NUCLEIC ACIDS

Nucleic Acids (14-marks) **05 Hours**

Types, components of nucleic acids - nitrogenous bases (A, G, C, U, T – structures only), sugars present in nucleic acids (ribose and deoxyribose) and phosphate group.

Nucleosides and nucleotides – nomenclature and structures. Partial structure of polynucleotide's, structure of DNA (Watson and Crick model), biological importance of DNA, RNA – types and their biological roles (structures not required).

Central dogma of molecular biology (basic principles only), genetic code and its features, replication (semi conservative mechanism), brief mention of transcription and translation.

Introduction:

Defination: Nucleic acids are long-chain polymeric molecules, the monomer (the repeating unit) is known as the nucleotides and hence sometimes nucleic acids are referred to as polynucleotides.

There are prominently two types of nucleic acids known to us.

Deoxyribonucleic Acid (DNA)

Chemically, DNA is composed of a pentose sugar, phosphoric acid and some cyclic bases containing nitrogen. The sugar moiety present in DNA molecules is β-D-2-deoxyribose. The cyclic bases that have nitrogen in them are adenine (A), guanine (G), cytosine(C) and thymine (T). These bases and their arrangement in the molecules of DNA play an important role in the storage of information from one generation to the next one. DNA has a double-strand helical structure in which the strands are complementary to each other.

Ribonucleic Acid (RNA)

RNA molecule is also composed of phosphoric acid, a pentose sugar and some cyclic bases containing nitrogen. RNA has β-D-ribose in it as the sugar moiety. The heterocyclic bases present in RNA are adenine (A), guanine (G), cytosine(C) and uracil (U). In RNA the fourth base is different from that of a DNA. The RNA generally consists of a single strand which sometimes folds back; that results in a double helix structure.

Functions of Nucleic Acids

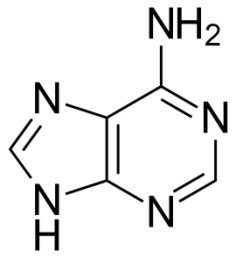
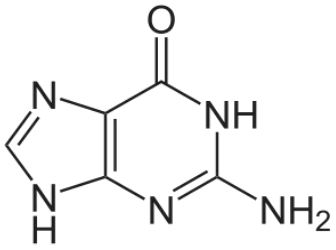
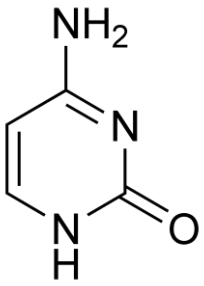
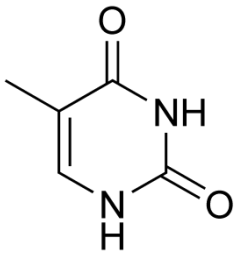
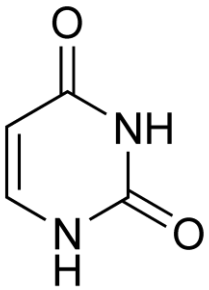
- Nucleic Acid is responsible for synthesis of protein in our body
- RNA is a vital component for protein synthesis.
- Loss of DNA content is linked to many diseases.
- DNA is an essential component required for transferring genes from parents to offspring.
- All the information of a cell is stored in DNA.
- DNA fingerprinting is a method used by forensic experts to determine paternity. It is also used for identification of criminals.

Components of Nucleic acid:

1) **Nitogenous bases :** Purines and pyrimidines are both organic compounds that take part in the synthesis of DNA and RNA, therefore they are called as the building blocks of the genetic materials.

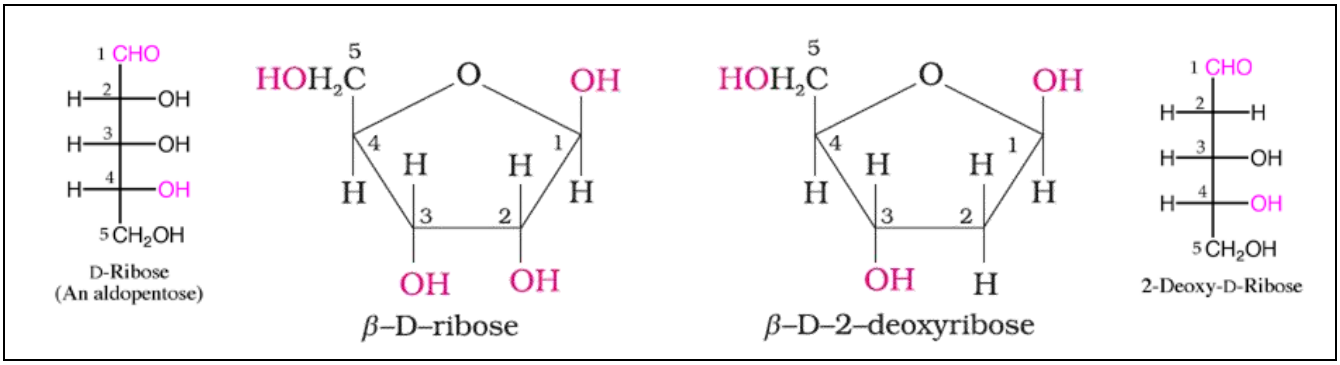
Purines	Pyrimidines
Purine is a heterocyclic aromatic organic compound composed of a pyrimidine ring fused with imidazole ring.	Pyrimidine is a heterocyclic aromatic organic compound that is composed of carbon and hydrogen.
It comprises adenine and guanine as nucleobases.	It comprises Cytosine, thymine, uracil as nucleobases
It consists of two hydrogen-carbon rings and four nitrogen atoms	It consists of one hydrogen-carbon ring and two nitrogen atoms
The melting point of purine is 214 °C	The melting point of pyrimidine is 20-22 °C

Structures:

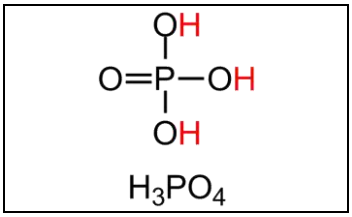
Purines		Pyrimidines		
Adenine	Guanine	Cytosine	Thymine	Uracil
				

2) Sugar: There are two types of sugars present in Nucleic acids.

The sugar moiety present in DNA molecules is β-D-2-deoxyribose where as RNA has β-D-ribose moiety.



3) Phosphoric acid residue: It is present in both DNA and RNA.



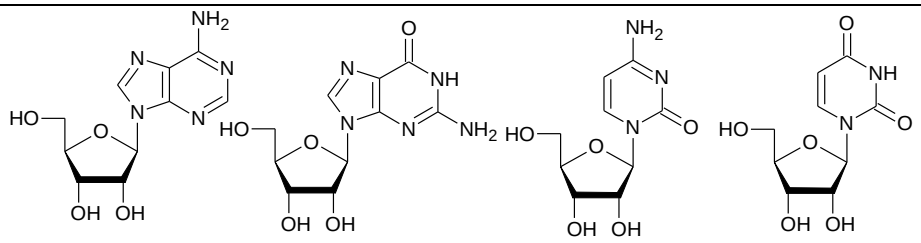
Nucleosides and Nucleotides:

- **Nucleosides**: a compound that consists of a purine or pyrimidine base combined with deoxyribose or ribose and is found especially in DNA or RNA. Examples: Adenosine, Guanosine.
- **Nucleotides**: Nucleotides are the building blocks of nucleic acids; they are composed of three sub unit molecules: a nitrogenous base (also known as nucleobase), a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group. Examples: AMP, ADT, ATP.

Nucleoside and Nucleotide nomenclature

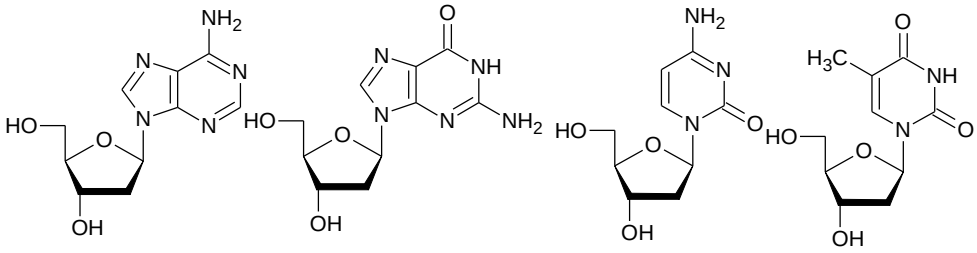
Base	Ribonucleoside	Ribonucleotide (5′-monophosphate)	Abbreviation
Adenine (A)	Adenosine	Adenosine 5′-monophosphate or adenylate	AMP
Guanine (G)	Guanosine	Guanosine 5′-monophosphate or guanylate	GMP
Cytosine (C)	Cytidine	Cytidine 5′-monophosphate or cytidylate	CMP
Uracil (U)	Uridine	Uridine 5′-monophosphate or uridylate	UMP
Base	**Deoxyribonucleoside**	**Deoxyribonucleotide (5′-monophosphate)**	**Abbreviation**
Adenine (A)	Deoxyadenosine	Deoxyadenosine 5′-monophosphate or deoxyadenylate	dAMP
Guanine (G)	Deoxyguanosine	Deoxyguanosine 5′-monophosphate or deoxyguanylate	dGMP
Cytosine (C)	Deoxycytidine	Deoxycytidine 5′-monophosphate or deoxycytidylate	dCMP
Thymine (T)	Deoxythymidine	Deoxythymidine 5′-monophosphate or deoxythymidylate	dTMP

Structures of Nucleosides of RNA



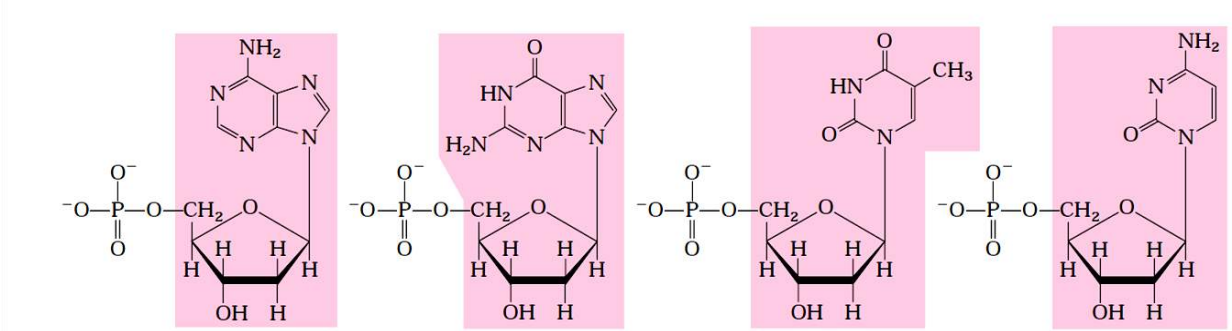
Adenosine Guanosine Cytidine Thymidine

Structures of Nucleosides of DNA



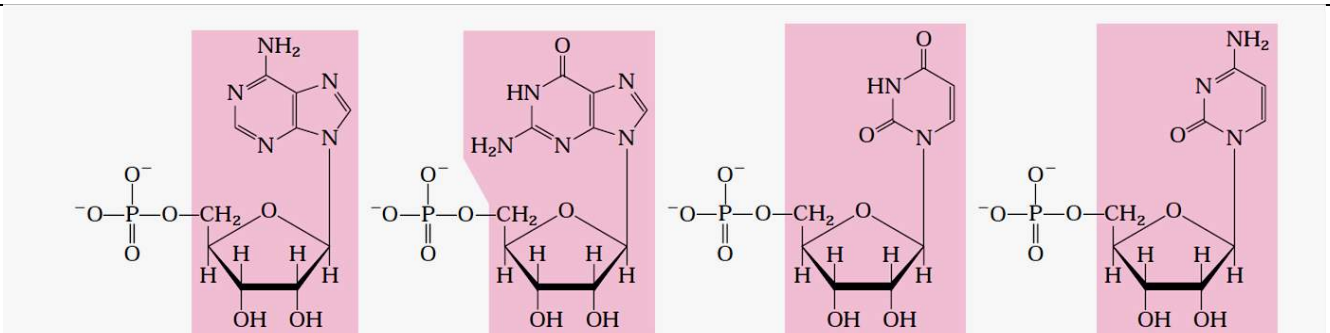
deoxy Adenosine deoxy Guanosine deoxy Cytidine deoxy Thymidine

Structures of Nucleotides of DNA

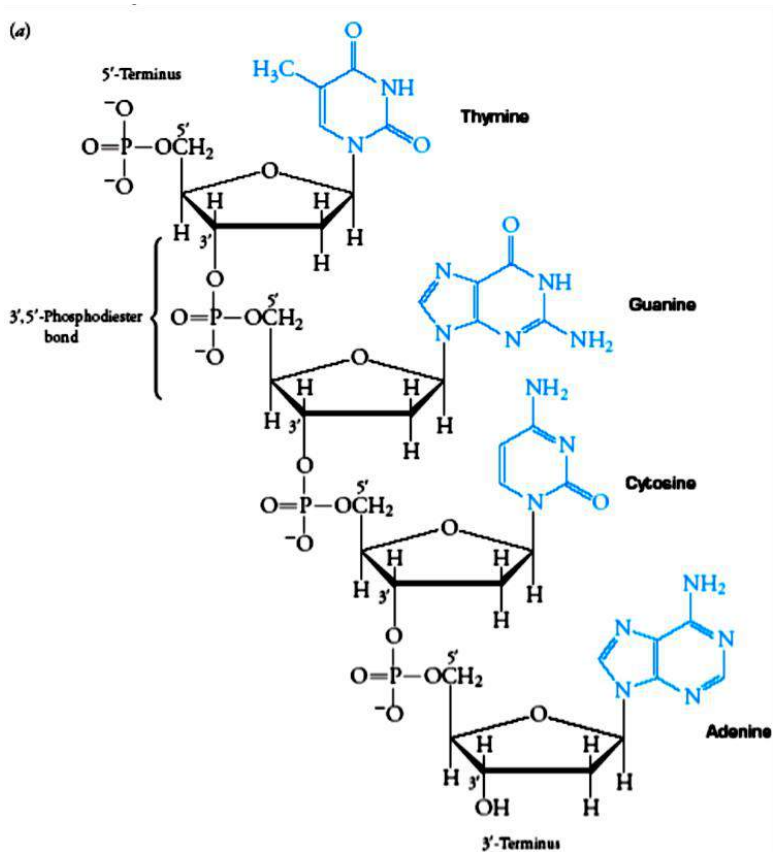
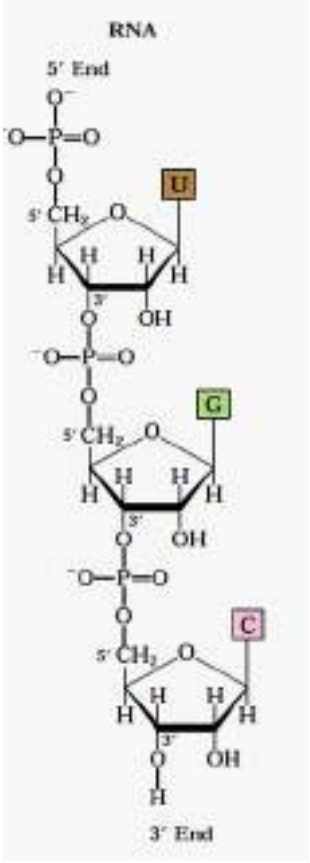


Nucleotide:	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

Structures of Nucleotides of RNA



Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

Partial structure of Polynucleotide of DNA:	Partial structure of Polynucleotide of RNA:
The monomeric deoxvnucleotides in DNA are held together by 3',5'-phosphodiester bridges.	The monomeric nucleotides in RNA are held together by 3',5'-phosphodiester bridges.
	

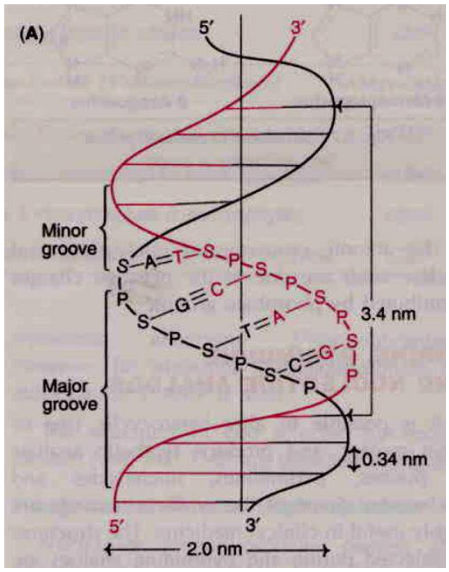
Watson – Crick Model:

In 1953, J.D. Watson (an American biologist) and F.H.C. Crick (a British Physicist) proposed the three-dimensional model of physiological DNA. For this Watson, Crick and Wilkins got Nobel Prize in medicine in 1962. Term DNA was given by Zaccharis.

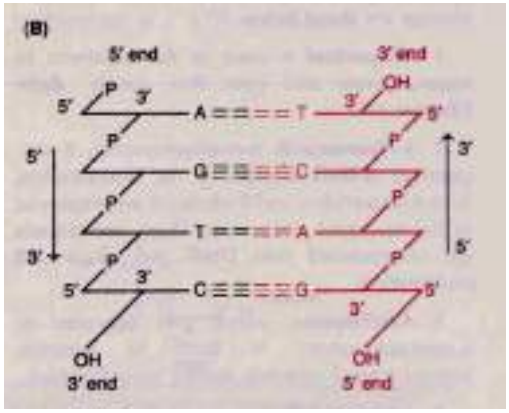
The important features of Watson – Crick Model or double helix model of DNA are as follows:

1. The DNA molecule consists of two polynucleotide chains or strands that spirally twisted around each other and coiled around a common axis to form a right-handed double-helix.
2. The two strands are antiparallel i.e. they ran in opposite directions so that the 3' end of one chain facing the 5' end of the other.
3. The sugar-phosphate backbones remain on the outside, while the core of the helix contains the purine and pyrimidine bases.
4. The two strands are held together by hydrogen bonds between the purine and pyrimidine bases of the opposite strands.
5. Adenine (A) always pairs with thymine (T) by two hydrogen bonds and guanine (G) always pairs with cytosine (C) by three hydrogen bonds. This complimentarily is known as the base pairing rule. Thus, the two stands are complementary to one another.
6. The base sequence along a polynucleotide chain is variable and a specific sequence of bases carries the genetic information.
7. The base compositions of DNA obey **Chargaff s rules**. According to which A=T and G=C; as a corollary purines (A+G) = pyrimidines (C+T); also (A+C) = (G+T). It also states that ratio of (A+T) and (G+C) is constant for a species.

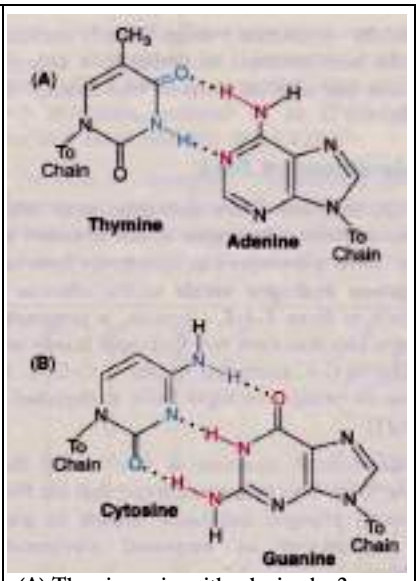
8. The diameter of DNA is 2 nm (20 A). Adjacent bases are separated 0.34nm (3.4 A) along the axis. The length of a complete turn of helix is 3.4nm (34 A) i.e. there are 10 base pairs per turn.
9. The DNA helix has a shallow groove called minor groove and a deep groove called major groove across.



(A) Watson-Crick model of DNA helix



(B) Complementary base pairing in DNA helix.



(A) Thymine pairs with adenine by 2 hydrogen bonds
(B) Cytosine pairs with guanine by 3 hydrogen bonds.

Complementary base paiing in DNA

Biological importance of DNA:

1. **Hereditary material:** The genetic information stored in the nucleotide sequence of DNA helps in synthesis of specific proteins or polypeptides and transmit the information to daughter cells or offspring's.
2. **Autocatalytic role DNA:**DNA undergoes replication (self-duplication) in the S-phase of cell cycle. During the process each DNA strand of a double helix can act as template for the synthesis of daughter strand.
3. **Hetero catalytic role:** During transcription any one strand of DNA acts as template for the synthesis of RNA. This is called the hetero catalytic role of RNA.
4. **Variations:** DNA undergoes recombination its meiosis and occasional mutation (changes in nucleotide sequences) which creates variations in population and ultimately contributes to evolution.
5. **DNA controls cellular metabolism, growth, and differentiation.**
6. **DNA finger printing (-DNA typing or profiling):** This technique is used to identify criminals, determine paternity, verification of immigrant etc.
7. **Recombinant DNA technology (Genetic engineering):** It involves the artificial cleaving and rejoining DNA sequences from two or more organisms to create recombinant DNA. This technology is employed for production of genetically modified organisms (GMOs), genetically modified foods (GMFs), vaccines, hormones, enzymes, clones etc.

Ribonucleic acid:

RNA is a polymer of ribonucleotides held together by 3',5'-phosphodiester bridges. Although RNA has certain similarities with DNA structure, they have specific differences

1. **Pentose:** The sugar in RNA is ribose in contrast to deoxyribose in DNA.
2. **Pyrimidine :** RNA contains the pyrimidine uracil in place of thymine (in DNA).
3. **Single strand:** RNA is usually a single stranded polynucleotide. However, this strand may fold at certain places to give a double stranded structure, if complementary base pairs are in close proximity.
4. **Chargaff's rule-not obeyed :** Due to the single-stranded nature, there is no specific relation between purine and pyrimidine contents. Thus the guanine content is not equal to cytosine (as is the case in DNA).

5. **Susceptibility to alkali hydrolysis:** Alkali can hydrolyse RNA to 2',3'-cyclic diesters.This is possible due to the presence of a hydroxyl group at 2' position. DNA cannot be subjected to alkali hydrolysis due to lack of this group.

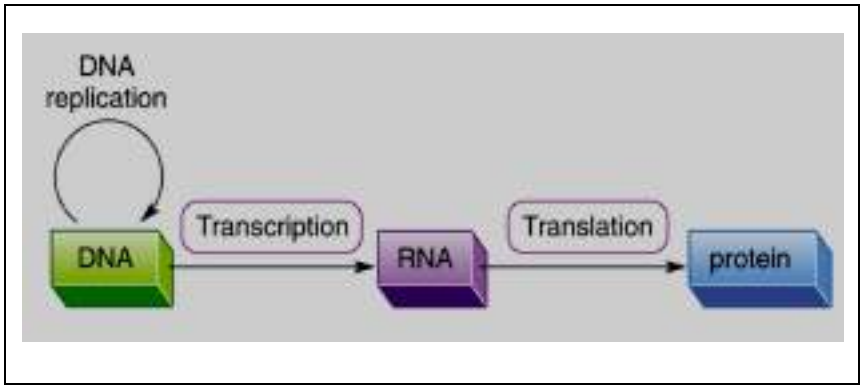
TYPES OF RNA:

The three major types of RNAs with respect to their cellular composition given below

1. **Messenger RNA** (**mRNA**):(5-1O %) functions as a carrier of genetic information from the DNA in the cell nucleus to the site of protein synthesis in the cytoplasm. mRNA has a short lifetime (usually less than one hour); it is synthesized as it is needed, then rapidly degraded to the constituent nucleotides.
2. **Ribosomal RNA** (**rRNA**): (80-85 %) the main component of ribosomes that are the site of protein synthesis. rRNA accounts for 80-85% of the total RNA of the cell. rRNA accounts for 65% of a ribosome's structure (the remaining 35% is protein).
3. **Transfer RNA** (**tRNA**) : (10-20%) delivers individual amino acids to the site of protein synthesis. tRNA is specific to one type of amino acid; cells contain at least one specific type of tRNA for each of the 20 common amino acids. tRNA is the smallest of the nucleic acids, with 73-93 nucleotides per chain.

Central dogma of molecular biology:

The **central dogma of molecular biology** states that ***"genetic information contained in the DNA is transferred to RNA molecules and then expressed in the structure of synthesized proteins".***



There are two steps in the flow of genetic information:

- **Transcription:** in eukaryotes, the DNA containing the stored information is in the nucleus of the cell, and protein synthesis occurs in the cytoplasm. The information stored in the DNA must be carried out of the nucleus by mRNA.
- **Translation:** mRNA serves as a template on which amino acids are assembled in the sequence necessary to produce the correct protein. The code carried by mRNA is translated into an amino acid sequence by tRNA.
- The communicative relationship between mRNA nucleotides and amino acids in a protein is called the **genetic code**.

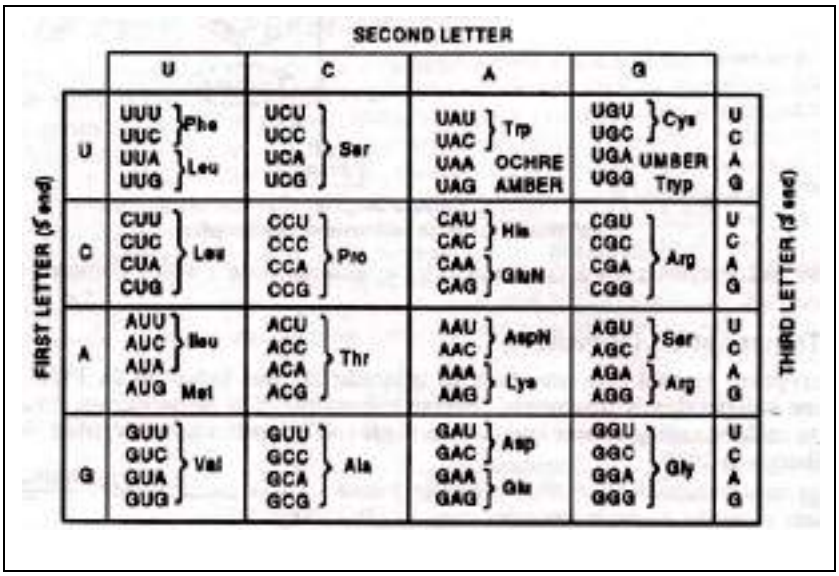
Genetic Code:

Genetic Code refers to the relationship between the sequence of nitrogenous bases (UCAG) in mRNA and the sequence of amino acids in a polypeptide chain. In other words, the relationship between the 4 letters language of nucleotides and twenty letters language of amino acids is known as genetic code.

Features of Genetic Code:

1. **Genetic code is triplet in nature:** The sequence of three nucleotides or nitrogen bases codes for one amino acid. Ex: AAA, UAC, AAU, etc.

2. **Genetic code is universal:** A particular codon codes for the same amino acid in all organisms from bacteria to higher plants and animals. Ex: AUG codes for *Methionine,* UUU codes for *phenylalanine*. (some exceptions in mitochondrial and protozoan codons)
3. **Genetic code is non-overlapping:** The nitrogen bases are read continuously in groups of three without sharing or overlapping.
4. **Genetic code is degenerate:** Most of the amino acids are coded by more than one codon, such codons are called degenerate or synonymous Codons and the phenomenon is called degeneracy. Ex: Alanine is coded by GCA, GCC, GCU and GCG.
5. **Genetic code is comma less:** The codons are read continuously from one end to other without any break or punctuation marks between the codons.

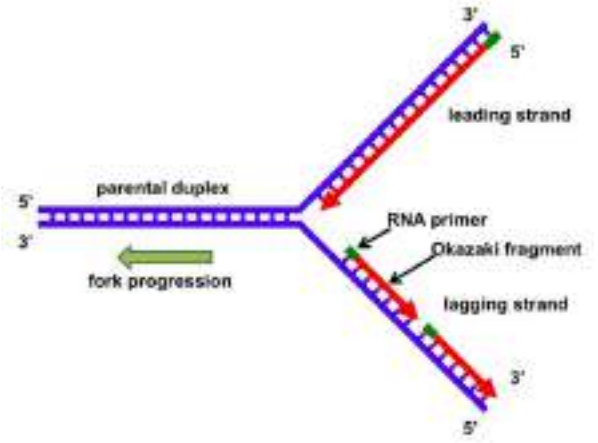


6. **Genetic code is non-ambiguous or specific:** A particular codon always codes for the same amino acid without any mistake this characteristic is called nonambiguity.
7. **Genetic code has an initiator codon:** The protein synthesis starts or initiates by a particular codon called initiator codon. Ex: AUG present near the 5' end of the m-RNA act as initiator codon in most of the organisms which codes for methionine. Therefore methionine is the first amino acid in most of the proteins. Rarely GUG act as initiator codon in some bacteria which codes for formyl-methionine.
8. **Genetic code has non-sense or terminator codons:** The codons which do not code for any amino acid and signal the termination of protein synthesis are called non-sense codons. Ex: UAA, UAG and UGA.
9. **Principle of co linearity:** The linear order of the nitrogen bases in DNA determines the linear order of m-RNA codons. This in turn determines the linear order of amino acids in a polypeptide. This principle is called co linearity.

Semi conservative replication of DNA:

The replication occurs during **S-phase** of Inter phase during cell cycle. The process of replication is proved qualitatively by **J.Herbert Tayler** and quantitatively by **Meselson and Stahl**.

Mechanism: The process of replication involves the following steps.



- **Activation of nucleotides:** The nucleotides of DNA such as d-AMP, d-TMP, d-GMP and d-CMP are activated and phosphorylated by ATP in to d-ATP, d-TTP, d-GTP and d-CTP respectively.
- **Unwinding of DNA helix:** The initiation of replication or uncoiling of the DNA helix starts at a specific point called origin of replication.The unwinding of DNA strands is catalyzed by Helicases. DNA Gyrases (Topoisomerases) remove the coils that accumulate in front of the replication fork. The separation of DNA strands during the initiation of replication forms a Y-shaped structure called **replication fork.** The separated DNA strands act as master strands or template strands for the formation of new strands.

- **Formation of RNA-primer:** The synthesis of new strand always proceeds in 5'"3' direction. During the initiation of replication a short segment of RNA is synthesized with the help of an enzyme RNA primase called RNA primer.
- **Initiation and elongation of DNA strand:** The DNA nucleotides are now added to exposed bases of parental DNA strand from the end of RNA primer. This process is catalyzed by DNA Polymerase III and Mg^{+2}. The addition of nucleotides of DNA proceeds only in 5'"3' direction. The two new strands of DNA produced in opposite or antiparallel direction called bidirectional replication. In one strand the synthesis of new DNA strand goes on continuously in 5'"3' direction and this new strand is called leading strand. In the opposite strand (3'"5') the addition of nucleotides proceeds as short segments away from the replication fork called lagging strand. The short single stranded fragments of DNA of the lagging strand are called Okazaki fragments. The lagging strand has many RNA primers. Later the RNA primers are removed and replaced by DNA nucleotides by an enzyme DNA polymerase I. The Okazaki fragments are joined by DNA Ligase enzyme.
- **Termination of replication:** The termination of replication is signaled by specific sequence of DNA nucleotides. After replication the DNA polymerase II takes an editing role to remove abnormal nitrogen bases and incorporate the normal bases **(proof reading**). This process is called **genetic repair mechanism**.

Transcription:

The process of copying genetic information from one strand of the DNA into RNA is called transcription. (The biosynthesis of RNA from DNA is called **transcription**.)

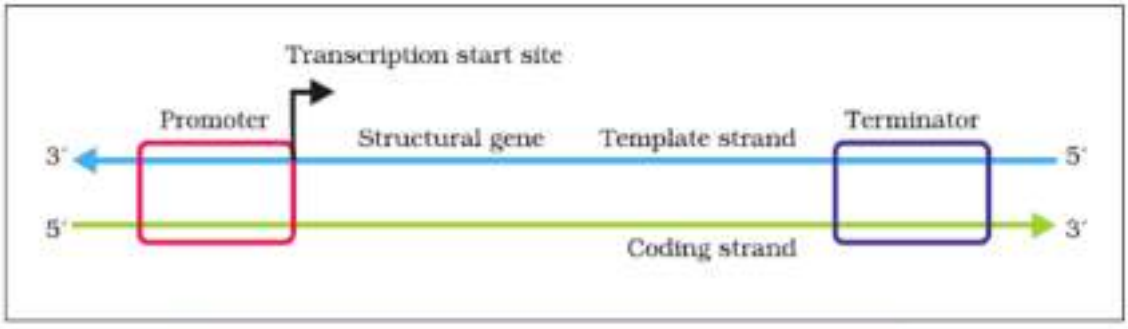


Figure 6.9 Schematic structure of a transcription unit

1. The transcription unit of DNA consists of three regions as a **promoter, structural gene** and **a terminator.**
2. The transcription begins by the uncoiling of DNA strands due to the breakage of hydrogen bonds.
3. After the unwinding **DNA dependent RNA polymerase** is only capable of catalyzing the process of elongation in association with **initiation – factor** (**σ**)**.** It binds to promoter and initiate transcription.
4. One of the strand of DNA (3'"5' strand) act as a template to produce RNA by complementary base arrangement is called **antisense strand**
5. The strand of DNA which bears the same sequence as the RNA and not used as template during transcription is called **sense strand** or **coding strand**.
6. The nucleotides of RNA are attracted and assembled complementary to template in the presence of DNA dependent **RNA-polymerase and Mg^{+}.** Only a short stretch of RNA remains bound to the enzyme.
7. The termination of RNA chain is brought about by certain terminator sequences on DNA & **termination factor** (**ϱ**).
8. Finally the new RNA formed and RNA-polymerase gets detached from the DNA. Again the two strands of DNA rewind by the hydrogen bonds.

Translation:

Translation refers to the process of polymerization of amino acids to form a polypeptide. The order and sequence of amino acids are defined by the sequence of bases in the mRNA.

1. The amino acids are joined by a bond which is known as a peptide bond. Formation of a peptide bond requires energy.
2. Therefore, in the first phase itself amino acids are activated in the presence of ATP and linked to their cognate tRNA–a process commonly called as charging of tRNA or aminoacylation of tRNA to be more specific. If two such charged tRNAs are brought close enough, the formation of peptide bond.
3. The presence of a catalyst would enhance the rate of peptide bond formation. The cellular factory responsible for synthesizing proteins is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins.
4. Ribosome exists as two subunits; a large subunit and a small subunit. Total number of triplet codons =64 Number of sense codons =61 Number of non-sense codons =03(UAA, UAG & UGA)
5. When the small subunit encounters an mRNA, the process of translation of the mRNA to protein begins. For initiation, the ribosome binds to the mRNA at the start codon (AUG) that is recognized only by the initiator tRNA.
6. There are two sites in the large subunit, for subsequent amino acids to bind to and thus, be close enough to each other for the formation of a peptide bond. Themribosome also acts as a catalyst (23S rRNA in bacteria is the enzyme- ribozyme) for the formation of peptide bond. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one.
7. At the end, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.

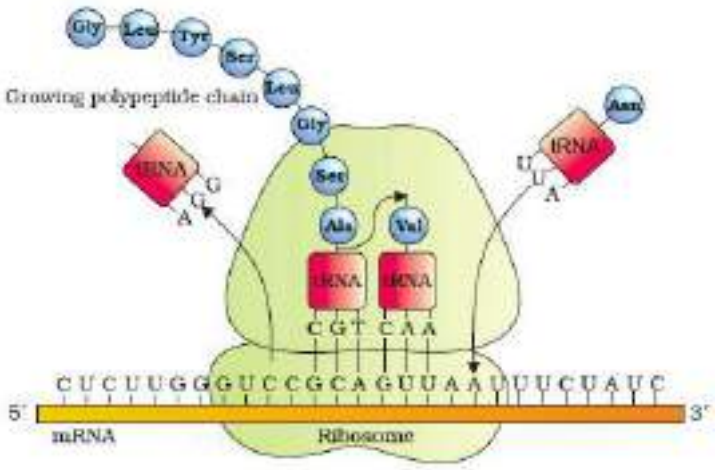


Figure 6.13 Translation

8. An mRNA also has some additional sequences that are not translated and are referred as untranslated regions (UTR). The UTRs are present at both 5′ –end (before start codon) and at 3′-end (after stop codon). They are required for efficient translation process.

Previous year questions:

1. Write the partial structure of polyribonucleotide. **[3M;2019]**
2. What are nucleosides and nucleotides? Write the structure of AMP and CMP. **[4M;2015,2016,2019]**
3. Explain central dogma of molecular biology. **[3M;2015]**
4. Discuss smemiconservative method of DNA replication. **[3M;2019]**
5. Write the structure of heterocycle presnt only in DNA. **[2M; 2015,2016]**
6. Two strands of DNA are complimentary. Justify. **[3M;2015]**
7. Explain Watson and Crick model of DNA. **[3M; 2014]**
8. What are polynucleotides? Give their classification. **[3M;2015]**
9. What is chargaff's rule of base equivalence? **[3M;2014,2016,2018]**
10. Write any two types of RNA and their functions? **[3M;2016,2018]**
11. Write the structures of polynucleotides? **[4M;2018]**
12. What is meant by transcription? **[2M;2018]**
13. Explain the terms leading strand and lagging strands in DNA replication. **[3M;2016,2018]**
14. 'Genetic code is said to be degenerate and universal" justify. **[3M;2016]**
15. What are Okazaki fragments? **[2M;2016]**

IV B

ENZYMES

Enzymes are biological catalysts, which catalyses the various biological reactions under physiological conditions.

Chemical Nature of Enzymes

Chemically enzymes are proteins in nature. Some enzymes are simple proteins and most of the enzymes are conjugated proteins. The amino acid part and the prosthetic group in conjugated proteins must be present together for the catalytic activity of the enzymes.

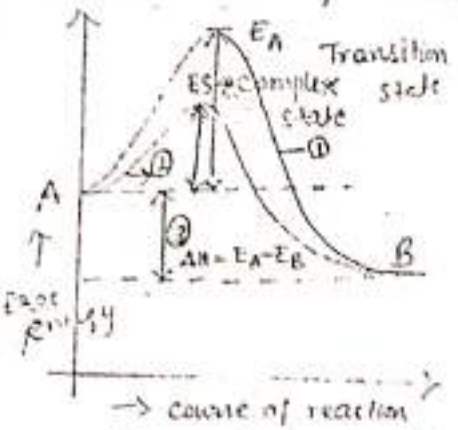
Nature of enzyme action

An enzyme is a catalyst which accelerates both forward and backward reaction to the same extent.

$$A \underset{B}{\overset{E}{\rightleftharpoons}} P$$

The reactant molecules must acquire sufficient energy called Energy of activation or Activation Energy (E_a) to attain an activated cond^n called Transition cond^n before they are converted into products.

Enzyme can act as substrate (reactant) molecule by lowering the Energy of activation with the formation of Enzyme–Substrate complex (ES–complex). This ES–complex breaks to form products and free energy.



curve (1) is the energy of activation for uncatalysed reaction. Curve (2) is the Energy of activation for catalyzed reaction.

$E_A - E_B$ ie (3) is the difference in energy levels between reactants & products.

$(E_A - E_B)$ is the heat of the reaction.

Enzyme Specificity :- The most striking property of an enzyme is its specificity.

There are 4 types of Enzyme specificities

I ABSOLUTE SPECIFICITY :-

Some enzymes can act only on one substrate, such enzymes are said to exhibit absolute specificity

EX: Succinic dehydrogenase (SDH) catalyses only the oxidation of succinate to fumarate.

Succinate

Fumarate.

II. HIGH SPECIFICITY

Some enzymes can act on a very small group of compds such enzymes are said to exhibit high specificity.

D- Glucose + ATP $\underset{\text{Hexokinase}}{\rightleftharpoons}$ D- Glucose - 6- phosphate + ADP

Hexokinase not only phosphorylate Glucose, but also other hexoses like Fructose, Galactose, mannose etc.

III LOW SPECIFICITY

Some enzymes act on fairly wide range of related compds such enzymes are said to exhibit low specificity.

EX: Lipases : catalyses the hydrolysis of ester bonds in large nos of oils & fats.

IV STEREO CHEMICAL SPECIFICITY

Some enzymes can act only on a particular stereo isomer of the compd, but not on the other isomers of the same compd. This type of specificity is called stereo chemical specificity.

EX: Lactate dehydrogenase (LDH) will catalyze the oxd^n of only L- Lactate, but not on D- Lactate.

L Lactate

Pyruvate

Active Sites

During the interaction of enzyme and substrate (reactant) these substrate interact at only a small specific portion or site on the surface of enzyme. This site is called active sites.

The characteristic features of active sites are,

1) The active site is a relatively small portion on the surface of the enzyme

2) It is a specific 3 dimensional region having unique arrangement of Amino-acid side chains.

3) Some side chains in the active site are involved in binding the substrate and some others bring out the catalysis

4) The substrate is bounded to enzyme active site by weak forces like hydrophobic forces, H_2 bonds electrostatic interaction etc.

Interaction of Enzyme & Substrate :-

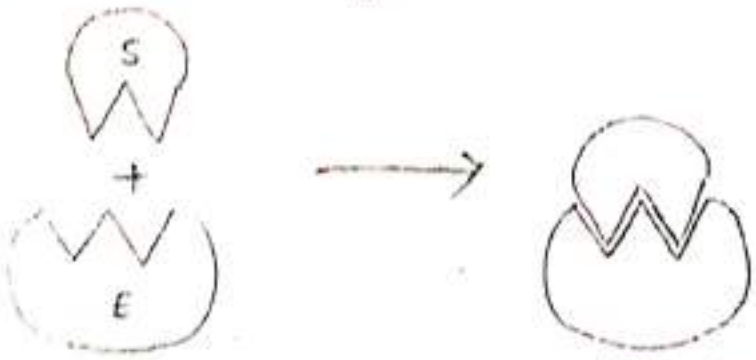
2 theories have been put forward to explain the interaction of enzyme & substrate.

I. Fisher lock & key Model

II. Koshland induced fit theory.

I. FISHER LOCK AND KEY MODEL :-

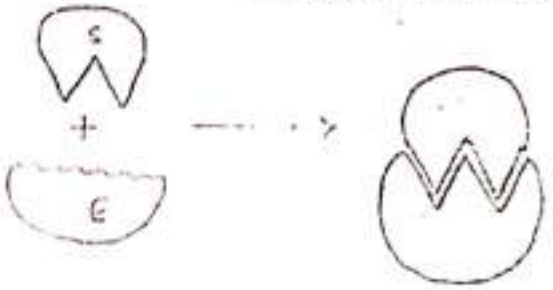
As a particular key fits into a particular lock, a particular substrate fit into specific enzymes to form ES-complex. A substrate must have matching shape to fit into active site. In this case the enzyme is considered to be rigid molecule which is having a structural resemblance as that of substrate. Then the substrate can easily fit into the active site of the Enzyme.



ES- Complex

II. KOSHLAND INDUCED FIT THEORY :-

According to this theory the active site of an enzyme is considered to be flexible and the shape of the active site is modified when the substrate binds to the active site takes the shape in resemblance as that of substrate.



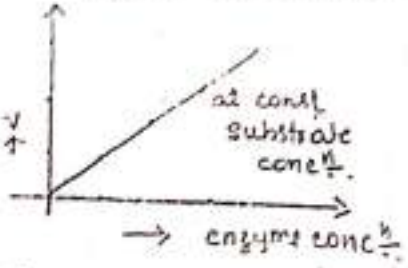
ES- complex

Factors affecting the rate of enzyme catalyse reaction :-

The important factors affecting the rate of enzyme catalyse reaction are

1) Effect of enzyme conc^{n} [E]
2) Effect of Tempr [T]
3) Effect of pH.
4) Effect of substrate conc^{n} [S]

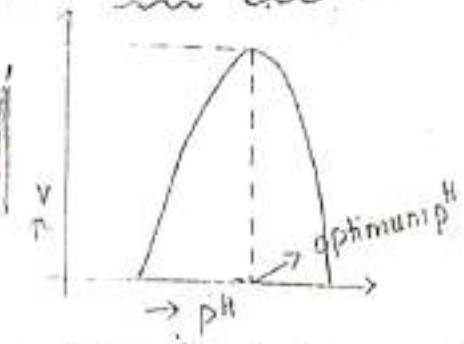
I Effect of enzyme conc^{n} :- As the conc^{n} of enzyme increases, the rate of the reaction also increases. This is due to the increase in no of active sites ie the rate of the reaction is directly $\propto^{nal}$ to the conc^{n} of Enzymes.



II Effect of Tempr :- increase in tempr, increases the rate of the reaction. This happens upto certain max. tempr called optimum tempr (30-35°C) beyond this tempr the activity of enzyme is destroyed due to denaturation of enzyme. This results in the decrease in rate of the reaction with increase in tempr further.

optimum temp^{n} of an enzyme is the temp^{r} at which the enzyme activity is maximum.

III. Effect of pH :-

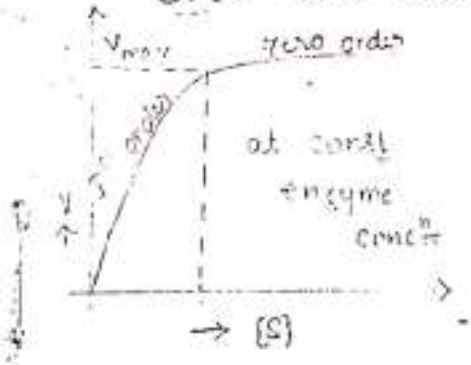


The rate of the enzyme catalyse reaction is dependent on pH of the medium. at extreme (low & high) pH values enzyme undergoes denaturation as a result the rate of the r^{n} is slow at very low & high pH values.

The rate of the reaction is max. at particular pH known as optimum pH. Each enzyme will have its own optimum pH

EX: Pepsin : 2 & Diastase : 7

IV Effect of substrate conc^{n} : The rate of the reaction is increases with increase in substrate conc^{n} upto certain level. After which the rate of the reaction is const with further increase in conc^{n}. ∵ the conc^{n} of the substrate is sufficient to saturate the enzymes.



Michaelis and menten's Eqn :-

According to michaelis - menten's hypothesis, substrate combines with enzyme to form ES- complex which breaks to form product & liberates the free enzyme.

$$E + S \rightleftharpoons ES\text{-complex} \longrightarrow E + P$$

Michaelis and menten derived an eqn called michaelis - menten Eqn which relates the initial velocity (V_o), maximum velocity (V_{max}) and initial substrate conc^{n} [s] thro' the michaelis-menten const (K_m)

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

If the initial velocity V_0 of the reaction becomes half of the V_{max}, ie $V_0 = \frac{1}{2} V_{max}$

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

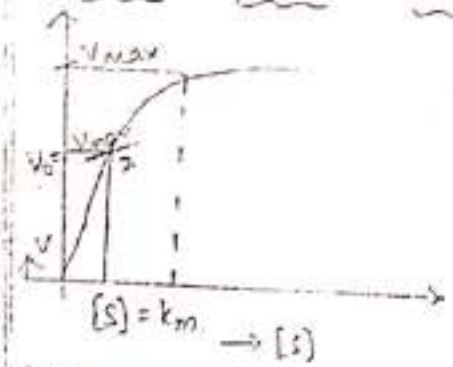
$$\Rightarrow K_m + [S] = 2[S] \Rightarrow \boxed{K_m = [S]}$$

Thus the michaelis-menten const K_m is equal to conc^n of substrate [S] at which the rate is half of the maximum velocity.

Significance of K_m :- K_m is the measure of the affinity of an enzyme by substrate. Lower the K_m value greater will be the rate of the reaction.

Determination of K_m :-

From the graph show V_0, V_{max}, K_m, [S] & mark.



The rate of the reaction at different concn^s of substrate are measured. The reaction rate increases with increase in concn^n in the beginning and remains const^t afterwards. Correspondingly is the max. velocity. If the reaction rate is plotted against substrate concn^n the above graph is obtained. The substrate concn^n where the velocity of the reaction is half of the maximum velocity will give the value of K_m.

Coenzymes :-

Some enzymes are simple proteins consisting only polypeptide chains (Amino acid)

Ex: Pepsin, Trypsin.

The name co-enzyme is applied to a non-protein (prothestic group) acting together with protein part in conjugated proteins such an enzyme is called Holoenzyme. Holoenzyme dissociates into a protein component called Apoenzyme and non-protein

component called co-factor.

Holoenzyme active : Apoenzyme as inactive + co-factor inactive inactive If the co-factor is covalently bonded to the enzyme then it is called prosthetic group.

If the co-factor is non-covalently bonded then it is called co-enzyme.

Biological Importance of co-enzyme :-

Coenzyme acts as carriers of chemical groups. The co-enzyme & the type of the reaction in which they participate are as follows

	VITAMIN	COENZYME	BIOLOGICAL FUNCTION
1)	Niacin [Nicotinic acid]	NAD^+, $NADP^+$	It acts as H_2 or $\bar{e}$ carrier
2)	Riboflavin [V_{B_2}]	FAD, FMN	——"——
3)	Thiamine [V_{B_1}]	TPP	oxidative decarboxylation
4)	Pentathonic acid	Coe. A	Acyl group carriers
5)	Pyridoxine	Pyridoxal-phosphate	Amino group transfer
6)	Folic acid	Tetra hydro folic acid	one carbon transfer
7)	Cobal amine (cobalt v--)	Cobamide co-enzyme	Transmethylation.

NAD^+ → Nicotinamide adenine dinucleotide in oxidised form.
$NADP^+$ → Nicotinamide adenine dinucleotide phosphate in oxidised form
FAD → flavin adenine dinucleotide
FMN → flavin mono Nucleotide
TPP → Thiamine pyro phosphate. - —

Activators and inhibitors :-

Substances which are on adding to an enzyme catalyze reaction increases the catalytic activity are known as activators and the phenomenon is called enzyme activation.

Eg : Mg^{2+} ions acts as activators in almost all the reaction involving ATP.

substances which are on adding to an enzyme catalysed rn decreases the catalytic activity are known as inhibitors and the phenomenon is known as inhibition.

Types of inhibition :-

Depending upon the nature of inhibitor the inhibition can be classified into 2 types.

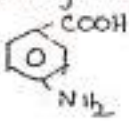
competitive inhibition

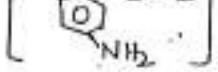
In competitive inhibition, the structure of the inhibitor closely resembles with that of substrate then the inhibitor combines with the active sites of the enzyme form EI- complex. This complex is inert and not converted into products. In this way the inhibitors competes with enzyme and thus inhibit the enzyme activity.

$$E + S \longrightarrow ES - complex \longrightarrow P + E$$

$$E + I \longrightarrow EI - complex \not\longrightarrow P$$

Inert

Eg: certain pathogenic bacteria uses PABA [Para amino Benzoic acid]  to synthesis folic acid which acts as a food for pathogenic bacteria.

Sulpha drugs such as sulphanilide  having the similar str. of PABA competes with it to combine with enzyme & thus prevents the formation of folic acid which acts as a food for pathogenic bacteria. hence the disease causing pathogenic bacteria will die due to lack of food. This is the mechanism of sulpha drug action. Thus competitive inhibition plays a very important role in chemotheraphy [Treatment and curing of diseases]

non - competitive inhibition :-

In non - competitive inhibition no competition occur b/n the substrate and inhibitors to combine with enzymes. The inhibitors has no resemblance with substrate. & the inhibitor bonds to enzyme other than the active site to form ESI complex or EI - complex which are inert and not converted into products.

$E + I \longrightarrow EI$ - complex $\not\rightarrow$ products

$E + S + I \longrightarrow ESI$ - complex $\not\rightarrow$ products.

USES of Enzyme inhibition studies :-

1) Enzyme inhibition studies and the Enzyme inhibitors are used as drugs, antibodies, preservatives, insecticides etc.

2) The inhibition of Enzyme activity can be used to regulate the activity of enzyme inside the cells.

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Respiratory chain phosphorylation and oxidative phosphorylation are same?

Respiratory chain corresponds to oxidative chain. Respiratory chain phosphorylation takes place in respiratory chain which is present in the mitochondria of mitochondria. So Respiratory phosphorylation and oxidative phosphorylation both are same.

Bioinorganic aspects of Sodium and Potassium

Sodium:

Sodium and potassium are present in enormous amounts in most foods and deficiencies due to these elements are rare. Na^+ is the principal extracellular cation, K^+ is the principal intracellular cation. These elements are very important in regulation of water and electrolyte balance and of acid-base balance in the body. They are regulated by the mineralocorticoid hormones of the adrenal cortex.

The actual nutritional requirement for sodium is only about 1 g/day and that of potassium is 4g/day. In take. We consuming sodium as Na sodium chloride salt. and potassium mainly present in tomato juice, citrus fruit and bananas.

In human body, Na^+ and K^+ are major components of urine. The solutes including NH_4^+, K^+ and phosphate occurs in relatively high concentration in urine compared with blood. These solutes are actively transported from the blood into the tubules, also against a concentration gradient. The transport of Na^+ and K^+ is especially important in the kidney which must preserve the proper concentration

of these vital cations in the body by conserving Na^+ and excreting K^+. All mammalian cells contain a relatively high concentration of K^+ and a low concentration of Na^+, whereas the blood plasma and most other extracellular fluids have a high concentration of Na^+ and low K^+. The plasma membranes of most cells contain $Na^+ K^+$ ATPase, which carries K^+ into cells and simultaneously transports Na^+ out. This energy-dependent process is coupled to hydrolysis of cytosolic ATP to ADP and phosphate. The $Na^+ K^+$ ATPase of the tubule cells functions in such a way as to allow constant loss of K^+ in the urine, whereas loss of Na^+ can be kept to very low levels.

Through the action of the Na^+/L^+ transporting ATPase as well as other energy requiring membrane transport systems for glucose and amino acids, the urine is so formed that those substances whose concentration in the blood must be lowered are excreted and those substances required are reabsorbed from the tubules.

~~The concentration of Na^+ ions in urine is~~

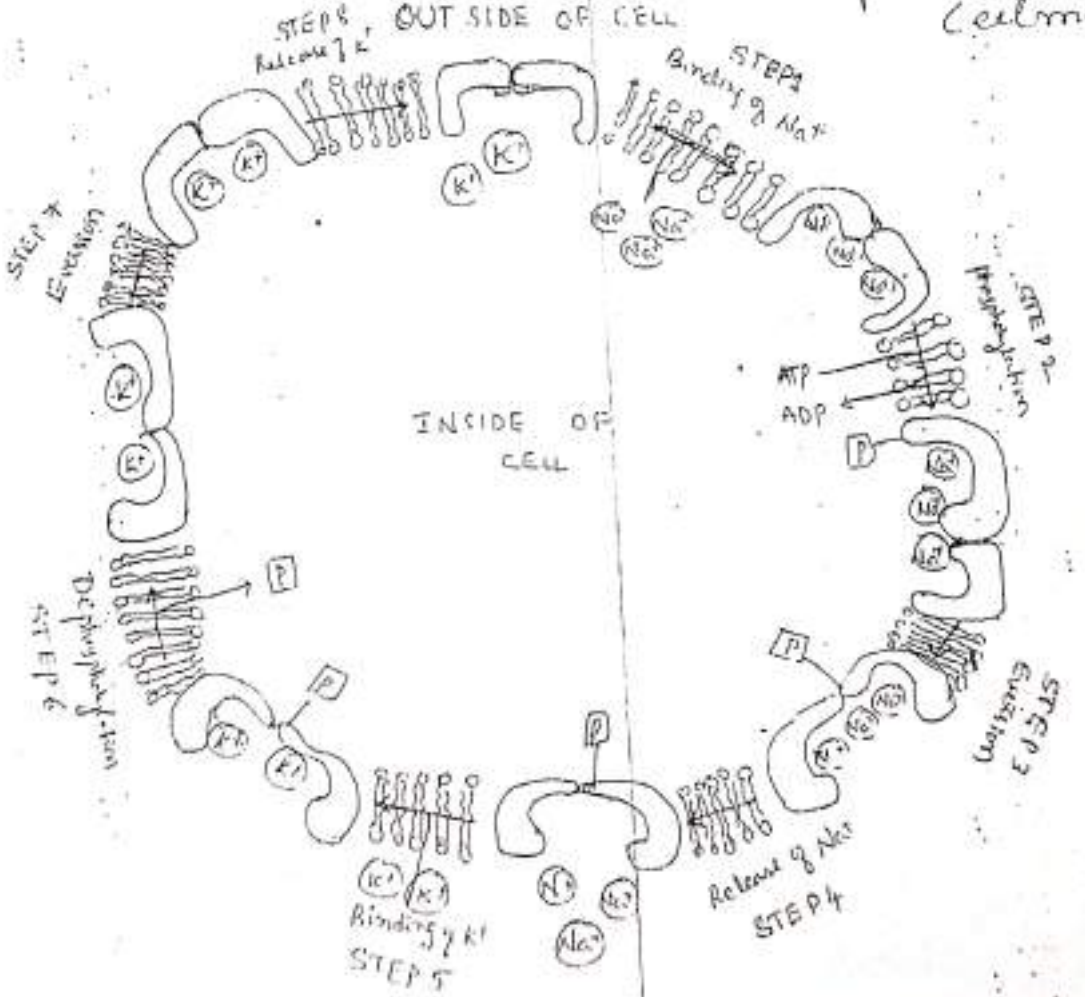
The Transport Mechanism [Na^+/K^+ pump]:

Gradients of ions across membranes are used to store energy and information. Two types of proteins are essential for the functioning of these systems, namely those involved in pumps and those comprising channels. Pumps function to couple the transport of ions across membranes with concomitant interconversion of ATP and ADP. Channels transport ions across membranes driven by ionic electrochemical gradients. They differ from pumps in that the transport of ions is not directly coupled to ATP hydrolysis or synthesis.

The generation of ionic gradients is an energy-dependent process, where the energy utilized is supplied by the hydrolysis of ATP to ADP & Pi. The mechanism responsible for generation of the Na^+ & K^+ gradients is the enzyme $Na^+ - K^+$ ATPase. The enzyme catalyzes the process as shown in below equation.

$$3\,Na^+_{in} + 2\,K^+_{out} + ATP + H_2O \rightleftharpoons 3\,Na^+_{out} + 2\,K^+_{in} + ADP + Pi$$

The outline of the mechanism is shown below



Scheme depicting the steps in the ATP-dependent export of three sodium ions and the concomitant import of two K^+ ions by the $Na^+ - K^+ - ATPase$

It involves the net transport of charge across the membrane three sodium ions are transported out for every two potassium ions pumped in. Sodium binding to the inner side of the membrane facilitates phosphorylation of a specific aspartic acid residue on the enzyme. This process is then induces a conformational change that leads to transport of the three Na^+ ions across the membrane. Next potassium binding to the on the outer membrane catalyzes dephosphorylation of the enzyme, followed by the inverse conformational change to transport the two K^+ ions into the cell.

Biological Functions and Toxicity of Na and K

Sodium:

Biological Functions: Important in nerve functioning in animals, major cation of extracellular fluid in animals.

Toxicity :- Relatively harmless except in excessive amounts. It is associated with some forms of hypertension.

Potassium:

Functions: Essential to all organisms with the possible exception of blue-green algae. Major cation in the intracellular fluid in animals; Essential for transmission of nerve impulse and cardiac function

Toxicity: Extremely toxic to mammals when injected intravenously

THE LIGANDS OF ALKALI CATIONS

The ligands of alkali metal ions may be classified into two broad categories. They are

(1) Synthetic complexing agents

(2) Naturally occurring ionophores.

Bioinorganic chemistry of Calcium and Magnesium

The adult human body contains more than a kilogram of calcium nearly all of which is in bones and teeth together with phosphate as the insoluble crystalline mineral calcium hydroxyapatite. Calcium also plays an important role in cells as an intracellular regulator or messenger. It helps to regulate the activity of skeletal muscle, the heart and many other tissues. Calcium is very abundant in foods, particularly in milk and cheese and also in cereal grains, legumes, nuts, and vegetables. Nevertheless it is of great importance in human nutrition because of the very high requirement for calcium during infancy and childhood, when the skeletal is actively growing, and during pregnancy and lactation.

Bone constitute a very large and labile reservoir of calcium that can be drawn upon when calcium is low in the diet. The calcium in bones is not permanently laid down, much of it is constantly undergoing turnover. About 700 mg to 800 mg of calcium may leave and enter the skeletal mass per day

The recommended daily allowance of calcium for adults is 800 mg/d and 1200 mg/d is recommended for women during pregnancy and lactation, and for teenagers.

Magnesium

The body contains about 25 g of magnesium, most of which is present in the bones. All cells contain rather high concentrations of magnesium. Mg^{2+} ions play a very important role in the action of many enzymes, particularly those

of glycolysis and many ATP-dependent reactions. The recommended magnesium intake is 350 mg/d for adults.

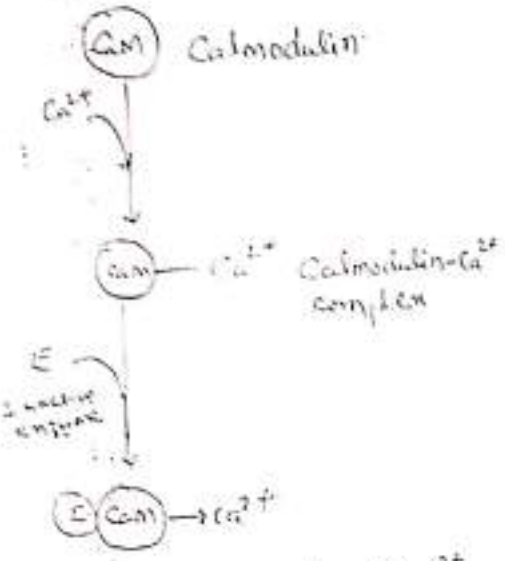
Binding of calcium

Calmodulin is a monomeric protein consisting of a chain of 148 amino acids that is capable of binding up to four Ca^{2+} ions. Each calcium is seven-coordinate, with three monodentate aspartate or aspara-gine residues, one bidentate glutamate residue, one coordinated peptide carbonyl group, and one bound water molecule. The nature of these coordination sites is important for metal-binding specificity. Calcium-responsive proteins must specifically recognize calcium in the presence of relatively high concentrations of other metal ions such as Mg^{2+}. The high coordination numbers produced by the structure of calmodulin favors Ca^{2+}-binding over Mg^{2+} binding.

The structure of the entire calmodulin molecule as determined by X-ray crystallographic methods is as shown in figure. The molecule is slightly strikingly dumbbell-shaped with a long, exposed alpha helix connecting the two pairs of EF-hand (α-helix) domains.

In overall calmodulin transmits the message called by an increase Ca^{2+} in the cytosol by forming Ca^{2+}-calmodulin complex, which then binds to the specific Ca^{2+}-regulated proteins & enzymes ie enzymes, such as protein-kinases, cAMP kinases, and phosphodiesterases and Ca^{2+} pumping ATPases and stimulating their activity.

Fig.



Active enzyme – Calmodulin-Ca^{2+} Complex

Calmodulin is mediator in many Ca^{2+} stimulated enzymatic and membrane transport systems.

Calcium → sending nerves signals
↳ muscle contraction

Calmodulin → detects the Ca^{+2} ion levels & sends signals to different enzymes, ion channels, or proteins

Calcium transport (Ca^{2+} ATPase) / Ca^{2+} pump]

Calcium ion plays an important role in the regulation of muscle contraction. Skeletal muscle contains an intricate network of membrane-bound tubules and vesicles. This membrane system called the sarcoplasmic reticulum regulates the Ca^{2+} concentration surrounding the contractile fibres of the muscle. At rest Ca^{2+} is pumped into the sarcoplasmic reticulum so that the Ca^{2+} concentration around the muscle fibers is very low. Excitation of the sarcoplasmic reticulum membrane by a nerve impulse leads to a sudden release of large amount of Ca^{2+}, which triggers muscle contraction. In other words, Ca^{2+} is the intermediate between the nerve impulse and muscle contraction.

The transport of Ca^{2+} by the sarcoplasmic reticulum is driven by the hydrolysis of ATP. There is an ATPase in the sarcoplasmic reticulum that is activated by Ca^{2+}. This Ca^{2+} ATPase is an integral part of the Ca^{2+} pump, just as the Na^+-K^+ ATPase is part of the Na^+-K-pump. The Ca^{2+} ATPase is also transiently phosphorylated by ATP.

$$E + ATP \underset{}{\overset{Ca^{2+},\, Mg^{2+}}{\rightleftharpoons}} E-P + ADP$$

$$E-P + H_2O \longrightarrow E + P_i$$

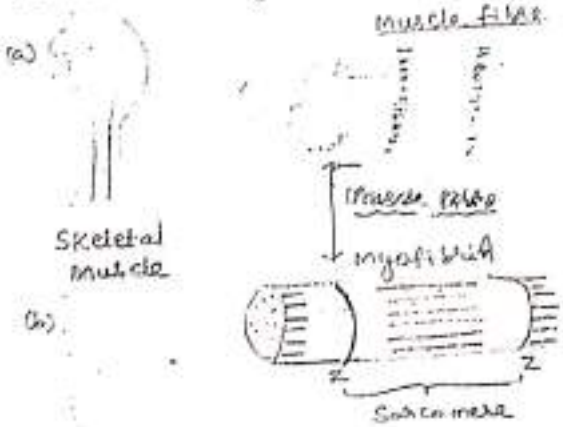
The very high affinity of this ATPases for Ca^{2+} ($K_m \approx 10^{-7}$ M) enables it to effectively transport Ca^{2+} from the cytosol (where $[Ca^{2+}] < 10^{-5}$ M) into the sarcoplasmic reticulum (where $[Ca^{2+}] \approx 10^{-2}$ M). Two calcium ions are transported for each ATP hydrolyzed. The maximal pump rate is about 10/sec. The Ca^{2+} ATPase is a large protein

... contd

with the sarcoplasmic reticulum membrane.

: Calcium and Muscle Contraction :

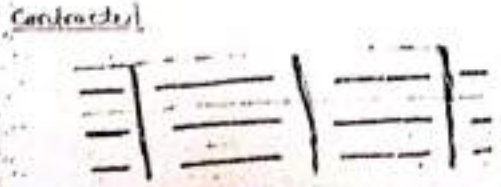
Skeletal muscle is composed of bundles of parallel muscle fibres. Each muscle fibre contains many myofibrils, parallel sets of contractile filaments. In the contractile system of skeletal muscle cells, there are two major types of filaments namely thick filaments and thin filaments. The thick are filaments are composed of bundles of parallel rodlike bundles of myosin, and the thin filaments consist of two F-actin strands twisted about each other. Each strand is made up of globular G-actin molecules. In muscle fibrils the thick and thin filaments are arranged in parallel, interdigitated sets called sarcomeres. During muscle contraction the thick filaments slide into spaces between the thin filaments in each sarcomere, causing shortening of the entire muscle fibre. The energy for this is obtained by the hydrolysis of ATP to ADP.



(a) Skeletal muscle composed of bundles of parallel muscle fibres.

(b) Each muscle fibre contains many myofibrils, parallel sets of contractile filaments.

(c) Each sarcomere is made up of regularly spaced thick and thin filaments.



(d) Thick fibre contains myosin

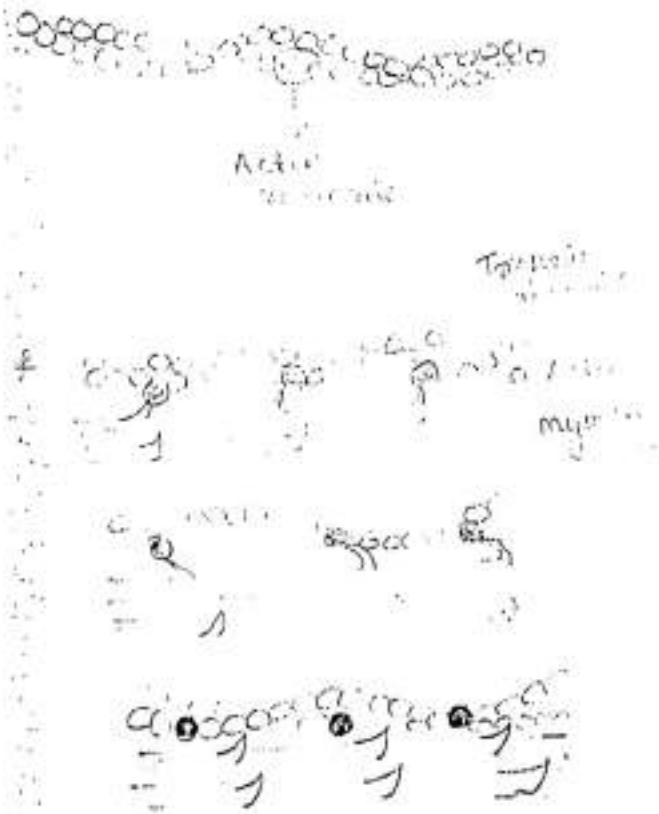
The contraction and relaxation of skeletal muscles is controlled by the Ca^{2+} concentration in the cytosol. Normally the Ca^{2+} concentration in resting muscle is very low. When the nerve impulse stimulates the muscle fibre, Ca^{2+} is released from membranous tubules (sarcoplasmic reticulum (SR)) that run across the muscle cell. The Ca^{2+} so released binds to a complex regulatory system protein, troponin located at intervals along the thin filaments. The troponin molecule serves as a trigger. It undergoes a conformational change that sets off the ATPase activity of the heads of the myosin molecule in the thick filament, thus initiating contraction. So long as free Ca^{2+} is present in the muscle cytosol, the troponin will remain active. Relaxation of muscle cannot takeplace when the nerve impulse cease and Ca^{2+} is is transported back from the sarcoplasm cytosol into the sarcoplasmic reticulum through action of a Ca^{2+} pumping ATPase activity in the membrane. ATP energy is required both for the contraction and relaxation of muscles.

Contraction and relaxation of smooth muscles are important in controlling different biological functions like maintenance of blood flow and blood pressure, urination, moving of matter through gastrointestinal tract, elimination of matter and sperm ejection etc.

(d) Thick filament-

(d) Thick filament- consist of bundles of myosin.

e Thin filament-

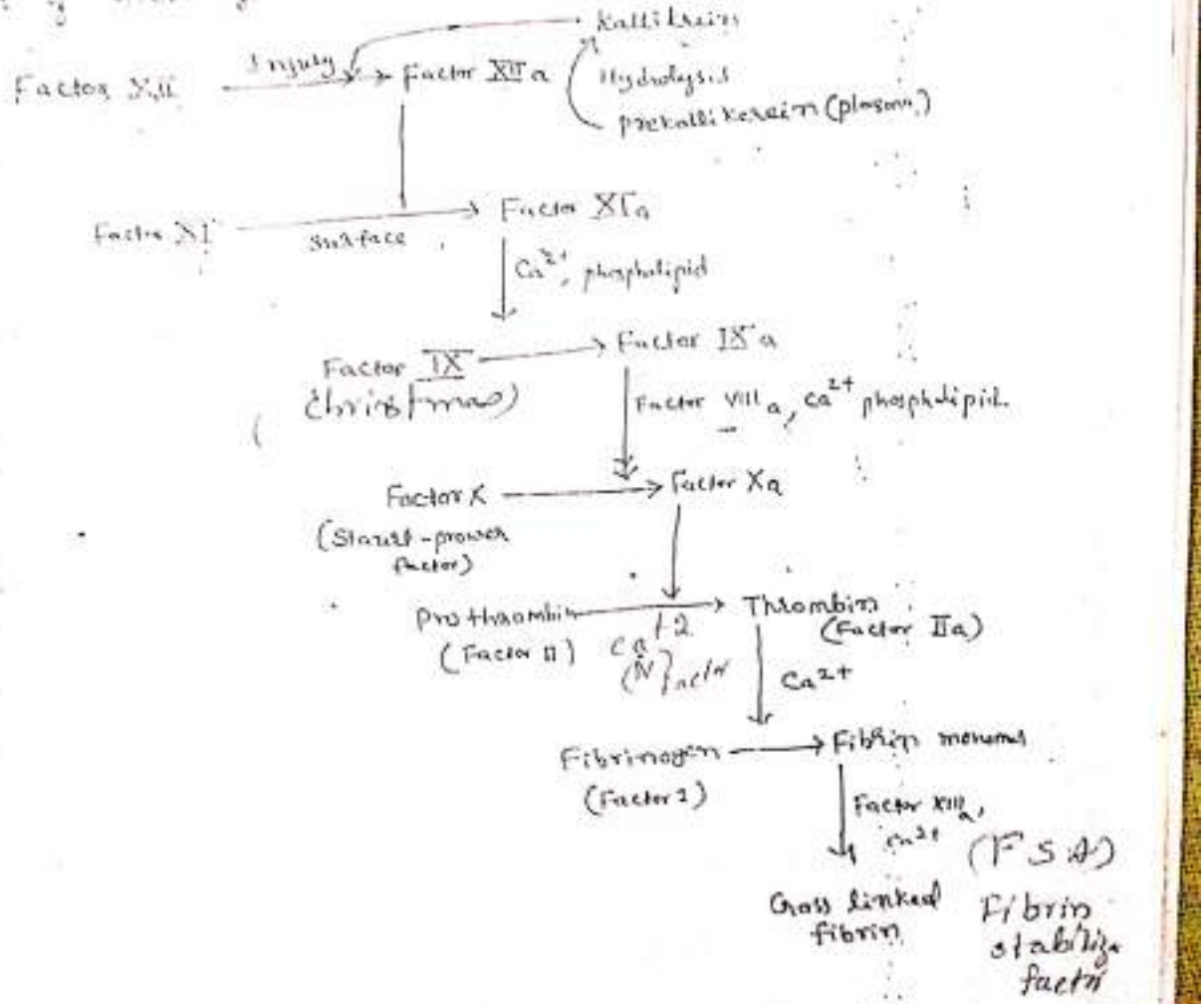


(e) Thin filament- consist of actin molecules.

(f) The contraction process is controlled by a Ca^{2+} binding protein troponin. which is attached to the actin filaments at intervals.

Calcium ion in Blood clotting

- the over all blood clotting process involves a cascade mechanism and many of the steps require the participation of Ca^{2+} ions. A no. of proteins (assigned by Roman numerals) participate in the process. The protein factors normally remain as inactive or precursor forms. When the tissues are damaged to start bleeding, these zymogens experience as sequential activation (scheme). The subscript a refers to the active form. The intrinsic pathway is initiated through the activation of Factor XII to Factor XIIa in the presence of surface and the process ends with the conversion of fibrinogen to cross linked fibrin.



Factors IX, X and prothrombin require vitamin K for their synthesis. In fact, vitamin-K Factor XIII is a tetramer ($\gamma_2 f_2$) and it is activated to factor XIIIa by thrombin through cleavage.

Formation of thrombin from prothrombin is a crucial step. Removal of protective groups on fibrinogen by thrombin initiates the fibrin formation. Thrombin does not exist in normal blood. Fragmentation of the large protein, prothrombin produces thrombin. Through the intermediatory of Ca^{2+} ions, prothrombin binds with the membranes of the injured blood platelets which contain the enzymes to catalyze the fragmentation of prothrombin. Prothrombin bears γ-carboxyglutamate (synthesized by vit K) moieties to bind about 10 Ca^{2+} ions per molecule. These Ca^{2+} ions also bind the platelet membranes. Thus the Ca^{2+} ion bridges between prothrombin and platelet membrane. This Ca^{2+} binding keeps prothrombin in contact with the enzyme responsible to produce thrombin from prothrombin. In this way Ca^{2+} ion facilitates the binding of one protein to another. Thus the thrombin produced clots the blood by conversion of the soluble fibrinogen to insoluble fibrin.

Chlorophyll and Its Role In Photosynthesis

photosynthesis

photosynthesis is a redox reaction where H_2O is oxidized to O_2 and CO_2 is reduced to carbohydrate (CH_2O) i.e.

$$H_2O + CO_2 \xrightarrow{\text{Light}} O_2 + (CH_2O)$$

In this process, solar energy is stored as chemical energy. In the respiration, the reverse reaction operates. photosynthesis in green plants occurs in chloroplasts which possess chlorophylls & absorb light. Then the light energy is converted into chemical energy through a series of reaction.

In common, the photosynthesis reaction means the involvement of H_2O as an electron donor and CO_2 as an electron acceptor.

The overall photosynthesis reaction occurs in two phases. ie Light-phase and Dark phase reaction

Light-phase reaction involves the capture of light by light absorbing pigments which lead to oxidation of H_2O to O_2 with the concomitant reduction of $NADP^+$ to NADPH. It also leads to the synthesis of ATP

$$2H_2O + 2NADP^+ + (2ADP + P_i) \xrightarrow[(8\ photons)]{\text{Light}} O_2 + 2NADPH + 2H^+ + (2\ ATP)$$

In the dark phase, NADPH reduces CO_2 to carbohydrate with the simultaneous conversion of ATP. This phase is also described as Calvin cycle. The overall dark-phase rxn is

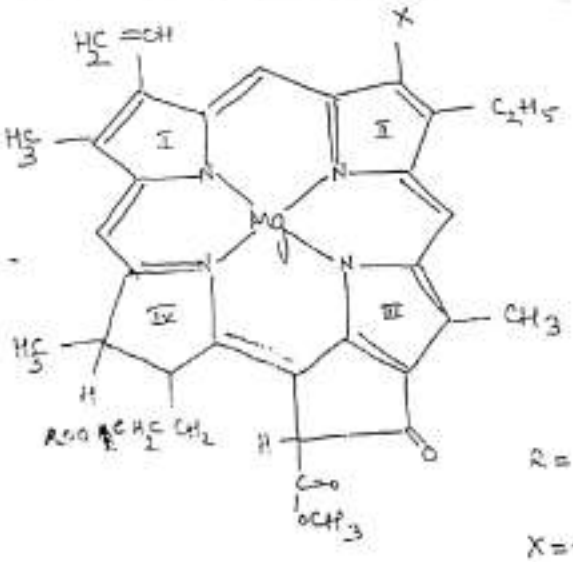
$$6CO_2 + 12\,NADPH^+ + 12H^+ + 18ATP \longrightarrow C_6H_{12}O_6 + 12NADP^+ + 6H_2O + 18(ADP + P_i)$$

Chlorophyll

In the photosynthesis the active component is the green pigment, Chlorophyll. Chlorophyll is a macrocyclic complex of $Mg^{(II)}$. Chlorophyll consists of a macrocyclic tetrapyrrole system called plus porphyrin ring belonging to porphyrin family of porphyrin ring with some modification. The macrocyclic ring in chlorophyll is referred as chlorin ring. There are four substituted pyrole rings in chlorin. In ring II, X differs for Chlorophyll - b ($X = -CHO$) and Chlorophyll - a ($X = CH_3$).

Mg II sits at the center of the chlorin ring and it lies above the macrocyclic plane by ~30 to 50 pm. Chlorophyll is also described as magnesium porphyrin. Chlorophyll is biosynthesized by iron through template reaction.

Str:



R = phytyl group

$X = -CH_3$ (Chlorophyll a)

$X = CHO$ (Chlorophyll b)

The Chlorophyll acts as the Chromophore in photosynthesis. The extensive conjugation in the chlorin ring allows the electron transition $\pi \longrightarrow \pi^*$ in the visible region.

Chlorophyll appears green because it absorbs blue and red light. Extensive conjugation in the chlorin rings of Chlorophyll allows the absorption to occur in the visible region. This conjugation mak the ring rigid and consequently less energy is wasted ... to molecular vibration.

The Role of Mg II in Chlorophyll.

(i) Without magnesium the chlorin ring is flourescent. ie the absorbed light energy is emitted back immediately. Due to Mg II present, Chlorophyll becomes phosphorescent so that absorbed light energy does not loss immediately. The stores energy is utilized for the chemical reaction.

(ii) Mg(II) (4° system) does not have crystal field stabilization energy to prefer the square planar geometry. But the rigid chlorin ring enforces Mg(II) to have the planar geometry.

(iii) Through coordination by the chlorophyll to the Mg(II) - center, rigidity of the macrocyclic str is further strengthened. The rigidity of the system minimizes the energy loss due to molecular vibration.

(iv) Staking of chlorophyll (ie polymerisation) is attained through the bridging action of Mg(II) b/w the adjacent chlorophyll moeities.

(v) The water molecule coordinate to the Mg(II) - center in the axial position direction in the chlorophyll active centre experiences the photoinduced splitting to generate the H-atom that provides the e^{n} to the photosynthetic process. Thus coordination of the water molecule to the Mg(II) - center plays a crucial role.

Electron Transport Chain in photosynthesis; Photosystem I and photosystem II (Z - Scheme)

Chlorophyll catalyzes the reduction of $NADP^+$ to NADPH and oxidation of H_2O to O_2 in the presence of light. The electron flows from H_2O to $NADP^+$ through an electron transport chain (P.680 to P-700*) which looks like Z when the electron carriers are placed in the order of their reduction potentials. Thus the chain is very often described as Z-Scheme.

The whole process is carried out by two kinds of photosystems. Photosystem I (abbreviated as PS-I or P-700, P stands for pigment) which is excited by the light of wavelength in the region 700 nm (or lower) generates a strong reductant to bring about the reduction of $NADP^+$ to NADPH. PS-I uses chlorophyll-a_1 (Chl-a_1) Photosystem-II (abbreviated as PS-II or P-680) uses the light of wavelength 680 nm or lower to produce a very strong oxidant to oxidize H_2O to O_2. PS-II uses chlorophyll-a_2 (Chl-a_2).

When the chlorophyll (present in PS-I or PS-II) is excited, its electron distribution pattern changes. On excitation, it can act both as a better reducing agent (because the excited e⁻s are easily removed) and also a better oxidizing agent (because the positive hole resulted from the excitation of electron can accept electron favorably). Thus the excited chlorophylls can initiate a series of redox reactions.

When P-700 is excited to P-700*, its reduction potential changes from +0.4V (at the ground state) to about −1V (at the excited state). P-700* becomes a better reducing agent and it transfers its electron to its primary electron acceptor P-430. It is a membrane bound ferradoxin of the Fe_4S_4 type characterized by an strong absorption maxima at 430 nm in the reduced form. Then the electrons flow or reach ultimately reach $NADP^+$ through a series of electron

Proteins

Definition: Proteins are polypeptides that are made up of 40-100 amino acids joined by amide linkage.

Structural organization of proteins

There are four distinct levels of protein structure.

Primary structure
Secondary structure
Tertiary structure
Quaternary structure

Primary Structure of Protein

'The primary structure of a protein describes the number, nature and sequence of amino acids in the chain'. The primary structure is held together by covalent bonds such as peptide bonds. The two ends of the polypeptide chain are referred to as the carboxyl terminus (C-terminus) and the amino terminus (N-terminus) based on the nature of the free group on each extremity.

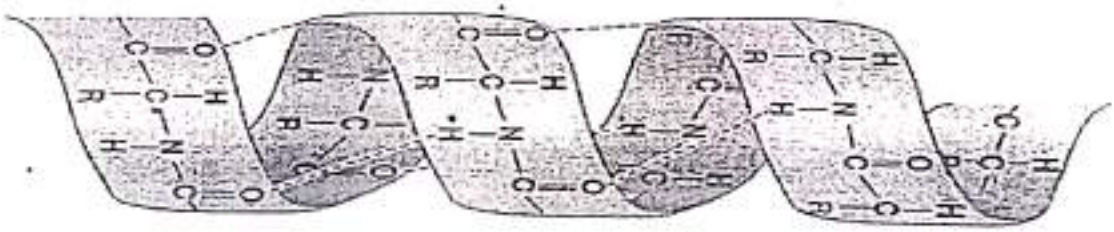
Insulin is the first protein whose amino acid sequence was determined.

Secondary Structure of Protein

'Secondary structure refers to folding patterns of polypeptide chain'. The partial double bond character of the peptide bond resulting in restricted rotation around the C-N bond makes it a planar arrangement. On either side of the planar, rigid peptide group rotation is possible around C_α-N and C_α-C bonds. The favourable rotation angles of these bonds define the secondary structure of the polypeptide chain. Many types of secondary structures exist in proteins. The most important are the ∝-helix, β-pleated sheet and the triple helix.

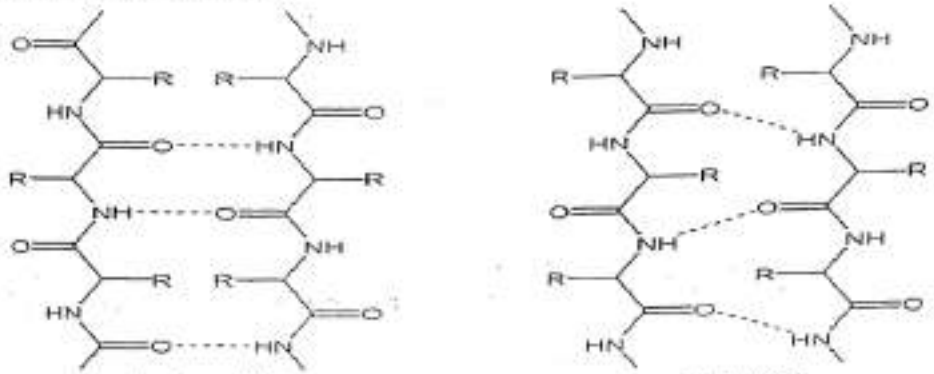
α-Helix:

The first type of secondary structure is α -helix, where the backbone coils around the long axis of the protein molecule. The substituent on the α-carbon of the amino acids protrude outward from the helix to minimize the steric hindrance. The H attached to amide nitrogen makes H-bonding with the carbonyl oxygen of an amino acid. Each turn consists of 3.6 amino acid residues.



β-Pleated Sheet

The second type of secondary structure is the β-pleated sheet, in which the backbone is extended in a zigzag structure resembling pleats. The H-bonding in a β-pleated sheet occurs between the adjacent peptide chains. It is of two forms.



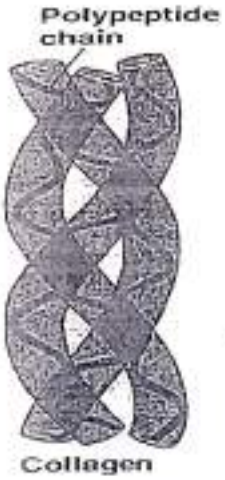
a. Antiparallel b. Parallel

Difference between Alpha Helix and Beta Pleated Sheet Shape

Alpha Helix	Beta Pleated Sheet
Alpha Helix is a right-handed coiled rod-like structure.	Beta sheet is a sheet-like structure.
Hydrogen bonds form within the polypeptide chain in order to create a helical structure.	Beta sheets are formed by linking two or more beta strands by H bonds.
Hydrogen bonds form between N-H group of one amino residue with C=O group of another amino acid, which is placed in 4 residues earlier	Hydrogen bonds are formed in between the neighbouring N-H and C=O groups of adjacent peptide chains.
-R groups of the amino acids are oriented outside of the helix.	-R groups are directed to both inside and outside of the sheet.
This has only one type.	This can be parallel, anti-parallel or mixed.
3.6 residues per turn.	3.5 A° rise between residues.

Triple helix:

The best example for triple helix is collagen which is found in skin, tendons, bone and cartilage. The structural unit of the collagen molecule is called tropocollagen. In tropocollagen, three left-hande helices are coiled around each other with aright twist to form a triple helix. They are stabilized by interchain hydrogen bonds and covalent cross-links between chains.



Tertiary Structure of Protein

'The tertiary structure of a protein is a description of the complex and irregular folding of the peptide chain in three dimensions'. It is essentially a picture of what the shape of the entire protein actually looks like. The side chains of amino acids present in the polypeptide chain interact with each other and folded in a compact manner gives exact shape to the protein.

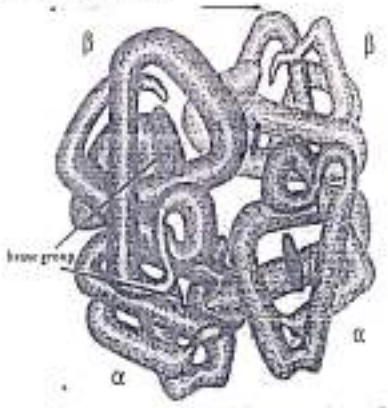
The tertiary structure of globular proteins is stabilized by four types of interactions such as:

1. hydrogen bonds between polar R- groups
2. ionic bonds between charged R-groups
3. hydrophobic interactions between nonpolar R-groups
4. Covalent bonds: The R-group of the amino acid cysteine contains a sulfur atom and this sulfur atom is capable of forming a covalent bond with another sulfur atom on a different cysteine molecule somewhere else on the chain. This bond is known as a **disulfide** bond and it acts as to stabilize the tertiary structure of those proteins that have such bonds.

Tertiary Structure of Protein

Quaternary Structure of Protein

'The quaternary structure of a protein describes the interactions between different peptide chains that make up the protein'. Some proteins (such as hemoglobin) have more than one peptide chain (these are **multimeric** proteins). The manner in which these chains fit together (sort of like a puzzle) is the quaternary structure. Obviously, if a protein is made up of only one chain (**monomeric**), there is no quaternary structure for that protein. The forces that hold different chains together are the same that hold the tertiary structure together, hydrogen bonding between polar R-groups, ionic bonds between charged R-groups, hydrophobic interactions between nonpolar R-groups, and disulfide bonds. The figure shows the structure of hemoglobin, a protein that has four subunits.



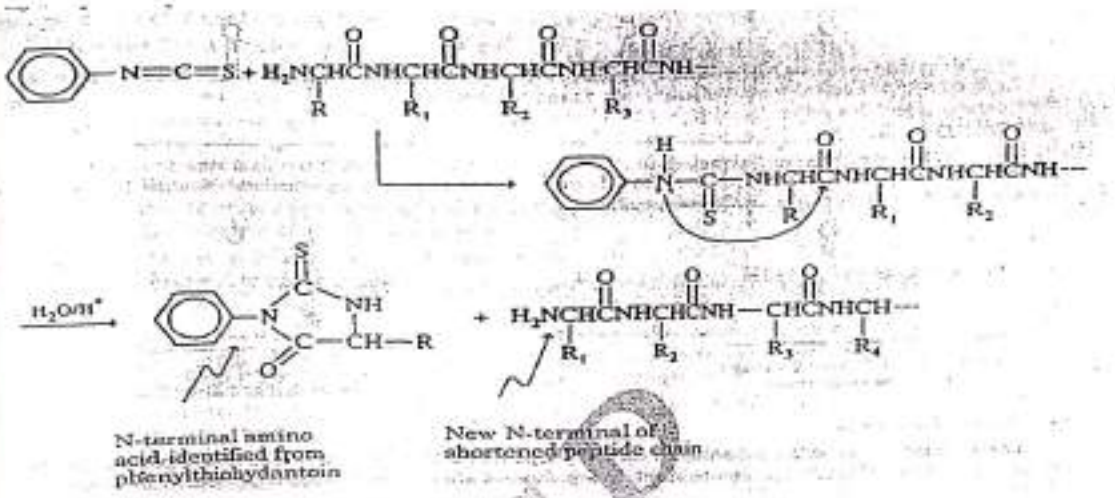
Quaternary Structure of Protein (e.g., haemoglobin)

N-Terminal Group Analysis

Determination of primary structure of peptides and proteins by degradation

Edman Degradation

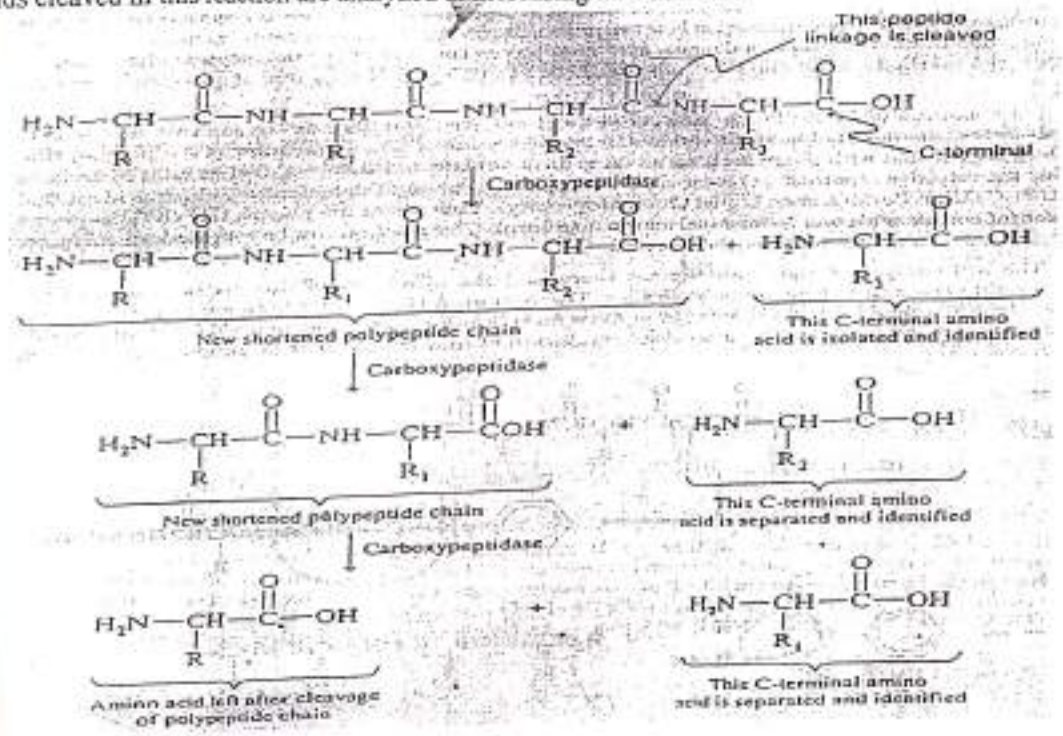
The phenyl isothiocyanate (Edman reagent) attacks and removes the N-terminal amino acid unit in the form of a substituted thiohydantoin heterocycle together with a shortened peptide chain. The N-terminal analysis may be repeated several times, characteristic hydantoin derivatives of all the amino acids have been made, thus providing the sequence of amino acids in the chain.



C-Terminal Group Analysis

Enzymatic C-terminal amino acid cleavage

Enzymatic C-terminal amino acid cleavage by carboxypeptidase enzymes is a fast and convenient method of analysis. Because the shortened peptide product is also subject to enzymatic cleavage,
A peptide having a C-terminal sequence is subjected to carboxypeptidase cleavage, and the free amino acids cleaved in this reaction are analyzed at increasing time intervals.



Pushala P.S

Lipids (Oils and Fats)

Lipids are the heterogeneous group of compounds such as oils, fats, fatty acids waxes, phosphoglycerides, sphingo lipids, steroids, terpenes etc These are hydrophobic in nature. As a result they are insoluble in water but soluble in non-polar solvents like benzene, ethers, CCl_4 CS_2 etc

Functions of Lipids

Due to the difference in their chemical nature, lipids are involved in wide variety of biological activities

① They are major storage energy contents of the body. They supply more amount of energy when compared to carbohydrates and proteins during their metabolic activity (oxidation)

② There are certain vitamins such as vitamin - A and vitamin E which are hydrophobic in nature. These are hydrophobic vitamins are transported from the point of absorption to site of their action with the help of lipids.

③ phospholipids, glycolipids, sphingolipids are the major components of cell membrane. These are responsible to maintain integrity of the cell membrane organelles.

④ Bile juice is itself a lipid. It facilitates emulsification of other lipids during their digestion.

⑤ lipids and lipid derivatives serve as anti-oxidants, harmones and vitamins.

⑥ A layer of fat deposited under the skin protects the skin from cold. Because it serves as a thermal insulation.

Classification of lipids

lipids are broadly classified into 3 types

① Simple lipids: These are the esters of fatty acids with alcohols which gives alcohol and acid on hydrolysis.

Ex: Triacyl glycerols, which are the esters of fatty acids with glycerol. They include oils and fats.

② Compound lipids: These are the esters of fattyacids with alcohols which also contain other groups such as

a) phosphate group ⇒ phospholipid

b) carbohydrate group → glycolipids

c) Amino alcohol → Sphingo lipids etc.

Derived lipids: These are the derivatives which are derived either from simple lipids or from compound lipids.

Ex:- Fatty acids, glycerol, steroids etc.

Note: waxes are the esters of fatty acids with long chain monohydric alcohols.

Fatty acids:

long chain aliphatic monocarboxylic acids are commonly called fatty acids. Fatty acids can be represented by the general formula R-COOH where 'R' may be any alkyl or aryl or alkenyl groups. The long hydrocarbon atom chain is non-polar and hydrophobic in nature. As a result, fatty acids are insoluble in water. All naturally occuring fatty acids contain even no of C-atoms. Fatty acids with 16, 18 and 20-Cat carbon atoms are more abundant in nature.

There are two types of fatty acids namely

a) Saturated fatty acids

b) unsaturated fatty acids

Saturated fatty acids can be represented by the general formula $C_nH_{2n+1}COOH$ where n is the number of carbon atoms

unsaturated fatty acids possess one or more double bonds in their hydrocarbon chain.

Note : with increase in chain length, melting point increases.

Structures of a few essential fatty acids

① palmitic acid - M.F → $C_{15}H_{31}COOH$

② stearic acid - M.F → $C_{17}H_{35}COOH$

③ oleic acid - M.F → $C_{17}H_{33}COOH$

④ linoleic acid - M.F → $C_{17}H_{31}COOH$

⑤ linolenic acid - M.F → $C_{17}H_{29}COOH$

⑥ Arachidonic acid - M.F → $C_{19}H_{31}COOH$

Essential fatty acids

Glucose when passes through metabolic pathway gives pyruvic acid. This pyruvic acid by the action of co-enzyme A gives acetyl co-enzyme A with the help of this acetyl CoA, a no of fatty acids are synthesized in the body itself. Such fatty acids need not be supplied to the body through the diet Hence such fatty acids are called non-essential fatty acids. But linoleic acid and linolenic acid are not synthesized in the body. But they are most esential to maintain in the normal health. These two fatty acids are called as essential fatty acids

Essential fatty acids must be supplied to body through the diet. The deficiency of essential fatty acid is characterized by scaly skin and poor healing of wounds.

Acyl glycerols

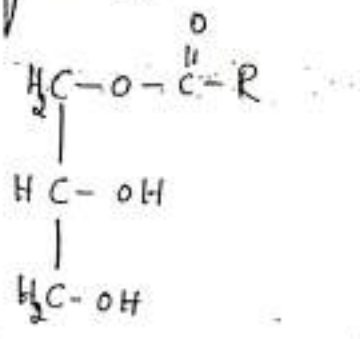
In nature, fatty acids does not occur in their free state. They are generally get esterified with glycerol.

Acyl glycerols are the derivatives of glycerol in which 1, 2, 3 or all the three -OH groups of glycerols are get esterified with fatty acids.

In nature, acyl glycerols generally occur as oils and or fats.

Monoacyl glycerols

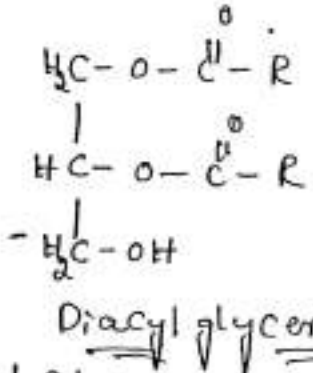
These are the derivatives of glycerol where only one -OH group of glycerol get esterified with fattyacids.



Mono-acyl glycerol

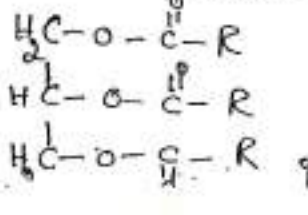
Diacyl glycerols

These are the derivatives of glycerol in which two -OH groups of glycerol get esterified with fattyacids



Diacyl glycerol

Triacyl Glycerols :- These are the derivatives of glycerols where all the three-OH groups of glycerol get esterified with fattyacids



Triacyl glycerol

Biological importance of triglycerides or triacyl glycerols (oils & fats)

① Triacyl glycerols stored in the form of fat serve as reserve metabolic fuels. They are stored in the body in adipose tissue.

② Being the poor conductor of heat, they protect the body from extreme cold, especially in aquatic mammals which live in cold region. ~~A thick layer of fat is also called~~
* stored in body as fats and oils
* protect the body extreme cold condition (poor conductor)
* supply energy during oxidation.

③ Triglycerides ~~is~~ are in the more reduced form compared to carbohydrates. As a result, they supply more amount of energy than carbohydrates during the oxidation through the metabolic pathway

④ oils and fats possess less density than water therefore, storing of triglycerides is not an extra load the organism.

⑤ Triglycerides are hydrophobic in nature, Hence they stored in the body in their anhydrous form. where as glycogen though it is water insoluble, it is hydrophilic and hence it stores in the body in the hydrated form.

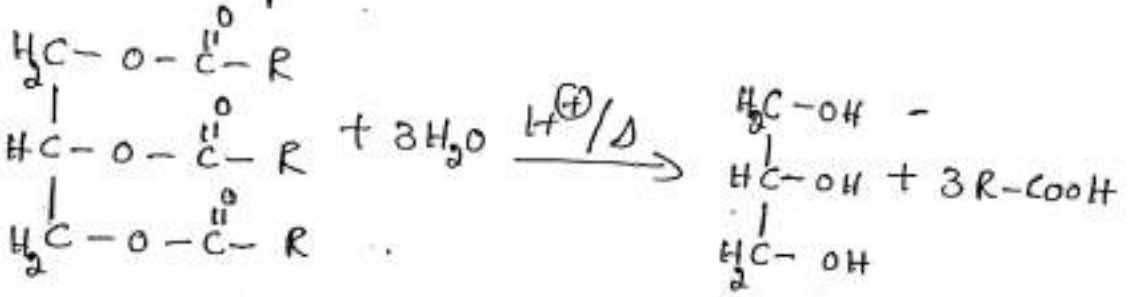
⑥ Triacyl glycerides, when they undergo oxidation in the biological system, they produce more water which can be used for other physio-logical activities.

⑦ Triglycerides are essential for the transportation of fat soluble vitamins such as vitamin A and vitamin E, K, D.

Chemical properties of triacyl glycerols or Triglycerides or oils & fats

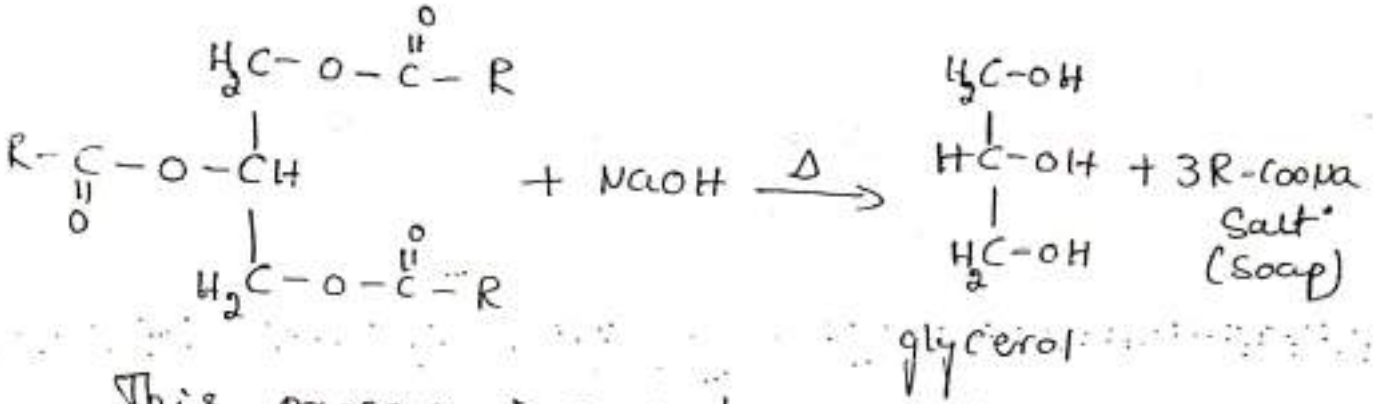
Hydrogenation: Triglycerides can be hydrolysed either by using an acid (or) base as an catalyst

ⓐ **Acid hydrolysis**: when oils and fats are heated with mineral acid (HCl, H_2SO_4) they undergo hydrolysis giving Glycerol and fatty acids.



ⓑ **Alkaline hydrolysis (Saponification)**: when triglycerides are heated with an alkaline like KOH or NaOH, they undergo hydrolysis to given glycerol (alcohol) and soap.

The sodium or potassium salts of fattyacids are called soaps.

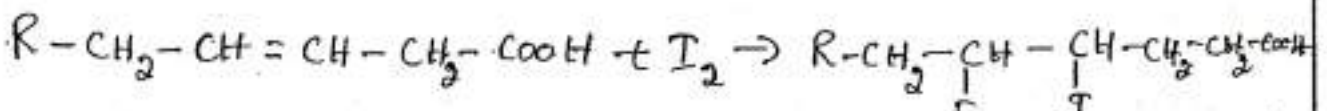


This process is called saponification.

Saponification number: (saponification value)
It is defined as "the number of miligrams of KOH required to completely saponify 1g of an oil or fat."

A high saponification number indicates that the given oil or a fat contains short chain fatty acids.. with low molecular wieght ie higher the saponification no, lower member is the fatty acid with low Molecular weight.

Addition reactions:- unsaturated fattyacids present in an oil or a fat undergoes addition reaction with hydrogen and halogens. Halogens like bromine and Iodine are decolourized by oils and fats



Iodine number

It is defined as "the no of grams of iodine required to saturate 100 g of an oil or a fat completely."

Greater the iodine number, greater is the unsaturation in the fattyacid portion of the lipid.

Rancidity: when an oil (or) a fat is exposed to atmospheric air for a long period, it develops an unpleasant odour and sour taste. This bad smelling oil is called Rancid oil or fat and the phenomenon is called Rancidity.

oxidative Rancidity

This is predominant in oils compared to fats. In presence of moist air, the fattyacid chain which contain double bonds in it undergoes oxidation at the position of double bonds. This results in the formation of a complex mixtures which contains aldehydes, ketones and esters etc.

This mixture is responsible to give bad smell which is called oxidative Rancidity.

Hydrolytic Rancidity: This is due to hydrolysis

This mixture is responsible to give bad smell which is called hydrolytic rancidity.

Prevention of Rancidity:

Rancidity can be prevented by any one of the following methods

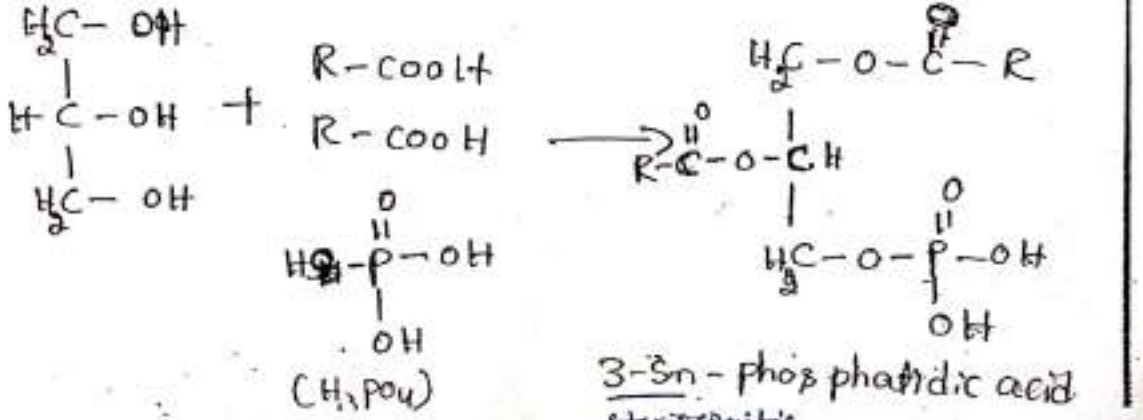
① By storing in storing in air tight containers

② By adding antioxidants such as vitamins A, E, K

③ By converting oil into fat by catalytic hydrogenation.

Phosphoglycerides (phospholipids)

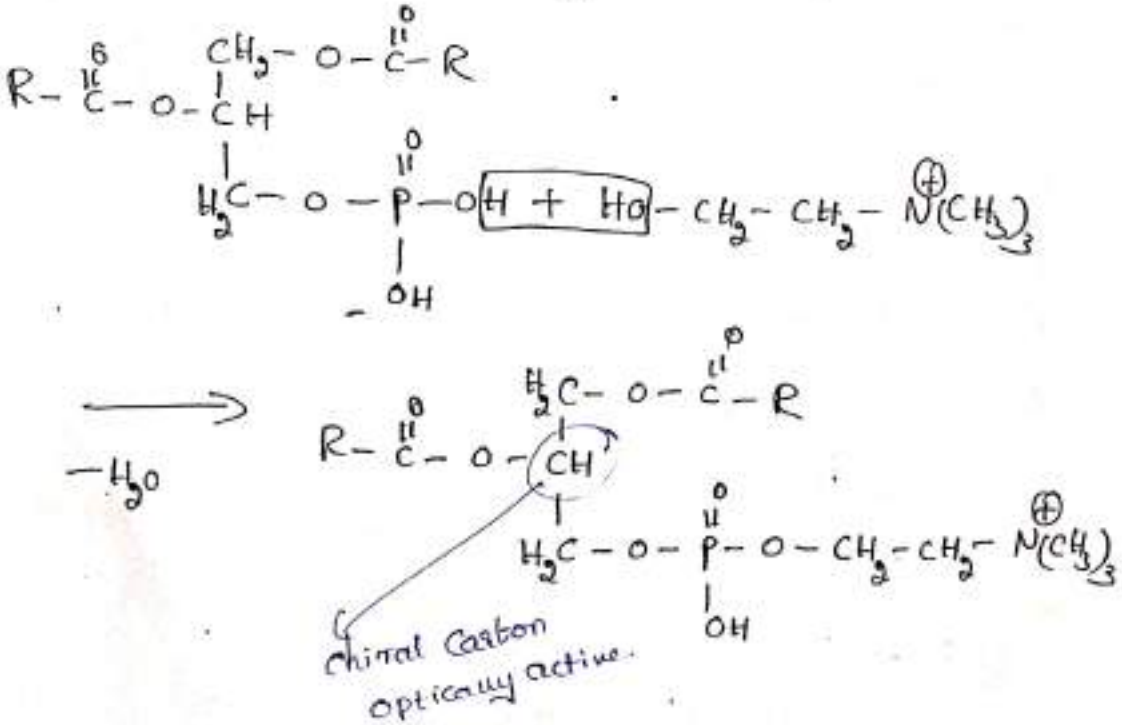
These are the derivatives of glycerol where one of the -OH group of glycerol is get esterified with phosphoric acid. when -OH group present on 3rd carbon atom of the glycerol is esterified with H_3PO_4 the resulting molecule is called 3-Sn-phosphatidic acid. This is the parent of all phosphoglycerides



The prefix Sn stands for stereospecific numbering and '3' indicates that the -OH group present on 3rd C-atom of glycerol is get esterified with H_3PO_4. In 3-Sn-phosphatidic acid, the 2nd C-atom of glycerol is a ~~was~~ asymmetric, ~~Hence~~ or chiral carbon atom. Hence it is optically active. Naturally occuring phosphoglycerides ~~have~~ have L-Configuration. The structure of a few biologically important phospholipids are given below

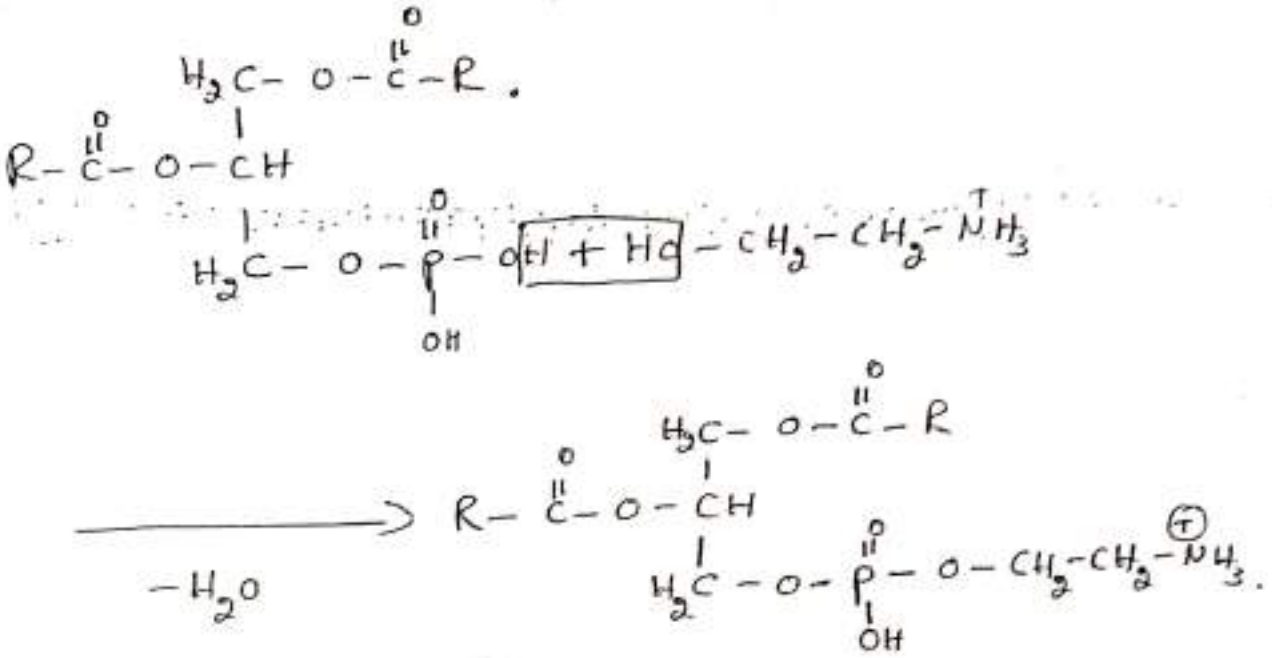
3-Sn-Phosphatidyl choline

$HO-CH_2-CH_2-N^{\oplus}(CH_3)_3$ → choline

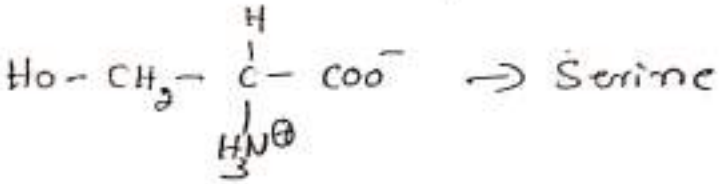


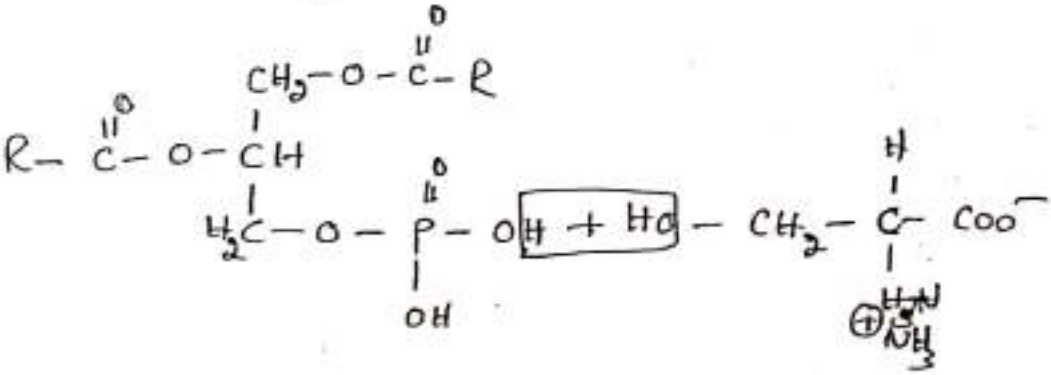
② 3-Sn-phosphatidyl ethanolamine (cephalin)

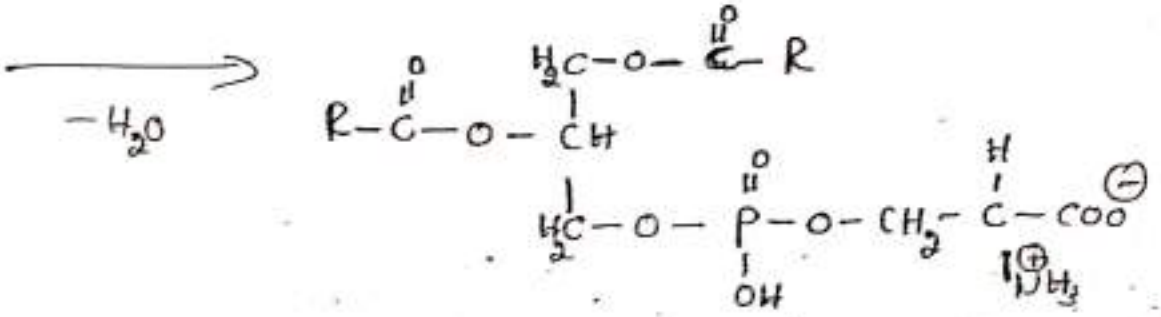
$HO-CH_2-CH_2-\overset{\oplus}{N}H_3$ → ethanolamine



③ 3-Sn-phosphatidyl serine







Biological importance of phospholipids

① Phosphoglycerides are the important components of all cell membrane. They influence, the structure and function of the cell. Phosphoglycerides which are arranged in a bilayer manner serves as a backbone of cell membrane. The cell membrane is the one which determines the permeability characteristics.

② Phosphoglycerides are required for the formation of lipoprotein in the liver. These lipoproteins help in the transportation of other hydrophobic lipids through the blood plasma.

③ Phosphoglycerides function as solvents to ~~dissolve~~ solubalise ~~the~~ cholesterol in bile duct and liver

Normal - 200 mg/dL
HDL - High density lipoprotein
LDL - Low density lipoprotein
It may effect adrenal