UNIT-III

PROTEIN

Points to be covered in this topic

- ❖ Introduction
- ❖ Amino acids
- ❖ Structure of proteins
- ❖ Regularities in protein pathways
- ❖ Cellular process
- ❖ Positive control & significance of protein synthesis

PROTEIN

❑ INTRODUCTION

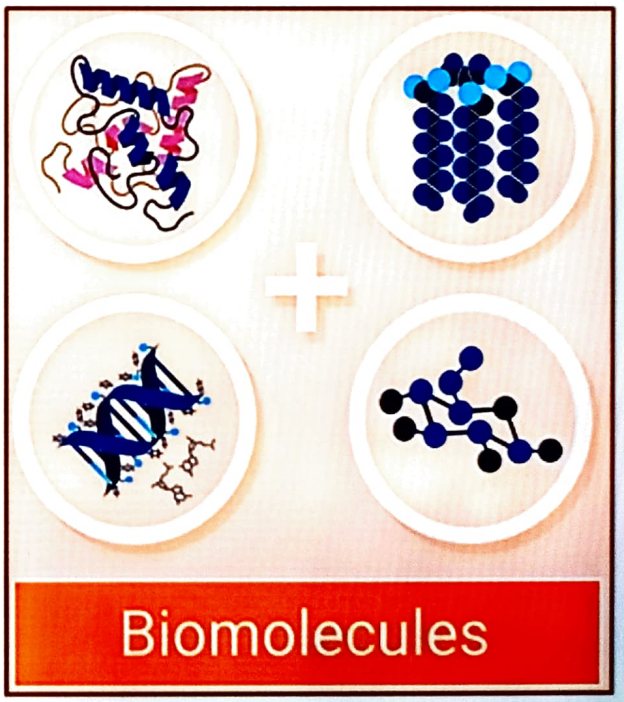
Proteins are the **most abundant organic molecules** of the living system. They occur in every part of the cell and constitute about **50% of the cellular dry weight**. Proteins form the fundamental basis of **structure and function of life.**

➢ Origin of the word 'protein'

- The term protein is derived from a **Greek word proteios**, meaning **holding the first place**. **Berzelius (Swedish chemist) suggested the name proteins** to the group of organic compounds that are utmost important to life. **Mulder (Dutch chemist) in 1838 used the term proteins for the high molecular weight nitrogen-rich** and most abundant substances present in animals and plants.

➢ Functions of proteins

These functions may be broadly grouped as static (structural) and dynamic.



- **Structural functions :** Certain proteins **perform brick and mortar roles** and are primarily responsible for **structure and strength of body**. These include **collagen and elastin found in bone matrix, vascular system and other organs and D-keratin** present in epidermal tissues.

- **Dynamic functions :** The dynamic functions of proteins are more diversified in nature. These include proteins acting as **enzymes, hormones, blood clotting factors, immunoglobulin, membrane receptors, storage proteins, besides their function in genetic control, muscle contraction, respiration** etc.

➢ Elemental composition of proteins

- Proteins are predominantly constituted by five major elements in the following proportion.
- Besides in the table, proteins may also contain other elements such as P, Fe, Cu, I, Mg, Mn, Zn etc.

Carbon : 50 – 55%
Hydrogen : 6 – 7.3%
Oxygen : 19 – 24%
Nitrogen : 13 – 19%
Sulfur : 0 – 4%

➢ Proteins are polymers of amino acids

- Proteins on complete **hydrolysis (with concentrated HCl for several hours) yield L-D-amino acids**. This is a common property of all the proteins. Therefore, proteins are the polymers of L-D-amino acids.

➢ Standard amino acids

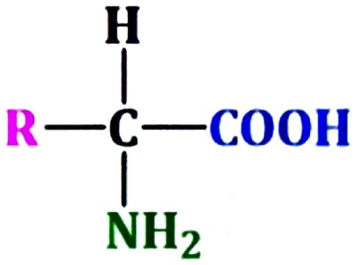
- As many as 300 amino acids occur in nature— Of these, **only 20— known as standard amino acids** are repeatedly found in the structure of proteins, isolated from different forms of life— **animal, plant and microbial.**
- This is because of the **universal nature of the genetic code** available for the incorporation of only 20 amino acids when the **proteins are synthesized in the cells.**
- The process in turn is **controlled by DNA,** the genetic material of the cell.
- After the synthesis of proteins, some of the **incorporated amino acids undergo modifications to form their derivatives.**

AMINO ACIDS

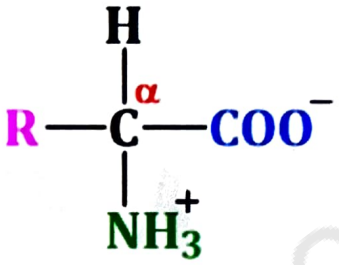
- Amino acids are a group of organic compounds containing **two functional groups— amino and carboxyl. The amino group (—NH_2) is basic while the carboxyl group (—COOH) is acidic in nature.**

➢ General structure of amino acids

- **The amino acids are termed as D-amino acids, if both the carboxyl and amino groups are attached to the same carbon atom,**



General structure



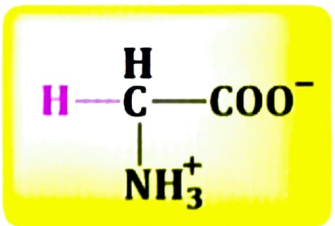
Exists as ion

- **The D-carbon atom binds to a side chain represented by R which is different for each of the 20 amino acids found in proteins.** The amino acids mostly exist in the ionized form in the biological system.

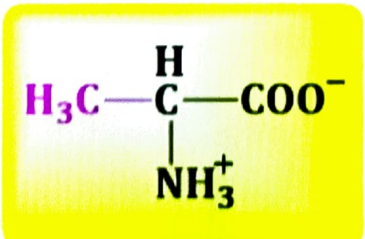
❖ Structural classification of L-D-amino acids found in proteins

I. Amino acids with aliphatic side chains

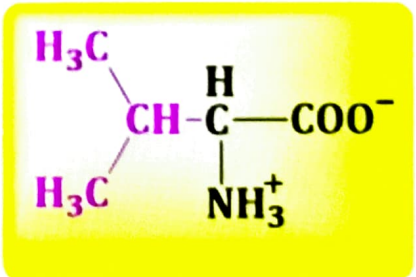
1. Glycine:-



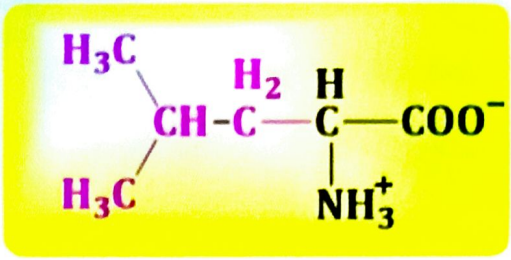
2. Alanine:-



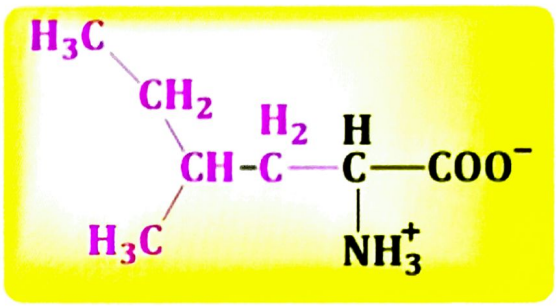
3. Valine:-



4. Leucine:-

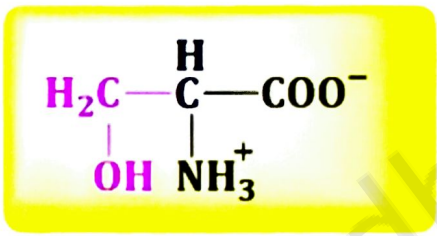


5. Isoleucine:-

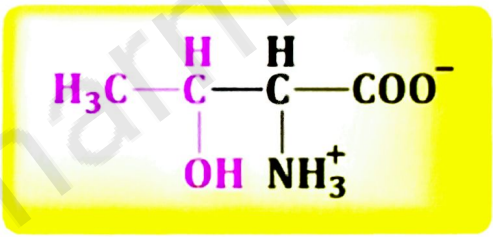


II. Amino acids containing hydroxyl (—OH) groups

6. Serine:-

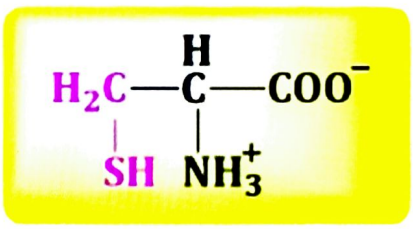


7. Threonine:-

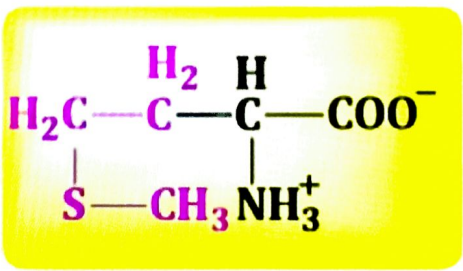


III. Sulfur containing amino acids

8. Cysteine:-

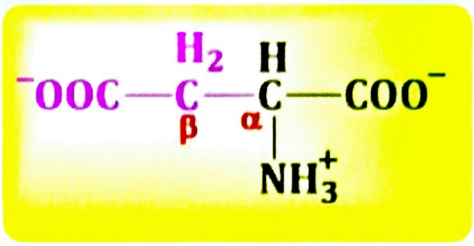


9. Methionine:-

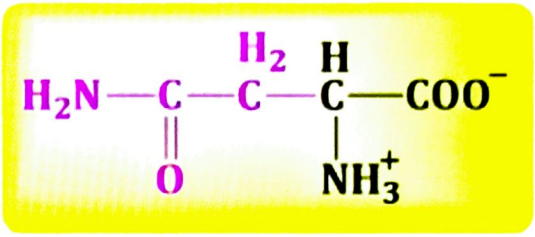


IV. Acidic amino acids and their amides

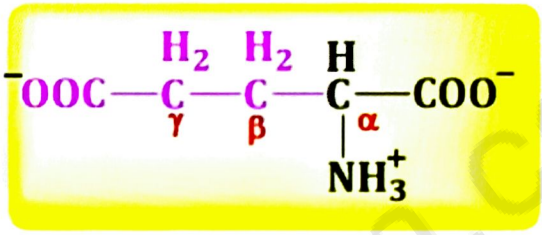
10. Aspartic acid:-



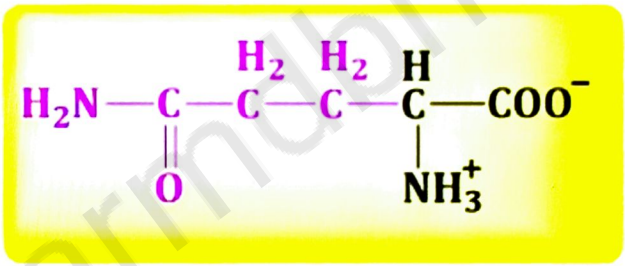
11. Asparagine:-



12. Glutamic acid:-

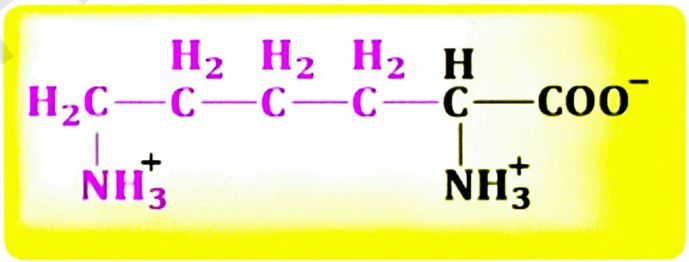


13. Glutamine:-

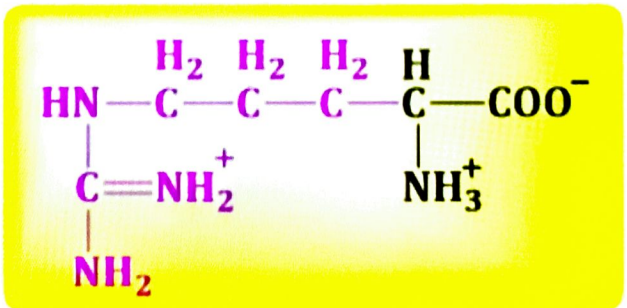


V. Basic amino acids

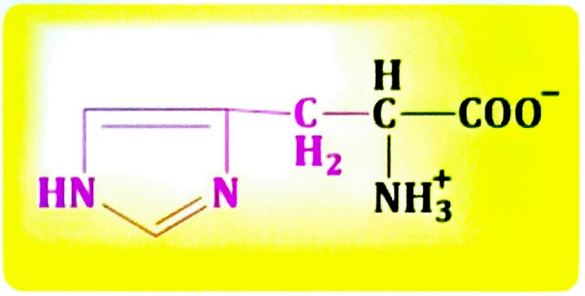
14. Lysine:-



15. Arginine:-

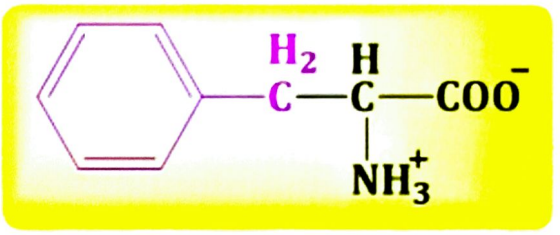


16. Histidine:-



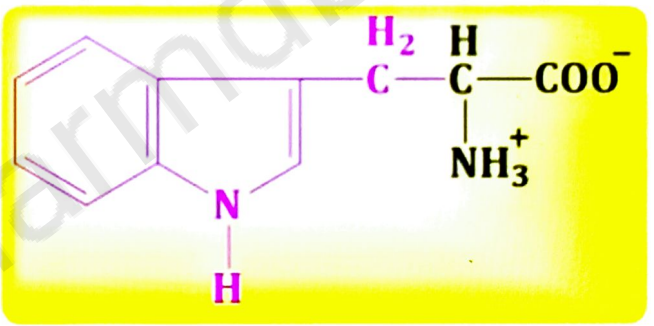
VI. Aromatic amino acids

17. Phenylalanine:-



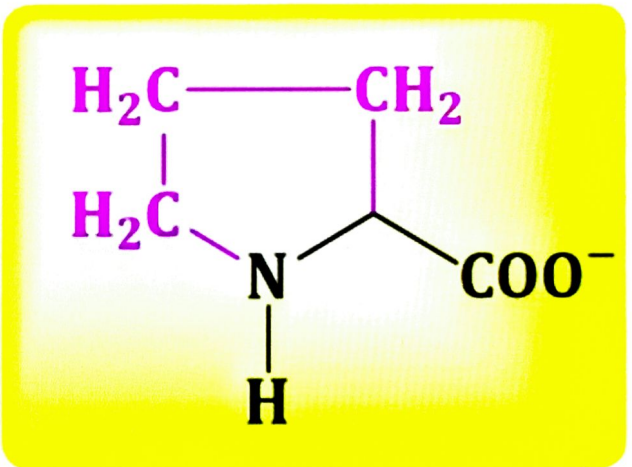
18. Tyrosine:-

19. Tryptophan:-



VII. Imino acid

20. Proline:-



➢ Properties of amino acids

The amino acids differ in their physico– chemical properties which ultimately determine the characteristics of proteins.

A. Physical properties

1. **Solubility :** Most of the amino acids are usually **soluble in water and insoluble in organic solvents.**
2. **Melting points :** Amino acids generally **melt at higher temperatures**, often above 200°C.
3. **Taste :** Amino acids may be **sweet (Gly, Ala, Val), tasteless (Leu)** or **bitter (Arg, Ile). Monosodium glutamate** is used as a flavoring agent in food industry, and Chinese foods to increase taste and flavor.
4. **Optical properties :** All the amino acids **except glycine possess optical isomers** due to the presence of asymmetric carbon atom. Some amino acids also have a second asymmetric carbon e.g. **isoleucine, threonine.**
5. **Amino acids as ampholytes :** Amino acids **contain both acidic (COOH) and basic (NH_2) groups.** They can donate a proton or accept a proton, hence amino acids are regarded as **ampholytes.**

   - **Zwitterion or dipolar ion :** The name zwitter is derived from the German word which **means hybrid**. Zwitter ion (or dipolar ion) is a hybrid molecule **containing positive and negative ionic groups.**
   - The amino acids rarely exist in a **neutral form with free carboxylic (COOH) and free amino (NH_2) groups.**
   - In strongly **acidic pH (low pH), the amino acid is positively charged** (cation) while in strongly **alkaline pH (high pH), it is negatively charged (anion).**
   - Each amino acid has a characteristic pH (e.g. leucine, pH 6.0) at which it carries both positive and negative charges and exists as zwitterion.

➢ **Isoelectric pH** (symbol pI) is defined as the **pH at which a molecule exists as a zwitterion or dipolar ion and carries no net charge.** Thus, the molecule is electrically neutral.

➢ Chemical properties

❖ Reactions due to COOH group

1. Amino acids **form salts** (COONa) with bases and esters (COOR') with alcohols.
2. **Decarboxylation :** Amino acids undergo **decarboxylation** to produce corresponding amines. These include **histamine, tyramine and γ-amino butyric acid (GABA)** from the amino acids histidine, tyrosine and glutamate, respectively.
3. **Reaction with ammonia :** The carboxyl group of dicarboxylic amino acids **reacts with NH_3 to form amide.**

Aspartic acid + NH_3 →Asparagine

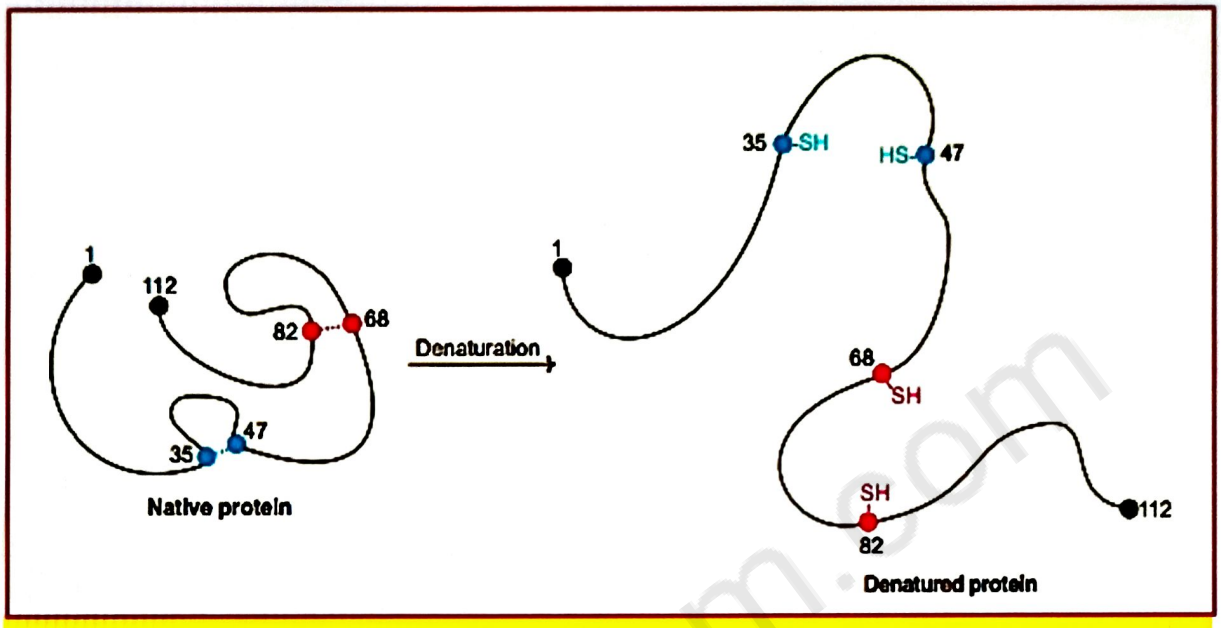
Glutamic acid + NH_3 → Glutamine

❖ Reactions due to NH_2 group

4. The amino groups behave as bases and combine with acids (e.g. HCl) to **form salts (NH_3^+ Cl^-).**
5. **Reaction with ninhydrin :** The D-amino acids react with ninhydrin to form **a purple, blue or pink colour complex (Ruhemann's purple).** Ninhydrin reaction is effectively used for the quantitative determination of amino acids and proteins. **(Note : Proline and hydroxyproline give yellow colour with ninhydrin).**
6. **Colour reactions of amino acids :** Amino acids can be identified by specific colour reactions.
7. **Transamination :** Transfer of an amino group from an amino acid to a **keto acid to form a new amino acid is a very important reaction** in amino acid metabolism.
8. **Oxidative deamination :** The amino acids undergo oxidative deamination to **liberate free ammonia.**

➤ Denaturation

- The phenomenon of **disorganization of native protein structure** is known as denaturation. Denaturation results in the **loss of secondary, tertiary and quaternary structure of proteins**. This involves a change in physical, chemical and biological properties of protein molecules.



Denaturation of a protein.

- **Agents of denaturation**

✓ **Physical agents :** Heat, violent shaking, X-rays, UV radiation.

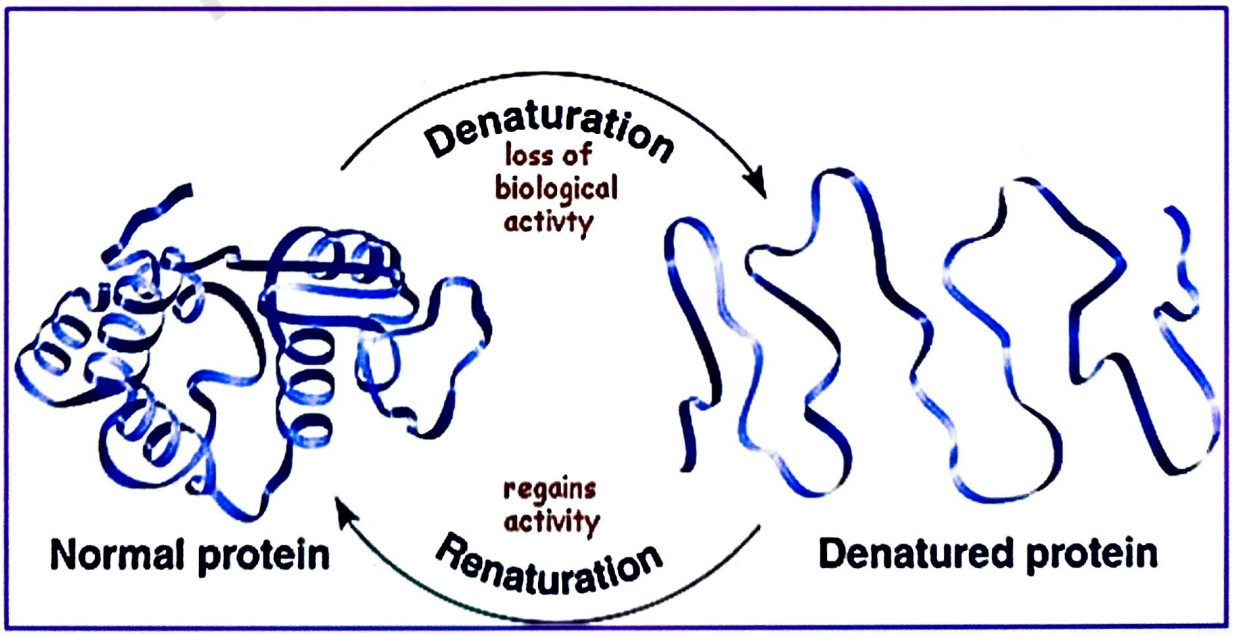
✓ **Chemical agents :** Acids, alkalies, organic solvents (ether, alcohol), salts of heavy metals (Pb, Hg), urea, salicylate, detergents (e.g. sodium dodecyl sulfate).

Characteristics of denaturation

1. The native **helical structure of protein is lost.**
2. The primary structure of a protein with **peptide linkages remains intact** i.e., peptide bonds are not hydrolysed.
3. The protein **loses its biological activity.**

4. Denatured protein becomes **insoluble in the solvent in which it was originally soluble.**
5. **The viscosity of denatured protein (solution) increases** while its surface tension decreases.
6. Denaturation is associated with **increase in ionizable and sulfhydryl groups of protein**. This is due to loss of **hydrogen and disulfide bonds.**
7. Denatured protein is more easily digested. **This is due to increased exposure of peptide** bonds to enzymes. **Cooking causes protein denaturation and therefore cooked food (protein) is more easily digested**. Further, denaturation of dietary protein by gastric HCl enchances protein digestion by pepsin.
8. Denaturation is usually **irreversible**. For instance, **omelet can be prepared from an egg (protein-albumin) but the reversal is not possible.**

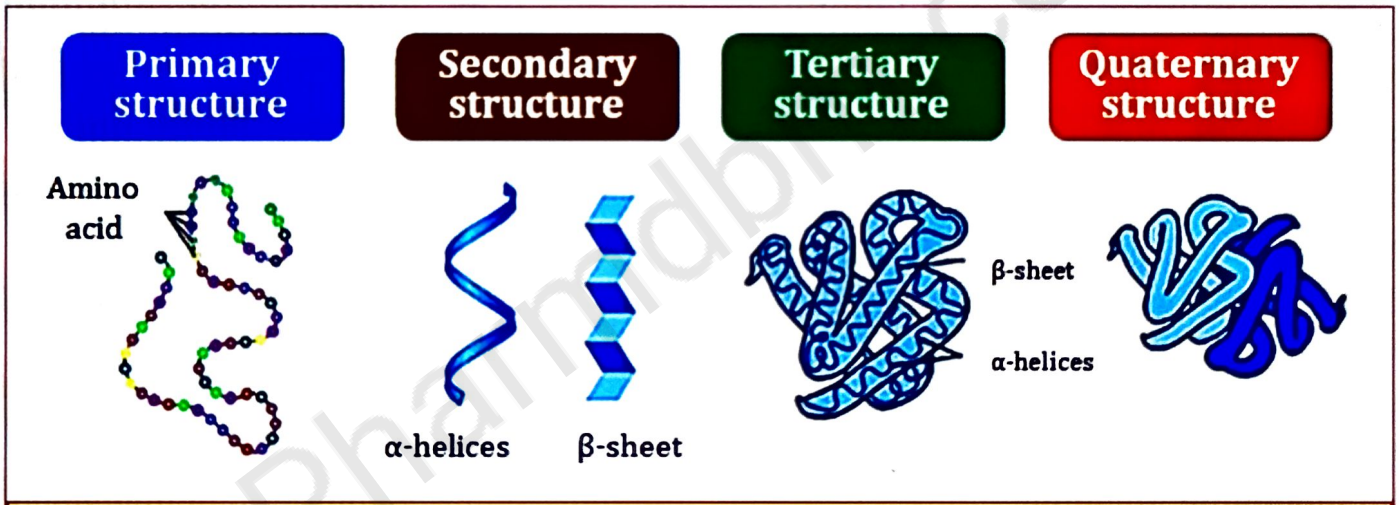
9. Careful denaturation is **sometimes reversible** (known as renaturation). Hemoglobin undergoes denaturation in the **presence of salicylate.** By removal of salicylate, hemoglobin is renatured.



STRUCTURE OF PROTEINS

Proteins are the polymers of L-D-amino acids. The structure of proteins is rather complex which can be **divided into 4 levels of organization:**

1. **Primary structure :** The **linear sequence** of amino acids forming the backbone of proteins (polypeptides).
2. **Secondary structure :** The spatial arrangement of protein by **twisting of the polypeptide chain.**
3. **Tertiary structure :** the **three dimensional structure** of a functional protein.
4. **Quaternary structure :** Some of the proteins are composed of **two or more polypeptide chains referred to as subunits.** The spatial arrangement of these subunits is known as quaternary structure.



Diagrammatic representation of protein structure

- The structural hierarchy of proteins is comparable with the structure of a building. The **amino acids may be considered as the bricks**, the wall as the **primary structure**, the **twists in a wall as the secondary structure**, a **full-fledged self-contained room as the tertiary structure. A building with similar and dissimilar rooms will be the quaternary structure.**
- The term protein is generally used for a polypeptide containing **more than 50 amino acids.**

❑ Primary structure of protein

- Each protein has a **unique sequence of amino acids** which is determined by the **genes contained in DNA**. The primary structure of a protein is largely responsible for its function.
- A vast majority of **genetic diseases are due to abnormalities in the amino acid sequences of proteins** i.e. changes associated with primary structure of protein. The amino acid composition of a protein determines its physical and chemical properties.

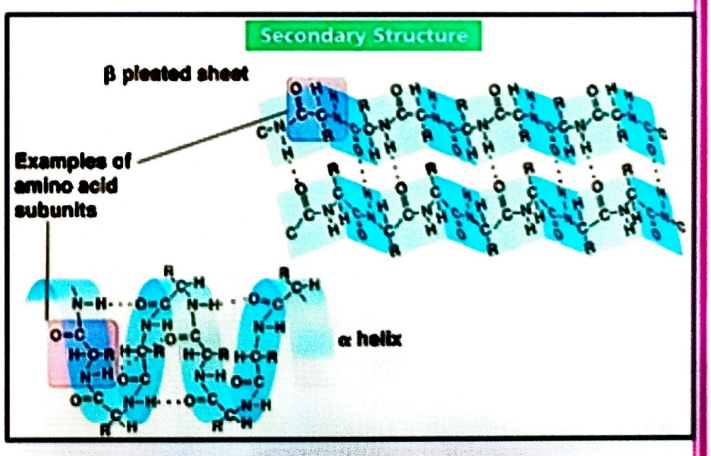
Peptide bond

The amino acids are held together in a protein by **covalent peptide bonds or linkages**. These bonds are **rather strong and serve as the cementing material** between the individual amino acids (considered as bricks).

- **Formation of a peptide bond :** When the **amino group of an amino acid combines with the carboxyl group** of another amino acid, a peptide bond is formed.
- **Characteristics of peptide bonds :** The peptide bond is **rigid and planar with partial double bond** in character. It generally exists **in trans configuration.** Both –C=O and -NH groups of peptide bonds are polar and are involved in hydrogen bond formation.

❑ Secondary structure of protein

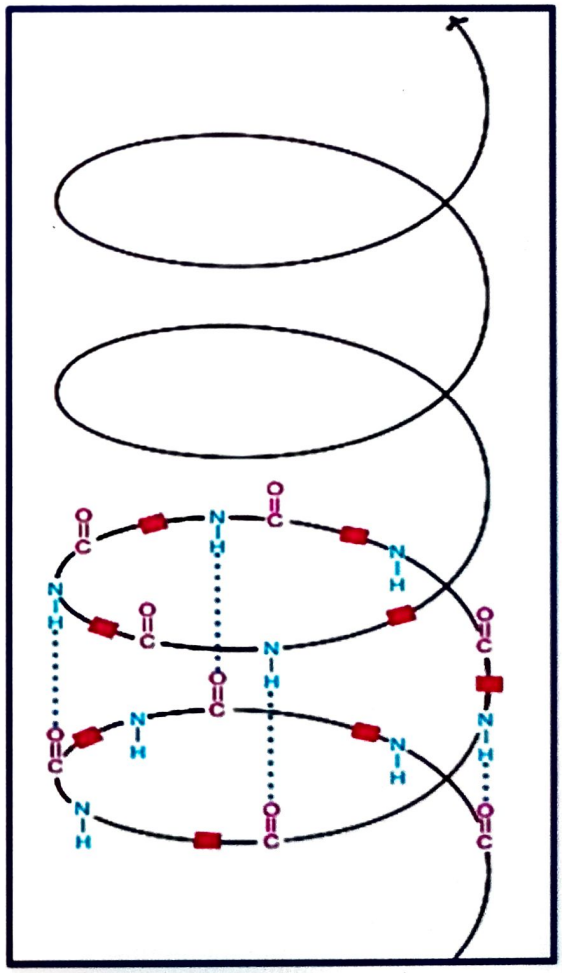
- The conformation of polypeptide chain by **twisting or folding is referred to as secondary structure.** The amino acids are located close to each other in their sequence.
- Two types of secondary structures, **α-helix and β-sheet**, are mainly identified. **Indian scientist Ramachandran** made a significant contribution in understanding the **spatial arrangement of polypeptide chains.**



α-Helix

α-Helix is the most common **spiral structure** of protein. It has a **rigid arrangement of polypeptide chain**. α-Helical structure was proposed by **Pauling and Corey (1951)** which is regarded as one of the milestones in the biochemistry research. The salient features of α-helix are given below

1. The α-helix is a **tightly packed coiled structure** with amino acid side chains extending outward from the central axis.
2. The α-helix is stabilized by **extensive hydrogen bonding**. It is formed between **H atom attached to peptide N, and O atom attached to peptide C**. The hydrogen bonds are individually weak but collectively, they are **strong enough to stabilize the helix.**
3. All the peptide bonds, except the first and last in a polypeptide chain, participate in hydrogen bonding.
4. Each turn of α-helix contains **3.6 amino acids and travels a distance of 0.54 nm**. The spacing of each amino acid is 0.15 nm.
5. α-Helix is a stable conformation formed spontaneously with the **lowest energy.**
6. The right handed α-helix is **more stable** than left handed helix.
7. Certain amino acids **(particularly proline) disrupt the α-helix**. Large number of **acidic (Asp, Glu) or basic (Lys, Arg, His)** amino acids also interfere with α-helix structure.



Diagrammatic representation of secondary structure of protein—a right handed α-helix

β-Pleated sheet

- This is the second type of structure (hence β after α) proposed by **Pauling and Corey.** β -Pleated sheets (or simply β -sheets) are composed of **two or more segments of fully extended peptide chains.**
- In the β -sheets, **the hydrogen bonds** are formed between the neighbouring segments of polypeptide chain(s).

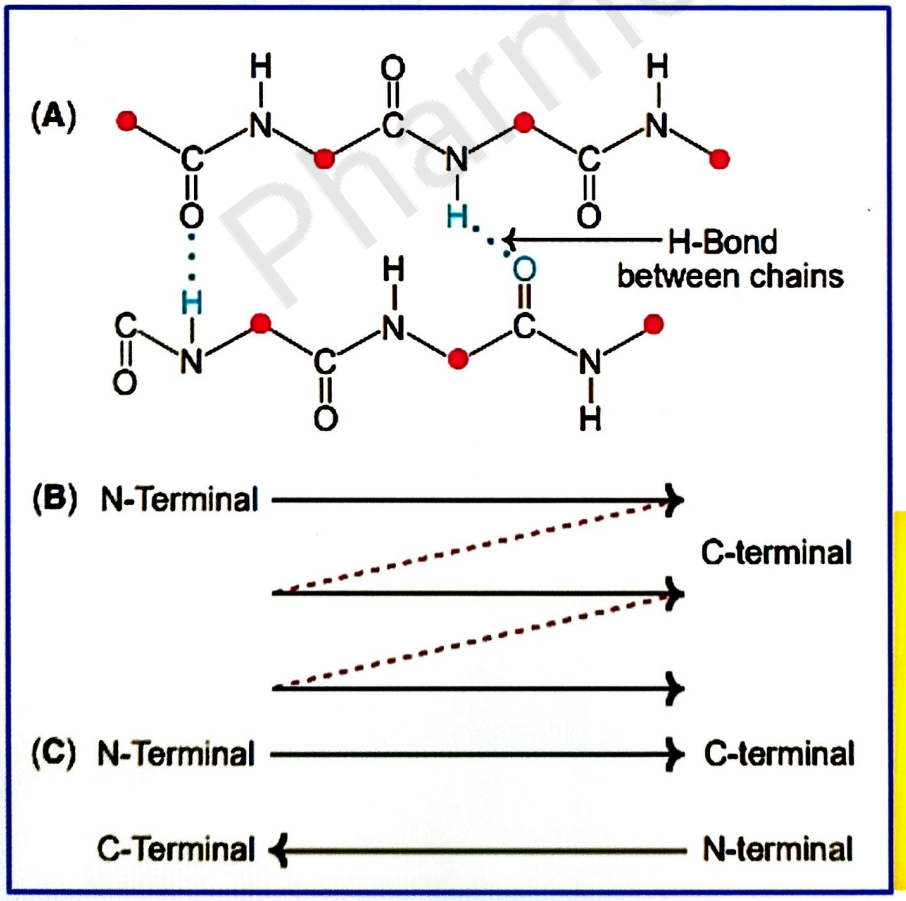
Parallel and anti-parallel β –sheets

- The polypeptide chains in the β -sheets may be arranged either in **parallel (the same direction) or anti-parallel (opposite direction).**
- β -Pleated sheet may be formed either by **separate polypeptide chains** (H-bonds are interchain) or a single polypeptide chain **folding back on to itself** (H-bonds are intrachain).

Occurrence of β-sheets :

Many proteins contain β -pleated sheets. As such, the **α -helix and β-sheet are commonly found in the same protein structure.**

In the globular proteins, **β-sheets form the core structure.**



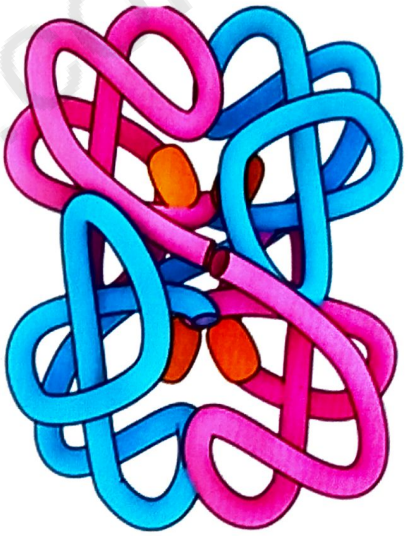
Structure of β-pleated sheet
(A) Hydrogen bonds between polypeptide chains
(B) Parallel β -sheet
(C) Antiparallel β -sheet

❑ Tertiary structure of protein

The three-dimensional arrangement of protein structure is referred to as tertiary structure. It is a compact structure with **hydrophobic side chains held interior while the hydrophilic groups are on the surface of the protein** molecule. This type of arrangement ensures stability of the molecule.

- **Bonds of tertiary structure :** Besides the hydrogen bonds, **disulfide bonds (-S-S-), ionic interactions (electrostatic bonds), hydrophobic interactions and van der Waals forces** also contribute to the tertiary structure of proteins.
- **Domains :** The term domain is used to represent the basic units of protein structure (tertiary) and function. **A polypeptide with 200 amino acids normally consists of two or more domains.**

❑ Quaternary structure of protein

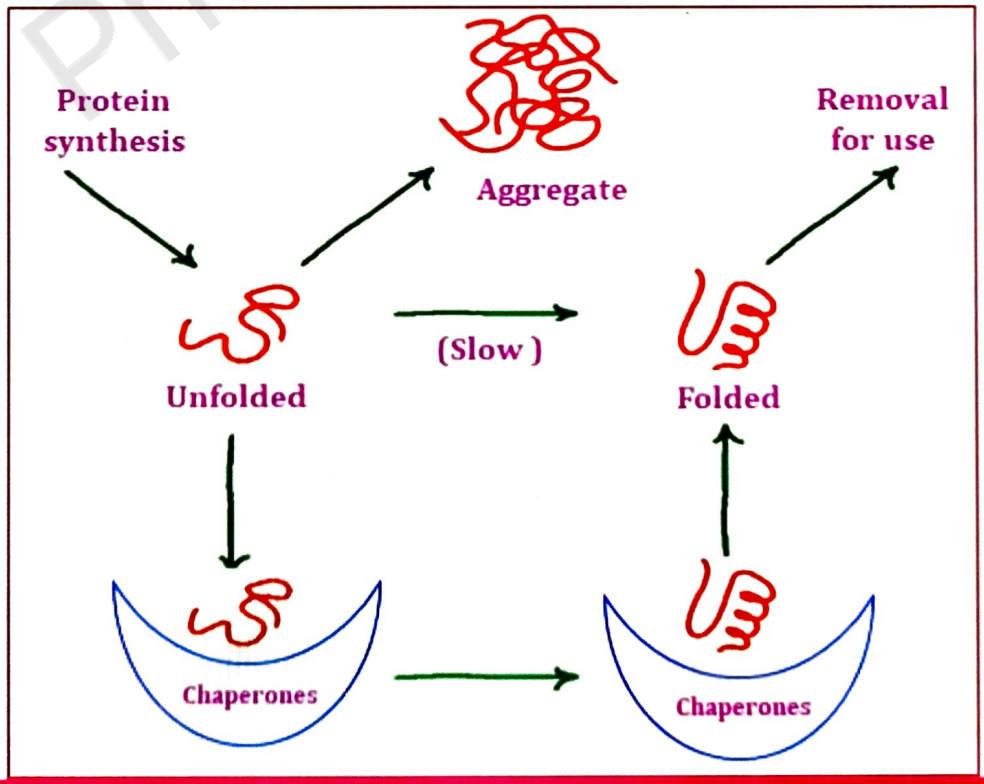


- A great majority of the proteins are composed of single polypeptide chains. Some of the proteins, however, **consist of two or more polypeptides which may be identical or unrelated.** Such proteins are termed as oligomers and possess quaternary structure.
- The individual polypeptide chains are known as **monomers, protomers or subunits.** A dimer consist of **two polypeptides while a tetramer has four.**
- Bonds in quaternary structure : The monomeric subunits are held together by **nonconvalent bonds namely hydrogen bonds, hydrophobic interactions and ionic bonds.**
- Importance of oligomeric proteins : These proteins play a significant role in the **regulation of metabolism and cellular function.** Examples of oligomeric proteins : **Hemoglobin, aspartate transcarbomylase, lactate dehydrogenase.**

REGULARITIES IN PROTEIN PATHWAYS

Chaperones and protein folding

- The **three dimensional conformation** of proteins is important for their biological functions.
- Some of the proteins can spontaneously generate the correct **functionally active conformation** e.g. **denatured pancreatic ribonuclease**. However, a vast majority of proteins can attain correct conformation, **only through the assistance of certain proteins referred to as chaperones.**
- **Chaperones are heat shock proteins** (originally discovered in response to heat shock). They **facilitate and favour the interactions on the polypeptide surfaces** to finally give the **specific conformation of a protein**.
- Chaperones can **reversibly bind** to **hydrophobic regions of unfolded proteins and folding intermediates.**
- They can **stabilize intermediates**, prevent formation of **incorrect intermediates**, and also **prevent undesirable interactions** with other proteins.
- All these activities of chaperones help the **protein to attain compact and biologically active conformation.**



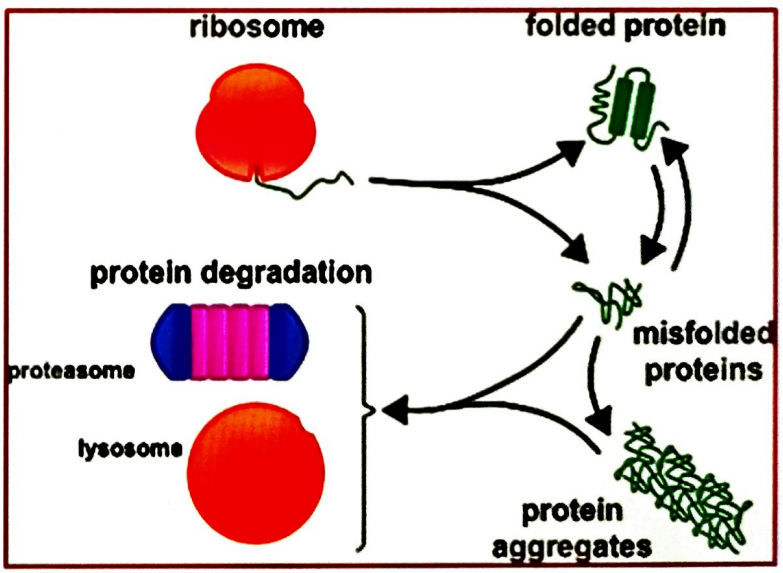
Types of chaperones

Chaperones are categorized into two major groups:-

1. **Hsp70 system:** This mainly consists of **Hsp70 (70-kDa heat shock protein) and Hsp40 (40-kDa Hsp)**. These proteins can bind **individually to the substrate** (protein) and help in the **correct formation of protein folding.**
2. **Chaperonin system:** This is a large oligomeric assembly **which forms a structure into which the folded proteins are inserted**. The chaperonin system mainly has **Hsp60 and Hsp10 i.e. 60 kDa Hsp and 10 kDa Hsp.** Chaperonins are required at a later part of the protein folding process, and often **work in association with Hsp70 system.**

Protein misfolding and diseases

- The failure of a protein to fold properly generally **leads to its rapid degradation.**
- Cystic fibrosis (CF) is a common **autosomal recessive disease**. Some cases of CF with mutations that result in **altered protein** (cystic fibrosis transmembrane conductance regulator or in short CFTR) have been reported.
- **Mutated CFTR cannot fold properly**, besides not being able to get glycosylated or transported. Therefore, **CFTR gets degraded.**
- Certain **neurological diseases which are due to cellular accumulation of aggregates of misfolded proteins** or their partially degraded products have been identified.
- The term **prions (proteinous infectious agents)** is used to collectively represent them.

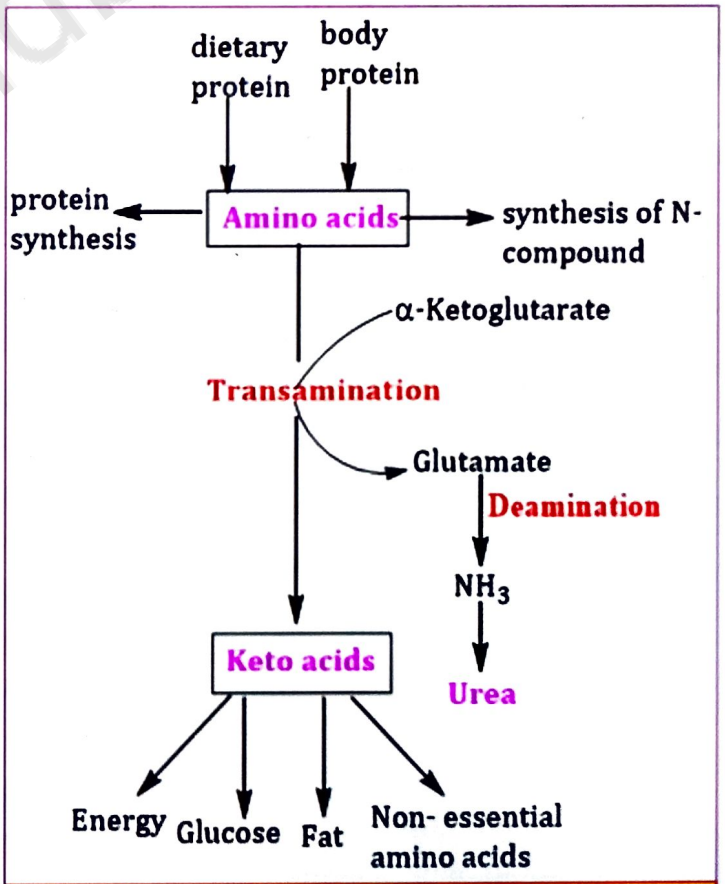


➢ Metabolism of amino acids

- The amino acids undergo certain common reactions like **transamination followed by deamination for the liberation of ammonia.**
- The amino group of the amino acids is utilized for **the formation of urea which is an excretory end product of protein metabolism.**
- The carbon skeleton of the amino acids is first converted to **keto acids (by transamination)** which meet one or more of the following fates.
  1. Utilized to **generate energy.**
  2. Used for the **synthesis of glucose.**
  3. Diverted for the **formation of fat or ketone bodies.**
  4. Involved in the **production of non-essential amino acids.**

❑ Transamination

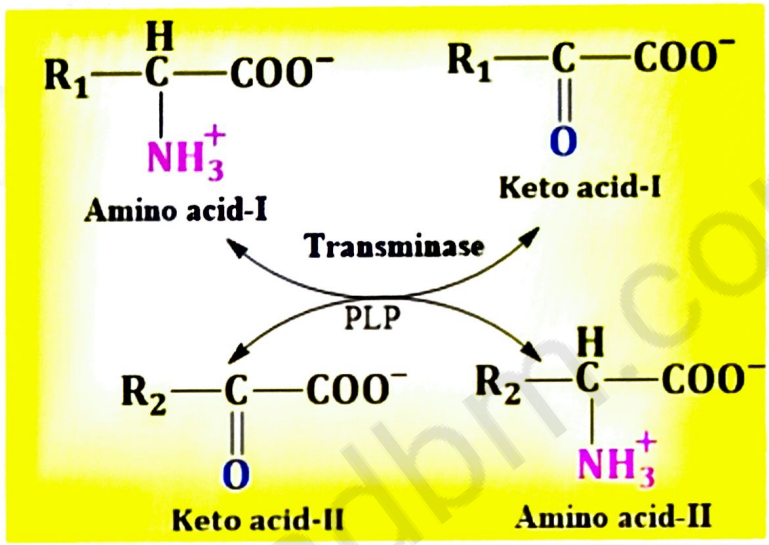
- The transfer of an **amino (NH_2) group from an amino acid to a keto acid is known as transamination.** This process involves the interconversion of a pair of amino acids and a pair of keto acids, **catalysed by a group of enzymes called transaminases** (aminotransferases).



An overview of amino acid metabolism

Salient features of transamination

1. All transaminases require **pyridoxal phosphate (PLP)**, a coenzyme derived from **vitamin B_6**.
2. Specific transaminases exist for **each pair of amino and keto acids**. However, only two— namely, **aspartate transaminase and alanine transaminase**—make a significant contribution for transamination.
3. There is **no free NH_3 liberated**, only the transfer of amino group occurs.
4. Transamination is **reversible.**



Transamination reaction

5. Transamination is very important for the **redistribution of amino groups and production of non-essential amino acids, as per the requirement of the cell.** It involves both catabolism (degradation) and anabolism (synthesis) of amino acids.
6. Transamination diverts the **excess amino acids towards energy generation.**
7. The amino acids undergo transamination to finally **concentrate nitrogen in glutamate**. Glutamate is the only amino acid that undergoes oxidative deamination to a **significant extent to liberate free NH_3 for urea synthesis.**
8. All amino acids **except lysine, threonine, proline and hydroxyproline participate in transamination.**

Mechanism of transamination

Transamination occurs in two stages

1. Transfer of the amino group to the **coenzyme pyridoxal phosphate** (bound to the coenzyme) to form pyridoxamine phosphate.
2. The amino group of **pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid** and the enzyme with PLP is regenerated.

- All the transaminases require **pyridoxal phosphate (PLP)**, a derivative of vitamin B_6. **The aldehyde group of PLP is linked** with Ɛ-amino group of lysine residue, at the active site of the enzyme forming a Schiff base (imine linkage).
- When an **amino acid (substrate) comes in contact with the enzyme**, it **displaces lysine and a new Schiff base linkage is formed**.
- The amino acid-PLP-Schiff base **tightly binds with the enzyme by noncovalent forces. Snell and Braustein proposed a Ping Pong Bi Bi mechanism** involving a series of intermediates (aldimines and ketimines) in transamination reaction.

❑ Deamination

- The **removal of amino group from the amino acids as NH_3 is deamination**. Transamination (discussed above) involves only the **shuffling of amino groups among the amino acids.** On the other hand, **deamination results in the liberation of ammonia for urea synthesis.** Simultaneously, the **carbon skeleton of amino acids is converted to keto acids**. Deamination may be either **oxidative or non-oxidative.**
- Although transamination and deamination are separately discussed, they occur simultaneously, often involving **glutamate as the central molecule.** For this reason, some authors use the **term transdeamination while describing the reactions of transamination and deamination, particularly involving glutamate.**

I. Oxidative deamination

- Oxidative deamination is the **liberation of free ammonia** from the **amino group of amino acids coupled with oxidation.** This takes place mostly in **liver and kidney**. The purpose of oxidative deamination is to **provide NH_3 for urea synthesis and α-keto acids for a variety of reactions**, including energy generation.

✓ **Role of glutamate dehydrogenase :** In the process of transamination, the **amino groups of most amino acids are transferred to α- ketoglutarate to produce glutamate**. Thus, glutamate serves as a **'collection centre'** for amino groups in the biological system. Glutamate rapidly **undergoes oxidative deamination, catalysed by glutamate dehydrogenase (GDH) to liberate ammonia**. This enzyme is unique in that it can utilize either NAD^+ or $NADP^+$ as a coenzyme. Conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, **α- iminoglutarate.**

✓ **Regulation of GDH activity : Glutamate dehydrogenase is a zinc containing mitochondrial enzyme.** It is a complex enzyme consisting of six identical units with a molecular weight of 56,000 each. **GDH is controlled by allosteric regulation. GTP and ATP inhibit— whereas GDP and ADP activate—glutamate dehydrogenase.** Steroid and thyroid hormones inhibit GDH.

- After ingestion of a protein-rich meal, **liver glutamate level is elevated.** It is converted to **α-ketoglutarate with liberation of NH_3.**
- Further, when the **cellular energy levels are low, the degradation of glutamate is increased to provide α-ketoglutarate** which enters TCA cycle to liberate energy.

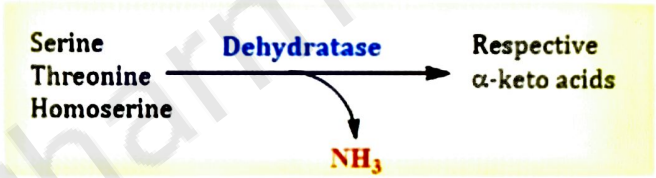
Oxidative deamination by amino acid oxidases:

- L-Amino acid oxidase and D-amino acid oxidase are **flavoproteins, possessing FMN and FAD, respectively**. They act on the corresponding amino acids (L or D) **to produce α-keto acids and NH_3**. In this reaction, oxygen is reduced to H_2O_2, which is later **decomposed by catalase**.
- The activity of L-amino acid oxidase is much low while that of D-amino acid oxidase is high in tissues (mostly liver and kidney). L-Amino acid oxidase **does not act on glycine and dicarboxylic acids.** This enzyme, due to its very low activity, **does not appear to play any significant role in the amino acid metabolism.**

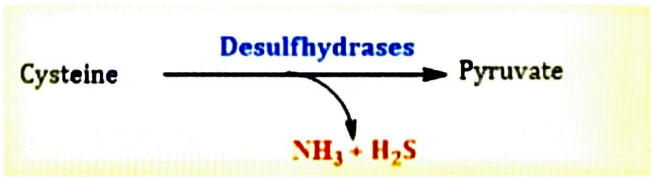
II. Non-oxidative deamination

- Some of the amino acids can be **deaminated to liberate NH_3 without undergoing oxidation**

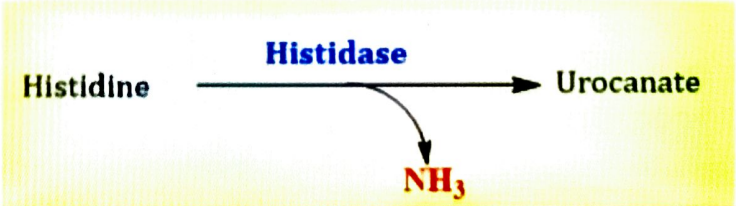
(a) Amino acid dehydrases : Serine, threonine and homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydrases (dehydratases).



(b) Amino acid desulfhydrases : The **sulfur amino acids, namely cysteine and homocysteine**, undergo deamination coupled with desulfhydration to give keto acids.



(c) Deamination of histidine : The **enzyme histidase acts on histidine** to liberate NH_3 by a non-oxidative deamination process.

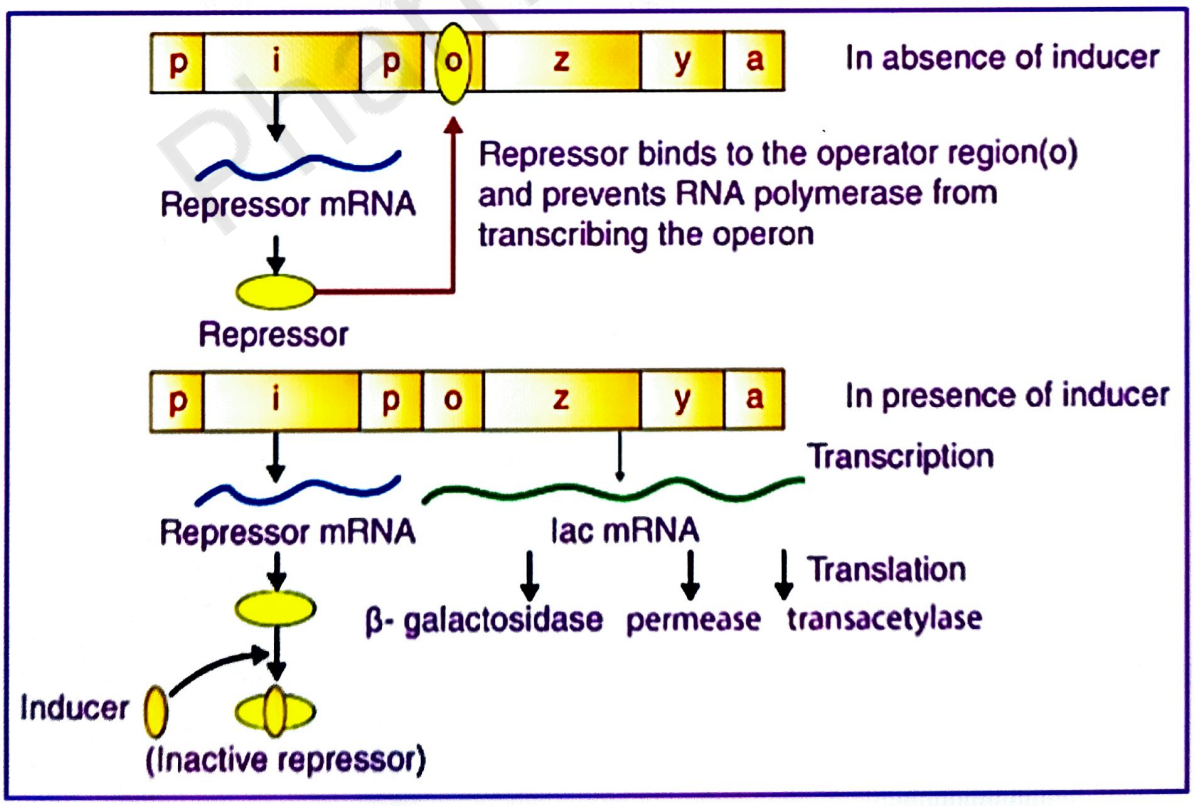


POSITIVE CONTROL & SIGNIFICANCE OF PROTEIN SYNTHESIS

- When a gene expresses in the presence of an **activator or inducer, it is said to be under positive control.**
- If positive regulatory protein is missing, the **operon is turned off.**
- For example, **lactose or allolactose operate as lac operon inducers.**
- The interaction with inducers **inactivates the repressor protein.**

Operons

- An **operon** is a **cluster of coordinately regulated genes.**
- It includes **structural genes** (generally encoding enzymes), **regulatory genes** (encoding, e.g. activators or repressors) and **regulatory sites** (such as promoters and operators).
- The type of control is defined by the **response of the operon when no regulatory protein is present.**
- In the case of **negative control**, the **genes in the operon are expressed unless they are switched off by a repressor protein.**
- Thus the operon will be **turned on constitutively** (the genes will be expressed) **when the repressor in inactivated.**



- In the case of **positive control**, the genes are expressed only when an **active regulator protein, e.g. an activator, is present.**
- Thus the operon will be turned off when the **positive regulatory protein is absent or inactivated.**

Catabolic versus Biosynthetic Operons

- Catabolic pathways **catalyze the breakdown of nutrients** (the substrate for the pathway) to **generate energy, or more precisely ATP**, the energy currency of the cell.
- In the **absence of the substrate,** there is no reason for the catabolic enzymes to be present, and the operon encoding them is repressed.
- In the presence of the substrate, when the enzymes are needed, **the operon is induced or de-repressed.**

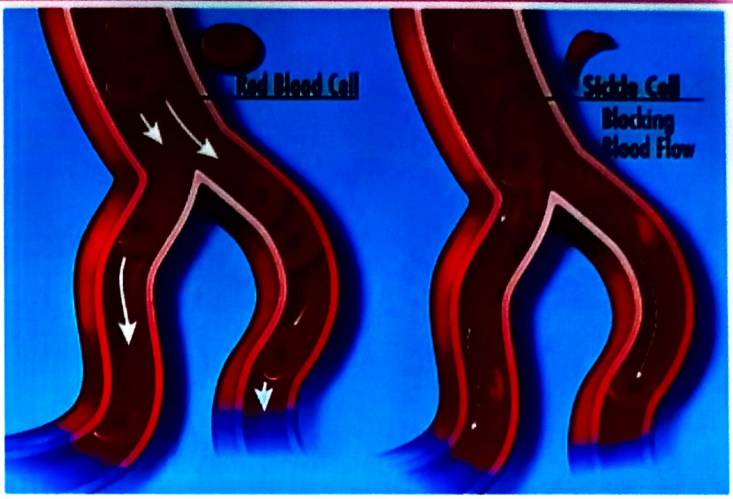
Inducible versus repressible Operons

- Inducible operons are **turned on in response to a metabolite** (a small molecule undergoing metabolism) that regulates the operon. E.g. **the lac operon is induced in the presence of lactose** (through the action of a metabolic by-product allolactose).
- **Repressible operons are switched off in response to a small regulatory molecule**. E.g., the ***trp*operon is repressed in the presence of tryptophan**.

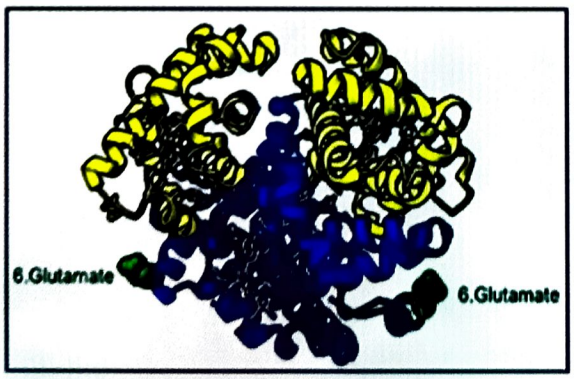
❑ Role of protein synthesis in disease

- Many diseases are caused by **mutations in genes, due to the direct connection between the DNA nucleotide sequence** and the **amino acid sequence of the encoded protein**.
- Changes to the **primary structure of the protein can result in the protein mis-folding or malfunctioning**. Mutations within a single gene have been identified as a cause of multiple diseases, including sickle cell disease, known as single gene disorders.

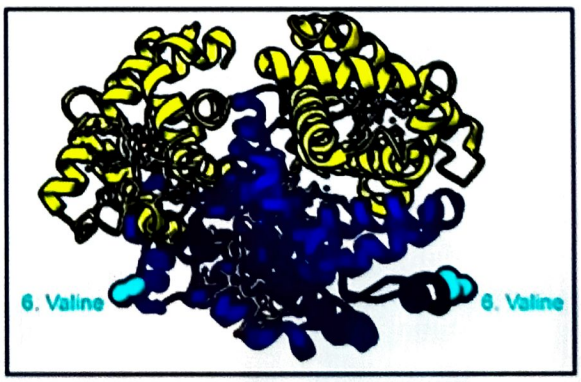
Sickle cell disease



- Sickle cell disease is a group of diseases caused by **a mutation in a subunit of hemoglobin, a protein found in red blood cells** responsible for transporting oxygen.
- The most dangerous of the sickle cell diseases is known as **sickle cell anemia.** Sickle cell anemia is the most common **homozygous recessive single gene disorder, meaning the affected individual must carry a mutation in both copies of the affected gene** (one inherited from each parent) to experience the disease.
- Hemoglobin has a **complex quaternary structure and is composed of four polypeptide subunits - two A subunits and two B subunits.**
- Patients with sickle cell anemia have a **missense or substitution mutation** in the gene encoding the hemoglobin B subunit polypeptide chain.
- A missense mutation means the **nucleotide mutation alters the overall codon triplet** such that a different amino acid is paired with the new codon.
- In the case of sickle cell anemia, the most common missense mutation is a single nucleotide mutation **from thymine to adenine in the hemoglobin B subunit gene.** This changes **codon 6 from encoding the amino acid glutamic acid to encoding valine.**

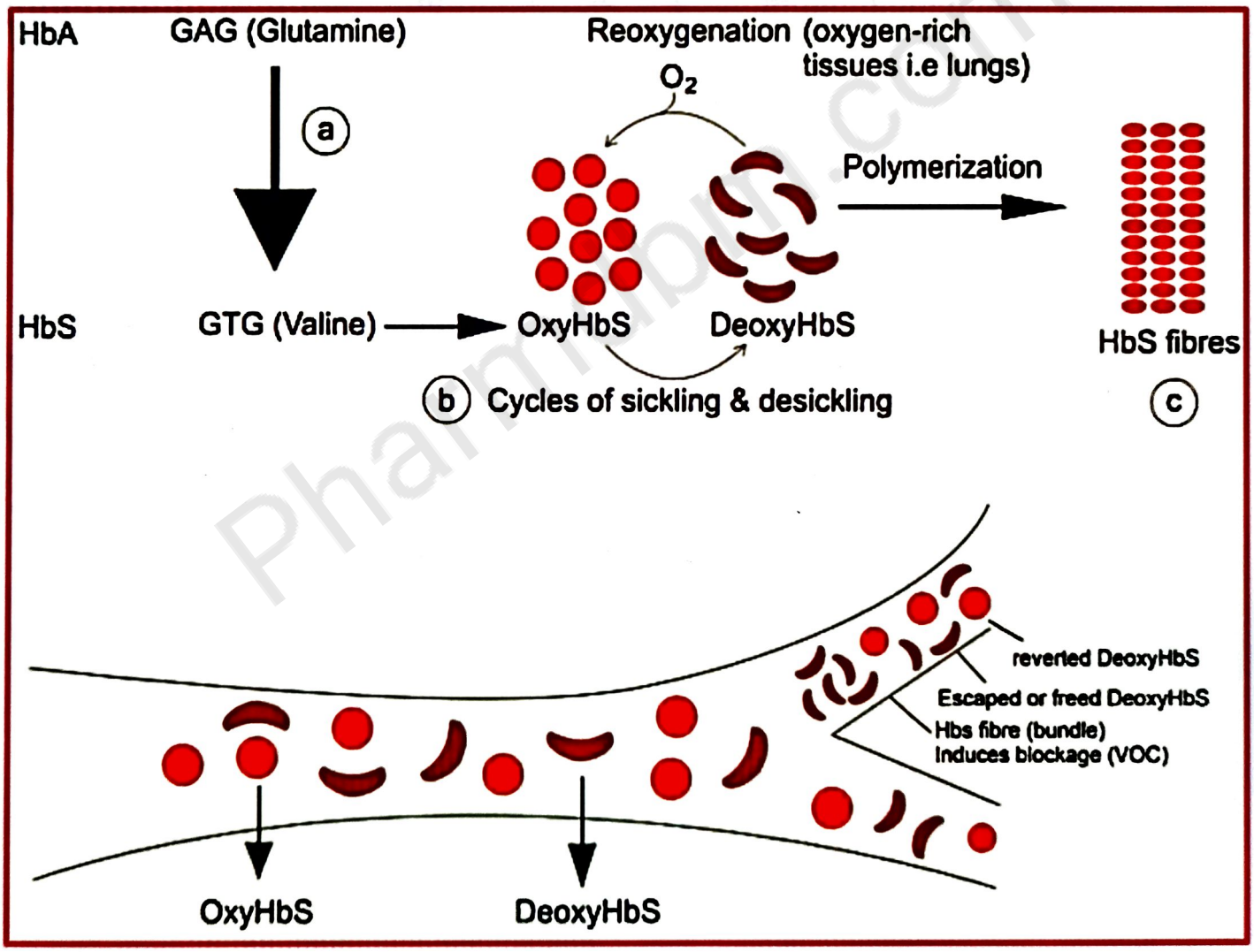


Normal Hemoglobin



Sickle Cell Hemoglobin

- This change in the primary structure of the **hemoglobin B subunit polypeptide chain alters the functionality** of the hemoglobin multi-subunit complex in low oxygen conditions.
- When red blood cells **unload oxygen into the tissues of the body**, the **mutated haemoglobin protein starts to stick together** to form a semi-solid structure within the red blood cell.
- This **distorts the shape of the red blood cell, resulting in the characteristic "sickle" shape**, and reduces cell flexibility.
- This rigid, distorted **red blood cell can accumulate in blood vessels creating a blockage.** The blockage prevents blood flow to tissues and can **lead to tissue death which causes great pain to the individual.**



Cancer

- Cancers form as a result of **gene mutations as well as improper protein translation.** In addition to cancer cells **proliferating abnormally, they suppress the expression of anti-apoptotic or pro-apoptotic genes** or proteins.
- Most cancer cells see a **mutation in the signaling protein RAS**, which functions as **an on/off signal transductor in cells.**
- In cancer cells, **the RAS protein becomes persistently active**, thus promoting the proliferation of the cell due to the absence of any regulation.
- Additionally, **most cancer cells carry two mutant copies of the regulator gene p53**, which acts as a gatekeeper for damaged genes and initiates apoptosis in malignant cells.
- In its absence, the **cell cannot initiate apoptosis or signal** for other cells to destroy it.
- As the tumor cells proliferate, they either remain confined to one area and **are called benign, or become malignant cells that migrate to other areas of the body.**
- Oftentimes, these malignant cells **secrete proteases that break apart the extracellular matrix of tissues.**
- This then allows the cancer to enter its terminal stage **called Metastasis**, in which the cells enter the bloodstream or the lymphatic system to travel to a new part of the body.