the atmospheric carbon dioxide to carbohydrates in the presence of water.
- Using photosynthesis, plants would convert the sunlight energy into the chemical energy being stored and thereby form carbohydrates using water and carbon dioxide and releases oxygen as a by-product of the reaction.
- Animal life is sustained with the aid of oxygen and carbohydrates.

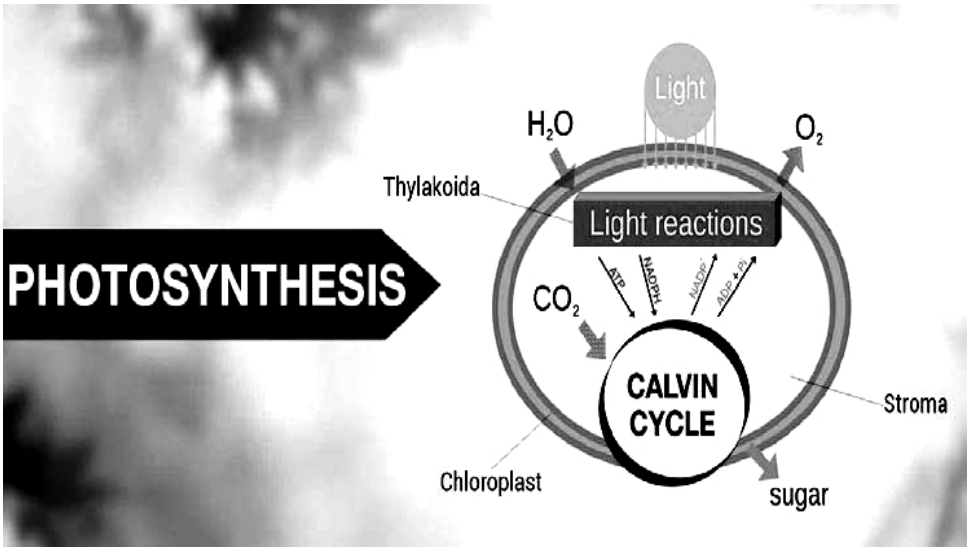

Fig.1.1 Photochemical Reaction in Photosynthesis

Photochemical reaction Examples :

The majority of processes on the other hand that we see in nature are photochemical ones. Our own ability to see the things in the world using the eyes is nothing but a photochemical reaction where a retinal that happens to be rhodopsin (photoreceptor cell molecule) changes its shape after sunlight or light absorption.

- The Vitamin D which is required for bone and teeth development and even functioning of Kidney while helping skin growth is the

chemical 7-dehydrocholesterol produced after exposure to sunlight.

- The ozone layer that is found in the earth's stratosphere is formed by the photochemical dissociation of molecular oxygen into oxygen atoms and these atoms reacting with molecules of oxygen to form ozone.
- The UltraViolet (UV) rays that are harmful to the human DNA and skin cancer likes are caused by photochemical reaction.
- Different sorts of commercial processes and devices are heavily influenced by photochemical reactions and their peak states.
- Activities that we encounter in our daily lives like xerography, photography and so on are based on photochemical processes whereas complex activities like manufacturing of semiconductor chips, the printing of newspapers are done by the aid of UV rays.
- The examples explained above would have given you a fair idea about how chemical reactions like the photochemical ones have a major impact on our daily lives without which it would be impossible for life to sustain on our planet.

In Plants, Two reaction centres act in tandem

The photosynthetic apparatus of modern cyanobacteria, algae, and vascular plants is more complex than the one-center bacterial systems, and it seems to have evolved through the combination of two simpler bacterial photocenters. The thylakoid membranes of chloroplasts have two different kinds of photosystems, each with its own type of photochemical reaction center and set of antenna molecules.

The two systems have distinct and complementary functions (**Fig.**1.2). Photosystem II (PSII) is a pheophytin-quinone type of system (like the single photosystem of purple bacteria) containing roughly equal amounts of chlorophylls *a* and *b*. Excitation of its reaction-center P_{680} drives electrons through the cytochrome b_6 *f* complex with concomitant movement of protons across the thylakoid membrane.

Photosystem I (PSI) is structurally and functionally related to the type I reaction center of green sulfur bacteria. It has a reaction center designated P700 and a high ratio of chlorophyll *a* to chlorophyll *b*. Excited P_{700} passes electrons to the Fe-S protein ferredoxin, then to $NADP^+$, producing NADPH. The thylakoid membranes of a single spinach chloroplast have many hundreds of each kind of photosystem. These two reaction centers in plants act in tandem to catalyze the light-driven movement of electrons from H_2O to $NADP^+$. Electrons are carried between the two photosystems by the soluble protein plastocyanin, a one-electron carrier functionally similar to cytochrome *c* of mitochondria. To replace the electrons that move from PSII through PSI to NADP, cyanobacteria and plants oxidize H_2O (as green sulphur bacteria oxidize H_2S), producing O_2 (Fig. 2). This process is called oxygenic

photosynthesis to distinguish it from the anoxygenic photosynthesis of purple and green sulfur bacteria.

All O_2-evolving photosynthetic cells—those of plants, algae, and cyanobacteria— contain both PSI and PSII; organisms with only one photosystem do not evolve O_2. The diagram in Figure (1.2), often called the Z scheme because of its overall form, outlines the pathway of electron flow between the two photosystems and the energy relationships in the light reactions. The **Z scheme** thus describes the complete route by which electrons flow from H_2O to $NADP^+$, according to the equation

$$2H_2O + 2NADP^+ + 8 \text{ photons} \quad \rightarrow \quad O_2 + 2NADPH + 2H^+$$

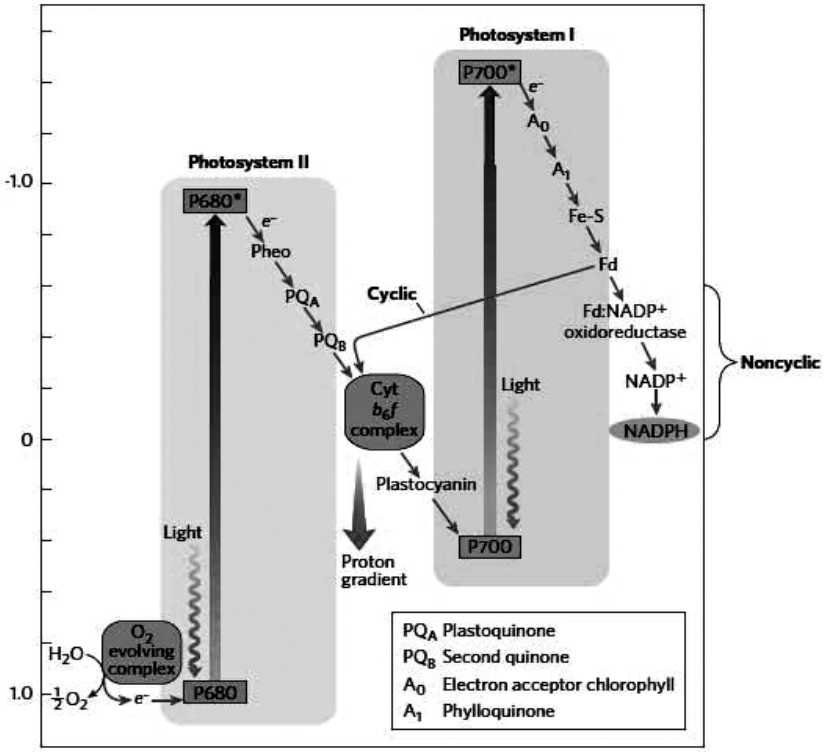

Fig. 1.2: Integration of photosystems I and II in chloroplasts.

This "Z scheme" shows the pathway of electron transfer from H_2O (lower left) to $NADP^+$ (far right) in noncyclic photosynthesis. The position on the vertical scale of each electron carrier reflects its standard reduction potential. To raise the energy of electrons derived from H_2O to the energy level required to reduce $NADP^+$ to NADPH, each electron must be "lifted" twice (heavy arrows) by photons absorbed in PSII and PSI. One photon is required per electron in each Photosystem. After excitation, the high-energy electrons flow "downhill" through the carrier chains shown. Protons move across the thylakoid membrane during the water-splitting reaction and during electron transfer through the cytochrome *b*6*f* complex, producing the proton gradient that is essential to ATP formation. An alternative path of electrons is cyclic electron transfer, in which electrons move from ferredoxin back to the cytochrome *b*6*f* complex, instead of

reducing $NADP^+$ to NADPH. The cyclic pathway produces more ATP and less NADPH than the noncyclic.

For every two photons absorbed (one by each photosystem), one electron is transferred from H_2O to $NADP^+$. To form one molecule of O_2, which requires transfer of four electrons from two H_2O to two $NADP^+$, a total of eight photons must be absorbed, four by each photosystems.

The mechanistic details of the photochemical reactions in PSII and PSI are essentially similar to those of the two bacterial photosystems, with several important additions. In PSII, two very similar proteins, D1 and D2, form an almost symmetric dimer, to which all the electron carrying cofactors are bound (Fig. 1.2). Excitation of P680 in PSII produces P680*, an excellent electron donor that, within picoseconds, transfers an electron to pheophytin, giving it a negative charge ($\bullet Pheo^-$). With the loss of its electron, P680* is transformed into a radical cation, $P680^+$. $\bullet Pheo^-$ very rapidly passes its extra electron to a protein-bound plastoquinone, PQA (or QA), which in turn passes its electron to another, more loosely bound plastoquinone, PQB (or QB). When PQB has acquired two electrons in two such transfers from PQA and two protons from the solvent water, it is in its fully reduced quinol form, $PQBH_2$. The overall reaction initiated by light in PSII is

$$4\ P680 + 4H^+ + 2\ PQ_B + 4\ photons \rightarrow 4\ P680^+ + 2\ PQ_BH_2$$

Eventually, the electrons in $PQBH_2$ pass through the cytochrome $b_6\ f$ complex (**Fig. 1.2**). The electron initially removed from P_{680} is replaced with an electron obtained from the oxidation of water, as described below. The binding site for plastoquinone is the point of action of many commercial herbicides that kill plants by blocking electron transfer through the cytochrome $b_6\ f$ complex and preventing photosynthetic ATP production.

The photochemical events that follow excitation of PSI at the reaction-center P_{700} are formally similar to those in PSII. The excited reaction-center P_{700}* loses an electron to an acceptor, A_0^- (believed to be a special form of chlorophyll, functionally homologous to the pheophytin of PSII), creating A_0^- and P_{700}^+ (Fig. 1.2, right side); again, excitation results in charge separation at the photochemical reaction center. P_{700}^+ is a strong oxidizing agent, which quickly acquires an electron from plastocyanin, a soluble Cu-containing electron-transfer protein. A_0^- is an exceptionally strong reducing agent that passes its electron through a chain of carriers that leads to $NADP^+$. First, phylloquinone (A_1) accepts an electron and passes it to an iron-sulfur protein (through three Fe-S centers in PSI). From here, the electron moves to ferredoxin (Fd), another iron-sulfur protein loosely associated with the thylakoid membrane. Spinach ferredoxin (*M*r 10,700) contains a 2Fe-2S center that oxidation and reduction reactions. The fourth electron carrier in the chain is the flavoprotein **ferredoxin : $NADP^+$ oxidoreductase**, which transfers electrons from reduced ferredoxin (Fd_{red}) to $NADP^+$:

$$2\ Fd_{red} + 2H^+ + NADP^+ \rightarrow 2Fd_{OX} + NADPH + H^+$$

This enzyme is homologous to the ferredoxin : NAD reductase of green sulfur bacteria shown Fig. 1.2.

1.6. Chemical Energy of Organic Substance:

In chemistry, an organic compound is generally any chemical compound that contains carbon. Due to carbon's ability to catenate (form chains with other carbon atoms), millions of organic compounds are known. The study of the properties, reactions, and syntheses of organic compounds comprises the discipline known as organic chemistry. For historical reasons, a few classes of carbon-containing compounds (e.g., carbonates and cyanide salts), along with a handful of other exceptions (e.g., carbon dioxide), are not classified as organic compounds and are considered inorganic. Other than those just named, little consensus exists among chemists on precisely which carbon-containing compounds are excluded, making any rigorous definition of an organic compound elusive.

Although organic compounds make up only a small percentage of the Earth's crust, they are of central importance because all known life is based on organic compounds. Living things incorporate inorganic carbon compounds into organic compounds through a network of processes (the carbon cycle) that begins with the conversion of carbon dioxide and a hydrogen source like water into simple sugars and other organic molecules by autotrophic organisms using light (photosynthesis) or other sources of energy. Most synthetically produced organic compounds are ultimately derived from petrochemicals consisting mainly of hydrocarbons, which are themselves formed from the high pressure and temperature degradation of organic matter underground over geological timescales. This ultimate derivation notwithstanding, organic compounds are no longer defined as compounds originating in living things, as they were historically.

Biological Resources for Energy

The chemical energy stored in biological resources can be converted into useful energy services such as heat, power, and transportation fuels. This article presents definitions such as energy crop, by-product and waste, and classifies biological materials according to their composition in four groups: lignocellulosic biomass, sugar and starches, oil biomass, and high-moisture biomass. Common primary and secondary conversion technologies for those groups are also briefly discussed. Biomass is seen as the renewable energy source with largest potential, but environmental and socio-economical impacts of bioenergy systems should be accurately evaluated in order to guarantee sustainable systems.

Primary Production

Chemosynthesis exploits chemical energy to convert inorganic carbon compounds into organic matter, in contrast with photosynthesis, which exploits the energy of light to produce organic matter. Chemosynthetic reactions are carried out by prokaryotic microorganisms,

principally bacteria and archaea (referred to as "bacteria" in the following). Energy is produced in chemosynthetic reactions from oxidizing reduced compounds. There are a variety of chemosynthetic bacteria that carry out these reactions including nitrifying bacteria (oxidizing NH_4 or NO_2), sulfur bacteria (oxidizing H_2S, S, and other sulfur compounds), hydrogen bacteria (oxidizing H_2), methane bacteria (oxidizing CH_4), iron and manganese bacteria (oxidizing reduced iron and manganese compounds), and carbon monoxide bacteria (oxidizing CO). This is not an exhaustive list and new modes of chemosynthesis as well as new chemosynthetic bacteria are still being discovered.

Animal Physiology

Metabolism and Digestion

The use of chemical energy is a fundamental characteristic of living animals. It is necessary to maintain cellular order and is vital to almost all physiological processes. Catabolic metabolism breaks down macromolecules for production of usable energy by cellular processes such as active transport, muscle contraction, ciliary movement, and production of heat, electricity, or light. Most cellular reactions need 20–40 kJ of energy per mole of reactants, which is much less than the energy yield of the complete oxidation of a typical metabolic substrate. Therefore high-energy phosphate compounds (phosphagens) are used as intermediary chemical energy stores. ATP is the most common phosphagen. Free energy is released by the hydrolysis of its terminal phosphate to form adenosine diphosphate (ADP) and inorganic phosphate (Pi) that is, $ATP \leftrightarrow ADP + Pi + 30.5\ kJ\ mol^{-1}$. There is a cyclic formation of ATP from ADP (by cellular metabolism) and subsequent breakdown of ATP by energy-requiring processes.

Animals are heterotrophs, and as such are unable to synthesize their own organic compounds from inorganic molecules and so rely on other organisms for nutrients. Energy is obtained from nutrients such as carbohydrates, lipids, and sometimes proteins (amino acids are required for protein systhesis but also produce energy when oxidized). Essential vitamins, minerals, and fatty acids are also needed for proper cell functioning and must also be obtained via the diet. Single-celled animals and sponges ingest food particles by phagocytosis. These are chemically and enzymatically reduced within a food vacuole to a few constituent substances (e.g., monosaccharides, fatty acids, and amino acids) that are transported into the cytoplasm. Most multicellular animals have a digestive system specialized for extracellular digestion. Food particles enter the digestive system where a series of physical and chemical digestive processes break down food particles into constituent molecules that are absorbed and distributed to the cells. These molecules can then be used for energy metabolism, or for cell maintenance or growth.

Metabolism may be aerobic or anaerobic. Aerobic metabolism is the oxidation of carbohydrates, lipids, and proteins by oxygen to provide

energy in the form of ATP. There are three major steps in the aerobic process: glycolysis, where glucose is converted to pyruvate with a net gain of 2 ATP (and 2 $NADH/H^+$), the citric acid (or Kreb's) cycle where pyruvate is converted to acetyl-CoA before undergoing a cycle of chemical reactions resulting in a further net gain of 2 ATP (and 6 NADH/H+ and 2 $FADH_2$), and finally the mitochondrial electron transfer system. Ninety-five percent of the ATP is generated by electron transfer, where electrons from $NADH/H^+$ and $FADH_2$ are transferred to electron carrier proteins, passing through several protein complexes and generating 34 ATP. Oxygen is the final electron receptor in the chain, and water is formed as the end product.

Anaerobic metabolism is an alternative to aerobic metabolism, but it is very inefficient by comparison, forming as little as 2 ATP per glucose molecule. Consequently most large and complex animals rely on aerobic metabolism to meet their resting requirements, but they may use anaerobic metabolism for supplemental energy, for example, during intense activity or anoxia. Build-up of lactate as an anaerobic end product of glycolysis is a major inhibitory factor in the long-term use of anaerobic metabolism in tetrapod vertebrates. However some (e.g., carp) can convert pyruvate to ethanol as the end product, which can be easily excreted to the environment and therefore does not inhibit glycolysis.

Many factors affect the metabolic rate (MR) of animals, including temperature, developmental stage, diet, photoperiod, taxonomy, habit, environment, activity, and circadian rhythm. Body size is a major determinant of MR and is probably the best studied but least understood topic in animal physiology. Larger animals have a higher overall MR than small animals but have a lower MR per gram of body mass, so the relationship (eqn [1]) between mass ($\boldsymbol{M}$) and MR does not scale isometrically (i.e., $b \neq 1$). Rather, $b < 1$ since small animals use proportionally more energy (i.e., per gram) than larger animals. This relationship is remarkably uniform for all animals, from single-celled protists to birds and mammals.

1.7. Summary

Living cells constantly perform work. They require energy for maintaining their highly organized structures, synthesizing cellular components, generating electric currents, and many other processes. Bioenergetics is the quantitative study of energy relationships and energy conversions in biological systems. Biological energy transformations obey the laws of thermodynamics. Activation Energy and transition state: For a biochemical reaction to proceed, the energy barrier needed to transform the substrate molecules into the transition state has to be overcome. The transition state has the highest free energy in the reaction pathway. The difference in free energy between the substrate and the transition state is termed the Gibbs free energy of activation ($\Delta G‡$). An enzyme stabilizes the transition state and lowers $\Delta G‡$, thus increasing the rate at which the

reaction occurs. A photon of visible light possesses enough energy to bring about photochemical reactions, which in photosynthetic organisms lead eventually to ATP synthesis. In the light reactions of plants, absorption of a photon excites chlorophyll molecules and other (accessory) pigments, which funnel the energy into reaction centers in the thylakoid membranes. In the reaction centers, photoexcitation results in a charge separation that produces a strong electron donor (reducing agent) and a strong electron acceptor. Plant photosystem I passes electrons from its excited reaction center, P_{700}, through a series of carriers to ferredoxin, which then reduces $NADP^+$ to NADPH. The reaction center of plant photosystem II, P_{680}, passes electrons to plastoquinone, and the electrons lost from P_{680} are replaced by electrons from H_2O (electron donors other than H_2O are used in other organisms). Flow of electrons through the photosystems produces NADPH and ATP. Cyclic electron flow produces ATP only and allows variability in the proportions of NADPH and ATP formed. The localization of PSI and PSII between the granal and stromal lamellae can change and is indirectly controlled by light intensity, optimizing the distribution of excitons between PSI and PSII for efficient energy capture. The light-driven splitting of H_2O is catalyzed by a Mn- and CO-containing protein complex; O_2 is produced. The reduced plastoquinone carries electrons to the cytochrome *b*6 *f* complex; from here they pass to plastocyanin, and then to P_{700} to replace those lost during its photoexcitation. Electron flow through the cytochrome b_6 *f* complex drives protons across the plasma membrane, creating a proton-motive force that provides the energy for ATP synthesis by an ATP synthase.

1.8. Terminal questions

Q.1. What do you understand by Bioenergetics process? Discuss briefly.

Answer:----------

Q.2. Define the role of photophosphorylation in bioenergetics process.

Answer:----------

Q.3. What is the activation energy? How transition state affects the conversion of substrate into products?

Answer:----------

Q.4. Photochemical Efficiency of Light at Different Wavelengths

The rate of photosynthesis, measured by O_2 production, is higher when a green plant is illuminated with light of wavelength 680 nm than with light of 700 nm. However, illumination by a combination of light of 680 nm and 700 nm gives a higher rate of photosynthesis than light of either wavelength alone. Explain.

Answer:----------

Q.5. Electron Flow through Photosystems I and II Predict how an inhibitor of electron passage through pheophytin would affect electron flow through (a) photosystem II and (b) photosystem I. Explain your reasoning.

Answer:----------

Q.6. Function of Cyclic Photophosphorylation When the [NADPH]/[NADP_] ratio in chloroplasts is high, photophosphorylation is predominantly cyclic (see Fig. 19–58). Is O_2 evolved during cyclic photophosphorylation? Is NADPH produced? Explain. What is the main function of cyclic photophosphorylation?

Answer:----------

1.9. Further readings

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UNIT : 2

THERMODYNAMICS

Structure

2.1. Introduction

Knowledge of thermodynamics, which is the description of the relationships among the various forms of energy and how energy affects matter, enables one to determine whether a physical process is possible. The first and second laws of thermodynamics are combined in the thermodynamic function, free energy (*G*). If the change in free energy (ΔG) of a reaction is negative, that reaction can occur spontaneously. If ΔG is positive, an input of energy is required to drive the reaction. The unit of energy is the Joule (J) or the calorie (cal). For a biochemical reaction to proceed, the energy barrier needed to transform the substrate molecules into the transition state has to be overcome. The transition state has the highest free energy in the reaction pathway. The difference in free energy between the substrate and the transition state is termed the Gibbs free energy of activation ($\Delta G‡$). An enzyme stabilizes the transition state and lowers $\Delta G‡$, thus increasing the rate at which the reaction occurs.

Objectives

- To explain about laws of thermodynamics
- To explain about state functions
- To know about quilibrium constant
- To know about Free energy charge and application to chemical reaction.

2.2. Thermodynamics Overview

The thermodynamics is the description of the relationships among the various forms of energy and how energy affects matter, enables one to determine whether a physical process is possible. The first and second laws of thermodynamics are combined in the thermodynamic function, free energy (*G*). If the change in free energy (ΔG) of a reaction is negative, that reaction can occur spontaneously. If ΔG is positive, an input of energy is required to drive the reaction. The unit of energy is the Joule (J) or the calorie (cal). For a biochemical reaction to proceed, the energy barrier needed to transform the substrate molecules into the transition state has to be overcome. The transition state has the highest free energy in the reaction pathway. The difference in free energy between the substrate and the transition state is termed the Gibbs free energy of activation ($\Delta G‡$). An enzyme stabilizes the transition state and lowers $\Delta G‡$, thus increasing the rate at which the reaction occurs.

The difference in energy level between the substrates and products is termed the change in Gibbs free energy (ΔG). A negative ΔG indicates that the reaction is thermodynamically favorable in the direction indicated, whereas a positive ΔG indicates that the reaction is not thermodynamically favorable and requires an input of energy to proceed in the direction indicated. An energetically unfavourable reaction is often driven by linking it to an energetically favorable reaction, such as the hydrolysis of ATP.

A chemical reaction often exists in a state of dynamic equilibrium. The equilibrium constant (*K*) defines the ratio of the concentrations of substrates and products at equilibrium. Enzymes do not alter the equilibrium position, but do accelerate the attainment of the equilibrium position by speeding up the forward and reverse reactions.

2.3. Biological Energy Transformations Obey the Laws of Thermodynamics

Many quantitative observations made by physicists and chemists on the inter conversion of different forms of energy led, in the nineteenth century, to the formulation of two fundamental laws of thermodynamics. The first law is the principle of the conservation of energy: *for any*

physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed. The second law of thermodynamics, which can be stated in several forms, says that the universe always tends toward increasing disorder: *in all natural processes, the entropy of the universe increases.*

Living organisms consist of collections of molecules much more highly organized than the surrounding materials from which they are constructed, and organisms maintain and produce order, seemingly immune to the second law of thermodynamics. But living organisms do not violate the second law; they operate strictly within it. To discuss the application of the second law to biological systems, we must first define those systems and their surroundings.

The reacting system is the collection of matter that is undergoing a particular chemical or physical process; it may be an organism, a cell, or two reacting compounds. The reacting system and its surroundings together constitute the universe. In the laboratory, some chemical or physical processes can be carried out in isolated or closed systems, in which no material or energy is exchanged with the surroundings. Living cells and organisms, however, are open systems, exchanging both material and energy with their surroundings. Living systems are never at equilibrium with their surroundings. The constant transactions between system and surroundings explain how organisms can create order within themselves while operating within the second law of thermodynamics.

A photochemical reaction is a **chemical reaction triggered when light energy** is absorbed by a substance's molecules. This response leads the molecules to experience a temporary excited state, thus altering their physical and chemical properties from the substance's initial molecule.

Photochemistry is the branch of chemistry **mainly concerned with rates and mechanisms of reactions resulting from the exposure of reactants to light radiations.** The photochemical reaction is in fact the thermal reaction of the electronically excited state of the molecule while the dark reaction of the molecule is the thermal reaction of the ground state.

2.4. State functions

Gibbs free energy, *G*, expresses the amount of an energy capable of doing work during a reaction at constant temperature and pressure. When a reaction proceeds with the release of free energy (that is, when the system changes so as to possess less free energy), the free-energy change, D*G*, has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and D*G* is positive.

Enthalpy, *H*, is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. When a chemical reaction releases heat, it is said to be exothermic; the heat

content of the products is less than that of the reactants and D*H* has, by convention, a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of D*H.*

Entropy, *S*, is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.

The units of Δ*G* and Δ*H* are joules/mole or calories/mole (recall that 1 cal 5 4.184 J); units of entropy are joules/ mole? Kelvin (J/mol?K)

Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation

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

in which Δ*G* is the change in Gibbs free energy of the reacting system, Δ*H* is the change in enthalpy of the system, *T* is the absolute temperature, and Δ*S* is the change in entropy of the system.

By convention, Δ*S* has a positive sign when entropy increases and Δ*H*, as noted above, has a negative sign when heat is released by the system to its surroundings. Either of these conditions, which are typical of energetically favorable processes, tend to make Δ*G* negative. In fact, Δ*G* of a spontaneously reacting system is always negative.

2.5. Activation energy and transition state

The energy changes that take place during the course of a particular biochemical reaction are shown in *Fig.2.1*. In all reactions there is an energy barrier that has to be overcome in order for the reaction to proceed. This is the energy needed to transform the substrate molecules into the transition state – an unstable chemical form part-way between the substrates and the products. The transition state has the highest free energy of any component in the reaction pathway.

Activation energy is the energy which must be provided to a chemical system with potential reactants to result in a chemical reaction. The activation energy (E_a) of a reaction is measured in joules (J) or kilojoules (kJ/mol) or kilocalories per mole (kcal/mol).

Activation energy can be thought of as the magnitude of the potential barrier (sometimes called the energy barrier) separating minima of the potential energy surface pertaining to the initial and final thermodynamic state. For a chemical reaction, to proceed at a reasonable rate, the temperature of the system should be high enough such that there exists an appreciable number of molecules with translational energy equal to or greater than the activation energy.

In some cases, rates of reaction *decrease* with increasing temperature. When following an approximately exponential relationship so the rate constant can still be fit to an Arrhenius expression, this results in a negative value of E_a. Elementary reactions exhibiting these negative activation energies are typically barrier less reactions, in which the reaction proceeding relies on the capture of the molecules in a potential well. Increasing the temperature leads to a reduced probability of the colliding molecules capturing one another (with more glancing collisions not leading to reaction as the higher momentum carries the colliding particles out of the potential well), expressed as a reaction cross section that decreases with increasing temperature. Such a situation no longer leads itself to direct interpretations as the height of a potential barrier.

Transition state:

The transition state is the transitory of molecular structure in which the molecule is no longer a substrate but not yet a product. All chemical reactions must go through the transition state to form a product from a substrate molecule. The transition state is the state corresponding to the highest energy along the reaction coordinate. It has more free energy in comparison to the substrate or product; thus, it is the least stable state. The specific form of the transition state depends on the mechanisms of the particular reaction.

In the equation S → X → P, X is the transition state, which is located at the peak of the curve on the Gibbs free energy graph.

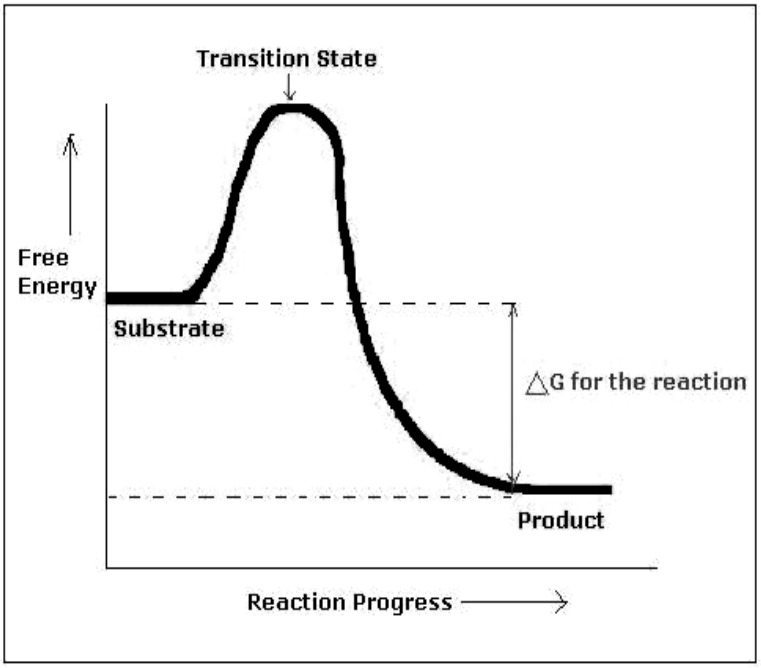

Fig. 2.1: The energy changes in biochemical reaction

2.6. Application to Enzymes

Enzymes are usually proteins that act like catalysts. The enzyme's ability to make the reaction faster depends on the fact that it stabilizes the transition state. The transition state's energy or, in terms of a reaction, the activation energy is the minimum energy that is needed to break certain bonds of the reactants so as to turn them into products. Enzymes decreases activation energy by shaping its active sites such that it fits the transition state even better than the substrate. When the substrate binds, the enzyme may stretch or distort a key bond and weaken it so that less activation energy is needed to break the bond at the start of the reaction. In many cases, the transition state of a reaction has a different geometry at the key atom (for instance, tetrahedral instead of trigonal planar). By optimizing binding of a tetrahedral atom, the substrate is helped on its way to the transition state and therefore lowers the activation energy, allowing more molecules to be able to turn into products in a given period of time. The enzyme stabilizes the transition state through various ways. Some ways an enzyme stabilizes is to have an environment that is the opposite charge of the transition state, providing a different pathway and making it easier for the reactants to be in the right orientation for reaction.

2.7. Free energy charge and application to chemical reaction.

The change in Gibbs free energy (ΔG) dictates whether a reaction will be energetically favorable or not. *Figure 2.2* shows an example where the overall energy change of the reaction makes it energetically favorable (i.e. the products are at a lower energy level than the substrates and ΔG is negative). It should be noted that ΔG is unrelated to $\Delta G‡$. The ΔG of a reaction is independent of the path of the reaction, and it provides no information about the rate of a reaction since the rate of the reaction is governed by $\Delta G‡$. A negative ΔG indicates

that the reaction is thermodynamically favorable in the direction indicated (i.e. that it is likely to occur without an input of energy), whereas a positive ΔG indicates that the reaction is not thermodynamically favorable and requires an input of energy to proceed in the direction indicated. In biochemical systems, this input of energy is often achieved by coupling the energetically unfavorable reaction with a more energetically favorable one (coupled reactions). It is often convenient to refer to ΔG under a standard set of conditions, defined as when the substrates and products of a reaction are all present at concentrations of 1.0 M and the reaction is taking place at a constant pH of 7.0. Under these conditions a slightly different value for ΔG is found, and this is called ΔG

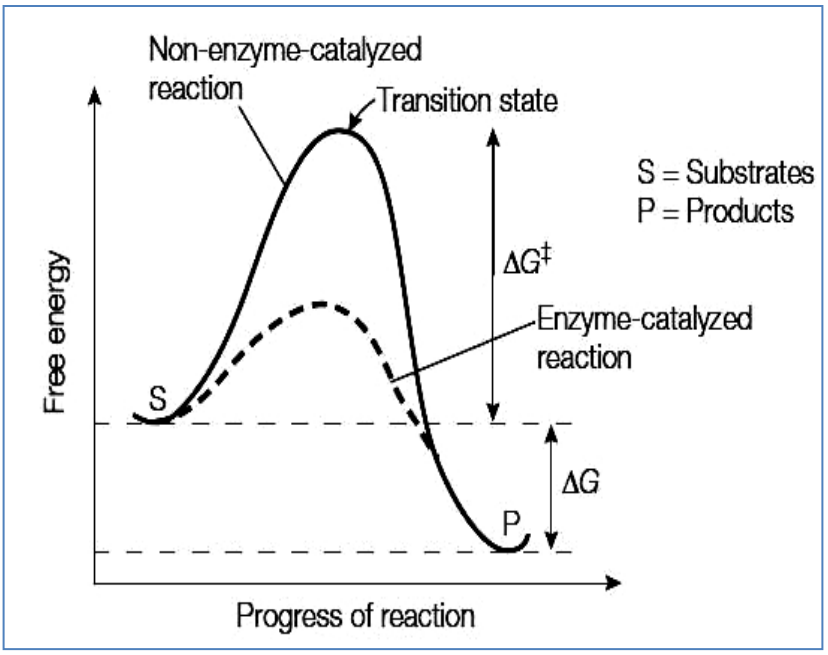

Fig. 2.2: The energy changes taking place during the course of a biochemical reaction.

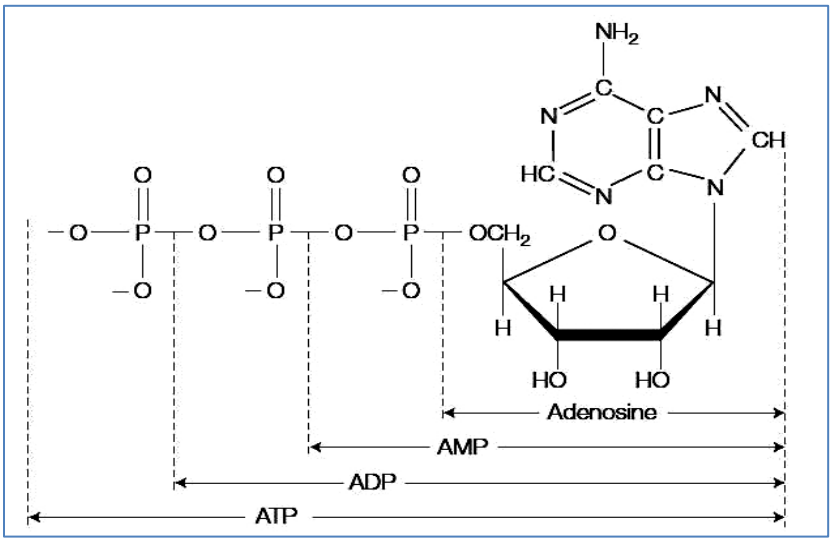

Fig.2.3: Structure of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenoscine monophosphate (AMP)

An example of an energetically favorable reaction which has a large negative $\Delta G^{0'}$ and is commonly used to drive less energetically favorable reactions is the hydrolysis of adenosine triphosphate (ATP; *Fig. 2.3*) to form adenosine diphosphate (ADP) and free inorganic phosphate (Pi):

$$ATP + H2O \rightarrow ADP + Pi \qquad \Delta G = -30.5 \text{ kJ mol}^{-1}$$

$$-7.3 \text{ kcal mol}^{-1}$$

2.8. Equilibrium Constant

2 A chemical reaction usually exists in a state of dynamic equilibrium, where although new molecules of substrate and product are continually being transformed and formed, the ratio of substrate to product remains at a constant value.

Consider the reaction:

$$A \underset{10^{-6}\ sec^{-1}}{\overset{10^{-4}\ sec^{-1}}{\rightleftharpoons}} B$$

where the rate of the forward reaction is 10^{-4} per second (sec^{-1}) and the rate of the reverse reaction is 10^{-6} sec^{-1}. At equilibrium the ratio of the concentrations of the substrate and product gives a constant value, known as the equilibrium constant (*K*). The equilibrium constant for a given reaction is defined as:

$$K = \frac{[\text{products}]_{eq}}{[\text{reactants}]_{eq}} = \frac{[B]_{eq}}{[A]_{eq}}$$

where square brackets indicate concentration. The equilibrium constant is also given by the ratio of the forward reaction rate (*k*f) and the reverse reaction rate (*k*b):

$$K = \frac{k_f}{k_b} = \frac{10^{-4}}{10^{-6}} = 100$$

Thus, for the above reaction at equilibrium, there is 100 times more of product B than there is of substrate A, regardless of whether there is enzyme presents or not.

This is because enzymes do not alter the equilibrium position of a reaction, but accelerate the forward and reverse reactions to the same extent. In other words, enzymes accelerate the attainment of the equilibrium position but do not shift its position. For the hypothetical reaction shown above, in the absence of added enzyme the reaction may take over an hour to reach the equilibrium position, whereas in the presence of enzyme the equilibrium position may be reached in less than 1 sec.

2.9. Coupled Reactions

In biochemistry, a **metabolic pathway** is a linked series of coupled reaction occurring within a cell. The reactants, products, and intermediates of an enzymatic reaction are known as metabolites, which are modified by a sequence of chemical reactions catalyzed by enzymes. In most cases of a metabolic pathway, the product of one enzyme acts as the substrate for the next. However, side products are considered waste and removed from the cell. These enzymes often require dietary minerals, vitamins, and other cofactors to function.

Different metabolic pathways function based on the position within a eukaryotic cell and the significance of the pathway in the given compartment of the cell. For instance, the, electron transport chain and oxidative phosphorylation all take place in the mitochondrial membrane. In contrast, glycolysis, pentose phosphate pathway, and fatty acid biosynthesis all occur in the cytosol of a cell.

There are two types of metabolic pathways that are characterized by their ability to either synthesize molecules with the utilization of energy (anabolic pathway) or break down of complex molecules by releasing energy in the process (catabolic pathway). The two pathways complement each other in that the energy released from one is used up by the other. The degradative process of a catabolic pathway provides the energy required to conduct a biosynthesis of an anabolic pathway. In addition to the two distinct metabolic pathways is the amphibolic pathway, which can be either catabolic or anabolic based on the need for or the availability of energy.

Pathways are required for the maintenance of homeostasis within an organism and the flux of metabolites through a pathway is regulated depending on the needs of the cell and the availability of the substrate. The end product of a pathway may be used immediately, initiate another metabolic pathway or be stored for later use. The metabolism of a cell consists of an elaborate network of interconnected pathways that enable the synthesis and breakdown of molecules (anabolism and catabolism).

Each metabolic pathway consists of a series of biochemical reactions that are connected by their intermediates: the products of one reaction are the substrates for subsequent reactions, and so on. Metabolic pathways are often considered to flow in one direction. Although all chemical reactions are technically reversible, conditions in the cell are often such that it is thermodynamically more favorable for flux to proceed in one direction of a reaction. For example, one pathway may be responsible for the synthesis of a particular amino acid, but the breakdown of that amino acid may occur via a separate and distinct pathway. One example of an exception to this "rule" is the metabolism of glucose. Glycolysis results in the breakdown of glucose, but several reactions in the glycolysis pathway

are reversible and participate in the re-synthesis of glucose (gluconeogenesis).

Glycolysis was the first metabolic pathway discovered:

1. As glucose enters a cell, it is immediately phosphorylated by ATP to glucose 6-phosphate in the irreversible first step.

2. In times of excess lipid or protein energy sources, certain reactions in the glycolysis pathway may run in reverse to produce glucose 6-phosphate, which is then used for storage as glycogen or starch.

-Metabolic pathways are often regulated by feedback inhibition.

-Some metabolic pathways flow in a 'cycle' wherein each component of the cycle is a substrate for the subsequent reaction in the cycle, such as in the Krebs Cycle.

Anabolic and catabolic pathways in eukaryotes often occur independently of each other, separated either physically by compartmentalization within organelles or separated biochemically by the requirement of different enzymes and co-factors.

Catabolic pathway (catabolism)

A catabolic pathway is a series of reactions that bring about a net release of energy in the form of a high energy phosphate bond formed with the energy carriers adenosine diphosphate (ADP) and guanosine diphosphate (GDP) to produce adenosine triphosphate (ATP) and guanosine triphosphate (GTP), respectively. The net reaction is, therefore, thermodynamically favorable, for it results in a lower free energy for the final products. A catabolic pathway is an exergonic system that produces chemical energy in the form of ATP, GTP, NADH, NADPH, FADH2, etc. from energy containing sources such as carbohydrates, fats, and proteins. The end products are often carbon dioxide, water, and ammonia. Coupled with an endergonic reaction of anabolism, the cell can synthesize new macromolecules using the original precursors of the anabolic pathway. An example of a coupled reaction is the phosphorylation of fructose-6-phosphate to form the intermediate fructose-1,6-bisphosphate by the enzyme phosphofructokinase accompanied by the hydrolysis of ATP in the pathway of glycolysis. The resulting chemical reaction within the metabolic pathway is highly thermodynamically favorable and, as a result, irreversible in the cell.

Cellular respiration

A core set of energy-producing catabolic pathways occur within all living organisms in some form. These pathways transfer the energy released by breakdown of nutrients into ATP and other small molecules used for energy (e.g. GTP, NADPH, FADH). All cells can perform anaerobic respiration by glycolysis. Additionally, most organisms can perform more efficient aerobic respiration through the citric acid cycle and oxidative phosphorylation. Additionally plants, algae and cyanobacteria

are able to use sunlight to anabolic ally synthesize compounds from non-living matter by photosynthesis.

Anabolic pathway (anabolism)

In contrast to catabolic pathways, anabolic pathways require an energy input to construct macromolecules such as polypeptides, nucleic acids, proteins, polysaccharides, and lipids. The isolated reaction of anabolism is unfavorable in a cell due to a positive Gibbs Free Energy (+ΔG). Thus, an input of chemical energy through a coupling with an exergonic reaction is necessary. The coupled reaction of the catabolic pathway affects the thermodynamics of the reaction by lowering the overall activation energy of an anabolic pathway and allowing the reaction to take place. Otherwise, an endergonic reaction is non-spontaneous.

An anabolic pathway is a biosynthetic pathway, meaning that it combines smaller molecules to form larger and more complex ones. An example is the reversed pathway of glycolysis, otherwise known as gluconeogenesis, which occurs in the liver and sometimes in the kidney to maintain proper glucose concentration in the blood and supply the brain and muscle tissues with adequate amount of glucose. Although gluconeogenesis is similar to the reverse pathway of glycolysis, it contains three distinct enzymes from glycolysis that allow the pathway to occur spontaneously. An example of the pathway for gluconeogenesis is illustrated in the image titled "Gluconeogenesis Mechanism".

Amphibolic pathway

An amphibolic pathway is one that can be either catabolic or anabolic based on the availability of or the need for energy. The currency of energy in a biological cell is adenosine triphosphate (ATP), which stores its energy in the phosphoanhydride bonds. The energy is utilized to conduct biosynthesis, facilitate movement, and regulate active transport inside of the cell. Examples of amphibolic pathways are the citric acid cycle and the glyoxylate cycle. These sets of chemical reactions contain both energy producing and utilizing pathways. To the right is an illustration of the amphibolic properties of the TCA cycle.

The glyoxylate shunt pathway is an alternative to the tricarboxylic acid (TCA) cycle, for it redirects the pathway of TCA to prevent full oxidation of carbon compounds, and to preserve high energy carbon sources as future energy sources. This pathway occurs only in plants and bacteria and transpires in the absence of glucose molecules.

Regulation

The flux of the entire pathway is regulated by the rate-determining steps. These are the slowest steps in a network of reactions. The rate-limiting step occurs near the beginning of the pathway and is regulated by feedback inhibition, which ultimately controls the overall rate of the pathway. The metabolic pathway in the cell is regulated by covalent or non-covalent modifications. A covalent modification involves an addition

or removal of a chemical bond, whereas a non-covalent modification (also known as allosteric regulation) is the binding of the regulator to the enzyme via hydrogen bonds, electrostatic interactions, and Van der Waals forces.

The rate of turnover in a metabolic pathway, also known as the metabolic flux, is regulated based on the stoichiometric reaction model, the utilization rate of metabolites, and the translocation pace of molecules across the lipid bilayer. The regulation methods are based on experiments involving 13C-labeling, which is then analyzed by Nuclear Magnetic Resonance (NMR) or gas chromatography-mass spectrometry (GC-MS)-derived mass compositions. The aforementioned techniques synthesize a statistical interpretation of mass distribution in proteinogenic amino acids to the catalytic activities of enzymes in a cell.

2.10. Summary

The first and second laws of thermodynamics are combined in the thermodynamic function, free energy (G). If the change in free energy (ΔG) of a reaction is negative, that reaction can occur spontaneously. If ΔG is positive, an input of energy is required to drive the reaction. The unit of energy is the Joule (J) or the calorie (cal). For a biochemical reaction to proceed, the energy barrier needed to transform the substrate molecules into the transition state has to be overcome. Activation energy is the energy which must be provided to a chemical system with potential reactants to result in a chemical reaction. The activation energy (E_a) of a reaction is measured in joules (J) and or kilojoules (kJ/mol) or kilocalories per mole (kcal/mol). The transition state is the state corresponding to the highest energy along the reaction coordinate. It has more free energy in comparison to the substrate or product; thus, it is the least stable state. Enzymes do not alter the equilibrium position of a reaction, but accelerate the forward and reverse reactions to the same extent. In other words, enzymes accelerate the attainment of the equilibrium position but do not shift its position. The reactants, products, and intermediates of an enzymatic reaction are known as metabolites, which are modified by a sequence of coupled reactions catalyzed by enzymes. In most cases of a metabolic pathway, the product of one enzyme acts as the substrate for the next.

2.11. Terminal questions

Q.1. What do you understand about thermodynamics, discuss briefly?

Answer:----------

Q.2. Which one of the following statements about the free energy change (ΔG) in a biochemical reaction is CORRECT?

a. If ΔG is negative, the reaction proceeds spontaneously with a loss of free energy.

b. In an exergonic reaction, ΔG is positive.

c. The standard free energy change when reactants are present in concentrations of 1.0 mol/L and the pH is 7.0 is represented as $\Delta G0$.

d. In an endergonic reaction, ΔG is negative.

e. If ΔG is 0, the reaction is essentially irreversible.

Answer:----------

Q.3. If the ΔG of a reaction is zero:

a. The reaction goes virtually to completion and is essentially irreversible.

b. The reaction is endergonic.

c. The reaction is exergonic.

d. The reaction proceeds only if free energy can be gained.

e. The system is at equilibrium and no net change occurs.

Answer:----------

Q.4. $\Delta G0'$ is defined as the standard free energy charge when:

a) The reactants are present in concentrations of 1.0 mol/L.

b) The reactants are present in concentrations of 1.0 mol/L at pH 7.0.

c) The reactants are present in concentrations of 1.0 mmol/L at pH 7.0.

d) The reactants are present in concentrations of 1.0 μmol/L.

e) The reactants are present in concentrations of 1.0 mol/L at pH 7.4.

Answer:----------

Q.5. Calculation of $\Delta G'^{0}$ from equilibrium constant : Calculate the standard free-energy change for each of the following metabolically important enzyme-catalyzed reactions, using the equilibrium constants given for the reactions at 25 8C and pH 7.0.

Answer:----------

Q.6. Dependence of ΔG on pH : The free energy released by the hydrolysis of ATP under standard conditions is 230.5 kJ/mol. If ATP is hydrolyzed under standard conditions except at pH 5.0, is more or less free energy released? Explain. Use the Living Graph to explore this relationship.

Answer:----------

2.12. Further readings

1. Lehninger- Principles of Biochemistry- David L. Nilson and Michael M. Cox, WH Freeman; 7th ed. 2017 edition.
2. TMH-Instant Notes of Biochemistry-2nd Edition.
3. Murray R K; Harper'S Illustrated Biochemistry, cbspd, 2006.
4. Voet D and Voet J.G., Biochemistry", 4th Edition,2010.
5. J L Jain et al., Fundamentals of Biochemistry; S Chand; Seventh edition ,2016.
6. U. Satyanarayana, Biochemistry; Elsevier India; 5 edition, 2017.

UNIT : 3

ATP

Structure

3.1. Introduction

Adenosine triphosphate(**ATP**) is a complex organic chemical that provides energy to drive many processes in living cells, e.g. muscle contraction, nerve impulse propagation, and chemical synthesis. ATP is often referred to as the "molecular unit of currency" of intracellular energy transfer. When consumed in metabolic processes, it converts either to adenosine diphosphate (ADP) or to adenosine monophosphate (AMP). Other processes regenerate ATP so that the human body recycles its own body weight equivalent in ATP each day. It is also a precursor to DNA and RNA, and is used as a coenzyme. The ATPases involved in DNA replication and other processes use ATP hydrolysis to cycle associated proteins between active and inactive forms. GTP-binding proteins that act in signaling pathways directly hydrolyze GTP to drive conformational changes that terminate signals triggered by hormones or by other extracellular factors.

The phosphate compounds found in living organisms can be divided somewhat arbitrarily into two groups, based on their standard free energies of hydrolysis. "High-energy" compounds have a $\Delta G'^0$ of hydrolysis more negative than 225 kJ/mol; "low-energy" compounds have a less negative $\Delta G'^0$. Based on this criterion, ATP, with a $\Delta G'^0$ of hydrolysis of 230.5 kJ/mol (27.3 kcal/mol), is a high-energy compound; glucose 6-phosphate, with a $\Delta G'^0$ of hydrolysis of 213.8 kJ/mol (23.3 kcal/mol), is a low-energy compound.

Objectives

- To explain about ATP cycle overview
- To explain about formation of ATP by phosphorylation
- To know about Importance of ATP and other compounds of high energy potential

3.2. ATP cycle overview

We know the nucleotides are the building blocks of nucleic acids; they are composed of three sub unit molecules: a nitrogenous base (also known as nucleobase), a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group. From the perspective of biochemistry, ATP is classified as a nucleoside triphosphate, which indicates that it consists of three components: a nitrogenous base (adenine), the sugar, and the triphosphate.

Structure

In terms of its structure, ATP consists of an adenine attached by the 9-nitrogen atom to the 1′ carbon atom of a sugar (ribose), which in turn is attached at the 5' carbon atom of the sugar to a triphosphate group. In its many reactions related to metabolism, the adenine and sugar groups remain unchanged, but the triphosphate is converted to di- and monophosphate, giving respectively the derivatives ADP and AMP. The three phosphoryl groups are referred to as the alpha (α), beta (β), and, for the terminal phosphate, gamma (γ). In neutral solution, ionized ATP exists mostly as ATP^{4-}, with a small proportion of ATP^{3-}.

Binding of metal cations to ATP

Being polyanionic and featuring a potentially chelatable polyphosphate group, ATP binds metal cations with high affinity. The binding constant for Mg2+ is (9554). The binding of a divalent cation, almost always magnesium, strongly affects the interaction of ATP with various proteins. Due to the strength of the ATP-Mg^{2+} interaction, ATP exists in the cell mostly as a complex with Mg^{2+} bonded to the phosphate oxygen centers.

A second magnesium ion is critical for ATP binding in the kinase domain. The presence of Mg^{2+} regulates kinase activity.

3.3. Chemical properties

Salts of ATP can be isolated as colourless solids. ATP is stable in aqueous solutions between pH 6.8 and 7.4, in the absence of catalysts. At more extreme pHs, it rapidly hydrolyses to ADP and phosphate. Living cells maintain the ratio of ATP to ADP at a point ten orders of magnitude from equilibrium, with ATP concentrations fivefold higher than the concentration of ADP. In the context of biochemical reactions, the P-O-P bonds are frequently referred to as high-energy bonds.

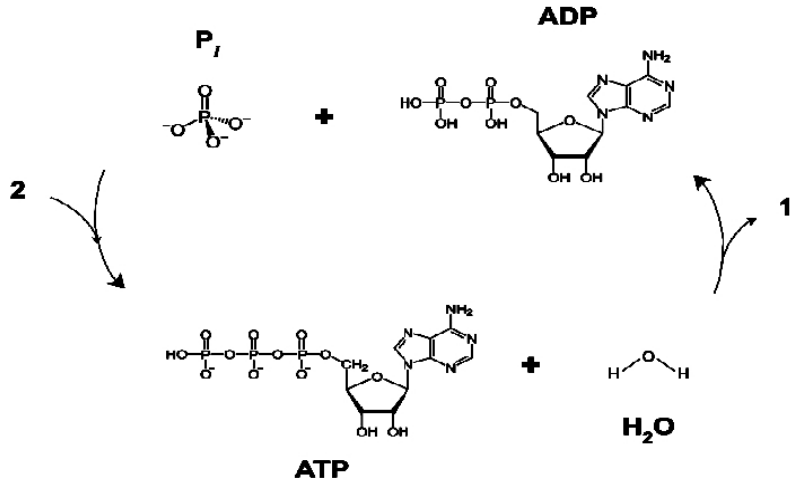

Fig 3.1: The cycle of synthesis and degradation of ATP; 1 and 2 represent output and input of energy, respectively

The hydrolysis of ATP into ADP and inorganic phosphate releases 30.5 kJ/mol of enthalpy, with a change in free energy of 3.4 kJ/mol. The energy released by cleaving either a phosphate (P_i) or pyrophosphate (PP_i) unit from ATP at standard state of 1 M are:

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G° = -30.5 \text{ kJ/mol } (-7.3 \text{ kcal/mol})$$

$$ATP + H_2O \rightarrow AMP + PP_i \quad \Delta G° = -45.6 \text{ kJ/mol } (-10.9 \text{ kcal/mol})$$

These abbreviated equations can be written more explicitly (R = adenosyl):

$$[RO\text{-}P(O)_2\text{-}O\text{-}P(O)_2\text{-}O\text{-}PO_3]^{4-} + H_2O \rightarrow [RO\text{-}P(O)_2\text{-}O\text{-}PO_3]^{3-} + [PO_4]^{3-} + 2\ H^+$$

$$[RO\text{-}P(O)_2\text{-}O\text{-}P(O)_2\text{-}O\text{-}PO_3]^{4-} + H_2O \rightarrow [RO\text{-}PO_3]^{2-} + [O_3P\text{-}O\text{-}PO_3]^{4-} + 2\ H^+$$

Production from AMP and ADP

A typical intracellular concentration of ATP is hard to pin down however reports have shown there to be 1–10 μM per gram of tissue in a variety of

eukaryotes. The dephosphorylation of ATP and rephosphorylation of ADP and AMP occur repeatedly in the course of aerobic metabolism.

ATP can be produced by a number of distinct cellular processes; the three main pathways in eukaryotes are (1) glycolysis, (2) the citric acid cycle/oxidative phosphorylation, and (3) beta-oxidation. The overall process of oxidizing glucose to carbon dioxide, the combination of pathways 1 and 2, is known as cellular respiration, produces about 30 equivalents of ATP from each molecule of glucose.

ATP production by a non-photosynthetic aerobic eukaryote occurs mainly in the mitochondria, which comprise nearly 25% of the volume of a typical cell.

3.4. Glycolysis

In glycolysis, glucose and glycerol are metabolized to pyruvate. Glycolysis generates two equivalents of ATP, by two enzymes, PGK and pyruvate kinase. Two equivalents of NADH are also produced, which can be oxidized via the electron transport chain and result in the generation of additional ATP by ATP synthase. The pyruvate generated as an end-product of glycolysis is a substrate for the Krebs Cycle.

Glycolysis is viewed as consisting of two phases with five steps each. Phase 1, "the preparatory phase", glucose is converted to 2 d-glyceraldehyde -3-phosphate (g3p). One ATP is invested in the Step 1, and another ATP is invested in Step 3. Steps 1 and 3 of glycolysis are referred to as "Priming Steps". In Phase 2, two equivalents of g3p are converted to two pyruvates . In Step 7, two ATP are produced. In addition, in Step 10, two further equivalents of ATP are produced. In Steps 7 and 10, ATP is generated from ADP. A net of two ATPs are formed in the glycolysis cycle. The glycolysis pathway is later associated with the Citric Acid Cycle which produces additional equivalents of ATP.

	Glycolysis: (Net yields)	
Stage I.	**ATP**	**2 ATP**
	2 NADH+H⁺ → 2 $FADH_2$ (to ETC)	**3 ATP**
Stage II.	**Conversion of pyruvate to ACoA**	
	2 NADH + H⁺ (to ETC)	**5 ATP**
Stage III.	**TCA cycle**	
	ATP (at one site)	**2 ATP**
	NADH+H⁺ at three steps (to ETC)	**15 ATP**
	$FADH_2$ at one step (to ETC)	**3 ATP**

Total ATP from one molecule of glucose = 30 ATP

Total ATP Production form the one Glucose molecule

Regulation

In glycolysis enzymes hexokinase is directly inhibited by its product, glucose-6-phosphate, and pyruvate kinase is inhibited by ATP itself. The main control point for the glycolytic pathway is phosphofructokinase (PFK), which is allosterically inhibited by high concentrations of ATP and activated by high concentrations of AMP. The inhibition of PFK by ATP is unusual, since ATP is also a substrate in the reaction catalyzed by PFK; the active form of the enzyme is a tetramer that exists in two conformations, only one of which binds the second substrate fructose-6-phosphate (F6P). The protein has two binding sites for ATP – the active site is accessible in either protein conformation, but ATP binding to the inhibitor site stabilizes the conformation that binds F6P poorly. A number of other small molecules can compensate for the ATP-induced shift in equilibrium conformation and reactivate PFK, including cyclic AMP, ammonium ions, inorganic phosphate, and fructose-1,6- and -2,6-biphosphate.

3.5. Citric acid cycle

In the mitochondrion, pyruvate is oxidized by the pyruvate dehydrogenase complex to the acetyl group, which is fully oxidized to carbon dioxide by the citric acid cycle (also known as the Krebs cycle). Every "turn" of the citric acid cycle produces two molecules of carbon dioxide, one equivalent of ATP guanosine triphosphate (GTP) through substrate-level phosphorylation catalyzed by succinyl-CoA synthetase, as succinyl- CoA is converted to Succinate, three equivalents of NADH, and one equivalent of FADH2. NADH and FADH2 are recycled (to NAD+ and FAD, respectively), generating additional ATP by oxidative phosphorylation. The oxidation of NADH results in the synthesis of 2–3 equivalents of ATP, and the oxidation of one FADH2 yields between 1–2 equivalents of ATP. The majority of cellular ATP is generated by this process. Although the citric acid cycle itself does not involve molecular oxygen, it is an obligately aerobic process because O2 is used to recycle the NADH and FADH2. In the absence of oxygen, the citric acid cycle ceases.

The generation of ATP by the mitochondrion from cytosolic NADH relies on the malate-aspartate shuttle (and to a lesser extent, the glycerol-phosphate shuttle) because the inner mitochondrial membrane is impermeable to NADH and NAD+. Instead of transferring the generated NADH, a malate dehydrogenase enzyme converts oxaloacetate to malate, which is translocated to the mitochondrial matrix. Another malate dehydrogenase-catalyzed reaction occurs in the opposite direction, producing oxaloacetate and NADH from the newly transported malate and the mitochondrion's interior store of NAD^+. A transaminase converts the oxaloacetate to aspartate for transport back across the membrane and into the intermembrane space.

In oxidative phosphorylation, the passage of electrons from NADH and $FADH_2$ through the electron transport chain pumps protons out of the mitochondrial matrix and into the intermembrane space. This pumping generates a proton motive force that is the net effect of a pH, gradient and an electric potential gradient across the inner mitochondrial membrane. Flow of protons down this potential gradient – that is, from the intermembrane space to the matrix – yields ATP by ATP synthase. Three ATP are produced per turn.

Although oxygen consumption appears fundamental for the maintenance of the proton motive force, in the event of oxygen shortage (hypoxia), intracellular acidosis (mediated by enhanced glycolytic rates and ATP hydrolysis), contributes to mitochondrial membrane potential and directly drives ATP synthesis.

Most of the ATP synthesized in the mitochondria will be used for cellular processes in the cytosol; thus it must be exported from its site of synthesis in the mitochondrial matrix. ATP outward movement is favored by the membrane's electrochemical potential because the cytosol has a relatively positive charge compared to the relatively negative matrix. For every ATP transported out, it costs 1 H^+. One ATP costs about 3 H^+. Therefore, making and exporting one ATP requires $4H^{+.}$ The inner membrane contains an antiporter, the ADP/ATP translocase, which is an integral membrane protein used to exchange newly synthesized ATP in the matrix for ADP in the intermembrane space. This translocase is driven by the membrane potential, as it results in the movement of about 4 negative charges out of the mitochondrial membrane in exchange for 3 negative charges moved inside. However, it is also necessary to transport phosphate into the mitochondrion; the phosphate carrier moves a proton in with each phosphate, partially dissipating the proton gradient. After completing glycolysis, the Citric Acid Cycle, electrons transport chain, and oxidative phosphorylation.

Regulation

The citric acid cycle is regulated mainly by the availability of key substrates, particularly the ratio of NAD+ to NADH and the concentrations of calcium, inorganic phosphate, ATP, ADP, and AMP. Citrate – the ion that gives its name to the cycle – is a feedback inhibitor of citrate synthase and also inhibits PFK, providing a direct link between the regulation of the citric acid cycle and glycolysis.

3.6. Beta (β) oxidation

In the presence of air and various cofactors and enzymes, fatty acids are converted to acetyl-CoA. The pathway is called beta-oxidation. Each cycle of beta-oxidation shortens the fatty acid chain by two carbon atoms and produces one equivalent each of acetyl-CoA, NADH, and $FADH_2$. The acetyl-CoA is metabolized by the citric acid cycle to generate ATP, while the NADH and $FADH_2$ are used by oxidative phosphorylation to generate

ATP. Dozens of ATP equivalents are generated by the beta-oxidation of a single long acyl chain.

Regulation

In oxidative phosphorylation, the key control point is the reaction catalyzed by cytochrome c oxidase, which is regulated by the availability of its substrate – the reduced form of cytochrome c. The amount of reduced cytochrome c available is directly related to the amounts of other substrates:

Which directly implies this equation?

Thus, a high ratio of [NADH] to [NAD^+] or a high ratio of [ADP][P_i] to [ATP] imply a high amount of reduced cytochrome c and a high level of cytochrome c oxidase activity. An additional level of regulation is introduced by the transport rates of ATP and NADH between the mitochondrial matrix and the cytoplasm.

Ketosis

Ketone bodies can be used as fuels, yielding 22 ATP and 2 GTP molecules per acetoacetate molecule when oxidized in the mitochondria. Ketone bodies are transported from the liver to other tissues, where acetoacetate and beta-hydroxybutyrate can be reconverted to acetyl-CoA to produce reducing equivalents (NADH and FADH2), via the citric acid cycle. Ketone bodies cannot be used as fuel by the liver, because the liver lacks the enzyme β-ketoacyl-CoA transferase, also called thiophorase. Acetoacetate in low concentrations is taken up by the liver and undergoes detoxification through the methylglyoxal pathway which ends with lactate. Acetoacetate in high concentrations is absorbed by cells other than those in the liver and enters a different pathway via 1,2-propanediol. Though the pathway follows a different series of steps requiring ATP, 1,2-propanediol can be turned into pyruvate.

Production, anaerobic conditions

Fermentation is the metabolism of organic compounds in the absence of air. It involves substrate-level phosphorylation in the absence of a respiratory electron transport chain. The equation for the oxidation of glucose to lactic acid is:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH(OH)COOH + 2\ ATP$$

Anaerobic respiration is respiration in the absence of O2. Prokaryotes can utilize a variety of electron acceptors. These include nitrate, sulfate, and carbon dioxide

ATP replenishment by nucleoside diphosphate kinases

ATP can also be synthesized through several so-called "replenishment" reactions catalyzed by the enzyme families of nucleoside diphosphate kinases (NDKs), which use other nucleoside triphosphates as a high-

energy phosphate donor, and the ATP: guanido-phosphotransferase family.

3.7. ATP production during photosynthesis

In plants, ATP is synthesized in the thylakoid membrane of the chloroplast. The process is called photophosphorylation. The "machinery" is similar to that in mitochondria except that light energy is used to pump protons across a membrane to produce a proton-motive force. ATP synthase then ensues exactly as in oxidative phosphorylation. Some of the ATP produced in the chloroplasts is consumed in the Calvin cycle, which produces triose sugars.

ATP recycling

The total quantity of ATP in the human body is about 0.2 moles. The majority of ATP is recycled from ADP by the aforementioned processes. Thus, at any given time, the total amount of ATP + ADP remains fairly constant.

The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily, which is around 50 to 75 kg. A human will typically use up his or her body weight of ATP over the course of the day. Each equivalent of ATP is recycled 500–750 times during a single day (100 / 0.2 = 500).

3.8. Formation of ATP by phosphorylation

Various biochemical reactions or processes for which ATP supplies energy, and the contribution of ATP to these reactions is commonly indicated as in, with a single arrow showing the conversion of ATP to ADP and Pi (or, in some cases, of ATP to AMP and pyrophosphate, PPi). When written this way, these reactions of ATP seem to be simple hydrolysis reactions in which water displaces Pi (or PPi), and one is tempted to say that an ATP-dependent reaction is “driven by the hydrolysis of ATP.” This is *not* the case. ATP hydrolysis per se usually accomplishes nothing but the liberation of heat, which cannot drive a chemical process in an isothermal system. A single reaction arrow such as that in (Fig. 1) almost invariably represents a two-step process (Fig. 2) in which part of the ATP molecule, a phosphoryl or pyrophosphoryl group or the adenylate moiety (AMP), is first transferred to a substrate molecule or to an amino acid residue in an enzyme, becoming covalently attached to the substrate or the enzyme and raising its free energy content. Then, in a second step, the phosphate containing moiety transferred in the first step is displaced, generating Pi, PPi, or AMP. Thus ATP participates *covalently* in the enzyme-catalyzed reaction to which it contributes free energy. Some processes *do* involve direct hydrolysis of ATP (or GTP), however. For example, noncovalent binding of ATP (or GTP), followed by its hydrolysis to ADP (or GDP) and Pi, can provide the energy to cycle some

proteins between two conformations, producing mechanical motion. This occurs in muscle contraction, and in the movement of enzymes along DNA or of ribosomes along messenger RNA. The energy-dependent reactions catalyzed by helicases, RecA protein, and some topoisomerases also involve direct hydrolysis of phosphoanhydride bonds. The AAA+ ATPases involved in DNA replication and other processes use ATP hydrolysis to cycle associated proteins between active and inactive forms. GTP-binding proteins that act in signaling pathways directly hydrolyze GTP to drive conformational changes that terminate signals triggered by hormones or by other extracellular factors.

The term "high-energy phosphate bond," long used by biochemists to describe the P ≈ O bond broken in hydrolysis reactions, is incorrect and misleading as it wrongly suggests that the bond itself contains the energy. In fact, the breaking of all chemical bonds requires an *input* of energy. The free energy released by hydrolysis of phosphate compounds does not come from the specific bond that is broken; it results from the products of the reaction having lower free-energy content than the reactants. For simplicity, we will sometimes use the term "high-energy phosphate compound" when referring to ATP or other phosphate compounds with a large, negative, standard free energy of hydrolysis.

As is evident from the additively of free-energy changes of sequential reactions, any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative free energy of hydrolysis. For example, because cleavage of Pi from phosphoenolpyruvate releases more energy than is needed to drive the condensation of Pi with ADP, the direct donation of a phosphoryl group from PEP to ADP is thermodynamically feasible:

3.9. Importance of ATP and other compounds of high energy potential

3.9.1. Biochemical functions

Intracellular signaling

ATP is involved in signal transduction by serving as substrate for kinases, enzymes that transfer phosphate groups. Kinases are the most common ATP-binding proteins. They share a small number of common folds. Phosphorylation of a protein by a kinase can activate a cascade such as the nitrogen activated protein kiase cascade.

ATP is also a substrate of adenylate cyclase, most commonly in G protein-coupled receptor signal transduction pathways and is transformed to second messenger, cyclic AMP, which is involved in triggering calcium signals by the release of calcium from intracellular stores. This form of signal transduction is particularly important in brain function, although it is involved in the regulation of a multitude of other cellular processes.

DNA and RNA synthesis

ATP is one of four "monomers" required in the synthesis of RNA. The process is promoted by RNA polymerases. A similar process occurs in the formation of DNA, except that ATP is first converted to the deoxyribonucleotide dATP. Like many condensation reactions in nature, DNA replication and DNA transcription also consumes ATP.

Amino acid activation in protein synthesis

Aminoacyl-tRNA synthetase enzymes consume ATP in the attachment tRNA to amino acids, forming aminoacyl-tRNA complexes. Aminoacyl transferase binds AMP-amino acid to tRNA. The coupling reaction proceeds in two steps:

1. aa + ATP ⟶ aa-AMP + PPi
2. aa-AMP + tRNA ⟶ aa-tRNA + AMP

The amino acid is coupled to the penultimate nucleotide at the 3′-end of the tRNA (the A in the sequence CCA) via an ester bond (roll over in illustration)

ATP binding cassette transporter

Transporting chemicals out of a cell against a gradient is often associated with ATP hydrolysis. Transport is mediated by ATP binding cassette transporters. The human genome encodes 48 ABC transporters, that are used for exporting drugs, lipids, and other compounds.

Extracellular signalling and neurotransmision

ATP serves as a neurotransmitter in many parts of the nervous system, modulates ciliary beating, affects vascular oxygen supply etc. ATP is either secreted directly across the cell membrane through channel proteins or is pumped into vesicles which then fuse with the membrane.

Protein solubility

ATP has recently proposed to act as a biological hydrotrope and has been shown to affect proteome-wide solubility.

ATP analogues

Biochemistry laboratories often use in vitro studies to explore ATP-dependent molecular processes. ATP analogs are also used in X-ray crystallography to determine a protein structure in complex with ATP, often together with other substrates.

Enzyme inhibitors of ATP-dependent enzymes such as kinases are needed to examine the binding sites and transition states involved in ATP-dependent reactions.

Most useful ATP analogs cannot be hydrolyzed as ATP would be; instead they trap the enzyme in a structure closely related to the ATP-bound state. Adenosine 5′-(γ-thiotriphosphate) is an extremely common ATP analog in which one of the gamma-phosphate oxygens is replaced by a sulfur atom; this anion is hydrolyzed at a dramatically slower rate than ATP itself and

functions as an inhibitor of ATP-dependent processes. In crystallographic studies, hydrolysis transition states are modeled by the bound vanadate ion.

Caution is warranted in interpreting the results of experiments using ATP analogs, since some enzymes can hydrolyze them at appreciable rates at high concentration.

Much of catabolism is directed toward the synthesis of high-energy phosphate compounds, but their formation is not an end in itself; they are the means of activating a very wide variety of compounds for further chemical transformation. The transfer of a phosphoryl group to a compound effectively puts free energy into that compound, so that it has more free energy to give up during subsequent metabolic transformations. We described above how the synthesis of glucose 6-phosphate is accomplished by phosphoryl group transfer from ATP.

Because of its intermediate position on the scale of group transfer potential, ATP can carry energy from high-energy phosphate compounds produced by catabolism to compounds such as glucose, converting them into more reactive species. ATP thus serves as the universal energy currency in all living cells. One more chemical feature of ATP is crucial to its role in metabolism: although in aqueous solution ATP is thermodynamically unstable and is therefore a good phosphoryl group donor, it is *kinetically* stable. Because of the huge activation energies (200 to 400 kJ/mol) required for uncatalyzed cleavage of its phosphoanhydride bonds, ATP does not spontaneously donate phosphoryl groups to water or to the hundreds of other potential acceptors in the cell. Only when specific enzymes are present to lower the energy of activation does phosphoryl group transfer from ATP proceed. The cell is therefore able to regulate the disposition of the energy carried by ATP by regulating the various enzymes that act on it.91

When the energy of ATP is used to drive a particularly unfavorable metabolic reaction, adenylylation is often the mechanism of energy coupling. Fatty acid activation is a good example of this energy-coupling strategy. The first step in the activation of a fatty acid— either for energy-yielding oxidation or for use in the synthesis of more complex lipids—is the formation of its thiol ester. The direct condensation of a fatty acid with coenzyme A is endergonic, but the formation of fatty acyl–CoA is made exergonic by stepwise removal of *two* phosphoryl groups from ATP. First, adenylate (AMP) is transferred from ATP to the carboxyl group of the fatty acid, forming a mixed anhydride (fatty acyl adenylate) and liberating PPi. The thiol group of coenzyme A then displaces the adenylyl group and forms a thioester with the fatty acid.

The activation of amino acids before their polymerization into proteins is accomplished by an analogous set of reactions in which a transfer RNA molecule takes the place of coenzyme A. An interesting use of the cleavage of ATP to AMP and PPi occurs in the firefly, which uses ATP as an energy source to produce light flashes.

3.10. Summary

- ATP is the chemical link between catabolism and anabolism. It is the energy currency of the living cell. The exergonic conversion of ATP to ADP and Pi, or to AMP and PPi, is coupled to many endergonic reactions and processes.
- Direct hydrolysis of ATP is the source of energy in some processes driven by conformational changes, but in general it is not ATP hydrolysis but the transfer of a phosphoryl, pyrophosphoryl, or adenylyl group from ATP to a substrate or enzyme that couples the energy of ATP breakdown to endergonic transformations of substrates.
- Through these group transfer reactions, ATP provides the energy for anabolic reactions, including the synthesis of informational macromolecules, and for the transport of molecules and ions across membranes against concentration gradients and electrical potential gradients.
- To maintain its high group transfer potential, ATP concentration must be held far above the equilibrium concentration by energy-yielding reactions of catabolism.
- Cells contain other metabolites with large, negative, free energies of hydrolysis, including phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine. These high-energy compounds, like ATP, have a high phosphoryl group transfer potential. Thioesters also have high free energies of hydrolysis.
- Inorganic polyphosphate, present in all cells, may serve as a reservoir of phosphoryl groups with high group transfer potential.

3.11. Terminal Question

Q.1. Which of the following statements about ATP is CORRECT?

a. It contains three high energy phosphate bonds.

b. It is needed in the body to drive exergonic reactions.

c. It is used as an energy store in the body.

d. It functions in the body as a complex with Mg2+.

e. It is synthesized by ATP synthase in the presence of uncouplers such as UCP-1 (thermogenin).

Answer:----------

Q.2. Which one of the following enzymes uses molecular oxygen as a ATP hydrogen acceptor?

a. Cytochrome *c* oxidase

b. Isocitrate dehydrogenase

c. Homogentisate dioxygenase

d. Catalase

e. Superoxide dismutase

Answer:----------

Q.3. The number of ATP molecules produced for each molecule of $FADH_2$ oxidized via the respiratory chain is:

a. 1

b. 2.5

c. 1.5

d. 2

e. 0.5

Answer:----------

Q.4. As one molecule of NADH is oxidized via the respiratory chain:

a. 1.5 molecules of ATP are produced in total.

b. 1 molecule of ATP is produced as electrons pass through complex IV.

c. 1 molecule of ATP is produced as electrons pass through complex II.

d. 1 molecule of ATP is produced as electrons pass through complex III.

e. 0.5 of a molecule of ATP is produced as electrons pass through complex I.

Answer:----------

Q.5. Which one of the following statement about cytochromes P450 is

INCORRECT?

a. They are able to accept electrons from either NADH or NADPH.

b. They are found only in the endoplasmic reticulum.

c. They are monooxygenase enzymes.

d. They play a major role in drug detoxification in the liver.

e. In some reactions they work in conjunction with cytochrome *b5.*

Answer:----------

Q.6. A number of compounds inhibit oxidative phosphorylation—the synthesis of ATP from ADP and inorganic phosphate linked to oxidation of substrates in mitochondria. Which of the following describes the action of oligomycin?

a. It discharges the proton gradient across the mitochondrial inner membrane.

b. It discharges the proton gradient across the mitochondrial outer membrane.

c. It inhibits the electron transport chain directly by binding to one of the electron carriers in the mitochondrial inner membrane.

d. It inhibits the transport of ADP into, and ATP out of, the mitochondrial matrix.

e. It inhibits the transport of protons back into the mitochondrial matrix through ATP synthase.

Answer:----------

3.12. Further readings

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Uttar Pradesh Rajarshi Tandon
Open University

UGBCH-103

Intermediary Metabolism

BLOCK

2

METABOLISM OF BIOMOLECULES

Course Design Committee

Prof. K.N. Singh — **Vice Chancellor**
Uttar Pradesh Rajarshi Tandon Open University

Dr. (Prof.) Ashutosh Gupta, — **Chairman**
School of Science, UPRTOU, Prayagraj

Prof. Prof. Umesh Nath Tripathi — **Member**
Department of chemistry
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. S.I. Rizvi — **Member**
Department of Biochemistry, University of Allahabad, Prayagraj

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Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. Sharad Kumar Mishra — **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Dr. Ravindra Pratap Singh — **Member**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh — **Course Coordinator**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Course Preparation Committee

Dr. Ajay Yadav — **Writer** — (Unit: 1,2 & 3)
Department of Zoology, Dr. B.R. Ambedkar
Gov. Girls P.G. College. Fatehpur. U.P.

Dr. Sushma Chauhan — **Author** — Unit: 4 & 9
Academic Consultant (Botany)
School of Sciences, UPRTOU, Prayagraj

Dr. Ravindra Pratap Singh — **Author** — Unit: 5
Academic Consultant (Biochemistry)
School of Sciences, UPRTOU, Prayagraj

Dr. Dharmveer Singh — **Author** — Unit: 6,7 &8
Academic Consultant (Biochemistry)

Dr. Ayodhya Prasad Verma — **Editor** — (All blocks and units)
Associate Professor,
Department of Botany
Bappa Sri Narain Vocational Post Graduate College, Lucknow

Dr. Dharmveer Singh — **(Course Coordinator)**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

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Introduction

This the second block of intermediary metabolism. It consists of following three units:

Unit-4 : In this unit we cover the introduction of metabolism of carbohydrates. The process of Glycolysis, Krebs's cycle, electron transport system involved in carbohydrate metabolism discuss briefly. Oxidative phosphorylation and mechanism of ATP synthesis also discuss in this unit.

Unit-5 : In this unit we will discuss about understanding of metabolism (catabolism & anabolism) and metabolism of lipids, fatty acids, sphingolipids, glycerophospholipids and glycerolipids etc. Fats, oils, hormones and certain components of living beings membranes are come under the category of lipids. The biosynthesis of cholesterol and B-oxidation of fatty acids discuss briefly.

Unit-6 : This unit covers the nitrogen fixation and their assimilation in plants. The role of various microorganisms for nitrogen fixation discussed. The degradation of nitrogen containing compounds release by various metabolic pathways also mentioned here. The various steps of urea cycle define excretion of nitrogenous compounds from living beings.

UNIT : 4

METABOLISM OF CARBOHYDRATES

Structure

4.1. Introduction

Carbohydrate metabolism is a fundamental biochemical process that ensures a constant supply of energy to living cells. The most important carbohydrate is glucose, which can be broken down via glycolysis; enter into the Kreb's cycle and oxidative phosphorylation to generate ATP. Glycolysis begins with the phosphorylation of glucose by hexokinase to form glucose-6-phosphate. This step uses ATP, which is the donor of the phosphate group. Glycolysis uses two ATPs but generates four ATPs, yielding a net gain of two ATPs and two molecules of pyruvate. In the presence of oxygen, pyruvate continues on to the Krebs cycle (also called the citric acid cycle or tricarboxylic acid cycle (TCA).

ATP is synthesized using the same strategy in oxidative phosphorylation and photophosphorylation. Oxidative phosphorylation and photophosphorylation are also mechanistically similar in some respects.

Objectives

- To describe the processes of glycolysis
- To describe the pathway of a pyruvate molecule through the Krebs cycle
- To describe the transport of electrons through the electron transport chain
- To describe the process of ATP production through oxidative phosphorylation

4.2. Glycolysis

Glycolysis is derived from the Greek words (glycose-sweet or sugar; lysis-dissolution). This pathway is often referred to as Embden-Meyerhof pathway (E.M, pathway) in honour of the two biochemists who made a major contribution to the knowledge of glycolysis. Glycolysis is defined as the sequence of reactions converting glucose (or glycogen) to pyruvate or lactate, with the production of ATP.

1. Glycolysis takes place in all cells of the body. The enzymes of this pathway are present in the cytosomal fraction of the cell.

2. It occurs in the absence of oxygen (anaerobic) or in the presence of oxygen (aerobic). Lactate is the end product under anaerobic condition. In the aerobic condition, pyruvate is formed, which is then oxidized to CO_2 and H_2O.

3. Glycolysis is a major pathway for ATP synthesis in tissues lacking mitochondria, e.g. erythrocytes, cornea, lens etc.

4. Glycolysis is very essential for brain which is dependent on glucose for energy.

The pathway of glycolysis can be divided into three distinct phases:

A. Energy investment phase or priming stage

B. Splitting phase

C. Energy generation phase

A. Energy investment phase

1. Glucose is phosphorylated to glucose 6-phosphate by hexokinase or glucokinase (both are isoenzymes).This is an irreversible reaction, dependent on ATP and Mg^{2+}. Glucokinase present in liver, catalyses the phosphorylation of only glucose, has high K_m

for glucose (10 mM) and is not inhibited by glucose 6-phosphate. Due to high affinity (low K_m), glucose is utilized by hexokinase even at low concentration, whereas glucokinase acts only at higher levels of glucose i.e., after a meal when blood glucose concentration is above 100 mg/dl.

2. Glucose 6-phosphate is impermeable to the cell membrane. lt is a central molecule with a variety of metabolic fates – glycolysis, glycogenesis, gluconeogenesis and pentose phosphate pathway.

3. Glucose 6 –phosphate undergoes isomerization to give fructose 6-phosphate in the presence of the enzyme phosphohexose isomerase and Mg^{2+}.

4. Fructose 6-phosphate is phosphorylated to fructose1,6-bisphosphate by phosphofructokinase (PFK). This is an irreversible and a regulatory step in glycolysis.

B. Splitting phase

5. The six carbon fructose 1,6- bisphosphate is split (hence the name glycolysis) to two three-carbon compounds, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate by the enzyme aldolase (fructose 1,6- bisphosphate aldolase).

6. The enzyme phosphotriose isomerase catalyses the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Thus, two molecules of glyceraldehydes 3-phosphate are obtained from one molecule of glucose.

C. Energy generation phase

7. Glyceraldehyde 3-phosphate dehydrogenase converts glyceraldehydes 3-phosphate to 1,3-bisphosphoglycerate. This step is important as it is involved in the formation of $NADH^+ H^+$ and a high energy compound 1,3-bisphosphoglycerate, lodoacetate and arsenate inhibit the enzyme glyceraldehyde 3-phosphate dehydrogenase. ln aerobic condition, NADH passes through the electron transport chain and 6 ATP (2 x 3 ATP) are synthesized by oxidative phosphorylation.

8. The enzyme phosphoglycerate kinase acts on 1,3-bisphosphoglycerate resulting in the synthesis of ATP and formation of 3-phosphoglycerate. This step is a good example of substrate Ievel phosphorylation, since ATP is synthesized from the substrate without the involvement of electron transport chain. Phosphoglycerate kinase reaction is reversible, a rare example among the kinase reactions.

9. 3-Phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase. This is an isomerization reaction.

10. The high energy compound phosphoenol pyruvate is generated from 2-phosphoglycerate by the enzyme enolase. This enzyme requires Mg^{2+} or Mn^{2+} and is inhibited by fluoride. For blood glucose estimation in the laboratory, fluoride is added to the blood to prevent glycolysis by the cells, so that blood glucose is correctly estimated.

11. The enzyme pyruvate kinase catalyses the transfer of high energy phosphate from phosphoenol pyruvate to ADP, leading to the formation of ATP. This step also is a substrate level phosphorylation. This reaction is irreversible.

4.3. Production of ATP in glycolysis

Under anaerobic conditions, 2 ATP are synthesized while, under aerobic conditions, 8 or 6 ATP are synthesized-depending on the shuttle pathway that operates.

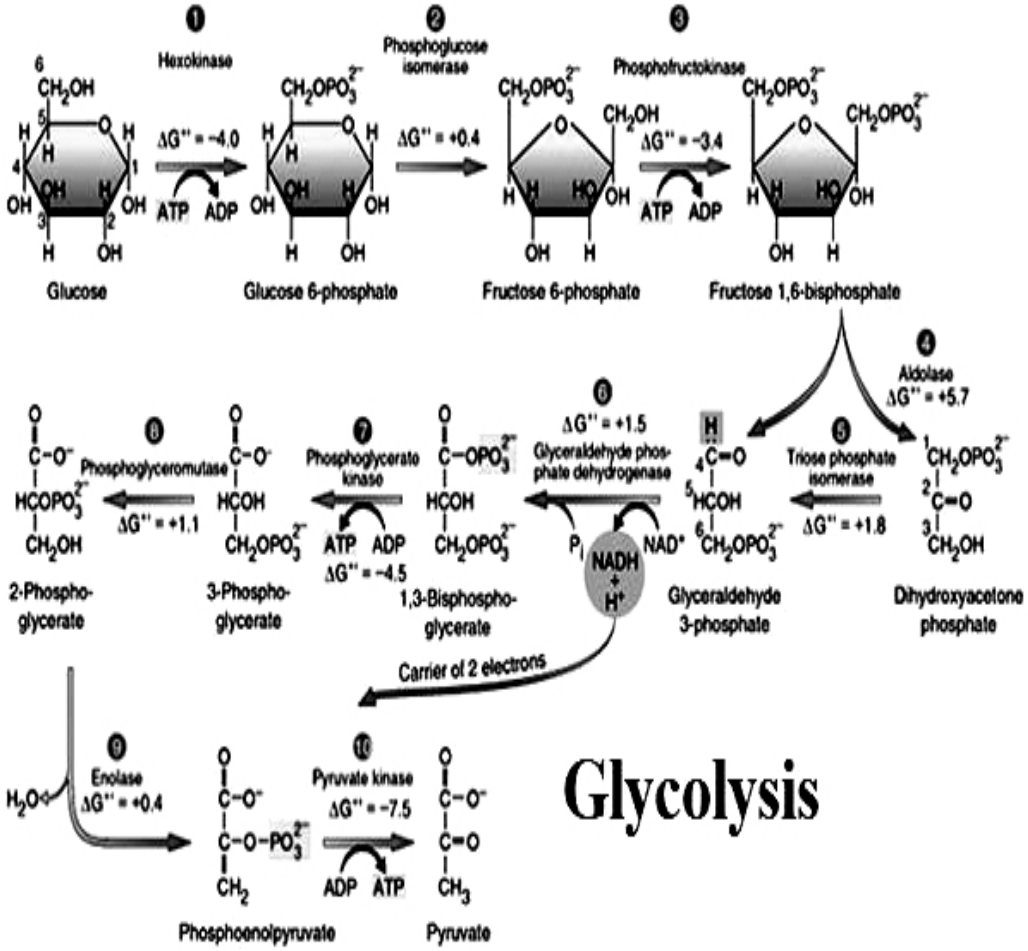


Fig.4.1: Production of ATP in glycolysis

Source: https://microbiologyinfo.com/glycolysis-10-steps-explained-steps-by-steps-with-diagram/

4.4. Krebs cycle

It was discovered by famous biochemist Sir Hans Krebs (1943) and hence, the pathway was termed as Krebs cycle. The Kreb cycle is also know as tricarboxylic acid cycle (TCA cycle) or citric acid cycle.

The Pyruvic acid produced in Glycolysis enters into mitochondrial matrix and converted to Acetyl-CoA.

Acetyl- CoA is the 'connecting link' between glycolysis and the krebs cycle. Its complete oxidation to Co_2 and H_2o involves a series of reactions of krebs cycle which are as follows :

- The 2-carbon acetyl co-A is added to a 4-cabon oxalo acetic acid to form a 6 carbon citric acid in presence of citrate synthetase enzyme.
- Citric acid is dehydrated to form cis-aconitic acid in presence of enzyme aconitase.
- Cis-aconitic acid reacts with one molecule of water to form iso-citric acid in presence of aconitase enzyme.
- Isocitric acid is oxidised to form oxalosuccinic acid in presence of isocitric dehydrogenase enzyme. At this stage one molecule of NAD+ is reduced to $NADH+H^+$.
- One molecule of H_2O is added to fumaric acid to form malic acid in presence of enyzme fumarase Malic acid is oxidised to oxaloacetic acid in presence of malic dehydrogenase enzyme. One molecule of NAD^+ is reduced to $NADH+H^+$ in the reaction.

(a) Significance of Krebs cycle.

1. Respiration provided energy for the reduction of nitrate to ammonia which is used in the synthesis of aminoacids.

2. α-ketoglutaric acid provides carbon skeleton for the biosynthesis of glutamic acid. Oxaloacetic acid is directly converted to aspartic acid by transamination reaction and alanine is formed from pyruvic acid. Alanine is impotant aminoacid which further gives rise to other aminoacids by transamination reactions.

3. Succinyl coenzyme- A used up for the synthesis of aromatic porphyrins which give rise to cytochromes, phytochromes and chlorophyll pigments.

4. The acetyl coenzyme-A synthesizes fatty acids which by combining with glycerol form fats.

(b) ATP produced in Aerobic Respiration

Each glycolytic $NADH+H^+$ yields 2 ATP molecules when oxidised by E.T.S. Thus 2 molecules of $NADH+H^+$ of glycolysis yield 4 ATP molecules. 4 ATP molecules are also produced in glycolysis by transphosphorylation out of which 2 ATP are consumed. Thus, the glycolysis contributes a total of 2+4=6 ATP molecules.

In krebs cycle 2 molecules of ATP are produced by transphosphorylation. Besides this 8 molecules of NADH and 2 molecules of $FADH_2$ are produced in krebs cycle. Each NADH of Krebs cycle yields 3 ATP molecules by oxidative phosphorylation thus total 8x3=24 ATP are produced. Each $FADH_2$ produces 2 ATP molecules by oxidative phosphorylation. Thus 2 $FADH_2$ produce 2x2=4 ATP molecules. The total ATP produced in krebs cycle are = 2+24+4=30 ATP.

Thus the total ATP produced in Respiration are:

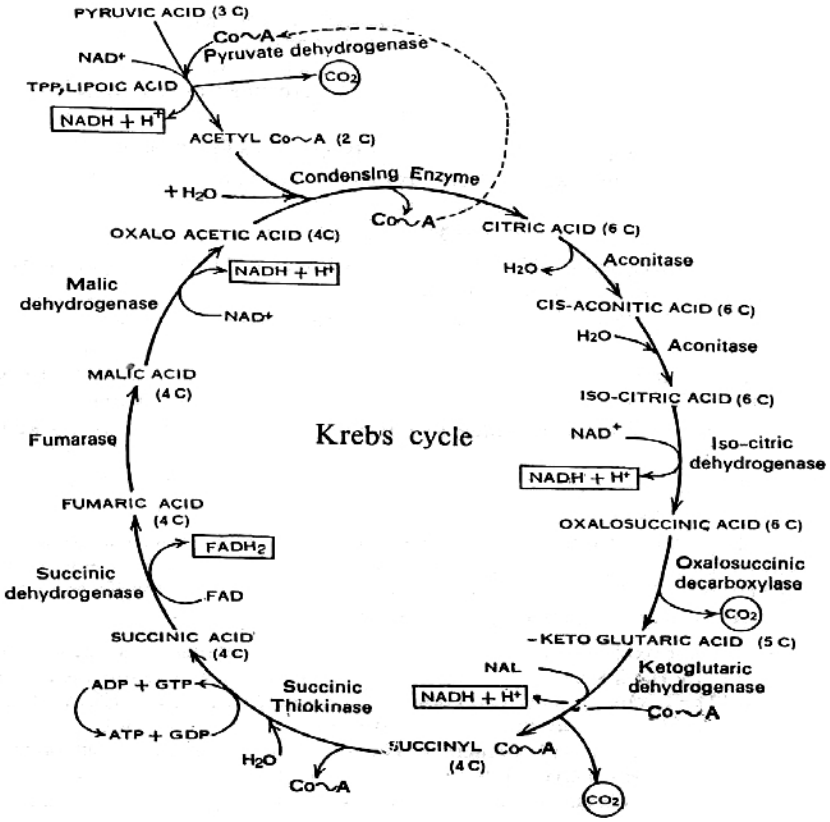
Glycolysis + Krebscycle

6 ATP + 30 ATP = 36 ATP

4.5. Electron transport system (ETS)

The various components of electron transport system include. Cytochrome b, 2 types of cytochrome c, ubiquinone, flavo protein (FMN or FAD) iron sulphur protein (Fe-S) and enzyme cytochrome oxidase which is ultimately associated with cytochrome a and a_3. These components are arranged in a sequence in the inner mitochondrial membrane. Reduced Coenzymes transfer their electrons and protons through E.T.S. in following ways-

- The 6-carbon oxalosuccinic acid is decarboxylated to 5-cabon, α-ketoglutaric acid in presence of oxalosuccinic dehydrogenase enzyme. One molecule of Co_2 is released in the reaction.
- The 5-carbon α-ketoglutaric acid is oxidatively decarboxylated to 4-carbon succinyl coenzyme-A in presence of δ-ketoglutaric dehydrogenase enzyme. In this reaction one molecule of Co-A is used up and one molecule of Co_2 is released. The coenzyme NAD+ is also reduced to $NADH+H^+$



.**Fig.4.2:** Krebs cycle (TCA cycle)

Source: https://www.expii.com/t/energy-balance-in-the-citric-acid-cycle-5730

- ❖ Succinyl Co-A is hydrolysed to succinic acid in presence of succinic thiokinase enzyme. In this reaction one molecule of H_2o is used up and Co-A releases one molecule of GDP (guanosine diphosphate) and is converted to GTP (guanosine triphosphate).
- ❖ Succinic acid is oxidized to fumaric acid in presence of succinic dehydrogenase enzyme. The coenzyme-FAD is reduced to $FADH_2$.

1. Transfer of hydrogen from $NADH+H^+$ (formed in TCA) to FMN (Metaloflavoprotein). The FMN get reduced to $FMNH_2$ and $NADH+H^+$ (coenzyme) get reduced to NAD.

2. Reduced FMN further transfers its electrons to Fe-S protein and two $2H^+$ into the inter membranal space.

3. Reduced Fe-S transfers its electron to ubiquinone (UQ). The UQ takes two electrons one after another from Fe-S and two protons ($2H^+$) from the matrix to become UQH_2.

4. Reduced UQH_2 transfers its electron to cytochrome b and two H^+ to outenside. $FADH_2$ reduced in kreb cycle also enters into ETS at this stage by transferring its $2H^+$ to UQ and UQ reduced to UQH_2.

5. $NADH+H^+$ reduced in glycolysis also enters in ETS. The NADH reduces a flavo protein (FP) (containing NADH-dehydrogense) located on the outer surface of inner mitochondrial membrane. The reduced FPH_2 (flavo protein) enters into main pathway by transferring 2H to UQ. The reduced UQH_2 transfers its electrons to cytochrome b and $2H^+$ to the outer side.

6. Reduced cytochrome b transfers its electrons to Fe-S protein $Fe^{+3} - s \rightarrow Fe^{+2} - s$. It transfers electrons to UQ which also takes $2H^+$ from inner matrix to become UQH_2.

7. The reduced UQH_2 transfers electron to cytochrome C, with the transport of a pair of H^+ outword.

8. Reduced cytochrome C, reduces cytochrome C by transferring electron.

9. Finally electrons from cytochrome C are transferred to O_2 via cytochrome and a_3.

This step is called terminal oxidation as it is catalysed by cytochrome oxidases (enzyme). The O_2 is reduced to H_2O by transferring electron from cytochrome a_3 and 2H+ from the medium in following way-

$$2cyt(Fe^{+2}) + \frac{1}{2}o_2 + 2H^+ \rightarrow H_2o + 2cy + (Fe^{+3})$$

The enzyme cytochrome oxidase is tightly bound to the inner mitochondrial membrane and inseparable to cyt a and cyt a_3' polypptides and two cu ions.

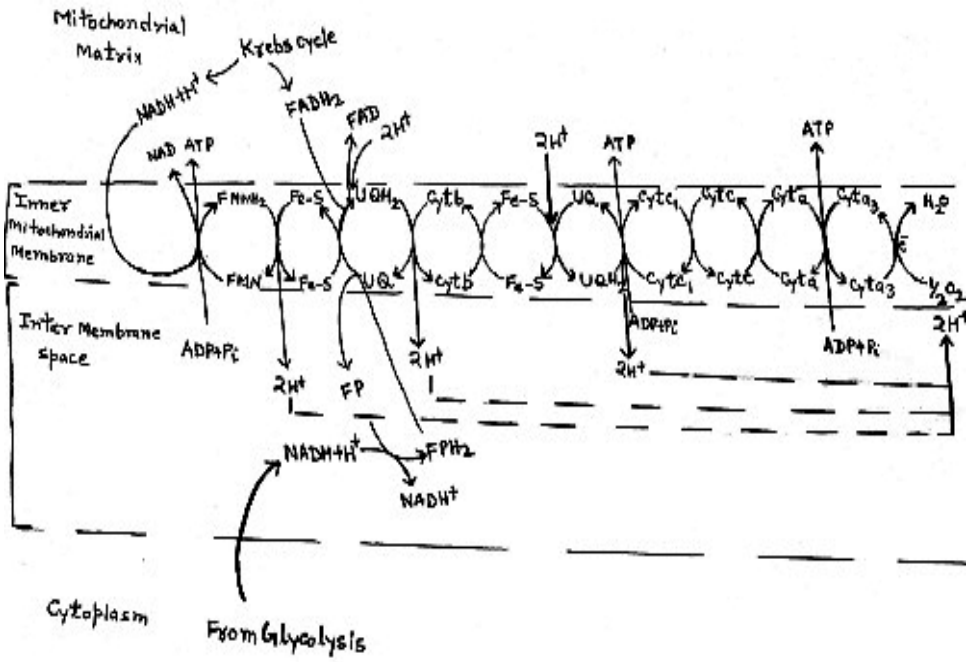


Fig.4.3: Cyclic representation of electron transport system

4.6. Oxidative phosphorylation

The NADH and $FADH_2$ formed in glycolysis are an energy rich molecule each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. In Oxidative phosphorylation ATP is formed as a result of the transfer of electrons from NADH or $FADH_2$ to O_2 by a series of electron carriers. These processes, which take place in mitochondria, is the major source of ATP in aerobic respiration.

The respiratory break down of simple carbohydrates in presence of oxygen is an oxidative process. During which many intermediates such as phosphoglyceraldehyde, pyruvic acid, iso-citric acid, α-ketoglutaric acid, succinic acid and malic acid are oxidised. The oxidation of all these is brought about by removal of a pair of hydrogen atoms (2H) from each one of them. The pair of hydrogen is usually picked from the substrate by $NADH^+$ or $FADH_2$ in the following manner.

These coenzymes are reduced by a pair of hydrogen (2H) in the following reactions of aerobic respiration.

NADH or $FADH_2$ released in glycolysis and krebs cycle, finally reduce O_2 to H_2o. The transfer of H^+ and e^- from reduced NAD^+ or FAD to O_2 is not a simple process. The NADH gets oxidised at redox potential of -032V and O_2 is reduced at redox potential of +0.82V. Thus there is a gap of +1.14V in redox potential which is too much. Therefore, NADH and $FADH_2$ connot directly combine with O_2 to form H_2O. Many intermediate cytochromes and other carriers having intermediate redox potential are arranged in a series which transport electrons from reduced NAD^+ or FAD to O_2 and form electron transport system (ETS). As electron transport down to energy gradient through electron transport system results in the formation of ATP (Adenosine triphosphate) from ADP (Adenosine diphosphate) and inorganic phosphate. The ATP produced here is due to oxidation reduction reaction therefore, known as oxidative phosphorylation.

4.7. Mechanism of ATP synthesis

Adenosine 5′-triphosphate (ATP) is the most important energy currency in cells in all living organisms. ATP is a nucleoside triphosphate containing adenine, ribose, and three phosphate groups. ATP is synthesized using the same strategy in oxidative phosphorylation and photophosphorylation. In oxidative phosphorlation ATP is generated as a result of electron flow from NADH or $FADH_2$ to O_2 via a series of membrane-bound electron carriers that is also known as the respiratory chain in which O_2 is reduced to H_2O the end. While in photophosphorylation the ATP is produced as of result of electron flow from H_2O to NADP+ via a series of membrane-bound electron carriers

where H_2O oxidizing to O_2 at the beginning. Oxidative phosphorylation and photophosphorylation are mechanistically similar in three respects.

- Both processes involve the flow of electrons through a chain of membrane-bound carriers. The free energy made available by this —downhill (exergonic) electron flow is coupled to the —uphill transport of protons across a proton-impermeable membrane, conserving the free energy of fuel oxidation as a. transmembrane electrochemical potential
- sThe transmembrane flow of protons down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP. These two way mediated by kinases and the other by ATP synthases. Kinase is the general name for enzymes that catalyze the transfer of 5′-terminal phosphate groups of ATP to substrates or vice versa.

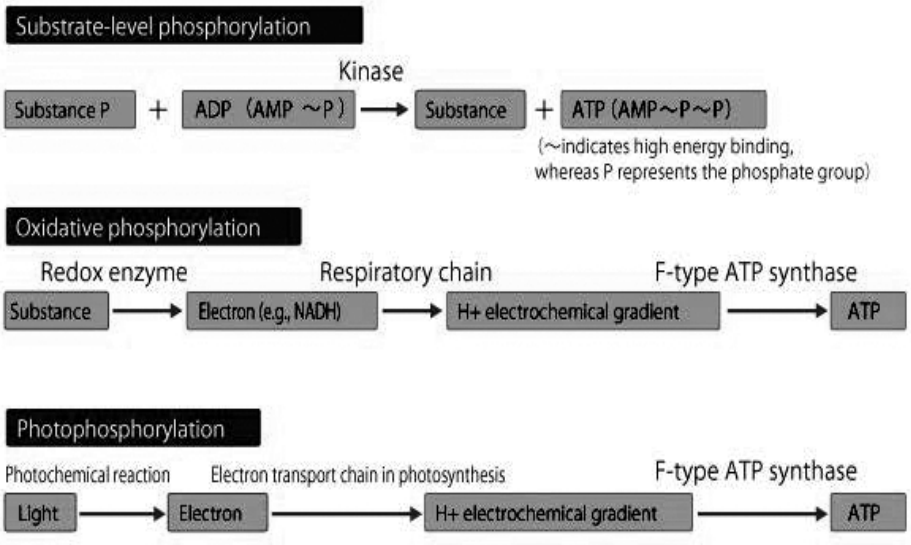


Fig4.4: Three mechanisms of ATP synthesis
Source: http://csls-text3.c.u-tokyo.ac.jp/inactive/16_01.html

The driving force for ATP synthesis is the H^+ gradient between the two sides of a membrane, which is generated by H^+ transport across the membrane using the energy from electron transport reactions in aerobic respiration and photosynthesis. Because H^+ has an electrical charge, the transport of H^+ across the biomembrane produces a membrane potential together with the concentration gradient. The combination of the two high-energy states is called a H^+ electrochemical gradient. This process is advantageous in that ATP can be synthesized by a common mechanism by using high-energy electrons extracted from a range of redox (reduction-oxidation) reactions.

ATP Generation

Glycolysis

- ❖ Conversion of glucose to pyruvate
- ❖ Net synthesis of 2 ATP by substrate level phosphorylation

Krebs Cycle

- ❖ Converts pyruvate to acetyl CoA & carbon dioxide
- ❖ 10 molecules of coenzymes NADH and 2 of $FADH_2$ are produced. Results in synthesis of 30 ATP and 4 ATP molecules, respectively in the respiratory chain.

Electron Transport (Respiratory) Chain

- ❖ The reduced coenzymes enter into the respiratory chain of the inner mitochondrial membrane
- ❖ Electron transport along the chain generates a proton electrochemical gradient and this is used to produce ATP

4.8. Summary

I this unit cover the metabolism of Carbohydrate and their relative by product in briefly. The production of ATP is discussed briefly in this unit that yield by different biochemical metabolic process. The process of glycolysis produces pyruvate or lactate along with the production of ATP. This process is cover of reactions and gives number of by product in their reactions. The glycolysis essential for brain which dependent on glucose for energy process. Glycolysis follow the pathway of glycolysis can be divided into three distinct phases: energy investment phase, splitting phase and energy generation phase. NADH and $FADH_2$ formed in glycolysis are an energy rich molecule each contains a pair of electrons having a high transfer potential. Production of ATP is occurs in process of glycolysis thus the process of glycolysis has significance role in metabolism living organisms. The Krebs cycle and electron transport system also generate the ATP. The driving force for ATP synthesis is the H^+ gradient between the two sides of a membrane, which is generated by H^+ transport across the membrane using the energy from electron transport reactions in aerobic respiration and photosynthesis. The total ATP produced in krebs cycle are 2+24+4=30 ATP. Thus the total ATP produced in Respiration are: Glycolysis + Krebscycle : 6 ATP + 30 ATP = 36 ATP**.**

4.9. Terminal questions

Q.1. What do you understand by phosphorylating? How it is useful in ATP generation.

Answer:----------

Q.2. Discuss briefly by about glycolysis process.

Answer:----------

Q.3. Discuss briefly about kerbs process and role in aerobic respiration.

Answer:----------

Q.4. Discuss briefly about oxidative phosphorylating and its role in metabdism of carbohydrate.

Answer:----------

Q.5. Discuss briefly electron transform system.

Answer:----------

Q.6. Write the mechanism of ATP synthesis.

Answer:----------

4.10. Further readings

1. David L. Nelson and Michael M. Cox, Lehninger Principles of Biochemistry 6th Edition
2. Jeremy M. Berg, John L. Tymockzo and Luber Stryer, Biochemistry 7th Edition
3. Reginald H. Garrett, Charles M. Grisham, Biochemistry by Reginald H Garrett 5th Edition .
4. U. Satyanarayana, U. Chakrapani, Biochemistry by U. Satyanarayana. 3rd Edition.

UNIT : 5

METABOLISM OF LIPIDS

Structure

5.1. Introduction

Metabolism is the set of life-sustaining chemical reactions in organisms. The three main purposes of metabolism are: the conversion of food to energy to run cellular processes; the conversion of food/fuel to building blocks for proteins, lipids, nucleic acids, and some carbohydrates; and the elimination of nitrogenous wastes. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The word metabolism can also refer to the sum of all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells, in which case the above described set of reactions within the cells is called intermediary metabolism or intermediate metabolism). Metabolic reactions may be categorized as catabolic - the breaking down of compounds (for example, the breaking down of glucose to pyruvate by cellular respiration); or anabolic - the building up of complex compounds (such as proteins, carbohydrates, lipids, and nucleic acids). Usually, catabolism releases energy, and anabolism consumes energy. The chemical reactions of metabolism are organized into

metabolic pathways, in which one chemical is transformed through a series of steps into another chemical, each step being facilitated by a specific enzyme.

Enzymes are crucial to metabolism because they allow organisms to drive desirable reactions that require energy that will not occur by them, by coupling them to spontaneous reactions that release energy. Enzymes act as catalysts - they allow a reaction to proceed more rapidly - and they also allow the regulation of the rate of a metabolic reaction, for example in response to changes in the cell's environment or to signals from other cells. The metabolic system of a particular organism determines which substances it will find nutritious and which poisonous. For example, some prokaryotes use hydrogen sulfide as a nutrient, yet this gas is poisonous to animals. The basal metabolic rate of an organism is the measure of the amount of energy consumed by all of these chemical reactions. A striking feature of metabolism is the similarity of the basic metabolic pathways among vastly different species. For example, the set of carboxylic acids that are best known as the intermediates in the citric acid cycle are present in all known organisms, being found in species as diverse as the unicellular bacterium Escherichia coli and huge multicellular organisms like elephants. These similarities in metabolic pathways are likely due to their early appearance in evolutionary history, and their retention because of their efficacy. During the breakdown of lipid metabolism involving or storage of fats for energy. The cholesterol biosynthesis pathway involves enzymes that are in the cytoplasm, microsomes (ER), and peroxisomes. The beta-oxidation is the catabolic process by which fatty acid molecules are broken down in the cytosol in prokaryotes and in the mitochondria in eukaryotes to generate acetyl-CoA.

Objectives

- To understand metabolism of lipids
- To the catabolism of triglycerides
- To know how cholesterol synthesize
- To understand beta-oxidation
- To understand the structure of triglyceride and its biosynthesis.

5.2. Lipids overview

Lipid sare diverse group of organic compounds including fats, oils, hormones, and certain components of membranes that are grouped together because they do not interact appreciably with water. One type of lipid, the triglycerides, is sequestered as fat in adipose cells, which serve as the energy-storage depot for organisms and also provide thermal insulation. Some lipids such as steroid hormones serve as chemical messengers between cells, tissues, and organs, and others communicate signals between biochemical systems within a single cell. The membranes

of cells and organelles (structures within cells) are microscopically thin structures formed from two layers of phospholipid molecules. Membranes function to separate individual cells from their environments and to compartmentalize the cell interior into structures that carry out special functions. So important is this compartmentalizing function that membranes, and the lipids that form them, must have been essential to the origin of life itself.

Fats, oils, hormones and certain components of living beings membranes are come under the category of lipids. The lipids provide thermal insulation to body of living organisms. The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids and prenol lipids. During the breakdown of lipid or storage fats used for energy. The cholesterol biosynthesis pathway involves enzymes that are in the cytoplasm, microsomes ER, and peroxisomes. The beta-oxidation is the catabolic process by which fatty acid molecules are broken down in the cytosol in prokaryotes and in the mitochondria in eukaryotes to generate acetyl-CoA.

Lipids are small biological molecules which are soluble in organic solvents, such as chloroform/methanol, and are sparingly soluble in aqueous solutions. They can be classified in a variety of ways. In one categorization, they can be divided into two major classes, saponifiable and nonsaponifiable lipids, based on their reactivity with strong bases. Saponifiable lipids contain long chain carboxylic (fatty) acids that are linked to an alcoholic functional group through an ester linkage. These fatty acids are released on based catalyzed ester hydrolysis. The nonsaponifiable classes include the fat-soluble vitamins (A, E) and cholesterol. Lipids are often distinguished from another commonly used word, fats. Some define fats as lipids that contain fatty acids that are esterified to glycerol.

It is defined as a water-insoluble biomolecule which has a high solubility in nonpolar organic solvents such as chloroform. The simplest lipids are the fats, which are triesters made up of one glycerol and three fatty acids. The term fats is also used as a general synonym for lipids, so the more precise terms triacylglycerols or triglycerides are preferable for the simplest lipids. Triacylglycerols are used primarily for energy storage in animals. More complex lipids, the phospholipids, glycolipids, and cholesterol, are the major constituents of biological cell membranes.

Water is the biological milieu-the substance that makes life possible-and almost all the molecular components of living cells, whether they will be found in animals, plants, or microorganisms, are soluble in water. Molecules such as proteins, nucleic acids, and carbohydrates have an affinity for water and are called hydrophilic (water-loving). Lipids, however, are hydrophobic (water-fearing). Some lipids are amphipathic-part of their structure is hydrophilic and another part, usually a larger section, is hydrophobic. Amphipathic lipids exhibit a unique behaviour in

water, they spontaneously form ordered molecular aggregates, with their hydrophilic ends on the outside, in contact with the water, and their hydrophobic parts on the inside, shielded from the water. This property is key to their role as the fundamental components of cellular and organelle membranes.

Although biological lipids are not large macromolecular polymers (e.g., proteins, nucleic acids, and polysaccharides), many are formed by the chemical linking of several small constituent molecules. Many of these molecular building blocks are similar, or homologous, in structure. The homologies allow lipids to be classified into a few major groups: fatty acids, fatty acid derivatives, cholesterol and its derivatives, and lipoproteins. This article covers the major groups and explains how these molecules function as energy-storage molecules, chemical messengers, and structural components of cells.

5.3. Types of Lipids

In biology and biochemistry, a lipid is a biomolecule that is soluble in nonpolar solvents.. Non-polar solvents are typically hydrocarbons used to dissolve other naturally occurring hydrocarbon lipid molecules that do not (or do not easily) dissolve in water, including fatty acids, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, and phospholipids. The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids have applications in the cosmetic and food industries as well as in nanotechnology. Scientists sometimes broadly define lipids as hydrophobic or amphiphilic small molecules. The amphiphilic nature of some lipids allows them to form structures such as vesicles, multilamellar/unilamellar liposomes, or membranes in an aqueous environment. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or building-blocks: ketoacyl and isoprene groups.

Using this approach, lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids,and polyketides (derivedfrom condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits). Although the term lipid is sometimes used as a synonym for fats. Fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as other sterol-containing metabolites such as cholesterol. Although humans and other mammals use various biosynthetic pathways, both to break down and to synthesize lipids. Some essential lipids can't be made this way and must be obtained from the diet.

- **Fatty acids**

Fatty acids, or fatty acid residues when they are part of a lipid, are a diverse group of molecules synthesized by chain-elongation of an acetyl-CoA primer with malonyl-CoA or methylmalonyl-CoA groups in a process called fatty acid synthesis. They are made of a hydrocarbon chain that terminates with a carboxylic acid group; this arrangement confers the molecule with a polar, hydrophilic end, and a nonpolar, hydrophobic end that is insoluble in water. The fatty acid structure is one of the most fundamental categories of biological lipids, and is commonly used as a building-block of more structurally complex lipids. The carbon chain, typically between 4 to 24 carbons long, may be saturated or unsaturated, and may be attached to functional groups containing oxygen, halogens, nitrogen, and sulfur. If a fatty acid contains a double bond, there is the possibility of either a cis or trans geometric isomerism, which significantly affects the molecule's configuration. Cis-double bonds cause the fatty acid chain to bend, an effect that is compounded with more double bonds in the chain. Three cis double bonds in 18-carbon α- linolenic acid ($C_{55}H_{98}O_6$). It is poly saturated fatty acids also called an omega-3 fatty acid, and is essential for all mammals in preventing and managing heart diseases. It helps to lower the blood pressure.

- **Glycerolipids**

Glycerolipids are composed of mono-, di-, and tri-substituted glycerols, the best-known being the fatty acid triesters of glycerol, called triglycerides. The word "triacylglycerol" is sometimes used synonymously with "triglyceride". In these compounds, the all three hydroxyl groups of glycerol are esterified, typically by different fatty acids. Because they function as an energy store, these lipids comprise the bulk of storage fat in animal tissues. The hydrolysis of the ester bonds of triglycerides and the release of glycerol and fatty acids from adipose tissue are the initial steps in metabolizing fat. Additional subclasses of glycerolipids are represented by glycosylglycerols, which are characterized by the presence of one or more sugar residues attached to glycerol via a glycosidic linkage. Examples of in this category are the digalactosyl and diacylglycerols found in plant membrane, and seminolipid from mammalian sperm cells.

- **Glycerophospholipids**

Glycerophospholipids, usually referred to as phospholipids (though sphingomyelins are also classified as phospholipids), are ubiquitous in nature and are key components of the lipid bilayer of cells, as well as being involved in physiological metabolism and cell signaling. Neural tissue (including the brain) contains relatively high amounts of glycerophospholipids, and alterations in their composition has been implicated in various neurological disorders. Glycerophospholipids may

be subdivided into distinct classes, based on the nature of the polar head group at the sn-3 position of the glycerol backbone in eukaryotes and eubacteria, or the sn-1 position in the case of archaebacteria. The popular fish like tuna, salmon and sardines are rich sources.

- **Sphingolipids**

Sphingolipids are a complicated family of compounds that share a common structural feature, a sphingoid base backbone that is synthesized de novo from the amino acid serine and a long-chain fatty acyl CoA, then converted into ceramides, phosphosphingolipids, glycosphingolipids and other compounds. The major sphingoid base of mammals is commonly referred to as sphingosine. Ceramides (N-acyl-sphingoid bases) are a major subclass of sphingoid base derivatives with an amide-linked fatty acid. The fatty acids are typically saturated or mono-unsaturated with chain lengths from 16 to 26 carbon atoms.

5.4. Metabolism of lipids

Lipid metabolism deals with the synthesis and degradation of lipids in cells, involving the breakdown or storage of fats for energy and the synthesis of structural and functional lipids, such as those involved in the construction of cell membranes. In animals, these fats are obtained from food or are synthesized by the liver. Lipogenesis is the process of synthesizing these fats. The majority of lipids found in the human body obtain from digesting food are triglycerides and cholesterol. Other types of lipids found in the body are fatty acids and membrane lipids. Lipid metabolism is often considered as the digestion and absorption process of dietary fat. However, there are two sources of fats that organisms can use to obtain energy, from consumed dietary fats and from stored fat.

Vertebrates (including humans) use both sources (plant and animal) of fat to produce energy for organs such as the heart to function. Since lipids are hydrophobic molecules, they need to be solubilized before their metabolism can begin. Lipid metabolism often the first step, begins with hydrolysis, which occurs with the help of various enzymes of the digestive system. The second step after the hydrolysis is the absorption of the fatty acids by the epithelial cells of the intestinal wall. In the epithelial cells, fatty acids are packaged and transported to the rest of the body. Lipid metabolism also occurs in plants, though the processes differ in some ways when compared to animals.

- **Lipid digestion**

Digestion is the first step to lipid metabolism, and it is the process of breaking the triglycerides into smaller monoglyceride units with the help of lipase enzymes. Digestion of fats begin in the mouth through chemical digestion by lingual lipase. Ingested cholesterol is not broken down by the lipase and stays intact until it enters the epithelium cells of small intestine. Lipids then continue to the stomach where chemical

digestion continues by gastric lipase and mechanical digestion begins (peristalsis). The major of lipid digestion and absorption, however, occurs when the fats reach within the small intestines. Chemicals from the pancreas (pancreatic lipase family and bile salt-dependent lipase) are secreted into the small intestines to help breakdown the triglycerides, along with further mechanical digestion until glycerol and free fatty acids unit are formed. The individual fatty acid units able are absorbed by the small intestine's epithelial cells. It is the pancreatic lipase that is responsible signaling for the hydrolysis of the triglycerides into separate free fatty acids and glycerol units.

Check your progress:

- ✓ What do you mean by cholesterol metabolism?
- ✓ Defineβ-Oxidation of fats.
- ✓ Define cholesterol with its structure.
- ✓ Write a short note on utilization of cholesterol.
- ✓ Define triglycerides with examples.

- ➢ **Lipid absorption**

The second step in lipid metabolism is the absorption of fats. Absorption of fats occurs only in the small intestines. Once the triglycerides are broken down into individual fatty acids and glycerols, along with cholesterol, they will aggregate into structures called micelles. Fatty acids and monoglycerides leave the micelles and diffuse across the membrane and enter the intestinal epithelial cells. In the cytosol of epithelial cells, fatty acids and monoglycerides are recombined back into triglycerides. In the cytosol of epithelial cells, triglycerides and cholesterol are packaged into bigger particles called chylomicrons which are amphipathic structures that transport digested lipids. Chylomicrons will travel through the bloodstream to enter adipose and other tissues in the body.

- ➢ **Transporting lipids**

Due to the hydrophobic nature of membrane lipids, triglycerides and cholesterols, they require special transport proteins known as lipoproteins. The amphipathic structure of lipoproteins allows the tryglycerols and cholesterol to be transported through the blood. Chylomicrons are one sub-group of lipoproteins which carry the digested lipids from small intestine to the rest of the body. The varying densities between the types of lipoproteins are characteristic to what type of fats they transport. For example, very-low-density lipoproteins (VLDL) carry the synthesized triglycerides by our body and low-density lipoproteins (LDL) transport cholesterol to our peripheral tissues. A number of these lipoproteins are synthesized in the liver, but not all of them originate from the organ.

➢ ***Tryglycerides catabolism***

Once the chylomicrons (or other lipoproteins) travel through the tissues, these particles will be broken down by lipoprotein lipase in the luminal surface of endothelial cells in capillaries to release triglycerides. Tryglycerides will then broken down into fatty acids and glycerol before entering cells and remaining cholesterol will again travel through the blood to the liver.

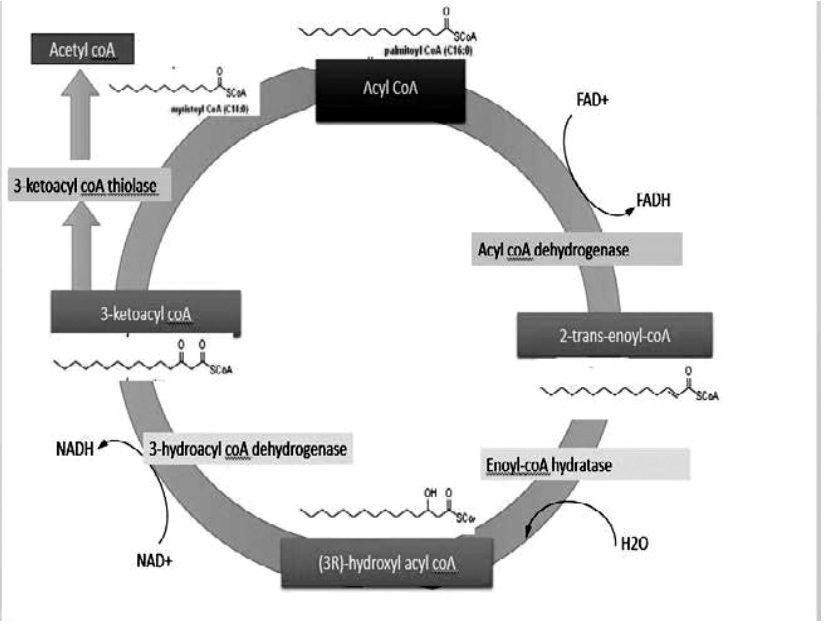


Fig.5.1:

Source:

In the cytosol of the cell (for example a muscle cell), the glycerol will be converted to glyceraldehyde 3-phosphate, which is an intermediate in the glycolysis, which is further oxidized and produce energy. However, the main steps of fatty acids catabolism occur in the mitochondria. Long chain fatty acids (more than 14 carbon) need to be converted to fatty acyl-CoA in order to pass across the mitochondria membrane.

Fatty acid catabolism begins in the cytoplasm of cells, as acyl-CoA synthetase uses the energy from cleavage of an ATP to catalyze the addition of coenzyme A to the fatty acid. The resulting acyl-CoA cross the mitochondria membrane and enter the process of beta oxidation. The main products, of the beta oxidation pathway are acetyl-CoA (which is used in the citric acid cycle to produce energy), NADH + H+ and FADH. The process of beta oxidation requires the following enzymes: acyl-CoA dehydrogenase 2,3 enoyl-CoA hydra, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase. The above figure shows how fatty acids are converted into acetyl-CoA. The overall net reaction, using palmitoyl-CoA (16:0) as a model substrate as under.

$$7\ FAD + 7\ NAD^{+} + 7\ CoASH + 7\ H_2O + H(CH_2CH_2)_7CH_2CO\text{-}SCoA \rightarrow 8\ CH_3CO\text{-}SCoA + 7\ FADH_2 + 7\ NADH + 7\ H^{+}$$

Check your progress:

- ✓ What do you mean by metabolism?
- ✓ Define lipids and its types.
- ✓ Define fatty acids with examples.
- ✓ Write a short note on sphingolipds.
- ✓ Explain catabolism of lipids.

5.5. Biosynthesis of lipids

In addition to dietary fats, storage lipids stored in the adipose tissues are one of the main sources of energy for living organisms. Triacylglycerols, lipid membrane and cholesterol can be synthesized by the organisms through various pathways.

❖ Membrane lipid biosynthesis

There are two major classes of membrane lipids: glycerophospholipids and sphingolipids. Although many different membrane lipids are synthesized in our body, pathways share the same pattern. The first step is synthesizing the backbone (sphingosine or glycerol). The second step is the addition of fatty acids to the backbone to make phosphatidic acid. Phosphatidic acid is further modified with the attachment of different hydrophilic head groups to the backbone. Membrane lipid biosynthesis occurs in the endoplasmic reticulum membrane.

❖ Triglyceride biosynthesis

The phosphatidic acid is also a precursor for triglyceride biosynthesis. Phosphatidic acid phosphotase catalyzes the conversion of phosphatidic acid to diacylglyceride, which will be converted to triacylglyceride by acyltransferase. Tryglyceride biosynthesis occurs in the cytosol.

❖ Fatty acid biosynthesis

The precursor for fatty acids is acetyl-CoA and it occurs in the cytosol of the cell. The overall net reaction, using palmitate (16:0) as a model substrate is as under.

8 Acetyl-coA + 7 ATP + 14 NADPH + 6H+ ----→ palmitate + 14 NADP+ + 6H2O + 7ADP + 7Pi

❖ Triglyceride

A triglyceride (TG, triacylglycerol, TAG, or triacylglyceride) is an ester derived from glycerol and three fatty acids (from tri- and glyceride). Triglycerides are the main constituents of body fat in humans and other vertebrates, as well as vegetable fat. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils.

There are many different types of triglyceride, with the main division between saturated and unsaturated types. Saturated fats are "saturated" with hydrogen-all available places where hydrogen atoms could be bonded to carbon atoms are occupied. These have a higher melting point and are more likely to be solid at room temperature. Unsaturated fats have double bonds between some of the carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. These have a lower melting point and are more likely to be liquid at room temperature.

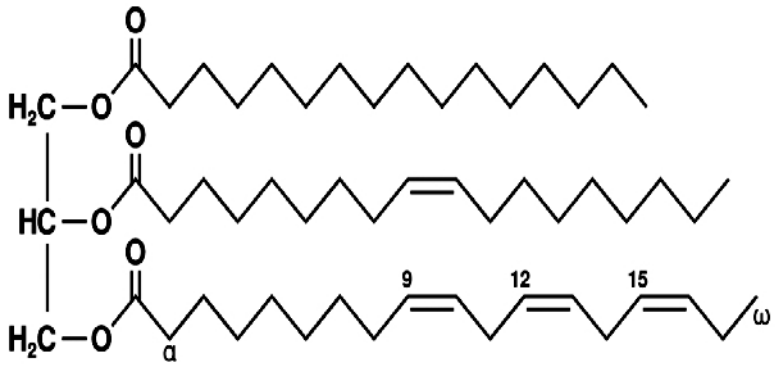


Fig. 5.2: An unsaturated fat triglyceride ($C_{55}H_{98}O_6$)

Chemical structure of Triglycerides

Triglycerides are tri-esters consisting of a glycerol bound to three fatty acid molecules. Alcohols have a hydroxyl (-HO) group. Organic acids have a carboxyl (-COOH) group. Alcohols and organic acids join to form esters. The glycerol molecule has three hydroxyl groups and each fatty acid has a carboxyl group. In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds:

HOCH2CH(OH)CH2OH + RCO2H + R′CO2H + RCO2H →
RCO2CH2CH(O2CR′)CH2CO2R + 3H2O

The three fatty acids (RCO2H, R′CO2H, RCO2H in the above equation) are usually different, but many kinds of triglycerides are known. The chain lengths of the fatty acids in naturally occurring triglycerides vary, but most contain 16, 18, or 20 carbon atoms. Natural fatty acids found in plants and animals are typically composed of only even numbers of carbon atoms, reflecting the pathway for their biosynthesis from the two-carbon building-block acetyl CoA. Bacteria, however, possess the ability to synthesise odd and branched chain fatty acids. As a result, ruminant animal fat contains odd-numbered fatty acids, such as 15, due to the action of bacteria in the rumen. Many fatty acids are unsaturated; some are polyunsaturated.

Most natural fats contain a complex mixture of individual triglycerides. Because of this, they melt over a broad range of temperatures. Cocoa butter is unusual in that it is composed of only a few triglycerides, derived

from palmitic, oleic, and stearic acids in the 1, 2, and 3 positions of glycerol, respectively.

5.6. Synthesis of triacylglycerols & catabolism(Lipolysis) in adipocytes

Adipose tissue and the adipocytes are characterized by accumulations of triacylglycerols, which acts as the main energy store for animals, although they also cushion and insulate the body. Thus, triacylglycerols stored when there is a surplus of nutrients are mobilized for energy production during starvation. Adipose tissue also functions as a reserve of bioactive lipids, such as eicosanoids and lipid-soluble vitamins, and when required provides structural components, including fatty acids, cholesterol and retinol, for membrane synthesis and repair. Large depots occur around internal organs such as the liver, and also subcutaneously. Brown and beige fat have special properties and are discussed below, while bone marrow adipocytes have distinctive functions also.

Similarly, within most other animal cells, even ganglia in the brain, a proportion of the fatty acids taken up from the circulation is converted to triacylglycerols as described above and incorporated into cytoplasmic lipid droplets (fat globules, oil bodies, lipid particles or adiposomes etc). By buffering against fatty acid accumulation that might exceed the capacity of non-adipose cells, they defend them against lipotoxicity while providing a rapid source of energy and essential metabolites. Acting in concert with other cellular organelles, they function in many different metabolic processes. The triacylglycerol droplets are surrounded by a protective monolayer that includes phospholipids, cholesterol and hydrophobic proteins. The phospholipid component of the monolayer consists mainly of phosphatidylcholine and phosphatidylethanolamine with fatty acid compositions distinct from those of the endoplasmic reticulum and plasma membrane.

Among the proteins are many that function directly in lipid metabolism, and they include acyltransferases, lipases, perilipins, caveolins and the Adipose Differentiation Related Protein (ADRP or adipophilin). In adipocytes, the lipid droplets can range up to 200 μm in diameter, while other cell types contain smaller lipid droplets of the order of 50 um in diameter. Cytosolic lipid droplets with similar metabolic activities are found in the fruit fly, *Drosophila melanogaster*, and in higher plants and yeasts (see below). Like adipose tissue cells, lipid droplets have a major function in that they sense and respond rapidly to changes in systemic energy balance. Within cells, lipid droplets facilitate the coordination and communication between different organelles of cell and act as vital hubs of cellular metabolism. They secrete important hormone-like molecules such as leptin, adiponectin and adipsin, and so influence food intake, insulin sensitivity, insulin secretion and related processes.

❖ Lipid droplet assembly

This process takes place in the endoplasmic reticulum, where at least one isoform of each of the enzymes of triacylglycerol biosynthesis, from acyl-CoA synthetases through to glycerol-3-phosphate acyltransferases, is located probably in a protein assembly or interactome. Triacylglycerols accumulate and so attract perilipins and other proteins that allow lipid droplets to grow as a lens-like swelling in patches of the membrane. A protein seipin stabilizes the nascent droplets with minimal disruption to the membrane and enables them to mature by a mechanism that is still uncertain but may involve regulation of protein and lipid trafficking into the droplet.

The growing lipid droplets bud toward the cytosol, a process that is believed to be directed and aided by surface proteins such as perilipin, while the triacylglycerol core attracts and is largely surrounded by phospholipids from the outer leaflet of the endoplasmic reticulum. Growth continues through an extended endoplasmic reticulum/droplet junction or bridge until finally with the involvement of membrane curvature-inducing coat proteins, trans-membrane (FIT) proteins that bind diacylglycerols, lysophospholipids and phosphatidic acid (non-bilayer forming lipids). The droplets bud off into the cytoplasm with their surface monolayer of phospholipids and proteins, including the enzymes of triacylglycerol biosynthesis. Subsequently, mitochondria, peroxisomes and other organelles may contribute or exchange lipids and effect changes in protein composition, although the lipid droplets remain close to the endoplasmic reticulum, and presumably this enables a dynamic response to any change in metabolic status sensed by the this organelle.

❖ Cholesterol metabolism

Cholesterol is an extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the steroid hormones, the bile acids, and vitamin D. Both dietary cholesterol, and that synthesized ***de novo***, are transported through the circulation in lipoprotein particles. The same is true for cholesteryl esters, the form in which cholesterol is stored in cells. Due to its important role in membrane function, all cells express the enzymes of cholesterol biosynthesis.

Cholesterol can be made from acetyl-CoA through a multiple-step pathway known as isoprenoid pathway. Cholesterols are essential because they can be modified to form different hormones in the body such as progesterone. 70% of cholesterol biosynthesis occurs in the cytosol of liver cells.

The synthesis and utilization of cholesterol must be tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. The particular clinical importance is the abnormal

deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries. Such deposition, eventually leading to atherosclerosis, is the leading contributory factor in diseases of the coronary arteries.

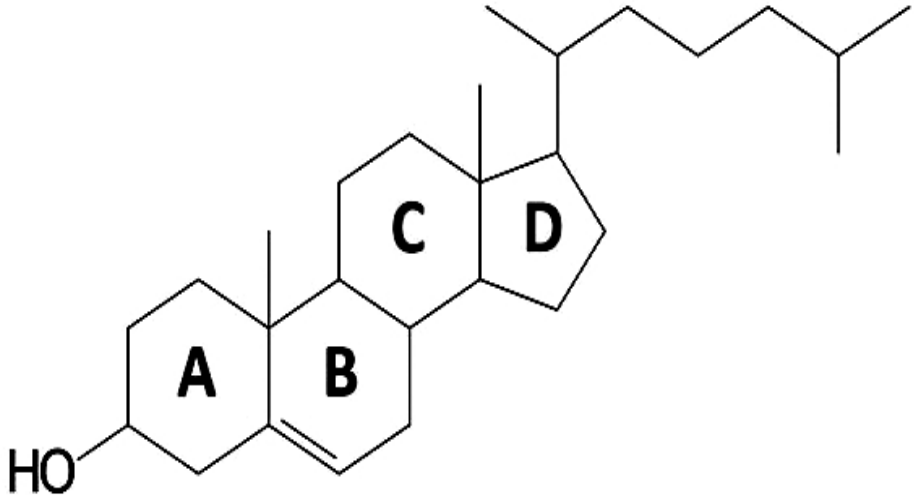


Fig. 5.3: Structure of cholesterol molecule

5.7. Biosynthesis of cholesterol

Slightly less than half of the cholesterol in the body derives from biosynthesis ***de novo***. Biosynthesis in the liver accounts for approximately 10%, and in the intestines approximately 15%, of the amount produced each day. The cholesterol biosynthesis pathway involves enzymes that are in the cytoplasm, microsomes (ER), and peroxisomes. Synthesis of cholesterol, like that of most biological lipids, begins from the two-carbon acetate group of acetyl-CoA. The initial steps in the pathway of cholesterol biosynthesis are collectively called the mevalonate pathway which itself culminates with the synthesis of the isoprenoid molecule, isopentenyl pyrophosphate (IPP).

The acetyl-CoA utilized for cholesterol biosynthesis is derived from an oxidation reaction (e.g., fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm by the same process as that described for fatty acid synthesis (see the Figure below). Acetyl-CoA can also be synthesized from cytosolic acetate derived from cytoplasmic oxidation of ethanol which is initiated by cytoplasmic alcohol dehydrogenase (ADH). All the reduction reactions of cholesterol biosynthesis use NADPH + H^+ as a cofactor. The isoprenoid intermediates of cholesterol biosynthesis can be diverted to other synthesis reactions, such as those for dolichol (used in the synthesis of ***N***-linked glycoproteins, coenzyme Q (of the oxidative

phosphorylation pathway) or the side chain of heme-***a***. Additionally, these intermediates are used in the lipid modification of some proteins.

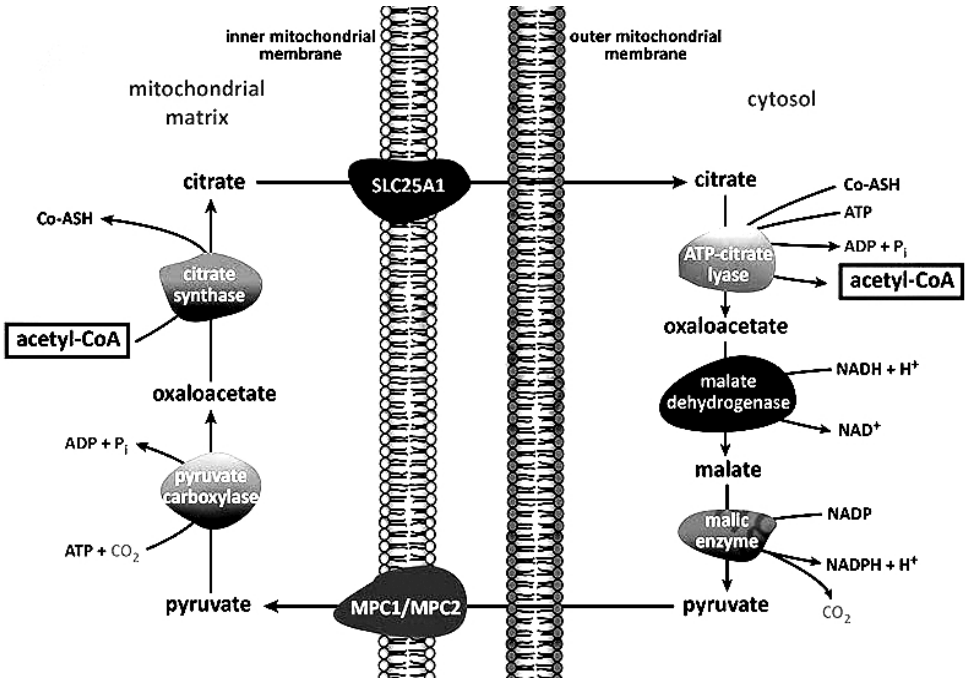


Fig. 5.4: **Pathway for the movement of acetyl-CoA units from within the mitochondrion to the cytoplasm.**

Source: http://themedicalbiochemistrypage.org/lipid-synthesis.php

The process of cholesterol synthesis can be considered to be composed of five major steps where the reactions that culminate in the synthesis of isopentenyl pyrophosphate, and its isomeric form dimethylallyl pyrophosphate, are commonly referred to as the mevalonate pathway.

- Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA).
- HMG-CoA is converted to mevalonate.
- Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP).
- IPP molecules are converted to squalene.
- Squalene is converted to cholesterol.

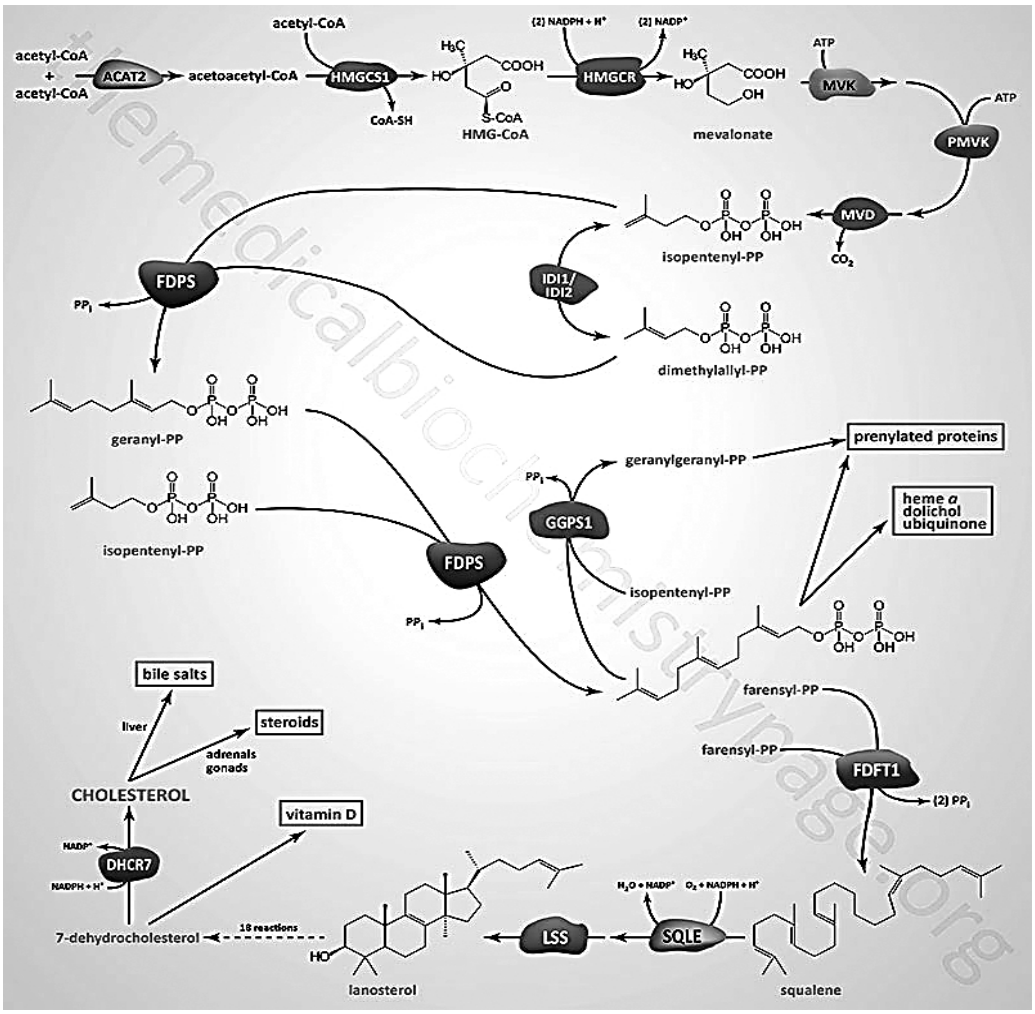


Fig. 5.5: **Pathway of cholesterol biosynthesis**
Source: https://themedicalbiochemistrypage.org/cholesterol.php

The Utilization of Cholesterol

Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with lipoproteins. Dietary cholesterol is transported from the small intestine to the liver within chylomicrons. Cholesterol synthesized by the liver, as well as any dietary cholesterol in the liver that exceeds hepatic needs, is transported in the serum within LDL (Low density lipoprotein). The liver synthesizes VLDL (Very Low density lipoprotein) and these are converted to LDL through the action of endothelial cell-associated lipoprotein lipase. Cholesterol found in plasma membranes can be extracted by HDL and esterified by the HDL (High density lipoprotein)-associated enzyme lecithin-cholesterol acyltransferase, LCAT. VLDL and LDL are considered as bad cholesterol and HDL id good cholesterol.

The cholesterol acquired from peripheral tissues by HDL can then be transferred to VLDL and LDL via the action of cholesteryl ester transfer protein (CETP) which is associated with HDL. **Reverse cholesterol transport** allows peripheral cholesterol to be returned to the liver in LDL. Ultimately, cholesterol is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids in the liver.

5.8. β-oxidation of fatty acids

In biochemistry and metabolism, beta-oxidation is the catabolic process by which fatty acid molecules are broken down in the cytosol in prokaryotes and in the mitochondria in eukaryotes to generate acetyl-CoA, which enters the citric acid cycle, and NADH + H^+ and $FADH_2$, which are co-enzymes used in the electron transport chain. It is named as such because the beta carbon of the fatty acid undergoes oxidation to a carbonyl group. Beta-oxidation is primarily facilitated by the mitochondrial trifunctional protein, an enzyme complex associated with the inner mitochondrial membrane. Although, very long chain fatty acids are oxidized in peroxisomes.

Fatty acids can be oxidised to Co_2 and H_2o with the production of large amount of ATP, NADH + H^+ and $FADH_2$. There are two mechanism viz. Alpha (α) oxidation and Beta (*β*) oxidation of fatty acid. The *β* oxidation is summarized in this unit.

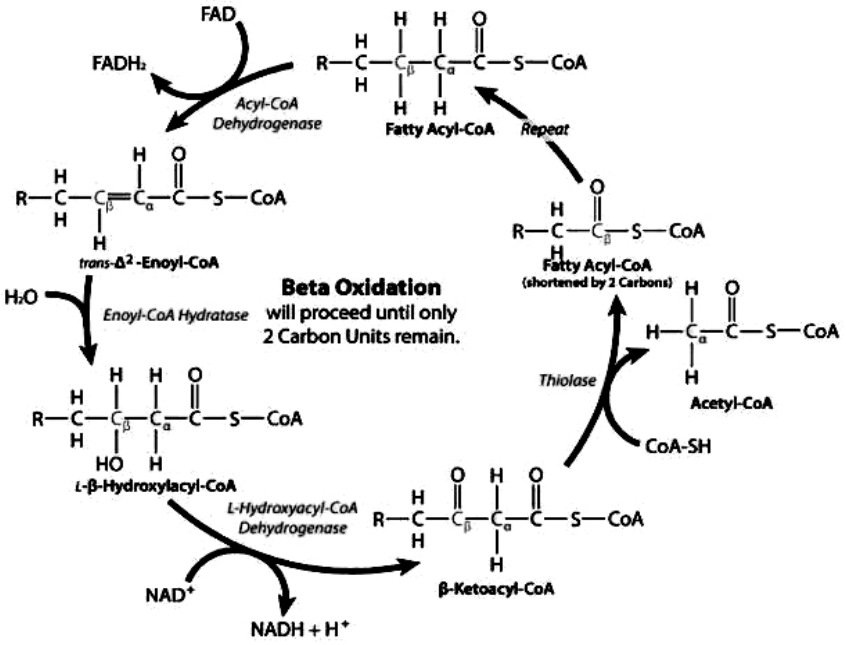


Fig. 5.6: Fatty acid oxidation

Source:https://bio.libretexts.org/Bookshelves/Biochemistry/Book%3A_Biochemistry_Free_and_Easy_(Ahern_and_Rajagopal)/06%3A_Metabolism_I/6.11%3A_Fatty_Acid_Oxidation

The overall reaction for one cycle of beta oxidation is:

$$C_n\text{-acyl-CoA} + FAD + NAD^+ + H_2O + CoA \rightarrow C_{n-2}\text{-acyl-CoA} + FADH_2 + NADH + H^+ + \text{acetyl-CoA}$$

- The fatty acids are activated, reacting with coenzyme A and ATP to form its Co-A derivative. The reaction is catalyzed by enzyme-thiokinase present in outer mitochondrial membrane.

- The second step involves removal of 2H atoms between α and β, unsaturated fatty acyl Co- A in presence of enzyme-acyle-Co- A dehydrogenase.
- A molecule of H_2O is added to trans-α-β-unsaturated fatty acyl Co- A across the double bond to form β-hydroxyacyl Co-A in presence of enzyme enoyl-CoA hydratase.
- The β-hydroxy acyle Co- A is dehydrogenated to form β-keto fatty acyl Co-A. NAD^+ is reduced in this step. The reaction is catalysed by enzyme-dehydrogenase. The β-C atom now bears a carbonyl function (β-oxidation).
- The β-keto fatty acyle Co- A finally undergoes thioclastic cleavage in presence of enzyme β-keto acyle thiolase to release a molecule of acetyl Co- A (2 C-unit) and a molecule of fatty acyl Co-A.

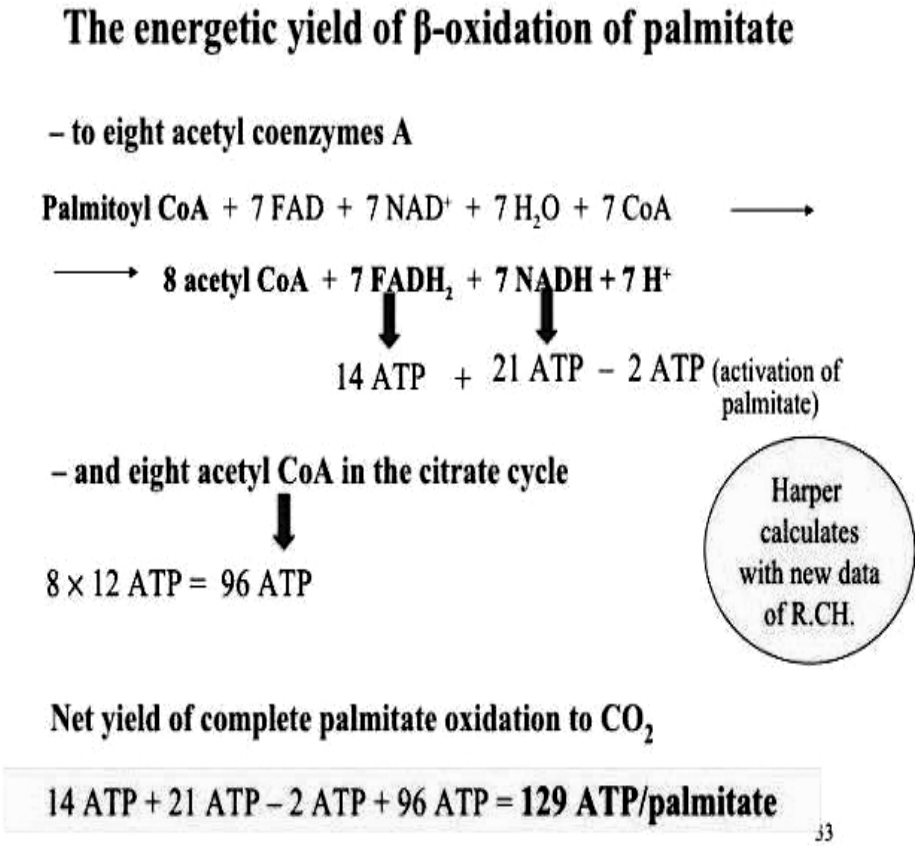


Fig.5.7: β- oxidation of palmitate

The fatty acyl Co-A produced in the reaction is shorter by 2 C atoms. It re-enters the β-oxidation spiral until 2 more carbon atoms are split out as acetyl Co-A. In this way, the long chain fatty acid released step by step 2C units and finally degraded to acetyl Co-A molecules. For example, the complete β-oxidation of one molecule of palmitate (16 carbon acid)

results in the formation of 8 acetyl CoA molecules as shown in the following summary equation.

The acetyl Co-A molecules enter the reactions of Krebs cycle and are completely oxidised to CO_2 and H_2O to release energy. During germination of fatty seeds the molecules of acetyl Co-A are converted into soluble sucrose through the glyoxylic acid cycle.

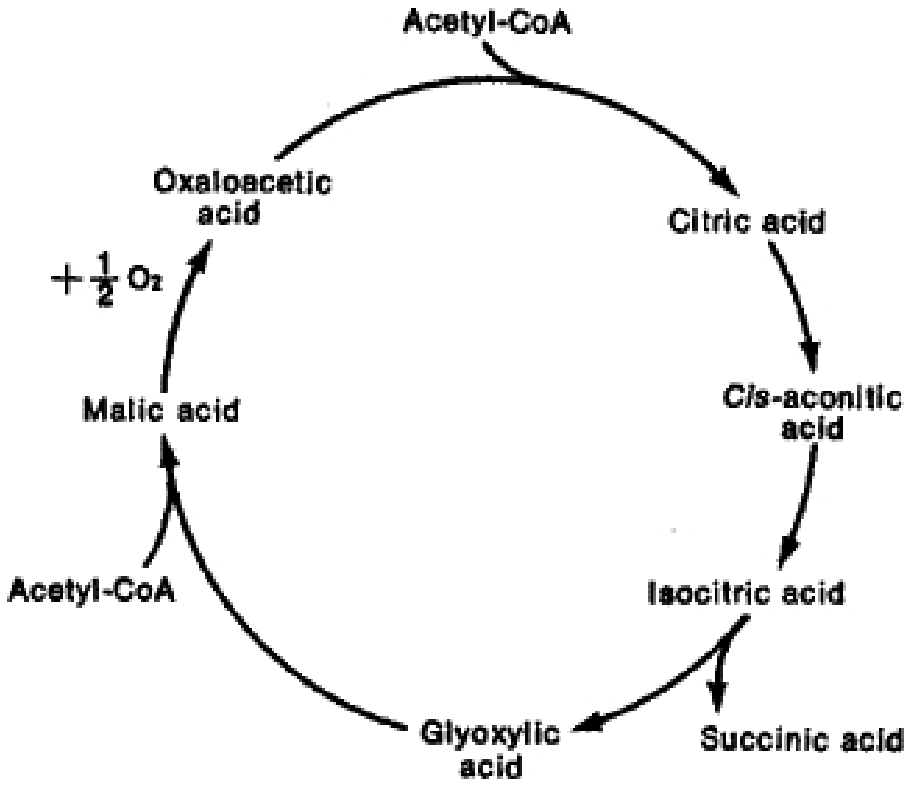


Fig. 5.8: The glyoxylate cycle

5.9. Summary

Fats, oils, hormones and certain components of living beings membranes are come under the category of lipids. The lipids provide thermal insulation to body of living organisms. Triglycerides a form of lipid is sequestered as fat in adipose cells that serve as the energy-storage depot for organisms. The membranes of cells and organelles are microscopically thin structures formed from lipids which separate individual cells from their environments and to compartmentalize the cell interior into structures that carry out special functions. The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids may be divided into eight categories: Fatty acids, Glycerolipids, Glycerophospholipids Sphingolipids, Saccharolipids, Polyketides , Sterol lipids and, Prenol lipids.

5.10. Terminal questions

Q.7. Describe lipids and their types.

Answer:----------

Q.8. Write a short note on fatty acids and glycolipids.

Answer:----------

Q.9. Discuss metabolism of lipids.

Answer:----------

Q.10. Describe lipid biosynthesis.

Answer:----------

Q.11. Explain biosynthesis of cholesterol and its utilization.

Answer:----------

Q.12. Describe β-oxidation of fatty acids types.

Answer:----------

Q.13. Explain catabolism of triglycerides.

Answer:----------

5.11. Suggested readings

1. Lehninger- Principles of Biochemistry- David L. Nilson and Michael M. Cox, WH Freeman; 7th ed. 2017 edition
2. Biochemistry –J.H.Weil
3. J L Jain et al., Fundamentals of Biochemistry; S Chand; Seventh edition ,2016
4. Voet D and Voet J.G., Biochemistry", 4th Edition,2010
5. U. Satyanarayana, Biochemistry; Elsevier India; 5 edition, 2017.
6. TMH-Instant Notes of Biochemistry-2nd Edition

UNIT : 6

NITROGEN METABOLISM

Structure

6.1. Introduction

Nitrogen is essential element for plant growth, development and production. It is a major component of chlorophyll, the most important pigment needed for photosynthesis, as well as amino acids, the key building blocks of proteins. It is also found in other important biomolecules, such as ATP and nucleic acids. Biological nitrogen fixation provides a natural means of supplying nitrogen for plants. It is an important part of many aquatic, as well as terrestrial ecosystems across our biosphere. This element is abundantly available in the earth's atmosphere

in the form of dinitrogen (N_2) gas, yet most organisms are unable to metabolize atmospheric N_2 because it is relatively inert. Hermann Hellriegel (1831-1895), a noted German agricultural chemist, discovered that leguminous plants took atmospheric nitrogen and replenished the ammonium in the soil through, the process now known as nitrogen fixation. In the process of amino acid degradation the nitrogen containing amino acid is ultimately converted to ammonia and excretes form body of living organism. Most of amino acids degradation occurs in liver while muscles also participate in the degradation of some other amino acids. We know during the nitrogen metabolism ammonia is released that is very toxic for living being. This ammonia is rapidly by converted into urea which takes place in the mitochondria of living cells. The urea forms, then enters the blood stream, is filtered by the kidneys and is ultimately excreted in the urine. Urea is the end product of protein metabolism.

Objective

- To know the process of nitrogen fixation.
- To know of biological organism in nitrogen fixation and assimilation
- To know the steps involve in urea cycle
- To know the process of amino acid degradation
- To know about chlorophyll and its structure and uses

6.2. Nitrogen fixation

Nitrogen is one of the more abundant elements in atmosphere plants cannot use nitrogen directly from the atmosphere and can only utilize reduced fixed form of this element. Therefore, nitrogen fixation, whether natural or synthetic, is necessary for all forms of plants. Fixation of atmospheric nitrogen the by micro organism through a reductive process into ammonia is called as biological nitrogen fixation. The number of prokaryotic organism has the ability to reduce the atmospheric N_2. The biological nitrogen fixation accounts for about 70% of the total N fixed in the biosphere. Nitrogen is an important element for all living organisms. Higher plant requires nitrogen in the form of nitrates. Ammonium and some organic nitrogenous compounds are also utilized to a lesser extent. Nitrogen is continuously decreasing in soil by the action of denitrifying bacteria and continuously increasing to the cycle through the action to nitrogen fixing microorganisms, light and artificial fertilizers. Microorganism plays a significance role in the operation of this nitrogen cycle by biological nitrogen fixation, ammonification, nitrification and denitrification. However, there are different process is found in biosphere that play an significant role in nitrogen fixation which are followings

- Atmospheric nitrogen fixation
- Industrial nitrogen fixation
- Biological nitrogen fixation

6.2.1. Atmospheric Nitrogen Fixation:

The atmospheric nitrogen fixation is carried out at certain atmospheric conditions through the electrical discharge in the troposphere and by cosmic radiation in the stratosphere. The atmospheric nitrogen fixation account about 10% of total nitrogen fixation.

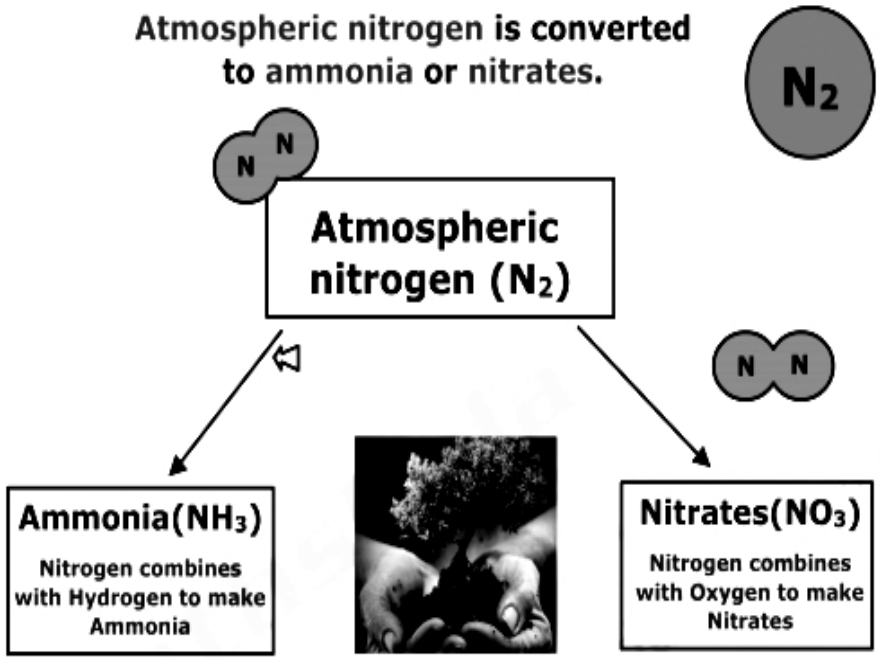


Fig.6.1: atmospheric nitrogen fixation

In atmosphere nitrogen molecules breaks down into nitrogen atoms, the nitrogen atoms combine with O_2 and formed nitrogen oxides. The nitrogen oxides when comes the contact with water, it forms nitrous acid (HNO_2) and nitric acid (HNO_3).

$N_2 + O_2$ Lightning → Thunder 2NO (Nitric Oxide)

$2NO + O_2 \rightarrow 2NO_2$ Oxidation (Nitrogen peroxide)

When nitrous acid or nitric fall on the soil along with rain water they react with the alkaline radicals of soil to form water soluble nitrates that are directly absorbed by plants.

$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$, HNO_3 + Ca or K salts → Ca or K nitrates

6.2.2. Industrial Nitrogen Fixation

The Haber-Bosch process: This process directly synthesizes ammonia from nitrogen and hydrogen. In 1909, the German chemist named Fritz Haber ascertained that atmospheric nitrogen could be combined with hydrogen under extremely high temperature and pressure condition which is catalyzed by an iron catalyst to yield an extremely high proportion of

ammonia, which is the starting point for the production of a wide range of nitrogen compounds. This process was made commercially feasible by Carl Bosch and now called as the Haber-Bosch method or the synthetic ammonia process. The Haber-Bosch process is now one of the largest and most-basic processes of the chemical industry throughout the world (Fig 6.2).

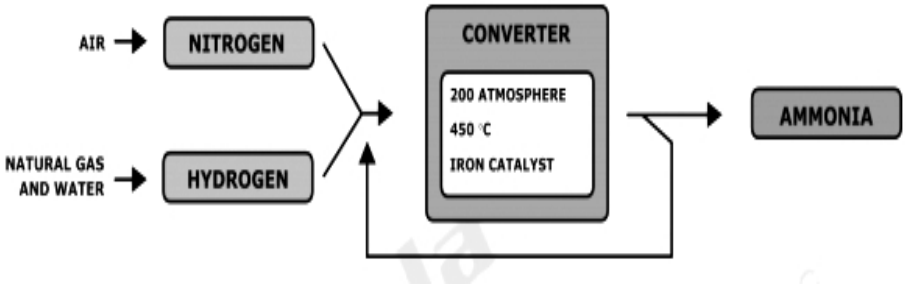


Fig.6.2: The Haber - Bosch process

6.2.3. Biological nitrogen fixation

In the biological nitrogen fixation the atmospheric nitrogen is converting in to nitrogenous compound by microorganisms. It can be divided into non symbiotic and associative symbiotic nitrogen fixation. Biological nitrogen fixation (BNF) was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. BNF contributes about 60% of the total N2 fixed in the biogeochemical nitrogen cycle. BNF is therefore called a key for sustenance of agriculture and reduction in soil fertility decline. These organisms utilize an enzyme called nitrogenase which catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Two kinds of nitrogen-fixing bacteria are known: free-living or non-symbiotic bacteria, including the cyanobacteria (or blue-green algae), Anabaena and Nostoc and genera such as Azotobacter, Beijerinckia, and Clostridium and mutualistic or symbiotic bacteria such as Rhizobium, associated with leguminous plants and certain Azospirillum species, associated with cereal grasses. Biological nitrogen fixation can be represented by the equation, in which two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions). Therefore, this fixation is costly process.

$$N_2 + 8H+ + 8e- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi$$

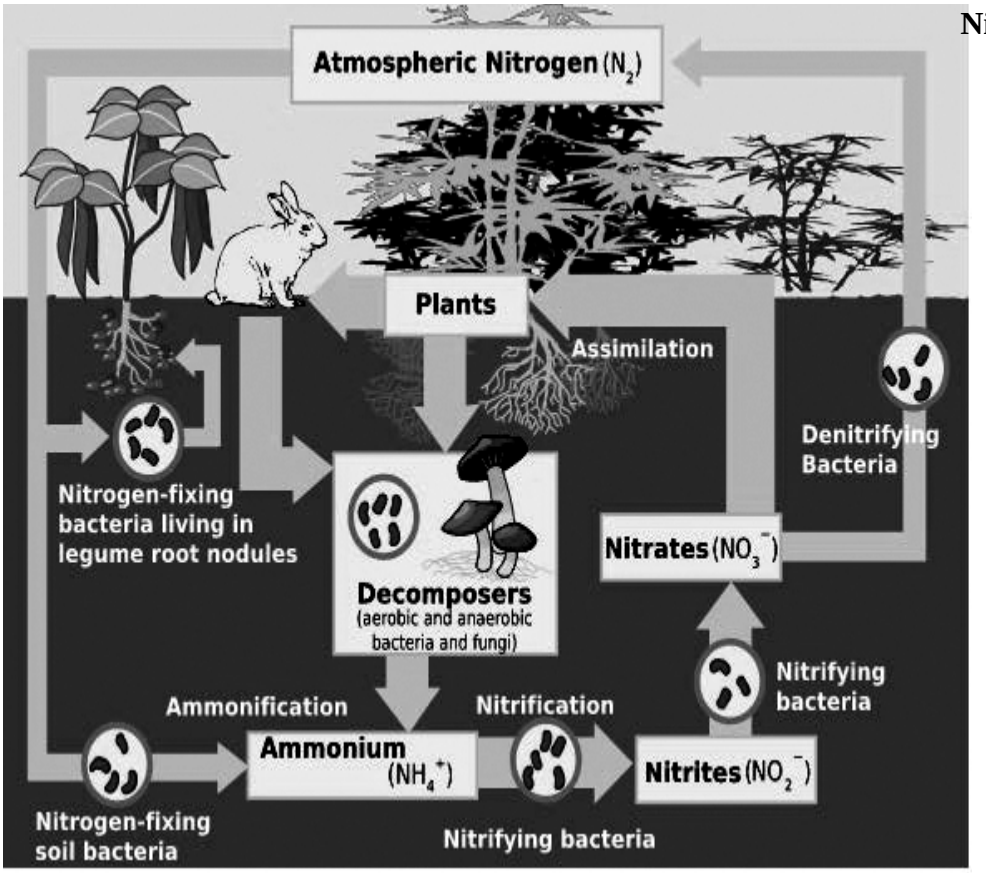


Fig. 6.3: The role of soil bacteria in the nitrogen cycle

Source: https://courses.lumenlearning.com/boundless-microbiology/chapter/nitrogen-fixation/

6.2.3.1. Non symbiotic nitrogen fixation

- Non symbiotic nitrogen fixation is done by free-living (nonsymbiotic) bacteria, includes the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and genera such as *Azotobacter*, *Beijerinckia*, and *Clostridium*
- In the roots of grass and cereal plants no nodules are formed like symbiotic bacteria. The bacteria azospirillum bransilense, pseudomonas, Bacillus grow in the rizosphere in close contact with the root and involved in the other cortical regions of the roots and fix nitrogen

6.2.3.2. Symbiotic nitrogen fixation

- Nitrogen is also fixed by microbes symbiotically including such as *Rhizobium*, associated with leguminous plants (e.g., various members of the pea family); *Frankia*, associated with certain dicotyledonous species (actinorhizal plants); and certain *Azospirillum* species, associated with cereal grasses.

- Root nodules are also found in the certain non leguminous plants which also fix nitrogen e.g. In alder and alnus the actinomyceties is involved in nodules and fix nitrogen quite efficiently and play significant role on the nitrogen balance of some forest ecosystem.

6.2.3.3. Associative symbiotic nitrogen fixation

- Nitrogen is also fixed by microorganism through non nodulation for example, the cyanobacteria, Anabaena azollae forms symbiotic association with Azolla Nostoc is found in the stem of Gunnera macrophylla. Azotobacctor paspali develops colonies below mucilaginous shealth of paspaum rotatum and fix atmospheric nitrogen.

- The process of N_2 fixation occurs in nodule is mediated by the enzyme , called nitrogenase and leghaemoglobin (which mediates the reduction of N_2 to ammonia) firstly, this enzyme was extracted from the anaerobic di nitrogen fixer Clostridum pasteurianum.

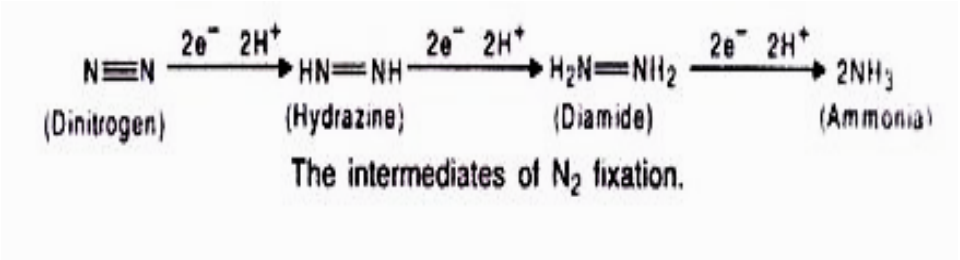
- The nitrogenase has 2 components i.e. Mo-Fe protein (molybdoferredoxin) and Fe-protein (azoferredoxin).The nitrogenase catalyzes the conversion of atmosphere di-nitrogen (N_2) to $2NH_3$. The ammonia is the first stable product of nitrogen fixation.

Latter, this enzyme has been isolated from most of other N_2 fixing bacteria.

The mechanism of N_2 fixation appears to be quite similar in most N_2 fixing prokaryotes. The enzyme has been fairly well characterized and the enzyme from these different systems share common properties allowing a unified single description of nitrogenase.

$$N_2 + 8_{e-} + 8H^+ \; 16\,ATP \xrightarrow[\text{nitrogenase}]{Mg^{2-}} 2NH_3 + H_2 \quad 16ADP + 16P_i$$

During nitrogen fixation, the free di-nitrogen first bound to MoFe protein and is not released until completely reduced to ammonia. The reduction of di-nitrogen is a stepwise reaction in which many intermediates are formed to form ammonia (NH_3) which is protonated at physiological pH to form $NH4^+$



The intermediates of N_2 fixation.

6.3. Nitrogen Assimilation

Nitrogen assimilation is the formation of organic nitrogen compounds like amino acids from inorganic nitrogen compounds present in the environment. In this process Nitrogen fixed by plant is converted into organic molecules such as protein, DNA, RNA etc. Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium ($NH4^+$). Ammonium ions are absorbed by the plant via ammonia transporters.

6.3.1. Ammonification

N_2 fixation results in NH_4^+ formation which reacts with organic acids and form amino acids that is mediated by ammonia assimilating enzyme.

GS–Glutaminesynthetase
GOGAT–Glutamatesynthese
GDH – Glutamate dehydrogenase

Protein and nucleic acid of dead plants and animal residue or excreted products of animal are degraded by microorganism with the liberation of ammonium. This is called ammonification. For this process, two steps are involved.

a. Proteolysis

$$\text{Protein} \longrightarrow \text{Amino acids}$$

Proteinase enzyme

$$\text{Protein} \rightarrow \text{Peptides} \longrightarrow \text{amino acids}$$

Clostridium, actinomycities, pseudomonas and many fungi cause proteolysis.

b. Amino acid degradation

Amino acids are degraded by microbial activity and ammonia is released.

$$\text{Alanine} + \frac{1}{2}O_2 \xrightarrow[\text{deaminase}]{\text{analine}} \text{Pyrubic acid} + NH_3$$

6.3.2. Nitrification:

Plants cannot take ammonia directly therefore; ammonia is converted rapidly (biological oxidation) to nitrate or nitrite by microbial activity by the process of nitrification. In the process of nitrification ammonia is firstly converted into nitrite by *nitrosomonas.* The nitrite is oxidized into nitrate by nitrogenous bacteria. However, different microbes are responsible for each steps of nitrification in marine environment.

6.3.3. Denitrifications

Transformation of nitrate to nitrogen gas by microorganism is called denitrification. A denitrification bacterium (micrococcus denitrificans) lives in deep in the soil and they like to live in oxygen free medium.

Denitrification is reverse of nitrogen fixation. It decreases soil fertility and reducing agriculture productivity.

Factors limiting Biological Nitrogen Fixation

There are three main factors effecting the process of biological nitrogen fixation these are:

- ❖ Edaphic factors for example extensive soil moisture, drought, salinity and Deficiency of P, Ca, Mo.
- ❖ Climatic factors for example extreme temperature and availability of sun light
- ❖ Biotic factors for example excessive defoliation of host plant, Crop competition, insects and nematodes

6.4. Urea Cycle

Ammonia produced is toxic to the animal include human body. Hence, if it is not reused to synthesize new amino acids or other nitrogen containing compounds, it is excreted out of the body as urea. This process of formation of urea occurs via the urea cycle for most terrestrial animals, also known as ureotelic species.

Ammonia is converted to urea in the hepatocytes of the liver in five steps via urea cycle in the mitochondria (first 2 steps) and cytosol (last 3 steps). The urea then travels through the blood stream to the kidney and is excreted out within the urine. The urea cycle involves a series of biochemical steps where nitrogen as a waste product of protein metabolism is removed from the blood and converted to a compound called urea in the blood. Normally, the urea is transferred into the urine and removed from the body. The urea cycle was discovered by Hans Krebs (who also discovered Citric acid or Krebs cycle) and his student associate Kurt Henseleit in 1932.

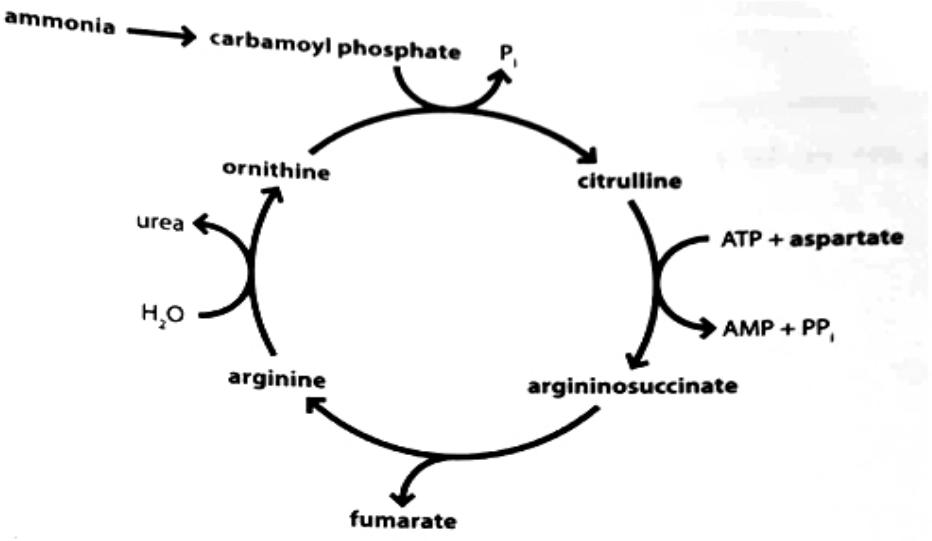
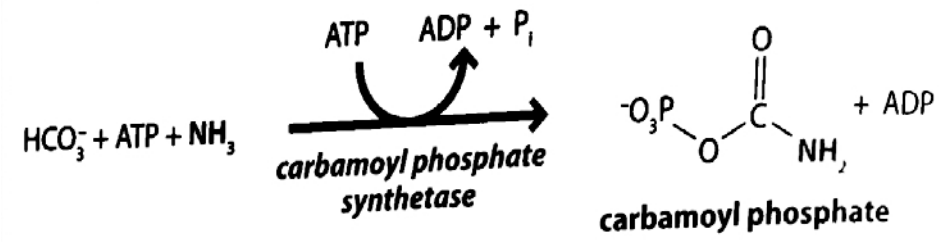


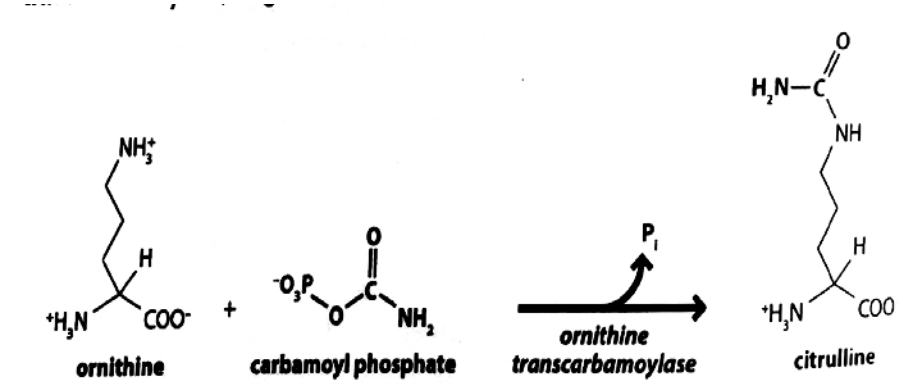
Fig. 6.4: Urea Cycle

1. Synthesis of carbamoyl phosphate : This step takes place in the mitochondria of the liver cells. Here the ammonium ions react with carbon dioxide (product of mitochondrial respiration) to form carbamoyl phosphate catalyzed by the enzyme carbamoyl phosphate synthetase I. This is an irreversible, rate-limiting, ATP-dependent reaction and consumes 2 ATP. Carbamoyl phosphate synthetase I (in mitochondria) is different from carbamoyl phosphate synthetase II (in cytosol) as the latter one has a different role to play and is involved in pyrimidine synthesis.

what is unique about this step is carbamoyl phosphate synthetase I, requires an obligate activator, N-acetyl-glutamate (NAG). NAG arises from glutamate and Acetyl-CoA via the enzyme NAG synthase, which can be upregulated by arginine. Of note, some sources may use NH and HCO+ as the initial reactants, but these are equivalent to CO+ HO + NH.

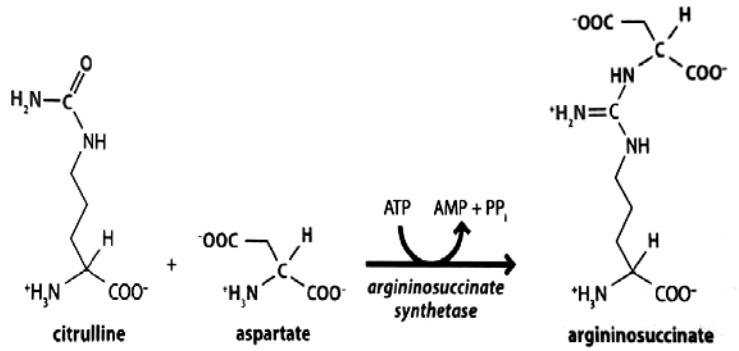


2. Synthesis of citrulline: Carbamoyl phosphate produced in the first step reacts with ornithine in the presence of ornithine transcarbamoylase to synthesize citrulline. Just like oxaloacetate in Kreb's cycle, ornithine plays a similar role acting as accepting substrate at each turn of the cycle. Via a transporter system this citrulline is now transferred to the cytosol of the liver cells.

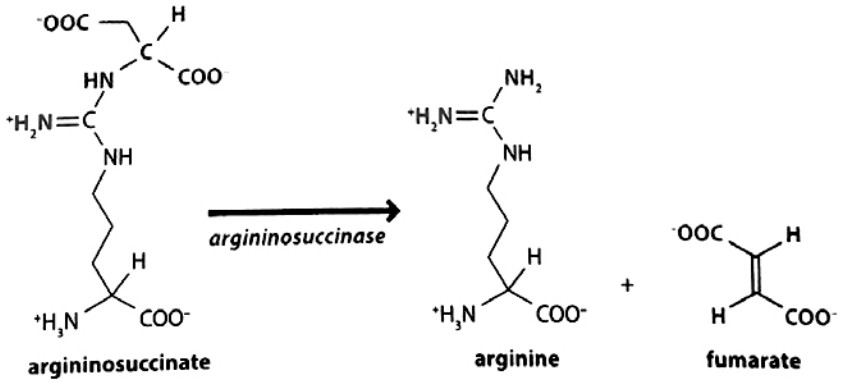


3. Formation of arginosuccinate: In this ATP dependent step, the carbonyl carbon of citrulline is attacked by the lone pair of the amine in aspartate to produce arginosuccinate in presence of argininosuccinate

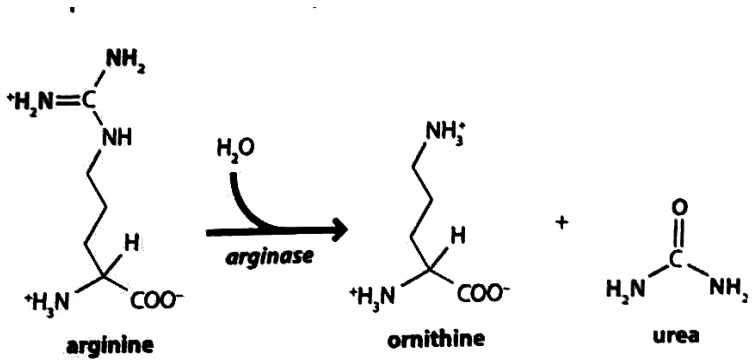
synthetase. In this step, the second nitrogen of urea is incorporated by condensation. ATP is broken down into AMP and pyrophosphate.



4. Breakdown of arginosuccinate: Arginosuccinase promotes the cleavage of arginosuccinate to give raise arginine and fumarate in a reversible manner. Fumarate formed here joins the citric acid cycle forming a link between urea cycle and citric acid cycle.



5. Formation of urea: Arginine produced in the earlier step is broken down by arginase to give urea and ornithine. Ornithine is recycled back to the mitochondria for the next cycle.



As we discussed, five enzymes took part in the formation of urea. Out of these the first four are found in all cells. But the last enzyme arginase is found only in the liver cells, thus assuring the formation of final product urea only in the liver despite the formation of arginine in other tissues. The liver is the only site where urea is synthesized and ultimately excreted by the kidneys.

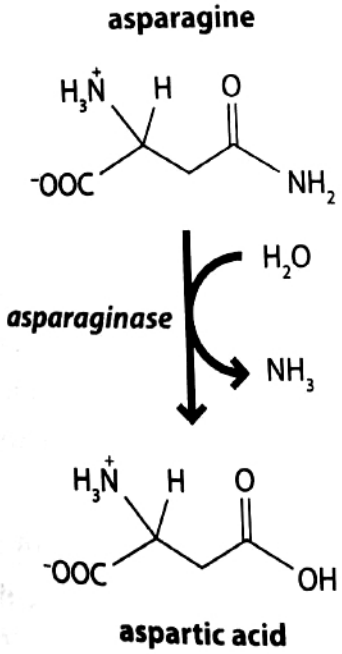
A urea cycle disorder is a genetic disorder caused by a mutation that results in a deficiency of one of the five enzymes of the urea cycle. These enzymes are responsible for removing ammonia from the blood stream.

6.5. Amino acid metabolism

We know that proteins are building block of our body, and the proteins are synthesized by the amino acid. The amino acids are nitrogenous compounds which consist of amino groups and carboxylic group. Plant and microorganism can synthesis all standard 20 amino acids in which some are essential and some are non essential. We have already discussed about types, amino acid structure and function in previous unit. Let us see the degradation of amino acids. Amino acid degradation, the nitrogen of the amino acid is ultimately converted to ammonia and excretes form body of living organism. Most of amino acids degradation occurs in liver while many amino acids like isoleucine, leucine and Valine are broken drown in muscles. In living organism, there is no store for excess of amino acid, because proteins are constantly turns over. Thus amino acids have to be continuously degraded. During brake down of amino acids, α- amino group of amino acid molecules change into carbon skelton, because the α- amino group of amino acids is removed. This carbon skeleton also going to convert into other metabolic intermediate product that would be useful in metabolic fuel. There are several pathway have adopted by standard 20 amino acids because of it broken complexity. These 20 amino acids have several pathway and finally produce in to seven product such as Pyruvate, acetyl coA, acetoacetyl coA, ∝-ketoglurate, succiny CoA, fumurate and oxilate shown in fig 6.5.

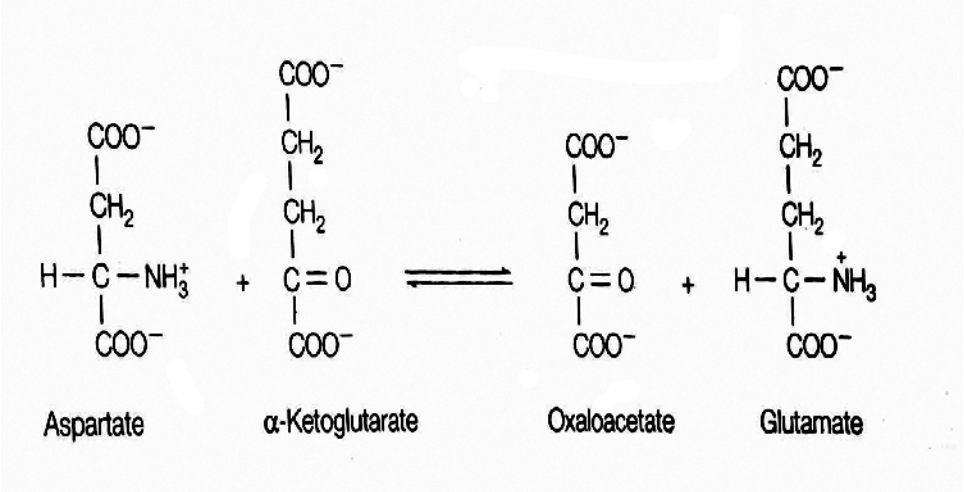
In amino acid degradation the side chain nitrogen reat with specific enzymes, for example asparagines remove the amide group from Asparagine and given raise new carbon skelton as aspartic acid and also produce ammonia. Thus the other amino acids are also deals by other enzymes. The removal of amino group from amino acid can we understood by more uniformed process involving transamination. Which is take place in liver of mammals. For each amino acid, this transamination moves the amino group to ∝-ketoglutrate generally known as glutamate. The amino acids that are degraded into Pyruvate, ∝-ketoglutarate succinyl coA, fumarate and oxalate are refers to glucogeic

that can we useful for next synthesis of glucose. However, other degraded product of amino acid such as acetyl coA or acetoacetyl coA are refers to ketogeic, because they give rise ketone body.



Transamination:

It is the first step to degradation of amino acid, where the ∝-amino group of amino acid is removed and formed new metabolic carbon skeleton. This process is reversible in nature. In this process the ∝-amino group of most of amino acids is transferred to ∝-ketoglutrate to form glutamate and corresponding ∝-keto acid. The enzymes that catalyze the reaction are called transaminases.



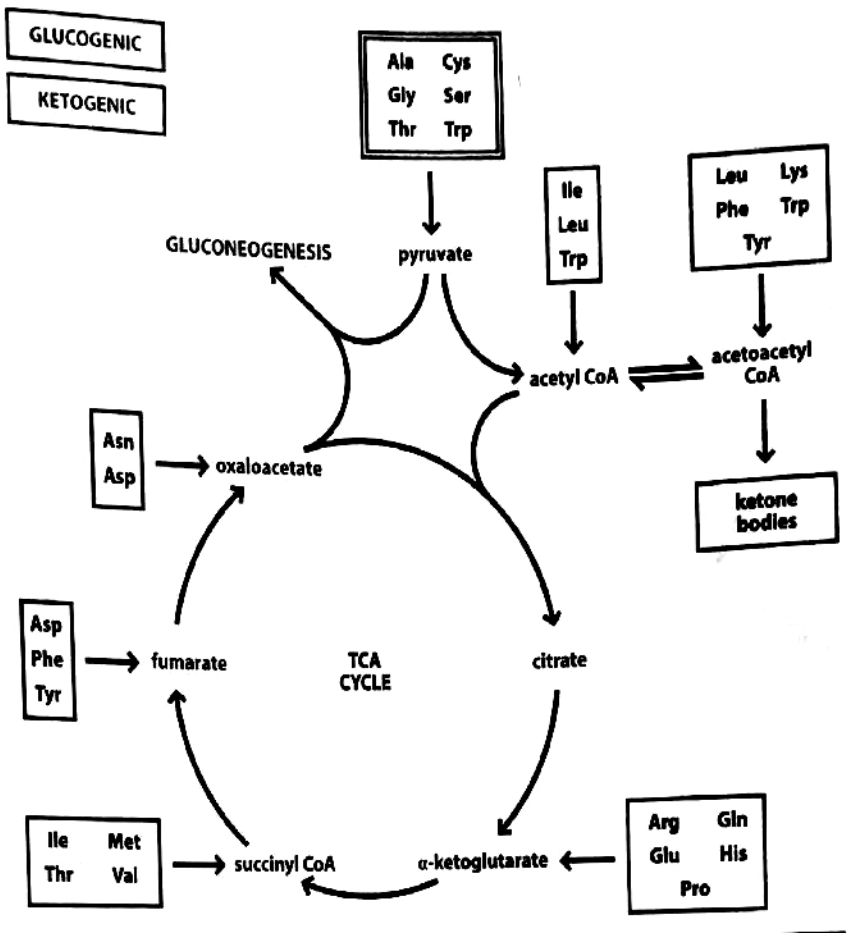


Fig.6.5: Formation of TCA

6.6. Chlorophyll

Chlorophyll is a naturally occurring molecule that gives plants their green color and is responsible for facilitating one of the most incredibly miraculous processes on earth – the process of photosynthesis. It is found in the chloroplasts of green plants cells. The chemical energy stored by photosynthesis in carbohydrates drives biochemical reactions in nearly all living organisms. As the chlorophyll in leaves decays in the autumn, the green colour fades and is replace by the oranges and reds of carotenoids. The basic structure of a chlorophyll molecule is a porphyrin ring, co-ordinated to a central atom. The chlorophyll is mixture of two compounds, chlorophyll-a and chlorophyll-b (Figure 1): There are five different types of chlorophyll molecules that are naturally present in photosynthetic organisms: Chlorophyll *a*, *b*, *c*, *d*, and *f*. All of the chlorophyll molecules have similar chemical structures. The small

differences in the chemical structures of the chlorophyll molecules cause variations in their light absorption properties.

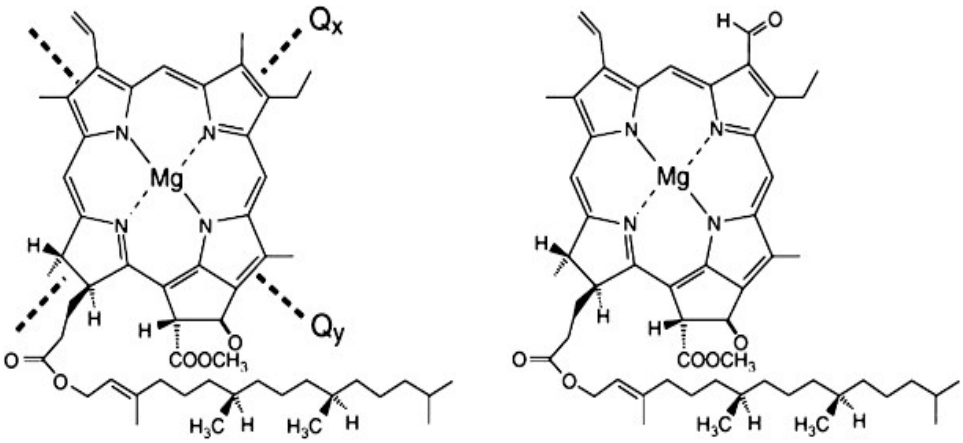


Fig.6.6: The molecular structure of (A) chlorophyll a and (B) chlorophyll b.

Source: https://www.sciencedirect.com/topics/earth-and-planetary-sciences/chlorophyll

In natural chlorophyll there is a ratio of 3 to 1 (of a to b) of the two components. Both of these two chlorophylls are very effective photoreceptors because they contain a network of alternating single and double bonds, and the orbitals can delocalise stabilising the structure. Such delocalised molecules have very strong absorption bands in the visible regions of the spectrum, allowing the plant to absorb the energy from sunlight. Due to presence of different side group, chlorophyll absorbs different wavelength of light. so that light that is not significantly absorbed by chlorophyll a, at, say, 460nm, will instead be captured by chlorophyll b, which absorbs strongly at that wavelength.

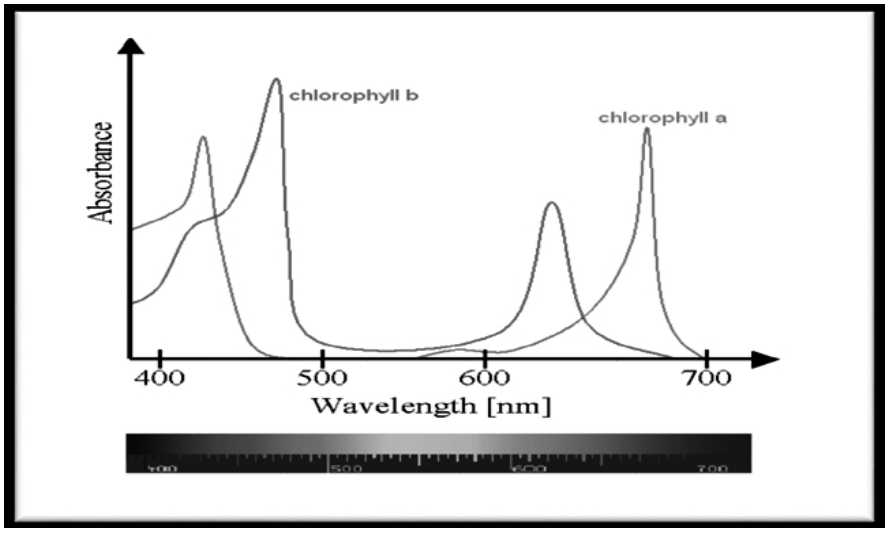


Fig.6.7: Absorption spectra of Chlorophyll a and b

In the photosynthetic reaction electrons, are transferred from water to carbon dioxide i.e. carbon dioxide is reduced by water. Chlorophyll assists this transfer as when chlorophyll absorbs light energy, an electron in chlorophyll is excited from a lower energy state to a higher energy state. In this higher energy state, this electron is more readily transferred to another molecule. This starts a chain of electron-transfer steps, which ends with an electron being transferred to carbon dioxide. The chlorophyll have numerous health benefits such as

- Cleanses and oxygenates, and builds the blood
- A powerful detoxification effect on the body
- Skin healing
- Rich in enzymes that promote quick rejuvenation of our cells
- High in Amino acids
- A natural deodorant

6.7. Summary

Nitrogen fixation is carried out for the conversion of elemental free nitrogen (N_2) into compound form that would easily available for the growth and development of plants. However, the fixed from of nitrogen is also an essential components of DNA and proteins. Therefore, it is needed for all life on earth. Nitrogen fixation is carried out by the enzyme nitrogenase, which is found in microbes. Nitrogen fixation, whether natural or synthetic, is necessary for agriculture and the production of fertilizer. Nitrogen fixation occurs in root nodules of plants belonging to the legume family. The root nodules of legumes contain symbiotic bacteria which contain the enzymes needed for nitrogen fixation. Urea (also known as carbamide) is a waste product of many living organisms, and is the major organic component of human urine. Urea cycle involves a series of biochemical steps where nitrogen as a waste product of protein metabolism is removed from the blood and converted to a compound called urea in the blood. Urea cycle enzymes change as a unit, and are largely influenced by dietary protein content. The urea cycle is closely linked to the citric acid cycle deriving one of its nitrogen through transamination of oxalacetate to form asparate and returns fumarate to that cycle. In living organism, there is no store for excess of amino acid, because proteins are constantly turns over, thus amino acids have to be continuously degraded. The amino acids break down into one of the following seven metabolic intermediates: pyruvate, acetyl-CoA, acetoacetate, a-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate. Those amino acids that are not used for building new proteins may be broken down further to enter the metabolic processes. Chlorophyll is the molecule that traps this 'most elusive of all powers' - and is called a photoreceptor. It is found in the chloroplasts of green plants, and is what makes green plants, green. Chlorophyll delivers a continuous energy

transfusion into our bloodstream, replenishing and increasing red blood cell count. Healthy blood flow and an abundance of oxygen also help the body to cleanse itself of toxic impurities, lending to chlorophylls detoxification properties.

6.8. Terminal questions

Q.1. What is symbiotic nitrogen fixation? Discuss the roles of microorganism in nitrogen fixation.

Answer:----------

Q.2. What do you understand by biological nitrogen fixation? Descries in brief.

Answer:----------

Q.3. Write the nitrogen assimilation and discuss how it useful in nitrogen cycle.

Answer:----------

Q.4. What is urea cycle? Discuss it roles in reduction of ammonia toxicity.

Answer:----------

Q.5. Role of liver and kidney in urea cycle .

Answer:----------

Q.6. Discuss about amino acid metabolism.

Answer:----------

6.9. Further readings

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Uttar Pradesh Rajarshi Tandon
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UGBCH-103

Intermediary Metabolism

BLOCK

3

PHOSPHORYLATION AND PHOTOSYNTHESIS

Course Design Committee

Prof. K.N. Singh — **Vice Chancellor**
Uttar Pradesh Rajarshi Tandon Open University

Dr. (Prof.) Ashutosh Gupta, — **Chairman**
School of Science, UPRTOU, Prayagraj

Prof. Prof. Umesh Nath Tripathi — **Member**
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Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

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Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Dr. Ravindra Pratap Singh — **Member**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh — **Course Coordinator**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Course Preparation Committee

Dr. Ajay Yadav — **Writer** — (Unit: 1,2 & 3)
Department of Zoology, Dr. B.R. Ambedkar
Gov. Girls P.G. College. Fatehpur. U.P.

Dr. Sushma Chauhan — **Author** — Unit: 4 & 9
Academic Consultant (Botany)
School of Sciences, UPRTOU, Prayagraj

Dr. Ravindra Pratap Singh — **Author** — Unit: 5
Academic Consultant (Biochemistry)
School of Sciences, UPRTOU, Prayagraj

Dr. Dharmveer Singh — **Author** — Unit: 6,7 &8
Academic Consultant (Biochemistry)

Dr. Ayodhya Prasad Verma — **Editor** — (All blocks and units)
Associate Professor,
Department of Botany
Bappa Sri Narain Vocational Post Graduate College, Lucknow

Dr. Dharmveer Singh — **(Course Coordinator)**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

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Introduction

This the third block of Intermediary metabolism. It consists of following three units:

Unit-7: this unit cover the electron transport chain and it organization in mitochondria. The structure and role of extra membrane and inner membrane of mitochondria is described briefly. The various steps of oxidative phosphorylation and their various by product discuss in details. Pentose phosphate pathway and Doudoroff pathway describes as a alternative respiratory pathway in plants.

Unit-8: this unit covers the Photophosphorylation in plants in which the structure of chloroplast and molecular architecture of Photosystem I and Photosystem II are discuss briefly. The role of Krebs and electron transport system also mentioned in this unit.

Unit-9: this unit generally covers the pigments of photosynthesis and its role in photosynthesis in briefly. The details study of oxygenic and anoxygenic photosynthesis, adsorption of light by chlorophyll, Calvin cycle, transcription, Role of ribosome's in protein synthesis are discussed in this unit.

UNIT : 7

OXIDATIVE PHOSPHORYLATION

Structure

7.1. Introduction

Mitochondria are most important cell organelles of cell that have a double membrane i.e. outer and inner membrane. The space between membrane is refers to in term embrace space. The apace which is present inside the organelle is called the matrix. The in folding's of inner membrane forms in cristae in, order to increase the surface area available for energy production via the electron transport chain and F1-F0 ATP-ase

in a process known as oxidative phosphorylation. The process by which ATP is formed by the use of the energy liberated by moving electron in electron transport system is known as oxidative phosphorylation. In Oxidative phosphorylation, ATP is formed as a result of the transfer of electrons from NADH $+H^+$ or $FADH_2$ to O_2 by a series of electron carriers. The number of mitochondria found in a cell is therefore a good indicator of the cell's rate of metabolic activity; cells which are very metabolically active, such as hepatocytes, will have many mitochondria. The various components of electron transport system include. Cytochrome b, 2 types of cytochrome c, ubiquinone, flavo protein (FMN or FAD) iron sulphur protein (Fe-S) and enzyme cytochrome oxidase which is ultimately associated with cytochrome a and a_3.

The breakdown of the simple sugar, glucose, in glycolysis provides the first 6-carbon molecule required for the pentose phosphate pathway. The pentose phosphate pathway is considered as alternative respiratory pathways in plants.

Objectives

- To understand the structural role of mitochondria in metabolic process
- To known how the oxidative phosphorylation take place in mitochondrial membrane
- To describe various components of electron transport chain
- To describe mechanism of pentose phosphate pathway in plants

7.2. Mitochondria:

7.2.1. Structure and its functions

Mitochondria are most important cell organelles present in cell, in constant number. The mitochondria are often preset near the structure that require ATP or near source of fuel. Mitochondria are covered by double unit membrane. Outer membrane has more phospholipids and cholesterol as compared to inner membrane. Phospholipids in inner membrane are generally diphosphatidly glycerol and have more protein. The outer membrane of the mitochondria smooth and somewhat elastic and inner membrane has number of inward fold called cistae. The structure and number of cistae depends on the cell types. The outer surface of inner membrane called C face and inner surface called M-face. Generally, the metabolically active mitochondria have more matrixes like structure that contain about 50% proteins. Some time these matrixes appear as reticular

network that attached to the inner surface of inner membrane. The outer and inner surface of mitochondrial membranes differs in ultra structure as reveal by negative contrast staining. The matrix is the internal space where the Krebs cycle takes place. All enzymes of the Krebs cycle are found in the matrix, ensuring high enzyme concentration and reduced loss of intermediates. The inner mitochondrial membrane is the site of oxidative phosphorylation and contains the electron transport chain. It is folded into cristae, creating a large surface area for oxidative phosphorylation to occur, increasing the rate of respiration. The selective permeability of the inner membrane prevents protons crossing the membrane, causing a high concentration of protons in the intermembrane space when they are pumped by the proton pump. This electrochemical gradient drives ATP synthesis and allowing oxidative phosphorylation to occur. During Oxidative Phosphorylation, protons are pumped into the intermembrane space and then flow down proton gradient through protein channels that are associated with ATP synthase, this is where ATP is synthesized.

Many electron carriers cytochromes are appeared in a definite sequence in inner membrane. They together formed electron transport system of energy rich molecules such as $NADH + H^+$ and $FADH_2$ for oxidation. Inner membrane has pin head partials called oxysomes, or elementary particles or F-F particles. These head of particles composed of ATPas enzymes and concern with oxidation of phosphorlation.

Mitochondria called power house of cell because oxidative ATP synthesis occurs in mitochondria. In this process, the organic compounds break down and to release stored metabolic energy in the form of ATP molecules.

Enzymes localizations in mitochondria

The outer, the inner, and the matrix have umber of compounds and enzymes. The inner membrane contains cytochromes b, c, c_1 a and a_3, the F_1 ATPhase associated with the mechanism of oxidative phosphorylation and certain dehydrogenase. The outer membrane have distinct monoamine oxidase, a flavoprotein that catalyze the oxidation of various monoamine. Outer membrane also contain 50% of lipids. Whereas, inner has 20% lipids. The enzymes like adenylate also found in space between membranes.

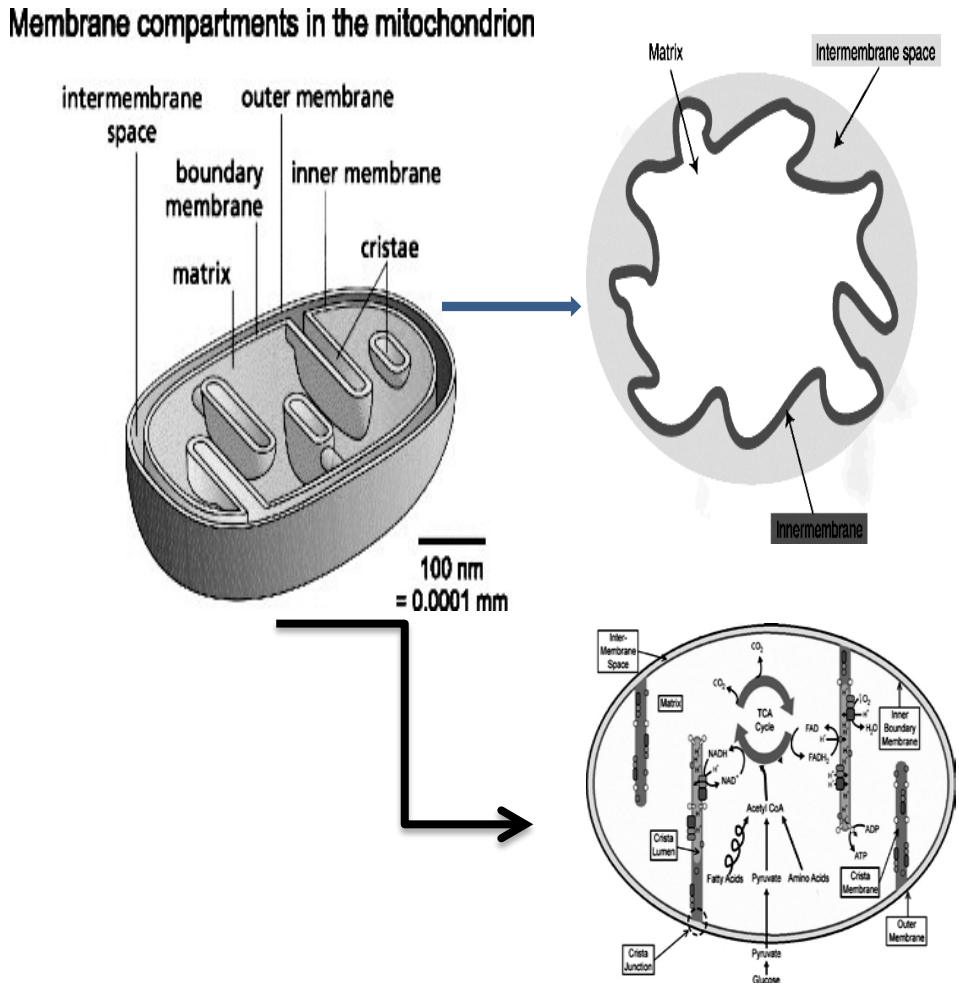

Fig.7.1: Structure of mitochondria

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7.3. Oxidative phosphorylation and ETS

7.3.1. Oxidative phosphorylation

The process (confined to the mitochondria of higher plant and animal cells) by which ATP is formed by the use of energy librated during the electron transport system known as oxidative phosphorlation. The NADH + H^+ and $FADH_2$ formed in TCA and NADH + H^+ in glycolysis are an energy rich molecule. Each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. Which is called oxidative phosphorlation or respiratory chain phosphorylation. In Oxidative phosphorylation, ATP is formed as a result of the transfer of electrons from NADH + H^+ or $FADH_2$

to O_2 by a series of electron carriers. This process, which takes place in mitochondria, is the major source of ATP in aerobic respiration.

1. It is a dehydrogenation reaction actually oxidation in which electron releases to form a molecules such electron cannot exist in free state and must received at once by hydrogen or electron acceptors.

2. The hydrogen atom and their electron are transferred to the first hydrogen acceptor, the NAD^+, which is then reduced to NADH + H^+. NADH + H^+ oxidize in presence O_2 and 3 molecules of ATP are formed.

3. $NADH + H^+ + 1/2O_2 + 3ADP + 2Pi \longrightarrow NADH + 3ATP + H_2O$

4. From the reduced NAD or NADH + H^+ the hydrogen atoms and their electrons are passed on the FAD, which to reduced to $FADH_2$

5. In the next step, $FADH_2$ is oxidized when it delivers the hydrogen atoms and their electrons transmit to the coenzyme Q. For example succinate dehydrogenase is an enzyme found in inner mitochondrial membrane. It also flavoprotein with FAD as the coenzymes.

6. Form reduced coenzyme Q hydrogen atoms are released in the cytoplasm and electron are now passed in a series of cytochromes (b, c, a and a_3). In this process cytochromes is alternatively reduced and oxidized.

7. In the last step the electrons are finally transferred from Cytochrome a_3 (also called Cytochrome oxidize) to the final acceptor or the molecular O_2.

8. The O_2 then units with hydrogen atoms (protons) to form water

9. 2 cytochromes a_3 (Fe^{++}) + $2H^+$ + ½ O_2 $\longrightarrow$ 2 Cytochromes a_3- Fe^{+++} + H_2O

The respiratory break down of simple carbohydrates in presence of oxygen is an oxidative process. During which many intermediates such as phosphoglyceraldehyde, pyruvic acid, iso-citric acid, α-ketoglutaric acid, succinic acid and malic acid are oxidised. The oxidation of all these is brought about by removal of a pair of hydrogen atoms (2H) from each one of them. The pair of hydrogen is usually picked from the substrate by NADH + H^+ or $FADH_2$ in the following manner.

NADH or $FADH_2$ released in glycolysis and Krebs cycle, finally reduce O_2 to H_2O. The transfer of H^+ and e^- from reduced NAD^+ or FAD to O_2 is not a simple process. The NADH gets oxidised at redox potential of 0-032V and O_2 is reduced at redox potential of +0.82V. Thus there is a gap of +1.14V in redox potential which is too much. Therefore, NADH and $FADH_2$ connot directly combine with O_2 to form H_2O. Many intermediate cytochromes and other carriers having intermediate redox potential are arranged in a series which transport electrons from reduced

NAD^+ or FAD to O_2 and form electron transport system (ETS). As electron transport down to energy gradient through electron transport system results in the formation of ATP (Adenosine triphosphate) from ADP (Adenosine diphosphate) and inorganic phosphate. The ATP produced here is due to oxidation reduction reaction therefore, known as oxidative phosphorylation.

For example metabolic in the cytoplasmic oxloacetic acid which can be reduced to malic acid by the intramitochondrial reduced NAD^+ malic acid enter into where it is reoxidized by the intramitochondrial NAD in the present of malic dehydrogenase with the regeneration of oxalic acid. Thus the reduced NAD^+ may be reoxidized by oxidised flavoproteins which accept the hydrogen from the NAD^+.

7.3.2. Electron Transport System (ETS)

The electron transport chain is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called chemiosmosis. Together, the electron transport chain and chemiosmosis make up oxidative phosphorylation. The electron transport chain involves a series of redox reactions that relies on protein complexes to transfer electrons from a donor molecule to an acceptor molecule. As a result of these reactions, the proton gradient is produced, enabling mechanical work to be converted into chemical energy, allowing ATP synthesis. In eukaryotes, the electron transport chain is located in the inner mitochondrial membrane. In prokaryotes, it is located within the plasma membrane. In electron transport, form organic subtract to molecular oxygen, four types of protein participate.

7.3.3. Steps of the Electron Transport Chain

Electron transfer chain over four steps where electrons move along a series of proteins to generate an expulsion type force to move hydrogen ions, or protons, across the mitochondrial membrane. The electrons begin their reactions in Complex I, continuing onto Complex II, traversed to Complex III and cytochrome c via coenzyme Q, and then finally to Complex IV.

Complex I

Complex is composed of flavin mononucleotide (FMN) and an enzyme containing iron-sulfur (Fe-S). FMN is one of several prosthetic groups or co-factors in the electron transport chain. I this reaction non-protein molecule is present as a prosthetic group required for the activity of a protein Prosthetic groups include co-enzymes. The enzyme in complex I is NADH dehydrogenase, a very large protein containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space; Complex I, also known as NADH dehydrogenase. It is in this way, the hydrogen ion gradient is

established and maintained between the two compartments separated by the inner mitochondrial membrane.

Q and Complex II

In this step another electron carrier and coenzyme like succinate is oxidize into fumarate. Where $FADH_2$ revive from oxidation of FAD (flavin-adenine dinucleotide). The transport molecule, FADH2 is then reoxidized, donating electrons to Q (becoming QH_2), while releasing another hydrogen ion into the cytosol. Q receives the electrons derived from $NADH + H^+$ from complex I and the electrons derived from FADH2 from complex II, including succinate dehydrogenase. While Complex II does not directly contribute to the proton gradient, it serves as another source for electrons.

Complex III

In this chain, the Q cycle is takes place and, this complex is also called Cytochrome oxidoreductases. Cytochrome proteins have a prosthetic heme group. The interaction between between Q and cytochromes occurs where the molecules composed of iron continue transfer the electrons. This electron fluctuating between different oxidation states: Fe^{2+} (reduced) and Fe^{3+} (oxidized). Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes. The Cytochrome c that moves the electrons to the last complex. In the process, another hydrogen ion is released into the cytosol to further create the proton gradient.

Complex IV

The fourth complex is composed of Cytochrome proteins c, a, and a_3. Electrons are transferred one at a time into the complex from Cytochrome c. The electrons, in addition to hydrogen and oxygen, then react to form water in an irreversible reaction. The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to produce water (H_2O). This is the last complex that translocates four protons across the membrane to create the proton gradient that develops ATP at the end. As the proton gradient is established, F1F0 ATP synthase, sometimes referred to as Complex V, generates the ATP.

The structure is a series of proteins embedded in a membrane that pump hydrogen ions in one direction to create a concentration gradient - the function is to generate ATP. The electron transport proteins accept high energy electrons from the electron carriers $NADH + H^+$ (in photosynthesis) and $NADH + H^+$ and $FADH_2$ (in cellular respiration), and through the action of transporting them from one to the other in a series of electron exchanges, small units of energy are extracted and used to pump hydrogen ions.

Phosphorylation and Photosynthesis

In cellular respiration they are pumped from the matrix into the intermembrane space of the mitochondria - in photosynthesis they are pumped from the stroma into the lumen of the thylakoids.

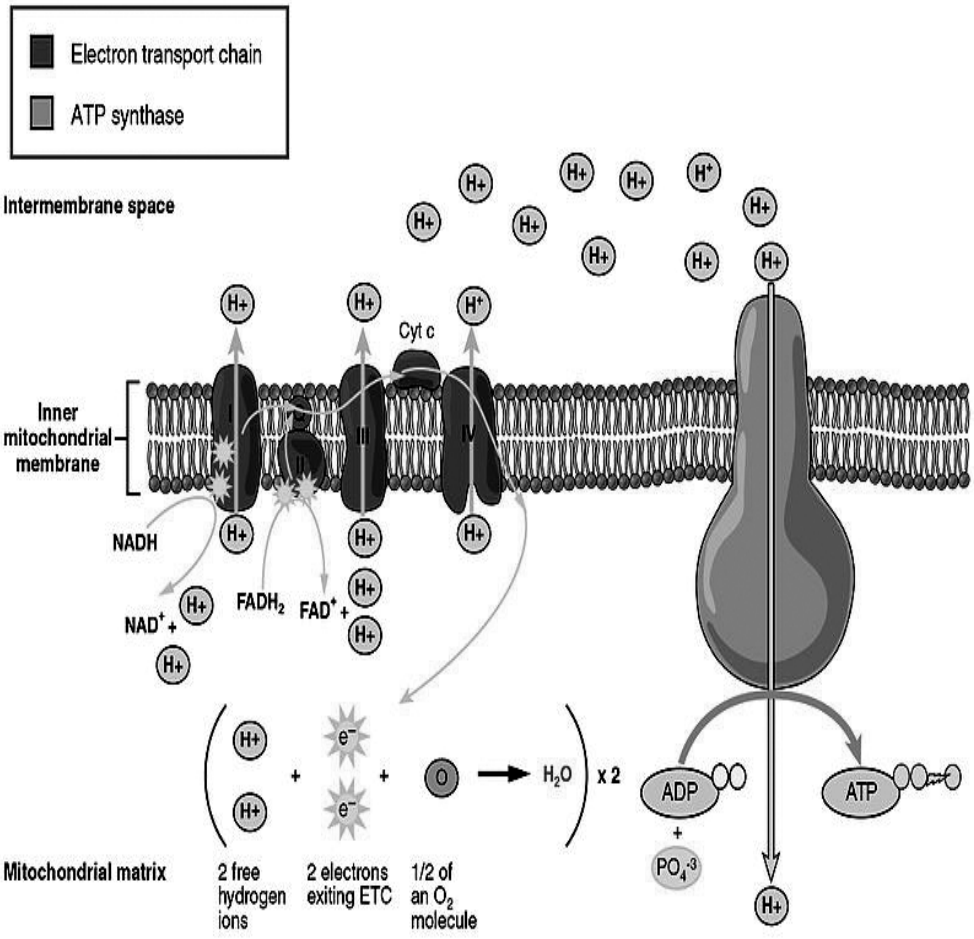


Fig. 7.2: Electron transport chain in mitochondria

Source: https://socratic.org/questions/what-is-the-structure-and-function-of-electron-transport-chain-in-chloroplast-an

In both cases, the high concentration of hydrogen ions can't cross the membrane (due to their charge), and place a great deal of osmotic pressure on the membrane. This pressure drives the hydrogen ions from [high] --> [low] through the enzyme ATP Synthase - using this energy to produce ATP molecules.

7.4. Alternative respiratory pathways in plants

There are generally two types of alternative pathway has been used in plants that is Pentose phosphate pathway and Entner doudoroff pathways. The more discussion about this pathway is as followings

7.4.1. Pentose phosphate pathway and it regulations

Pentose phosphate pathway is an alternative pathway to glycolysis and TCA cycle for oxidation. This pathway has two distinct phases two oxidative phases and the non-oxidative phase. In the oxidative phase, two molecules of $NADP^+$ are reduced to NADH + H^+, utilizing the energy from the conversion of glucose-6-phosphate into ribulose-5-phosphate. These NADPH + H^+ molecules can then be used as an energy source elsewhere in the cell. The non-oxidative phase generates 5-carbon sugars, which can be used in the synthesis of nucleotides, nucleic acids, and amino acids.

Pentose phosphate pathway occurs in cytoplasm of both prokaryotes and eukaryotes. It starts with glucose and it is a multi-steps reaction. This pathway also known as hexose monophosphate pentose shunt. Through glycolysis is the most common pathway of glucose break down, there exists an alternate pathway called pentose pathway (PPP) in many organisms. This was described by Warburg's et al in 1935 and dickens in 1938. According to them the pathway involves direct oxidation of glucose-6-phosphate without entering into glycolysis. The pentose phosphate pathway and its partially reversible reactions are shown in Fig. 7.3 which are given below:

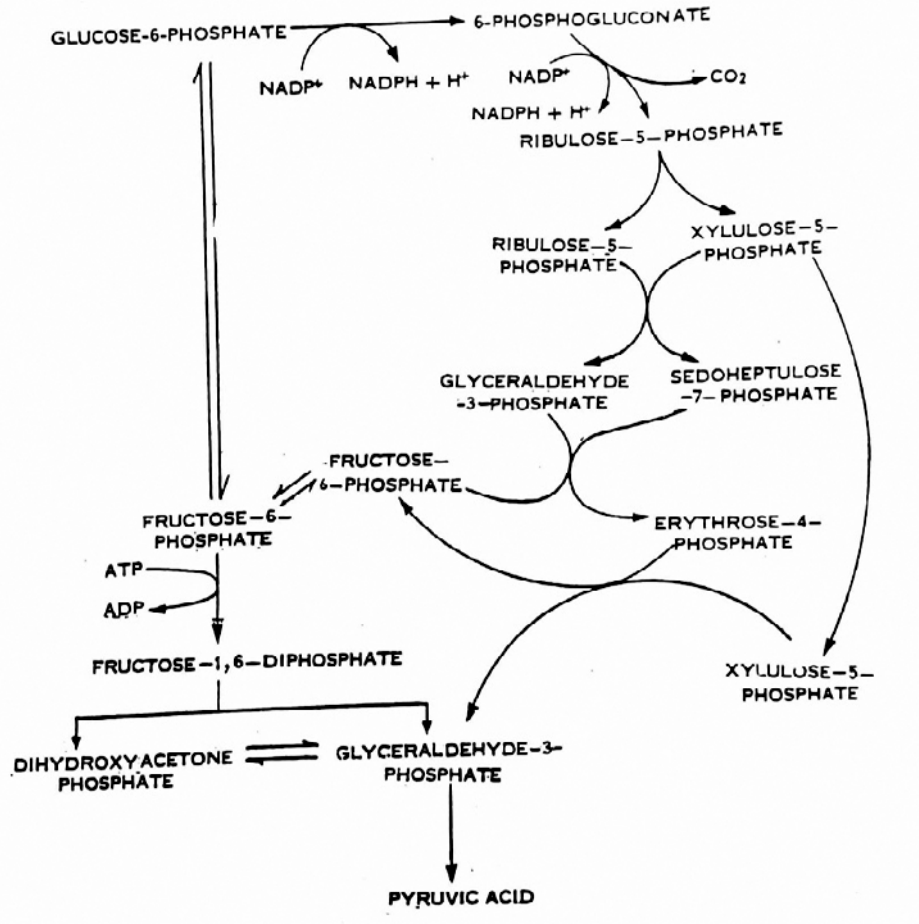


Fig. 7.3: Demonstration of pentose phosphate pathway

1. Glucose-6-phosphate is oxidized to gluconate-6-phosphate, accompanied by reduction of coenzyme NADP+ to NADPH + H^+.
2. Glucose- 6-phasphate is oxidatively decarboxylated to ribulose-5-phasphate. One molecule of Co_2 is released and the coenzyme $NADP^+$ is reduced in the reaction.
3. Ribulose-5-phasphate undergoes isomerization either to form ribose-5-phasphate or xylulose-5-phosphate.
4. A molecule of ribose-5-phosphate interacts with a molecule of xylulose-5-phosphate to form sedoheptulose-7 phosphate and glyceraldehydes-3- phosphate.
5. Sedoheptulose-7 phosphate and glyceraldehydes-3- phosphate combine to produce fructose-6-phasphate and erythrose-5-phosphate.
6. The molecules of fructose-6-phasphate are isomerized to glucose-6-phasphate.
7. The glyceraldehydes-3-phasphate is broken down to pyruvic acid in glycolysis.

7.4.1.1. Oxidative phase

First four reactions are irreversible and oxidative in which glucose molecule is oxidized twice generating two molecules of NADPH + H^+ and glucose is converted into Ribose-5 phosphate.

Step 1: conversion of glucose to glucose-6 phosphate.

This reaction is catalyzed by the enzyme hexokinase and a molecule of ATP is utilized.

Step2: conversion of glucose-6 phosphate to 6-phosphogluconolactone.

This reaction is catalyzed by an enzyme glucose-6 phosphate dehydrogenase (G6PD) in the presence of Mg++ ion. In this reaction a molecule of NADPH + H^+ is produced.

Step 3: conversion of 6-phosphogluconolactone to 6-phosphogluconate

This reaction is a hydrolysis reaction catalyzed by hydrolase enzyme

Step 4: conversion of 6-phosphogluconate to ribose-5 phosphate

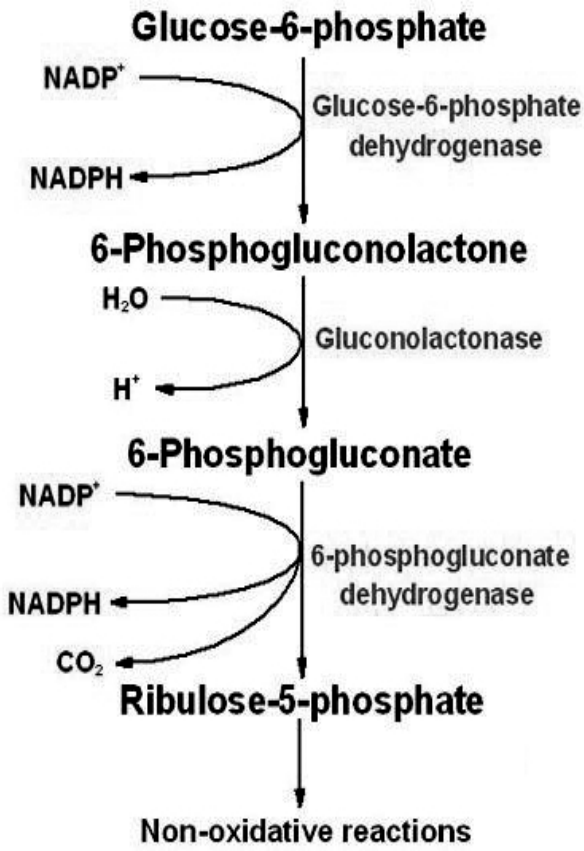


Fig.7.4: Steps of oxidative phase

7.4.4.2. Non oxidative phase

Oxidative reactions are followed by a series reversible sugar phosphate inter-conversion reaction.

Step 1: Ribulose-5-phosphate is epimerized to produce xylulose 5-phosphate in the presence of enzyme phosphorpentose epimerase. Similarly ribulose-5-phosphate is also keto-isomerized into ribose 5-phosphate.

Step 2: Xylulose-5-phsphate transfer two carbon moiety to ribose 5-phospahate in the presence of enzyme transketolase to form sedoheptulose-7-phosphate and glyceraldehyde 3—phosphate.

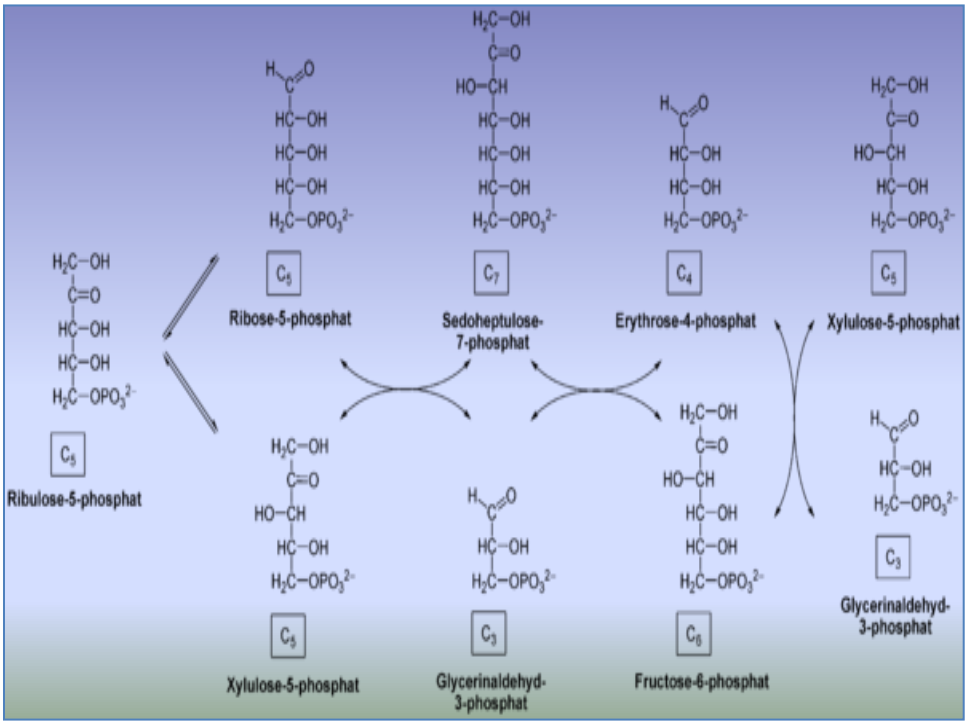
Step 3: Sedoheptulose -7-phosphate transfer three carbon moiety to glyceraldehyde -3-phosphate to form fructose 6-phopsphate and erythrose 4-phosphate in the presence of enzyme transaldolase.

Step 4: Transketolase enzyme catalyze the transfer of two carbon moiety from Xylulose-5-phsphate to erythrose-4- phosphate to form fructose-6-phosphate and glyceraldehyde-3-phosphate.

Step 5: Fructose-6-phosphate and glyceraldehyde-3-phosphate is later enter into glycolysis and kreb's cycle.

Step 6: The rate and direction of reversible reaction depends upon the needs of cell.

Step 7: If cell needs only NADPH + H^+ then fructose-phosphate and glyceraldehyde-3-phosphate are converted back to glucose by reverse glycolysis, otherwise converted to pyruvate and enter TCA cycle generating ATPs.



Pentose phosphate pathway has following significance

1. Coenzyme NADPH + H^+ for some synthetic process.
2. Ribose sugar for nucleic acid synthesis
3. Erythrose for lignin and other aromatic compounds
4. Co_2 for fixation in photosynthesis.
5. Ribose, tetrose, pentose, hexose and heptose sugar are generated as intermediate products in pentose phosphate pathway.
6. NADPH + H^+ is also used to reduce (detoxify) Hydrogen peroxide in cell.

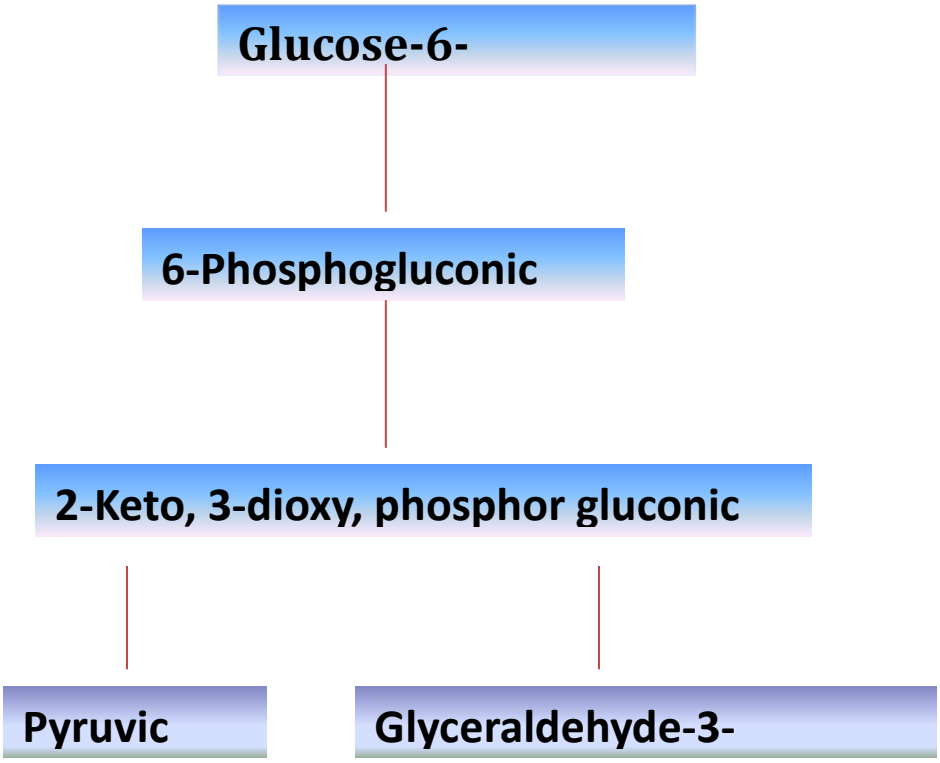
7.4.2. Entner doudoroff pathways

A third pathway of glucose breakdown occurs in number of bacteria is known as enter Doudoroff pathway. It is summaries as follows

Step 1: Glucose-6-phasphate is oxidized to 6-phosphogluconic acid. The coenzyme $NADP^+$ is reduced to NADPH + H^+ in the reaction.

Step 2: The 6-phosphogluconic acid is the converted to 2-keto, 3-dioxy, phosphogluconic acid, which is then converted to pyruvic acid and glyceraldehyde-3-phosphate.

Step 3: The glyceraldehyde-3-phosphate is converted to pyruvic acid by glycolysis.



Key Points

- The Entner-Doudoroff pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.
- There are a few bacteria that substitute classic glycolysis with the Entner-Doudoroff pathway.

7.5. Summary

Mitochondria are organelles have double membranes. The inner membrane folds into cristae which divide the organelle into three compartments: the intermembrane space (between outer and inner membranes), cristae space (formed by infoldings of the inner membrane), and the matrix (within the inner membrane). The matrix of the mitochondria is the site of Krebs Cycle reactions. On oxidative phosphorylation, oxygen *must* be present to receive electrons from the protein complexes. This allows for more electrons and high energy molecules to be passed along, and maintains the hydrogen pumping that produces ATP. During glycolysis, only two ATP molecules are produced.

$NADH + H^+$ is then oxidized to synthesized ATP. The pentose phosphate pathway takes place in the cytosol of the cell, the same location as glycolysis. It is special because no energy in the form of ATP, or adenosine triphosphate, is produced or used up in this pathway. The various components of electron transport system include. Cytochrome b, 2 types of cytochrome c, ubiquinone, flavo protein (FMN or FAD), iron sulphur protein (Fe-S) and enzyme cytochrome oxidase which is ultimately associated with cytochrome a and a_3. These components are arranged in a sequence in the inner Mitochondrial membrane.

7.6. Terminal Questions

Q.1. Define the Structure and function of mitochondria and it role in oxidative phosphorylation.

Answer:----------

Q.2. Define the electron transport chain in briefly.

Answer:----------

Q.3. What is oxidative phosphorylation, define it.

Answer:----------

Q.4. Briefly define the role of oxidative phosphorylation.

Answer:----------

Q.5. Define the alternative respiration pathways in plants.

Answer:----------

Q.6. Define the role of Entner doudoroff pathways in respiration**.**

Answer:----------

7.7. Further readings

1. Lehninger- Principles of Biochemistry- David L. Nilson and Michael M. Cox, WH Freeman; 7th ed. 2017 edition

2. J L Jain et al., Fundamentals of Biochemistry; S Chand; Seventh edition ,2016

3. Voet D and Voet J.G., Biochemistry", 4th Edition,2010

4. U. Satyanarayana, Biochemistry; Elsevier India; 5 edition, 2017.

5. Jeremy M. Berg, John L. Tymockzo and Luber Stryer, Biochemistry 7th Edition

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UNIT : 8

PHOTOPHOSPHORYLATION

Structure

8.1. Introduction

Phosphorylation is a biochemical process that involves the addition of phosphate to an organic compound, by this process energy-rich molecule called ATP is formed. Phosphorylation regulates the cell cycle, growth, apoptosis and signal transduction in living organism. However, the regulation of protein functions and signal transmission is well known mechanism in phosphorylation. It is also involves in the oxidation of H_2O to O_2, where NADP+ work as electron acceptor. In the process of photosynthesis, the phosphorylation of ADP to form ATP using the energy of sunlight is called Photophosphorylation. The structure and size of chloroplast has played important role because it absorbs sunlight and use it conjunction with water and carbon dioxide gas to produce food for

plant. Chloroplasts captured light energy from the sun to produce the ATP and NADPH H^+ through a process called light phase of photosynthesis. Photosystems are functional and structural units of protein complexes involved in photosynthesis that together carry out the primary photochemistry of photosynthesis. Photosystems are the functional units for photosynthesis. The photosystem consists of two pigment protein such as antenna complex and core complex which is useful in photosynthesis. Photoinhibition occurs in all organisms capable of oxygenic photosynthesis, from *cynobacteria* to vascular plants . Inhibition of photosynthesis caused by excessive radiance, affects field production to a great extent.

Objectives

- To discuss the role of Phosphorylation in photosynthesis
- Significance of photosynthesis.
- Significance of photoinhabitation
- To discuss the role of photosystems in the light dependant reactions

8.2. Photophosphorylation

We know about photosynthesis occurs in plants that is the biological process of converting light energy into chemical energy. In this process, light energy is captured and used for converting carbon dioxide and water into glucose and oxygen gas. The complete process of photosynthesis is carried out by light reaction and by dark reaction. The photosynthesis of light reaction is associated with presence of phosphate molecules. The phosphate in the presence of light or the synthesizing of ATP play significant role in photosynthesis. Thus, the phosphorylation can be defined as the process of converting the energy of light into the energy of chemical bonds. It takes place in the chloroplast of cells, specifically in the thylakoid membrane. In the process of phosphorylation, light is absorbed by the chlorophyll and convert ADP into ATP molecule. Phosphorylation is an important process occurring in the living cell because by this process energy-rich molecule called ATP is formed. In this process, the phosphate group is added to ADP to form ATP by the enzyme kinases and phosphorylases.

Photophosphorylation involves the oxidation of H_2O to O_2, with NADP+ as electron acceptor. Therefore, the oxidation and the phosphorylation of ADP are coupled by a proton gradient across the membrane. Photophosphorylation process may be occurs either by cyclic process or non cyclic process.

8.2.1. Cyclic Photophosphorylation

Cyclic Photophosphorylation occurs where same electron is recycled. The chlorophyll absorbed the light energy that stimulates the electrons. The electron is then passed towards an electron acceptors protein which passes

it along with an electron transport channel. Cyclic photophosphorylation involves the use of only **one** photosystem (PS I) and does **not** involve the reduction of NADP+. As the electron is passed along the transport channel, the electron loses energy, which is then used to make ATP from ADP and Pi. The electron is then recycled and again enters into the photosystem again. As the electron returns to the Photosystem (PSI), NADP+ is not reduced and water is not needed to replenish the electron supply.

In this Photophosphorylation process, only photosystem I operates. So no photoxidation of water take place, Therefore, no evolution of O_2 and no formation of NADPH + H^+ occurs. But when shorter wave length is given simultaneously, PS II also comes in operation and photoxidation of water relases H^+ which reduces $NADP^+$ to NADPH + H^+ and photosynthetic enhancement takes place. In cyclic photophosphorylation, the high energy electron is free from P700 to ps1 flow down to a cyclic pathway.

In bacterial photosynthesis, a single photosystem is used, and therefore is involved in cyclic photophosphorylation. It is favored in anaerobic conditions and conditions of high irradiance and CO_2 compensation points.

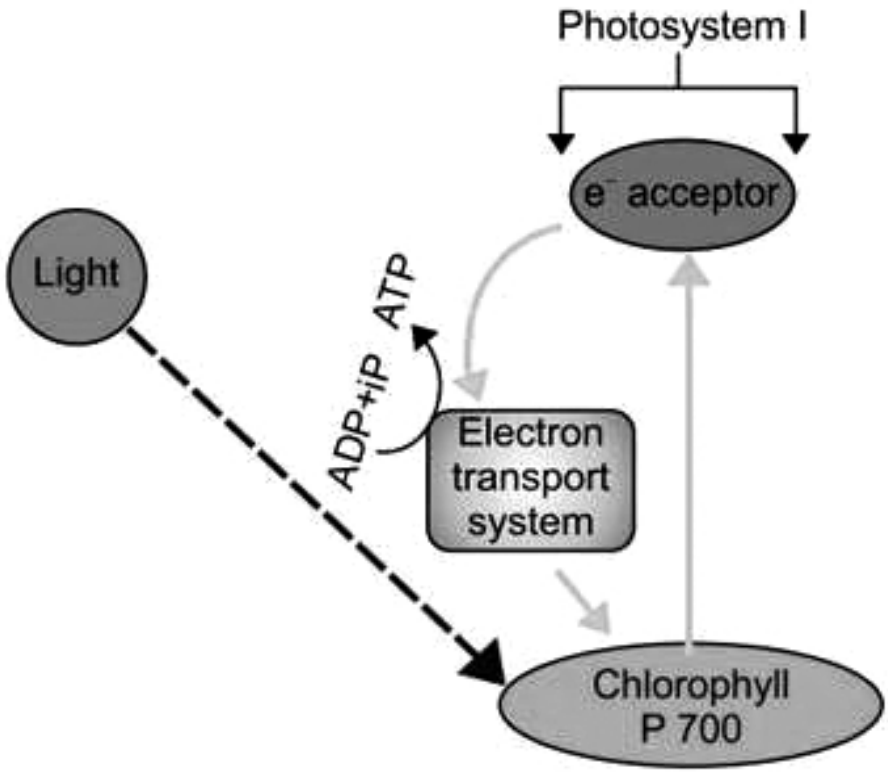

Fig.8.1: Cyclic Photophosphorylation

Source: https://byjus.com/biology/cyclic-and-non-cyclic-photophosphorylation/

8.2.2. Non cyclic photophosphorylation

Non-cyclic photophosphorylation is two step process occurs as a result of an interaction between photosystem I and photosystem II. Non-cyclic photophosphorylation helps in the formation of ATP as a result of electron flow from water to $NADP^+$. Being a light reaction, non-cyclic photophosphorylation happens in the thylakoid membrane. Light of longer wave length hits pigment of photosystem-I as a result P700 gets excited and releases electrons which are accepted by an unknown primary electron acceptor and are finally passed on to NADP . Here, the electrons combine with the protons – H+ which is produced by splitting up of the water molecule and reduces $NADP^+$ to NADPH + H^+. Where a water molecule is broken down into $2H^+ + 1/2\ O_2 + 2e^-$ by a procedure called photolysis (light splitting).

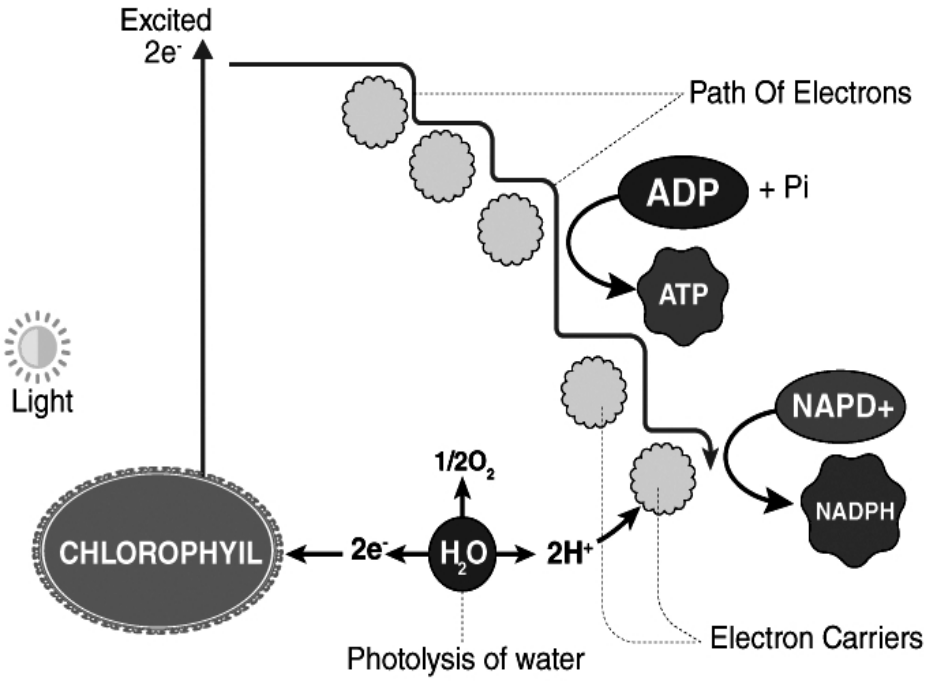

Fig.8.2: Non-cyclic Photophosphorylation

Source: https://byjus.com/biology/cyclic-and-non-cyclic-photophosphorylation/

When a lower wave length of light is received by PS II, P680 looses an electron which is accepted by quinone. The electron then travels down the hill through a series of carriers B, PQ, cyt and plastocyanin. The energy released in the transfer of electron from PQ to cytf is utilised to convert ADP into ATP and electron goes to PS I. At this stage water dissociates into H^+ and OH^- ions. The hydroxyl ion (OH) looses electrons and transferred to PS II. The H^+ are taken up by NADP+ which get reduced to $NADPH+H^+$. Thus, in non-cyclic photophosphorylation the electron is not cycled back. Therefore, it is called non cyclic photophosphorylation.

Table 8.1 : Difference between Cyclic and non Cyclic Photophosphorylation

Cyclic Photophosphorylation	Non-Cyclic Photophosphorylation
Only Photosystem I are involved in the process.	Both Photosystem I and II are involved in the process
In cyclic photophosphorylationP700 is the active reaction center.	In non-cyclic photophosphorylationP680 is the active reaction center.
Electrons passes in a cyclic manner.	Electrons passes in a non -cyclic manner.
ATP molecules are generated.	Both NADPH + H^+ and ATP molecules are formed.
NADPH + H^+ is not produced.	NADPH + H^+ is produced.
This process is ideal only for bacteria.	This process is ideal in all green plants.

8.3. Chloroplast

Chloroplasts are the site of photosynthesis in eukaryotic cells. They are only present in photosynthetic cells like plant cells and algae. There are no chloroplasts in animal or bacterial cells. They are semiautonomous as they can grow and their DNA contains a portion of genetic information needed for the synthesis of chloroplast. It is said that chloroplast was once an independent cell which was engulfed into another cell as an endosymbiont. In higher plants it is usually discoid, ovoid, ellipsoidal or biconvex lens shaped. Chloroplasts are a type of plastid a round, oval, or disk-shaped body. Some chloroplasts are in shape of club, they have a thin middle zone and the ends are filled with chlorophyll. The size of the chloroplast also varies from species to species and it is constant for a given cell type. In higher plants, the average size of chloroplast is 4-6 µ in diameter and 1-3 µ in thickness. Chloroplasts are distinguished from other types of plastids by their green colour, which results from the presence of two pigments, chlorophyll a and chlorophyll b. The chloroplast is a heterogenous structure.

8.3.1. Parts of Chloroplasts

- **Outer membrane**: It provide passage to small molecules and ions due to permeable nature. The outer membrane is not permeable to larger proteins.

- **Intermembrane Space**: It 10-20 nanometers intermembrane space that is present between the outer and the inner membrane of the chloroplast.

- **Inner membrane** : The inner membrane of the chloroplast forms a border to the stroma. It regulates passage of materials in and out of the chloroplast.

- **Stroma:** Stroma is a alkaline, aqueous fluid which is protein rich and is present within the inner membrane of the chloroplast. It is the colourless matrix of protenacious nature. It contains enzymes of photosynthesis, salts, starch grain, osmophilic granules RNA and DNA. Grana are embedded in stroma.

- **Grana :** Grana is small disc like structure. About 10-100 disc like grana or thylacoid are super imposed just like pil of coin and are called granum. The grana are interconnected by stroma lamellae which are extension of the membrane of grana into the matrix. Grana is the site of light reaction of photosynthesis where as stroma is the site of dark reaction of photosynthesis.

- **Thylakoid System:** The thylakoid system is suspended in the stroma. The thylakoid system is a collection of membranous sacks called thylakoids. The chlorophyll is found in the thylakoids and is the sight for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as grana. One thylakoid stack is called a granum. Each granum contains around 10-20 thylakoids.

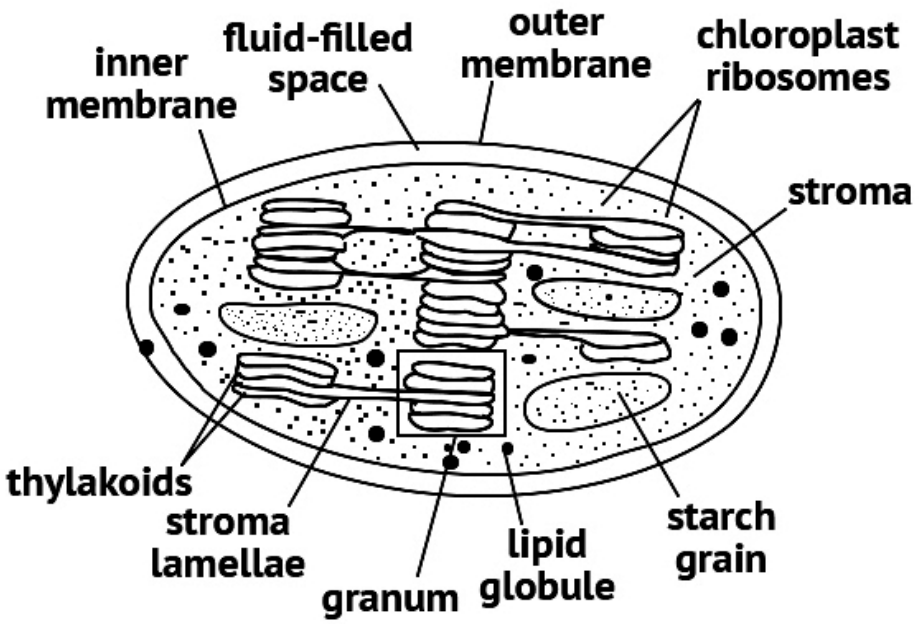

Fig 8.3: Structure of chloroplast
Source: https://www.philpoteducation.com/mod/book/view.php?id=805&chapterid=1076#/

8.4. Photo inhibition

Photoinhibition is the inhibition of photosynthetic activity due to visible light(400-700 nm). Photoinhibition also occurs under high temperature stress, drought stress, salinity stress etc. When leaves are exposed to more light than they can utilize, the reaction center of PS II is inactivated and damaged in a phenomenon called as photoinhibition. Even when plants are not stressed photoinhibition takes place. It occurs in all organisms which evolve oxygen, for example from cyanobacteria to higher plants. The characteristic of photoinhibition in the intact leaf depends on the amount of light to which the plant is exposed. Photoinhibition is defined as the light dependent decrease in photosynthetic rate that may occur whenever the irradiance is in excess of that required either for the photosynthetic evolution of oxygen or photosynthetic assimilation of carbon dioxide. The PSII reaction centre (P680) is specifically inactivated by photoinhibition. Photooxidation is the light and oxygen dependent destructive reactions, like pigment bleaching and lipid peroxidation which occurs in the thylakoid membrane. Ultimately photoinhibition leads to a loss of plant productivity. There are two types of photoinhibition like Dynamic and Chronic photoinhibition that discuss briefly below.

8.4.1. Dynamic Photoinhibition

Under moderate excess light, dynamic photoinhibition is observed. Quantum efficiency decreases, but the maximum photosynthetic rate remains unchanged. It is caused by diversion of absorbed light energy towards heat dissipation and hence the decrease in quantum efficiency. This decrease is often temporary and quantum efficiency can return to initial higher value when photon flux decreases below saturation levels.

8.4.2. Chronic photoinhabitation:

It results from exposure to high levels of excess light that damage the photosynthetic system and decrease both quantum efficiency and maximum photosynthetic rate. It is associated with the damage and replacement of the D1 protein from reaction center of PS II. In contrast to dynamic photoinhibition, these effects are relatively long lasting, persisting for weeks or months.

8.4.3. Effect and Significance of photoinhibition

Cumulative effects of daily depression in photosynthetic rate caused by photoinhibition decreases biomass by 10% at the end of growing season. It

is significant in natural plant populations competing for limited resources conditions.

Significance of photoinhibition Dynamic photoinhibition occurs normally in midday, when leaves are exposed to maximum amounts of light and there is corresponding reduction in carbon dioxide fixation. This effect becomes larger at low temperatures, and photoinhibition can shift to more severe chronic form under more extreme climatic conditions.

8.5. Photosystem

Photosystems are the functional units for photosynthesis. The light absorption processes associated with photosynthesis occur in large protein complexes present in thylakoid membrane known as photosystems. The photosystem consists of two pigment protein such as antenna complex and core complex. The antenna complex also known as light harvesting complex (LHC). The two definite types of photosystem like photosystem-I (PS I plastocyanin-ferredoxin oxidoreductase) and Photosystem II (PS II, water-plastoquinone oxidoreductase) are found in nature. The PS I consists of LHC I and core complex I (CC I) located in the stroma lamella of thylakoids. The PS II consists of LHC II and CC II located in stacked grana domain. The PS I uses the absorbed energy to transfer an electron to low potential acceptor that via intermediates reduces NAD+ (Nicotinamide adenine dinucleotide phosphate). The PS II oxidizes water releasing O2, H+ and e_ and reduce PS I reaction centres (P700).

8.5.1. Photosystem I

Photosystem I is the light-driven plastocyanin-ferredoxin oxidoreductase present in the thylakoid membranes of cyanobacteria and chloroplasts. PSI is involved in the cyclic and non-cyclic photophosphorylation. PS I receive the electrons from photosystem II. This system produces a strong reductant which reduces NADP+ to NADPH + H^+. The reaction center of this photosystem contains chlorophyll a molecules (P700) that absorb light of 700 nm wavelength. Molecular oxygen is not evolved in this system. The structure of photosystem I in a cyanobacterium has been provided in Fig. 8.4. It is a homotrimer with each subunit in the trimer containing 12 different protein molecules bound to 96 molecules of chlorophyll a, 22 molecules of carotenoid, 4 lipid molecules, 3 clusters of Fe_4S_4 and 2 phylloquinones.

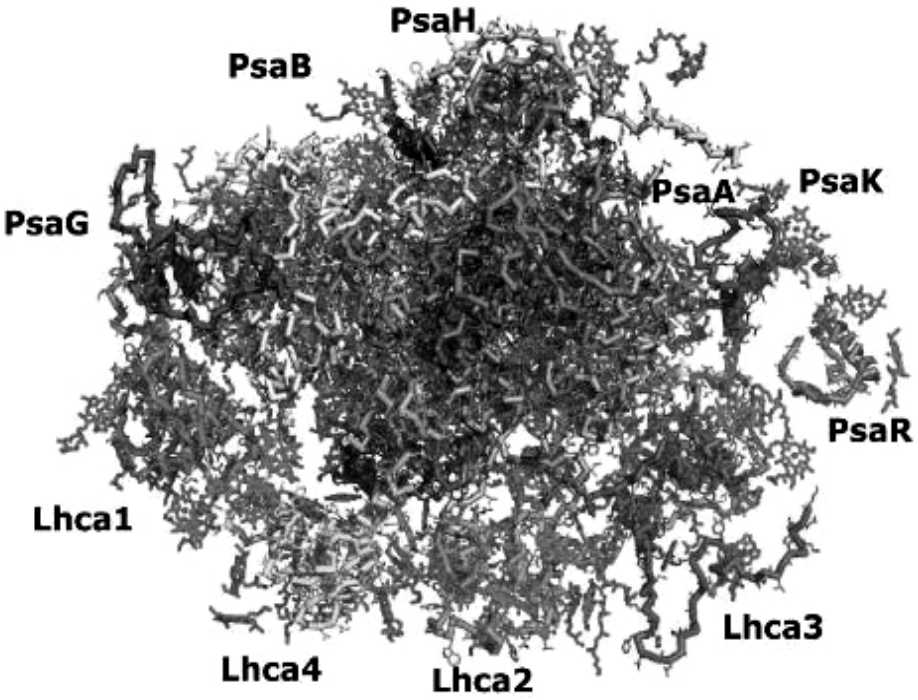

Fig.8.4: Structure of Photosystem I

The function of PS I is as followings:

- **Excitation of electron in PS I:** Photoexcited electrons enter photosystem I via an electron transport chain set in the thylakoid membrane where it waits until the electron is excited by another photon.
- **Chemiosmosis:** The energy fall is harvested to transport hydrogen (H+) through the membrane, into the thylakoid lumen to generate ATP
- **Conversion of NADP + to NADPH:** The excited electrons in photosystem I oxidize NADP + to NADPH which will be needed in the Calvin Cycle.

8.5.2. Photosystem II

Photosystem II" is the first link in the photosynthesis chain is a multi subunit pigment-protein complex (water-plastoquinone oxidoreductases) embedded in the lipid environment of the thylakoid membranes of plants, algae and cyanobacteria. At the heart of this photosytem, is a reaction center (RC) core containing chlorophyll a molecules (P 680) that absorbs light of 680 nm. Driven by light, this enzyme catalyzes the chemically and thermodynamically demanding reaction of water splitting. While doing so, it harnesses solar irradiation to oxidize two molecules of water to molecular oxygen, liberating electrons which provide the reducing equivalents required for the conversion of CO2 into the organic molecules of life.

PhotosystemII (PSII) is involved only in non-cyclic photophosphorylation. Photosystem II (PSII) donates electrons to photosystem I where NADP+ is reduced. This system is responsible for the photolysis of water and involves the evolution of molecular oxygen. Photosystem II (Fig. 8. 5) is also a complex assembly of more than 20 different protein molecules bound to: 50 or more chlorophyll a molecules, Some half dozen carotenoid molecules and 2 molecules of plastoquinone.

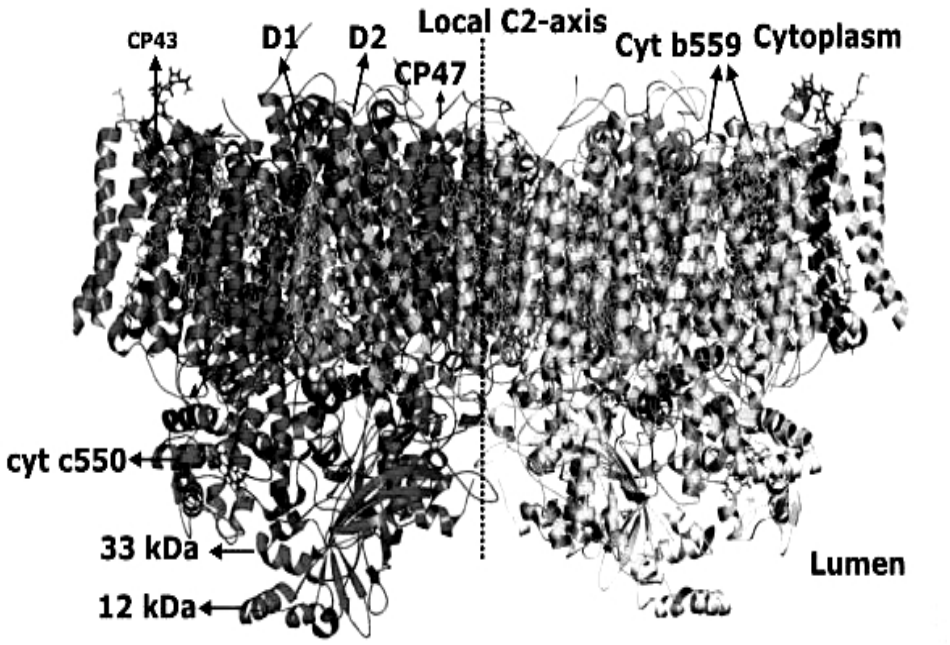

Fig.8.5: Structure of Photosystem II

The function of PS I is as followings:

- **Light absorption:** Absorption of light energy by photosystem II (P680).
- **Electron capture:** Excitation of electron from a low energy state to a high energy state. The electron travels down the electron transport system (ETS). Along the way, more H^+ is pumped into the thylakoid compartment.
- **Splitting of water and releasing oxygen:** Meanwhile the photosystem's lost electron is replenished by photolysis, or the splitting of H_2O to form H+ and O_2 . The O_2 resulting is the source of all oxygen in our atmosphere

8.6. Summary

In this unit you have learnt that the downhill movement of electrons from an electron acceptor to P700 results in the formation of ATP and this is termed as cyclic photophosphorylation. It is very important to note that oxygen and NADPH $+H^+$ are not formed during

Photo phosphoryalation

cycle photophosphorylation.The photosynthesis which occurs in presence of light is used high-energy electrons to reduce NADP+ to $NADPH^{+} + H^{+}$. It also indirectly used high-energy electrons through an electron-transport chain to generate a proton-motive force across a membrane. Chloroplasts are green, so you can identify them by color under a microscope, but it is easier to identify them by their fluorescence, if you have the right equipment (a blue light and a red optical filter). The plant photosynthetic reactions occur in two stages namely "light reactions" involving electron-proton transfer processes and dark reactions involving the reduction of CO_2 for the biosynthesis of carbohydrates. During the light reactions, the solar energy is converted into ATP and $NADPH^{+}$ with the help of multi-pigment protein complexes known as photosystem I and photosystem II. Photosystem I (P700) utilizes light energy to generate high energy electrons which eventually reduce $NADP^{+}$ to produce $NADPH + H^{+}$ that subsequently enters the Calvin cycle. Photosystem II (P680) performs the light-induced electron transfer reactions in photosynthesis that is responsible for splitting of water into hydrogen ions and oxygen. Photosystem II is damaged by light irrespective of light intensity.

8.7. Terminal Questions

Q.1. Define the photosynthesis? How it is useful in plants.

Answer:----------

Q.2. Define the structure of chloroplast briefly.

Answer:----------

Q.3. What is cyclic photophosphorylation? Discuss briefly.

Answer:----------

Q.4. What is non cyclic photophosphorylation? Discuss briefly.

Answer:----------

Phosphorylation and Photosynthesis

Q.5. What do you understand about photosystem?

Answer:----------

Q.6. Discuss the role of photo inhibition in plants.

Answer:----------

8.9. Further Readings

1. Lehninger- Principles of Biochemistry- David L. Nilson and Michael M. Cox, WH Freeman; 7th ed. 2017 edition
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UNIT : 9

PHOTOSYNTHESIS

Structure

9.1. Introduction

Most of us are familiar with the concept of a factory, where raw materials enter and after due processing finished products exit. Imagine photosynthesis occurring in two connecting factories. The product of first factory is energy carrying molecules ATP (adenosine triphosphate) and NADPH $+H^+$ (reduced nicotinamide adenine dinucleotide phosphate) and second factory to make sugar, the final product. All organisms, including human, need energy to fuel the metabolic reactions of growth, development and reproduction. But organisms cannot use light energy

directly for their metabolic needs. The green plants have chloroplast and can synthesize their own food by the process of photosynthesis. The entire humanity depends on plants for food. Every year about 200 billion tons of carbon is utilized in the process of photosynthesis. Thus the photosynthesis is the most massive chemical event going on the earth. The plants take up 7×10^{11} tons of CO_2 to produce roughly 5×10^{11} tons of solid plant material.

Objectives

After studying this unit you should be able to :

- To differentiate the structure and functions of chloroplast.
- To differentiate the of photosynthesis in plant.
- To differentiate the effect and cyclic and non cyclic photophosphorylation.
- C_3, C_4 and CAM cycle of dark fixation of CO_2.
- To differentiate the C_3 and C_4 plant.

9.2. Photosynthesis fundaments

All photosynthetic organisms used water as electron and hydrogen H^+ donor except bacteria. The 90% of the world's photosynthesis in carried out by marine and fresh water algae. Thus the photosynthesis is a very important metabolic process because it supplies food to the biological world and purifies the atmosphere through taking up Co_2 from the atmosphere and releasing O_2 in the atmosphere.

Photosynthesis is the synthesis of carbohydrates from Co_2 and H_2o in presence of sunlight on chlorophyll.

$$6Co_2 + 12H_2o \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}o_6 + 6\ O_2 + 6H_2O$$

In this equation carbohydrate is produced as a end product of photosynthesis. However, this equation does not apply to the entire photosynthetic organism. Instead of water, some other organism used other compounds as electron donors. For example green and purple bacterial used hydrogen sulfide that show in equations.

$$Co_2 + 2H_2S \xrightarrow{Light} CH_2O + H_2O + 2S$$

Photosynthesis taking place in water labed with oxygen isotope does not fact yield labeled O_2.

9.2.1. Structure of Chloroplast

A chloroplast is a type of organelle known as a plastid, characterized by its two membranes and a high concentration of chlorophyll. Other plastid types, such as the leucoplast and the chromoplast, contain little chlorophyll and do not carry out photosynthesis. It is found in all photosynthesizing cells except prokaryotes. Chloroplasts have two membranes. Inside, there are little disks known as thylakoids, which carry out light part of photosynthesis. Stacks of thylakoids are known as granum, and the space outside of the granum is the stroma, which is where the rest of photosynthesis occurs (dark phase). Chloroplasts are semiautonomous as they can grow and their DNA contains a portion of genetic information needed for the synthesis of chloroplast. It is said that chloroplast was once an independent cell which was engulfed into another cell as an endosymbiont.

Shape and size : In higher plants it is usually discoid, ovoid, ellipsoidal or biconvex lens shaped. The average diameter of chloroplast in higher plants is 4-6 μm. Chloroplasts are a type of plastid a round, oval, or disk-shaped body. Chloroplasts are distinguished from other types of plastids by their green colour, which results from the presence of two pigments, chlorophyll a and chlorophyll b. The chloroplast is a heterogenous structure. It has two distinct structures -

(1) Grana

(2) Stroma

1. Grana : Grana is small disc like structure. About 10-100 disc like grana or thylacoid are super imposed just like pil of coin and are called granum. The grana are interconnected by stroma lamellae which are extension of the membrane of grana into the matrix.

With in the membrane of grana layers of particles called quantasomes are present. Quantasomes were discovered by Park and Biggins. Photosynthetic pigments are arranged within each quantasome.

A monolayer of chlorophyll along with carotenoid and phycobilines are present between lipid and protein layer. They are arranged in such a way that head of chlorophyll is towards the protein layer and tail towards the lipid layer.

2. Stroma : It is the colourless matrix of protenacious nature. It contains enzymes of photosynthesis, salts, starch grain, osmophilic granules RNA and DNA. Grana are embedded in stroma.

Grana is the site of light reaction of photosynthesis where as stroma is the site of dark reaction of photosynthesis.

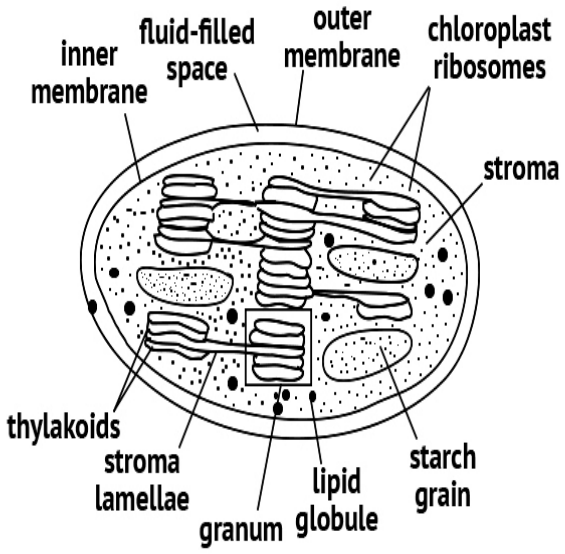


Fig.9.1: Structure of chloroplast

9.2.2. Absorption of light by chlorophyll

We know the plant is appeared in green color because the present of special pigment that is called chlorophyll. Plants contain chlorophyll a and b. These two types of chlorophyll differ only slightly, in the composition of a single side chain. Chlorophyll is a compound that is known as a chelate because it form chelates like structure in which central metal ion bonded to a large organic molecule. Chlorophyll has magnesium as its central metal ion, and the large organic molecule to which it bonds is known as a porphyrin. Chlorophyll absorbs certain wavelengths of light within the visible light spectrum As shown in detail in the absorption spectra, chlorophyll absorbs light in the red (long wavelength) and the blue (short wavelength) regions of the visible light spectrum. Light with a wavelength of 460 nm is not significantly absorbed by chlorophyll a, but will instead be captured by chlorophyll b, which absorbs strongly at that wavelength. The two kinds of chlorophyll in plants complement each other in absorbing sunlight. Plants are able to satisfy their energy requirements by absorbing light from the blue and red parts of the spectrum.

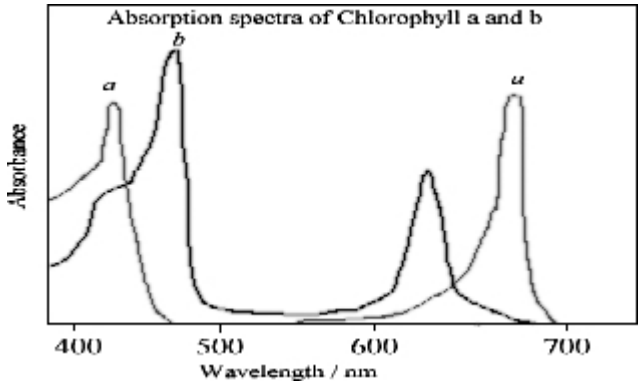


Fig.9.2: Absorption spectra chlorophyll a and chlorophyll b result in slightly different absorptions of visible light.

9.3. Mechanism of Photosynthesis

The process of photosynthesis gets completed in two steps

- Light reaction or photochemical reaction.
- Dark reaction or light independent biochemical reaction or Dark Fixation of CO_2 or Black man's reaction.

Light reaction or photochemical reaction is directly depends on the light energy and the dark reaction which can occur in absence of light. The light and dark phase of photosynthesis firstly observed by D.I. Arnon and his colleagues in 1958. They show that the light and dark phase could be separated temporarily. Today we know that the light reactions of photosynthesis are primarily responsible for converting light into chemical energy in the form of ATP and NADPH $+H^+$, whereas, the dark reactions involved in the utilization of chemical energy of ATP and NADPH $+H^+$ to bring about the reaction of carbon dioxide to hexose. The light and dark reaction depends on the following evidence that are discussed briefly here.

- **Evidence from intermittent light Experiment, Warburg's Experiment**

The rate of photosynthesis was found greater in intermittent light. In continuous light the product of light reaction, $NADPH+H^+$ and ATP are not consumed at the same rate at which they are produced. Thus they get accumulated. But in intermitted light the product of light reaction is quickly utilized.

- **Evidence from Temperature Co-efficient or Q_{10}**

Q_{10} = Rate of reaction at given temperature

Rate of reaction exactly at 10^0C lower temperature

The value of Q_{10} is 2 or more for purely chemical reaction and Q_{10} is unity for a photo-chemical reaction.

Blackman demonstrated that Q_{10} 2 or more for a well illuminated plant and unity under low intensity of light.

- **Emerson effect and two pigment system**

Quantum yield or photosynthetic yield

$$= \frac{No\ of\ oxygen\ molecule\ evolved}{Per\ quanta\ of\ light\ absorbed} = \frac{1}{8} = 12\%$$

Emerson and his co workers exposed chlorella plant to only one wavelength of light at a time and measured quantum yield. This was carried out just to find out that at which wave length, of light quantum yield is more.

They observed a decline in photosynthetic yield at a wavelength above 680mμ i.e. was in the red region of visible sepctrun (380-760 mμ). This fall in photosynthesis is called Emerson's red drop (first experiment).

Again in another experiment, Emerson and his Co-workers supplied shorter wavelength (red light) along with long wave length (far red light). They observed enhancement in photosynthesis. This is called Emerson's enhancement effect.

The quantum yield from the two combined beam of light (red + far red) was found to be greater than the sum effect of both beams used separately.

Emerson's effect clearly shows the existence of two photo chemical process. One is driven by wave length exceeding 680 μm and other by shorter wave length. The two photochemical processes are believed to be associated with two specific group of pigments, called pigment system-I and pigment system-II.

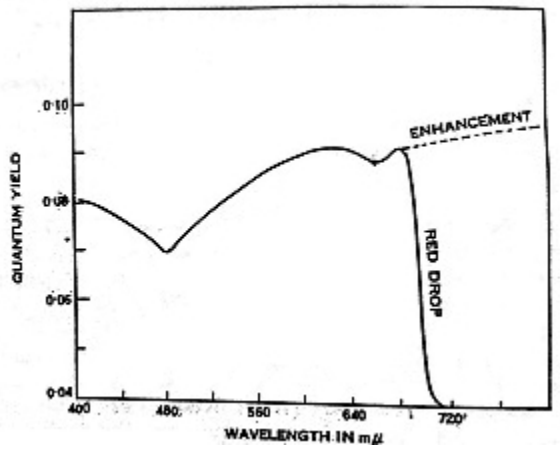


Fig.9.3: Graph showing Red Drop and Enhancement.

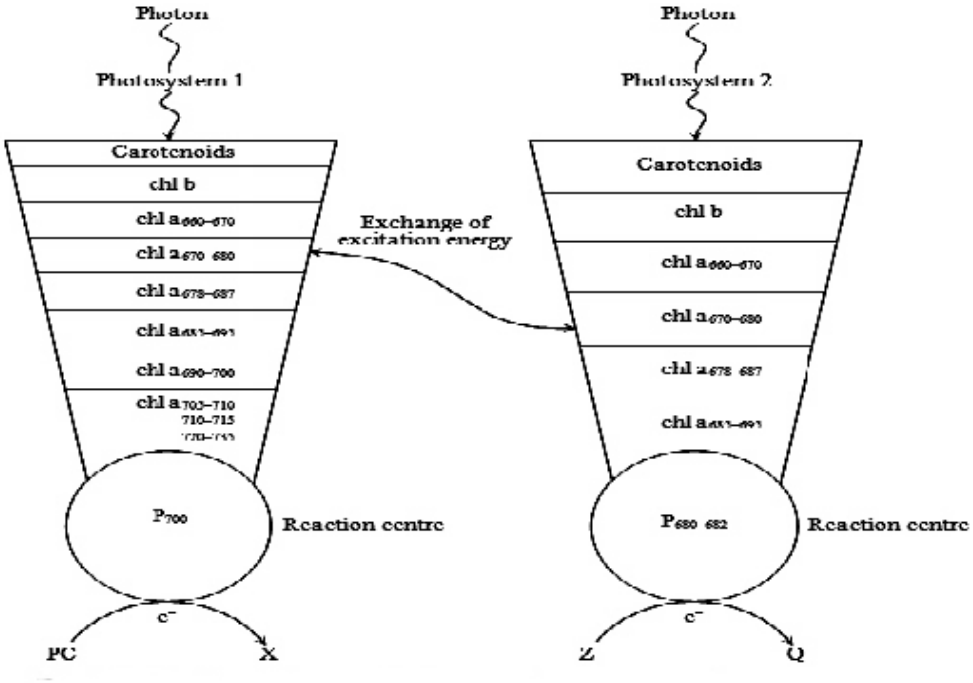
- **Pigment System**

Photosynthetic units occur in the form of two distinct groups called photosystems or pigment systems. Green plants and cyanobacteria possess two photosystems, I and II. But bacteria possess only one photosystem (Fig. 9.4).

Pigment System-I

It contains pigment like chlorophyll,a and b, canoten oils, xanthophyll and phycobilins. The reaction center of PSI is P700. The special chlorophyll a molecule which absorbs light near 700 ml. It is active both in red light and far red light and carried out reduction of NADP+. It is associated with cyclic electron transport. Photosystem I has FeS, ferredoxin, plastoquinone, cytochrome complex and plastocyanin. It takes part in both cyclic and non-cyclic photophosphorylation.

It absorbs shorter wave length its reaction center is P680 and pigments are chlorophyll (chlorophyll) a, b, and carotenoid. It is involved in non cyclic electron transport and causes photo oxidation of H_2O and evolution of O_2.



Pigment System I Pigment System II

Fig.9.4: Pigment System

9.3.1. Light reaction mechanism

In the light reaction following events occur -

1. Photooxidation of water take peace and O_2 is released.

2. Synthesis of ATP taking place by photo phosphorylation.

3. NADP+ is reduced to NADPH+H^+ Nicotinamide adenine dinucleotide phosphate.

Ruben et al-used radioactive O_2 in water supplied to the plant and found that O_2 released in photosynthesis comes from water-

$$6Co_2 + 12H_2{}^{18}o \rightarrow C_6H_{12}O6 + 6H_2O + 6^{18}O_2$$

The photophosphorylation is of two types:

- Non cyclic photophosphorylation or scheme or Z scheme.
- Cyclic photophosphorylation.

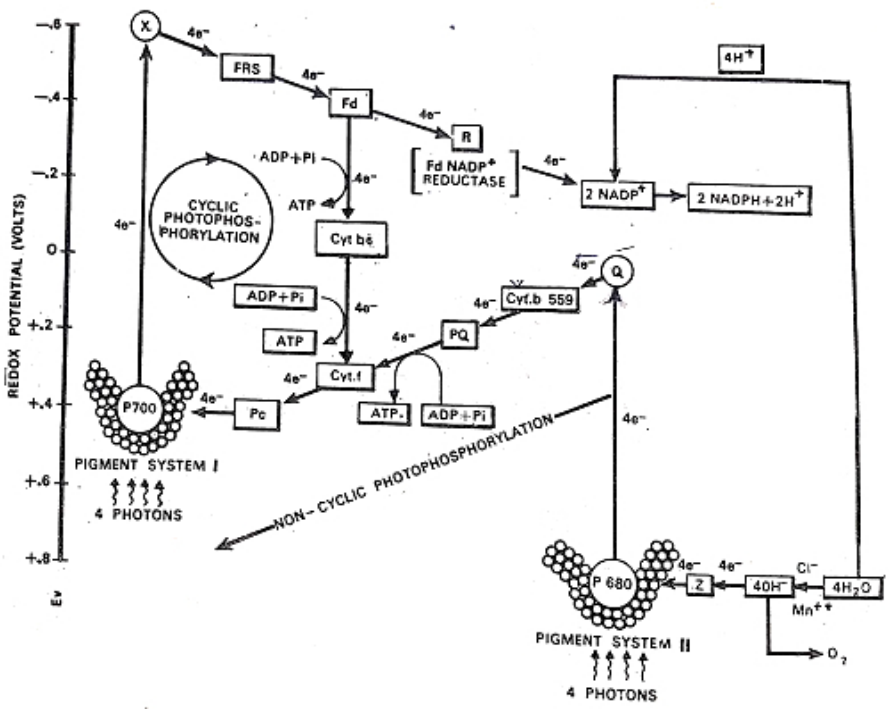


Fig. 9.5: Cyclic and Non Cyclic phoyophosphorylation.

9.3.1.1. Oxygenic photosynthesis or Non cyclic photophosphorylation

Oxygenic photosynthesis or Non-cyclic photophosphorylation occurs as a result of an interaction between photosystem I and photosystem II. Non-cyclic photophosphorylation helps in the formation of ATP as a result of electron flow from water to NADP. As this is a unidirectional flow, and does not follow any cyclic procedure, is called as non-cyclic photophosphorylation. The electron released from one particular pigment system does not return back to same pigment system. So electron deficiency of this pigment system satisfied by other pigment system.

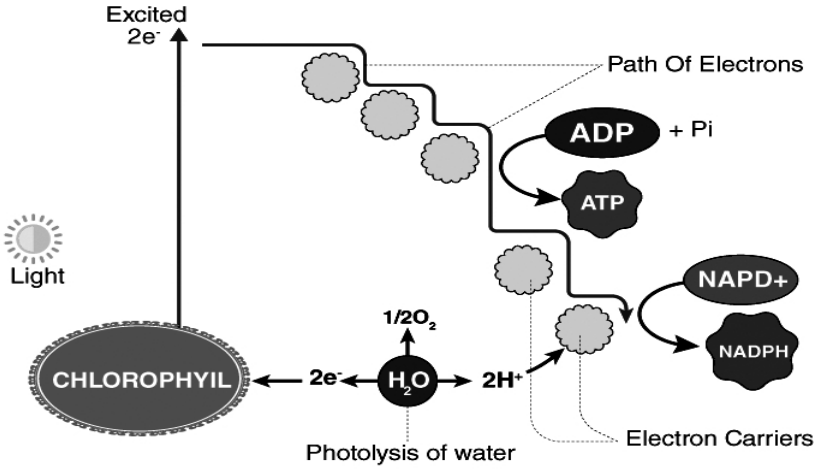


Fig.9.6: Non-cyclic Photophosphorylation

Source: https://byjus.com/biology/cyclic-and-non-cyclic-photophosphorylation/

Light of longer wave length hits pigment of photosystem-I as a result P700 gets excited and releases electrons which are accepted by an unknown primary electron acceptor A (Fe-5) believed to be an iron sulphur protein complex and gets reduced. From reduced A (Fe-S) the electrons are accepted by a nonhemeiron protein called ferrodoxin (Fd) and from reduced Fd. The electrons from reduced fd are transferred to NADP+ and NADP+ get reduced to $NADPH+H^+$. This causes deficit of electron in photo-system I. This deficit is made up by photo excitation of P680 of Photosystem I. Electron deficit created in photosystem II is filled by electrons derived by the photo-oxidation of water in presence of Mn^{++} and cl^- ions.

When a lower wave length of light is received by PS II, P680 looses an electron which is accepted by quinone. The electron then travels down the hill through a series of electron carriers B, PQ, cytf and plastocyanin. The energy released in the transfer of electron from PQ to cytf is utilised to convert ADP into ATP and then electron goes to PS I. At this stage water dissociates into H^+ and OH^- ions. The hydroxyl ion (OH^-) looses electrons and transferred to PS II. The H^+ are taken up by NADP+ which get reduced to $NADPH+H^+$.

Thus, in non-cyclic photophosphorylation the electron is not cycled back. Therefore, it is called non cyclic photophosphorylation.

9.3.1.2. Anoxygenic photosynthesis or cyclic photophosphorylation

Anoxygenic photosynthesis or cyclic photophosphorylation takes place under certain conditions. It operates when CO_2 assimilation is curtailed and $NADPH +H^+$ starts accumulating. The cyclic transport is for more production of ATP which is needed by chloroplast.

In this process, only photosystem I operates. So no photoxidation of water take place. Therefore, no evolution of O_2 and no formation of $NADPH+H^+$ occurs.

The electron flows from P_{700} to A (Fe-s), then to Fd which is unable to pass electron to NADP+. The electron passes to $cytb_6$ and cytf and then to PS I. Thus, here electron is cycled back. In this only one PSI operates. Therefore no $NADPH+H^+$ is formed and Co_2 fixation is curtailed. This results in decline in quantum yield.

But when shorter wave length is given simultaneously PS II also comes in operation and photoxidation of water relases H+ which reduces NADP+ to $NADPH+H^+$ and photosynthetic enhancement takes place.

9.3.2. Dark Reaction mechanism

The dark reaction mechanism is also known as light independent enzyme controlled biochemical reaction or dark fixation of Carbon dioxide. Dark reaction is purely enzymatic and does not require light. The site of dark reaction is stroma of chloroplast. Various enzymes required for fixation of

CO_2 are present in the stroma of chloroplast. The CO_2 absorbed by the plants from the environment combines with certain compounds in sequential steps to form intermediate compounds and ultimately results in the formation of sugar and starch. There are three pathways of CO_2 fixation.

- Calvin cycle or C_3 cycle.
- hatch-slack pathway or C_4 cycle.
- CAM cycle.

9.3.2.1. Calvin Cycle or C_3 cycle

It was discovered by Calvin. He used 14C and green alga chlorella and scendesmus and discovered C_3 cycle of Co_2 fixation using radioactive tracer technique. Sixmolecules of Co_2 combine with six molecules of ribulose 1,5 diphosphate in presence of water to form 12 molecules of 3-phosphoglyceric acid in presence of enzyme carboxydismutase. 3-phosphoglyceric acid (PGA) is first stable and detectable compound of calvin cycle which is 3 carbon compound. The 12 molecules of PGA react with 12 ATP molecules to produce 12 molecules of 1, 3-Diphosphoglyceric Acid in presence of Phosphoglycerokinase.

The 12 molecules of diphosphoglyceric acid is reduced to 12 molecules of phosphoglyceraldehyde by 12 molecules of NADPH $+H^+$. 12 NADPH $+H^+$ and 12 H_3PO_4 are regenerated in the process in presence of enzyme 3-phosphoglyceraldehyde dehydrogenate.

The 5 molecules of 3-phosphoglyceraldehyde get isomerised to form dihydroxy acetone phosphate. The 3 molecules of dihydroxy acetone phosphate combines with 3 molecules of 3-phosphoglyceraldehyde to form 3 molecules of Fructose 1, 6 diphosphate in presence of enzyyme aldolase.

Each molecules of Fructose 1, 6-diphosphate loses one phosphate in presence of enzyme phosphatase to form 3 molecules of Fructose-6 phosphate. One molecule of Fructose 6-phosphate forms one molecule of Hexose sugar.

2 molecules of frustose 6- phosphate react with 2 molecules of 3-phosphoglyceraldehyde to produce 2 molecules of xylulose 5-phosphate and 2 molecules of erythrose-4-phosphate in presence of enzyme Transketolase.

2 molecules of Erythrose-4-phosphate combines with 2 molecules of dihydroxy acetone phosphate to produce 2 molecules of sedoheptulose-1, 7, diphosphate in presence of transaldolase enzyme.

Each molecule of sedoheptulose-1, 7 diphosphate loses one phosphate group in presence of phosphate enzyme to form sedoheptulose-7 phosphate.

2 molecules of sedoheptulose-7 phosphate react with 2 molecules of 3-phosphoglyceraldehyde in presence of enzyme transketolase to produce 2 molecules of Ribose-5-phosphate and 2 molecules of xylulose-5-phosphate.

2 molecules of ribose-5-phosphate are converted to two molecules of ribulose-5-phosphate in presence of phosphoribose isomerase enzyme.

4 molecules of xylulose-5-phosphate are isomerised to 4 molecules of ribulose-5-phosphate in presence of enzyme ribulose phosphate isomerase.

At the end of cycle all the six molecules of ribulose-5-phosphate get converted to ribulose-1, 5- diphosphate.

Thus the whole process of calvin cycle begins with the absoption of 6molecules of CO_2 by 6 molecules of RUBP and ends with the formation of 1 molecule of hexose sugar with the regeneration of 6 mol. of RUBP.

The energy required in this reaction is supplied by 12 $NADPH+H^+$ and 18 ATP formed in the light reaction of photosynthesis.

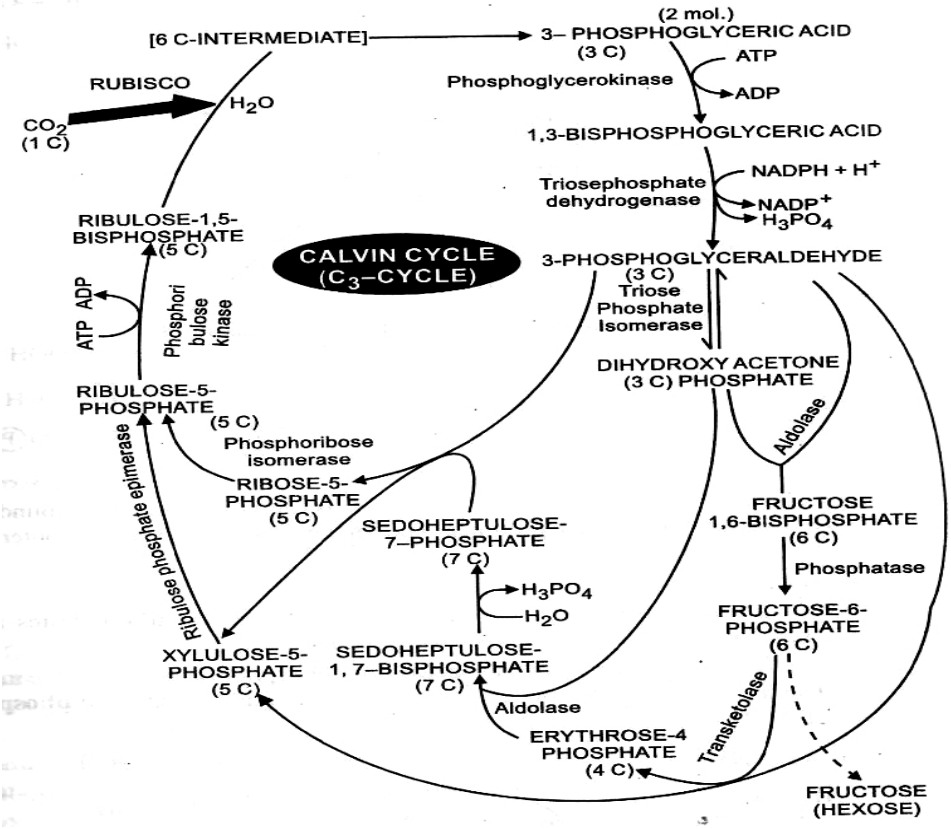


Fig.9.7: Calvin Cycle(C3 cycle)

9.3.2.2. C4 cycle

C4 is the alternative pathway of Calvin cycle (C3 cycle). It is just order to avoid photorespiration because photorespiration is a wasteful reaction that occurs when plants take in oxygen and give out carbon dioxide instead of

taking in carbon dioxide and releasing oxygen. In the C4 cycle the first stable compound is 4 carbon compounds, namely oxaloacetic acid. Hence it is called C4 cycle. It is a process of carbon dioxide fixation. This pathway was work out by Hatch and slack 19 66 so that this cycle is also known as Hatch-Slack cycle. For example, corn,sorghum and sugar cane etc. f

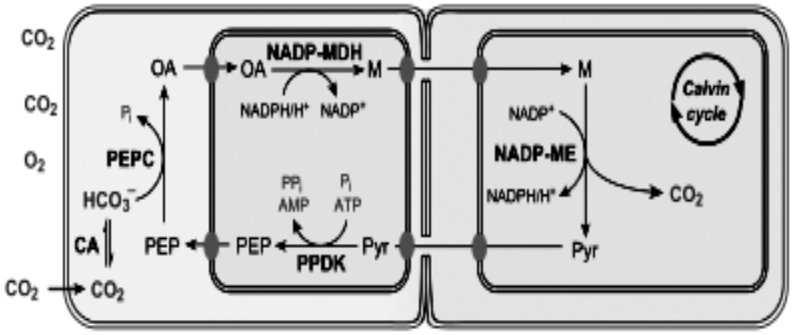


Fig. 9.8: C4 Cycle

Table9.1: Basic deference between C3 anC4 Cycle

C3 cycle	C4 cycle
C3 cycle operates in all plants that occurs in Mango, Apple etc.	C4 cycle operates only in C4 plants that occurs in maize, Sorghum and Cane etc
The primary CO_2 acceptor is Ribulose bi phosphate (RUBP a 5 Carbon compound).	The primary CO_2 acceptor is Phosphoenol pyruvic acid (PEP, a 3 Carbon compound).
The carboxylase enzyme is Rubisco.	The carboxylase enzyme is PEP carboxylase and Rubisco.
Single CO_2 fixation. Cannot operate under very low CO2 concentration.	Two CO2 fixations.
Fixation of 1 molecule of CO_2 requires 3 ATP and 2 NADH.	Fixation of 1 molecule of CO_2 requires 5 ATP and 3 NADH.
Only granal types of chloroplasts are involved.	Granal and agranal (bundle sheath chloroplast) type of chloroplasts are involved are dimorphic in nature.
Oxygen has inhibitory effect on photosynthesis.	Oxygen has no inhibitory effect on photosynthesis.

9.3.2.3. CAM Cycle

CAM is stand for Crassulacean acid metabolism. Some plants that are adapted to dry environments, such as cacti and pineapples, these plants adopt CAM pathway to minimize photorespiration. This name comes from the family of plants, the Crassulaceae, in which scientists first discovered with pathway. CAM plants minimize photorespiration and save water by separating these steps in time, between night and day. This allows the plants to conserve their water by closing their stomata during the hot daytimes. CAM plants separate these processes in time. At night, CAM plants open their stomata, allowing CO_2 to diffuse within the leave. This Co2 is fixed into oxaloacetate by PEP carboxylase then converted to malate or another type of organic acid. It is resorted in vacuole of cell. During day time, its come out from vacuole into cytoplasm, where it is decarboxylated. The relased CO_2 fixed by Calvin cycle.

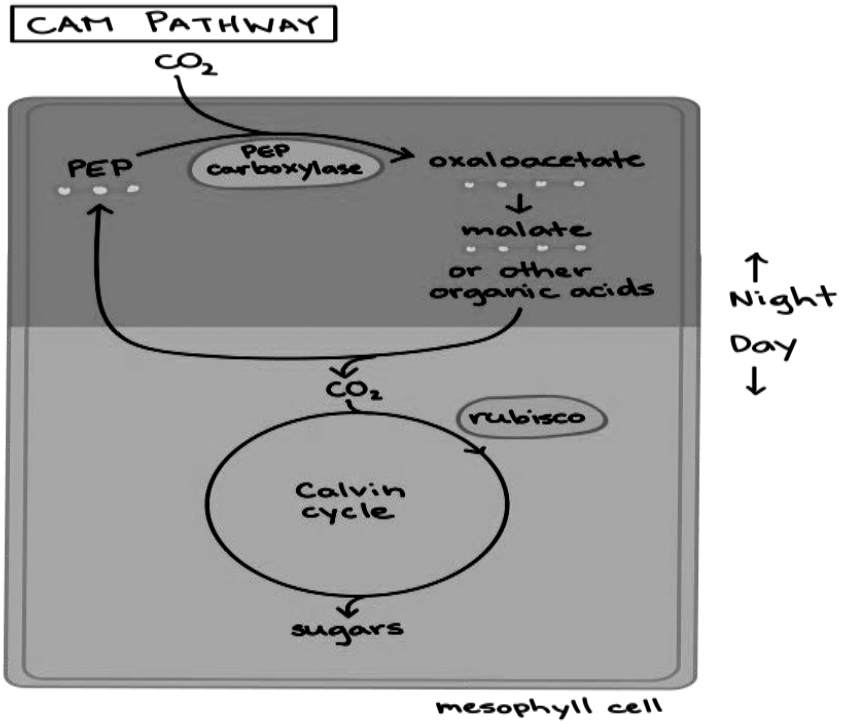


Fig.9.9: CAM pathway

Source:https://www.khanacademy.org/science/biology/photosynthesis-in-plants/photorespiration--c3-c4-cam-plants/a/c3-c4-and-cam-plants-agriculture

9.4. Summary

In this unit you have learnt that:

Plants synthesize their own food from raw materials by the process of photosynthesis. The photosynthesis is a very important metabolic process which provides food and O_2 to living organism. Photosynthesis occurs in

chloroplast. Light reaction occurs in grana and dark reaction in the stroma of chloroplast. In the light reaction, synthesis of ATP (Adenosine triphosphate), NADPH+H+ (reduced Nicotinamide adeninedinucleotide phosphate) and evolution of oxygen from water takes place. In the dark reaction fixation of CO_2 takes place and final product carbohydrate is formed by C_3 cycle, C_4 cycle and CAM cycle only fix Co_2 in day and night time, respectively. C_3 cycle occurs in C_3 plants, C_4 cycle occurs in C_4 plants and CAM cycle occurs in succulent plants. The C_4 cycle and CAM cycle is a kind of adaptation in C_4 and succulent plant respectively. Photorespiration or C_2 cycle occurs in C_3 plants therefore, photosynthetic yield of C_3 plant is less than C_4 plant. C4 cycle is just order to avoid photorespiration because photorespiration is a wasteful reaction that occurs when plants take in oxygen and give out carbon dioxide instead of taking in carbon dioxide and releasing oxygen. CAM pathways are two adaptations beneficial features arising by natural selection that allow certain species to minimize photorespiration.

9.5. Terminal Questions

Q.1. Describe the structure of chloroplast.

Answer:----------

Q.2. Describe the mechanism of light reaction of photosynthesis.

Answer:----------

Q.3. Give an account of calvin (C_3) cycle.

Answer:----------

Q.4. Discuss the mechanism and significance of Hatch-Slack Pathway (C_4 cycle).

Answer:----------

Q.5. Differentiate between C_3 and C_4 plant.

Answer:----------

Short Questions

Q.6. Write short note on :

(1) Emerson Effect

(2) PS I

(3) PS II

(4) Q_{10} or temperature co-efficient.

(5) CAM cycle

Q.7. Multiple choice questions MCQ.

(1) Light reaction of photosynthesis occurs in :

(a) Grana of chloroplast (b) Stroma of chloroplast

(c) Both (d) None of the above

(2) Dark reaction of photosynthesis occurs in :

(a) Stroma of chlorplast (b) Grana of chloroplast

(c) Both (d) None of the above

(3) Oxidation of water is catalysed by :

(a) Photosystem-I (b) Photosystem-II

(c) ATP Synthetase (d) None of these

(4) Photosynthesis is :

(a) Fixation of Co_2 and H_2o (b) Fixation of carbohydrates

(c) Fixation of sugar (d) None of these

(5) The O_2 released in photosynthesis comes from :

(a) Co_2 (b) H_2O (c) Both (d) None of these

Answer :

(1) a (2) a (3) b (4) a (5) b

9.6. Further readings

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