Paper IX

Bio - Organic, Bio - Inorganic and Bio - Physical Chemistry

Bio – Organic Chemistry

Unit I : Nucleic Acids

Purine and pyrimidine bases of nucleic acids. Nucleosides, Nucleotides, Base pairing via H – bonding. Primary and secondary structures of DNA and RNA. Forces responsible for holding double helix structure. Transcription and translation DNA sequencing, Enzymatic method, PCR technique in chain amplification.

Unit II : Cobohydrates and Lipids

A. Carbohydrates :

Storage polysaccharides – starch, chitin and glycogen. Structure and biological functions of glucosaminoglycans of mucopolysaccharide. Carbohydrates of glyeoproteins and glycolipids. Role of sugars in biological recognition. Blood group substances.

Carbohydrate metabolism:

Kreb's cycle, glycolysis, glycogenesis and glycogenolysis, gluconeogenesis, Pentose phosphate pathway.

B. Lipids:

Fatty acids, essential fatty acid, structure and function of triacylglycerols glycerophospholipids, sphingolipids, cholesterol, bile acids, prostoglandins. Lipoproteins – composition and function, role in atherosclerosis. Biological membranes. Fluid mosaic model of membrane structure.

Unit – III Bioinorganic Chemistry I

Non – metals and metals in biological system; essential and trace elements. Classification of metallobiomolecules – non – proteins, proteins and enzymes. Metallobiosite, its coordination environment and entatic state.

Dioxygen binding and transport – heme proteins: myoglobin and hemoglobin, their structure function and physiology ; non – heme proteins: hemerythrine and hemocyanin.

Biochemical iron storage and transport by Transferrins and siderophores; metal ion exchange activity of siderophores.

Unit IV

Bioinorganic Chemistry II

Electron transfer in biological sytems – Prophyrins and cytochromes ; Fe – S – cluster proteins (Ferredoxins and rubredoxins) and their synthetic models; blue copper proteins. Photosynthetic pathway – chlorophyll, PSI, PSII and involvent of Mn complex and Cytochrome C oxidase.

Unit V : Biophysical Chemistry

Thermodynamics in biology – energy flux – transfer of potentials and coupled reactions – role of single oxygen in biology – general principles of function and structural organization in bioenergetic fundamental reactions – structure of membranes (introductory aspects only) – solute transport across membranes – membrane potentials – ion pumps – biophysical applications of Moussbauer effect.

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References

Unit 1 & II

1. G.L. Zubay, W.W. Parson and D.E. Vance, Principles of Biochemistry, Wm.C. Brown Publishers 1995.
2. G.L. Zubay, Biochemistry, Wm.C. Brown Publishers, Chicago, 1998.
3. L. Stryer, Biochemistry (4th edn.) W.H. Freeman and Company, 1995.

Unit III & IV

1. D.E. Fenton, Biocoordination Chemistry, Oxford Chemistry, Primer series, Oxford Science Publications, Oxford, 1995.
2. W.L. Jolly, Modern Inorganic Chemistry, Mc Graw Hill Company 2nd edn. 1991.
3. J.E. Huheey, E.A. Keiter and R.L. Keiter, Inorganic Chemistry, Harper and Row, 4th edn. 1993.
4. B. Douglas and D. McDaniel and J. Alexander Concepts and Models of Inorganic Chemistry, John Wiley & Sons, 2nd edn. 1983.

Unit V

1. A.G. Marshall, Biophysical Chemistry, John Wiley and Sons, New York, 1978.
2. K.J. Laidler, Physical Chemistry with Biological Applications, Benjamin, 1980.
3. A.L. Lehninger, D.I. Nelson, and M.M. Cox, Principles of Biochemistry, Worth Publishers Inc. USA. 1993.
4. G.L. Zubay Biochemistry, Wm.C. Brown Publishers, Chicago, 1998.

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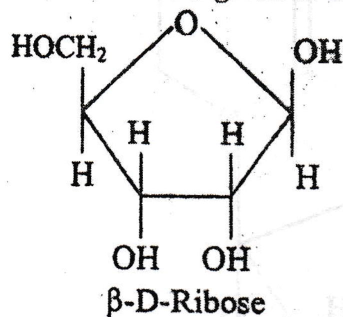
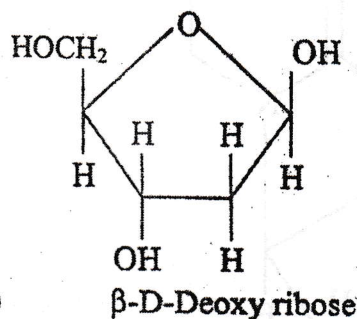
UNIT – I Bio Organic Chemistry

NUCLEIC ACIDS

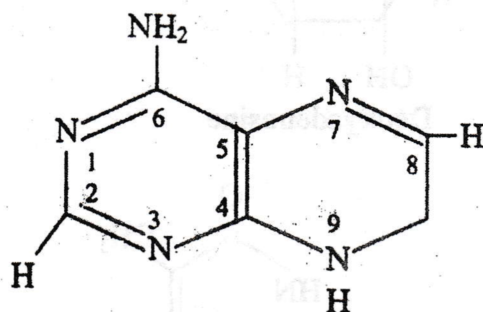
Nucleosides are simple molecules of pentoses whose anomeric hydroxy groups (C_1) are replaced with purine or pyrimidine bases. In biosystems, the pentoses are usually β-D-ribose or β-2-deoxy D-ribose and the bases are adenine, guanine, cytosine, uracil and thymine. Their structures are given as under:

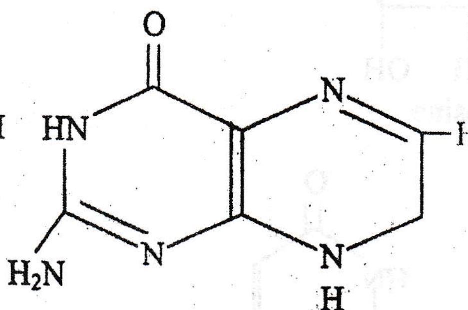


β-D-Ribose

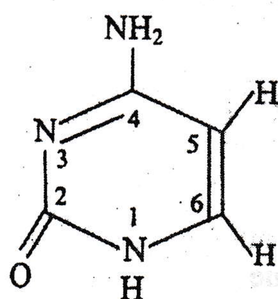


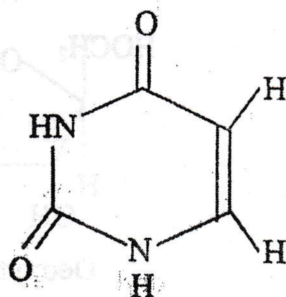
(Pentoses) β-D-Deoxy ribose

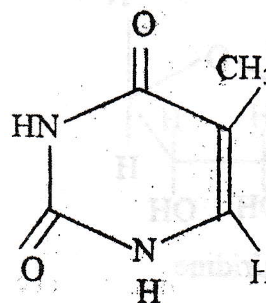




Adenine (Purines) Guanine



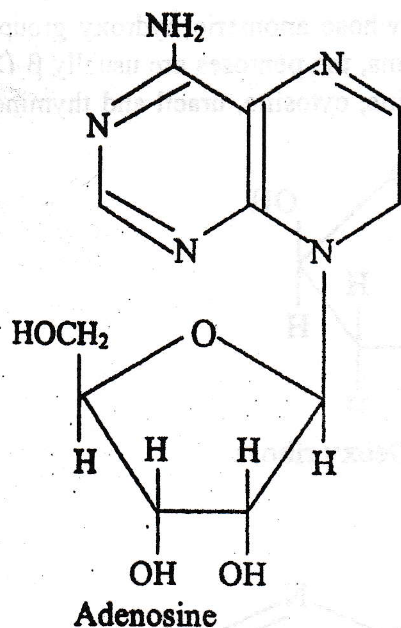




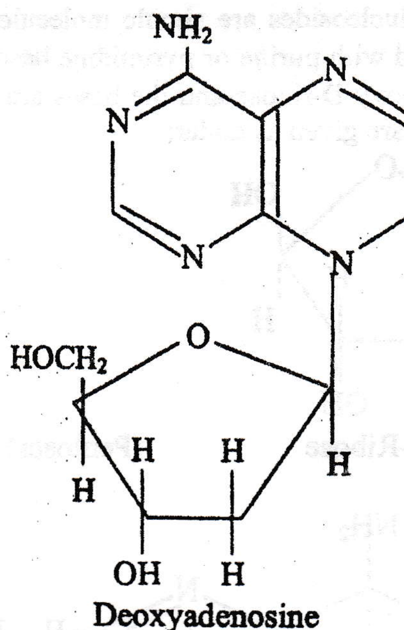
Cytosine Uracil Thymine

(Pyrimidines)

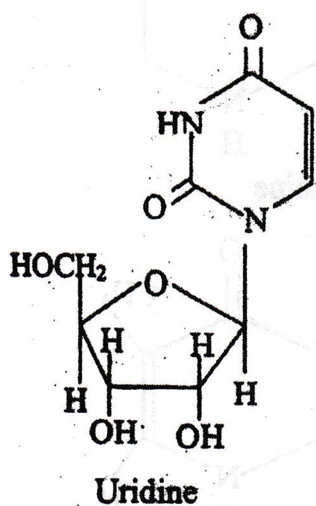
The C_1 carbon of the ribose is bonded to N_1 of the pyrimidine or N_9 of the purine base. The ribonucleosides are known as adenosine, guanosine, uridine and cytidine. The deoxyribonucleosides are also named accordingly but thymine replacing uracil. Hence they are deoxyadenosine, deoxyguanosine, deoxythymidine and deoxycyctidine. Their structures are given as under:



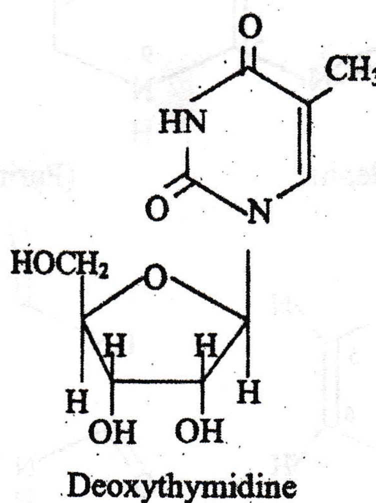
Adenosine



Deoxyadenosine

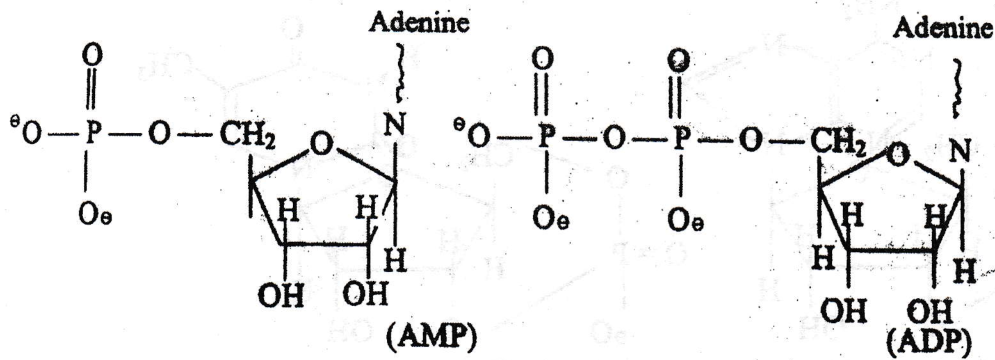


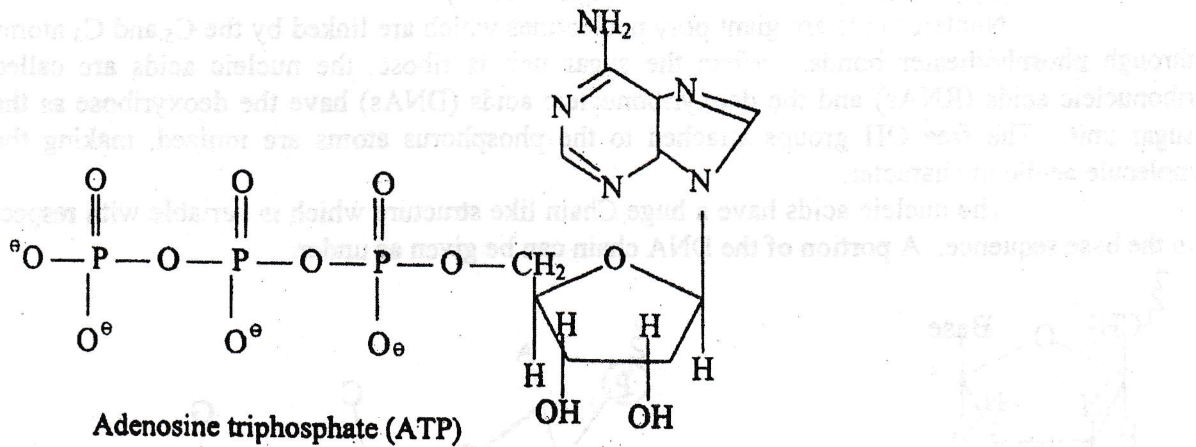
Uridine



Deoxythymidine

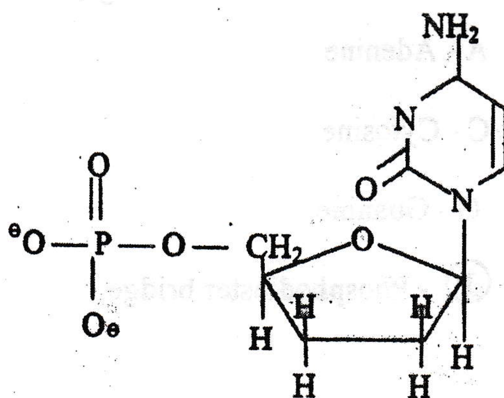
Nucleotides are nucleosides phosphorylated at C_5 position, hence known as phosphate esters of nucleosides. (e.g) Adenosine 5-phosphate (Adenosine monophosphate better known as AMP). This nucleotide could be further phosphorylated or esterified to give adenosine diphosphate (ADP) and adenosine triphosphate (ATP), generally called adenlyates.





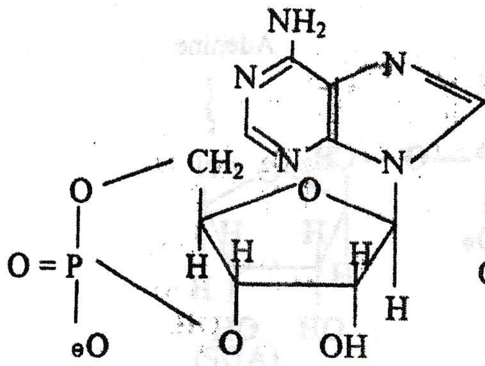
Adenosine triphosphate (ATP)

Other ribonuleotides such as guanylate (GMP), uridylate (UMP) and cytidate (CMP), along with the deoxy ribonucleotides such as deoxyadenylate (dAMP), deoxy guanylate (dAMP), deoxy thymidylate (dTMP) and deoxy cytidylate (dCMP) are known.

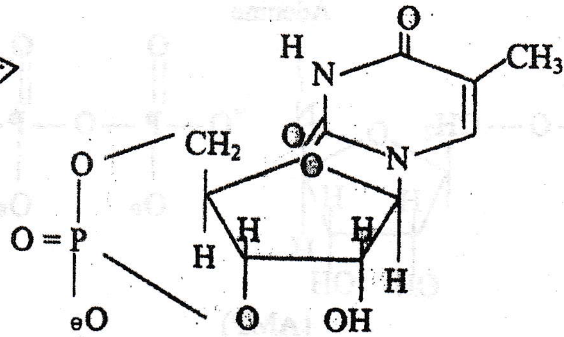


Deoxy cytidylate (dCMP)

Cyclic ribonucleotides and deoxy ribonucleotides are also common (e.g) cyclic AMP is formed by phosphorylation through C_3 and C_5-OH groups in the pentose.



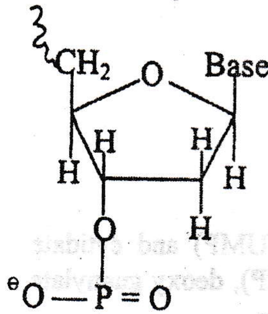
(Cyclic AMP)

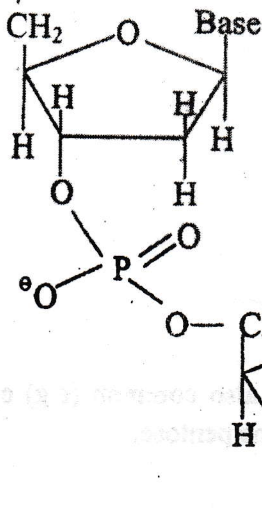


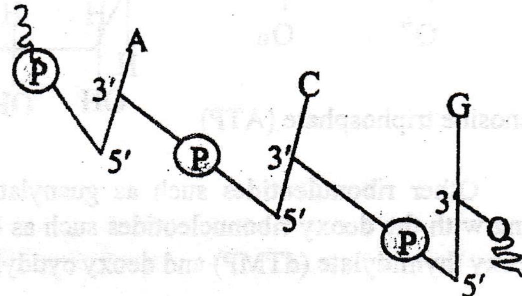
(Cyclic TMP)

Nucleic acids are giant poly nucleotides which are linked by the C_5 and C_3 atoms through phosphodiester bonds. When the sugar unit is ribose, the nucleic acids are called ribonucleic acids (RNAs) and the deoxyribonucleic acids (DNAs) have the deoxyribose as the sugar unit. The free OH groups attached to the phosphorus atoms are ionized, making the molecule acidic in character.

The nucleic acids have a huge Chain like structure which is variable with respect to the base sequence. A portion of the DNA chain can be given as under:







A - Adenine

C - Cytosine

G - Guanine

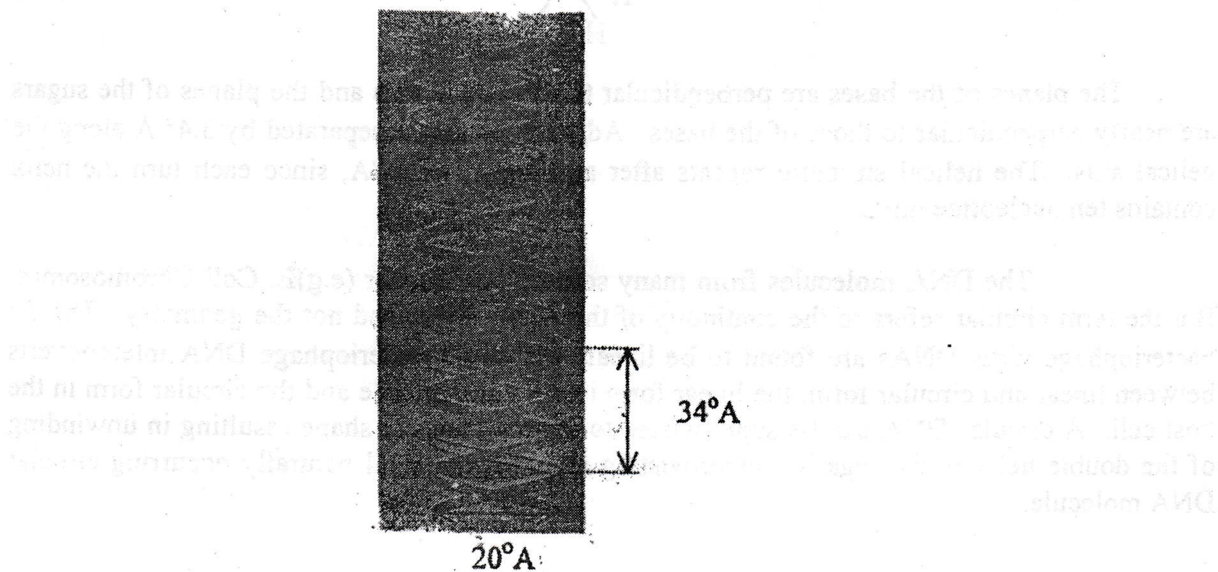
(P) - Phosphaodiester bridge.

By convention, the base sequence for instance, ACG means that the bases are linked in the 5' ⟶ 3' direction, starting from adenine, through cytosine to guanine.

Primary and Secondary Structures

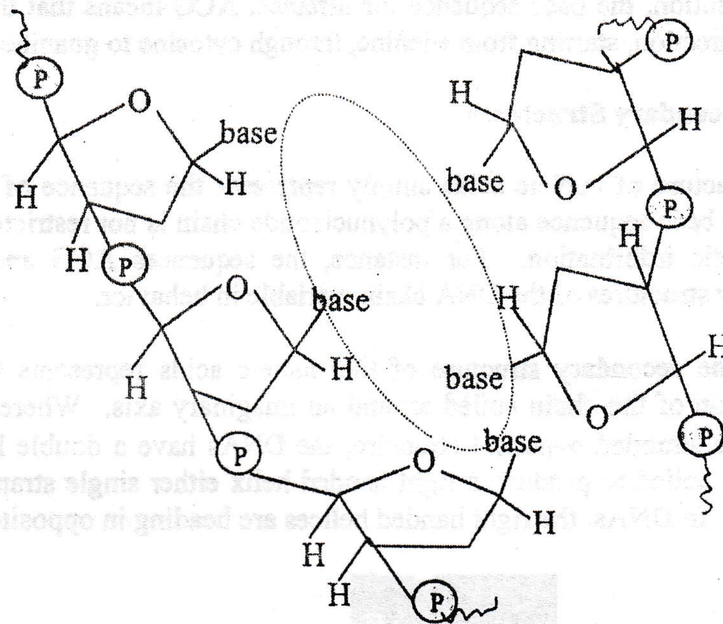
The primary structure of nucleic acids simply represents the sequence of bases attached to the sugar units. The base sequence along a polynucleotide chain is not restricted in any way but that carries the genetic information. For instance, the sequences ACG and GCA represent the differing primary structures of the DNA chain, variable in behavior.

The secondary structure of the nucleic acids represents the three dimentional helical distribution of the chain coiled around an imaginary axis. Whereas the RNA takes the shape of a single stranded α-helical structure, the DNAs have a double helical structure. The chains are found coiled to produce a right handed helix either single stranded (RNA) or double stranded (DNA). In DNAs, the right handed helices are heading in opposite directions.



Watson-Crick model of DNA

The two strands are 20°A diameter apart, and each turn in the helix has approximately ten nucleotide residues. The purine and pyrimidine bases are inside of the helix, whereas the phosphate and sugar units are on the outside as shown

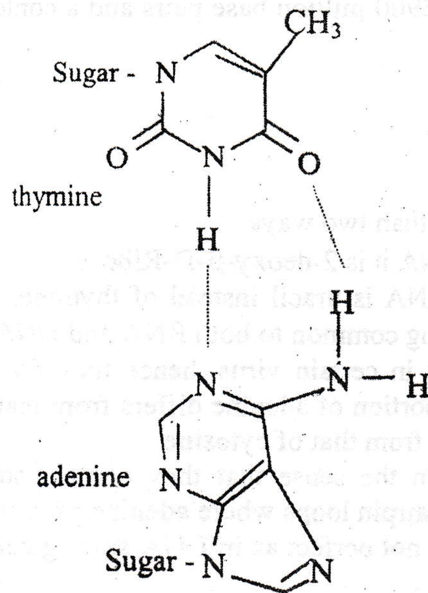


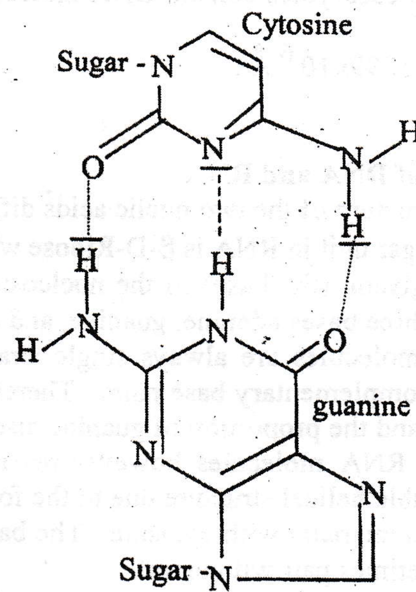
The planes of the bases are perpendicular to the helical axis and the planes of the sugars are nearly perpendicular to those of the bases. Adjacent bases are separated by 3.4° A along the helical axis. The helical structure repeats after an interval of 34°A, since each turn the helix contains ten nucleotide units.

The DNA molecules from many sources are circular (e.g)E. Coli Chromosomes. But the term circular refers to the continuity of the DNA chains and not the geometry. The T7 bacteriophage virus DNAs are found to be linear whereas λ bacteriophage DNA interconverts between linear and circular form, the linear form in the virus particle and the circular form in the host cell. A circular DNA can be supertwisted to a more compact shape resulting in unwinding of the double helix with, negative supertwisting as observed in all naturally occurring circular DNA molecule.

Forces Operating in a DNA Molecule (Base Pairing)

The sugar units and phosphate links form the back bone of the polynucleotide chains. The two chains are held together by specific hydrogen bonding between a pair of bases., on the opposite sides of the chains which form the inside of the helix. The most important aspect of the DNA double helix, is the specificity, of pairing of bases. Adenine in always found to be paired through Hydrogen bonding with thymine and guanine is paired with cytosine as indicated below.





(A–T) (C–G)

Due to the steric requirement necessitated by the regular helical model, the glycosidic bonds attached to the hydrogen bonded bases, should be 10.85°A apart. Moreover, because of steric factors one of the pairing bases must be a pyrimidine and the other a purine. The Hydrogen bonding requirement also restricts the base pairing, since adenine cannot pair with cytosine nor guanine with thymine. Whenever adenine forms two hydrogen bonds with thymine, guanine forms three such bonds with cytosine. The base pairing scheme receives strong support from the proposed Watson-crick model, since the adenine/thymine and guanine /cytosine ratios in most of the species studied, are found to be around one.

Replication of DNA molecule

If the actual order of the bases on one of the chains is known, it is possible to write down the order of bases on the other chain, because of specific pairing. In other words, one chain is the complement of the other, since the sequence of pairs of bases are duplicated exactly in order to facilitate specific pairing through hydrogen bonding. Thus each chain acts as a template or mould for the formation of a companion chain (e.g) The complementary sequence of the chain 5′GATCAA3′ could be written based on the base pairing mechanism as, 3′ TTGATC5′ since adenine pairs with thymine and guomine pairs with cytosine. The double helical DNA is found to be the replicative form of all known genes. However, the base pairs and their length differ (e.g).

(1) E. Coli bacteria Chromosome is a single molecule of DNA with four million base pairs and a length of 14×10^{6} A°.

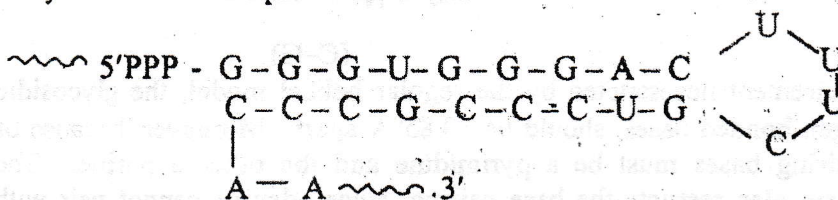
(2) Human eucaryotes contain DNA molecules having 2900 million base pairs and a contour length of 99×10^{6} Å.

Comparison of DNA and RNA

The structure of the two nuclic acids differs in more than two ways

1. The sugar unit in RNA is β-D-Ribose whereas in DNA it is 2-deoxy-β-D-Ribose.
2. The aglycon (the base) in the nucleoside part of RNA is uracil instead of thymine, the other three bases adenine, guanine, and cytosine being common to both RNA and DNA.
3. RNA molecules are always single stranded except in certain virus, hence they do not have complementary base pairs. Therefore the proportion of adenine differs from that of uracil and the proportion of guanine also is different from that of cytosine.

RNA molecules however resemble DNA in the sense that they contain some regions of double helical structure due to the formation of hairpin loops where adenine pairs with uracil and guanine pairs with cytosine. The base pairing is not perfect as in DNA since guanine may also sometimes pair with uracil.



The two nuclic acids also differ with respect to their functions. There are at least three different types of RNAs, which perform a concerted action in protein synthesis. There is a messenger RNA (mRNA) corresponding to every gene or group of genes, hence their heterogeneous nature. There is at least one transfer RNA (tRNA) for each of the twenty α-amino acids since it is the tRNA (refer – 'Translation' for the structure of tRNA) which carries the amino acid in the active form to the reaction site (ie) ribosomes. The ribosomal RNA (rRNA) is the major component of the ribosomes, where protein synthesis takes place. The relative % of the 3 RNAs differ in E. Coli, along with other differences. The smallest among the RNAs are t RNA With only about 75 nucleotides, whereas the largest ones are among the m RNAs containg more than 5000 nucleotides.

Type of RNA	Relative %	Sedimentation coeff. ($1S = 10^{-13}$ sec)	No of nucleotides
rRNA	80	235 165 55	3700
t RNA	15	45	75
m RNA	5	Heterogeneous	>5000

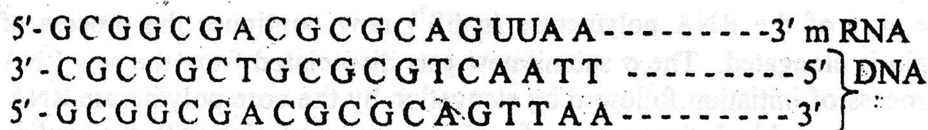
Transcription and Translation

DNAs provide the source of genetic information of cells and are termed as molecules of heredity. Normally the purine and pyrimidine bases carry the genetic information whereas the sugar and phosphate groups perform only a structural role. The DNA template gives the necessary instructions to the information-carrying RNA molecules (mRNAs) in protein synthesis. This process is known as transcription. Following the process of transcription by the DNAs, the transfer RNAs read out the message from the messenger RNAs and carry out the protein synthesis by transferring the specified amino acid to the ribosomes. This process of execution of the transcription is known as translation.

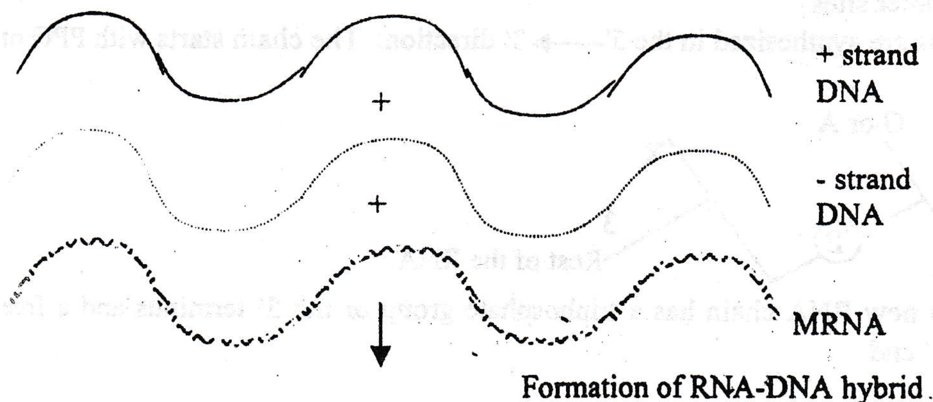
The m RNA involved in transcription, must be a very short-lived intermediate associated with ribosomes and heterogeneous in nature, since each gene (DNA) has a m RNA. The base composition of a m RNA should reflect the base composition of the DNA on whose template the m RNA is made.

Mechanism of Transcription

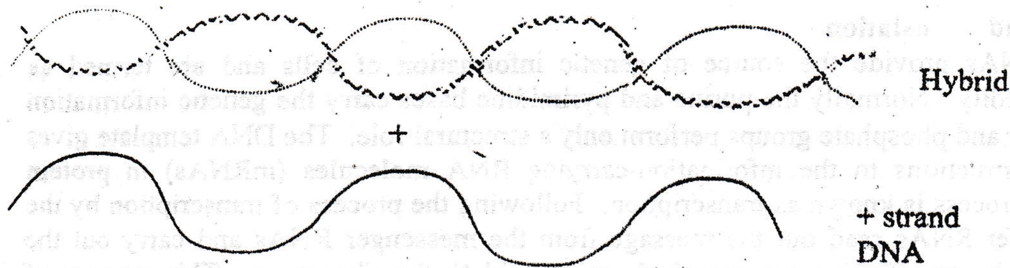
The complementary relationship between the DNA template and the RNA product has been established through hybridization experiments from a mixture of single stranded DNA and RNA when their base sequences were complementary. A portion of the base sequence in m RNA on its complement DNA template is as shown.



It has also been established that only one strand of DNA serves as a template for transcription as shown by hybridization studies on E. Coli infected with Ø x174 phage.



Formation of RNA-DNA hybrid.



The process of transcription starts at specific sites called promoters on the DNA template. The DNA in E. Coil shows a sequence of seven base pairs centered about ten bases, which constitutes the promoter sites. The process of transcription involves 3 stages (i) initiation (ii) elongation and (iii) termination.

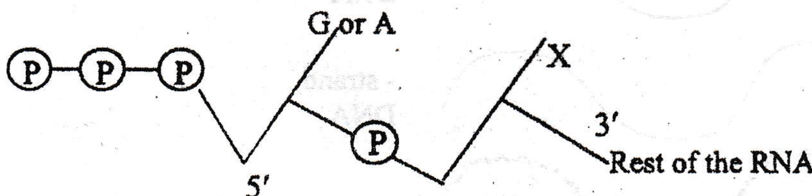
Initiation

At the start of the transcription, the RNA polymerase enzyme from E. Coli having a number of subunits or holoenzymes α, β, β, and σ, gets dissociated. The σ subunit first dissociates selecting the initiation sites for transcription. The σ submit also contributes to specific initiation by decreasing the affinity of the RNA polymerase for the general regions of DNA by a factor of 10^4. It also participates in opening the DNA double helix, by unwinding one of the strands of the helix, which serves as the template. Hence the stage is set for the formation of the first phosphodiester bond of the new RNA chain.

Elongation

The core part of the RNA polymerase ($\alpha_2\beta\beta^1$), now continues the process of transcription and he chain is elongated. The σ submit now gets dissociated from the new RNA chain and repeats the process of initiation followed by elongation by the core polymerase RNA. Certain proteins called repressors, block the process of initiation by interfering with the binding RNA polymerase(e.g) (1) lac repressor, a tetrameric protein, is present in an infected E. Coli (2) Antibiotics such as Rifamycin and Actinomycin also inhibit transcription by different mechanisms, getting bound to the DNA. However the presence of some positive regulatory factors are found to enhance the process of initiation. (e.g) cyclic AMP is found to stimulate transcription either alone or in combination with catabolite gene activator proteins (CAP), by binding to certain promoter sites :

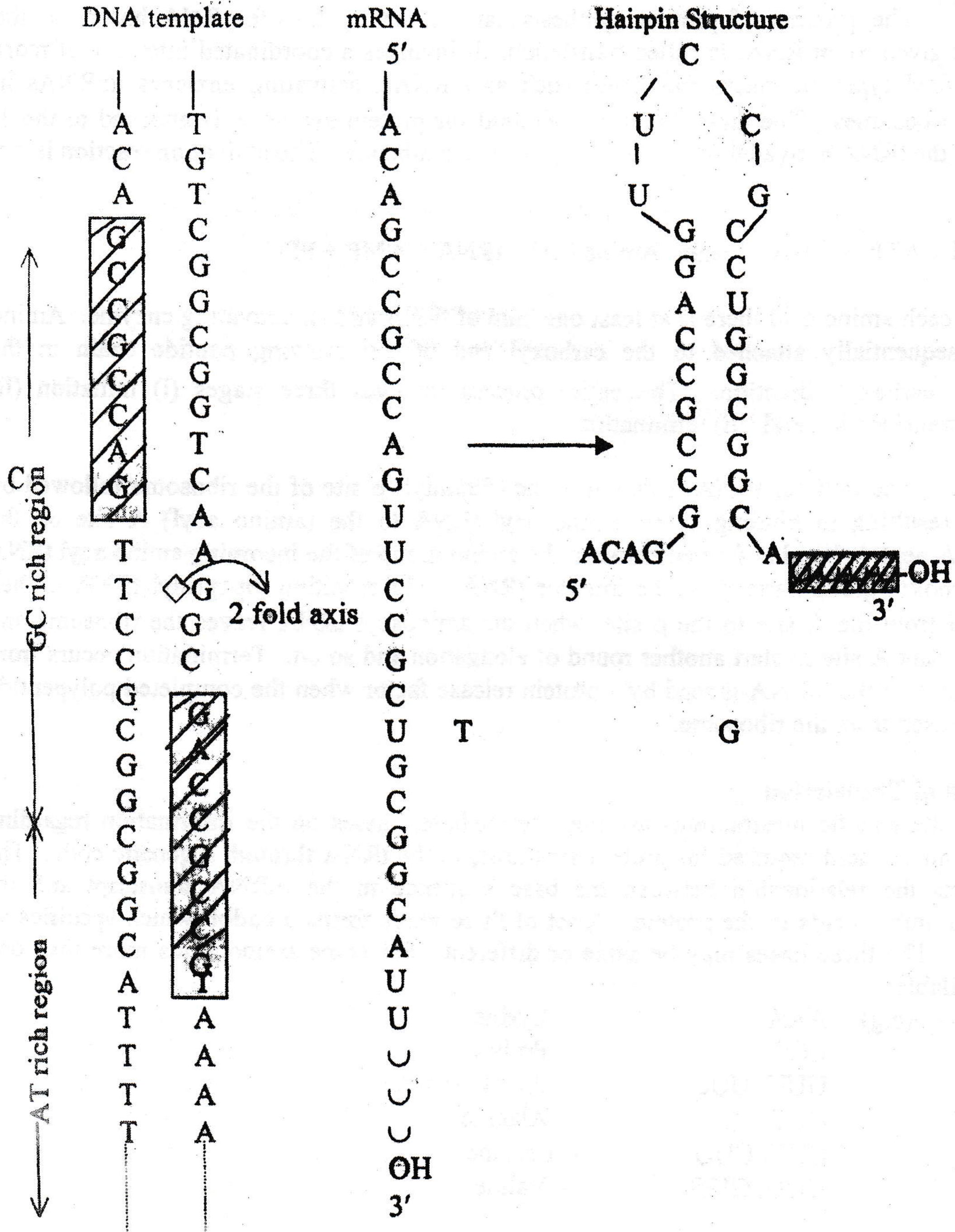
The RNA chains are synthesized in the 5′⟶3′ direction. The chain starts with PPG or PPPA as shown:



Hence a new RNA chain has a triphosphate group or the 5′ terminus and a free hydhoxyl group at its 3′ end.

Termination

The DNA template contains stop signals for transcription which is controlled in the same way as its initiation. The template is characterized by a GC-rich region and an AT-rich region. The former region is distinguished by the presence of a two fold axis of symmetry and the RNA transcriprt of this region can form a hairpin structure. In addition the RNA chain ends with several ∪ residues. One or all these structural features give a stop signal and the chain terminates. *



Evidence has also been obtained for the participation of ρ (rho protein) in termination of transcription. RNAs synthesized in the presence of rho protein are found to be shorter than that in its absence. These RNAs have sedimentation coefficients of 135,175 and 235 when rho proteins are added a few seconds, 2 minutes and ten minutes respectively after the initiation of synthesis.

Translation

The process of protein synthesis carried out by transfer RNA based on the instructions given by m RNA, is called translation. It involves a coordinated interplay of more than a hundred types of macro molecules such as t RNAs, activating enzymes, mRNAs in addition to ribosomes. The amino acid etc required for protein synthesis is attached to the 3′ terminus of the tRNA activated by an aminoacyl tRNA synthetase. The activation reaction is run by ATP.

$$\text{Amino acid} + \text{ATP} + \text{tRNA} \rightleftharpoons \text{Amino acyl} - \text{tRNA} + \text{AMP} + \text{PPi}$$

For each amino acid there is at least one kind of tRNA and an activating enzyme. Amino acids are sequentially attached to the carboxyl end of the growing peptide chain in the amino → carboxyl direction. The entire process involves three stages (i) initiation (ii) elongation translocation and (iii) termination.

The initiator tRNA is bound to the (Peptidyl) p site of the ribosome followed by elongation resulting in binding of an amino acyl tRNA to the (amino acyl) A site on the ribosome. A peptide bond is formed between the amino group of the incoming amino acyl tRNA and the carboxyl group carried by the initiator tRNA. The resulting dipeptidyl tRNA is then translocated from the A site to the p site, when the aminoacyl tRNA leaves the ribosome and binds the vacant A site to start another round of elongation and so on. Termination occurs from the stop signal on the mRNA is read by a protein release factor when the completed polypeptide chain is released from the ribosome.

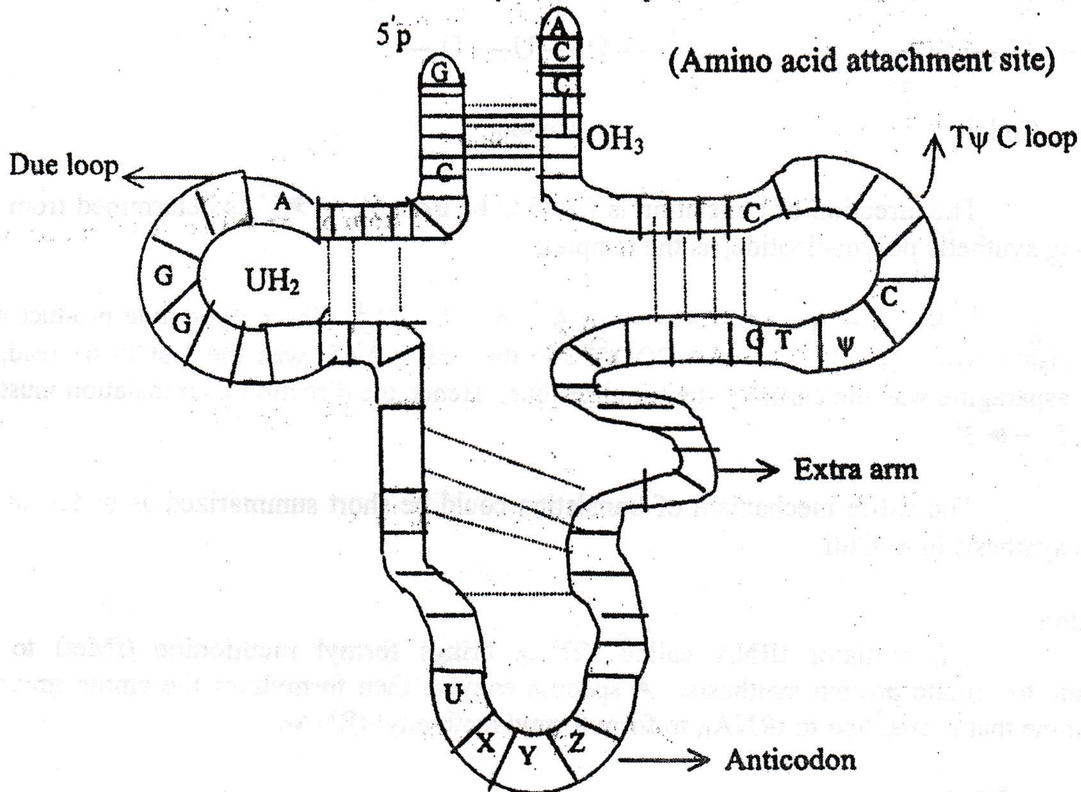
Mechanism of Translation

A mRNA, the genetic information-carrying intermediate, passes on the information regarding the type of amino acid required for protein synthesis, to the tRNA through a genetic code. The code reflects the relationship between the base sequence in the mRNA transcript and the sequence of amino acids in the protein. A set of three bases forms a codon which specifics an amino acid. The three bases may be same or different. For some amino acids more than one code is available.

(e.g)	AAA	- Lysine
	CCC	- Proline
	UUU, UUC	- Phenyl alanine
	GCC	- Alanine
	CUC, CUU	- Leucine
	UGU, GUG	- Valine

Structure of tRNA

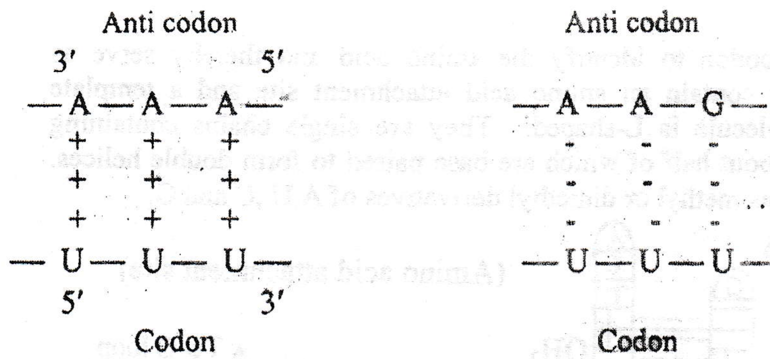
The tRNAs read the codon to identify the amino acid and thereby serve as adaptors in protein synthesis. They contain an amino acid attachment site and a template recognition site as shown. The molecule is L-shaped. They are single chains containing between 73 and 93 ribonucleotides, about half of which are base paired to form double helices. They also contain unusual bases such as methyl or dimethyl derivatives of A,U ,C and G.



Five groups of bases in the non helical regions are not paired. These five groups are,

1) 3′ CCA terminal which do not interact with the rest of the molecule.
2) T ψ C loop (ribo thymine-pseudouracil-cytosine)
3) Extra arm containing variable residues
4) DHU loop (dihydho uracil)
5) Anticodon loop containing seven bases, three of which are anticodon,

The anticodon on tRNA is the translating site for the codon on mRNA. The translation or recognition of the required amino acid for protein synthesis, is done by base pairing with the complementary base on the anticodon. Now the codon and anticodon are lined up in an antiparallel fashion. The anticodon bases are A, G,U ,C and I (Inosine), the corresponding complementary codon bases being, U,C orU, A or G, G and U, C or A respectively. Some tRNA molecules can recognize more than one codon (e.g) For phenylalanine, the anticodon GAA recognizes (translates) the condons UUU as well as UUC.



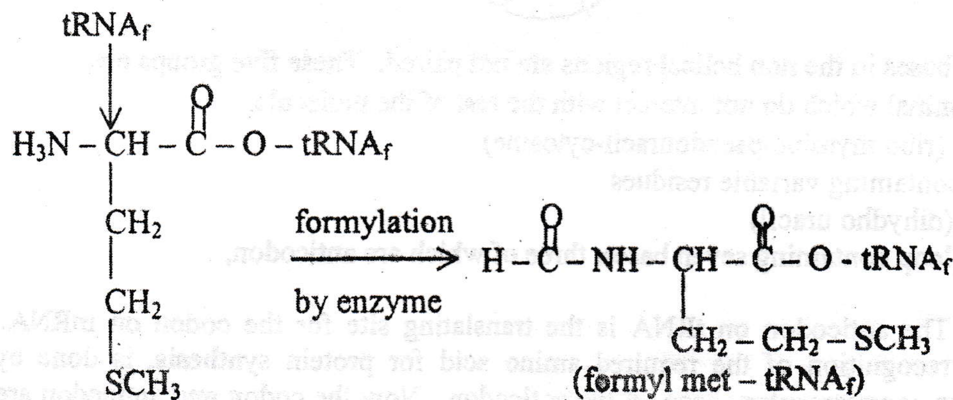
The direction of translation is found to be from 5′ ⟶ 3′, as determined from the following synthetic polynucleotide, as the template

$^{5'}A - A - A - (A - A - A)_n - A - A - A - C^{3'}$. The polypeptide product was $\overset{\oplus}{H_3N}$– Lys – $(Lys)_n$ – Asn – $COO^{\ominus}$ and the codon AAC was the last to be read, as asparagine was the carboxyl-terminal residue. Hence the direction of translation must be 5′ ⟶ 3′.

The entire mechanism of translation could be short summarized as under, in the protein synthesis in E. Coli.

Initiation

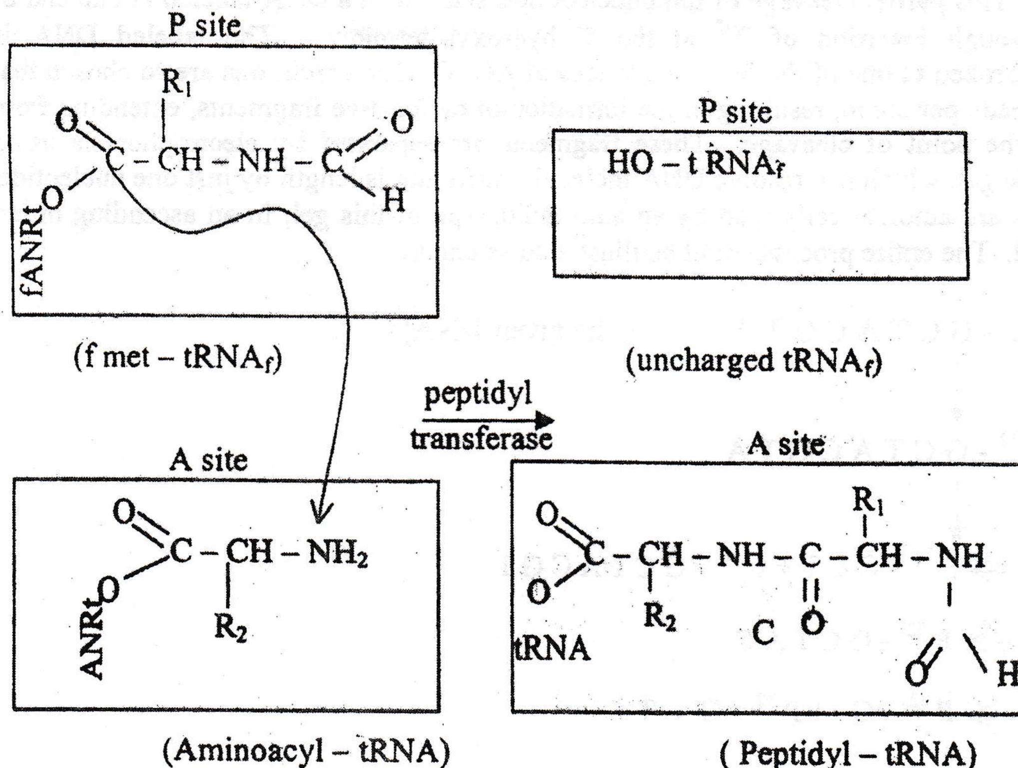
The initiator tRNA called $tRNA_f$, brings formyl methionine (fMet) to the ribosome to initiate protein synthesis. A specific enzyme then formylates the amino group of methionine that is attached to $tRNA_f$, to form formyl methionyl-$tRNA_f$.



Now mRNA, formyl met-$tRNA_f$ and a 30S ribosomal subunit come together to form a 30S initiation complex. The start signal on mRNA is AUG (meaning methionine) preceeded by a purine-rich sequence that can base pair with 16S rRNA. A 50S ribosomal sub unit then joins this complex to form a 70S initiation complex, while the formyl met- $tRNA_f$ molecule occupying the p site of the ribosome, the A site remaining empty.

Elongation and Translocation

The amino acyl t-RNA is delivered into the empty A site of the ribosome (codon recognition) by a protein elongation factor EF – Tu. The GTP (guanine Triphosphate) bound to the elongation factor, is hydrolysed while the aminoacyl – t-RNA is positioned on the A site of the ribosome, and f Met – t-RNAf. on the P site. Now a peptide bond is formed by a reaction catalysed by the enzyme peptidyl transferase, when



the activated formyl methionine unit is transferred from the P site, to the amino group on the A site. This is followed by translocation when the uncharged $tRNA_f$ leaves the P site, the pep-tRNA moves to the P site and the m RNA moves a distance of three nucleotides. The GTP bound to the EF, is hydrolysed and released from the ribosome.

Termination

Protein synthesis is terminated by release factors such as $RF_1$and RF_2 which recognize stop signals produced by the termination codons UAA, UGA or UAG, with high specificity. This leads to the hydrolysis of the bond between the peptide – tRNA in the P site and the polypeptide chain leaves the ribosome..

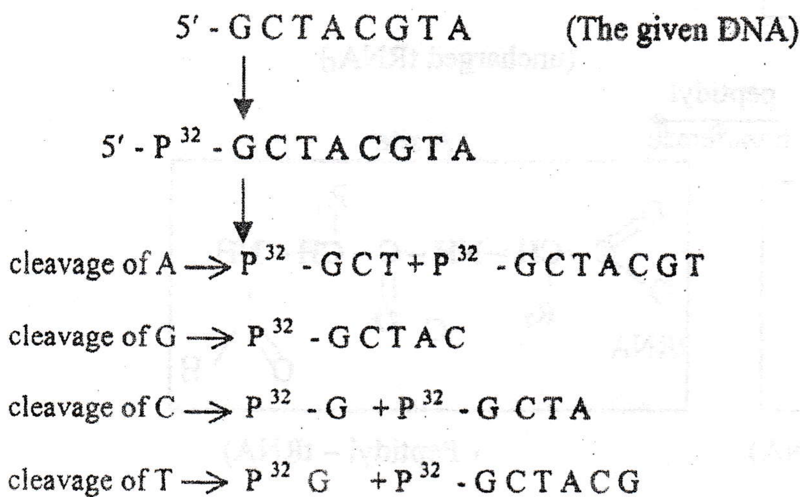
Antibiotics such as streptomycin inhibits initiation, causing misreading of mRNA whereas Puromycin causes premature chain termination as it mimics Amino acyl t-TNA.

DNA Sequencing

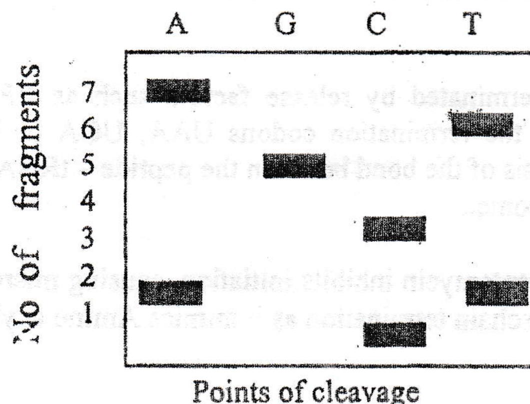
The importance of base sequence in DNA and its relationship with genetic code have facilitated the development of several techniques to determine the base sequence. At least two such methods are currently applied to determine the DNA sequencing.

1. PCR Technique

This partial cleavage of radionucleotides, starts with a DNA labeled at one end of the strand through insertion of P^{32} at the 5′ hydroxyl terminus. The labeled DNA is preferentially broken at one of the four nucleotides of AGTC. The conditions are so chosen that one break is made per chain, resulting in the formation of radioactive fragments, extending from P^{32} label, to the point of cleavage. These fragments are separated by electrophoresis using polyacrylamide gel, which can resolve DNA molecules differing is length by just one nucleotide. The fragments are automatically read by an auto radiograph of this gel, in an ascending order, and sequenced. The entire process could be illustrated as under:



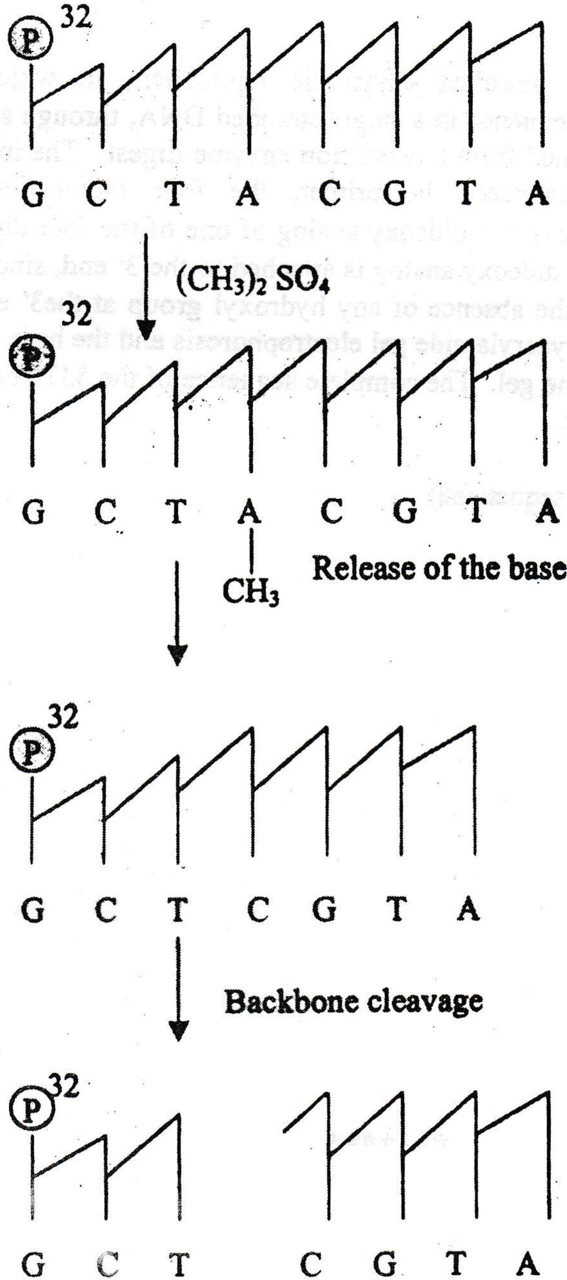
The seven fragments due to cleavage at A, G, C and T nucleotides are read by the auto radiograph as under:



Reading all the seven bands in the ascending order gives the following sequence
5'G - C TACGTA3'

The partial clearage can be effected as follows: when treated with dimethyl sulphate, the purine bases (A & G) are methylated at N_3 and N_7 positions respectively. When the glycosidic bond of a methylated purine is readily broken by heating at P_H^7, the backbone of the nucleotide is easily cleaved by heating with alkali.

Cleavage of A occurs as follows



Resolving the fragments on the gel gives a pattern of light and dark bands. Since the guanine is methylated more rapidly than adenine, the dark bands correspond to cleavage at guanine. Similarly the pyrimidine bases (C& T) are split by hydrazine and the back bone is cleaved by piperidine which displaces the products of the hydhazine reaction, and catalyses the elimination of phosphates. Cleavages at thymine and cytocine give a series of bands. Thymine and cytosine cleavage is distinguished by hydrazinolysis in the presence of 2M Nacl, which suppresses, the reaction with thymine. It can be expected that the shortest fragment viz. P^{32} – G in the given example, will form the bottom of the gel, as it has the highest electrophoretic mobility, and hence the sequence starts in the ascending order.

Enzymatic Method

This method involves a controlled enzymatic replication, in which DNA polymerase I is used to get a copy of the sequence in a single stranded DNA, through synthesis. This synthesis make use of a primer obtained from a restriction enzyme digest. The incubation mixture contains,,the DNA to be sequenced, the primer, the four radioactive deoxy ribonucleoside triphosphates, in addition to a 2′3′ dideoxy analog of one of the four nuclosides. Now fragments are produced in which the dideoxy analog is attached to the 3′ end, since further growth of the chains are avoided due to the absence of any hydroxyl group at the3′ end. The four sets of fragments are separated by polyacrylamide gel electrophoresis and the base sequence may be read from the auto radiograph of the gel. The complete sequence of the 5375 bases in Ø x174DNA was determined by Fred.Sanger.

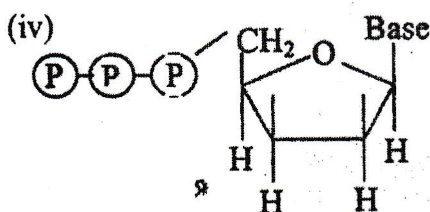
3′ - GAATTC GCTAATGC (DNA to be sequenced)

(i) 5′ - C TTAA

(ii) DNA Polymerase I

(iii) DATP, dTTP, dCTP, DGTF

(iv)



(2′3′ dideoxy analog)

4 set of fragments.

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UNIT – II Bio Organic Chemistry

CARBOHYDRATES AND LIPIDS

A. CARBOHYDRATES :

It is a group of organic compounds now considered as polyhydroxy alcdehydes or polyhydroxyketones and their derivatives. Carbohydrates are classified into two main groups such as sugars and polysaccharides.

STORAGE OF POLYSACCHARIDES:

Polysaccharides are composed of more than six monosaccharide residues united by glycosidic linkages. It is also called as glycons. Polysaccharides are further classified into two groups such as homo and hetero polysaccharides. Among these several polysaccharides are storage materials. For example, starch, chitin and glycogen etc.

1. STARCH:

It is a homopolysaccharide of D-glucose. Starch is storage materials of plants. It is deposited in the cytoplasm of plant cells as insoluble granules known as starch grains. Starch occurs in Vegetables, substance. It is insoluble in Water and gives a blue colur with iodine solution. It forms colloidal solution when it boiled with water. It can be separated into two major fractions by treatment with hot water. 1) α -amylose or α-fraction and 2) β-amylose or amylopectain or β-fraction.

α - Amylose :

It is an unbranched helical polymer of few thousands of glucose residues united by α - (1 - 4) bonds as shown in Fig.1. Thus its molecular weight is in the range of few thousand to million. It is soluble in water and gives colour reaction with iodine solution. α -Amylose gives maltose by hydrolysis with the enzyme diastase. It also gives D-glucose by the hydrolysis with dilute acids. It makes in the range of 10-20% of starch.

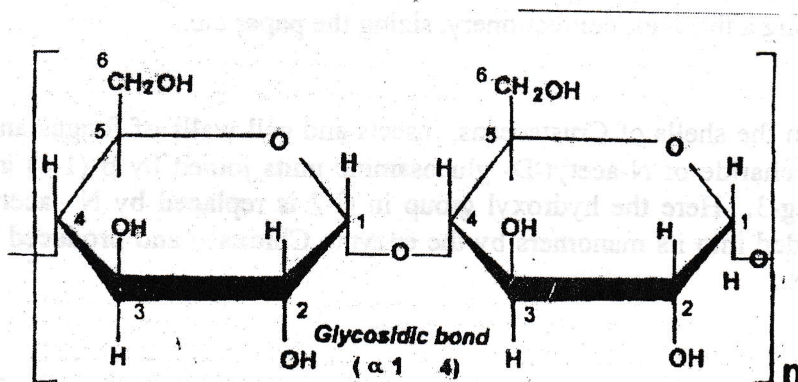


Fig 1

β - fraction:

The β- amylose makes in the range of 80 – 90% starch. It contains α (1-4) linked glucose residues, but it is a branched polysaccharide fraction, through α (1-6) linkages as shown in Fig – 2. The branching occurs at every 24 to 30 glucose residues. The molecular weight ranges between fifty thousand to several lac. It is insoluble in water. Amylopectin gives colour reaction with iodine. It gives maltose by the hydrolysis with enzyme, diastase. It also gives D-glucose with dilute acids.

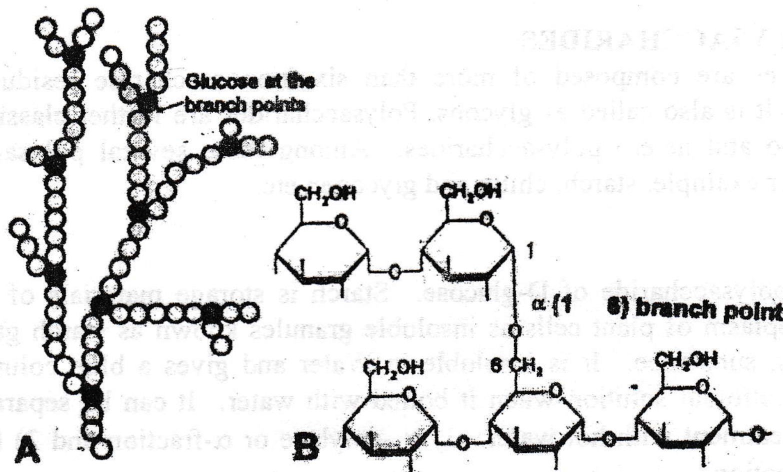


Fig 2

Biological importance of starch:

a) Storage of glucose as starch reduces the large intracellular osmotic pressure.

b) Starch storage in the form of glucose units will increase of the osmotic pressure in the cell.

c) It is the main source of carbohydrate in the human diet.

d) Starch is hydrolysed by boiling with water at about 250° C to give dextrin. Which is used for making adhesives, confectionery, sizing the paper etc.,

2.CHITIN:

Chitin is found in the shells of Crustaceans, insects and cell walls of fungus and certain bacteria. It is a polysaccharide of N-acetyl-D- glucosamine units joined by β (1-4) glycosidic linkages as shown in Fig-3. Here the hydroxyl group in C-2 is replaced by N –acetyl amino group. It may be degraded into its manomers by the enzyme Chitinase and produced by many bacteria and streptomyces.

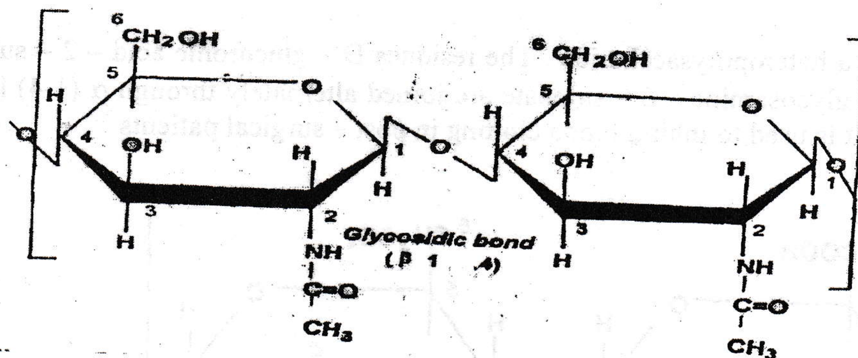


Fig 3

GLYCOGEN:

It belongs to homopolysaccharides of D-glucose. It is also known as animal starch. Glycogen is present in all the cells but is abundant in the skeletal muscles and the liver. The structure of glycogen resembles β -fraction as shown in Fig –2. But it has more branches than β - amylose. It occurs at every 8 to 12 glucose residues. These residues are joined by α (1-4) linkages and at the branching point α (1-6) linkages. It is soluble in water and gives purple – red colour with iodine solution. On hydrolysis it gives D-glucose.

Biological importance of glycogen.

a) It is the storage polysaccharide of animals.
b) The conversion of glucose to glycogen maintains the glucose level in the blood.
c) It occurs in the muscle and used up during muscular activity.

GLYCOSAMINOGLYCANS:

Glycosaminoglycans are also called mucopolysaccharides. It is an important group of heteropolysaccharides. These type of carbohydrates are gel-like and extracellular. These mucopolysaccharides are unbranched and they consist of alternative arrangements of uronic and hexosamic residues. For example, hyaluronic acid, dermatan sulphate, Heparin, Chondroitin – 4- sulphate etc., Few of the glycosaminoglycans are discussed below:

(i) Chondroitin – 4- sulphate:

It is a component of cartilage and other connective tissues. It is joined about 50 to 1000 units through β (1-4) linkages Each digaceharide unit consists of a D – glucuronic acid and N – acetyl – d – galactoamine – 4 – sulphate linked by β (1-3) bond as shown in fig 4.

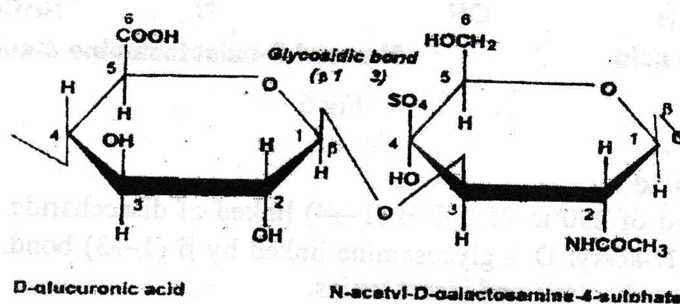


Fig 4

ii) Heparin:

Heparin is a heteropolysaccharide . The residues D – glucuronic acid – 2 – sulphate and N – sulpho – D – glycosamine – 6 – sulphate are joined alternately through α (1-4) linkages as shown in fig -5. It is used to inhibit blood clotting in post – surgical patients

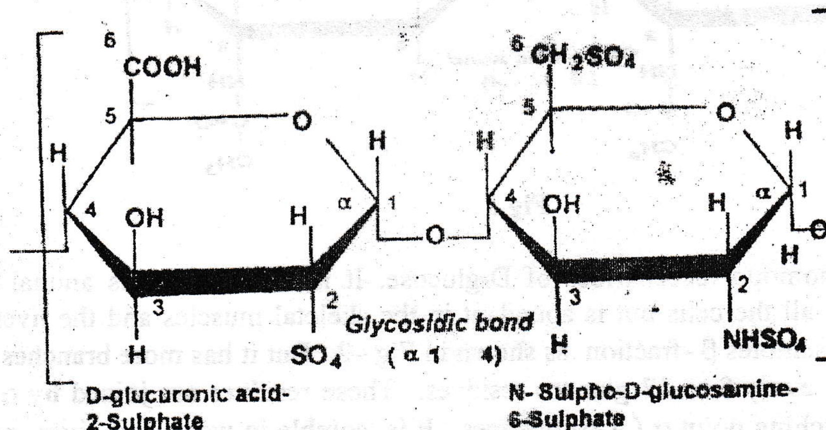


Fig 5

iii) Dermatan sulphate :

Dermatan sulphate resembles with chondroitin – 4- sulphate but differs only by an inversion of configuration about C – 5 of the β - D – glucuronic acid residues of L – iduronic acid as shown below]

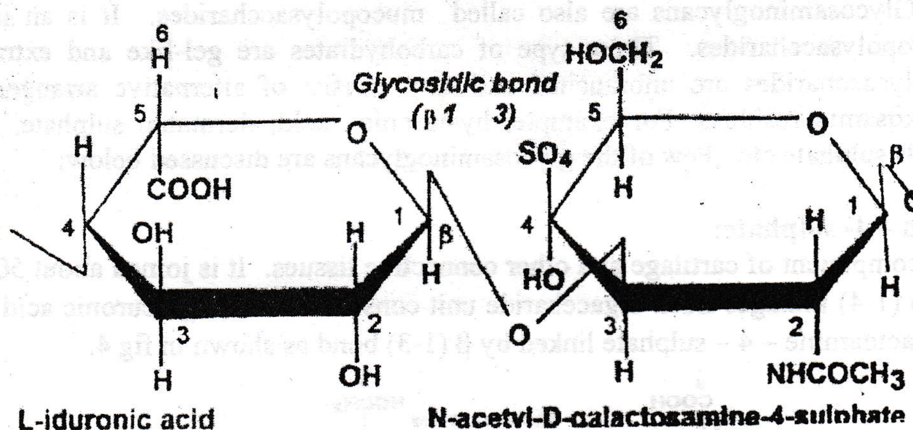


Fig 6

iv) Hyaluronic acid :

It is composed of 250 to 25,000 B (1→4) linked of disaccharide units consisting of D-glucuronic acid and N-acetyl D – glycosamine linked by β (1→3) bond. It is occurs in many types of animal tissues, bacteria and insect toxins.

CARBOHYDRATES OF GLYCOPROTEINS AND GLYCOLIPIDS :

Proteins which are associated with carbohydrates through covalent bond are called glyoproteins. It occurs in the mucous secretions, for example ovalbumin, hormones, urinary glycopeptides, ovomucoid, hormones, mucins, enzymes etc. The carbohydrate content in glycoproteins vary from 1 to 90% by weight.

Lipids which are linked with carbohydrates are called glycolipids.

1. Mucins or muscus :

The secretions of muscous membranes have glycoproteins known as mucins. It has protectine and lubricatine functions. It also contains a number of negatively charged oligosacharides.

2. Glycocalyx of plasma membrane :

The outer surface of the plasma membrane is covered by carbohydrates. It forms cell coating known as glycocalyx. The carbohydrate chains are either bound with the protein part or the lipid part of the plasma membrance. The carbohydrate groups associated with membranes are located only on the external surface of the cell membrance. The carbohydrate protein or lipid complex are called glycoprotein or glycolipids. Such carbohydrates are important in the cell to cell recognition. Such type of behaviour is related in processes such as fertilisation, organ formation, infection of cells by bacteria and viruses and cellular differentiation.

3. Peptido glycon :

The cell walls of bacteria consist of covalently linked polysaccharide and polypeptide chains. The polysaccharide component consists of linear chains of alternating β (1→ 4) linked N- acetyl – glucosamine and N-acetyl muranic acid.

BLOOD GROUP SYBSTANCES :

A. B and H antigens are the A B O blood group substances. These are components of erythrocyte surface sphingo glycolipids.

BIOLOGICAL FUNCTIONS OF CARBOHYDRATES

Carbohydrates have many functions. All of them can be grouped into the following three categories :

a) Carbohydrates are the important components of many macromolecules which have important structural and functional roles in the cells.

b) They are the sources of energy in the living systems.

c) They give structure and support to the organism

Functions of Carbohydrates are discussed below :

(i) Ribose sugar is a constituent of adenosine triphosphate (ATP) which is the universal currency of free energy in biological systems.

(ii) Glyceraldehyde is a triose. Its derivatiave, glycerol is a major component of lipids in plants and animals.

(iii) D - ribose and 2 - deoxy - D - ribose are pentoses. These sugars form the back bone of the nucleic acids. Thus they have an important hereditary role.

(iv) Carbohydrates, especially glucose, are the fuel of the cells.

(v) D – ribose sugar is an important component of certain co-enzymes. It is also a component of several other important biomolecules of physsiological importance such as NADH, NADPH, FAD which are electron carriers. Ribose sugar forms part of several vitamins like, riboflaving and niacin.

(vi) Carbohydrates are the important storage food materials in both plants and animals. Starch (in plants) and glycogen (in animals) are important food-storage Polysaccharides.

(vii) Glucose is the main sugar in the blood. It is the major metobolic fuel of the cells. Conversion of glucose into glycogen maintains the glucose level in the blood.

(viii) Sugars containing an amino group are called amino sugar. Many antibiotics contain amino sugars, eg., erthromycin and carbomycin. The amino-sugars are related to the entibiotic activity of these drugs.

(ix) Storage of carbohydrates in the form of glucose will increase the osmotic pressure of the cell. Hence storage of glucose as starch reduces the large intracellular osmotic pressure.

(x) In plants cell walls are made up of celluclose which is a homopolysaccharide of D-glucose. Cell walls give shape and rigidity to the cells and the plant body as a whole.

(xi) Mucilage is a mucoprotein. It can bind enarmous amount of water. This is much useful in the xerophytic plants to store water.

(xii) Heparin is a sulphated glycosaminoglucan. Heparin has wide clinical use to inhibit blood clotting, eg., in post- surgical patients.

(xiii) Hyaluronic acid is viscoelastic in nature. Hence it is an excellent biological shock absorber and lubricant.

(xiv) The outer membranes of certain bacteria contain repeating oligosaccharide units which are attached to the lipid part of the membrane. These have indefinite length and protrude as fibers from the outer surface of the membrane. Such lipopoly saccharides have antigenic properties. These are called oantigens.

(xv) The outer surface of the plasma membrane is covered by carbohydrate chains. These are either bound with the protein part or the lipid part of the plasmamembrane. These carbohydrates are important in the cell to cell recognition.

CARBOHYDRATE METABOLISM

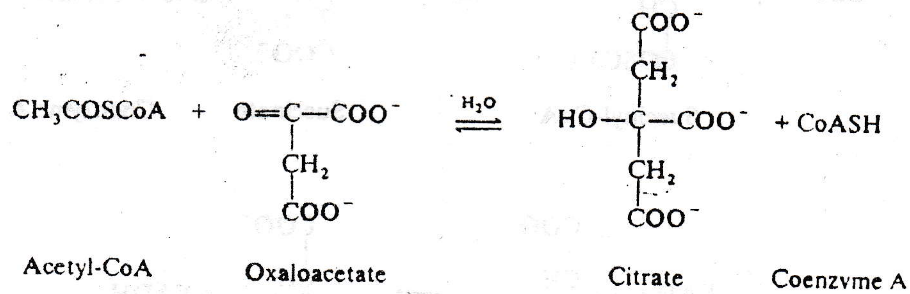
Kreb's cycle (citric acid cycle)

It is a sequence of reactions in which the two carbon atoms of acetyl – CoA (Coenzyme A) are completely oxidised to CO_2. Kreb's cycle is also called citric acid cycle or tricarboxylic cycle. It is a central pathway for the release of energy from acetyl CoA, which is produced from

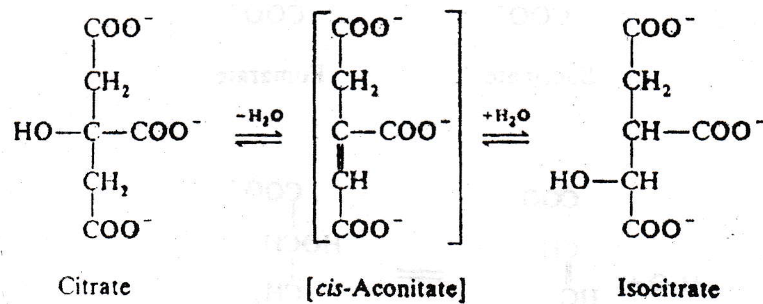
the catabolism of carbohydrates, fathy acids and some amino acids and involued two other processes such as electron trnasport and oxidatine phosphorylation.

Kreb's cycle consists of the following eight steps.

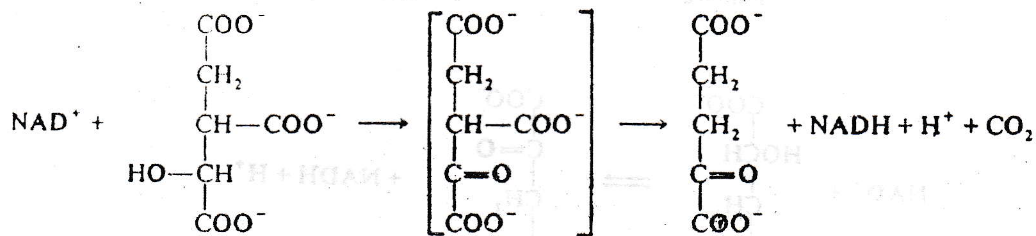
Step 1



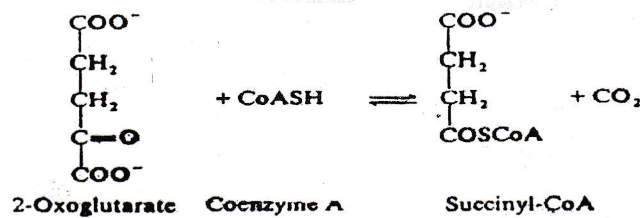
Step 2



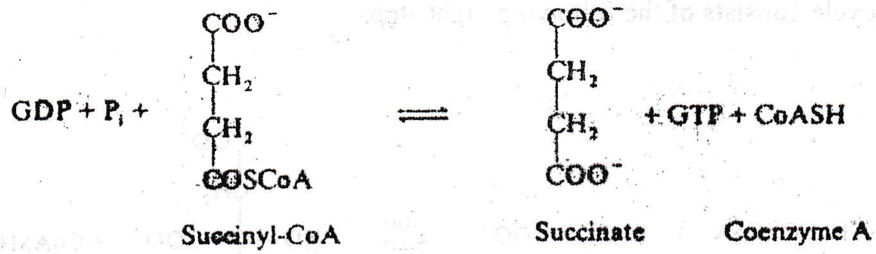
Step 3



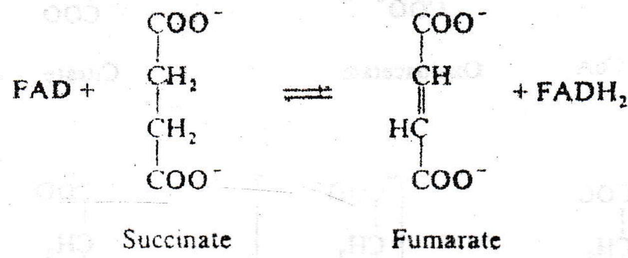
Step 4



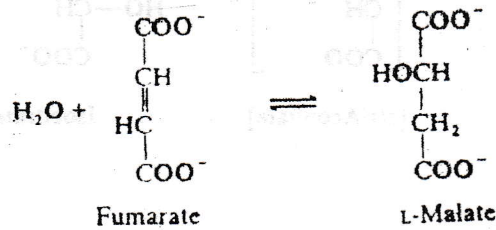
Step 5



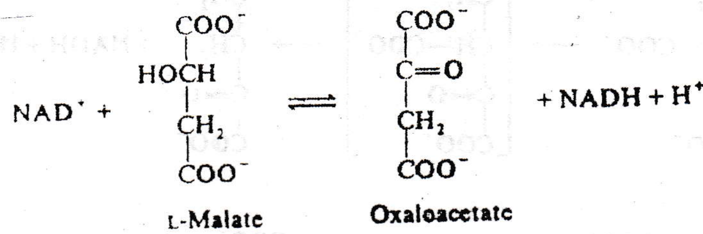
Step 6



Step 7



Step 8



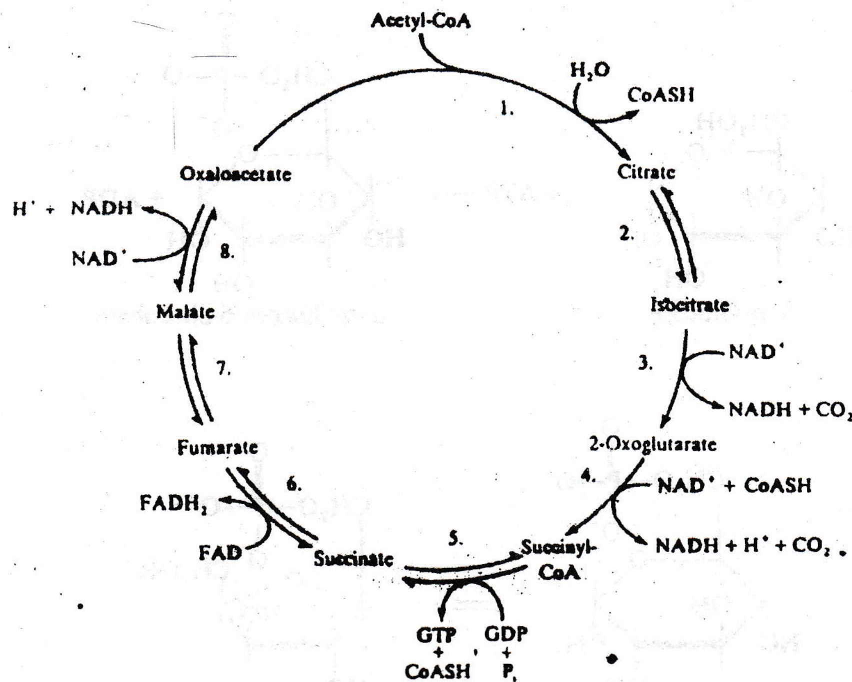


Fig. The citric acid cycle. The numbered reactions refer to the steps given in the text.

Fig 7

The regenerated of oxaloacetate in step – 8, condenses with another molecule of acetyl – CoA to commence another turn of the cycle. The carbon atoms are liberated at steps 3 and 4 as CO_2.

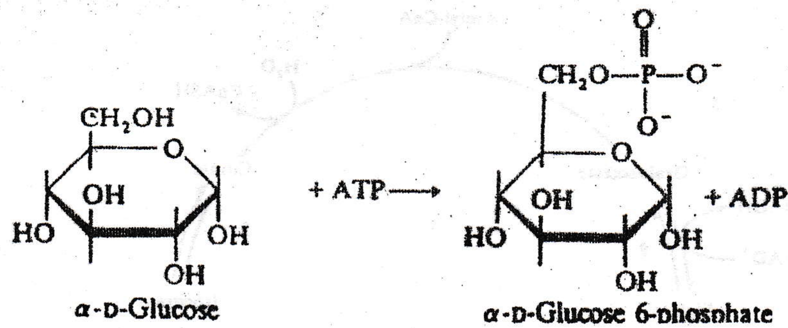
Glycolysis

It is a process that results in the conversion of a molecule of glucose to two molecules of pyruvate. The pathway of glycolysis performs the following functions in the cell.

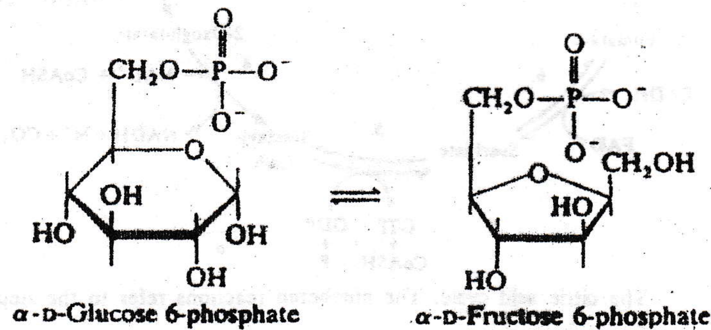
a) Glucose converted to pyrurate, which can be oxidised in the citric acid cycle.
b) Many compounds other than glucose can enter the pathway at intermediate stages.
c) In some cells the pathway of emzymes is modified to enable glucose to be syntherised.
d) The pathway contains intermediates that are involved in alternative metabolic reactions.
e) For each glucose that is consumed, two molecules of ADP are phosphorylated by substrate level phosphorylation to produce two molecules of ATP.

Glycolysis consists of the following ten steps.

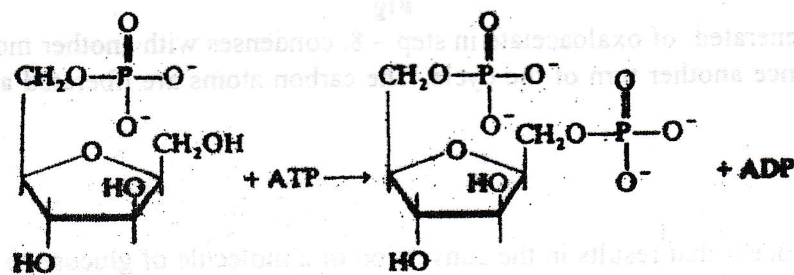
Step 1



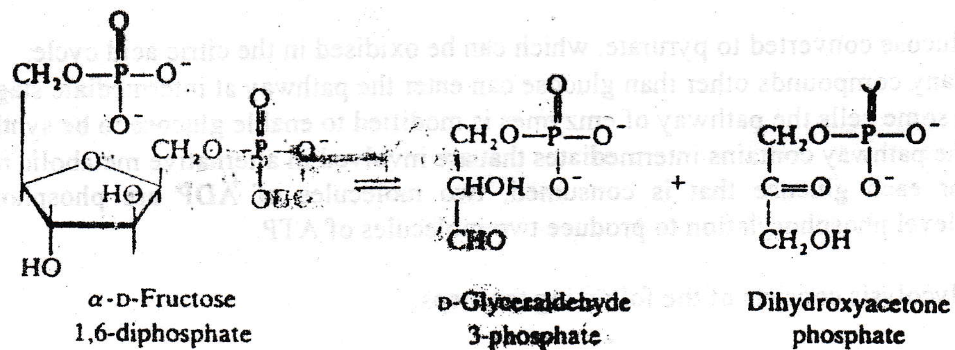
Step 2

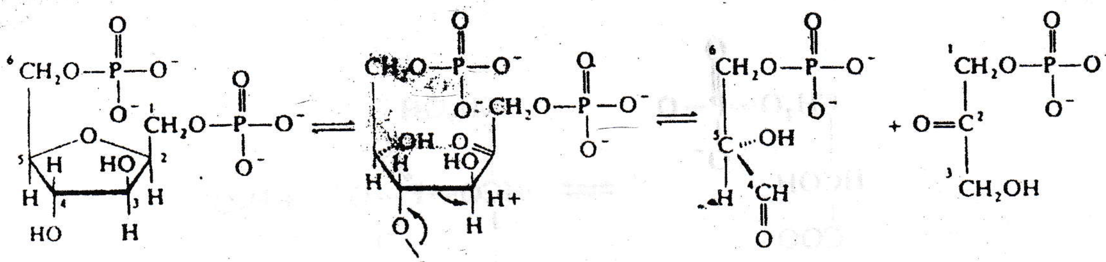


step 3

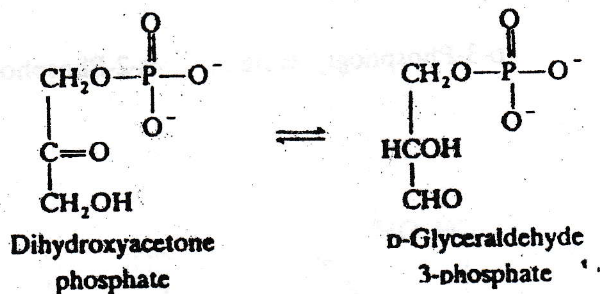


Step 4

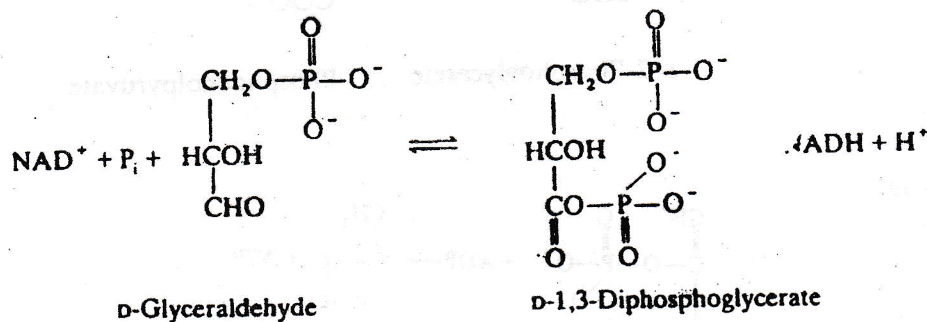




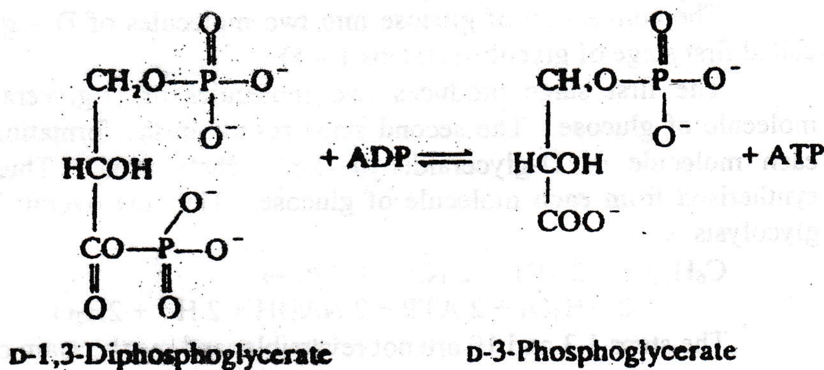
Step 5



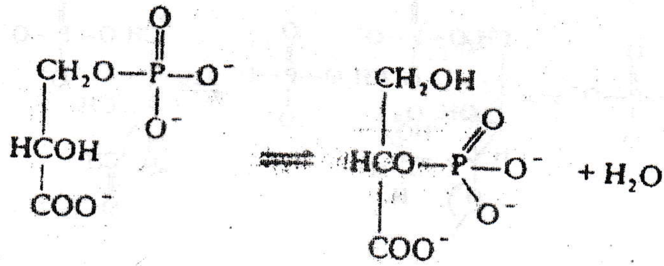
Step 6



Step 7

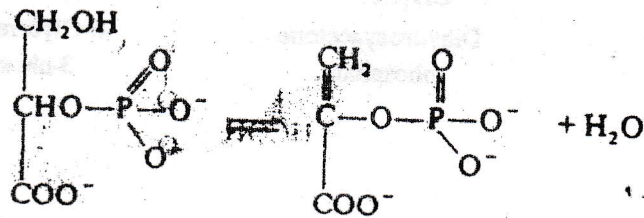


Step 8



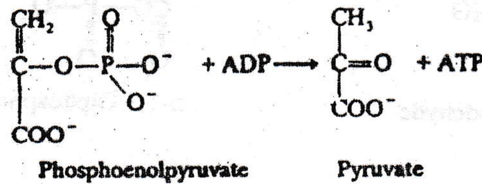
D-3-Phosphoglycerate ⇌ D-2-Phosphoglycerate

Step 9



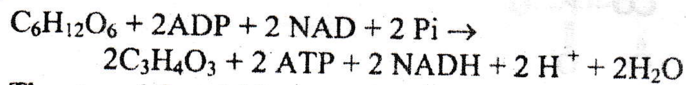
D-2-Phosphoglycerate Phosphoenolpyruvate

Step 10



The conversion of glucose into two molecules of D – glyceraldehyde -3 - Phosphate is called first stage of glycolysis (steps 1 – 5)

The first stage produces two molecules of D-glyceraldehyde-3-phosphate from one molecule of glucose. The second stage results in the formation of two molecules of ATP for each molecule of D-glyceraldehyde-3-phosphate used. Thus two molecules of ATP are synthesised from each molecule of glucose. Thus the overall balanced chemical equation for glycolysis is



$$C_6H_{12}O_6 + 2ADP + 2\ NAD + 2\ Pi \rightarrow$$
$$2C_3H_4O_3 + 2\ ATP + 2\ NADH + 2\ H^+ + 2H_2O$$

The steps 1,3 and 10 are not reversible, and me the main control points in the pathway.

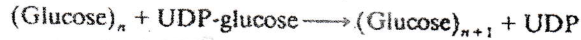
Glycogen

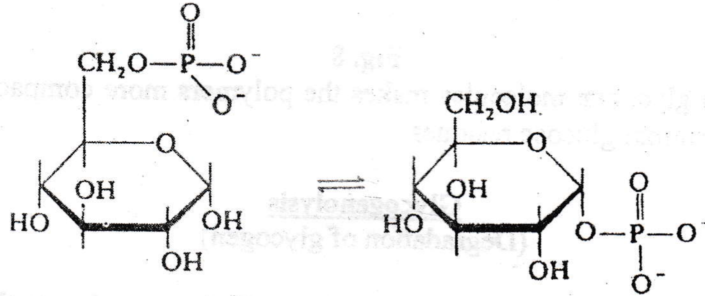
Glycogen is a polymer of glucose, It is an energy store that can be repidly broken down to glucose – 6 phosphate entering the glycolytic pathway. It is syinthesised from glucose – 6 – phosphate.

Synthesis of glycogen from glucose – 6 – phosphate as follows.

Step : 1

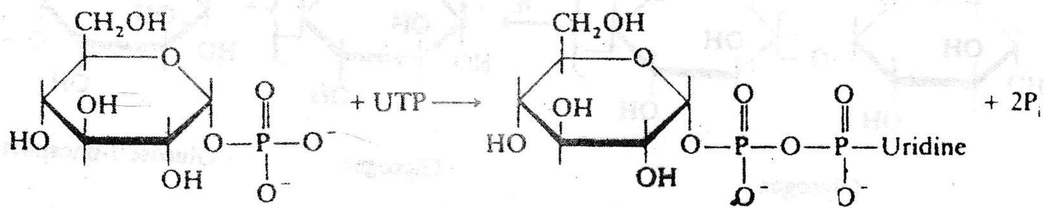
The modified glucose molecule is a substrate for the enzyme ***glycogen synthase***:





Glucose 6-phosphate   Glucose 1-phosphate

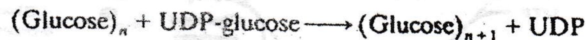
Step : 2



Glucose 1-phosphate   Uridine diphosphoglucose (UDP-glucose)

Step : 3

The modified glucose molecule is a substrate for the enzyme ***glycogen synthase***:



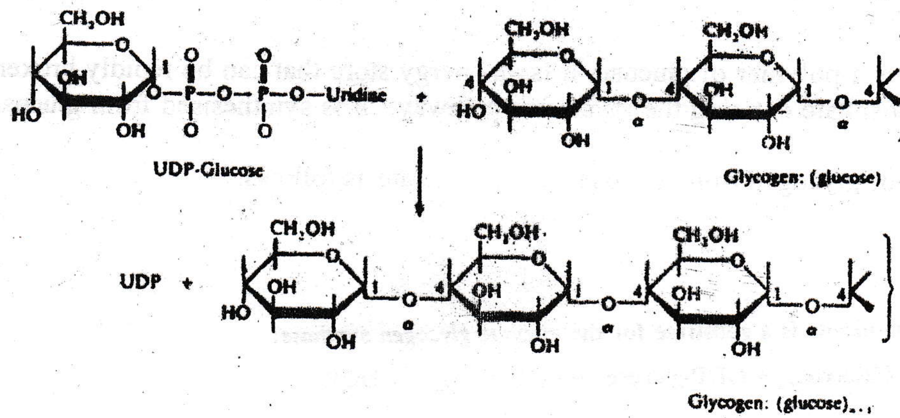


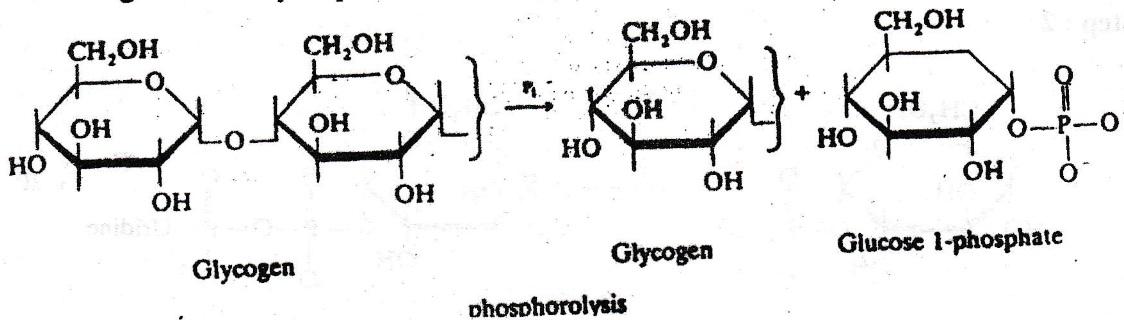
Fig. 8

The branching within glycogen molecules makes the polymers more compact and more soluble and produces more terminal glucose residues.

Glycogenolysis

(Degradation of glycogen)

Glycogen is degraded to glucose - 6 - phosphate by a pathway that differs from its synthesis. The first step with inorganic phosphate, catalyses the cleavage of a terminal & (1 → 4) bond, provided (1 → 6) linkage is not attached, to produce glycogen with one residue less and a molecule of glucose -1 - phosphate.



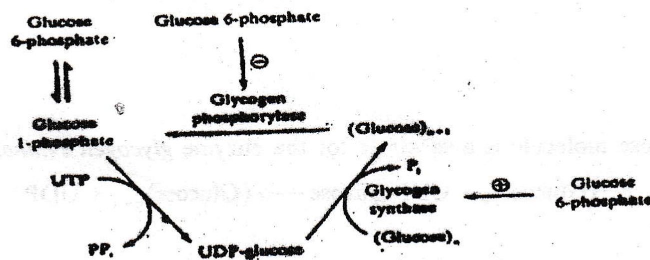


Fig 9

Gluconeogenesis :

The conversion of pyruvate into glucose pathway can't operate directly in reverse direction because of the three irreversible steps, but pyruvate can be converted into glucose because of additional reactions. The summary of the process is given below.

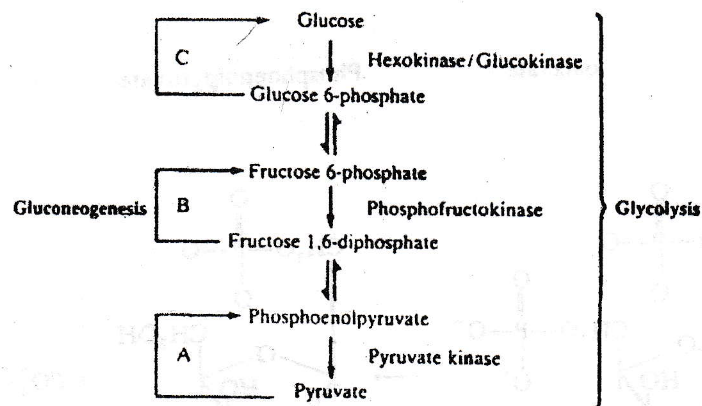
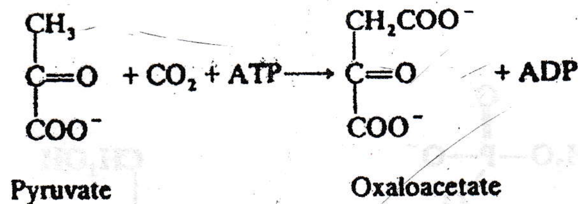
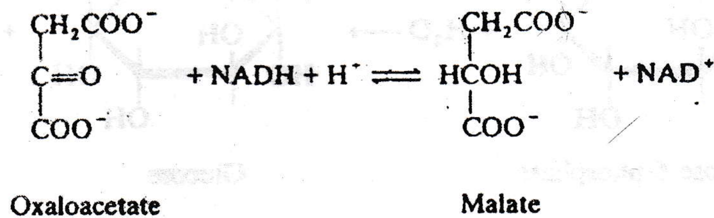
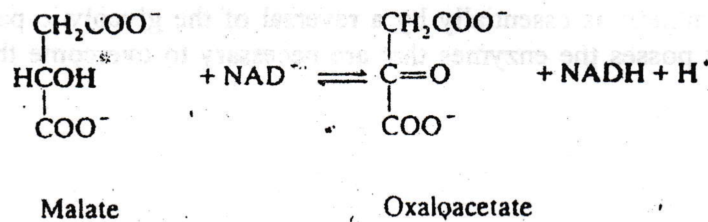
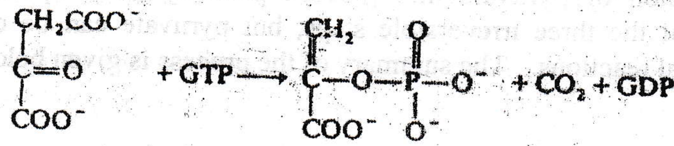


Fig 10

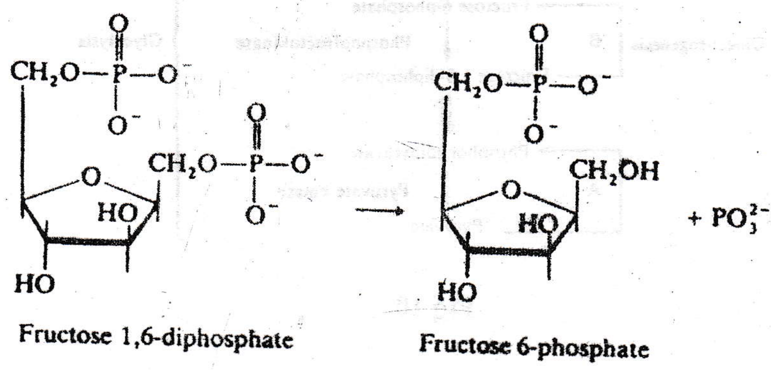




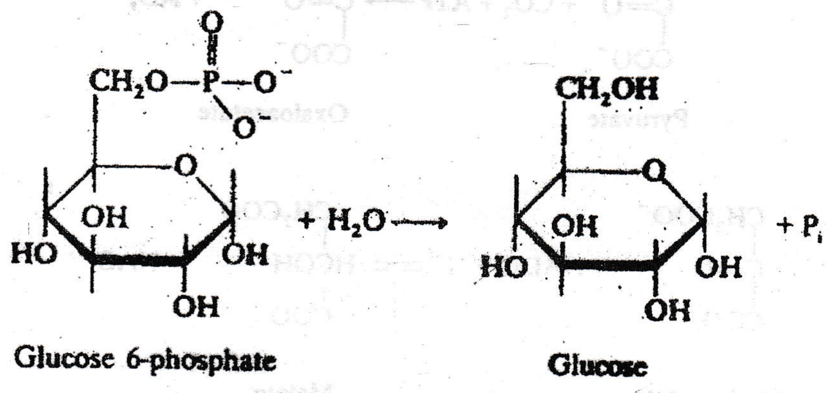




.oacetate Phosphoenolpyruvate



Fructose 1,6-diphosphate Fructose 6-phosphate



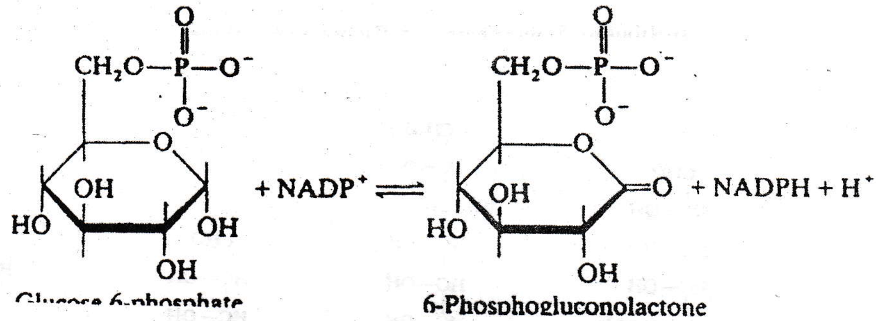
Glucose 6-phosphate Glucose

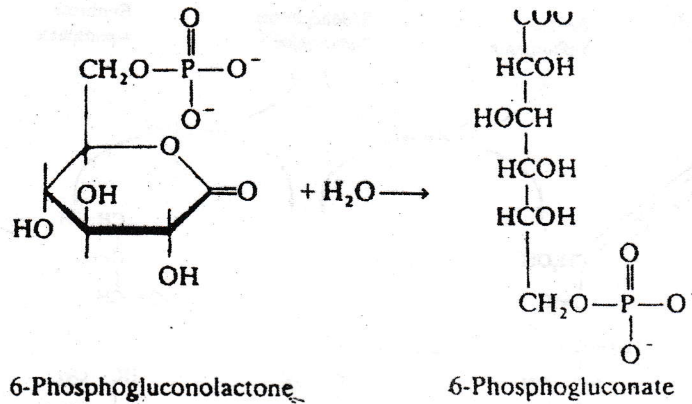
The above syntheris is essentially by a reversal of the glycolytic pathway, because the cells of these tissues posses the enzymes that are necessary to overcome the three irreversible glycolytic steps.

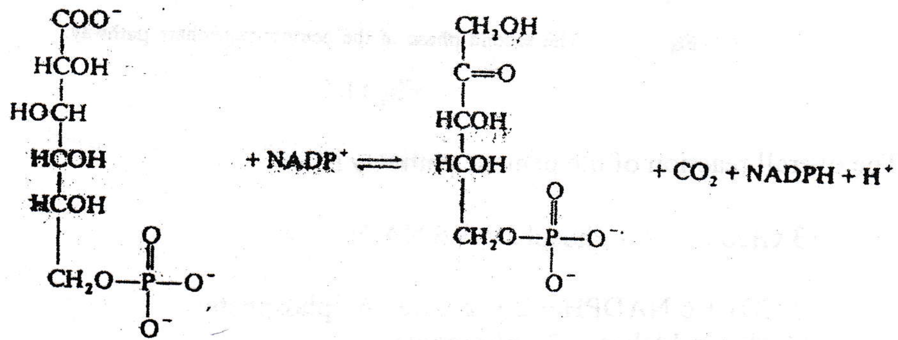
Pentose Phosphate pathway :

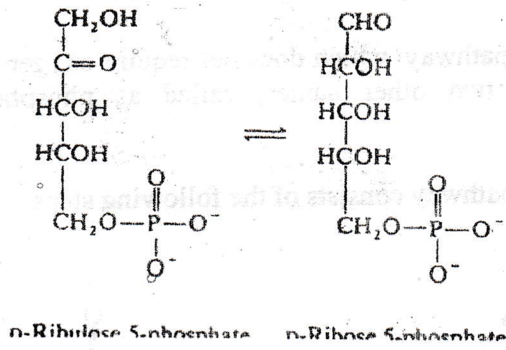
The pentose phosphate pathway, which does not require oxygen and which occurs in the cytoplasm of the cells, has two other names, called as phosphogluconate pathway or menophosphate shunt.

The pentose phosphate pathway consists of the following steps.









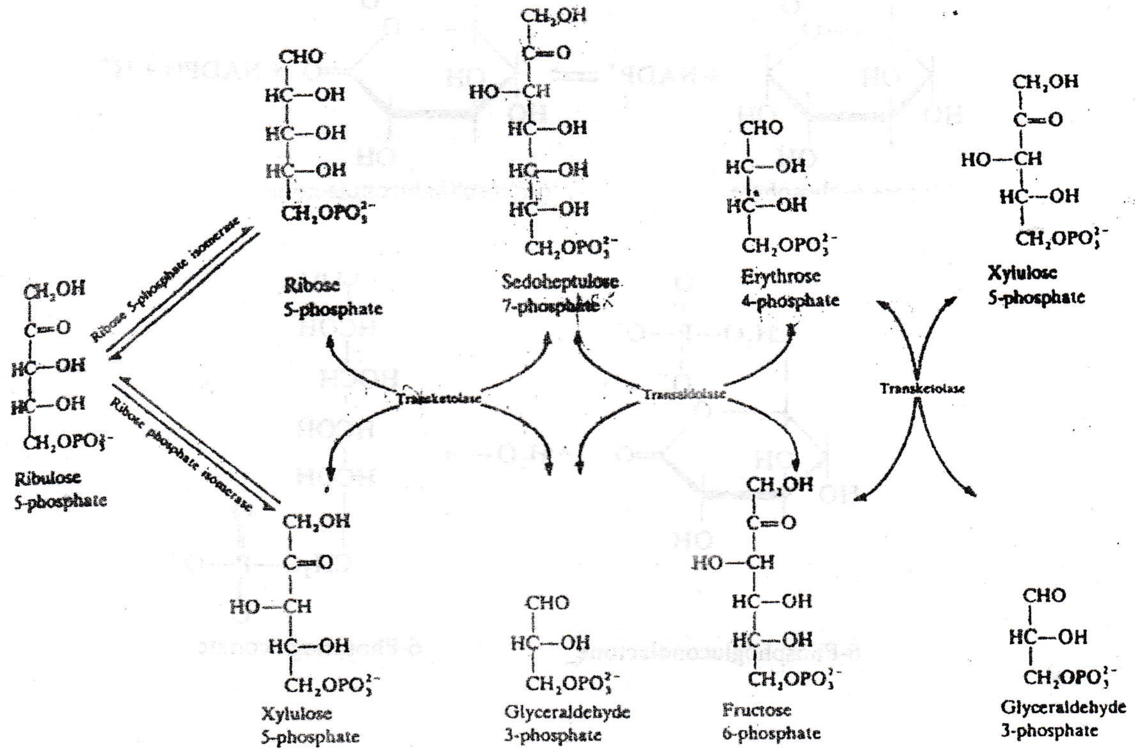
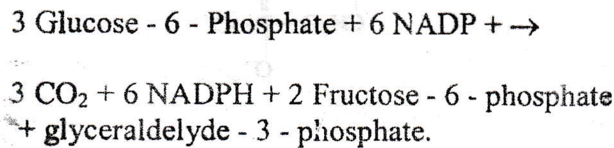


Fig The second phase of the pentose phosphate pathway.

Fig 11

The overall reaction of the pentose pathway is



3 Glucose - 6 - Phosphate + 6 NADP + →

3 CO_2 + 6 NADPH + 2 Fructose - 6 - phosphate + glyceraldelyde - 3 - phosphate.

The above reactions show the production of NADPH in the pathway and its return of all but one of the carban atoms of glucose-6-phosphate to the glycolytic pathway.

FATTY ACIDS

A fatty acid consists of a hydrocarbon chain and a terminal carboxylic acid group (Fig.1). Most fatty acids found in biology have an even number of carbon atoms arranged in an unbranched chain. Chain length usually ranges from 14 to 24 carbon atoms, with the most common fatty acids containing 16 or 18 carbon atoms. A saturated fatty acid has all of the carbon atoms in its chain satuarated with hydrogen atoms (Fig.1a). This gives the general formula $CH_3 (CH_2)_n COOH$, where n is an even number. **Mono-unsaturated fatty acids have one** double bond in their structure (Fig.1b and 1c), while polyunsaturated fatty acids have two or more double bonds (Fig.1d). The double bonds in polyunsaturated fatty acids are separated by at least one methylene group.

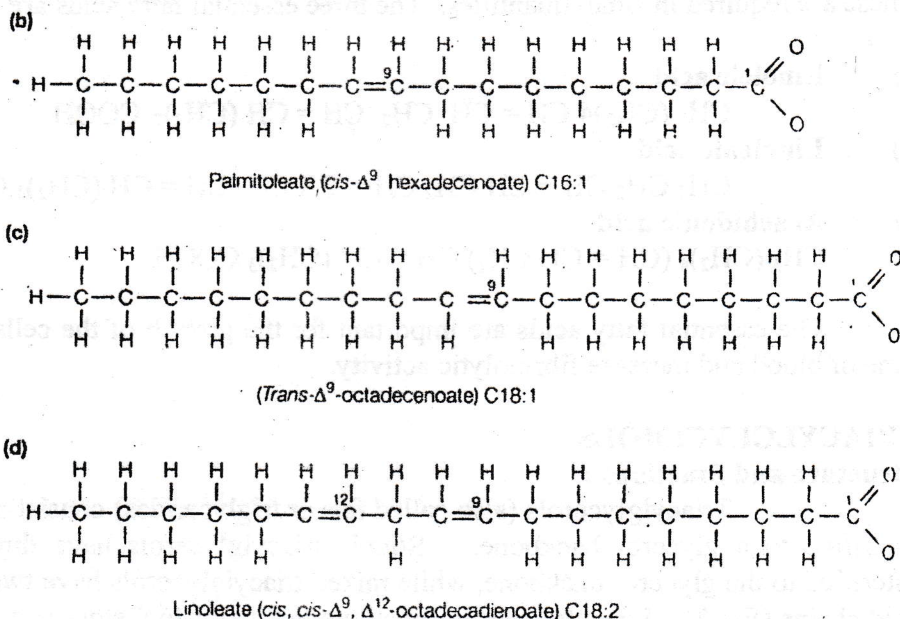


Fig. 1. Structures of (a) a saturated fatty acid (palmitate, C16:0); (b) a mono-unsaturated fatty acid with the double bond in the cis configuration (palmitoleate, C16:1); (c) a mono-unsaturated fatty acid with the double bond in the trans configuration (C18:1); and (d) a unsaturated fatty acid (linoleate, C18:2).

The properties of fatty acids depend on their chain length and the number of double bonds. Shorter chain length fatty acids have lower melting temperatures than those with longer chains. Unsaturated fatty acids have lower melting temperatures than satulrated fatty acids of the same chain length, whilst the corresponding polyunsaturated fatty acids have even lower melting temperatures.

Biological Roles of Fatty Acids :

1. They are used to make glycerophospholipids and sphingolipids that are essential components of biological membranes.

2. Numerous proteins are covalently modifed by fatty acids Myristate (C14:0) and palmitate (C16:0) are directly attached to some proteins, while phosphatidylinositol is covalently linked to the C terminus of other proteins via a complex glycosylated structure;
3. Fatty acids act as fuel molecules, being stored as triacylglycerols, and broken down to generate energy;
4. Derivatives of fatty acids serve as hormones (such as the prostaglandins) and intracellular second messengers (such as DAG and IP_3)

Essential of fatty acids :

Fatty acids which cannot be synthesised by mammals are called essential fatty acids. There have specific nutriotional importance and hence should be supplied through the diet. These are required in small quantities. The three essential fatty acids are :

a) **Linoleic acid**

$CH_3\ (CH_2)4\ CH = CH\ \ CH_2\ \ CH = CH\ (CH_2)_7\ COOH$

b) **Linolenic acid**

$CH_3\ CH_2\ CH = CH\ CH_2\ CH = CH\ CH_2\ CH = CH\ (CH_2)_3\ COOH$

c) **Arachidonic acid**

$CH_3\ (CH_2)_4\ (CH = CH\ CH_2)\ CH = CH\ (CH_2)_3\ COOH$

The essential fatty acids are important for the growth of the cells. They prolong clotting time of blood and increase fibrinolytic activity.

TRIACYLGLYCEROLS

Structure and functions :

Triacylglycerols (also called fats or triglycerides) consist of three fatty acid chains esterified to a glycerol backbone. Simple triacylglycerols have three identical fatty acids esterified to the glycerol backbone, while mixed triacylglycerols have two or three different fatty acid chains (Fig 2). Triacylglycerols constitute the major fuel store and the major fuel store and the major dietary lipid in humans. Triacylglycerols are a highly concentrated energy store. The energy yield from the complete oxidation of fatty acids is about 39 KJ g^{-1} of carbohydrate of carbohydrate or protein. The hydrophobic properties of fats make them insoluble in water, and fats are stored in specialized cells called adipose cells (fat cells), which consist almost entirely of triacylglycerol. These cells are specialized for the synthesis and storage of triacylglycerols and for their mobilization into fuel molecules. Triacylglycerols are transported round the body in large lipid-protein particles called lipoproteins.

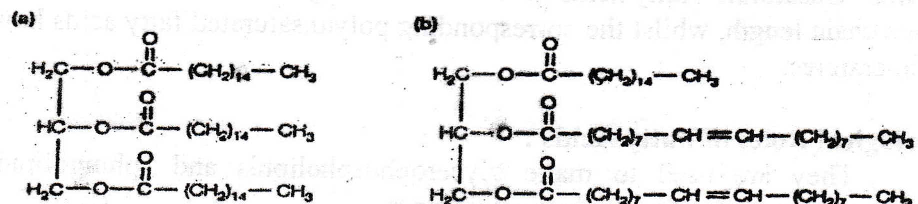


Fig. 2

Synthesis :

Triacylglycerols are synthesized from fatty acyl CoAs and glycerol 3-phosphate (Fig 3) as follows :

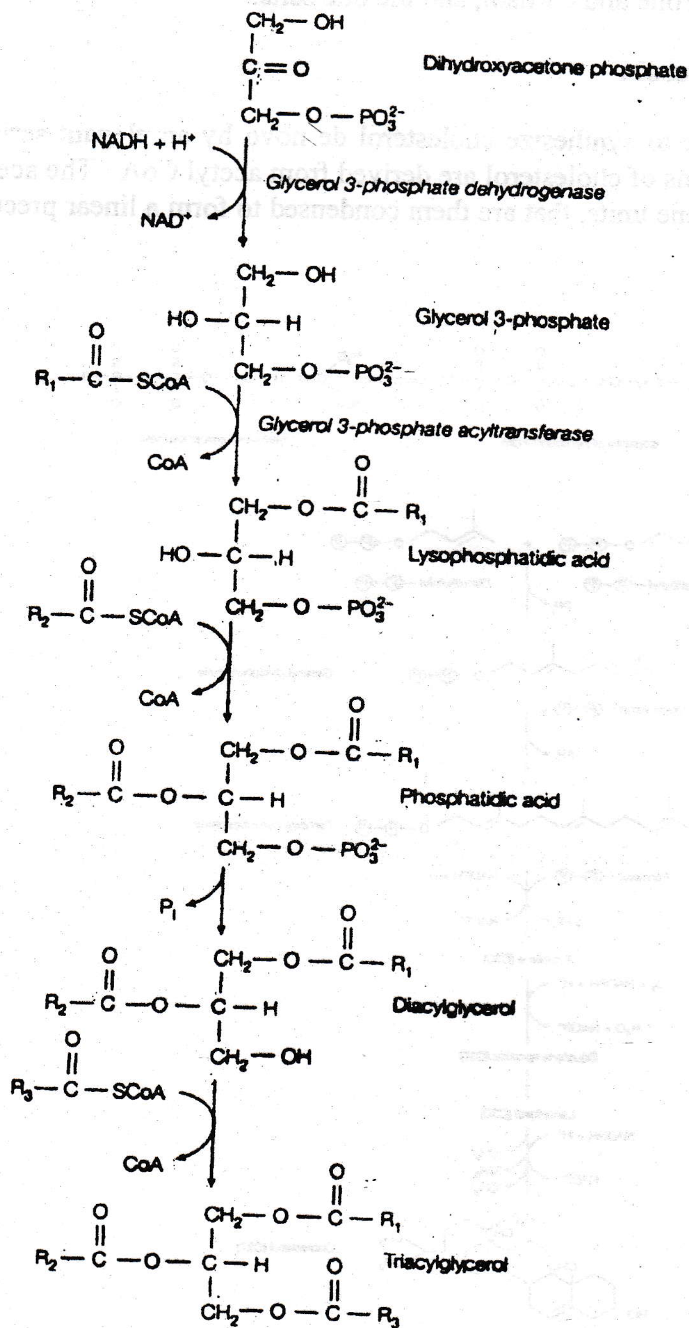


Fig 3

CHOLESTEROL :

Functions of cholesterol :

Cholesterol is a steroid. It is an important constituent of cell membranes, where, in mammals, it modulates their fluidity. Cholesterol is also the precursor of sterioid hormones such as progesterone; testosterone and cortisol, and the bile salts.

Biosynthesis of Cholesterol :

Animals are able to synthesize cholesterol de novo by an elegant series of reactions in which all 27 carbon atoms of cholesterol are derived from acetyl CoA. The acetate units are first converted into C5 isoprene units, that are them condensed to form a linear precursor to the cyclic cholesterol as follows.

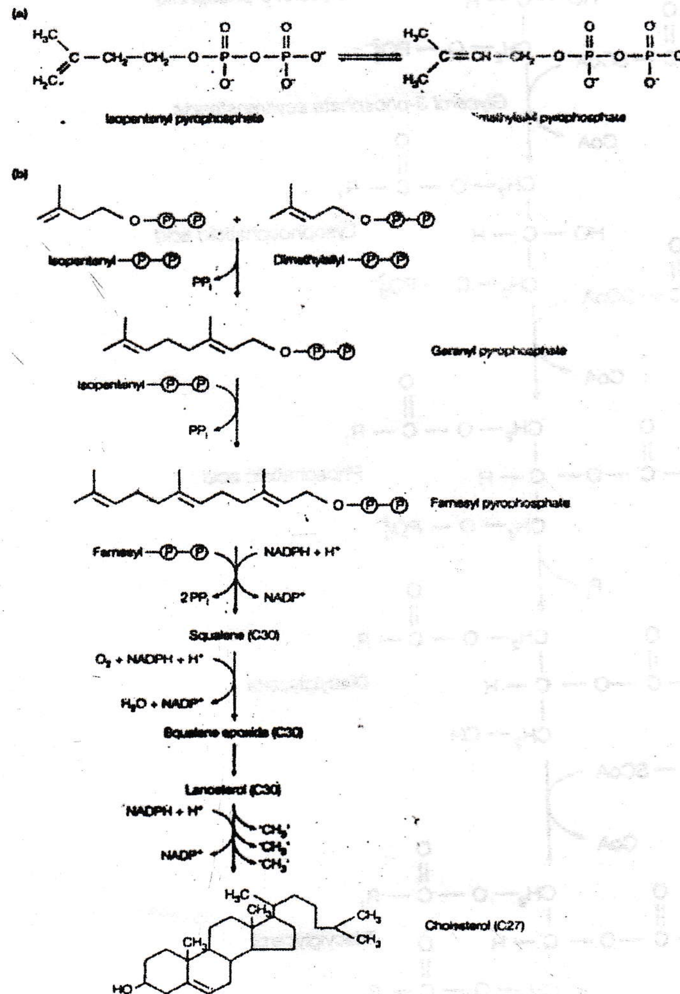


Fig 4

Bile Acids:

Bile salts (or bile acids) are polar derivatives of cholesterol and constitute the major pathyway for the excretion of cholesterol in mammals. In the liver, cholesterol is converted into the activated intermediate cholyl CoA which then reacts either with the amino group of glycine to form glycocholate (Fig.5a), or with the amino group of taurine (H2N-CH2-CH2-SO3, a derivative of cysteine) to form taurocholate (Fig.5b). After synthesis in the liver, the bile salts glycocholate and taurocholate are stored and concentrated in the gall bladder, before release into the small intestine. Since they contain both polar and nonpolar regions (that is are amphipathic molecules), the bile salts are very effective detergemts and act to solubilize dietary lipids. The resulting increase in the surface area of the lipids aids their hydrolysis by lipases and their uptake into intestinal cells. The intestinal absorption of the lipid-soluble vitamins A, D, E and K also requires the action of the bile salts

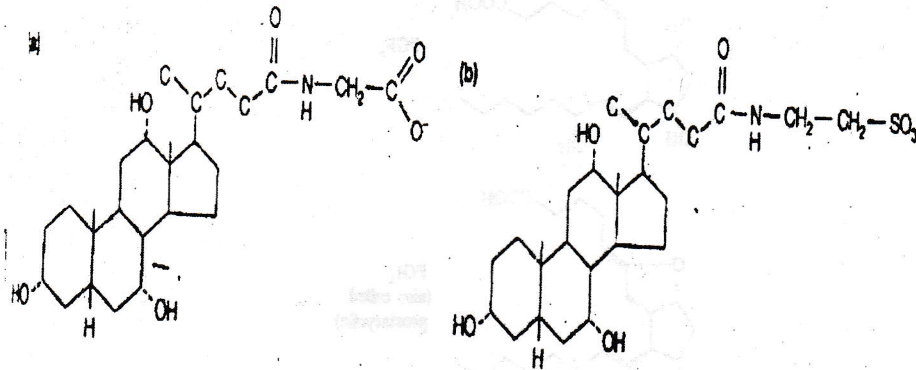
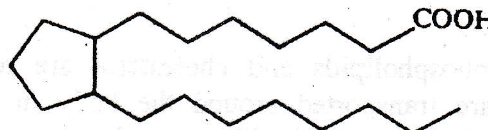


Fig 5 a & b

PROSTAGLANDINS

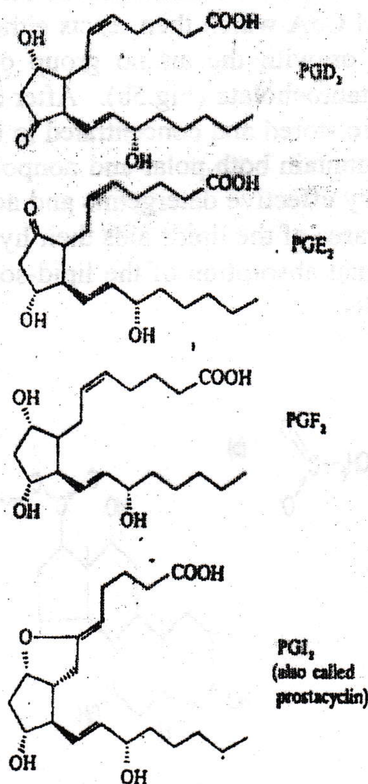
The prostaglandins are Czo unsaturated hydroxy acids with a substitued cyclopentane ring and two aliphatic side chains.

Consider the carbon skeleton of the prostaglandins.



Prostanoic acid

The structures and abbreviated names of some prostaglandins are :



Biological Role of the Prostaglandins

The Prostaglandins occur in all tissues but in very small amount. They act on loci in the same cells as those in which they are synthesized, and their biological roles are diverse; e.g., they function in the female reproductive system during ovulation, menstruation, pregnancy, and parturition, and they stimulate uterine muscle contraction.

LIPOPROTEINS

Structure and Functions :

Triacylglycerols, phospholipids and cholesterol are relatively insoluble in aqueous solution. Hence, they are transported around the body in the bloood as components of llipoproteins. These globoular, micelle-like particles consist of a hydrophobic core of trilacylglycerols and cholesterol esters surrounded by an amphipathic coat of proten, phospholipid and cholesterol. The protein components of lipoproteins are called apolipoproteins (or apoproteins). At least 10 different apoproteins are found in the different human lipoproteins. Their functions are to help solubilize the hydrophobic lipids and to act as cellular targeting signals. Lipoproteins are classified into five groups on the basis of their physical and functional properties (Table 1):

Table 1. Characteristics of the five classes of lipoproteins

Lipoprotein	Molecular mass (kDa)	Density (g ml^{-1})	% Protein	Major lipids	Apoproteins
Chylomicrons	> 400 000	< 0.95	1.5–2.5	TG	A, B-48, C, E
VLDLs	10 000–80 000	<1.006	5–10	TG, PL, CE	B-100, C, E
IDLs	5000–10 000	1.006–1.019	15–20	CE, TG, PL	B-100, C, E
LDLs	2300	1.019–1.063	20–25	CE, PL	B-100
HDLs	175–360	1.063–1.210	40–55	PL, CE	A, C, D, E

C, cholesterol; CE, cholesterol ester; TG, triglyceride; PL, phospholipid.

* Chylomicrons are the largest and least dense lipoproteins. They transport dietary (exogenous) triacylglycerols and cholesterol and cholesterol from the intestines to other tissues in the body.
* Very low density liporoteins (IDLs) and low density lipoproteins (LDLs) are a group of related lipoproteins that transport internally lproduced (endogenous) triacylglycerols and cholesterol from the liver to the tissues.
* High density lipoproteins (HDLs) transport endogenous cholesterol from the tissues to the liver.

ATHEROSCLEROSIS

Atherosclerosis, the most common type of hardening of the arteries, is characterized by the presence of cholesterol-rich arterial thickenings (atheromas). This progressive disease begins with the intracellular deposition of lipids, mainly cholesterol esters, in the smooth muscle cells of the arterial wall. These lesions become fibrous, calcified plaques that narrow and can eventually block the arteries. Blood clots are also more likely to occur which may stop the blood flow and deprive the tissues of oxygen. If these blockages occur in the coronary arteries, those supplying the heart, the result is a mylocardial infarction or heart attach, which is the most common cause of death in Western industrialized countries. Blood clots in cerebral arteries cause stroke, while those in peripheral blood vessels in the limbs can lead to possible gangrene and amputation.

BIOLOGICAL MEMBRANES

Membranes form boundaries both around the cell (the plasma membrane) and around distinct subcellular compartments (e.g. nucleus, mitochondria, lyso somes, etc.) They act as selectively permeable barriers allowing the inside environment of the cell or organelle to differ from that outside Membranes are involved in signaling processes; they contain specific receptors for external stimuli and are involved in both chemical and electrical signal generation. All membranes contain two basic components : lipids and proteins. Some membranes also contain carbohydrate. The composition of lipid, protein and carbohydrate varies from one membrane to another. For example, the inner mitochondrial membrane has a large amount of protein than lipid due to the presence of numerous protein complexes involved in oxidative phosphorylation and electron transfer, whereas the myelin sheath membrane of nerve cells, which serves to electrically insulate the cell, has a larger lproportion of lipid.

Membrane lipids

Lipids were originally classified as biological substances that were insoluble in water but soluble in organic solvents such as chloroform and methanol. In addition to being structural components of membranes, lipids have several other biological roles. They serve as fuel molecules, as concentrated energy stores (e.g. triacylglycerol) and as signaling molecules. Within membranes there are three major types of lipid : the glycerophospholipids, the sphingolipids and the sterols.

Glycerphospholipids

The glycerophospholipids are made up of three components: a phosphorylated headgroup, a three-carbon glycerol backbone and two hydrocarbon fatty acid chains (Fig.1). The phosphorylated head group is attached to carbon-3 of the glycerol backbone, while the two fattyacid chains are attached to the other two carbon atoms. The simplest glycerophospholipid is phosphatidate (diacylglycerol 3-phosphate) which has only a phosphoric acid group esterrified to carbon-3 of the glycerol. Although phosphatidate itself is present in small amounts in membranes, the major glycerophospholipids are derived from it. In thesel other lipids the phosphate is further esterified to the hydroxyl group of one of several aalcohols (choline, ehanolamine, glycerol, inositol or serine). The major glycerophospholipids found in membranes include phosphatidylcholine; phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylserine (Fig 6) Diphosphatidylglycrol is found predoninatly inth the inner mitochondrial membrance.

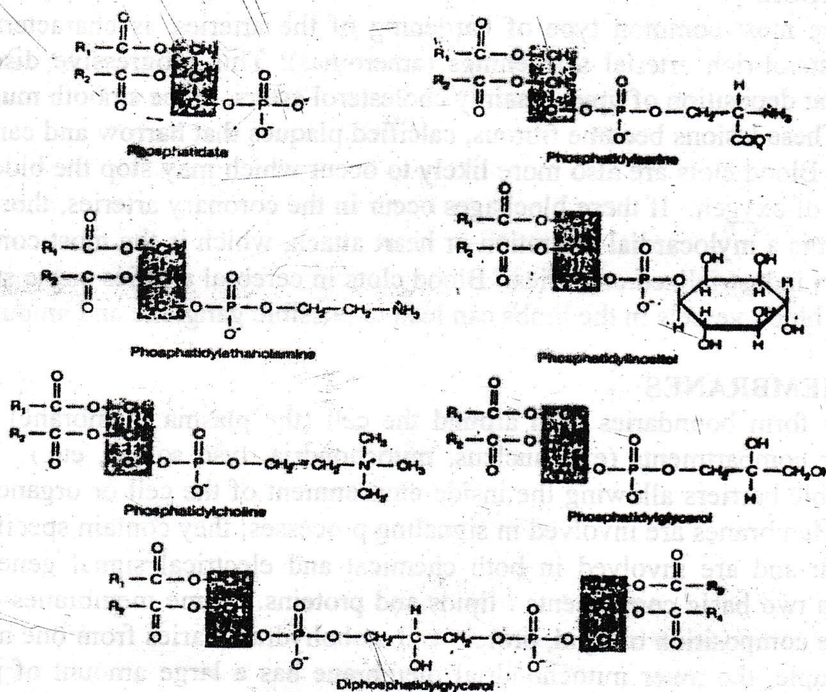


Fig. 6. Structures of membrane glycerophospholipids. R_1 and R_2 represent hydrocarbon chains of fatty acids

Sphingolipids

Sphingomyelins, the commonest shingolipids, have a sphingosine backbone (Fig. 7a) in place of the glycerol in glycerophosphilipids. Like the glycerophospholipids, they also have a phosphorylated headgroup (either choline or ethanolamine) and two hydrocarbon chains (Fig . 7a) One of the hydrocarbon chains comes from the shingosine molecule, the other is a fatty acid as found in the glycerophospholipids except that it is bonded via an amide bond in sphingolipids. The sphingomyelins are particularly abundant in the myelin sheath that surrounds nerve cells. The glycosphingolipids, such as the cerebrosides and gangliosides, are also derived from sphigosine, but in place of the phosphorylated headgroup they have one or more sugar residues. The galactocerebrosides have a single galactose residue (Fig. 7a) and are found predominantly in the neuronal cell membranes of the brain. The ganglioside.

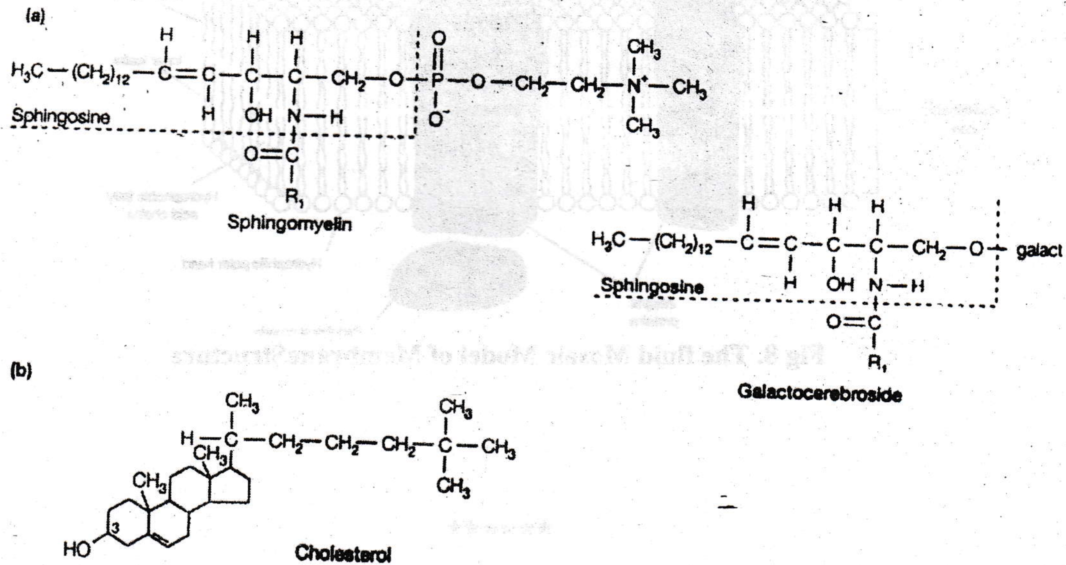


Fig. . Structures of (a) the sphingolipids sphingomyelin and galactocerebroside; (b) cholesterol. R_1 represents the hydrocarbon chain of fatty acids.

have several sugar residues including at least one sialic acid (N-acetylneuraminic acid) residue and are a major constituent of most mammalian plasma membranes, being particularly abundant in brain cells.

Fluid Mosaic Model of Membrane Structure :

In 1972 S.Jonathan Singer and Garth Nicholson proposed the fluid mosaic model for the overall structure of biological membranes, in which the membranes can be viewed as two-dimensional solutions of oriented lipids and globular proteins (Fig.8). The integral membraane proteins can be considered as 'icebergs' floating in a two-dimensional lipid 'sea'. They proposed that the bilayer organization of the lipids would act both as a solvent for the amphipathic integral membrane proteins and as a permeability barrier. They also proposed that

some lipids may interact with certain membrane proteins, that these interactions would be essential for the normal functioning of the protein, and that membrane proteins would be free to diffuse laterally in the plane of the bilayer unless restricted in some way, but would not be able to flip from one side of the bilayer to the other. This model is now [illegible] a wide va of experimental observations.

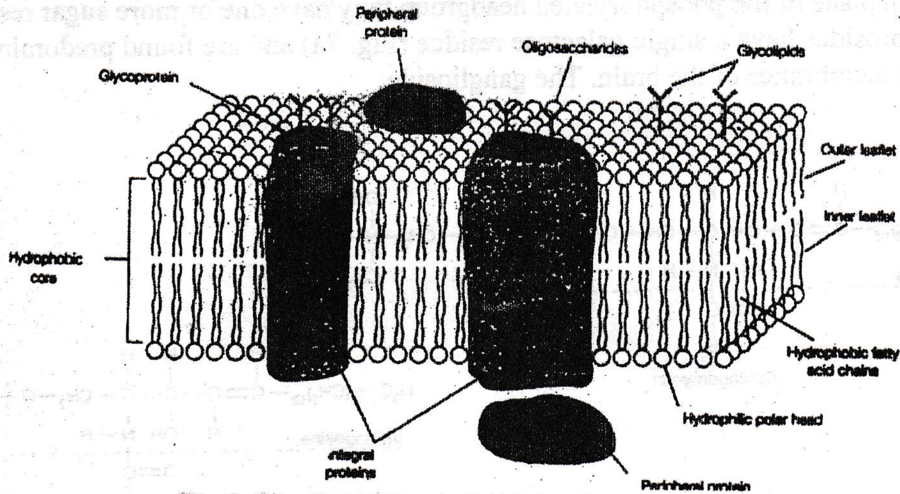


Fig 8. The fluid Mosaic Model of MembraneStructure

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UNIT - III BIOINORGANIC CHEMISTRY

NON – METALS IN BIOLOGICAL SYSTEMS

The essential non-metals comprise a much more heterogeneous group than do the transition metals, whose properties are all closely related. The two essential halogens, Fluorine and Iodine are highly specific and very different. Fluoride has a remarkable anti-dental carries effect. This may be related to its ability to replace OH, thereby stabilizing the structural matrix of bones and teeth. Fluoride also can inhibit strongly certain key enzymes enclose pyrophosphatase.

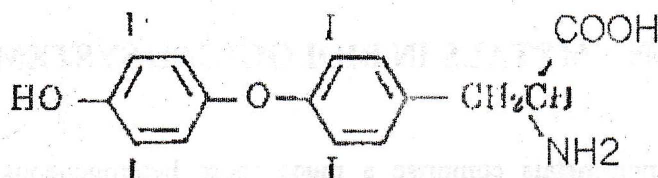
Among the non-metals the most important is selenium. Despite its high toxicity (0.2 mg/l) selenium has been shown to be a component of several enzymes involved in essential oxidation-reduction reactions. One enzyme, gluta thionine peroxidase (GSH) appears to play a major role in the protection of red blood cells against the effects of hydrogen peroxide, which is readily generated inside the cell. This is prime example of an enzyme that relies on an ultra trace non-metal iron for its biological activity.

Very little is known about the three remaining non-metals, Silicon, Arsenic, Bismuth, emphasizes the probability of their mechanisms, as well as the previous metals, being independent for all six non metals. Silicon has a structural role in connective tissue and osteogenic cells. No specific biological function for arsenic is known till date. Arsenic affects arginine membrane phospholibid and zinc metabolism. It is less toxic than selenium and ultra trace element with an established role. The primary focus on boron has been on its essential role in plants, possibly involving membrane function and nucleic acid biosynthesis.

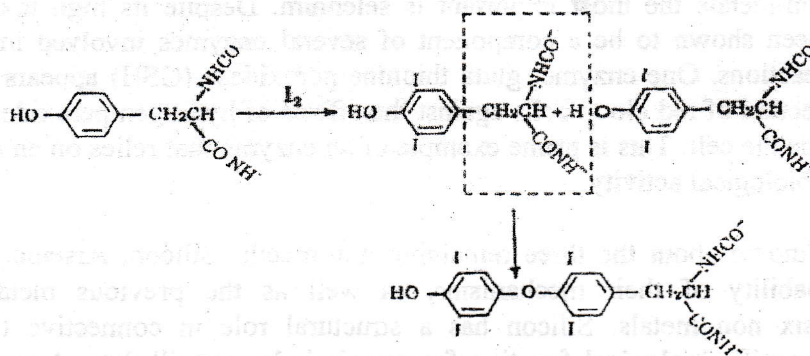
Iodine and the Thyroid Hormones

Iodine was established as an indispensable constituent of the thyroid gland and the presence of iodine has dominated consideration of thyroid hormone structure and function, with special emphasis on the properties of iodine as an electron sink. It was assumed that 3,5 and 3' positions on thyronine had to be filled by halogens, particularly iodine or bromine, in order to satisfy the minimum electronic and structural requirements for thyroid hormone activity.

The unique relationship of iodine to the thyroid hormones is to be found in the biosynthesis and metabolism of thyroxine and triiodothyronine. The presence of iodine along with H_2O_2 peroxide systems afforded a convenient mechanism for introducing iodine ortho to the phenolic group of tyrosine in or out of proteins to form mono and diiodotyrosine. The introduction of groups of similar size, e.g. methyl or isoprophyl, ortho to a phenolic hydroxyl group is extremely rare in vertebrate metabolism. Under oxidative conditions, these single-ring iodo compounds were favoured to undergo coupling reactions to form the iodothyromines, especially thyroxine. Ample proteases were available for the hydrolytic release of iodothyronines from their peptide linkages into the serum. Specific serum binding proteins, thyroxine binding globulin and prealbumin evolved to provide protective transport of the iodo amino acids.



The ability to preferentially trap iodine permitted reutilization of the iodine after metabolic deiodination, further contributing to the economy of thyroid harmone synthesis. All these circumstances combined to favour the biosynthesis of Thyroxin and triodothyronine with their unique function in the vertebrates.



The formation of a thyroxyl residue from the iodination

Essential and trace elements in biological systems

The functions of metalloporphyrins and metallo enzyme systems have indicated the importance of certain metals in chemical reactions within living organisms. Certain elements are essential in that they are absolutely necessary (perhaps in large, perhaps in small quantities) for life process. Other elements are non-essential since if they are absent other elements may serve the same function. The term 'trace element' although widely used is not precisely defined. For example molybdenum averages about 1-2 ppm in rocks, soils, plants and marine animals and even lower in land animals, yet is an essential trace element. At the other extreme iron, which averages about 5% in rocks and soils and 0.02-0.04% in plants and animals, might or might not be considered a 'trace' metal.

The number of elements that are known to be biologically important comprises a relatively small fraction of the 108 known elements. Natural abundance limits the availability of the elements for such use. Molybdenum (Z = 42) is the heaviest metal and iodine (Z = 53) is the heaviest non-metal of known biological importance. The metals of importance in enzymes are principally those of the first transition series and the other elements of importance are relatively light; sodium, potassium, magnesium, calcium, carbon, nitrogen, phosphorous, oxygen, chlorine and of course, hydrogen. Some of the elements that have been found to be essential or to be poisonous are listed with their actions in the following table.

Element	Function	Toxicity
Hydrogen	Molecular hydrogen metabolized by some bacteria	–
Boron	Essential for green algae and higher plants	Moderately toxic to plants, slightly toxic to mammals
Carbon	Synthesis of all organic molecules and of biogenetic carbonates	Carbon monoxide is slightly toxic to plants and highly toxic to mammals CN^- is toxic to all organisms.
Oxygen	Structural atom of water and most organic molecules in biological systems; required for respiration by most organisms.	Very toxic as ozone, superoxide, peroxide and hydroxyl radicals.
Fluorine	Essential element, 2.5ppm in diet for optimal growth. Strengthens teeth in mammals and used as CaF_2 in some mollusks.	Moderately toxic may cause mottled teeth.
Sodium	Important in nerve functioning in animals. Major component of Vertebrate blood plasma. Activates some enzyme.	Relatively harmless in excessive amounts. Associated with some forms of hypertension.
Magnesium	Essential to all organisms present in all chlorophylls has other electrochemical and enzyme activating functions.	
Aluminium	May activate succinic dehydrogenase and δ aminolevulinate hydrolase. The latter is involved in porphyrin synthesis.	Moderately toxic to most plants and slightly toxic to mammals.
Silicon	Essential element for growth and skeletal development in chicks and rats.	Silica is injurious to the mammalian lung.
Sulfur	Essential element in most proteins important in tertiary structure (through s-s links) of proteins involved in vitamins, fat metabolism, and detoxification process. H_2SO_4 in digestive fluid in ascidians. H_2S replaces H_2O in photosynthesis of some bacteria. H_2S and S_8 are oxidised by other bacteria.	Elemental sulfur is highly toxic to most bacteria and fungi relatively harmless to higher organisms. H_2S is highly toxic to mammals. SO_2 is highly toxic.

Chlorine	Essential for higher plants and mammals. NaCl electrolyte, HCl in digestive juices.	Highly toxic in oxidizing forms Cl_2, ClO^-, and $ClO3^-$.
Potassium	Essential to all organism with the possible exception of blue green algae; major cation of cytoplasm; important in nerve action and cardiac function.	Moderately toxic to mammals when injected intravenously; otherwise harmless.
Calcium	Essential for all organisms used in cell walls, bones and some shells as structural component; important electro chemically and involved in blood clotting.	Relatively harmless.
Vanadium	Essential to ascidians, chicks and rats.	Highly toxic to mammals
Chromium	Essential, functioning as a glucose tolerance factor. It is related to insulin in its biological role and thus to sugar metabolism and diabetes.	Highly toxic as Cr(IV), moderately so as Cr(III).
Manganese	Essential to all organism; activate numerous enzymes; deficiencies in solids lead to infertility in mammals, bone malformation in growing chicks.	Moderately toxic.
Iron	Essential to all organisms as Hemoglobin, myoglobin (respiration) Fe-S proteins electron carriers.	Normally only slightly toxic. Excess cause siderosis and damage to organs.
Cobalt	Essential for many organisms including mammals; activates a number of enzymes; vitamin B12.	Very toxic to plants and moderately so injected intravenously in mammals.
Nickel	Essential trace elements to chicks and rates.	Very toxic to most plants and slightly to mammals.
Copper	Essential to all organisms; constituent of redox enzymes and hemocyanin	Very toxic to most plants and highly toxic to invertebrates and moderately so to mammals.
Zinc	Essential to all organisms; used in enzymes, stabilized coiled ribosomes, plays a role in sexual maturation and reproduction.	Moderately to slightly toxic and causes vomiting.

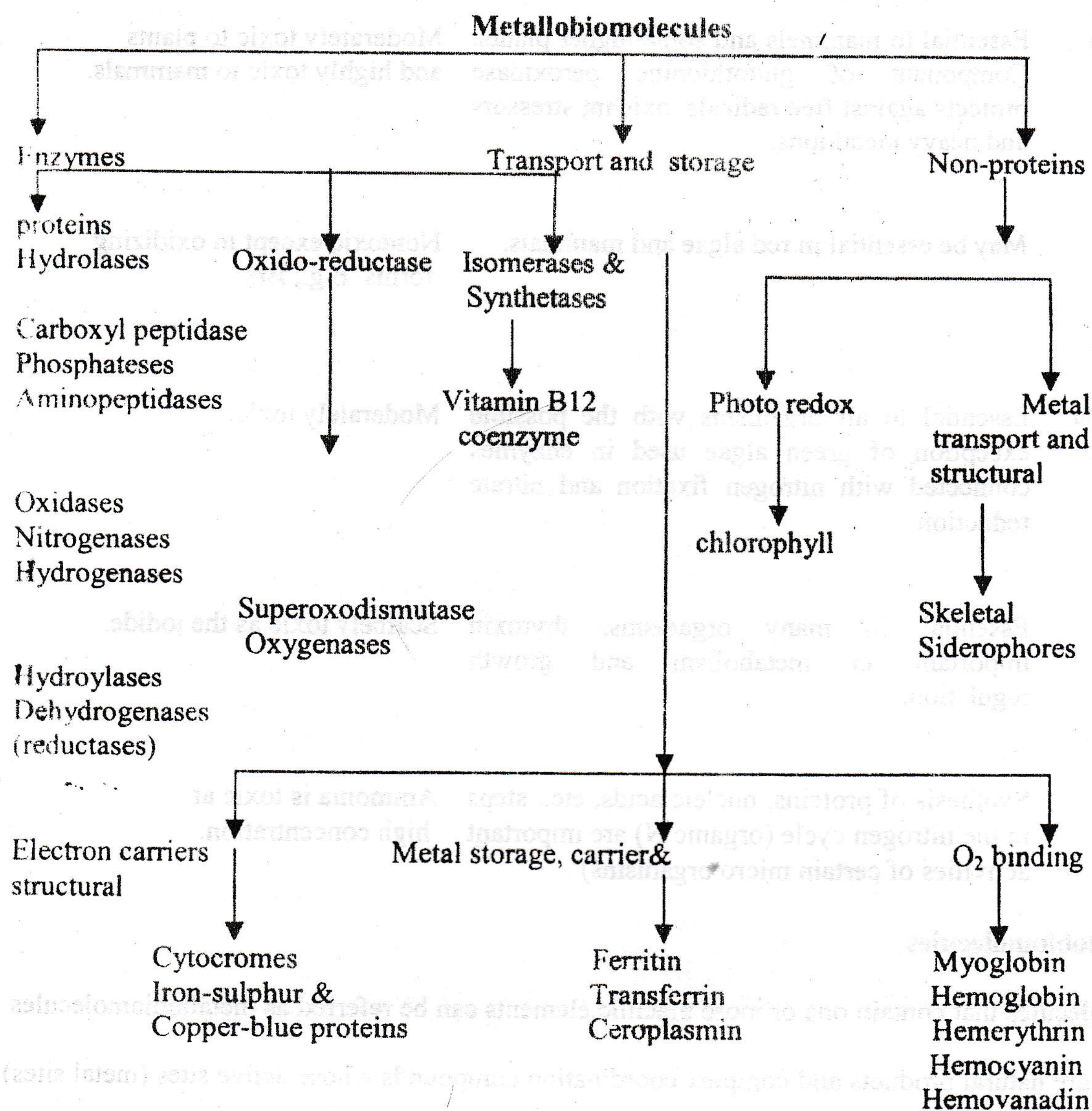
Moderately toxic to plants and highly toxic to m
Nontoxic except in oxidizing forms. E.g., Br_2
Moderately toxic.
Scarcely toxic as the iodide.

Ammonia is toxic at high concentration.

Selenium	Essential to mammals and some higher plants. Component of glutothionine peroxidase protects against free radicals, oxidant stressors and heavy metal ions.	Moderately toxic to plants and highly toxic to mammals.
Bromine	May be essential in red algae and mammals.	Nontoxic except in oxidizing forms. E.g., Br_2
Molybdenum	Essential to all organisms with the possible exception of green algae used in enzymes connected with nitrogen fixation and nitrate reduction.	Moderately toxic.
Iodine	Essential in many organisms, thyroxin important in metabolism and growth regulation.	Scarcely toxic as the iodide.
Nitrogen	Synthesis of proteins, nucleic acids, etc., steps in the nitrogen cycle (organic N) are important activities of certain micro organisms) .	Ammonia is toxic at high concentration.

Metallobiomolecules

Biomolecules that contain one or more metallic elements can be referred as metallobiomolecules. These are natural products and complex coordination compounds whose active sites (metal sites) are usually involved in different biochemical processes such as electron transfer, binding of exogenous molecules and catalysis. Based upon the function of these biomolecules, they are classified into three groups.



Non-protein metallobiomolecules

Chlorophyll: Radient solar energy enters the biosphere using magnesium porphyrin complex i.e., through chlorophyll. The process is also referred as photosynthesis and almost all life processes depends upon chlorophyll and photosynthesis. Here the energy is stored in carbohydrate and when carbohydrate molecules breakup into small molecules energy is released and the life processes use solar energy thus by photosynthesis.

Siderophores: Siderophores are responsible for iron transport into and within bacteria cells. They form chelates with iron and transport the iron into the cells. In some cases it has been observed that siderophores release iron at the cell wall and iron alone passes through, whereas in

other cases entire complex coordination compound enters the cell and releases iron when required.

Calcium phosphate in skeleton: Calcium is present in the body in large amounts than any other mineral elements. About 99% of the body calcium is in the skeleton. The major inorganic constituents of bone is comprised of a crystalline form of calcium phosphate, however, noncrystallline, amorphous calcium phosphate is also present.

Silicon in mucopolysaccharides: Silicon appears to be an integral component of acid mucopolysaccharides and may have structural role in connective tissues, cartilage, skin and bone. Silicon is an essential element for growth and skeletal development in rat and chick.

Phophorous: About 10% of phosphorous is in combination with proteins, lipids and carbohydrates and in other compounds in blood and muscle. The remaining 10% is widely distributed in various chemical compounds. The greater important of the phosphate ester in energy transfer is also known. 80% of the phosphorous is used in bone formation.

Metallobiomolecules-proteins

1. Electron carriers

a) **Cytocromes:** Cytocromes are heme proteins those act as electron carrier involved in photosynthesis. It consists of a porphyrin ring chelated to iron atom. Cytocromes are found in all aerobic forms of life. The oxidation state of iron may either II or III and they exist as redox intermediates in electron transfer reactions.

b) **Iron-Sulphur proteins:** Several non-heme iron sulphur proteins are involved in electron transfer. They are made up of peptide chains having cysteine(amino acid) moiety where cysteine sulphur is coordinated to iron or cluster of iron-sulphur atoms. E.g., rubredoxins and ferridoxins.

c) **Copper blue proteins:** They act as elecron carriers in photosynthesis and mainly occur in algae, green leaves and other plants. E.g., plastocyanin and azurin.

2. Metal storage

a) **Ferritin:** Iron can be stored and released in epithelial cells by a protein called ferritin. The excess of iron can be stored within cell in non-toxic form by binding to protein called apoprotein in the mucosal cells. iron(II), once within the mucosal cells is oxidized to iron(III) and then after combining with apoprotein called ferritin.

b) **Trnsferrin:** Nearly all the iron released from the mucosal cell enters the portal blood, mostly in iron(II) state. In the plasma iron (II) is oxidized to iron(III) andthen picked upby iron binding protein, transferrin.

.3. **Oxygen binding metallobiomolecule**

a) **Hemoglobin and Myoglobin:** The function of oxygen transport and storage in higher animals is provided by iron-porphyrin complexes, hemoglobin and myoglobin. The former transport the oxygen from lungs to site of its use inside the muscle cell and there the oxygen is transferred to myoglobin for use in respiration.

b) **Hemerythrin:** It is a non-heme, oxygen binding iron protein used in great variety of marine worm and phyla of marine invertebrates. It also contains iron(II) which binds

oxygen reversibly and when oxidized to iron(III) (methmerythrin) show no tendency to bind oxtgen.

c) **Hemocyanin:** Hemocyanin contains copper and bind one molecule of oxygen for every pair of copper (I) ions. When oxygen is bound to hemocyanin its colour is blue otherwise unbound hemocyanin is colourless. They are found in molluscus and ortropods.

d) **Hemovanadins:** The vacuoles in the blood cells (vanadocytes) of the acidians contain a vanadium protein complex dissolved in acid medium. It has been established that carefully prepared vanadocytes uptake oxygen reversibly.

Enzymes

Enzymes are catalysts that enhance the rates of biochemical reaction from 10^6 to 10^{12} times that of uncatalysed reations. All enzymes are proteins and contain a functional site, called the active site, where reactants are converted into products. Each enzyme is highly specific, catalyzing one at most a few reactions. Enzymes are of interest to inorganic chemists composed of protein structure (called an apoenzyme) and a small prosthetic group, which may be either a simple metal ion or a complexed metal ion. For example, zinc is the prosthetic group in carbonic anhydrase and carboxy peptidase. A reversibly bound group that contains with an enzyme for a particular reaction and then is released to combine with another is termed a coenzyme. Both prosthetic groups and coenzymes are sometimes called cofactors.

Classification of enzymes

Class	Action	Examples
Oxido-reductase	Enzymes catalyzing oxidation-reduction reactions. The hydrogen donor is regarded as the substrate.	Oxygenase, Reductase phenolase, trogenase, superoxide dismutase,.
Transferases	Enzymes catalyzing the general form $X\text{-}Y + Z \rightleftharpoons X + Y\text{-}Z$	Cyanocobalamine
Hydrolases	Enzymes catalyzing a hydride cleavage of C-O, C-N, C-C and other bonds	Carboxypeptidase, Carbonicanhydrase
Lyases	Enzymes catalyzing cleavage of C-O, C-N, C-C and other bonds by elimination, leaving double bonds, or alternatively by the addition of groups to double bonds.	Lyases
Isomerases	Enzymes catalyzing a change in the geometric or spatial configuration of a molecule.	Cyanocobalamine
Ligases	Enzymes catalyzing the joining together of two molecules with the accompanying hydrolysis of a high-energy bond.	Ligases

Oxido-reductase

These enzymes catalyses oxidation and reduction process between two substrates A and B.

$A_{reduced} + B_{oxidised} \quad B_{reduced} + A_{oxidised}$

This large and important class include examples such as oxygenase, hydrogenase, nitrogenase etc.

Oxidases:

These are the enzymes catalyze the removal of hydrogen from a substrate but use only oxygen as hydrogen acceptor. Two important examples are cytocrome oxidase and phenolase.

Cytocrome oxidase

Detailed in unit IV under cytocromes.

Phenolases

It is a copper-containing enzyme that is specific for more than one type of reaction. It is able to convert monophenol or o-diphenols to quinones.

Transferase

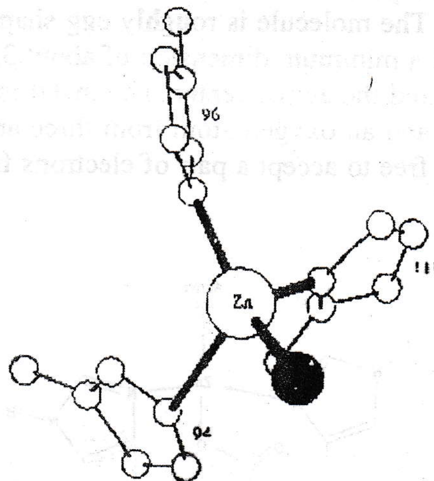
Detailed in unit IV under vitamin B12

Hydrolases

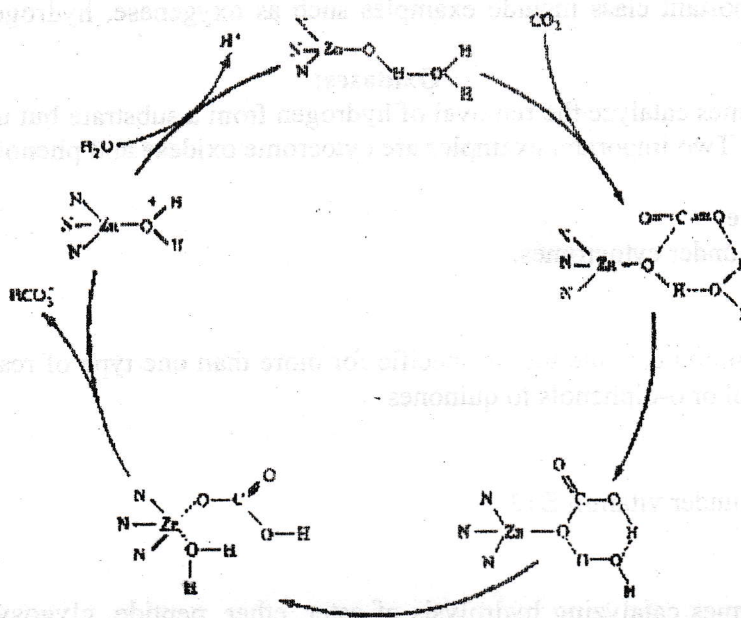
These are the enzymes catalyzing hydrolysis of ester, ether, peptide, glygosyl, acid anhydride, carbon-carbon double bond, carbon-halogen bond and phosphorous-nitrogen bond. The important enzymes among them are carbonic anhydrase and carbonic peptidase.

Carbonic anhydrase

It catalyzes the interconversion of carbon dioxide and carbonates. It contains a zinc atom and the zinc atom coordinated to three histidine residues (His94, His96, His119) and a water molecule or hydroxide ion. The active site contains other amino acids that may function through hydrogen bonding, proton transfer etc.

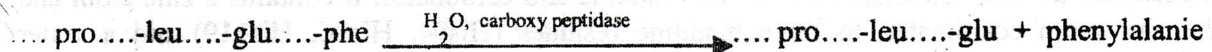


The mechanism of the reversible hydrogen of carbon dioxide to carbonic acid (actually the hydrogen carbonate ion at physiological pHs) thought to follow the pathway shown by the following closed loops like all truly catalytic process.

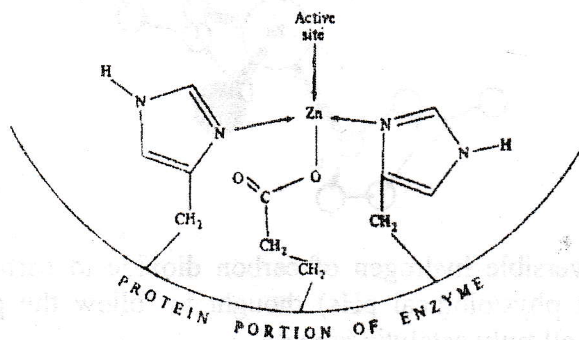


Carboxy peptidase

Carboxy peptidase is a pancreatic enzyme cleaves the carboxyl terminal amino acid from a peptide chain by hydrolyzing the amide linkage.

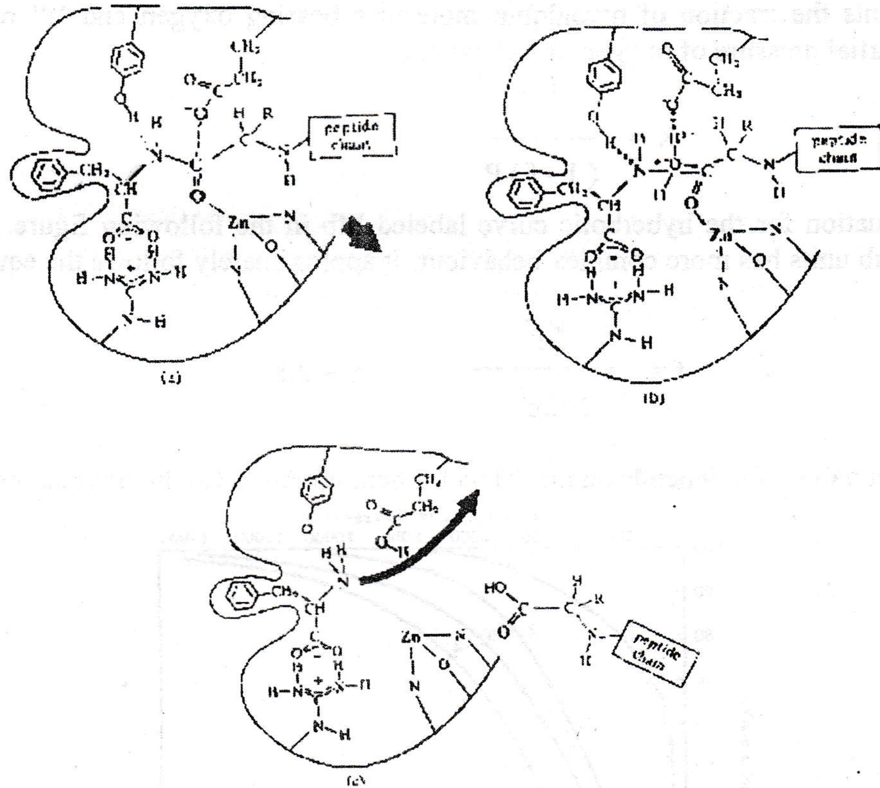


The enzyme consists of a protein chain of 30% amino acid residues plus one Zn^{2+} ion for a molecular weight of 34,600. The molecule is roughly egg shaped with a maximum dimension of approximately 5000 pm and a minimum dimension of about 3800 pm. There is a cleft on one side that contains the zinc ion and the active center. The metal ion is coordinated approximately tetrahedraly to nitrogen atoms and an oxygen atom from three amino acids in the protein chain. The fourth coordination site is free to accept a pair of electrons from a donor in the substance to be cleaved.



The enzyme is thought to act through coordination of the zinc atom to the carbonyl group of the amide linkage. In addition, a nearby hydrophobic pocket envelops the organic group of the amino acid to be cleaved and those amino acids with aromatic side groups react most readily. The arginine side chain moves about 200 pm closer to the carboxylate group of the substrate, and the phenolic group of the tyrosine comes within hydrogen bonding distance of the imido group of the C-terminal amino acid, a shift of 200 pm. The hydrogen bonding to the free carboxyl group (by arginine) and the amide linkage (by tyrosinase not only holds the substrate to the enzyme but helps to break the N - C bond. Nucleophilic displacement of the amide group by an attacking carboxylate group from a glutamate group could form an anhydride link to the remainder of the peptide chain. Hydrolysis of this anhydride link to the remainder of the peptide chain could then complete the cycle and regenerate the original enzyme. More likely, the glutamate acts indirectly by polarizing a water molecule that attacks the amide linkage.

Suggested mode of action of carboxy peptidase in the hydrolysis of amide linkage in a polypeptide chain is shown below.



Hemoglobin – Myoglobin

The function of oxygen transport and storage in higher animals is provided by hemoglobin and myoglobin. Hemoglobin has two functions

i) It binds oxygen molecule to its iron atoms and transports them from the lungs to muscle where they delivered to myoglobin.

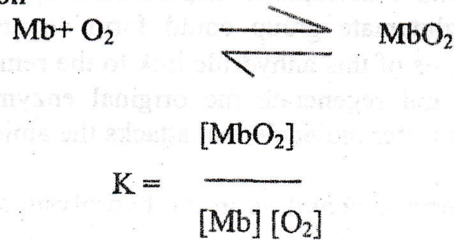
ii) The hemoglobin then uses certain amino groups to bind carbon dioxide and carry it back to the lungs.

Myoglobin may serve as a simple storage reservoir. It also facilitates the flow of oxygen within the cell and buffers the partial pressure of oxygen within the cell.

Physiology of blood (Bohr effect)

Myoglobin must have greater affinity for oxygen than hemoglobin, in order to effect the transfer of oxygen to the cell.

The equilibrium contact for myoglobin-oxygen complexation is given by the simple equilibrium expression



$$Mb + O_2 \rightleftharpoons MbO_2$$

$$K = \frac{[MbO_2]}{[Mb]\,[O_2]}$$

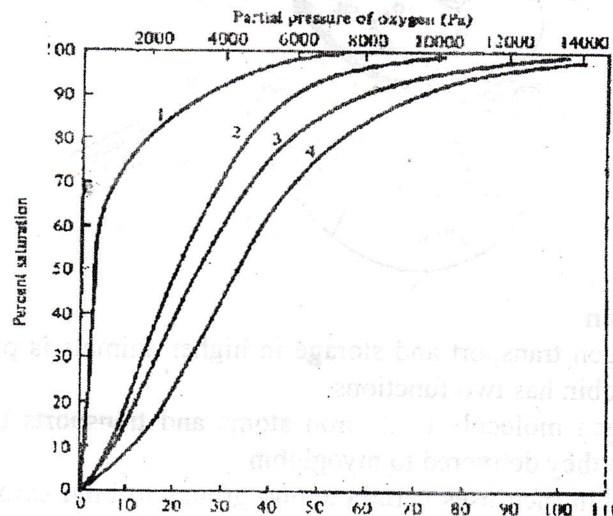
If 'f ' represents the fraction of myoglobin molecules bearing oxygen and 'P' represents the equilibrium partial pressure of oxygen, it follows that

$$K = \frac{f}{(1-f)P}$$

This is the equation for the hyberbolic curve labeled Mb in the following figure. Hemoglobin with its four sub units has more complex behaviour. It approximately follows the equation.

$$f = \frac{Kp^n}{1+kp^n} \qquad n \sim 2.8$$

where the exact value of n depends on pH. Thus for hemoglobin (Hb) the oxygen binding



1) Dioxygen binding curves for Mb and Hb at various partial pressure of CO_2 2) 20 mm Hg 3) 40 mm Hg 4) mm Hg

curves are sigmoidal as shown above. The fact that 'n' exceeds unity can be ascribed physically to the fact that attachment of O_2 to one heme group increases the binding constant for the next O_2 which in turn increases the constant for the next one and so on.

It will be seen that, while Hb is about as good an O_2 binder as Mb at high O_2 pressure, it is much poorer at the lower pressures prevailing in muscle and hence passes its oxygen on to the Mb as required. Moreover, the need for O_2 will be greatest in tissues that have already consumed oxygen and simultaneously produced CO_2 lowers the pH thus causing the Hb to release even more oxygen to the Mb. The pH sensitivity (called the Bohr effect) as well as progressive increase of the binding constants in Hb are due to interactions between the subunits, Mb behaves more simply because it consists of only one unit. From the above discussion it is clear that each of the two is essential in the complex oxygen-transport process.

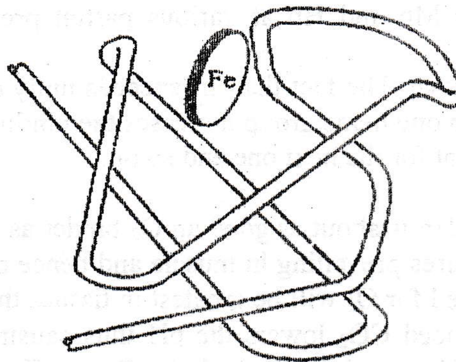
Cooperativity effect

The difference between hemoglobin and myoglobin in their behaviour towards oxygen is related to the structure and movements of the four chains. If the tetrameric hemoglobin is broken down into dimers or monomers, these effects are lost. Upon oxygenation of hemoglobin, two of the heme groups move apart 100 pm toward each other while two others separate by about 700 pm these movements seem responsible for the cooperative effects observed.

Perutz has suggested a mechanism to account for the cooperative effects observed. Deoxyhemoglobin contains iron(II) in a highspin state. With two electrons occupying e_g orbitals, the bonding radius of the iron atom is so large that it cannot fit into the plane of the four nitrogen atoms of the heme porphyrin. It therefore lies some 70-80 pm out of the plane, the Fe-N distance being about 220 pm. The iron is thus penta coordinate with square pyramidal coordination provided by four porphyrin nitrogen atoms in the basal position and an imidazolenitrogen from histidine in the apical position. When an oxygen molecule is bound in the position opposite to the histidine the iron atom goes in to a low spin state. The e_g orbital are than empty and the radius of the iron atom decreases by (60 pm) so much that it now fits into the plane of the porphyrin system. Thus the iron atom moves some 75 pm when deoxyhemoglobin becomes oxygenated. Since it remains attached to the side chain of histidine this shift is transmitted to various parts of the sub unit causing. Particularly important movement of the entire helical in which, a substantial change in the position of tyrosine and the other amino acid residues attached to it. These changes in one subunit then cause changes in other subunits since the interface between the subunits is altered. When the fully saturated (four O_2 molecules) hemoglobin molecule reaches the tissues, the reverse takes place. The change can be represented diagrammatically as follows.

Structure and Mechanism of hemoglobin

Myoglobin has a molecular weight of 17,000 of which most is a protein chain folded about the heme reducing access to the iron simultaneously producing a hydrophobic environment which is shown below.



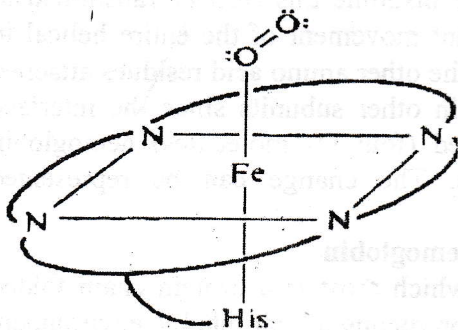
(a)

(a) myoglobin

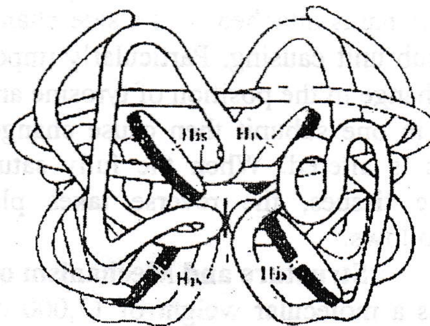
The microenvironment of the iron in Hemoglobin and myoglobin is similar to that found in cytochrome 'C'. The heme group is coordinated to the nitrogen atom of a histidine group in the protein chain. Unlike cytochrome 'C' there are no links from the outer, organic portions of the heme to the chain; also unlike cytochrome C, there is no sixth ligand from the chain to complete the coordination sphere of the iron atom. This provides a site for the coordination of oxygen molecule.

Hemoglobin may be considered approximate tetramer of myglobin. It has a molecular weight of 64500 and contains four heme groups bound to four protein chains, which is shown below. If the tetrameric hemoglobin is broken down into dimers or monomers, these effects are lost.

Upon oxygenation of hemoglobin two of the heme groups more about 100 pm towards while two others separate by about 700 pm. Hemoglobin has a channel 2000 pm wide, lined with polar groups.The radius of Fe^{2+} is 78 pm. The Fe-N bond distance in heme is 218 pm. Since there is only room for bond length of about 200-205 pm, the iron atom sit about 80 pm above the plane of the heme group. Low spin Fe^{2+} is 17 pm smaller than high spin Fe^{2+}. The Iron –Nitrogen bond distance should therefore be almost exactly 200 pm and the low spin iron atom should fit snugly in the porphyrin hole. Coordination with oxygen will therefore cause the iron to drop about 60 pm into the plane of the heme group.

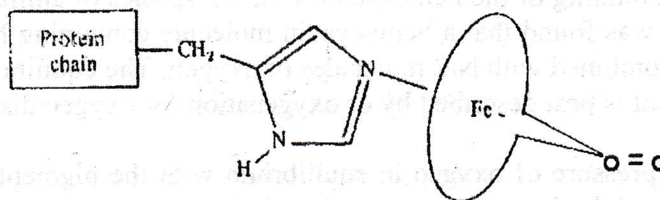


(a) Dioxygen heme group



(b) Hemoglobin molecule

Mechanism: Write down the Bohr effect and cooperative effect.



(b)

(b) Close view of the heme environment

Hemocyanin: The Copper Blood

The name is misleading. Hemocyanin, unlike hemoglobin, has no heme group; the copper is bound directly to the protein and not to any cyanide ion.

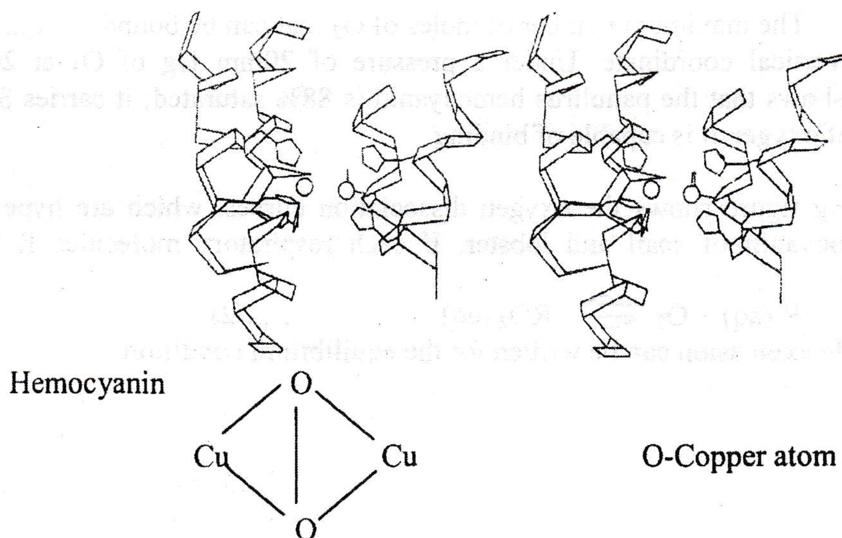
The occurrence of Hemocyanin

Hemocyanin occurs in only two phyla of animals, mollusks and among arthropods it is found in all decapods crustaceans (lobsters, crabs, shrimps).

Molecular structure of Hemocyanins

The gross molecular structures of the hemocyanins in the two phyla are quite different though both bind dioxygen co-operatively and spectroscopic evidence indicates that the dioxygen-binding centers are similar. The dioxygen binding site appears to be a pair of copper atoms, each bound by three histidine ligands. The copper is in the +1 oxidation state in the deoxy form and +2 in the oxy form.

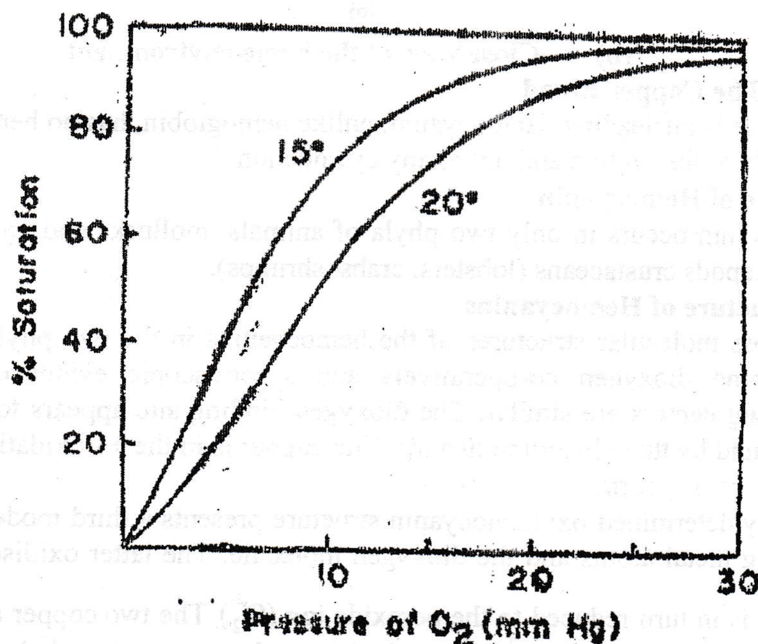
Recently determined oxyhemocyanin structure presents a third mode of binding between oxygen-carrying metal atoms and the dioxygen molecule. The latter oxidises each copper (I) to copper (II) and is in turn reduced to the peroxide ion (O_2^{2-}). The two copper atoms are bridged by the peroxide ion with unusual $\mu\eta^2$: η^2 bonds, i.e., each oxygen atom is bonded to both copper atoms.



Oxygen and Hemocyanin

The oxygen binding of the hemocyanin from 13 species of animals had been investigated and in each case it was found that a hemocyanin molecule containing N atoms of copper, when fully oxygenated, combined with N/2 molecules of oxygen. The equilibrium between oxygen and a respiratory pigment is best described by or oxygenation (or oxygen dissociation) curve which is shown below.

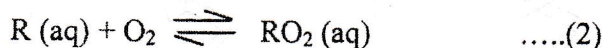
The partial pressure of oxygen in equilibrium with the pigment solution is indicated on the horizontal axis and the % saturation, which is defined as



$$\%\text{Saturation} = \frac{\text{The Number of moles of } O_2 \text{ actually bound to the pigment}}{\text{The maximum number of moles of } O_2 \text{ that can be bound}} \times 100 \quad ....(1)$$

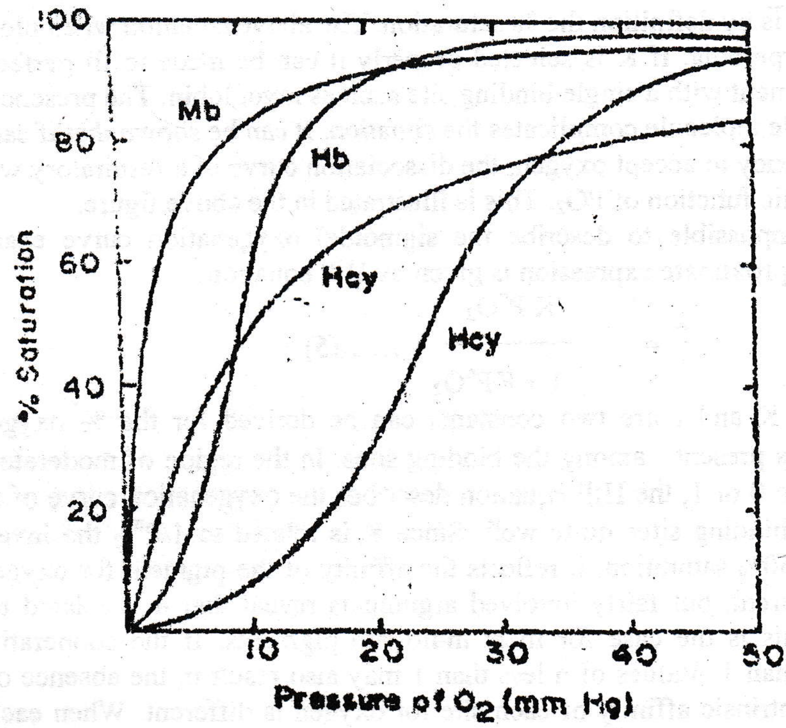
is marked on the vertical coordinate. Under a pressure of 20mm Hg of O_2 at 20 °C the oxygenation curve shows that the panulirus hemocyanin is 88% saturated; it carries 88% of the maximum amount of oxygen it is capable of binding.

The following figure shows the oxygen dissociation curves, which are hyperbolic and sigmoidal for hemocyanin of snail and lobster. If each respiratory molecules R binds one molecule of oxygen.



$$R\,(aq) + O_2 \rightleftharpoons RO_2\,(aq) \quad .....(2)$$

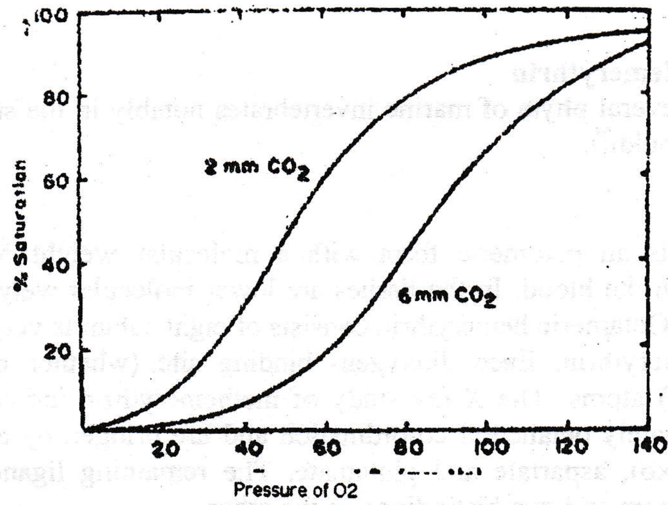
Then a simple expression can be written for the equilibrium condition.



$$K = \frac{[RO_2]}{[R]\,PO_2} \quad .....(3)$$

The rearrangement of this equation yields

$$\bar{\gamma} = \frac{[RO_2]}{[R] + [RO_2]} = \frac{K\,PO_2}{1 + KPO_2} \quad .....(4)$$



where $\gamma \times 100$ is by definition the % saturation. The above equation when plotted as γ versus PO_2 describes a hyperbola. If K is selected properly it can be made to fit perfectly the dissociation curve of a pigment with a single binding site such as myoglobin. The presence of several binding sites on a single molecule complicates the situation. It can be shown that if each site has the same intrinstic tendency to accept oxygen, the dissociation curve of a respiratory with many binding is still a hyperbolic function of PO_2. This is illustrated in the above figure.

It is impossible to describe the sigmoidal oxygenation curve exactly with a simple formula the approximate expression is given by Hill equation.

$$\bar{\gamma} = \frac{K P^n O_2}{1 + KP^n O_2} \quad ......(5)$$

Where K and r are two constants can be derived for the % oxygenation even when cooperativity is present among the binding sites. In the region of moderate pressure when γ is not too close to 0 or 1, the Hill equation describes the oxygenation curve of respiratory pigment with multiple binding sites quite well. Since K is related to $1/P^n_{50}$ the inverse of the pressure necessary for 50% saturation, it reflects the affinity of the pigment for oxygen. The meaning of 'n' is not apparent, but fairly involved arguments reveal that it is related to the extent of co-operativity. This is the case for most multi-site pigments. If the cooperatively is negative, n becomes less than 1. Values of n less than 1 may also result in the absence of any cooperativity effects if the intrinsic affinity of each site for oxygen is different. When each site has the same affinity and no cooperativity effects are present n becomes 1 and the Hill expression reduced to equation 4. In hemocyanin n varies between 1 and 5 indicating that the extent of cooperativity is much less than what one would expect from a molecule containing more binding sites. The extents of cooperativity changes with temperature, pH and the presence of salts.

Hemerythrin

Hemirtythrin is a nonheme, dioxygen-binding pigment utilised by four phyla of marine invertebrates it is of interest because it has certain similarities and differences with hemoglobin and myoglobin.

The occurrence of Hemerythrin

It occurs in several phyla of marine invertebrates notably in the sipunculid worms (e.g., the worm glofingia gouldii).

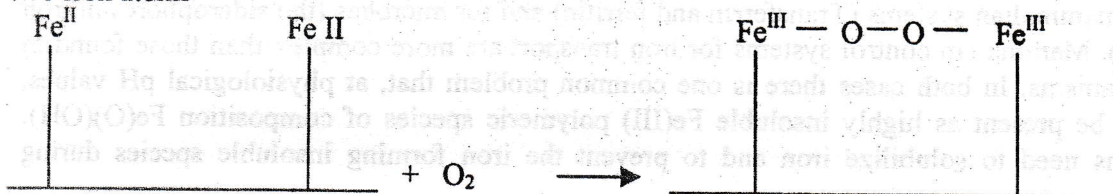
Molecular structure

Hemerythrin is an octomeric form with a molecular weight of about 108,000 that transports dioxygen in the blood. In the tissues are lower molecular weight monomers, dimers, trimers, or tetramers. Octameric hemerythrin consists of eight subunits very similar in quaternary structure to myohemerythrin. Each dioxygen- binding site (whether monomer or octamer) contains two iron (II) atoms. The X-ray study of methemerythrin indicates that the two iron atoms have approximately octahedral co-ordination and are bridged by an oxygen atom (from water, hydroxo or oxo), aspartate and glutamate. The remaining ligands are three histidine residues on one iron atom and two histindines on the other.

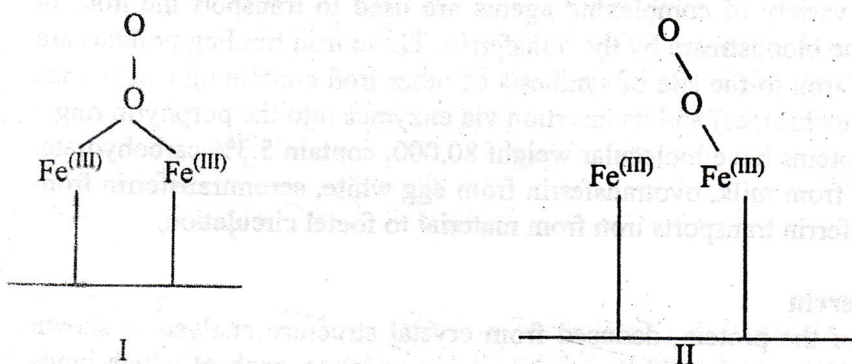
Oxygen and Hemerythrin

The oxygen binding reaction takes place via a redox reaction to form iron (III) and peroxide (O_2^{2-}) oxyhemerythrin is diamagnetic, indicating spin coupling of the odd electrons on the two iron (III) atoms.

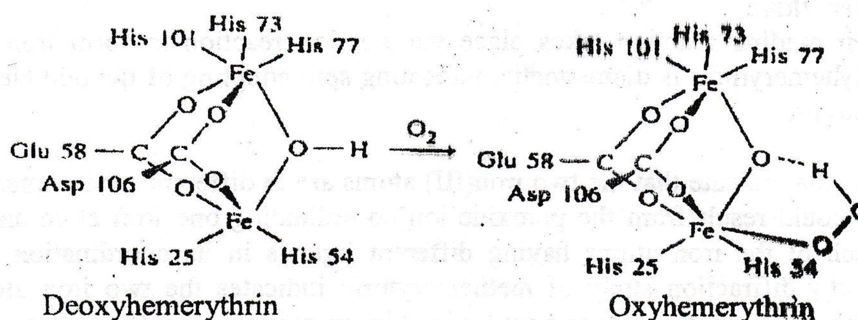
Mossbauer data indicate that the two iron(III) atoms are in different environments in oxy hemerythrin. This could result from the peroxide ion co-ordinating one iron atom and not the other, or from each of the iron atoms having different ligands in its coordination sphere. Evidence from X-ray diffraction study of methemerythrin indicates the two iron atoms have approximately octahedral coordination and are bridged by an oxygen atom (from water, hydroxo or oxo), aspartrate and glutamate. The remaining ligands are three histidine residues on one iron atom and two histidine on the other. Therefore this favours a simple peroxo bridge between the two iron atoms:



where the continuous line connecting the two iron atoms is a simplified representation of the coordination spheres and the protein chain holding the iron atoms in place. Against this simple structure the Mossbauer spectrum does not distinguish the iron atoms in deoxyhemerythrin. Further the Raman spectrum of oxy(O^{16} O^{18}) hemerythrin, which shows the two oxygen atoms to be nonequivalent positions. Among the various alternative structures the Raman data compatible with the following two:



O^{16} O^{18} data, as well as other spectroscopic evidence, are compatible with structure (II) but the X-ray structure of oxyhemerythrin requites further refinement. The proposed strucures of deoxyhemerythrin and oxyhemerythrin are:



Transport and storage of Iron

Iron is the fourth most abundant element in the earth's crust, but is not always readily available for use. Transport and storage processes involving iron are by for the best understood, both for mammalian systems (Transferrin and ferritin) and for microbes (the siderophores in iron transport). Mammalian control systems for iron transport are more complex than those found in microorganisms. In both cases there is one common problem that, at physiological pH values, iron will be present as highly insoluble Fe(III) polymeric species of composition Fe(O)(OH). Organisms need to solubilize iron and to prevent the iron forming insoluble species during storage.

In an adult human some 65% of the total iron is found in hemoglobin and myoglobin, and the bulk of the remainder is found in the storage proteins. A small amount is utilized in iron enzymes.

It is interesting that biologically functioning iron compounds such as hemoglobin, myoglobin, cytochromes and ferredoxins employ iron (II) compounds, but the transferrins and siderophores coordinate iron (III).

Transferrins

Within the organism a variety of complexing agents are used to transport the iron. In higher animals it is carried in the bloodstream by the transferrin. These iron-binding proteins are responsible for the transport of iron to the site of synthesis of other iron containing compounds (such as hemoglobin and the cytochromes) and its insertion via enzymes into the porphyrin ring. The transferrins are all glycoproteins have molecular weight 80,000, contain 5.3% carbohydrate. Transferrin includes lactoferrin from milk, ovotransferrin from egg white, serumtransferrin from a range of organisms, and uteroferrin transports iron from material to foetel circulation.

Molecular structure of transferrin

A schemetic structure of the protein, deduced from crystal structure analysis is shown below. Transferrin is an elipsoidal protein with two subdomains or lobes, each of which binds iron. The iron centres in transferrin, are of approximately octahedral geometry with two tyrosinate, two imidazole, a carbonate (or bicarbonate) and a water molecule (or hydroxide iron) as ligands. This is consistent with the loss of three protons on binding of iron to apotransferrin, namely, from two tyrosines and the aqua group. The two monoiron (III) half fragments differ slightly from each other. The two sites in transferrin have different affinities for iron, which may be dependent on the anion used. The two sites release iron at different rates in a pH dependent

manner. The site in the C- terminal half of human serum transferrin retains its iron at pH 6.0 and so is the acid- stable site. The site on N- terminal half is the acid-labile site. The four principal species of transferrin are iron(III), N terminal monoiron (III), C-terminal monoiron(III) and the apotransferrin.

Mechanism of iron intake and transport

The coordiration environment of iron in transferrin involve two tyrosine and two or three histidine residues as ligands. The coordination is completed by an aqua or hydroxo group and the obligate anion. The anion binds weakly to the apoprotein as a prerequisite for the binding of iron. It is reasonable that the anion binds to positively charged group, which otherwise would hinder the binding of iron. The anion-binding group has been proposed to be a protonated imidazole of a histidine residue or a guanidinium group of an arginine residue. The former suggestion is supported by the fact that the conserved residues in both domains include three histidines. Two of these are ligands to iron, while the third could be the anion-binding site.

The release of iron into the cell could follow several pathways. The iron could be released from the transferrin at the receptor site and be carried into the cell. Alternatively, the whole transferrin receptor complex could be taken into an acidic compartment, where the iron is released, passed out of the compartment and stored in ferritin.

The final step in the biosynthesis of heme takes place within the inner membrane of the mitochondria where the ferrochelatase catalyses the insertion of Fe(II) into protoporphyrin. Thus, the major part of the iron entering the cell is used in the mitochondria.

Siderophores

The extreme insolubility of iron in aerobic environment at physiological pH values presents a remarkable difficulty for the microorganisms. However, microorganisms accomplish iron gain by synthesizing special ligands, the siderophores, which are low molecular weight compounds with remarkable affinity for Fe(III).These ligands are synthesized in the cell in response to low levels of iron and are the secreted. They bind and solubilise insoluble Fe(III) to give siderophore iron complex, which are taken back into the cell via specific receptor sites on the cell surface.

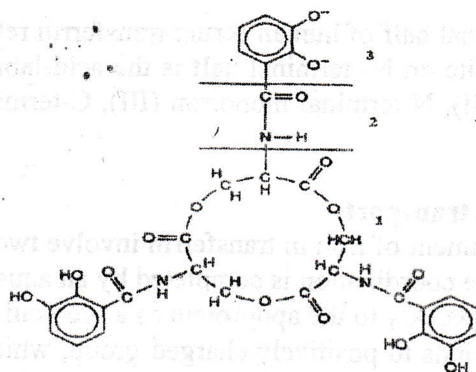
Types of sideropnores

The siderophores are of two main chemical types:

1) Catecholate – phenolate donar groups
2) Hydroxamate donar groups.

The catecholate siderophores

The best known example of this type is enterobactin(also called enterochelin), which is produced apparently by all enteric bacteria. It has three 2,3-dihydroxybenzoyl (3) groups attached to a macro cyclic lactone(2) derived from three residues of L-serine condensed head to tail. Six phenolate(1) oxygen atoms in an octahedral environment bind the iron. Enterobactin has the highest known affinity for Fe(III). The iron (III) complex can exist in isomeric forms, which may be associated with selectivity in binding to the receptor site.



Enterobactin

Linear catecholate siderophores are also known. They contain oxazoline ring, which is subject to acid hydrolysis. The opening derivatives are agrobactin A and parabactin A. In these siderophores there is the possibility of coordination of the ring N atom, although agrobactin can provide six phenolate groups. While the ligand exists in three separate conformers, only cis chelate is formed in the complex.

Hydroxamate siderosphores

Ferrichrome was the first ligand of this type to be isolated. Ferrichrome is a cyclic peptide with three hydroxamic acid side chains. It gives a neutral complex with Fe(III). A number of variations having substituents on the hexpeptide or on the acyl group are also known. The ferrichromes are synthesised by fungi, but many bacteria also use them as a source of iron, even though they do not synthesise the siderophore themselves.

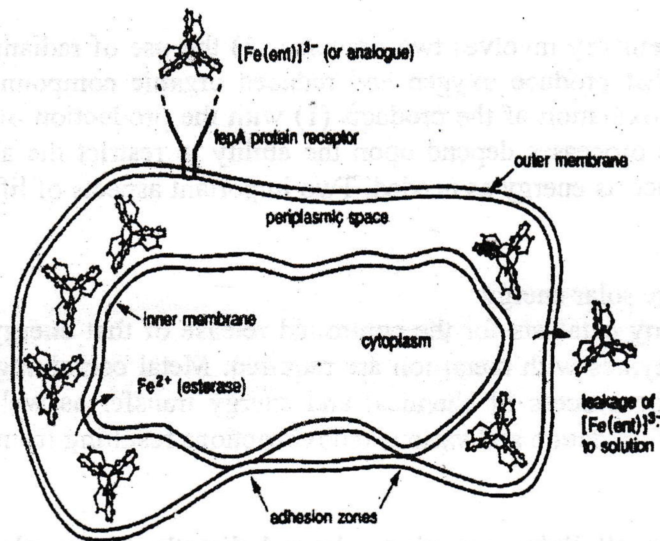
The fundamental structural unit of the ferrichromes is N^{δ}–hydroxyornithine. The diversity of structure and biological activity of these siderophores results from the various substitutions present in the peptide and acyl groups. These ferrichromes all show similar conformations of the peptide backbone and similar stereochemistry.

Binding of Iron (III) siderophore complexes to receptors and the release of iron in to the cytoplasm

The Fe(III) siderophore complex is too big in size to permeate the water-filled channels in the outer membranes of Gram-negative bacteria. It is therefore, a transport mechanism is necessary, involving proteins and expenditure of energy. The first stage in transport involves the recognition of the iron(III) sideropore by receptor proteins in the cell membranes. These proteins are synthesized at low levels of iron. Specific receptor proteins are detected in E.coli for the binding of iron(III) complexes of enterobactin, citrate, ferrichrome and aerobactin. The receptor for Fe(III)-enterobactin retains its affinity for the complex and for colicin B in invitro after extraction. The gene for synthesis of the receptor is ferrioxamine A.

The iron gets transferred from the siderophore complex at the outer membrane to another membrane-bound protein. Example in the uptake of iron by rhodoturulic acid in Rhodoturula. The other mechanism is that in which intact Fe(III)-siderophore complex is taken up into the cell.

The mechanism of release of the iron, in view of its high affinity for the siderophore involves the reduction of the Fe(III)-siderphore complex. Iron (II) has much lower affinity for the siderophore, and so can be released. Example reduction of Fe(III)- hydroxamate siderophores can occur readily under physiological conditions.



The release of iron from catechol siderophores involves reduction of Fe(III)-enterobactin, which undergoes hydrolysis before reduction.

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UNIT-IV BIOINORGANIC CHEMISTRY

ELECTRON TRANSFER IN BIOLOGICAL SYSTEMS-PORPHYRINS

Introduction

On our earth life of chemistry involves two processes 1) the use of radiant solar energy to drive chemical reactions that produce oxygen and reduced organic compounds, from carbon dioxide and water (2) The oxidation of the products (1) with the production of carbon dioxide, water and energy. The life processes depend upon the ability to restrict the above process by controlled kinetics to produce as energy as needed. Two important aspects of life will be interest to us.

1. The ability to capture solar energy
2. The ability to employ catalysts for the controlled release of that energy. For the above processes many enzymes with metal ion are required. Metal containing compounds are also important in the process of chemical and energy transfer as well as to transport oxygen to the site of oxidation and various redox reactions resulting from its use.

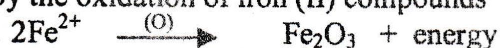
Energy Sources for Life

Even though almost all living organisms depend directly (green plant) or indirectly (Saprophytes and animals) upon photosynthesis to capture the energy of the sun, there are a few reactions, relatively, unimportant but interesting in terms of chemistry, utilizing inorganic sources of energy. These are known as non-photosynthetic process.

Non-photosynthetic process

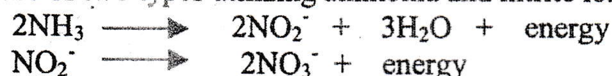
Even these may be indirectly dependent upon photosynthesis, since it is believed that all free oxygen on earth has been formed by photosynthesis.

Eg. Chemolithotrophic bacteria obtain energy from various sources. For e.g., iron bacteria produces energy by the oxidation of iron (II) compounds



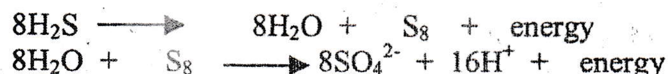
$$2Fe^{2+} \xrightarrow{(O)} Fe_2O_3 + \text{energy}$$

Nitrifying bacteria are of two types utilizing ammonia and nitrite ion as nutrients.



$$2NH_3 \longrightarrow 2NO_2^- + 3H_2O + \text{energy}$$
$$NO_2^- \longrightarrow 2NO_3^- + \text{energy}$$

There are species or sulfur bacteria that obtain energy form the oxidation of various states of sulfur.

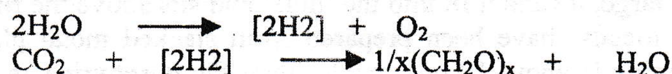


$$8H_2S \longrightarrow 8H_2O + S_8 + \text{energy}$$
$$8H_2O + S_8 \longrightarrow 8SO_4^{2-} + 16H^+ + \text{energy}$$

The later reactions are the source of energy for a fauna on the floor of the ocean.

Photosynthetic reaction

The photosynthetic processes in green plants consist of splitting the elements of water, followed by reduction of carbon dioxide.

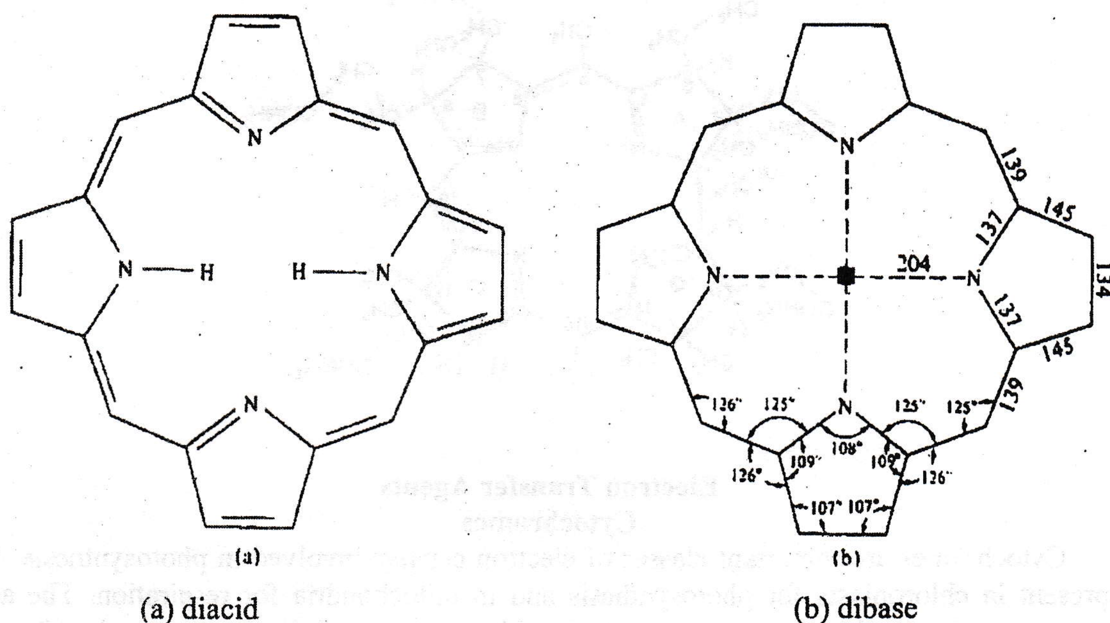


$$2H_2O \longrightarrow [2H2] + O_2$$
$$CO_2 + [2H2] \longrightarrow 1/x(CH_2O)_x + H_2O$$

Where $[2H_2]$ does not imply free hydrogen, but the reducing capacity formed by the oxidation-reduction of water. In green plants there are two photosynthetic systems. The two differ in the type of chlorophyll present and in the accessory chemicals for processing the trapped energy of the photon. Chlorophyll is the first example of bioinorganic compounds containing metals, the metalloporphyrins.

Porphyrin ring system

It is a macrocyclic tetrapyrole system with conjugated double bond. Depending upon the nature and variety of the substitution in the pyrole ring (Electron donating or with drawing), the ring can tune the delocalised molecular orbitals of the complex and varies the properites of the complex.



(a) diacid (b) dibase

The porphyrins can accept two hydrogen ions to form a +2 diacid or donate two protons and becomes –2 dianion. The dianion forms metallo porphyrin complexes.

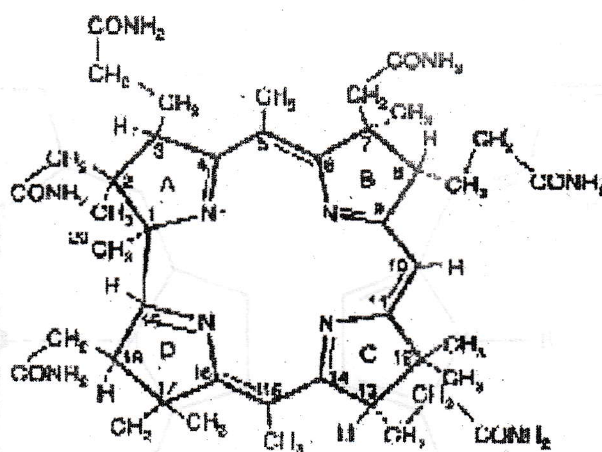
From covalent radial it is estimated that a bond between a nitrogen atom and an atom of the first transition series should be about 200 pm long. The size of the 'hole' in the centre of the porphyrin ring is ideal for accomadating metals of the first transtion metals. The porphyrin system is fairly rigid and the metal- nitrogen bond distance does not vary greatly from 193-196

pm in nickel porphyrins to 210 pm in high spin iron (II) porphyrins. The rigidity of the ring derives from the delocalisation of the π electrons in the pyrole rings. If the metal is small the ring becomes ruffled to allow closer approach of the nitrogen atoms to the metal. At the other extreme, if the metal atom is large, it cannot fit into the 'hole' and sits above the ring.

Recently 'molecular metals' have been prepared from stacked metal phthalocyanines. The hole in the phtahalocyanine is about 10 pm smaller than the porphyrins as a result of the smaller size of nitrogen atom compared with carbon. The order of stability of complexes of porphyrins with +2 metal ions is that excepted on the basis of the Irving- Williams series except that the square planar lignad favours the d^8 configuration of Ni^{+2}. The order is $Ni^{+2} > Cu^{+2} > Co^{+2} > Fe^{+2} > Zn^{+2}$. The kinetics of formation of these metalloporphyrins have also been measured and found to be in the order $Cu^{2+} > Co^{2+} > Fe^{+2} > Ni^{+2}$.

Corrin Ring System

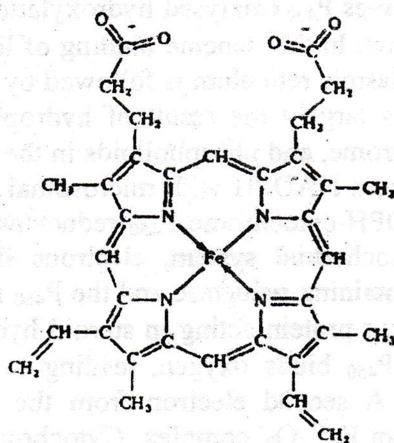
The corrin ring is a modified prophyrin ring in which one of the =CH- bridges between two of the pyrrole- type rings is missing contracting the ring. The simplest known corrinoid natural product is cobyric acid, which can be represented as follows.



Electron Transfer Agents

Cytochromes

Cytochromes are important classes of electron carriers involved in photosynthesis. They are present in chloroplasts for photosynthesis and in mitochondria for respiration. The active centre in cytochrome is a heme group. The oxidation state of the Iron atom is +2 or +3. cytochrome 'C' has a polypeptide chain contains a variable number of aminoacids ranging from 103 in some fish, and 104 other fish, and 112 in green plants and vertebrates. A nitrogen atom from a histidine segment and a sulphur atom from a methionine segment of this chain are



The structure of the heme group.

coordinated to the fifth and sixth coordination sites. Therefore there is no position for further coordination like the iron in haemoglobin or myoglobin. Cytochrome 'C' therefore cannot react by simple coordination but must react indirectly by an electron- transfer mechanism. It can reduce the oxygen and transmit its oxidising power towards the burning of food and release of energy in respiration. There is quite a variety of cytochrome 'C' depending upon the ligands present, the redox potential of a given cytochrome can be tailored to meet the specific need in the electron transfer scheme (Photosynthesis or respiration). The potentials are such that the electron flow is b $\rightarrow$ c $\rightarrow$ a $\rightarrow$ O_2. Some of the 'a' type is thought to be capable of binding oxygen. They are thus normally 5 co-ordinates in contrast to cytochrome 'C'.

Cytochrome P_{450}

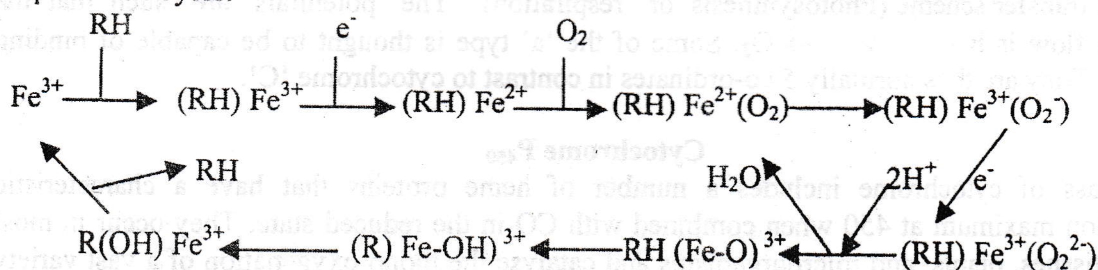
This class of cytochrome includes a number of heme proteins that have a characteristic absorption maximum at 450 when combined with CO in the reduced state. They occur in most animal tissues, plants, and microorganisms and catalyse the mono oxygenation of a vast variety of hydrophobic substances. Cytochrome P_{450} serves as the oxygenating catalyst in the presence one or more electron-transfer proteins or redox enzymes in reactions of the following form, where RH is the substrate.

$$RH + O_2 + NAD(P)H + H^+ \longrightarrow ROH + H_2O + NAD(P)^+$$

The total number of forms that occur is unclear, but liver, for example, contains at least three forms inducible by photochemical that differ in size (MW = 48,000) from those induced by methylchloroanthrene (53,000).

Irrespective of their structural and enzymic differences, the cytochoromes P_{450} appear to have similar active sites. Each contains an iron-protophorphyrin (heme) group in a rather hydrophobic cleft, which is open to the substrate. The heme is bound to the apoprotein by a combination of noncovalent interactions as well as by a coordinate bond to the iron atom. The iron has four bonds to the pyrrole N atoms and a fifth to a thiolate group of cysteine in the ferrous polypeptide chain. The sixth coordination position is open in the ferrous form, but in the substrate free, ferric state is occupied by water. Upon reduction, the resulting Fe^{2+} atoms are the binding sites for dioxygen.

The proposed mechanism for cytochoromes P_{450} catalysed hydroxylation reactions of the type given above (RH → ROH) is shown below. In this scheme binding of lipophilic substrate (step 1) to the oxidized Fe^{3+} -P_{450} on the endoplasmic reticulum is followed by reduction (step 2) to the reduced Fe^{3+} -P_{450}. Substrate binding is largely the result of hydrophobic interactions between substrate, the membrane bound cytochrome, and phospholipids in the membrane. The 1 electron reduction (step 2) ultimately comes from NADPH with microsomal and mitochondria P_{450}. In liver the electron is provided by NADPH-cytochrome P_{450} reductdase, a flavo protein containing both FMN and FAD. In the mitochodrial system, electrons from NADPH are transferred to Fe-sulphur redoxin by a FAD containing reductase and the P_{450} is then reduced by the redoxin; e.g., adrenodoxin is the iron-sulphur protein acting in steroid hydroxylation by the adrenal gland. After reduction, the heme of P_{450} binds oxygen, leading to formation of the treatment of the transient Fe^{3+}-O_2^- complex. A second electron from the reductase is then accepted by the latter complex, giving rise to an Fe^{3+}-O_2^- complex. Cytochrome b5 also supply the second electron in some cases. In the absence of substrate, this complex dissociates to give H_2O_2 accounting for the oxidase activity of the system. Even in the presence of substrate as much as half of the O_2 consumed by the system converted may be converted to peroxide. Splitting of the oxygen-oxygen bond occurs in step 5 with the release of one atom of oxygen in water; the oxygen atom is activated oxygen, which accepts a H atom from RH (step 6), giving rise to ROH in step 7. Hydroxylation involves abstraction of a hydrogen from RH and binding of the carbon radical, R^-, to the $Fe(OH)^{3+}$ intermediate. Dissociation of ROH from the active site completes the cycle.



Other functions of Cytochrome P_{450}

Cytochrome P_{450} system not only catalyses hydroxylation of aliphatic and aromatic carbons, but also is involved in N-oxidation, sulpoxidation, epoxidation, N-,S- and O-dealkylation, peroxidation, deamination, desulphuration and dehalogenation reactions. Substrates may include normal metabolites; such as fatty acids and prostoglands; a vast array of naturally occurring compounds present in foodstuffs; as well as xenobiotics, foreign synthetic compounds that are drugs, insecticides, anesthetics, or petroleum products including many carcinogens. In addition, synthesis of many steroids are also requires action of Cytochrome P_{450}.

Cytochrome 'a' Toxicity

In cytochrome 'a' the sixth coordination is unusually occupied by CN^- during cyanide poisoning. The cyanide binds tightly to the sixth position and stabilizes the Fe(III) to such an extent that it can no longer be readily reduced and takes part in the electron shuttle. The inhibition of cytochrome 'a' by the cyanide ion is much more serious than the interference with

oxygen transport. In fact the standard treatment for cyanide poisoning is inhalation of amyl nitrite or injection of sodium nitrite to oxidise some of the hemoglobin to methmoglobin. The methnoglobn although useless for oxygen transport, binds cyanide even more tightly than hemoglobin or cytochrome 'a' and removes it from the system.

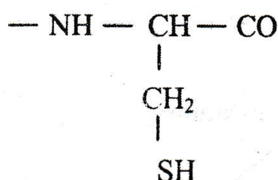
Iron-sulphur proteins

There are several non-heme iron sulfur proteins that are involved in electron transfer. They are ferredoxin, Rubredoxin, chromatium HIPIP, hemerythrin, haemosidesin and transferrin. They are found in all plants including algae, in all photosynthetic bacteria, protozoa, in some fermentative anaerobic bacteria. The Iron sulphur proteins contain cluster of iron-sulphur clusters, composed of iron atoms, sulfhydryl groups from cysteine residues and labile or inorganic sulphur. The inorganic sulphur can be readily removed by washing with acid.

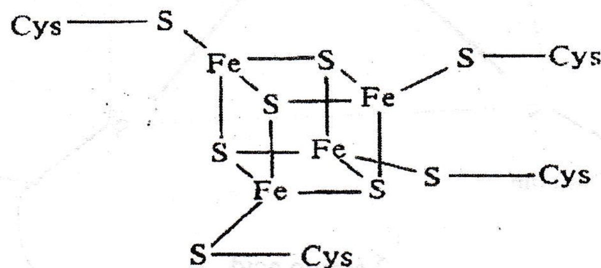
$$(RS)_4Fe_4S_4 + 8H^+ \longrightarrow [(RS)_4Fe_4]^{4-} + 4H_2S$$

Ferredoxins

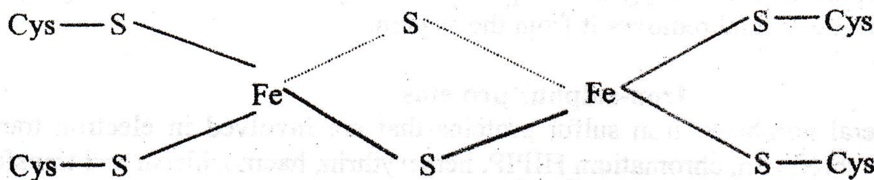
They are relatively small proteins (with molecular weight ranging from 6000-12000) contain non-heme iron, cysteine sulphur and inorganic or liable sulfur. They are found in all plants including algae, in all photosynthetic bacteria, protozoa, in some fermentative anaerobic bacteria. There are several ferredoxins with similar redox properties but the iron content varies from two to eight in a molecule. From the X-ray crystallography study the structure of 8-iron atoms ferredoxin from M-aerogenes is found to be



The molecular wt of the above molecule is 6000, contains 8 Iron atoms, 8 labile sulphur atoms and eight cysteine residues. The Iron atom and sulphur atom from two separate clusters, with a cysteine sulphur atom form separate clusters, with a cysteine sulphur atom coordinated to each iron atom along a 3 fold axis of the tetrahedron of iron atoms. The center of the clusters is about 12 Å apart.



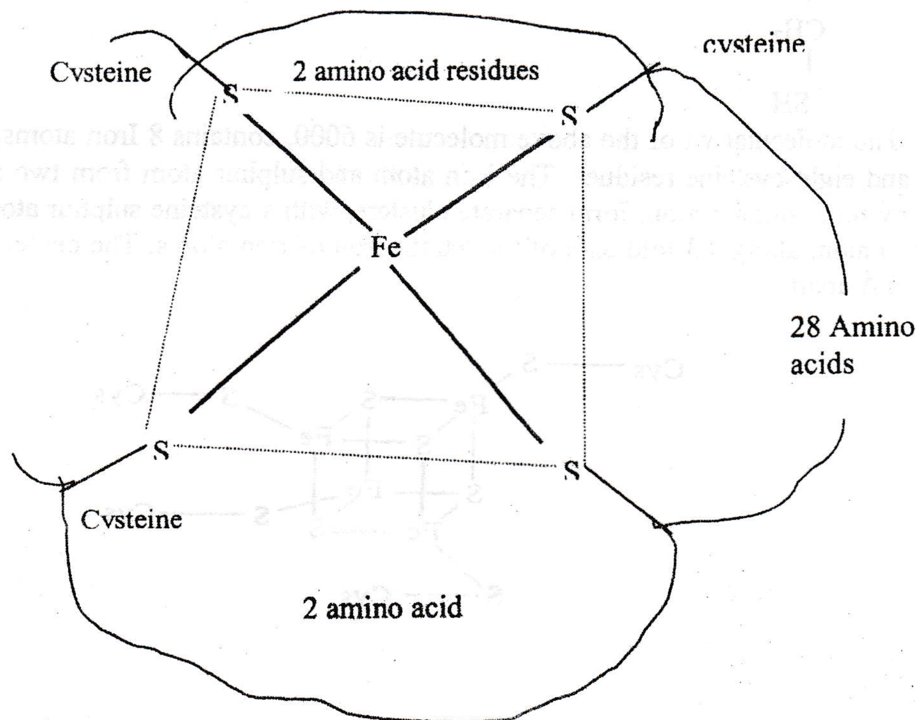
The clusters in the ferredoxin molecule associated with photosynthesis in higher plants are thought to have the bridged structure Fe_2S_2 as shown below.



These ferredoxin molecules play an essential role as electron transfer agents at the low potential end of the photosynthetic processes but an exact chemical specification of their activity is still lacking. They are also involving in anaerobic metabolism (e.g., bacterial ferredoxin).

Rubredoxin

This sort of substance was first isolated from C-pasteurianum. It appears to participate in a number of biological reaction in which ferredoxin is also active. It contains only one iron atom, four-cysteine sulphur and no inorganic sulphur. Its molecular weight is 6000. Four cysteine sulphur atoms tetrahedrally coordinate the iron atom. The tetrahedron is a distorted one. The Fe-S distance is 2.39,2.93, 2.31 and 1.97 Å. The angles range between 101° and 108°, The structure of the tetra hedran is given below.



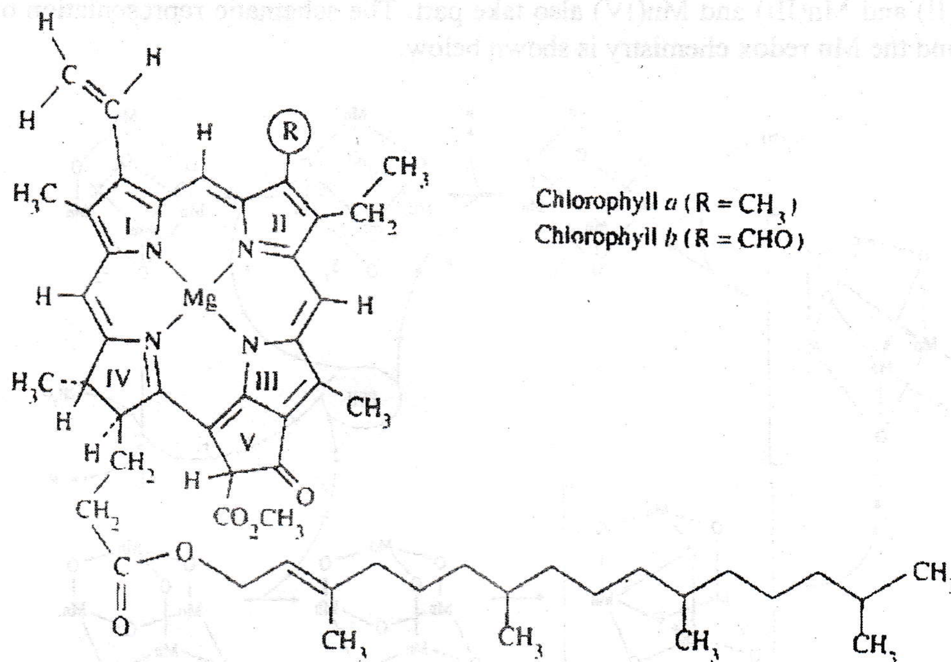
Chlorophyll

The chlorophyll ring system is a porphyrin ring system has been reduced. A fused cyclopentanone ring is also present. The Magnesium metal atom is at the centre, coordianted to four nitrogen atoms. It absorbs low-energy light in the far infrared region (~ 700 nm). The exact frequency depends on the nature of the substituents on the chlorophyll. In addition other pigments such as carotenoids are present which absorb higher energy light. This serves two purposes.

1) The energy may be passed along to the chlorophyll system and used in photosynthesis.

2) It protects the biological systems from photochemical damage

A photon of light hitting a molecule of chlorophyll in either of the photosynthetic system mentioned above provides the energy for a series of redox reaction.

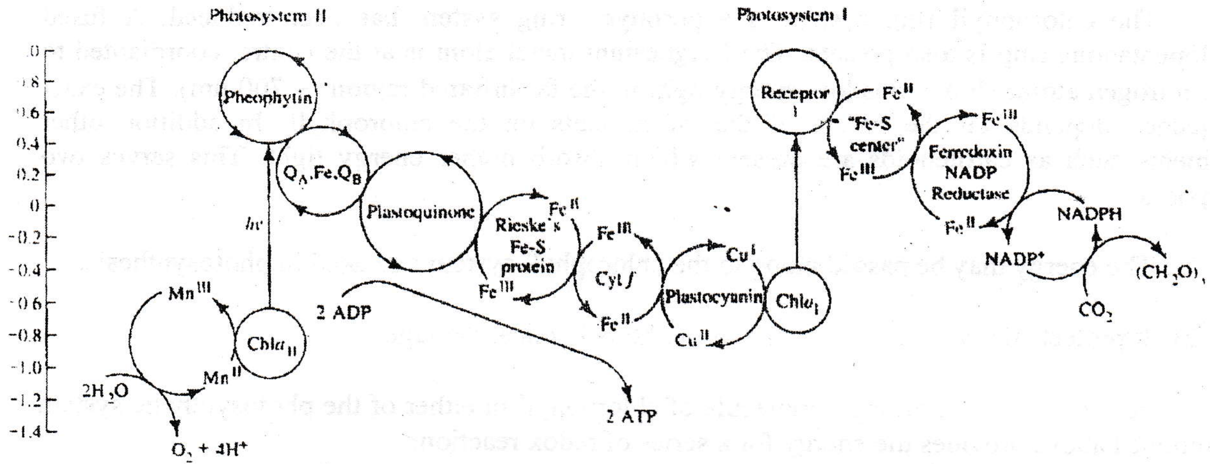


Photochemical reactions

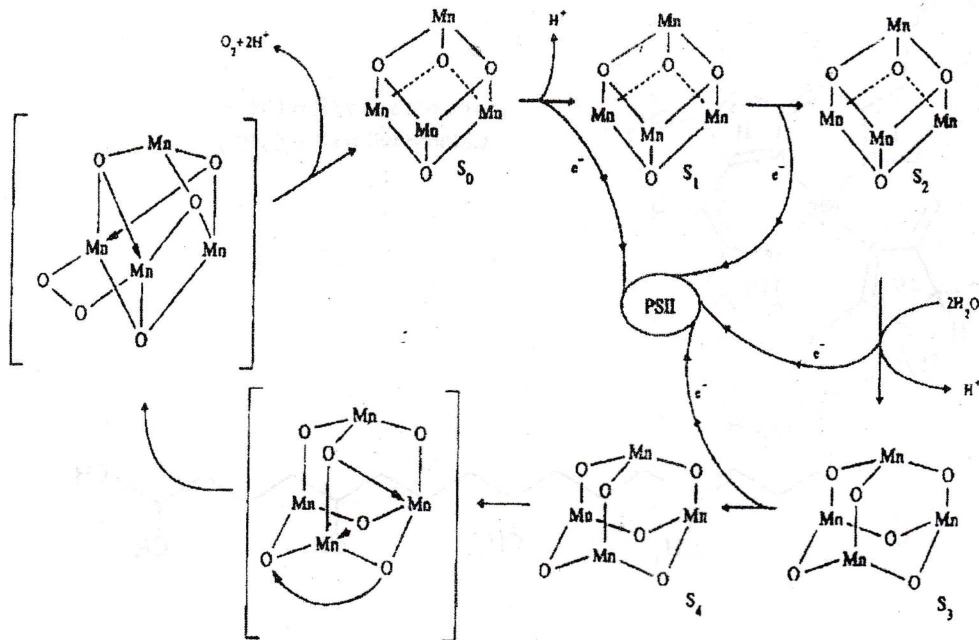
System I produces a moderately strong reducing species (REDI) and a moderately strong oxidizing species (OX_1)

System II produces a moderately strong oxidizing agent (OXII) but weaker reducing agent (REDII).

(OXII) is responsible for the production of molecular oxygen. A manganese complex probably containing two atoms of manganese per molecule reduces (OXII) which is recycled for use by another excited chlorophyll. In the redox reactions the manganese cycles



between Mn(II) and Mn(III) and Mn(IV) also take part. The schematic representation of system I, System II and the Mn redox chemistry is shown below.



RED I transfer its electron through carriers such as ferredoxin (Fe^{2+}/ Fe^{3+}), eventually to form NADPH. The latter serves as a stable source of reducing capacity to convert CO_2 to carbohydrate.

The acceptor in photosystem (II) is pheophytin. The initial product of photon excitation, the chla$^+$ PheO$^-$ radical ion pair, has been observed, although its lifetime is only 10 nonoseconds. Actually this has been described as a dramatically long time and is the single most unusual aspect of PS I or PS II reaction centres. OX I and RED II react with each other to complete the regeneration of the original species prior to irradiation. The electron flows through

plastoquinone, a series of cytochromes iron-sulphur compounds, and plastocyanin (Cu^{+} / Cu^{2+}). Tow moles of energy rich ATP are synthesised from the energy released in the process.

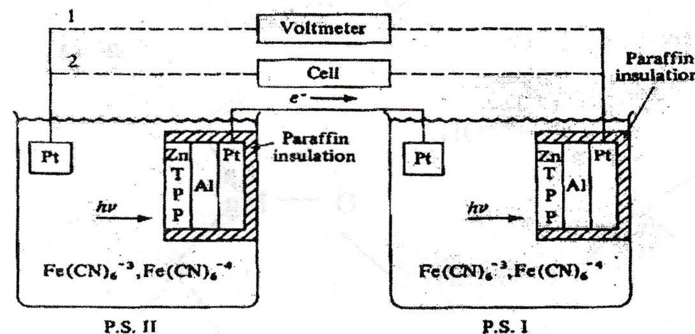
From the above study on the photo behaviour of chlorophyll invitro it is known that chlorophyll is the main pigment in photosynthesis. The important features of chlorophyll in photosynthesis can be summarized as follows.

1) The extensive conjugation of the porphyrin ring lowers the energy of the electronic transition and shifts the absorption maximum into the region of visible light. Conjugation also helps make the ring rigid and less energy is wasted in internal thermal degradation.

2) A second important factor is the phosphorescent behaviour of chlorophyll. In order for phosphorescence to occur there must be an excited state with a finite lifetime. The free porphyrin shows only fluorescent emission. Spin orbit coupling by the metal ion allows mixing of the excited singlet and triplet states and promotes the formation of the relatively stable triplet state which is the source of the phosphorescence (and of the energy for photosynthesis).

Synthetic leaf

Here the Photon- capturing pigment is zinc tetra phenyl porphyrin deposited on a clean aluminium surface. The electron carriers in solution are potassium ferricyanide and pottassium ferrocyanide. The Zinc TPP was activated by orange light, and the captured energy was used to reduce NADP and oxidise water to oxygen gas.



If the circuit shown is closed to the voltammeter a potential of 2.2- 2.6 volt can be obtained. If the circuit is closed to an electrolytic cell containing NADP and NADP- reductase the NADP reduced and oxygen gas is evolved. In practice a micro cell is used.

Studies of a 'synthetic leaf' similar to that shown in the above figure but utilizing natural chlorophyll provided further insight into the photosynthetic process. A chlorophyll water adduct is either impregnated in a polymer membrane or deposited on one side of a metal foil. The

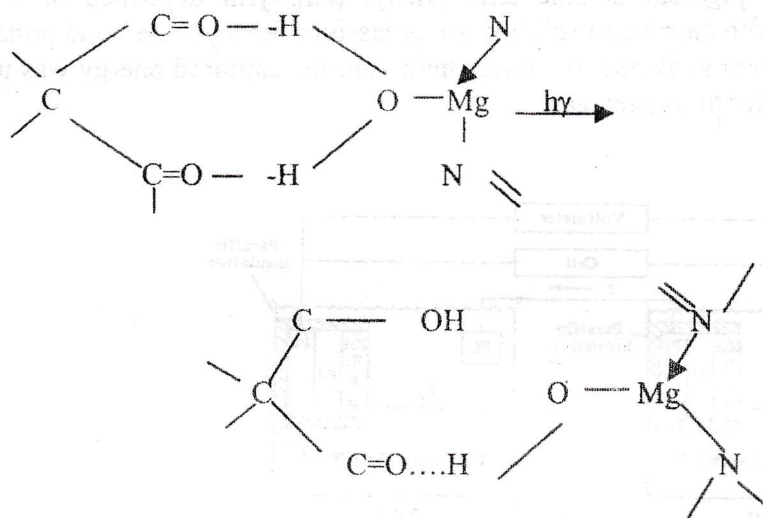
membrane or foil is then placed in the cell with an electron accepting solution on one side and an electron donating solution on the other side. Irradiation with light produces a potential. It is postulated that invivo photosynthesis involves hundreds of 'antena' molecules that capture photons and pass the energy along to the "reactive centre". There is no universal agreement on the structures of the antenna molecules or the reactive centre.

Role of Magnesium metal

In chlorophyll one water molecule is coordinated to the magnesium atom and forms a hydrogen bond with the ring (5) keto group of an adjacent molecule, which in turn has a magnesium coordinated water molecule hydrogen bonded to a third molecule and so on. This forms a one-dimensional array of partially overlapping chlorophyll molecule that has been suggested as a model for the aggregation in the antenna system.

It also appears that 5 coordination is preferred over co-ordination as in the structure of the Phenylporphyrin hydrate, where the Mg atom is out of the plane of the N. atom, and is approximately sausage pyramids. Although Mg and other metalloporphyins can undergo oxidation by one-electron changes, for Mg it is the macro cycle and not the metal that is involved.

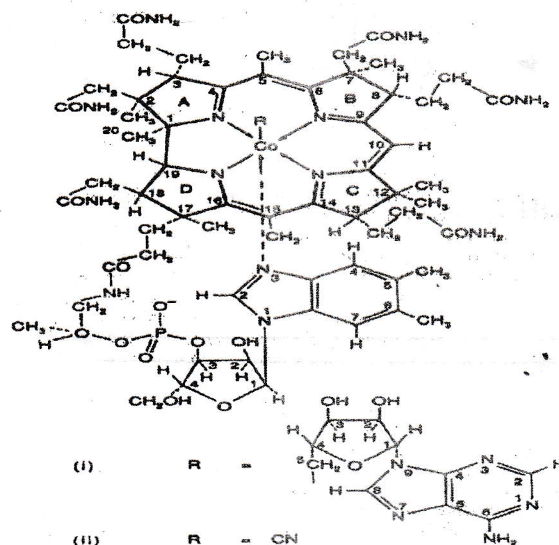
The role of the cholorophyll in the photosynthetic reduction of CO_2 by water in plants to provide a source of electrons that may continue to be supplied for a time in the dark. Electron



spin resonance studies of light irradiated chlorophyll show that radicals are frothed. They are probably of the above type.The electrons are transmitted through chlorophyll micells to other intermediates involved in the reduction of CO_2.

Vitamin B12

This molecule is built around a corrin ring containing a cobalt (III) atom. The corrin ring is a modified porphyrin ring in which one of the =CH- bridge between two of the pyrole- type rings is missing, contracting the ring. The fifth and sixth coordination sites of the cobalt are filled by a nitrogen atom from an imidazole ring and a cyanide ion.



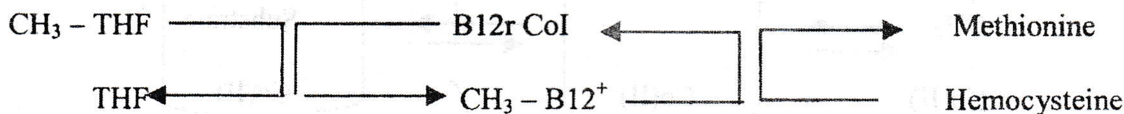
In the isolated Vitamin, R is a cyanide ion. However, in invivo, the cyanide ion is not present, and R is probably a loosely bound water molecule.

Vitamin B12 may be reduced by one electron (VitaminB12 r) or two electrons (Vitamin B 12 S) to form the Co^{II} and Co^{I} complexes, respectively. The two-electron reduction may be accomplished by NADH and Flavin Adenine Dinucleotide (FAD).

The cobalt I complex is strongly nucleophilic and may be important in the biological functioning of B12. It readily undergoes alkylation via oxidative addition.

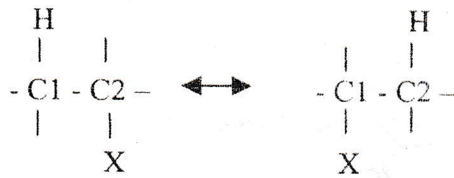
$$[B12(Co^{I})] + CH_3I \longrightarrow [B12(Co^{III}) - CH_3]^+ + I^-$$

Two important recognized functions of vitamin B12, involve the reduced Co^{I} form. The first is reduction of organic species. The second involves the acceptance of a methyl group from N-Methyltetrahydrofolate (CH_3–THF). The cobalt (III) methyl corrinoid can then partake in biomethylation reactions.

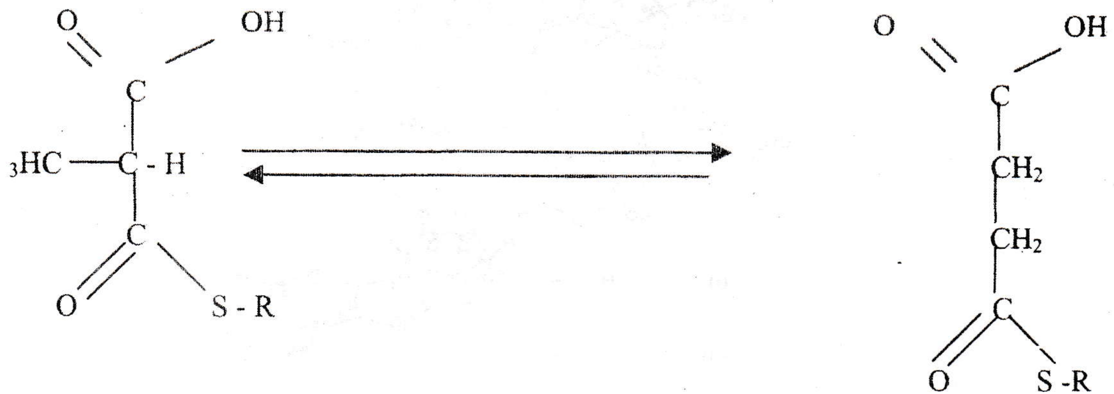


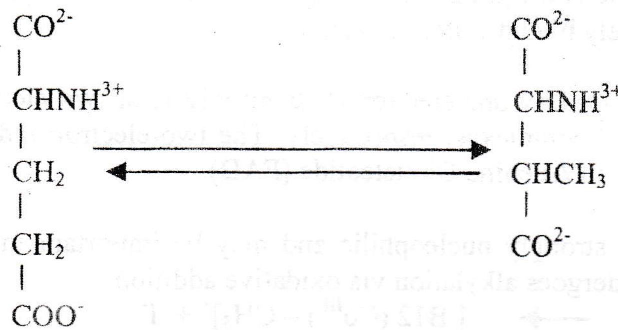
Vitamin B12 coenzyme

When vitamin B12S reacts with adenosine triphosophate (ATP), alkaylation takes place with the formation of a direct carbon cobalt bond between adenosine and cobalt. This species is called B12 coenzyme and is involved in acting in contact with several other enzymes to effect 1,2 shifts of the general type.

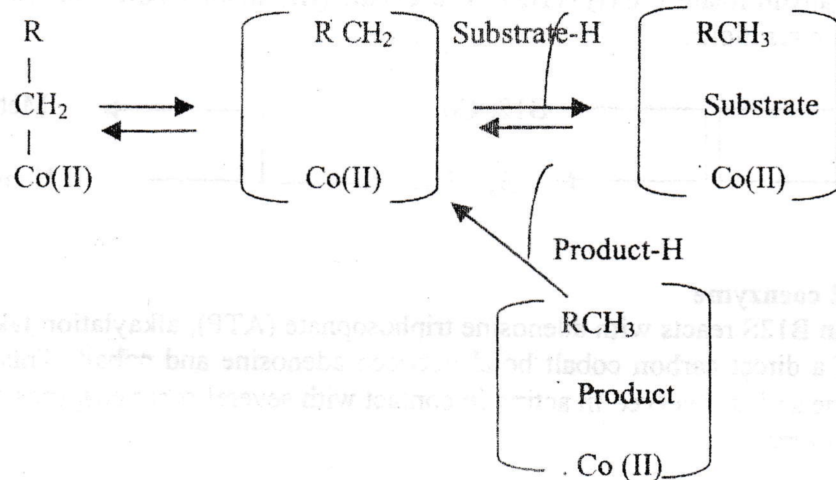


E.g.,



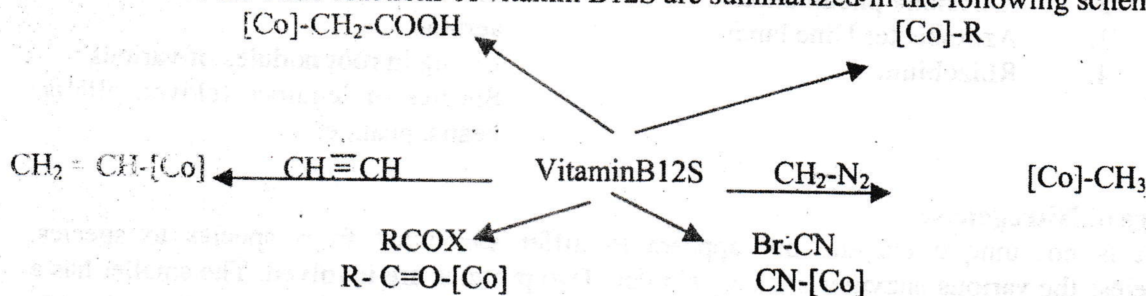


One possible mechanism of the hydrogen transfer in such reaction is shown in the following scheme.



Here RCH_2 represents the adenosyl group of the coenzyme.

Some of the other reactions of vitamin B12S are summarized in the following scheme.



Fitness of Vitamin B12

The 'Fitness' of the B12 system is performing the above duties has been attributed to the fact.

1. The geometric stage of the CoIII can be such that one of the six ligands is held weakly or is absent.
2. The electronic state of the cobalt is such that oxidative addition and reductive elimination reactions take place without large activation energies.
3. The corrin ring is flexible and can take up of number of conformations to fit the environment.

Nitrogen fixation

Nitrogen is the most important key nutrient element for plant growth and its productivity. The atmosphere is a vast reservoir of nitrogen. The atmosphere contains 78% nitrogen (by weight) which amounts to about 4×10^{12} tons. But unfortunately this triple bonded molecular and gaseous nitrogen is so stable (N = N) inert and unreactive that it cannot be utilised by plants. Therefore it requires to be transformed into plant available form i.e., (NH_4^+ or NO_3^-) by the several processes of nitrogen fixation. E.g., Biological nitrogen's fixation, Industrial nitrogen fixation, lightning, ionization and combustion etc.,

Nitrogen fixation on earth

Source	Quantum of 'N' fixed million-tons/ year
Biological 'N' Fixation	170
Lightning	10
Combustion	10
Ozonization	10
Industrial Nitrogen fixation INF	50

Biological Nitrogen Fixation [BNF]

Invivo Nitrogen Fixation

The enzymes turn nitrogen (N_2) from the air into ammonia (NH_3). The compound which living cells need in order to make their amino acid and essential organic bases. Both free-living species and symbiotic species are involved. The bacteria involved in the BNF are

1.	Clostridium pasteurianum	-	an aerobic
2.	Klebsiella pneumoniae	-	aerobic
3.	Azotobacter Uine landi	-	aerobic
4.	Rhizobium	-	Living in root nodules of various Species of legumes (clover, alfalfa, beans, peak, etc.

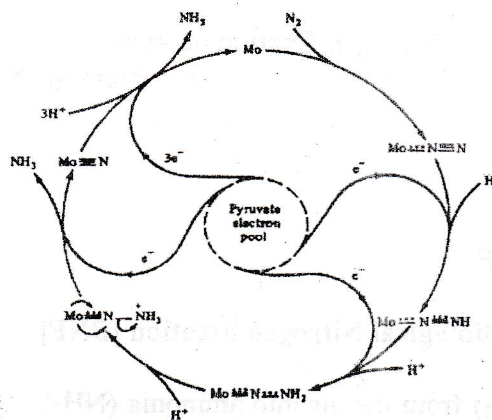
Structure of Nitrogenase

It is not unique enzyme but appears to differ somewhat from species to species. Nevertheless the various enzymes are very similar. Two proteins are involved. The smaller has a molecular weight of 57,000- 73,000. It contains a Fe_4S_4 cluster. The larger protein is an $\alpha_2\beta_2$ tetramer with a molecular weight of 2,200,000- 2,40,000 containing two molybdenum atoms about 30, iron atoms and about 30 labile sulfide ions. The iron sulfur clusters probably at as redox centres. A suggested arrangement of these components that accounts for observed properties is shown below

Neither protein by itself shows any nitrogen-fixing ability, but recombination of them gives immediate activity, indicating that no slow winding of protein chains of the two components is necessary.

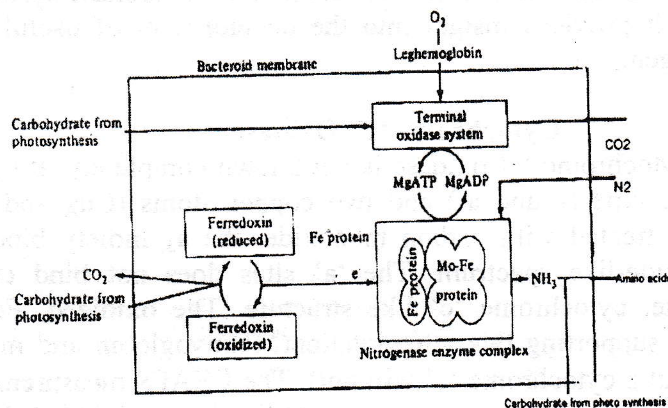
Structural studies show that the coordination sphere of the molybdenum consists of several sulfur atoms at distance about 235 pm.

The ultimate source of reductive capacity in pyruvate and the electros are transferred via ferredoxin to nitrogenase. Two Mo (III) atoms cycling through Mo(VI) would provide the six electrons necessary for reduction of dinitrogen. Alternatively, since the enzyme is rich in ferredoxin type clusters, there should be a ready flow of electrons and the molybdenum may stay in the one or two oxidation states that most readily bind dinitrogen and its intermediate reductants. The overall catalytic cycle is shown below.



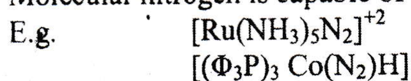
A schematic diagram for the production of fixed nitrogen compounds, including the sources of material and energy, and the overall reactions are given below. It is felt that

leghemoglobin a monomeric oxygen-binding molecule that is present in nitrogenase tightly protects the nitrogenase, which cannot operate in the presence of oxygen. On the other hand it allows a reservoir of oxygen for respiration to supply energy to keep the fixation process going.



Invitro nitrogen fixation

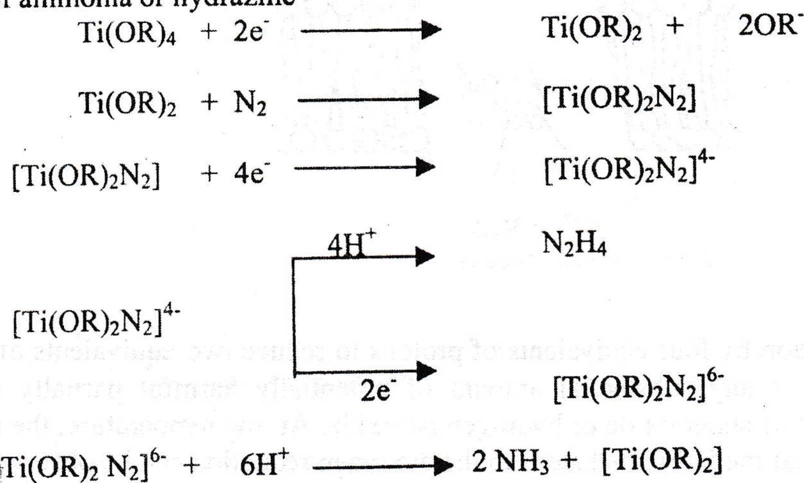
Molecular nitrogen is capable of forming stable complexes with transition metals.



E.g. $[Ru(NH_3)_5N_2]^{+2}$

$[(\Phi_3P)_3Co(N_2)H]$

Of the various systems investigated, that employing titanium(II) was the first to be successful. Titanium(II) alkoxides form dinitrogen complexes which may then be reduced with subsequent release of ammonia or hydrazine



$$Ti(OR)_4 + 2e^- \longrightarrow Ti(OR)_2 + 2OR^-$$

$$Ti(OR)_2 + N_2 \longrightarrow [Ti(OR)_2N_2]$$

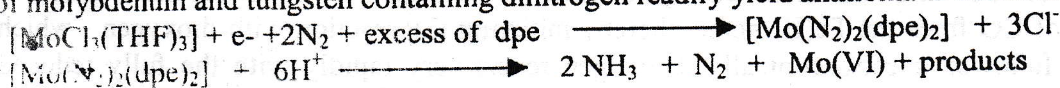
$$[Ti(OR)_2N_2] + 4e^- \longrightarrow [Ti(OR)_2N_2]^{4-}$$

$$[Ti(OR)_2N_2]^{4-} \xrightarrow{4H^+} N_2H_4$$

$$[Ti(OR)_2N_2]^{4-} \xrightarrow{2e^-} [Ti(OR)_2N_2]^{6-}$$

$$[Ti(OR)_2 N_2]^{6-} + 6H^+ \longrightarrow 2 NH_3 + [Ti(OR)_2]$$

Until recently, all methods for converting dinitrogen complex into ammonia required very powerful reducing agents. The dinitrogen in the complex was almost as unreactive as atmospheric N_2. An important development was the discovery that certain phosphine complexes of molybdenum and tungsten containing dinitrogen readily yield ammonia in acidic media.



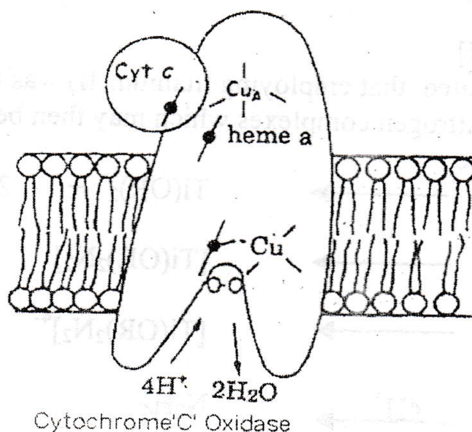
$$[MoCl_3(THF)_3] + e- + 2N_2 + \text{excess of dpe} \longrightarrow [Mo(N_2)_2(dpe)_2] + 3Cl^-$$

$$[Mo(N_2)_2(dpe)_2] + 6H^+ \longrightarrow 2 NH_3 + N_2 + Mo(VI) + \text{products}$$

THF-tetrahydrofuran; dpe-1,2 bis (diphenylphosphino)ethane = $\Phi_2PCH_2\ CH_2P\ \Phi_2$ Φ-phenyl group.Both reactions take place at room temperature and atmospheric pressure. The reaction sequence is important for two reasons. 1) It models the invivo nitrogenase systems that appear to employ molybdenum. 2) It provides insight into the development of useful catalysis for the industrial fixation of nitrogen.

Cytochrome 'C' Oxidase

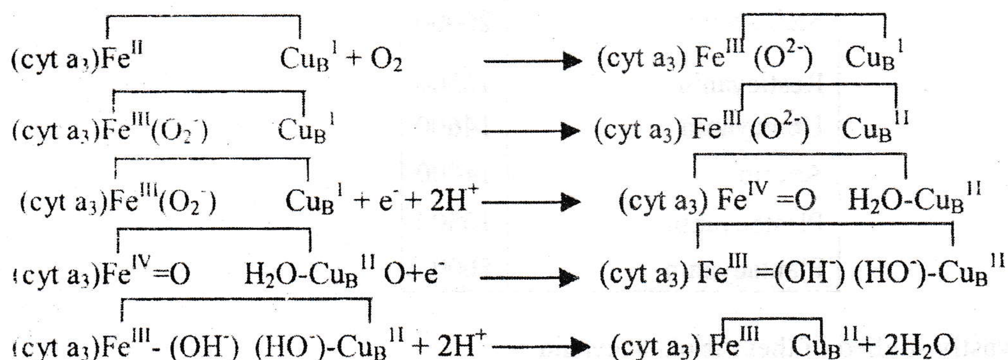
The structure of cytochrome 'c' oxidase is not known completely. It contains two heme groups of the cytochrome type (a and a_3) and two copper atoms (Cu_A and Cu_B). When the reduced Fe(II) oxidase is treated with carbon monoxide, the a_3, moiety binds it and gives a myoglobin-carbon monoxide-like spectrum. The 'a' sites does not bind carbon monoxide, indicating a six-coordinate, cytochrome 'c' like structure. The oxidized, Fe(III) form binds cyanide at a_3, but not 'a', supporting this interpretation(Metmyoglobin and methmoglobin will also bind a cyanide ion, but a cytochrome 'c' will not). The EXAFS measurements indicate that these Fe and Cu atoms are about 370 pm apart, compatible with a sulphide bridge. The electron flow is probably: (1) reduction of the four metal centers by four equivalents of reduced cytochrome 'c', (2) binding of dioxygen to the partially or fully reduced enzyme,(3) transfer of four electrons to dioxygen,



Cytochrome'C' Oxidase

coupled with (4) protonation by four equivalents of protons to reduce two equivalents of water, all without the leakage of any substantial amount of potentially harmful partially reduced dioxygen byproducts such as super oxide or hydrogen peroxide. At low temperature, the reaction can be slowed down, so that the individual steps in the dioxygen reduction can be observed. Such experiments are carried out using the fully reduced enzyme to which CO has been bound. Binding of CO to the Fe(II) heme center in reduced cytochrome 'c' oxidase inhibits the enzyme and makes it to unreactive to dioxygen. The CO-inhibited derivative then can be mixed with dioxygen and then the mixture cooled. Photolysis of metal-CO complexes always almost leads to dissociation of CO and CO-inhibited cytochrome 'c' oxidase is no exception. Photlytic dissociation of CO frees the Fe(II) heme, thereby initiating the reaction with dioxygen , which can then be followed spectroscopically. Dioxygen reacts very rapidly with the fully reduced

enzyme to produce a species that appears to be the dioxygen adduct of cytochrome a_3. Such species is assumed to be similar to other mononuclear oxyheme derivatives. The dioxygen ligand in this species is then rapidly to reduced to peroxide by the nearby CuB, what is believed to be a binuclear μ-peroxo species. These steps represent a two-electron reduction of dioxygen to the peroxide level and are entirely analogous to the model reactions discussed above except that the binuclear intermediates contain one copper and one heme iron. The μ-peroxo Fe(III)-O_2^{2-}-Cu(II) species reduced by a third electron, resulting in the cleavage of the O-O bond. One of the oxygen atoms remains with the iron in the form of a ferryl complex, i.e., an Fe(VI) oxo, and the other is protonated and bound to copper in the form of Cu(II) aquo complex. Reduction by another electron leads to hydroxo complexes of both Fe(II) and the Cu(II) centers. Protonation then causes dissociation of two water molecules from the oxidized cytochrome a_3-Cu_B center.



Several important questions remain to be resolved in cytochrome 'c' oxidase research. One is the nature of the ligand bridge that links cytochrome a_3 Cu_b in the oxidized enzyme. EXAFS measurements of metal- metal separation and the strength of the magnetic coupling between the two metal centres provide evidence that a single atom bridges the two metals.

Blue copper proteins

These low molecular weight proteins have been isolated from a variety of sources. They cycle between Cu(II) and Cu(I). Blue copper centers are found in many different organisms, from bacteria to humans. The only known substrate is the electron (and in some cases, perhaps the proton) and the primary function appears to be electron transfer.

The most distinctive characteristic of a blue copper site-and the basis of term "blue copper"- is a strong absorbance in the region of 600 nm ($\varepsilon = 500\ M^{-1}cm^{-1}$). The intensity of absorbance that is so unusual in the blue proteins, about 400 timees as intense as that of $[Cu(H_2O)_6]^{2+}$.

The tetrahedral coordination geometry for the blue copper site was proposed in order to rationalize the intense visible absorption. Since there is no centre of symmetry in a tetrahedron, the ground state wave function of the copper center could have mixed 3d-4p orbital characters. This would intensify the legand-field or "d-d" transitions because they assume d→ p character. It has now become clear that the intense visible absorption is due to low-lying charge- transfer transitions, vide infra.

The near infrared bands are prominent in the magnetic circular dichroism spectrum, consistent with their assignment as magnetic dipole–allowed, d-d transitions. It is important to note that the d-d bands of cobalt (II)-substituted derivatives of azurin, plastocyanin, and stellacyanin also indicated that the site was distorted tetrahedral. Since a singled-d transition is expected from Cu(II) in pure tetrahedral symmetry, the fact that several d-d bands are observed indicates that the site has distorted symmetry. If the distortion is modeled in terms of single angular deformation, the transition energies can best be explained in terms of a D_2 type flattening along one of the two fold axes of the original tetrahedron. Some of the blue copper proteins with their molecular weight is given in the following table.

Blue Copper proteins	MW
Stellacyanin	20000
Resticyanin	16500
Umecyanin	14600
Azurin	14000
Plastocyanin	10500
Plantacyanin	8000

The most vastly studied of these is plastocyanin.

Plastocyanins

The plastocyanins are found in plant chloroplasts and other photosynthetic organisms. They act as membrane-bound electron carriers between photo systems II and I in the photosynthetic pathway of higher plants, green algae and some blue- green algae.

Plastocyanin consists of a single polypeptide chain of molecular weight around 10500 and one copper atom. It is synthesized in the cytoplasm as a precursor of higher molecular weight, with an additional polypeptide that is essential for transport of the protein into the chloroplast. The protien is cylinder like in overall structure with the metal ions site buried 6 Å in the interior towards one end. The copper site, involves as ligands, the imidazole groups of His-37 and His-87 (2.10 Å) and Met-92 (Cu – S = 2.13 Å). The second N in the imidazole of His-87 is hydrogen bonded to a water molecule. The geometry of the metal site is distorted tetrahedral with bond angles differing by up to 50 ° from tetrahedral geometry.

The structure of the reduced protein involves displacement of the Cu atom by about 0.4 Å in a direction such that the Cu-S(Met) bond length decreases to 2.74 Å, while the imidazole of His-87 is displaced about 0.4 Å, away from the copper. The copper appears to move within the ligand cage.

The blue1copper site thus provides a set of ligand donor atoms. A stereochemistry that is a compromise between the preference of Cu(I) (soft ligands and tetrahedral geometry) and Cu(II) (hard ligands and square planar geometry). The structural restriction placed upon the copper by the protein results in a diminishing of the Frank- Condon reorganization barrier to electron transfer.

The role of the protein in this context supported strongly by comparison with the structure of the apoplastocyanin. The structures of apoplastocyanin and Cu-plastocyanin are very similar, showing that the irregular geometry of the blue copper site is imposed upon the metal by the polypeptide. Only five residues in plastocyanin undergo significant changes when the copper atom is removed. His-87 is one such residue. In this case, the orientation of the imidazole ring is changed by a rotation of 180° about Cβ - Cγ bond. The N atom of His-87 is then hydrogen bonded to a different water molecule form the one involved in the structure of Cu-Plastocyanin. The imidazole ring of His-87 accordingly acts as a ' revolving door' to give access to the copper site. The reversible binding of Cu(II) at the exposed N atom of His-87 will be followed by the rotation of the imidazole ring and the formation of the other three Cu-protein links.

The structure of plastocyanin offers several hydrophobic channles for the quantum mechanical tunnelling of electrons. However, residue His-87 provides a short pathway to the solvent and so an outer-sphere mechanism seems more probable. The protein surface contains two patches of highly conserved residues. One of these consists of two groups of acidic side-chains surrounding the exposed side-chain of a tyrosine, Tyr-83. The second patch is made up of hydrophobic side chains surrounding the exposed edge of the imidazole ring of His-87.

Other Blue Copper Proteins

The azurins are electron-transfer proteins in the respiratory chains of certain bacteria. They have been particularly well studied from Pseudomonous aeruginsoa and other Pseudomonads. They contain one blue copper bound to a single polypeptide chain of molecular weight about 16000. Amino acid sequence data for a number of azurins show that all contain three cysteine residues.

The structure of *Ps.* Aeruginosa azurin has been determined by X-ray crystallography. The structure is similar to that of plastocyanin, and involves two imidazoles from His-46 and His-117 (Cu-N = 1.97 Å) and sulfur atoms from Cys-112 and Met-121. Again there is a short Cu-S (Cys) distance (2.10Å) and a long Cu-S (Met) distance of 2.25 Å

Stellacyanin from Rhus vernicifera is also well studied. The polypeptide does not contain a methioine residue showing that the ligands of the blue copper site may vary. There is evidence for a short Cu-S (Cys) bond. It has been suggested that methionine is replaced as a ligand by an –S-S- group.

The other small blue proteins are only poorly characterized. It is assumed their function is that of electron transfer. Ruysticyanin from *Thiobacillus ferroxidans* is thought to be the initial electron acceptor from iron (III) in the respiratory chain at pH 2. Rusticyanin contains 159 residues, with one cysteine, three methionine and five histidine residues. The protein is unusually stable at low pH, in accord with its presence in an acidophilic organism. The midpoint potential of rusticyanin is high (+680mV).

Hemocyanin and Hemoerythrin

Refer Unit 3.

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UNIT – V: BIO-PHYSICAL CHEMISTRY

Thermodynamics in biology-energy flux-transfer of potentials and coupled reactions-role of singlet oxygen in biology-general principles of function and structural organization in bioenergetics fundamental reactions –structure of membranes (introductory aspects only)-solute transport across membranes-membrane potentials-ion pumps –biophysical applications of Mössbauer effect.

Thermodynamics in biology:

Energy is vital in the microorganism of a living cell and cells are models for the development of useful new energy-transforming devices, particularly for the capture of solar energy.

Bioenergetics is the field of biochemistry concerned with the transformation and use of energy by living cells. All living systems obey the laws of thermodynamics.
The two fundamental laws of thermodynamics are :

The first law of thermodynamics:

In any physical or chemical change the total amount of energy in the universe remains constant and the statement is given by the equation

$$\Delta E = q - w$$

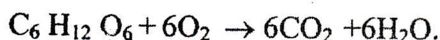
The second Law of thermodynamics:

"In any spontaneous process the total entropy of the system and the surroundings increases." There are two kinds of useful energy. (1) Free energy, the kind of energy that can do work at constant T and P. (2) heat energy, which can do work only through a change in temperature. Entropy is energy in a state of randomness or disorder, it is unavailable, useless energy. In the biological system, reactants do exchange energy and they exchange matter with their surroundings.

Changes in free energy, heat and entropy in chemical reactions at constant T and P, the conditions existing in biological systems, are related by the equation.

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

Living organisms preserve their internal order by taking in free energy of nutrients (or light) from their surroundings and returning to their surroundings an equal amount of energy in a less useful form, largely heat that becomes randomized throughout the rest of the universe. The increase in entropy of the universe during biological processes, because it is reversible, provides their driving force and gives direction to all biological activities. Living organisms constantly produce entropy in their surroundings as a necessary cost of maintaining their own internal order. Aerobic cells carry out the oxidation of glucose ($C_6H_{12}O_6$) to CO_2 and H_2O at constant T and P.



$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O.$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} = \frac{-673,000-(-686,000)}{298} = \frac{13000}{298} = +44 \text{ cal/deg/mole of glucose}$$

The molecular disorder or entropy is increase for the above reaction.

Cells Require free energy:

Cells can and must use free energy which can do work at constant T and P. The two kinds of cells like heterotrophic and photosynthetic transform free energy inputs into chemical energy and use them to do their work. Cells are chemical engines that function at constant T and P.

Applications of the free energy function:

Every chemical reaction has a characteristic standard free energy change ΔG^0. This can be calculated from the equilibrium constant (K_{eq}) of reaction under standard conditions that is, T = 298K and P=1.00 atm (760mm Hg). By using the relationship, $\Delta G^0 = -2.303RT \log K_{eq}$

The standard free energy change is calculated by another way and which is the difference between the free energy content of the products and the reactants under standard conditions.

EX: Oxaloacetate $+H^+(10^{-7}M) \rightarrow CO_2\,(g)$ + pyruvate

ΔG^0 for the above reaction as

$$\Delta G^0 = -113.44-94.45-(-9.87-190.62)$$
$$= -207.89+200.49$$
$$= -7.4 \text{kcal/mol}$$

The bio-chemical reactions take place near pH 7.0 and involves H^+ formation, the standard free energy change at pH 7.0 in biochemical systems is designated by $\Delta G^{0'}$. Each chemical reaction has a characteristic standard free energy change $\Delta G^{0'}$. The free energy change $\Delta G^{0'}$ is given by the equation.

$$\Delta G = \Delta G^{0'} + 2.303RT \log \frac{[Products]}{[Reactants]}$$

The $\Delta G^{0'}$ values of sequential chemical reactions are additive and which is the algebraic sum of the individual standard free energy change of the reactions.

Ex $\Delta G^{0'}$ **(kcal/mol)**

(i)Glucose + $ATP^{4-} \rightarrow$ Glucose -6-physphate+H^+ +ADP^{3-} - 5.4

(ii) Glucose -6-phosphate+$H_20 \rightarrow$ Glucose +HPO_4^{2-} -3.0

$ATP^{4-} + H_20 \rightarrow ADP^{3-} + HPO_4^{2-} + H^+$ -8.4

Biological systems perform various kinds of work:

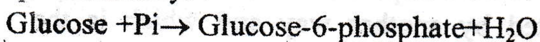
To sustain and propagate life, cells do various types of work. This work takes three major forms,

(1) Mechanical work: changes in location or orientation and cell movement against the force of gravity or friction
(2) Concentration and electrical work: movement of molecules and ions across the membranes.
(3) Synthetic Work: Changes in chemical bonds. It is necessary for the formation of the complex organic molecules of which cells are composed.

Coupled Reactions:

The living organisms to synthesize complex molecules with high enthalpies and low entropies. This is against the laws of thermodynamics. Coupling them to favorable processes can drive thermodynamically unfavorable reaction.

Ex: The formation of glucose -6-phosphate and water from glucose and inorganic phosphates ion (Pi) has unfavorable, ΔG^0=+3.0k cal/mol at 298, and this reaction does not occur spontaneously.

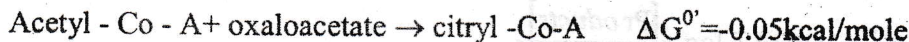


Glucose +Pi→ Glucose-6-phosphate+H_2O

The reaction ATP +H_2O → ADP +H+ has highly favorable, $\Delta G^{0'}$ is -8.4 kcal/mol. If two reactions are combined to give the reaction. Glucose G-6-P +H_2o

Glucose +ATP → G-6-P+ ADP+H^+, and the $\Delta G^{0'}$ is 3.0-8.4 = -5.4. kcal/mol The combined reaction is thermodynamically favorable. In many cells, the coupled reaction is catalyzed by an enzyme that facilities the transfer of phosphate from ATP directly to glucose. This is a common motif in biosynthetic processes. Another common mechanism for coupling reaction is simply to arrange for one of the reactions to proceed or follow the other.

EX:



Acetyl - Co - A+ oxaloacetate → citryl -Co-A $\Delta G^{0'}$=-0.05kcal/mole

Citryl-Co-A →citrate + Coenzyme A. $\Delta G^{0'}$=-8.4kcal/mole

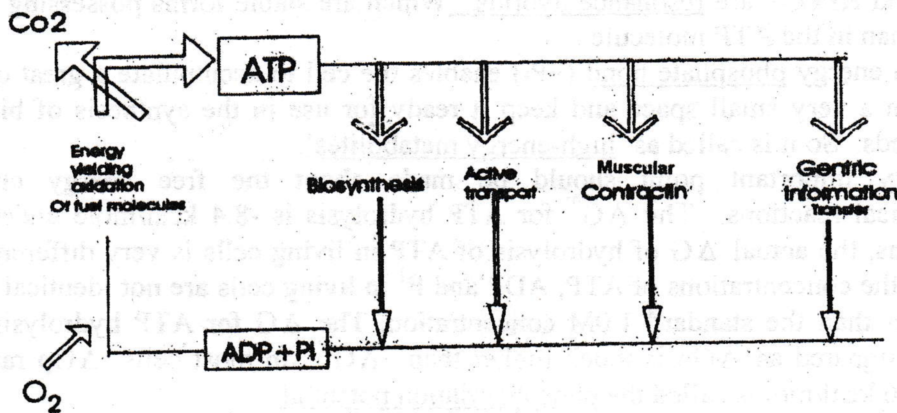
These two reactions are catalyzed by the enzyme citrate synthase. The important point to keep in mind is that ΔG values determine whether a process to occur spontaneously or not but not by ΔG^o. The actual free energy change depends on the concentration of reactants and products.

ATP as the main carrier of free energy in biochemical bystems:

Virtually all living organisms use ATP for transferring free energy between energy producing and energy-consuming systems. Processes that proceed with large negative changes in free energy, such as the oxidative degradation of carbohydrates or fatty acids, are used to derive the formation of ATP, and the hydrolysis of ATP is used to drive biosynthetic reactions and other processes. In human body, about 2.3kg of ATP is formed and consumed every day.

The ATP cycle in cells as shown in figure 1.

Fig.1

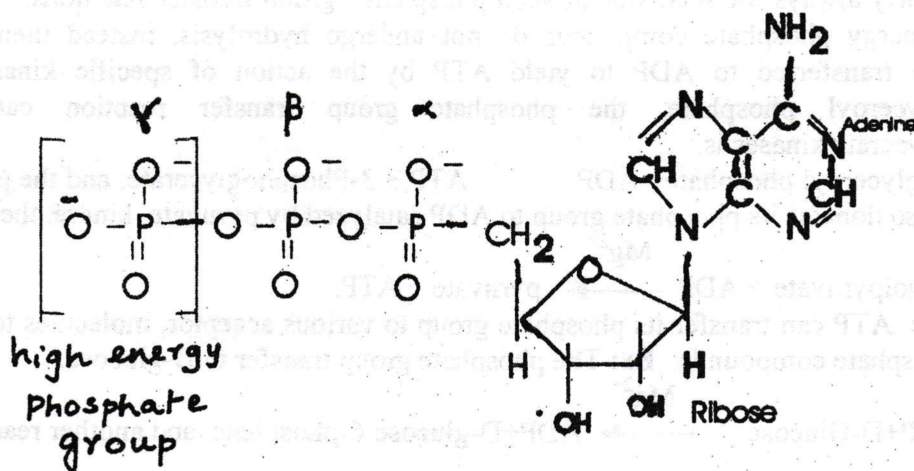


ATP and its successive hydrolysis products like adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are nucleotides. The nucleotides contains a purine base adenosine, the sugar D-ribose and one, two and three phosphate units respectively. ATP is the high-energy metabolite, because

(1) The ionization of ATP and its hydrolysis products occur at pH 7.0; almost completely ionized as the ATP^{4-} ion, which yields three products namely ADP^{3-}, $HPO4^{2-}$ and H^+. The reaction is $ATP^{4-}+H_2O \rightarrow ADP^{3-} + HPO_4^{2-}+H^+$.

(2) The higher $\Delta G^{0'}$ for ATP is that at pH 7.0 ATP molecules have closely spaced negative charge which repel each other strongly. (Fig.2)

Fig. –2



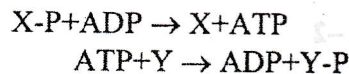
(3) The large negative $\Delta G^{0'}$ value of ATP hydrolysis is the fact that each of the two products ADP^{3-} and HPO_4^{2-} are resonance hybrids. Which are stable forms possessing much less energy than in the ATP molecule .
The high energy phosphate bond (~Pi) enables the cell to accumulate a great quantity of energy in a very small space and keep it ready for use in the synthesis of biochemical compounds. So it is called as 'high-energy metabolites'.

(4) The very important point should be made about the free energy changes of biochemical reactions. The $\Delta G^{0'}$ for ATP hydrolysis is -8.4 kcal/mole under standard conditions, the actual ΔG of hydrolysis of ATP in living cells is very different. This is because the concentrations of ATP, ADP and P^1 in living cells are not identical and much are lower than the standard 1.0M concentration. The ΔG for ATP hydrolysis in intact cells, designated as ΔGp is much higher than $\Delta G^{0'}$, in most cells ΔGp ranges from -12 to -16 kcal/mol is called the phosphorylation potential.

Transfer of phosphorylation potential:

The ATP molecules serve as an intermediate carrier of phosphate groups from super high-energy compounds. The reactions of metabolism take place via chains of consecutive or sequential enzyme catalyzed reactions linked by common intermediates. The successive transformation products in a pathway are known as metabolic intermediates or metabolites.

ATP functions as an energy-carrying common intermediate in the cell. During energy-yielding catabolic reactions super high-energy phosphate compounds are generated at the expense of energy released on degradation of organic cell nutrients. The transfer of a phosphate group from such a super high-energy phosphate compound as X-P to ADP to form ATP., and in the second step the transfer of the terminal phosphate group from ATP to an acceptor molecule.



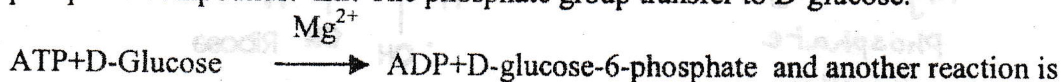
$$X\text{-}P + ADP \rightarrow X + ATP$$
$$ATP + Y \rightarrow ADP + Y\text{-}P$$

ATP is nearly always the mediator of such phosphate –group transfer reactions. In the cells, the high-energy phosphate compounds do not undergo hydrolysis, instead their phosphate groups are transferred to ADP to yield ATP by the action of specific kinases. For 3-phosphoglyceroyl phosphate, the phosphate group transfer reaction catalyzed by phosphoelycerate kinases is.

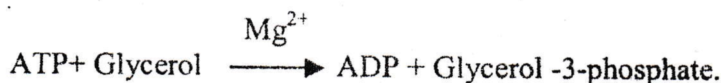
3-phosphoglyceroyl phosphate +ADP ATP + 3-Phosphoglycerate, and the phosphoenol pyrurate also donates its phosphate group to ADP catalyzed by pyruvate kinase, the reaction is

$$\text{Phosphoenolpyruvate} + ADP \xrightarrow{Mg^{2+}} \text{pyruvate} + ATP.$$

The ATP can transfer its phosphate group to various acceptor, molecules to yield low-energy phosphate compounds. **Ex:** The phosphate group transfer to D-glucose.



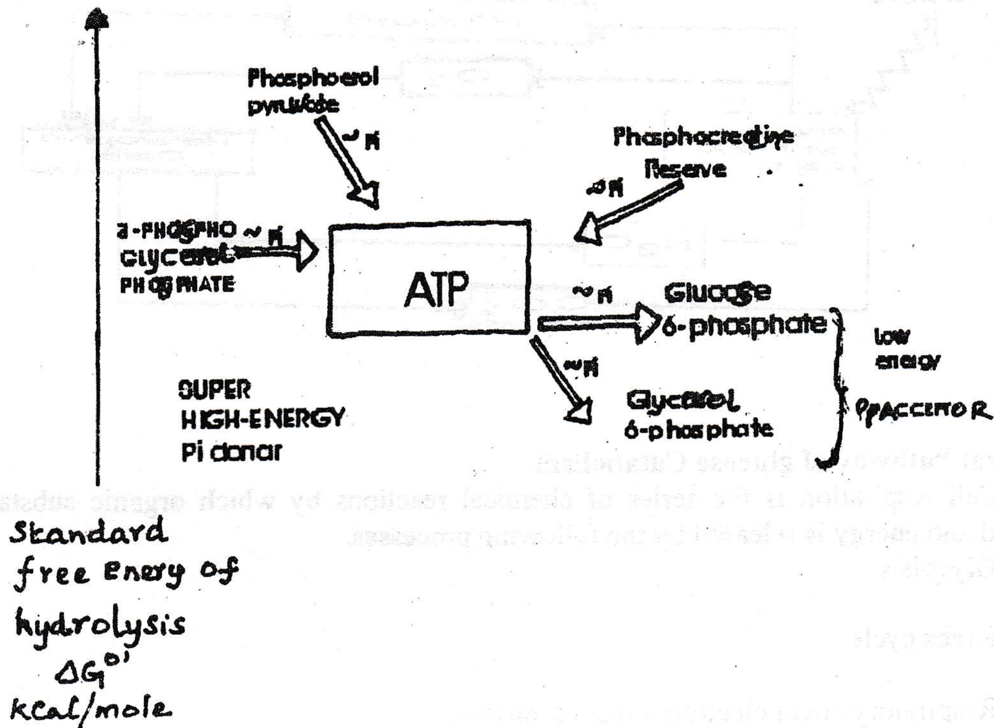
$$ATP + D\text{-}Glucose \xrightarrow{Mg^{2+}} ADP + D\text{-}glucose\text{-}6\text{-}phosphate$$ and another reaction is



$$ATP + Glycerol \xrightarrow{Mg^{2+}} ADP + Glycerol\text{-}3\text{-}phosphate.$$

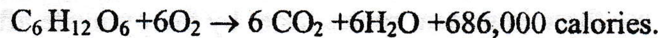
Glucose-6-phosphate and glycerol-3-phosphate have a higher energy content, which are activated building block for bio-synthesis process. The flow sheet of enzymatic phosphate transfer reactions in the cell is given in figure 3.

Fig. -3



General Principles of function and structural organization in bio-energetic fundamental reactions:

The chemical energy is released by combustions of glucose is useful to perform various kinds of biological work in cells.



$$C_6H_{12}O_6 + 6O_2 \rightarrow 6\,CO_2 + 6H_2O + 686{,}000 \text{ calories.}$$

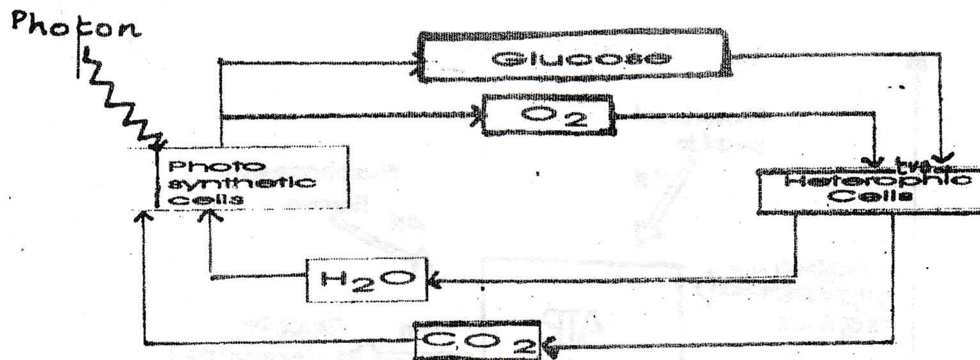
Within the living cells this enormous amount of energy is not released suddenly and it proceeds in a stepwise and controlled manner requiring a great number of enzymes that finally convert the fuel into CO_2 and H_2O.

All cells and organism can be grouped into two main classes, differing in the mechanism of extracting energy for their own metabolism. The first cell type is autotrophs. CO_2 and H_2O are transformed into glucose by the process of photosynthesis from which more complex molecules are made.

The second class of cells, called heterotrophs obtain energy from the different foodstuff. The energy in the organic molecules is released mainly by combustion with O_2 from the

atmosphere in a process called aerobic respiration. The release of H_2O and CO_2 by heterotrophic cells completes this cycle of energy (Fig. 4).

Fig -4



A Central Pathway of glucose Catabolism

Cell respiration is the series of chemical reactions by which organic substances are degraded and energy is released by the following processes.

(1) Glycolsis

(2) Krebs cycle

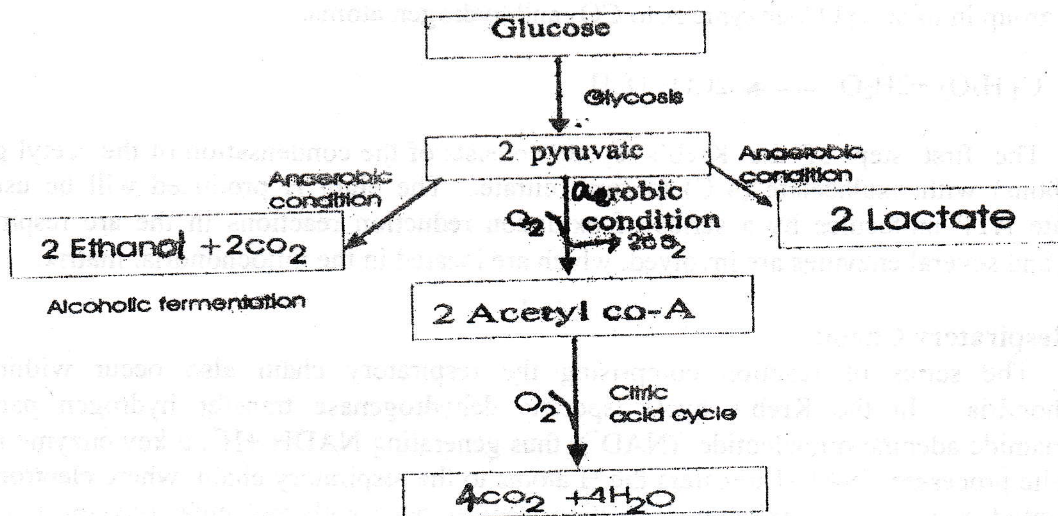
(3) Respiratory chain electron transport process.

Glycolsis:

Glycolsis is an almost universal central pathway of glucose catabolism. The glycoltic sequence of reaction, which involves in the absence of oxygen is called anaerobic glucoses, on the other hand, in the presence of O_2 by the process of aerobic respiration, glucose is oxidized to CO_2 and H_2O. There are three important routes taken by the pyruvate after glycolsis. In the first pathway, the pyruvate is oxidized, with loss of its carboxyl as CO_2, to from the acetyl group of <u>acetyl- Coenzyme A.</u> The acetyl group is completely to CO_2 and H_2O by the citric acid cycle.

The second pathway is in which the pyruvate is reduced to lactate. This process in skeletal muscle is called aerobic glycolysis, this is an important source of ATP energy in which is very intense physical activity. Lactate is also the product of glycolysis in aerobic microorganism that carryout lactic acid fermentation. (Figure.5)

Fig. 5



The third major pathway of pyruvate leads to ethanol, the pyruvate formed from glucose by glycolysis is converted aerobically into ethanol and CO_2, This process is called alcoholic fermentation. During glycolysis much of the free energy of the glucose molecule is conserved in the form of ATP and the overall standard-free energy change, for this process is -32.4 kcal/mol.

Glucose +2Pi+2 ADP $\rightarrow$ 2 lactate $+2H^{+}$+2ATP+$2H_2O$ ΔG=-32.4kcal/mol. The two molecules of lactate formed by glycolysis contain most of the biologically available energy of the glucose molecule.

Glycolysis has two phases

(1) Preparatory phase: In this reaction, glucose is enzymetically phosphorylated by ATP to yield fructose -1,6-diphosphate which is cleaved into two-molecules of glyceraldehyde-3-phosphate.

(2) In the second phase of glycolysis the two molecules of glyceraldehyde3-phosphate are converted into two molecules of pyruvate which is conserved by the coupled phosphorylation of four molecules of ADP to ATP, the net over all yield is two molecules of ATP per molecule of glucose since two molecules of ATP were used in the first phase.

Three different types of chemical transformations take place. They are

1. The degradation of the glucose into pyruvate
2. The phosphorylation of ADP to ATP by high energy phosphate compounds
3. The transfer of hydrogen atoms or electrons.

The Krebs cycle: (or) Citric acid cycle:

The Krebs cycle, also called the tricarboxylic acid cycle is the common final pathway of cellular catabolism, the fuel molecules undergo an oxidative process. This cycle degrades the acetyl group in to acetyl Coenzyme A to CO_2 and hydrogen atoms.

$$C_2 H_4O_2 + 2H_2O \longrightarrow 2CO_2 + 8\,H$$

The first step of the Kreb's cycle consists of the condensation of the acetyl group (2-carbons) with oxalacetate (4-C) to form citrate. The eight H produced will be used to generate ATP molecules by a series of oxidation reduction reactions in the are respiratory chain, and several enzymes are involved, which are located in the mitochondrial matrix.

The Respiratory Chain:

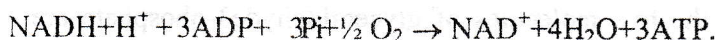
The series of reaction comprising the respiratory chain also occur within the mitochondria. In the Kreb's cycle, specific dehydrogenase transfer hydrogen pairs to nicotinamide adenine dinucleotide (NAD^+), thus generating $NADH + H^+$, a key enzyme in the catabolic processes. NADH transfers the H atoms to the respiratory chain, where electrons are transported in a series of oxidation-reduction steps to react with molecular oxygen involving several cytochromes. In many biological oxidations electrons are transferred via hydrogen atoms. The cytochromes are iron containing molecules, in which electrons are transferred by the reaction

$$Fe^{2+} \quad Fe^{3+} + e^-.$$

The final cytochrome (cytochrome 'A' or cytochrome oxidase) transfer the H atoms to O_2 to produce H_2O. The respiratory chain enzymes are located in the inner mitochondrial membrane. (Fig. 6)

Oxidative phosphorylations:-

The respiratory chain is coupled with the process of oxidative phosphorylation in the inner membrane of the mitochondrion and ATP is formed at three steps of the electron transport chain. The equation for this process is



$$NADH + H^+ + 3ADP + 3Pi + \tfrac{1}{2} O_2 \rightarrow NAD^+ + 4H_2O + 3ATP.$$

The energy balance of aerobic respiration shows that 36 ATP molecules are produced from each glucose molecule. The overall equation can be written as

$$C_6H_{12}O_6 + 6O_2 + 36Pi + 36\,ADP \rightarrow 6CO_2 + 36ATP + 6H_2O$$

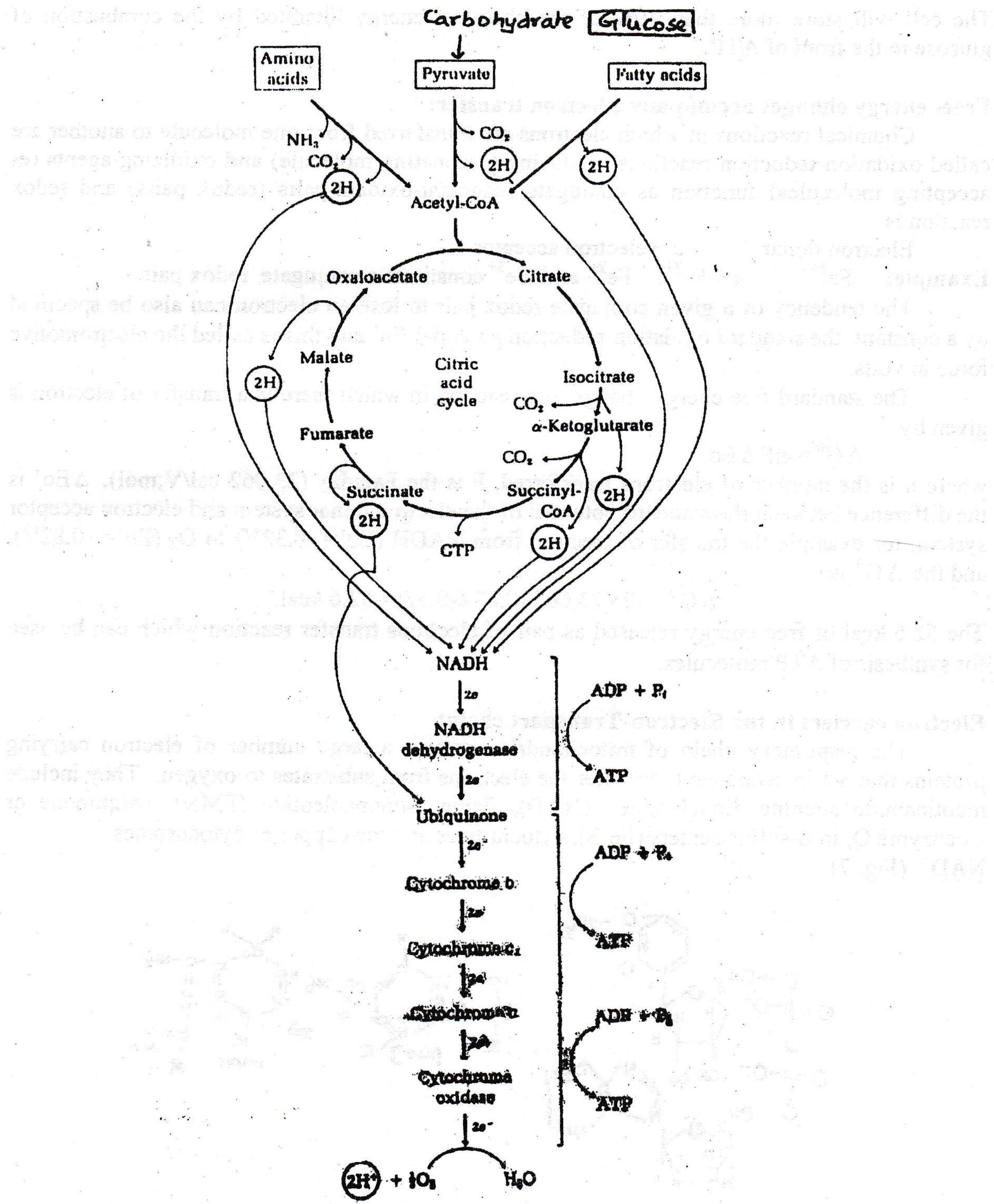


Fig.6. Flow Sheet - Respiration

The cell will store more than 40% of the chemical energy liberated by the combustion of glucose in the from of ATP.

Free- energy changes accompany Electron transfer:

Chemical reactions in which electrons are transferred from one molecule to another are called oxidation-reduction reactions. Reducing (e-donating molecule) and oxidizing agents (e-accepting molecules) function as conjugate reductant-oxidant-pairs (redox pairs) and redox reaction is

Electron donar e^- +electron acceptor

Example: Fe^{2+} $e^- + Fe^{3+}$, Fe^{2+} and Fe^{3+} constitute a conjugate redox pair.

The tendency of a given conjugate redox pair to lose an electron can also be specified by a constant, the standard oxidation-reduction potential Eo' and this is called the electromotive force in volts.

The standard free energy change of a reaction in which there is a transfer of electron is given by

$$\Delta G^{0'} = -nF\Delta Eo'$$

where n is the number of electrons transferred, F is the Faraday (23,062 cal/V;mol), $\Delta Eo'$ is the difference between the standard potential of the electron donar system and electron acceptor system, for example the transfer of electron from NADH (Eo'= -0.32V) to O_2 (Eo'= -0.82V), and the $\Delta G^{0'}$ is

$$\Delta G^{0'} = -2 \times 23{,}062\ (0.82-(-0.32) = -52.6\ \text{kcal.}$$

The 52.6 kcal of free energy released as pair of electrons transfer reaction which can be used for synthesis of ATP molecules.

Electron carriers in the Electron-Transport chain:

The respiratory chain of mitochondria contains a large number of electron carrying proteins that act in sequence to transfer the electrons from substrates to oxygen. They include nicotinamide adenine dinucleotide (NAD), flavin mononucleotide (FMN), uniquinone or Coenzyme Q, iron-sulfur centers (Fe-S), cytochromes and the copper of cytochromes.

<u>NAD</u> $^+$ (Fig. 7)

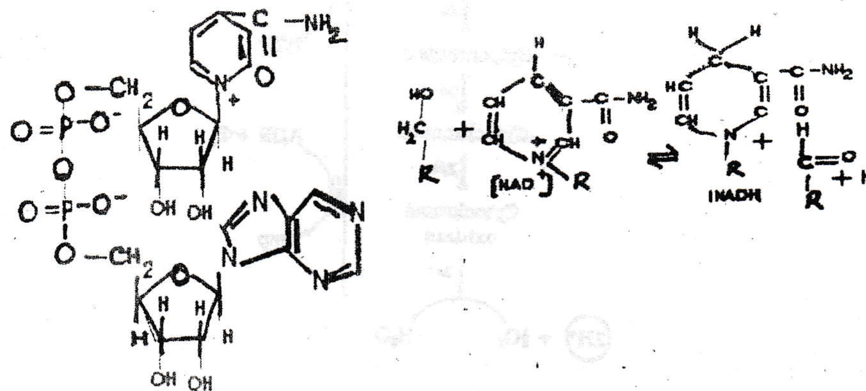


Fig. 7

Membranes:

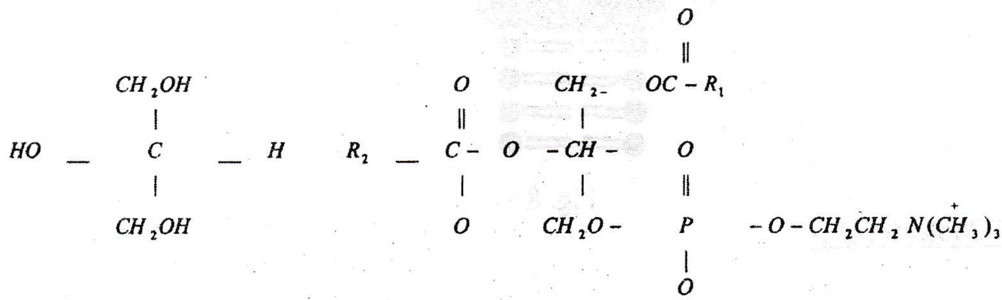
Properties of Membranes:

1. Biological membranes are impermeable to polar molecules or ions.
2. Membranes are not rigid but rather adapt flexibly to changes in cellular or organelle shape and size.
3. They are durable
4. Membranes contain proteins of simple structure in nature, but have a variety of enzymatic activities.
5. Membranes have a trilingual appearance of two dark lines separated by a lighter space in the order of 40A°.

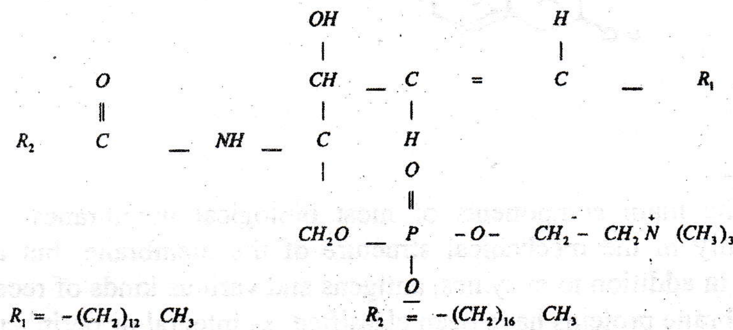
The Structure of Biological Membranes:

The plasma membrane consists approximately 52% protein, 40% lipids and 8% carbohydrates of its mass. The main lipid components of the plasma membrane are phospholipids, cholesterol and galactolipids, their proportion varies in different cell membranes.

The two main types of lipids, which occur in biological membranes phospholipids and sterols. The phospholipids in most membranes are phosphoglycerides, which are phosphate esters of the three carbon alcohol, glycerol. A typical structure of phosphatidyl choline (lecithin)



Here R_1 and R_2 are long fatty acid side chains. The animal cell membranes contain a second group of phospholipids, sphingolipids. Ex: sphingomyclin_ has the structure.



The second major type of lipid in some biological membranes is cholesterol. It occurs in membranes both in its free form and esterified with long chain fatty acids. Plant cell membranes have no cholesterol, but contain relatively large amounts of glycolipids .

The phospholipids can form a bilayer the hydrocarbon tails of two monolayers of phospholipids pack together while the polar head-groups face outward and remain protein in contact with water on either side. The phospholipids are agitated in the presence of excess water, they tend to aggregate spontaneously to from bilayers. (Fig. 8)

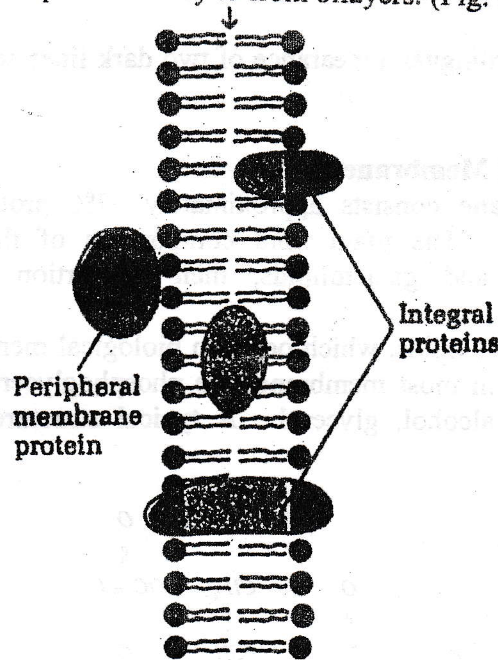
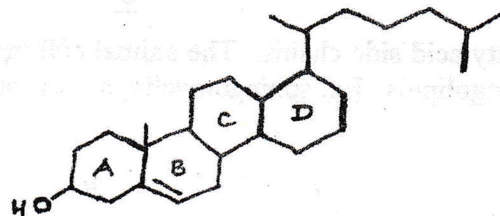


Fig. 8

Structure of Cholesterol :-



Membrane Proteins:-

Proteins are the main components of most biological membranes. They play an important role, not only in the mechanical structure of the membrane, but also as carriers serving for transport. In addition to enzymes, antigens and various kinds of receptor molecules are also present. Membrane proteins have been classified as integral or peripheral according to the degree of their association and solubility.

- Peripheral proteins separated by mild treatment are soluble in aqueous solution and free of lipids. EX Cytochrome 'C'
- Integral proteins are insoluble in water solutions and need the presence of detergents to be maintained in a non-aggregated form. These proteins may be attached to oligosaccharides, forming glycoproteins.

Cell Permeability:-

Permeability is the fundamental property for the function of the living cells, and to the maintenance of satisfactory intracellular physiological conditions. The membrane establishes a net difference between the in intracellular fluid and the extra cellular fluid. One of the function of the cell membrane is to maintain a balance the osmotic pressure between the intracellular fluid and that of the interstitial fluid. In higher organisms the kidney regulates the osmotic pressure of the whole body.

Solute transport across Membranes:-

The membranes engage inselective transport processes in all living cells, which depends on an influx of phosphate and other ions, and of nutrients such as carbohydrates and amino acids. They extrude certain ions such as Na^+ and metabolic end products. The biological membranes contain proteins that act as specific transporters. They bind substrates and release products. The primary function is not to catalyze chemical reactions but to move materials from one side of a membrane to the other.

Most solutes are Transported by Specific carriers:-

The movement of glucose passes through the porous membrane by the diffusion process. Glucose and water molecules can diffuse from either side of the membrane to other. The solute diffuses from the more concentrated solution side to the more dilute solution side. The net rate of diffusion is proportional to the difference between the two concentrations ($\Delta C = C_2 - C_1$)

The protein has a specific binding site for the material that it transports. The number of these sites in the membrane limits the overall rate of transport and the biological transport systems are highly specified.

The solutes are uncharged, the free energy change associated with moving of the material from a solution with concentration (C_1) to a solution with concentration (C_2) is

$$\Delta G_{1\to2} = 2.303RT \log [C_2]/[C_1]$$

The solute diffuses spontaneously from solution1 to solution 2, if $[C_1] > [C_2]$, $\Delta G_{1\to2}$ is negative. The net flux across the membrane ceases when the concentrations are equal on both sides, $[C_1] = [C_2]$, $\Delta G_{1\to2} = 0$.

If the solute carries a net charge, there is an additional thermodynamic effect of moving the charge across the membarane, which has any difference in electric potential, which exists between the solutions on the two sides of the membrane. The free energy change is

$$\Delta G_{1\to2} = 2.303 RT \log [C_2]/[C_1] + n F \Delta E$$

The equilibrium condition is, $\log \frac{[C_2]}{[C_1]} = \frac{-np\Delta E}{2.303RT}$

Among the transporters that facilitate diffusion of charged species, which down the electrochemical potential gradients are the Na^+ and K^+ channels of nerve cells.

Cells can transport some materials against gradients of concentration or electric potential or both. The transport against such concentration and electric gradient is called active transport. To drive the active transport, a cell must couple transport process to another process, which is thermodynamically favorable, and the total ΔG is negative. Some transport system including the Na^+ -K^+ pump of animal cells, which drive active transport process by the hydrolysis of ATP. (Fig. 9)

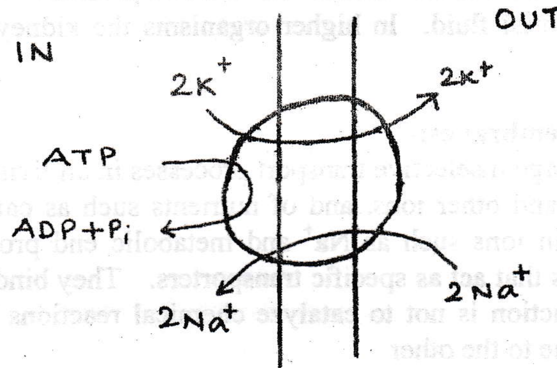


Fig. 9

The active transport of a solute against a concentration gradient also can be driven by a flow of an ion, which reduces the concentration gradient. Many eukaryotic cells take up neutral amino acids by coupling the inward movement of Na^+ and another example is the β -galactoside transport system of E.coli , which couples uptake of lactose to the inward flow of protons. Some bacterial cells can energize sugar transport by group translocation. They modify the sugar by phosphorylation as they transport it. (Fig 10).

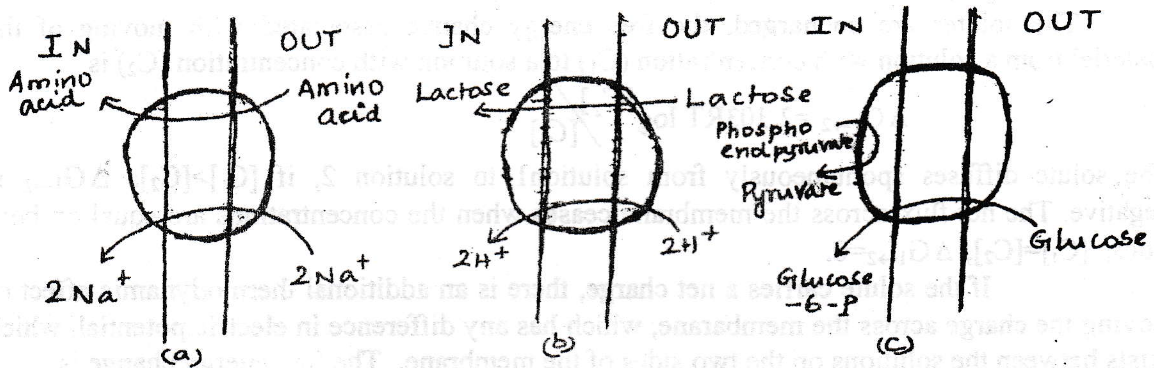


Fig .10

Molecular Models of Transport Mechanisms:

The lactose perm ease could couple the movement of protons and lactose across the membrane. In this system (model) the transmembrane channel is never fully open, but is always plugged at one end or the other. The lactose binding site is centrally located and is accessible to either the extra cellular solution or intracellular solution depending on where the channel is open. The transition between these two states might occur by relatively minor conformational changes in the protein and the binding of a proton from the solution on either side, which favors the binding of lactose from the same side. The transporter would tend to pick up both a proton and lactose from the solution with the lower pH or the more positive electrical potential and to release them on the other side.

Membrane Potentials:-

In all cells there is a difference in ionic concentration in the extra cellular medium and intracellular medium, there is an electrical potential across the membrane. The electrical potential depends on an unequal distribution of the ion on both sides of the membrane. The interstitial fluid has a high concentration of Na^+ (145 mEq) and Cl^- (120 mEq) with potential zero, and the intracellular fluid has a high concentration of K^+ and large organic anions (A^-) (155 mEq) with potential -90 mV. Using the fine microelectrodes is able to penetrate through the membrane into a cell and into the cell nucleus and to measure the electrical potential (or resting, or steady potential), which is always negative inside. The membrane potential in different tissues varies between -20 and -100mV. The membrane potential is negative on inside and is accompanied by a high K^+ and a low Cl^- concentration. The relationship between the concentration gradient and the resting membrane potential is given by the equation, $E=2.303RT \log [C_1]/[C_2]$ This equation for K^+ and Cl^- is given by

$$E=2.303RT \log \frac{[K^+]_{in}}{[K^+]_{out}} = 2.303RT \log \frac{[Cl^-]_{out}}{[Cl^-]_{in}}$$

According to the above equation, any increase in the membrane potential will cause an increase in the ion asymmetry across the membrane and vice versa. (Fig.11)

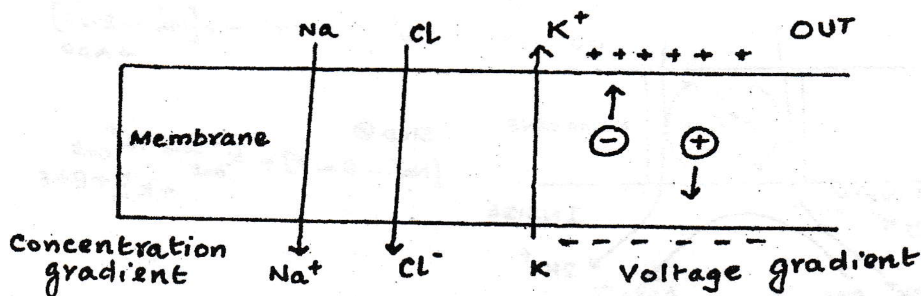


Fig. 11

The action potential of the cell sweeps down due to electric depolarization of the membrane caused by Na^+ flowing into the cell followed by the depolarization as K^+ flows out. The Na^+ influx is thermodynamically downhill, because the intracellular Na^+ concentration

(12mM) is much lower than the extra cellular concentration (145mM) and the internal electric potential is negative relative to the external potential by about 60 mV. The membrane Na+-K+ pump sets up these conditions. The tendency of the negative potential difference (ΔE) to pull K^+ into the cell is nearly balanced by the higher intracellular concentration that favors K^+ efflux. {$[K^+]$= 140mM inside and 4mM outside}. When the sudden influx of Na^+ causes $\Delta E=0$, and change sign, this balance is upset and K^+ starts flowing out.

IONS PUMP:- (Na^+-K^+pump)

The electric potential of the inside of the cell membrane is negative relative to the external potential by about 60 mV. The membrane Na^+ - K^+ pump sets up this condition. The eukaryotic cells pump out Na^+ ion and the electric potential inside is more negative than the potential outside. The Na^+ - K^+ pump of animal cells may consume 70% of the ATP hydrolysis. The Na^+ influx is down hill thermodynamically, because the Na^+-K^+ pump keeps the intra cellular concentration of Na^+ lower than the extra cellular concentration.

The eukaryotic Na^+-K^+ pump or Na^+-K^+ATPase has two polypeptide subunits. The larger subunit contains the cation binding sites and the catalytic site for ATP hydrolysis, the smaller subunit is a glycoprotein, the function of which is unknown. ATP binds to the enzyme only from the intracellular side of the membrane and the enzyme releases ADP and Pi on the same side, Na^+ also binds most strongly from the intra cellular side, but K^+ binds best from the extra cellular side.

The ion pumping and ATPase activities of the enzyme are tightly coupled the ATP hydrolysis occurs at an appreciable rate only if Na^+ and K^+ both are present on appropriate sides of the membranes.

For each ATP that it splits, the Na^+-K^+ pump moves three Na^+ ions out the cell and bring two K^+ ions inside the membrane. This means that the pump is intrinsically electrogenic and it creates an electric potential gradient across the membrane because it moves more +ve charges out than it brings in. (Fig. 12).

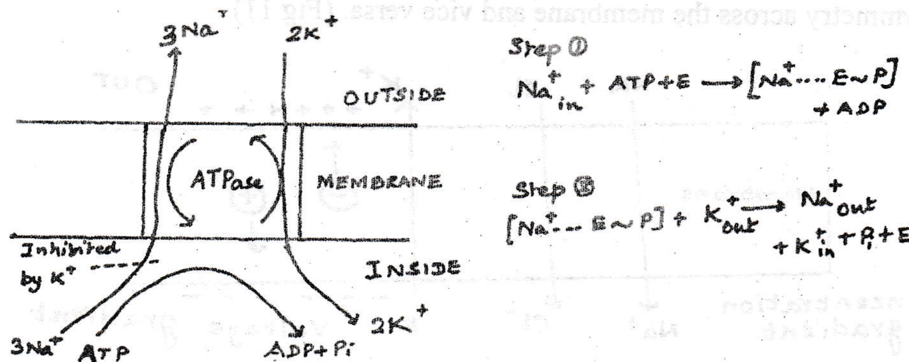
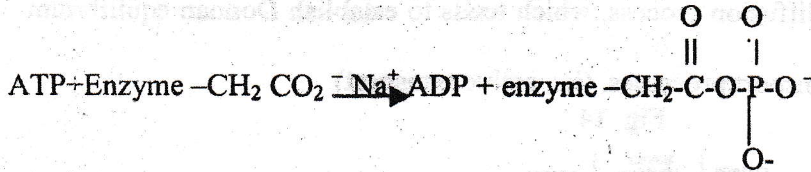


Fig . 12

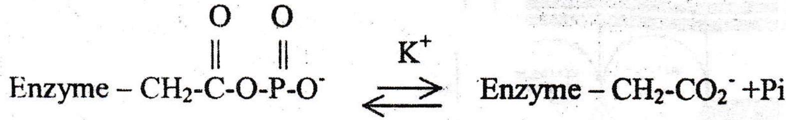
The ΔE is about -60mV, and its built-up across the plasma membrane of a nerve cell, which owes more leakage of K^+ back out of the cell through other types of channels. The K^+ tends to leak out, because the Na^+ - K^+ pump elevates the internal K^+ concentration above the extra cellular concentration.

In the catalytic cycle of the Na^+-K^+ATPase, the terminal phosphate group of ATP is transferred to the enzyme. It forms a carboxylic-phosphoryl anhydride with an aspartyl residue.



$$ATP + Enzyme - CH_2\,CO_2^- \xrightarrow{Na^+} ADP + enzyme - CH_2\text{-}\overset{\overset{O}{\|}}{C}\text{-}O\text{-}\overset{\overset{O}{|}}{\underset{\underset{O^-}{|}}{P}}\text{-}O^-$$

Na^+ promotes this step of the reaction and K^+ on the other hand, promotes hydrolysis of the phosphorylated enzyme into Pi.



$$Enzyme - CH_2\text{-}\overset{\overset{O}{\|}}{C}\text{-}O\text{-}\overset{\overset{O}{\|}}{P}\text{-}O^- \overset{K^+}{\rightleftarrows} Enzyme - CH_2\text{-}CO_2^- + Pi$$

According to the diagram, Na^+-K^+ATPase enzyme has binding sites for Na^+-K^+ and release these ions at opposite sides of the membrane by the complex carrier mechanism.

The Na^+ -K^+ pump drives ions extruding Na^+ and taking in K^+, and at the same time the passive Na^+ influx depends on a large driving force resulting from the concentration and voltage gradients, and only a slight permeability of the membrane. Similarly the net passive K^+ influx results not from a small driving force but from a greater permeability. (Fig. 13).

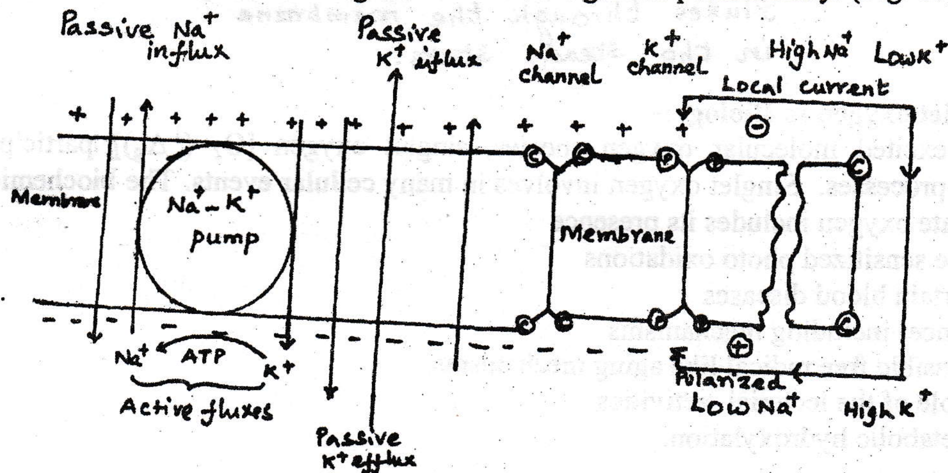


Fig.13

The active transport of ions is fundamental to maintenance of the osmotic equilibrium of the cell, the required concentration of anions and cations and the special ions needed for the functioning, of Na+ is pumped out by the cell, There is an excess of water molecules and the cell keeps its osmotic pressure constant.

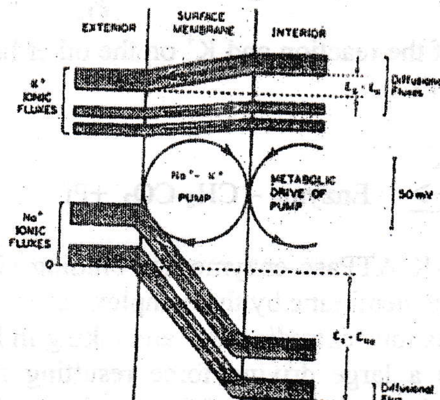
The potassium ions, which are concentrated inside the cell, must pass against a concentration gradient. This can be achieved by the pumping mechanism at the expense of energy and also an active process, which is called the sodium pump which, transports Na^+.

The following figure.14 summarizes the relationship existing between the transfer of K^+ and Na^+ by passive and active mechanism and the resulting steady state potential. The passive

fluxes are distinguished from the active fluxes. It demonstrates that distribution of ions across the membrane depends on the summation of two distinct processes.

1. Simple electrochemical diffusion process, which tends to establish Donnan equilibrium (passive transport), and
2. Energy dependent ion transport processes. (ie., active transport)

Fig. 14



Active and Passive Na^+ and K^+ fluxes through the membrane in the steady state.

Role of singlet oxygen in Biology:-

The excited molecular oxygen species, singlet oxygen [O_2 ($^1\Delta_g$)] participates in biochemical processes. Singlet oxygen involves in many cellular events. The biochemical role of excited state oxygen includes its presence

(1) in dye sensitized photo oxidations
(2) in certain blood diseases
(3) in cancer including mechanisms
(4) in possible free radical like aging mechanisms
(5) the role of the lecterial activities
(6) in metabolic hydroxylation.

Physical and chemical nature of singlet oxygen:-($^1O_2$)

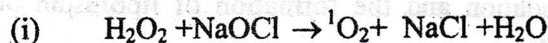
The two highest energy electrons in ground state O_2 are in degenerate π molecular orbital, whereas in singlet O_2, the highest energy electrons are in the same orbital with their spin opposed. A second excited state of oxygen involving the highest energy electrons are in different orbitals with their spins opposed. The two excited states of oxygen can be represented

State	Symbol	Energy	orbitals
2nd Es	$^1\Sigma$	37 kcal	↑↑
1st Es	$1\,\Delta_g$	22 kcal	↑↓ __
Gs	$^3\Sigma$	0	↑↑

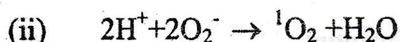
The lifetime of the $^1\Sigma$ state is very short, 10^{-9}s and to decay to the $^1\Delta$ state before appreciable chemistry can occur. The lifetime of the $^1\Delta$ state in the order of 10^{-6}-10^{-5}s. Relaxation of singlet oxygen to the grond state can occur with the production of 'dimol' chemiluminescences at 6334 A° and 7032 A°

The singlet oxygen can be produced via a variety of chemical and physical methods. The reactivity of singlet $^1O_2$ resembles that of ethylene; it is an electrophilic reactant.

Production of $^1O_2$ (Singlet)

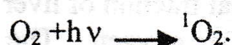


(i) $H_2O_2 + NaOCl \rightarrow {}^1O_2 + NaCl + H_2O$



(ii) $2H^+ + 2O_2^- \rightarrow {}^1O_2 + H_2O$

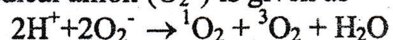
(iii) $O_2 + sens \rightarrow {}^1O_2 + sens$



$O_2 + h\nu \longrightarrow {}^1O_2$.

The different biomolecules that react readily with singlet oxygen under dye sensitized photoOxidative conditions include amino acids, proteins, enzymes, nucleic acids and cell membrane components.

The important source of singlet oxygen in biochemical systems, via dismutation of super oxide radical anion (O_2^-) is given as



$$2H^+ + 2O_2^- \rightarrow {}^1O_2 + {}^3O_2 + H_2O$$

Role of singlet oxygen, $^1O_2$, in Biology:

(1) Role of $^1O_2$ in dye sensitized photo oxidations:

The bacteria are killed in the presence of light. The porphyrin containing molecules such as chlorophyll and hemoglobin are capable of sensitizing oxygen to its excited singlet state. Foote, Chang and Denny have suggested that plants are protected from oxidative damage by oxygen sensitization from their own chlorophyll due to the presence of carotenoid pigments, which are excellent singlet oxygen quenchers, riboflavin, vitamin-A and α -tocopherol react chemically with singlet oxygen.

(2) Role of $^1O_2$ in blood diseases:

The singlet oxygen ($^1O_2$) is one important species in photo induced erythrocyte hemolysis and this hemolysis is induced by cell membrane, photo-peroxidation via singlet oxygen which weakens the membrane and results in the lysis of the erythrocyte .

(3) Role of singlet oxygen in cancer inducing processes:

Kahn and Kasha have proposed the "optical residue" theory in which the polynuclear aromatic is bound to a cellular constituent, and it undergoes excitation, which generates singlet oxygen. The excited oxygen is capable of intracellular damage leading to tumor initiation. The malignant cells take up and bind the $^1O_2$ to a greater

extent than normal tissue; and that irradiation selectively kills the tumor cells due to $^1O_2$ exposure, which links with skin cancer.

(4) Role of singlet oxygen in aging processes:

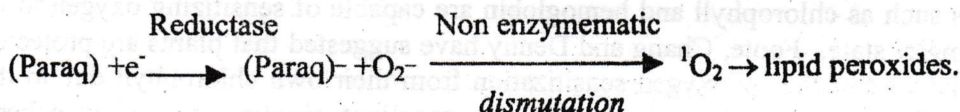
Aging of skin is correlated with oxidative polymerization of elastin, which might be a singlet oxygen mediated process. Lipofuscin fluorine lipid containing granules is to accumulate in heart, brain and muscle of aged persons. The accumulation of lipofuscin is to be involved in the deficiency of memory and learning functions. The singlet oxygen not trapped by a biological antioxidant is capable of leading to lipid peroxidation and the formation of lipofuscin bodies, which results in aging of the system.

(5) Role of singlet oxygen in metabolic hydroxylations:

Enzymatically induced hydroxylations or oxygenations of biomolecules by singlet oxygen. The metabolic hydroxylations of aromatic compounds by nonspecific enzyme systems located in the microsomal fraction of liver cells involve the incorporation of an oxygen atom from molecular oxygen. The oxidation of NADPH by O_2 with both adrenal and spinach systems involves the super oxide anion radical. The resulting super oxide anion radical undergoes dismutation to yield singlet oxygen, which could be inhibited by the addition of enzyme superoxide dismutase (SOD). The $^1O_2$ production is increased two hundred times in the presence of cytochrome 'c'.

Conclusion:

Recent work has also intimated that the intermediacy of $^1O_2$ in certain types of drug sensitivity. The herbicide paraquet, which is toxic to the man due to the formation of pulmonary lesions to be involved with singlet O_2 production <u>in vivo</u> by the following scheme.



The singlet oxygen is also generated via an atmospheric photochemical processes. Pine pollen undergoes oxidation when exposed to singlet oxygen yielding lipid peroxide. The hydro peroxides produced mimics the effects of ozone in leading to pulmonary edema and lung congestion in rats. The recent increase in atmospheric concentration of pollutants, can serve as oxygen sensitizers.

Biophysical applications of Mössbauer Spectroscopy

Itroduction:

The Mossbauer effect is a technique that studies the transition between the various energy states of a material as reflected in the electromagnetic radiation that is emitted or absorped by the material. It is different, unique because the frequency or energy distribution of the radiation utilized is extremely relative to the actual energy of the radiation. The Mossbauer spectroscopy is closely related to NMR spectroscopy because both involve transitions between

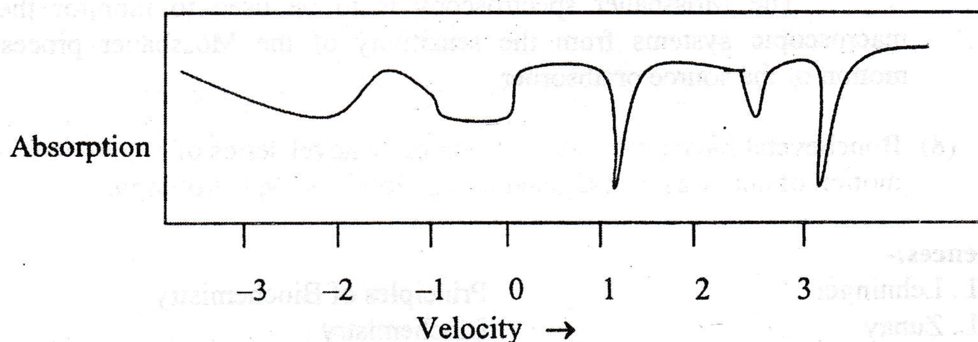
nuclear states. Basically the Mossbauer-effect sources consist of a radioactive material to yield through radioactive decay an isomeric nuclear excited state. The precursor sources are available for both Fe-57, Sn-119 and Co-61. The information contained in a Mossbauer spectrum may be divided into two types. (1) The energetic of the nuclear states and how the electronic and magnetic environment surrounding the nucleus influences them.
(2) The dynamics of the nuclear transition and the lattice containing the nucleus.

Biophysical applications:

Mossbauer spectroscopy has important applications in the study of biological systems because of the presence of the element iron in certain biomolecules. Most Mössbauer investigations of biological molecules have involved the use of ^{57}Fe isotope and which is optimum for the technique.

(1) The main groups of biological molecules, which contain Fe, are the heme proteins, the iron-sulfur proteins, the iron-transport compounds and the iron-storage compounds. Certain hormones and related compounds, which contain iodine and vitamin B_{12}, which contains cobalt, also be studied.

(i) The Mössbauer data indicate that the iron is in the low-spin ferrous state in oxyhemoglobin, the oxygen-carrying constituent of arterial blood. The application of an external magnetic field leads to a splitting which corresponds to an effective field at the Fe exactly equal to the applied field (6T) to the gamma-ray beam and the spectrum is



(ii) In deoxyhemoglobin, the iron is in the high-spin ferric state. It shows a large chemical shift. (≈0.9mm/s at 4.2K)

(iii) In hemoglobin cyanide, azide and hydroxide, the iron is in the low-spin ferric state and at about 50K, the spectrum obtained shows a small chemical shift.

(iv) In hemoglobin fluoride, the iron is in the high-spin ferric state and has zero orbital magnetic moment and the Mossbauer spectra are broadened by magnetic hyperfine interactions.

(v) From the Mossbauer spectra the ferric ion in two spin-state in the ferricytochrome 'c' probably S=3/2 and S=5/2.

(2) The iron-sulfur proteins are formed in a wide range of living organisms and take part in many important biological processes. Mössbauer spectroscopy had played an important part in characterizing and identifying the different types of active centers of iron in iron-sulfur proteins.

(3) From the Mössbauer spectra that the two iron binding sites are present is the iron transport proteins found in higher organisms are different.

(4) Iodine compounds and hormones play an important part in certain physiological processes.

a. By using ^{129}I Mössbauer spectroscopy to study iodine bonding in some hormones.

b. The quaternary structure of the tobacco mosaic virus can be obtained from the information of ^{129}I spectroscopy.

(5) Measurement of vibration and movement

The Mössbauer spectrum consists spectral intensity, which depends on the recoil free fraction, line width and the second-order Doppler shift. From these parameters it is possible to obtain information concerned the movement of the Mössbauer atom with in a molecule, movement of the molecule within a whole system.

(6) Protein Dynamics:-

The dynamics of iron atoms within protein molecules have been made using Mössbauer spectroscopy.

(7) Macroscopic movement:-

The Mössbauer spectroscopy is to be used to monitor the motion of macroscopic systems from the sensitivity of the Mössbauer process using the motion of the source or absorber.

(8) Boncheve tal have performed an extremely novel series of experiments in which the motion of ants was investigated using Mössbauer spectroscopy.

References:-

(1) A.L. Lehninger _ Principles of Biochemistry

(2) G.L. Zubay _ Biochemistry

(3) K.J. Laidler _ Physical chemistry with Biological Applications

(4) E.D.P. Derobertis, E.M.F. DeRobertis – Cell and Molecular Biology

(5) J. Blaud, Journal of Chemical Edn. 274, (1976)
(Singlet O_2 in Biochemistry)

(6) G.L.Long, Mössbauer Specetroscopy, Vol-1 (1984)

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Code No. : 6134 **Sub. Code: DCH 24**

M.Sc. (DD & CE) DEGREE EXAMINATION

APRIL 2009

Second Year - Non-Semester

CHEMISTRY

BIO - ORGANIC, BIO - INORGANIC AND BIO - PHYSICAL CHEMISTRY

(For those who joined in July 2003 and afterwards)

Time : Three hours **Maximum : 100 Marks**

SECTION A - (5x5=25 Marks)

Answer any FIVE questions

1. Explain the bioligical importance nucleic acids.

2. Discuss how DNA is sequenced enzymatically.

3. Write briefly on the role of sugars in biological recognition.

4. Describe the structure of cholestrol.

5. What are the different types of blue copper proteins? Describe their characteristics.

6. The convertion of carbonic acid to CO_2 and HO_2 is a natural process. Why is carbonic anhydrase needed Explain.

7. Describe how active transport across membrances takes place.

8. Discuss the significance of metallic enzymes.

SECTION B - (5X15=75 marks)

Answer any FIVE questions

9. Discuss in detail the mechanism of RNA transcription.

10. (a) Describe the major metabolic pathways of glycolysis.

    (b) Explain how fructose-6-phosphate is synthesised form ribose-5-phosphate in the biological system.

11. Discuss the structure and biological function of

    (a) Hemoglobin

    (b) Hemocyamin and

    (c) Rubredoxin

12. Compare porpyrin and Corrin rings. Mention some of the coenzyme B_{12} dependant enzymes and the conversion of each enzymes.

13. Give a critical account on the principles of function and structural organisation in bioenergetic fundamental reactions.

14. Write notes on:

    (a) Biophysical applications of Mossbauer effect.

    (b) PCR technique in chain amplification.

    (c) Metal in exchange activity of siderophores.

15. Discuss the structure of Cholorophyl. Bring out the role of PS(I) and PS(II) of cholophyl in photosynthesis.

16. (a) Discuss the invitro nitrogen fixation brought out by Ti and Mu complexes.

    (b) Explain the sequence of reaction taking place in the Kreb's cycle.

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