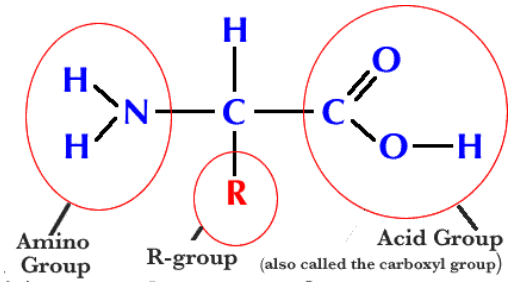
Protein
Basic Introduction

1 *Introduction:*

Proteins are the most widely distributed biological macromolecules, occurring in all type of cells. Amino acids are the smallest structure unit of proteins. All proteins, whether from the most earliest lines of bacteria or from the complex forms of life, are built from the same fixed 20 amino acids. Most notable is that cells can produce proteins with unusually different properties and activities by constructing the same 20 amino acids in different combinations and orders. From these building blocks units different organisms can make such widely diverse products as enzymes, hormones, antibodies, transporters, muscle fibers, the lens protein of the eye, feathers, milk proteins, antibiotics, and mushroom poisons and other substances which have different biological activities. While proteins contain only L-α-amino acids, microorganisms made up of more complex peptides that contain both D- and L-α-amino acids.

1.1 *Definition:*

Proteins may be defined as high molecular mass compounds containing entirely chains of amino acids. The general formula of a naturally occurring amino acid may be represented with the following formula. In this structure an amino group is present on the carbon atom adjacent to carboxyl group.



The amino acids having this common formula are known as alpha (α) amino acids. In its structure four bonds of a carbon atom (C_α) are occupied by NH_2, COOH, H and R molecules. R may be any alkyl chain which define the properties of an amino acid and is called as the side chain of amino acid.

1.2 *Function of Protein*

Proteins are the agents of biologically importance. Nearly all cellular activities are depending on one or more specific proteins. Thus, a useful way to categorize the vast number of

proteins is by the biological function they fulfill. The various functions of proteins are as follows:

Enzymes

Enzymes are also classified as proteins. More than 3000 different enzymes are recorded in enzyme nomenclature, the standard reference book on enzyme classification. **Enzymes** are catalysts that speed up the rate of biological reactions. Each enzyme is very specific in their activity and has a specific function and involve in a particular metabolic reaction. Almost every step in metabolism is catalyzed by an enzyme. Enzymes are scientifically classified according to the nature of the reaction that they catalyze, such as the transfer of a phosphate group (Phosphotransferase) or an oxidation–reduction (Oxidoreductase).

The proper names of enzymes come from the particular reaction within the class that they catalyze, as in ATP: D-fructose-6-phosphate 1-phosphotransferase.

Regulatory Proteins

A number of proteins do not perform any chemical transformation but can regulate the ability of other proteins to carry out their physiological functions. Such proteins are termed as regulatory proteins. A well-known example is Insulin, this hormone regulate glucose metabolism in animals. Insulin is a comparatively small protein and contains two polypeptide chains linked together by disulfide cross-bridges.

Transport Proteins

These proteins involve in transportation of a specific substances from one place to another. One type of transport is represented by the transport of oxygen from lungs to the tissues by haemoglobin or transport of fatty acids from adipose tissue to various organs by the blood protein (serum albumin). Membrane transport proteins involve in movement of metabolite across the biological membrane. For examples the Transport Proteins are responsible for the absorption of essential nutrients into the cell, such as glucose or amino acids.

Storage Proteins

Proteins which provide a reservoir of an essential nutrient are called storage proteins. Because proteins are amino acid polymers and nitrogen is generally a limiting nutrient for growth, organisms have utilized proteins as a source to provide sufficient nitrogen in times of need. For example, ovalbumin, the protein of egg white, provides the developing bird embryo with a source of nitrogen during its isolation within the egg.

Casein is the most abundant protein of milk and thus the major source of nitrogen for mammalian infants. The seeds of higher plants frequently contain 60% storage protein to make the germinating seed nitrogen-sufficient during this critical period of plant development.

Ferritin is a protein found in animal tissues that binds with iron, retaining this essential metal so that it is available for the synthesis of hemoglobin.

Contractile and Motile Proteins

Certain proteins give cells with unique abilities for movement. Cell division, muscle contraction, and cell movement represent some ways in which cells perform motion. Examples include actin and myosin, the filamentous proteins form the contractile mechanism of cells, and tubulin, the major component of microtubules.

Structural Proteins

A very important role of proteins is creating and maintaining biological structures. Structural proteins provide strength and protection to cells and tissues. Monomeric units of structural Proteins normally polymerize to generate long fibers (as in hair). α-*Keratins* are insoluble fibrous proteins that make hair, horns, and fingernails.

Collagen, another insoluble fibrous protein, generally found in bone, connective tissue, tendons, and cartilage. Where it forms inelastic fibrils of great strength. One-third of the total protein in a vertebrate animal is collagen. A structural protein having elastic properties is, appropriately, elastin, an important component of ligaments. Certain insects make a structurally useful protein, fibroin the major constituent of cocoons (silk) and spider webs.

Scaffold Proteins (Adapter Proteins)

Some proteins play a just discovered role in the complex pathways of cellular effect to hormones and growth factors. These proteins are known as scaffold or adapter proteins, have a integrated organization in which specific portion (modules) of the protein's structure recognize and bind with certain structural components in other proteins through protein–protein interactions.

Protective and Exploitive Proteins

In contrast to the passive protective nature of some structural proteins, another group can be appropriately classified as protective or exploitive proteins because of their active role in cell defense, protection, or exploitation. Outstanding among the protective proteins are the immunoglobulins or antibodies produced by the lymphocytes of vertebrates. Antibodies have ability to definitely recognize and neutralize "foreign" molecules resulting from the attack of the organism like as bacteria, viruses, or other infectious agents.

Other group of protective proteins is the blood-clotting proteins, thrombin and fibrinogen, which prevent the loss of blood when the circulatory system is damaged.

Another class of exploitive proteins includes the toxins produced by bacteria, such as diphtheria toxin and cholera toxin.

1.3 Physiochemical properties of proteins

1.3.1 Physical Properties of Proteins

- ***Colour and Taste***

  Proteins are colourless and usually tasteless. These are homogeneous and crystalline.
- ***Shape and Size***

  The proteins vary in shape from simple crystalloid spherical structures to long fibrillar structures. Two different arrangements of shape have been recognized:

  Globular proteins- These are spherical in shape and occur mainly in plants, esp., in seeds and in leaf cells. These are bundles formed by folding and crumpling of protein chains. e.g.,pepsin, edestin, insulin, ribonuclease etc.

  Fibrillar proteins- These are thread-like or ellipsoidal in shape and occur generally in animal muscles. Most of the studies related to protein structure have been conducted using these proteins. e.g. fibrinogen, myosin etc.

- ***Molecular Weight***
  The proteins generally have large molecular weights ranging between 5 × 103 and 1 × 106. It might be noticed that the values of molecular weights of many proteins lie close to or multiples of 35,000 and 70,000.
- ***Colloidal Nature***
  Because of their big size, the proteins show many colloidal properties, such as their diffusion rates are very slow and they may produce considerable light-scattering in solution, thus resulting in visible turbidity (Tyndall effect).
- ***Denaturation***
  Denaturation refers to the changes in the properties of a protein. In other words, it is the loss of biologic activity. In many instances the process of denaturation is followed by coagulation— a process where denatured protein molecules tend to form large aggregates and to precipitate from solution.
- ***Amphoteric Nature***
  Like amino acids, the proteins are amphoteric, i.e., they act as acids and alkalies both. These can be migrating in an electric field and the direction of movement depends upon the net charge possessed by the molecule. The net charge is affected by the pH value. Each protein has a fixed value of isoelectric point (pl) at which it will move in an electric field.
- ***Ion Binding Capacity***
  The proteins can form salts with both cations and anions based on their net charge.
- ***Solubility***
  The solubility of proteins is influenced by pH. Solubility is lowest at isoelectric point and increases with increasing acidity or alkalinity. This is because when the protein molecules exist as either cations or anions, repulsive forces between ions are high, since all the molecules possess excess charges of the same sign. Thus, they will be more soluble than in the isoelectric state.
- ***Optical Activity***
  All protein solutions rotate the plane of polarized light to the left, i.e., these are levoratotory.

1.3.2 Chemical Properties of Proteins

- **Hydrolysis**
  Proteins are hydrolyzed by a variety of hydrolytic agents.
  A. By acidic agents: Proteins, upon hydrolysis with conc. HCl (6–12N) at 100–110°C for 6 to 20 hrs, yield amino acids in the form of their hydrochlorides.
  B. By alkaline agents: Proteins may also be hydrolyzed with 2N NaOH.
- **Reactions involving -COOH Group**
  A. Reaction with alkalies (Salt formation)
  B. Reaction with alcohols (Esterification)
  C. Reaction with amines
- **Reactions involving -NH2 Group**
  A. Reaction with mineral acids (Salt formation): When either free amino acids or proteins are treated with mineral acids like HCl, the acid salts are formed.
  B. Reaction with formaldehyde: With formaldehyde, the hydroxy-methyl derivatives are formed.
  C. Reaction with benzaldehyde: Schiff's bases are formed

D. Reaction with nitrous acid (Van Slyke reaction): The amino acids react with HNO_2 to liberate N_2 gas and to produce the corresponding α-hydroxy acids.
E. Reaction with acylating agents (Acylation)
F. Reaction with FDNB or Sanger's reagent
G. Reaction with dansyl chloride

- **Reactions involving both -COOH and -NH2 Group**

A. Reaction with Triketohydrindene hydrate (Ninhydrin reaction)
B. Reaction with Phenyl Isocyanate: With Phenyl Isocyanate, Hydantoic acid is formed which in turn can be converted to hydantoin.
C. Reaction with Phenyl Isothiocyanate or Edman reagent
D. Reaction with Phosgene: With phosgene, N-carboxyanhydride is formed
E. Reaction with carbon disulfide: With carbon disulfide, 2-thio-5-thiozolidone is produced

- **Reactions involving -R Group or Side Chain**

A. Biuret test
B. Xanthoproteic test
C. Millon's test
D. Folin's test
E. Sakaguchi test
F. Pauly test
G. Ehrlich test

1.4 Classification of Proteins

Like carbohydrates and lipids, proteins could not be classified only on the basis of structural similarities, because protein molecules possess great structural complexities. These can be classified on the basis of physical properties like solubility and composition.

Accordingly, proteins are classified as follow:

I. Simple protein
II. Conjugated proteins
III. Derived proteins

Above groups are further subdivided into a number of subgroups:

I. Simple protein

- Albumin
- Globuline
- Gluteline
- Prolamines
- Sclera proteins
- Histones
- Protamine

II. Conjugated proteins

- Nucleoprotein
- Phosphoproteins
- Glycoproteins

Lipoproteins
Metallo proteins
Chromoproteins

III. Derived proteins

Primary derived proteins: Metaproteins.
Secondary derived proteins: Proteoses, peptones, peptides.

In earlier days, proteins were classified as following:

(1) Fibrous—elongated proteins, e.g., silk fibroin, keratin etc.

(2) Globular—spherical, compact proteins, e.g., egg albumin, caesin and most enzymes. Fibrous proteins tend to be insoluble in water and other solvents, whereas globular proteins are soluble in water and in solutions of salt and water.

Nowadays, there are two methods for protein classification:

(1) According to the composition of the protein and

(2) According to the function of the protein.

These two classifications are given here:

1. Classification according to the composition:

In this process, proteins are classified into two groups:

(a) Simple proteins, and

(B) Conjugated proteins.

A. Simple proteins:

A simple protein is composed of only α-amino acids. Thus, produces completely α-amino acids on hydrolysis. These proteins are further subdivided according to their solubility in various solvents. Different simple proteins are described here.

(i) Albumins:

These are soluble in water and in dilute salt solutions. Albumins constitute the most important and the most common group of simple proteins. These are present in egg white (egg albumin) and in blood (serum albumin).

(ii) Globulins:

These are insoluble in water but are soluble in dilute salt solutions. They are widely distributed group of simple proteins. They are present as antibodies in blood serum and as blood fibrinogen.

(iii) Histones:

They are soluble in water and insoluble in dilute ammonium hydroxide. Histones contain a high proportion of basic amino acids (lysine and/or arginine). These are found in combination with nucleic acids in the nucleoprotein of the cell.

(iv) Scleroproteins (albuminoids):

These are characterized by their insolubility in water and other solvents. Scleroproteins have structural and protective functions in the body. The examples of scleroproteins are keratin (present in hair, skin and nails), collagen (present in bone, tendon and cartilage) and elastin (elastic fibers of connective tissues).

B. Conjugated proteins:

These are formed of α-amino acids and a non-protein material. The non-protein material of the conjugated protein is called prosthetic group. Different types of conjugated proteins are subdivided on the basis of their prosthetic group.

Different conjugated proteins are as follows:

(i) Phosphoproteins:

These are composed of α-amino acids and phosphoric acids. So, their prosthetic group is phosphoric acid. Caesin, present in milk, is an important member of this group.

(ii) Glycoproteins:

They contain a carbohydrate or a carbohydrate derivative as prosthetic group. Mucin, a constituent of saliva, is a glycoprotein.

(iii) Chromo-proteins:

Their prosthetic group is a pigment compound. Example: Haemoglobin. It possesses the iron-containing pigment haeme coordinated to the simple protein globin.

(iv) Necleoproteins:

Here prosthetic groups are complex polymers of high molar masses and are called nucleic acids (DNA and RNA). Nucleoproteins are present in all the cells of plants and animals.

(v) Lipoproteins:

They consist of cholesterol esters and phospholipids attached to the protein molecules. They are frequently classified as compound lipids. Most of the lipid in mammalian blood is transported in the form of lipoprotein complexes. The electron transport system in the mito-

chondria contains large amount of lipoproteins. These are also found in egg yolk, myelin sheath of nerves and different cell organelles.

C. Derived proteins

These proteins are derived by partial or complete hydrolysis from the simple or conjugated proteins by the action of acids, alkalies or enzymes. Derived protein includes two types of derivatives,

a) Primary-derived proteins

b) **Secondary-derived proteins**

a) Primary-derived proteins

- These protein derivatives are formed by processes causing only slight changes in the protein molecule and its properties.
- There is little or no hydrolytic cleavage of peptide bonds.

I) Proteans

Proteans are insoluble products formed by the action of water, dilute acids and enzymes. These are particularly formed from globulins but are insoluble in dilute salt solutions e.g., myosan from myosin, fibrin from fibrinogen.

II) Metaproteins

These are formed by the action of acids and alkalies upon protein.
They are insoluble in neutral solvents.

III) Coagulated proteins

Coagulated proteins are insoluble products formed by the action of heat or alcohol on natural proteins e.g., cooked meat and cooked albumin.

b) Secondary-derived proteins

These proteins are formed by the hydrolytic cleavage of the peptide bonds of protein molecule. They are roughly grouped into proteoses, peptones and peptides according to average molecular weight.

- Proteoses are hydrolytic products of proteins, which are soluble in water and are not coagulated by heat.
- Peptones are hydrolytic products, which have simpler structure than proteoses.
- They are soluble in water and are not coagulated by heat.
- Peptides are composed of relatively few amino acids.
- They are water-soluble and not coagulated by heat.
- The complete hydrolytic decomposition of the natural protein molecule into amino acids generally progresses through successive stages as follows:
  Protein -----> Protean -------Metaprotein

  Proteoses ------>Peptones ------->Peptides ------amino acids

1.5 Amino Acids

Proteins are the important agents of biological function, and amino acids are the smallest structural unit of proteins. The range of the thousands of proteins occurred in nature composed of mainly by 20 amino acids. Proteins are polymeric structure of amino acids, with each amino acid residue joined to its adjacent amino acid by a specific type of peptide bond (covalent bond). Proteins can be hydrolyzed to their constituent amino acid and produce free amino acids. About 300 amino acids naturally occurred, 20 establish the monomer units of proteins. All 20 amino are considered as biologically essential. Human body can synthesize 12 amino acids (nutritionally nonessential) out of the 20 common amino acids from the amphibolic intermediates of glycolysis and of the citric acid cycle. Out of 12 nutritionally nonessential amino acids, nine are synthesized from amphibolic intermediates and three (cysteine, tyrosine and hydroxylysine) from nutritionally essential amino acids.

List of Essential and Nonessential Amino acids

Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Aspartic acid
Lysine	Cysteine
Methionine	Glutamic acid
Phenylalanine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
	Tyrosine
	Asparagine
	Selenocysteine
	Pyrrolysine

Essential amino acids are "essential" not because they are important to life than compare to others, but because the body does not synthesize them. They must be present in the diet. In addition, the amino acids ***arginine***, ***cysteine***, ***glycine***, ***glutamine***, ***histidine***, ***proline***, ***serine*** and ***tyrosine*** are considered as essential, means they are not generally required in the diet, but must be provided exogenously to specific people that do not synthesize them in sufficient amounts.

Selenocysteine, whereas not normally considered an amino acid present in proteins, selenocysteine occurs at the active sites of several enzymes. Examples include the human enzymes thioredoxin reductase, glutathione peroxidase, and the deiodinase that converts thyroxine to triiodothyronine. Pyrrolysine considered “the 22nd amino acid”, is not mentioned here as it is not used by humans.

1.5.1 Structure of a Typical Amino Acid

An α-amino acid contain a central carbon atom, called the α carbon, connected to an amino group, a carboxylic acid group, a hydrogen atom, and a distinct -R group. Such type of amino acids is termed as alpha amino acids.

R represents the side chain of amino acids and the nature of alkyl chain (R) determines the type of amino acids. R may be simple as H in case of Glycine or it may be aliphatic, aromatic or heterocyclic.

Amino acids in solution at neutral pH exist mostly as dipolar ions (also called zwitterions). Amino acids can exist as zwitterions - substances containing equal numbers of positive and negative charge due to their carboxyl and amine groups, which can be negatively and positively charged, respectively. In the dipolar form, the amino group is protonated (NH3+) and the carboxyl group is deprotonated (COO–). The ionization state of an amino acid varies with pH. They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and which affect the solubility of the amino acids in water.

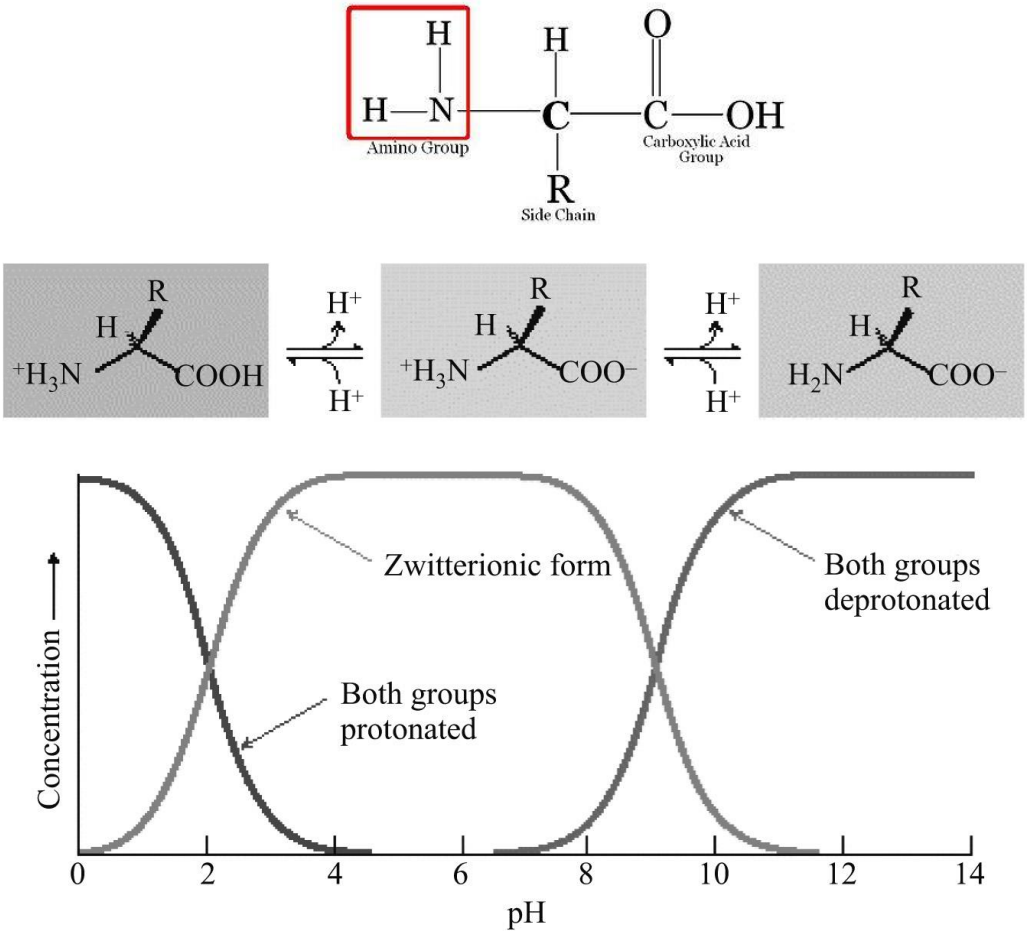


Fig. 1.5.1: Ionization State as a Function of pH. The ionization state of amino acids is altered by a change in pH. The zwitterionic form predominates near physiological pH.

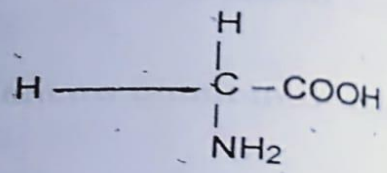
Amino Acids linkage via Peptide Bonds

The important feature of amino acids that permits them to polymerize to form peptides and proteins is the presence of their two identifying chemical groups: the amino (NH_3^+) and carboxyl (COO^-) groups. The amino and carboxyl groups of amino acids can react in a head-to-tail fashion, releasing a water molecule and form a covalent amide linkage, which, in the case of peptides and proteins, is normally stated as a peptide bond.

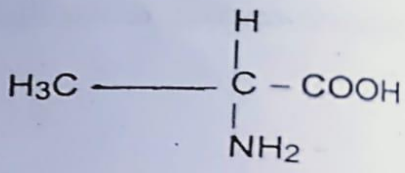
1.5.2 Structure of various Amino acids

I. Neutral amino acids

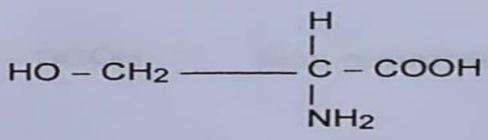
1. Glycine



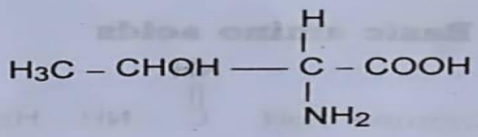
2. Alanine



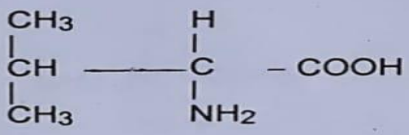
3. Serine



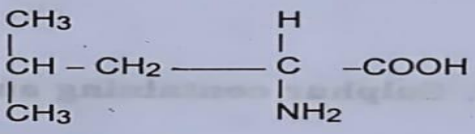
4. Threonine



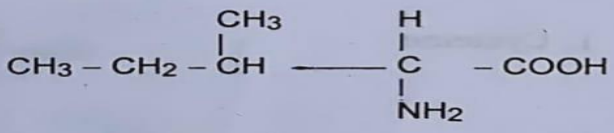
5. Valine



6. Leucine



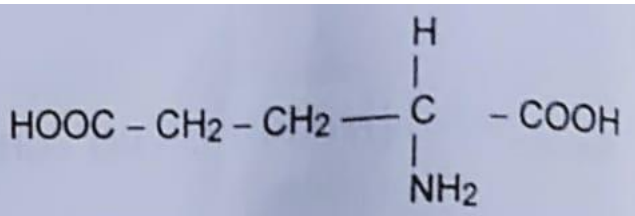
7. Isoleucine



II. Acidic amino acids

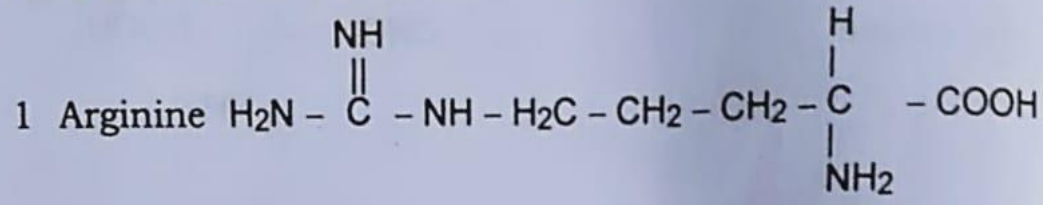
1. Aspartic acid

2. Glutamic acid

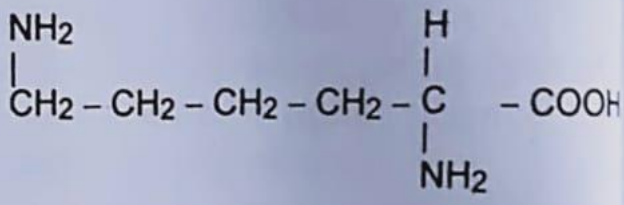


III. Basic amino acids

1 Arginine

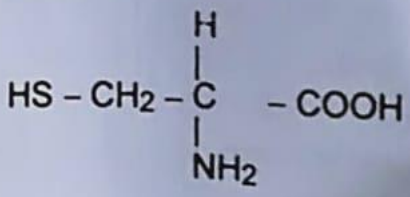


2. Lysine

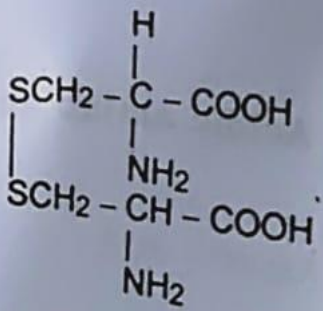


IV. Sulphur containing amino acids

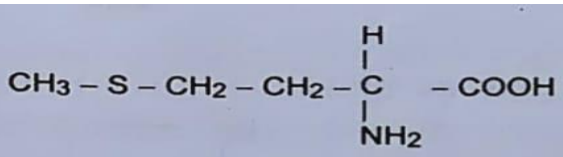
1. Cysteine



2. Cystine

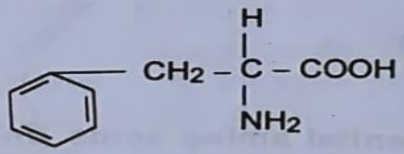


3. Methionine

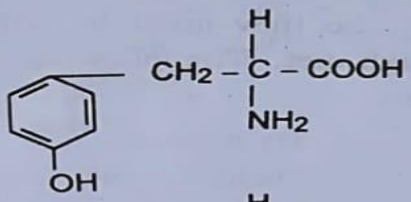


V. Aromatic amino acids

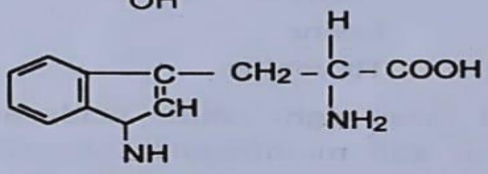
1. Phenylalanine



2. Tyrosine

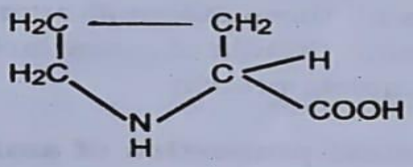


3. Tryptophan

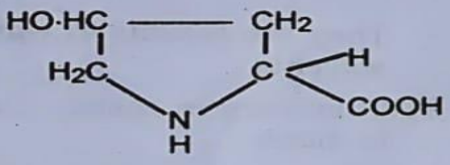


VI. Heterocyclic amino acids

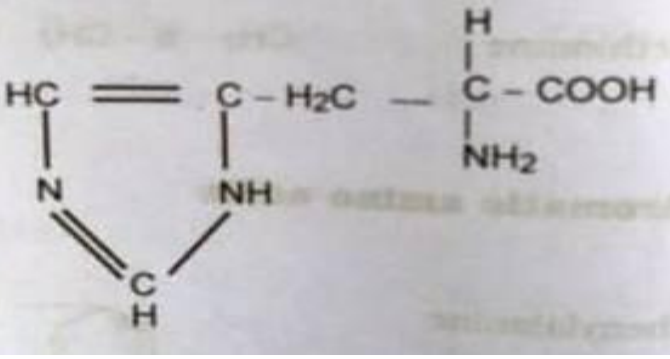
1. Proline



2. Hydroxyproline



3. Histidine



1.5.3 Classification of Amino acids

The structures and abbreviations for the 20 amino acids which are found in proteins are depicted in Figure 1.5.3.1. The amino acids except proline have both free amino and free carboxyl groups.

Amino acids are classified on the basis of polarity of the side chains.

(1) Nonpolar or hydrophobic amino acids,

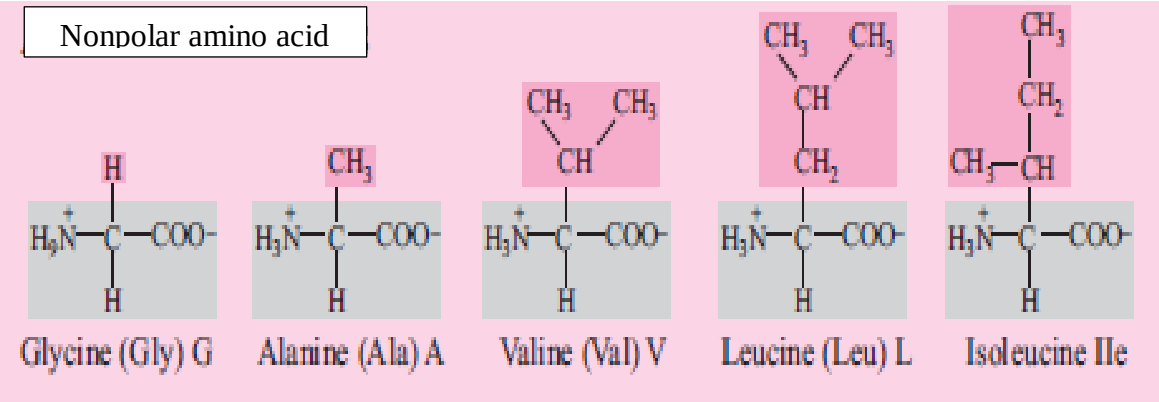
(2) Neutral but polar amino acids,

(3) Acidic amino acids

(4) Basic amino acids

1. Nonpolar Amino Acids

It include all those amino acids with alkyl chain R groups (Alanine, Valine, Leucine, and Isoleucine), as well as proline (unusual cyclic structure), methionine (sulfur-containing amino acids), and two aromatic amino acids, phenylalanine and tryptophan.

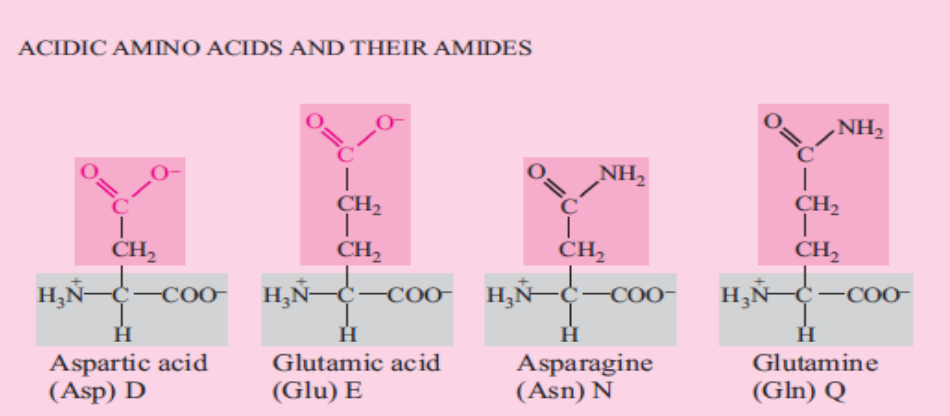


2. Polar, Uncharged Amino Acids

The polar amino acid (uncharged amino acid) except glycine contains R groups that form hydrogen bonds with water. These amino acids are generally more soluble in water than compare to nonpolar amino acids. Tyrosine shows the lowest solubility in water out of 20 amino acids. Glycine's solubility mainly affected by its polar amino and carboxyl groups, and glycine is best considered a member of the polar, uncharged group. It should be observed that tyrosine has nonpolar characteristics.

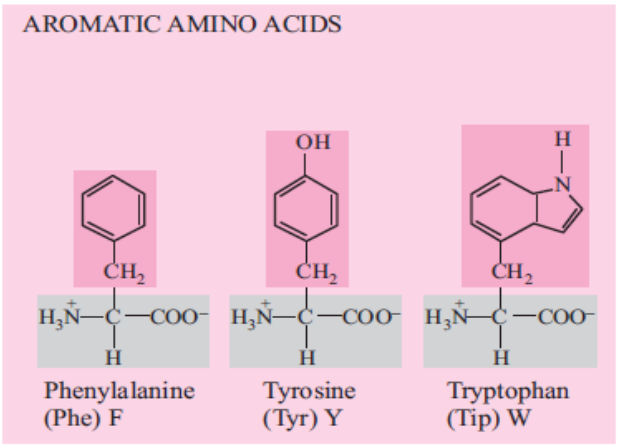
3. Acidic Amino Acids

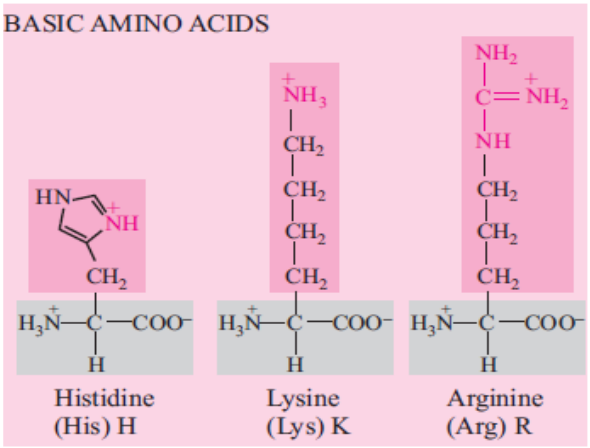
There are two acidic amino acids – Aspartic acid and Glutamic acid – who's R-Groups contain a carboxyl group. Aspartic acid and glutamic acid have a negative charge at pH 7. Proteins that bind with metal ions for structural or functional purposes have metal binding sites containing one or more aspartate and glutamate side chains.

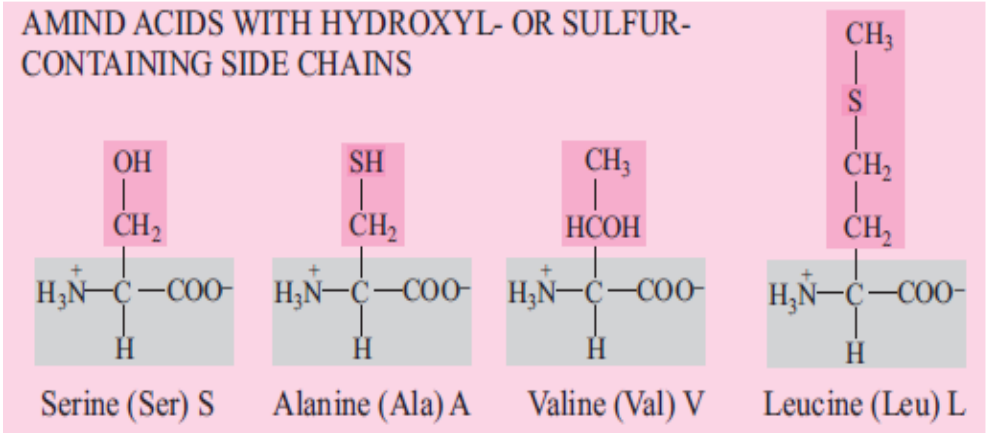


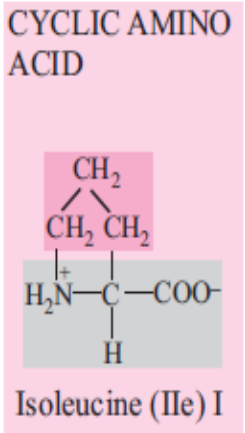
4. Basic Amino Acids

Three amino acids have side chain with net positive charges at neutral pH: Histidine, Arginine, and Lysine. Ionized group of Histidine is an Imidazolium, that of Arginine is a Guanidinium, and lysine has a protonated alkyl amino group. Arginine and lysine side chains are protonated under physiological conditions and participate in electrostatic interactions in proteins.









1.5.4 Physio Chemical Properties of Amino acids:

- ***Solubility:***
  Most of the amino acids are generally soluble in water, and insoluble in organic solvents.

- **Melting Point:**
  Amino acids are generally melted at higher temperature out of ten above 200^0C.

- **Taste:**
  Amino acids may be sweet (Gly, Ala & Val), tasteless (Leu) or Bitter (Arg & Ile).

- **Optical Properties:**
  All amino acids possess optical isomers due to the presence of chiral α-carbon atoms.

- **Zwitter ion and Isoelectric point:**
  Amino acids behave as a zwitter ion at isoelectric pH.

<u>Chemical Properties of Amino acids:</u>

Chemical reactions of amino acids are due to carboxyl and amino groups:

I) Reaction due to Carboxyl group:

a) Decarboxylation:

The amino acids will undergo alpha decarboxylation to form the respective "amines". Thus important amines are produced from amino acids.

Histidine → Histamine + CO_2

Tyrosine →Tyramine + CO_2

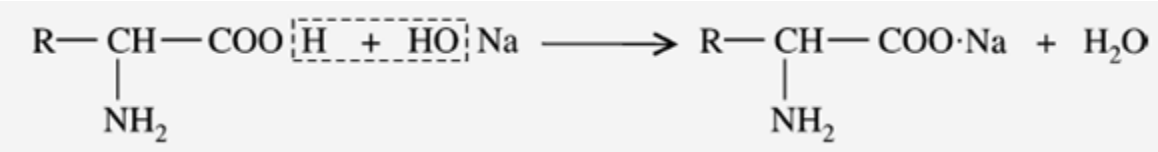
Tryptophan →Tryptamine + CO_2

Lysine →Cadaverine + CO_2

Glutamic acid → Gamma Amino Butyric Acid (GABA) + CO_2

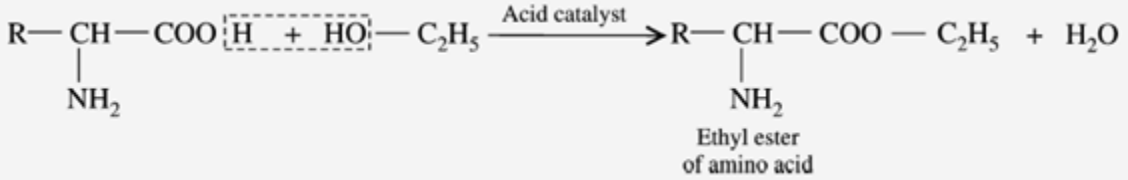
b) Reaction with Alkalies (Salt formation):

The carboxyl group of amino acids can release H^+ ion with the formation of Carboxylate (COO^-) ions. These may be neutralized by cations like Na^+ and Ca^{+2} to form Salts. Thus amino acids react with alkalies to form "Salts".



c) Esterification (Reaction with Alcohols):

Amino acids is react with alcohol to form, "Ester". The esters are volatile in comparison to the form amino acids.



d) Reaction with Amines:

Amino acid reacts with Amines to form "Amides".

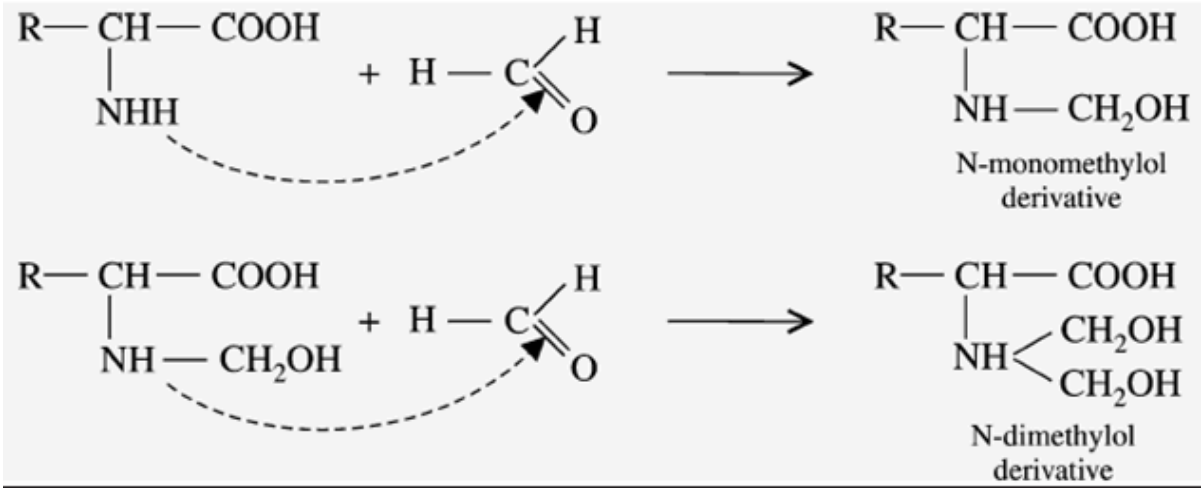
II) Due to Amino group:

a) Reaction with Mineral acids (Salt formation)

When the amino acids are react with mineral acids (like HCl), it forms "Acid Salts".

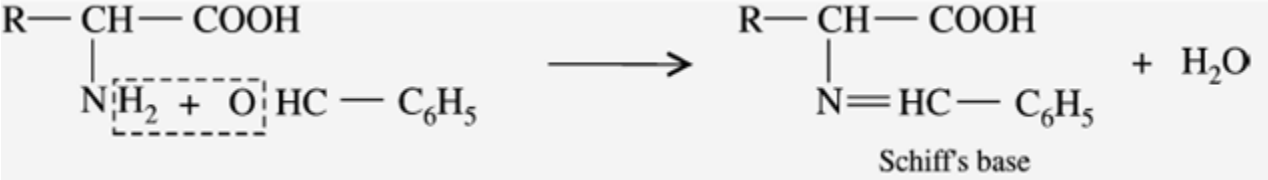
b) Reaction with Formaldehyde:

Amino acid reacts with two molecules of Formaldehyde to forms "N-dimethylol derivative" (Hydroxy-methyl derivative). This reaction is completed in two steps. These derivatives are insoluble in water and resistant to attack by microorganisms.



c) Reaction with Benzaldehyde:

Amino acid reacts with Benzaldehyde and gives "Schiff's base".

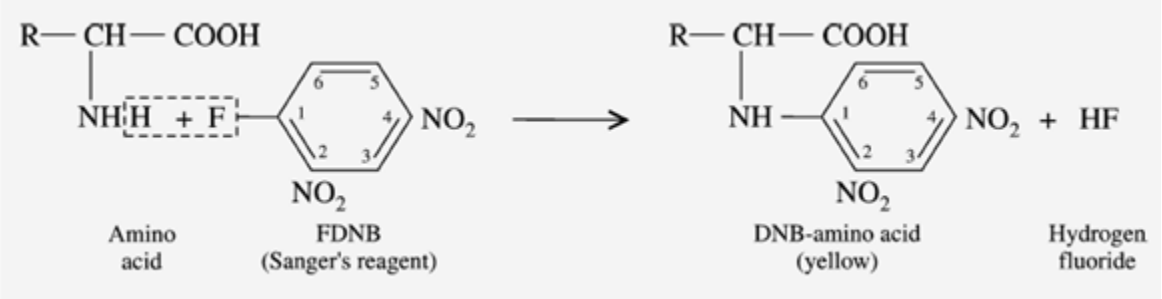


d) Reaction with Nitrous acid (Van slyke reaction):

Amino acids react with Nitrous acid (HNO_2) to liberate N_2 gas and to produce the corresponding "α-hydroxyl acid". The amino acids Proline and Hydroxyproline do not give any reaction.

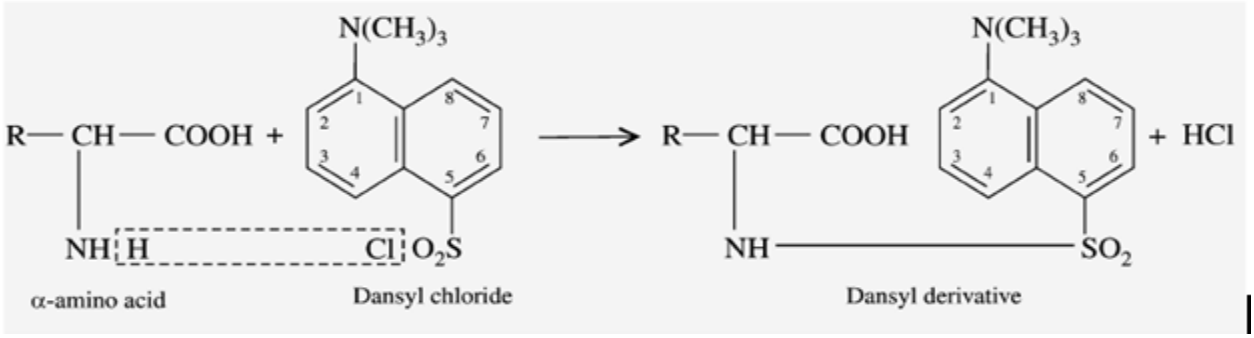
e) Reaction with Sanger's reagent:

"1-flouro-2,4-dinitrobenzene" is called Sanger's reagent (FDNB). In mildly alkaline solution, sanger's reagent reacts with α-amino acid to give Yellow colored derivative, DNB-amino acid.



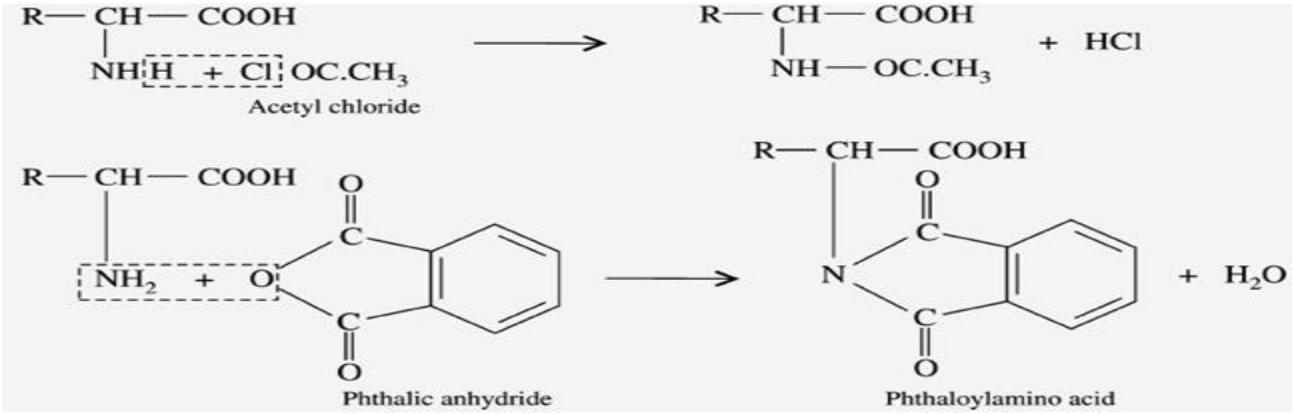
f) Reaction with DANSYl Chloride:

Dansyl chloride means "Dimethyl Amino Naptha Sulphonyl Chloride". Amino acid reacts with Dansyl chloride reagent; and "Flourescent Dansyl derivative".



g) Reaction with Acylating agents (Acylation):

Amino acids react with "Acid chloride" and acid anhydride (Pthalic anhydride) in alkaline medium and provide "pthaloyl amino acid".

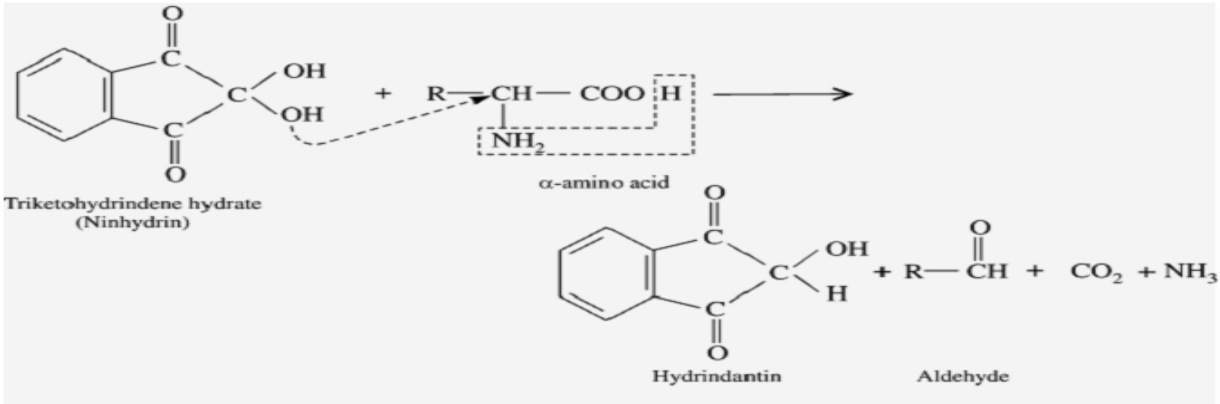


c) Reaction due to amino & carboxyl group:

Ninhydrin reaction:

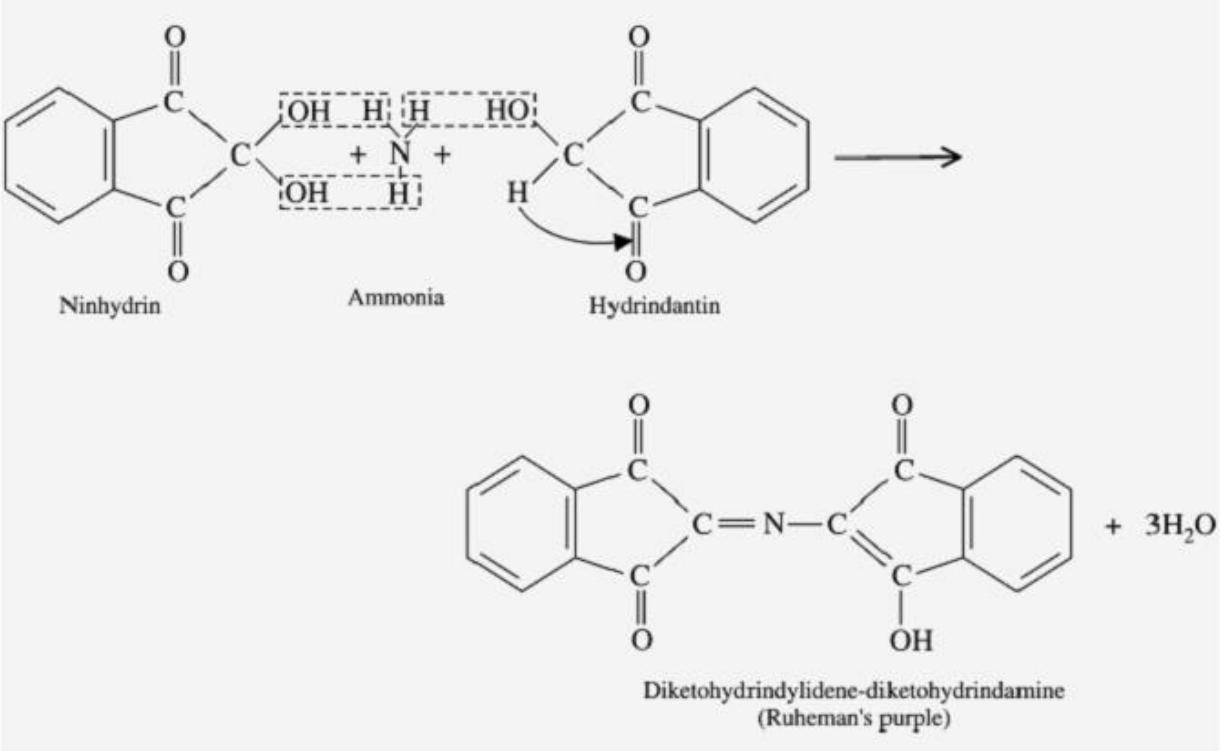
Step (1):

Ninhydrin is a powerful oxidizing agent which cause oxidative decarboxylation of α-amino acids and gives CO_2, NH_3 and an aldehyde with one less carbon atom than the parent amino acid.



Step (2):

The reduced ninhydrin then reacts with the released NH_3 and a mole of ninhydrin, forming Blue-colored Rhumann's complex.

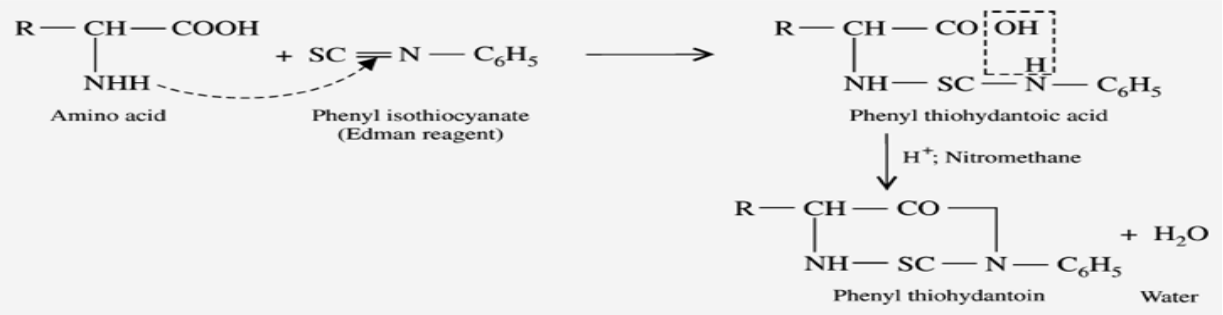


This reaction is very sensitive reaction used for amino acid and imino acid detection.

When Amino acids (or) Imino acid reacts with Ninhydrin molecule and gives **Purple color Rhumann's Complex**, the Unknown sample is Amino acids (Which have primary amine $–NH_2$) or gives Yellow color, the Unknown sample is Imino acid (**-NH-**).

Reaction with Edmann's degradation:

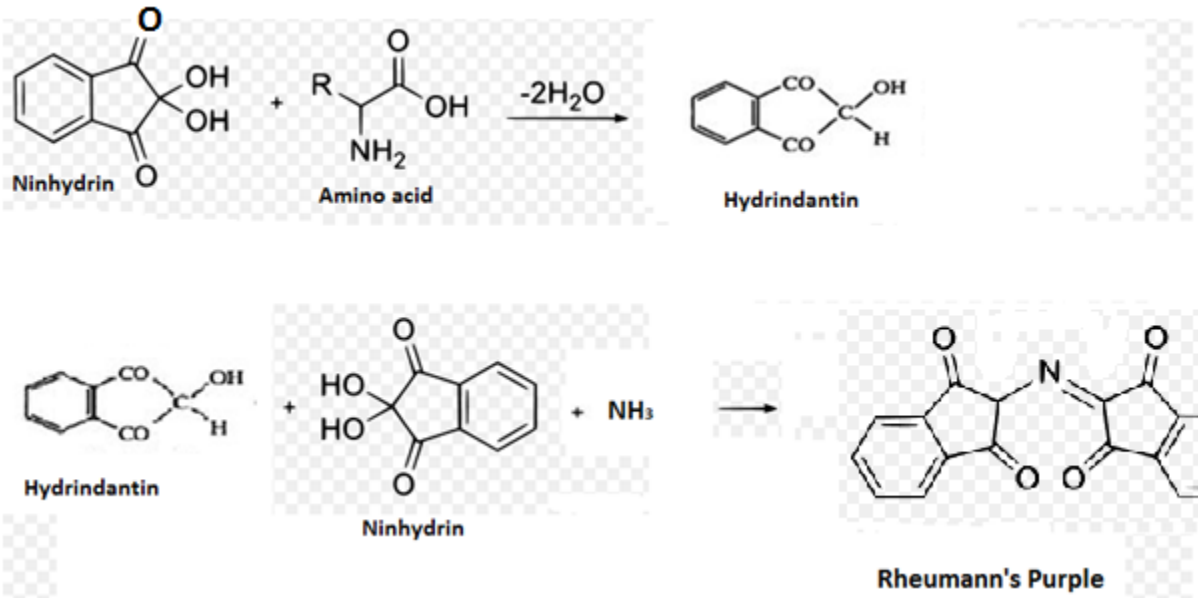
Edmann's reagent is **"phenylisothiocyanate"**. When amino acids react with Edmann's reagent it gives "*phenyl thiohydantoic acid*" lastly it turns into cyclized form "*Phenyl thiohydantoin*".



1.5.5 Colour reactions of amino acids

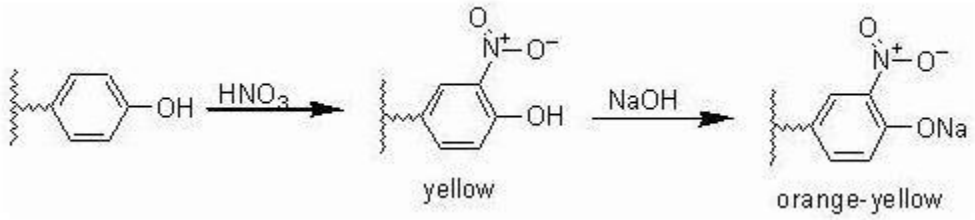
Ninhydrin test

All α- amino acids in the pH range of 4-8, react with ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent who give a purple colored product (diketohydrin) named as Rhuemann's purple. All primary amines and ammonia react in similar fashion but without release of carbon dioxide. The imino acids proline and hydroxyproline also react with ninhydrin reagent and give a yellow colored complex instead of a purple color. Further amino acids, other complex structures such as peptides, peptones and proteins react with the ninhydrin reaction.



Xanthoproteic acid test

Aromatic amino acids, such as Phenyl alanine, tyrosine and tryptophan, give this test. In the presence of concentrated nitric acid, the aromatic phenyl ring is nitrated to produce yellow colored nitro-derivatives. Due to the ionization of the phenolic group, the color changes to orange, at alkaline pH.



Pauly's diazo Test

This test is definite for the identification of Tryptophan or Histidine. The reagent used for this test has sulphanilic acid dissolved in hydrochloric acid. Sulphanilic acid on diazotization in the presence of sodium nitrite and hydrochloric acid gives a diazonium salt. The diazonium salt

formed bind with either tyrosine or histidine in alkaline medium to produce a red coloured chromogen (azo dye).

Millon's test

Phenolic amino acids like as Tyrosine and its derivatives give this test. Compounds with a hydroxybenzene radical react with Millon's reagent to produce a red colored complex. Millon's reagent is a solution of mercuric sulphate in sulphuric acid.

Histidine test

Histidine test was discovered by Knoop. This reaction involves bromination of histidine in acid solution, followed by neutralization of the acid with excess of ammonia. Heating of alkaline solution give a blue or violet coloration.

Hopkins cole test

It is a specific test for detecting tryptophan. The indole moiety of tryptophan reacts with glyoxilic acid in the presence of concentrated sulphuric acid to produce a purple colored product. Glyoxilic acid is prepared from glacial acetic acid in the presence of sunlight.

Sakaguchi test

In alkaline condition, α- naphthol (1-hydroxy naphthalene) reacts with a mono-substituted guanidine compound like arginine, which on treatment with hypobromite or hypochlorite, produces a characteristic red color.

Lead sulphide test

Sulphur containing amino acids, such as cysteine and cystine. On boiling with sodium hydroxide (hot alkali), produce sodium sulphide. This reaction is due to incomplete conversion of the organic sulphur to inorganic sulphide, which can determine by precipitating it to lead sulphide, using lead acetate solution.

$$\text{S. (Proteins)} + 2\,NaOH \longrightarrow Na_2S$$

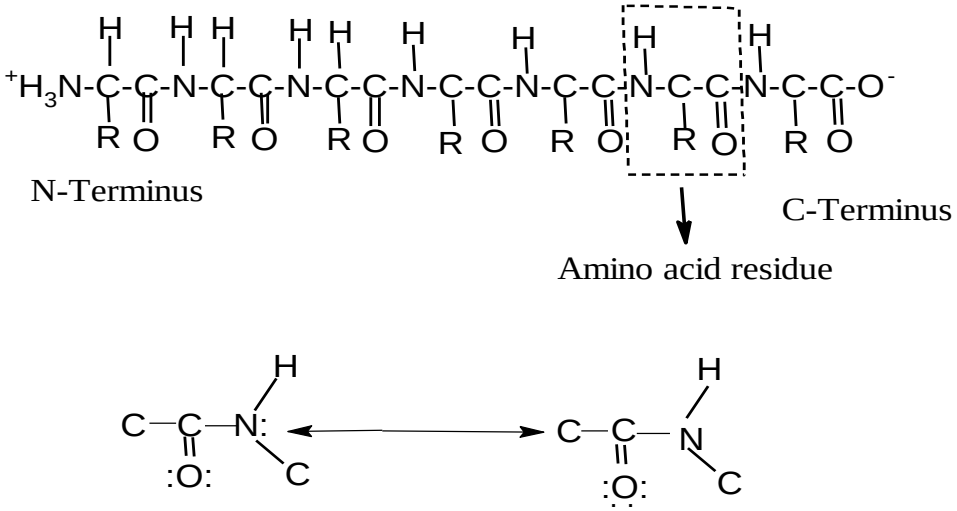
$$Na_2S + (CH_3COO)_2Pb \longrightarrow PbS + 2CH_3COONa$$

1.5.6 Biological importance of amino acids

- Amino acids are connected with each other by peptide bond and form peptides and protein.
- Amino acids like Glycin and alanine are converted to carbohydrate in human body.
- Some amino acids can give rise to specialized biological products in the body. That is tyrosine give rise to adrenaline and Histidine give rise to histamine.
- Some amino acids are necessary for detoxification of toxic substances like as glycine and cysteine.
- Methyl group of methionine is transferred to various substances by Transmethylation.

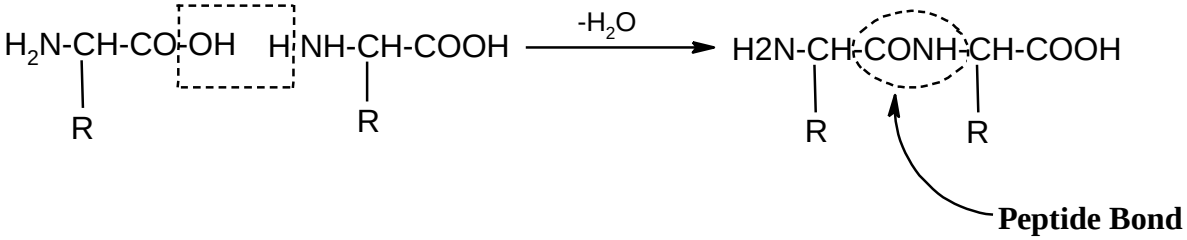
1.5.7 Polypeptide

These molecules are composed of chains of amino acids. Every amino acid is joined to the next through an amide or *peptide* bond from the carbonyl carbon of one amino acid "residue" to the α-amino group of the next amino acids. At one end of the chain there will be a free or protonated amino group: the N-terminus. At the other end there will be a free carboxyl or carboxylate group: the C-terminus. By convention the N-terminus is drawn at the left end, as shown below in a generic hexapeptide (6 amino acid residues).



Peptide formation:

Amino group of amino acid can be react with carboxyl group of other amino acid to form a peptide (CONH) bond as shown below



Biological function of peptides

- **Precursor of protein:** Peptides are precursor of almost all protein.
- **As alkaloids:** Peptides are also the chemical constituents of alkaloids (Alkaloids are group of secondary metabolites such as Nicotin, Caffeine, Terpentine, Ergotamine etc).
- **Antimicrobial agent:** some peptides possess antibacterial properties. Secondary metabolites of bacteria and fungi possess antimicrobial activity. Eg. Penicillin G (valine-cystein-phenylacetic acid)
- **Hormones:** Peptides also acts as hormones eg. Insulin, Somatostatin, vasopressin etc
- **Peptides as growth factors**. Eg. Ascorbic acid (Vit. C)
- **Anti-oxidant:** Peptide also act as an antioxidant. They scavenge free radicals. Eg. Carnosine

- **Clinical diagnosis:** Hyper secretion of certain peptide in urine is indicator for mental disturbance like depression, schizophrenia etc.
- **Structural component:** peptides form long chains of protein which provides support to body. Eg. Keratin, collagen

1.5.8 Structure of protein

Protein possesses a complex structure but it arranged in a well-organized way. The structure of protein can be studied easily under following levels of organization:

1. Primary structure
2. Secondary structure
3. Tertiary structure
4. Quaternary structure

1. Primary structure (formation of peptide chain):

Primary structure deals with sequence of amino acids which are joined to each other via peptide bond. The peptide bond form between the carboxyl group (COOH) of one amino acid and the amino group (NH_2) with another amino acid as represented below:

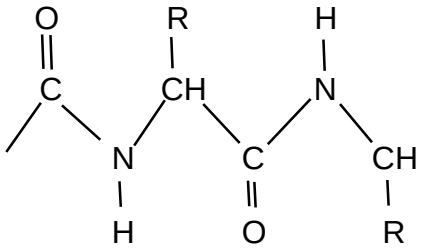
$$H_2N\text{-}CH_2\text{-}CONH\text{-}CH_2\text{-}COOH$$

A lot of amino acids can be joined in such a fashion by peptide linkage as shown in give sequence:

—Ala—Gly—Gly—His — Leu

—Ala — Gly — His — Gly — Leu

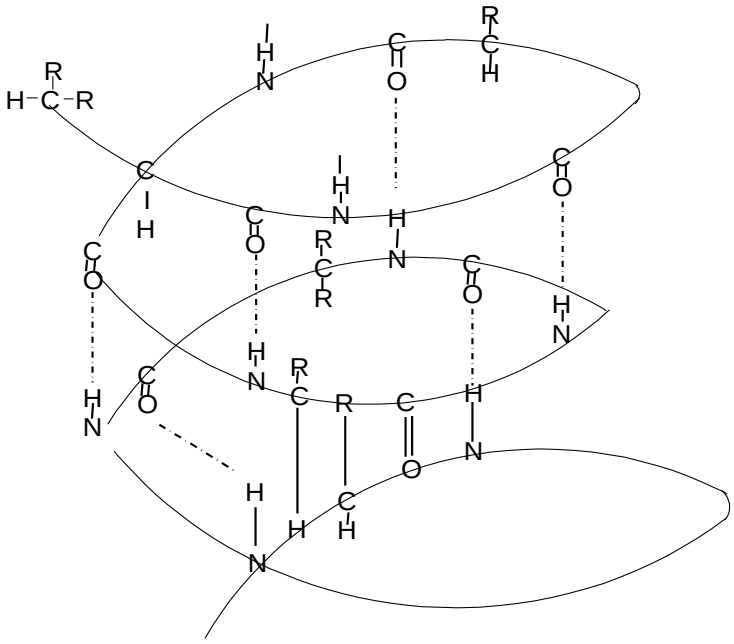
Each protein possesses unique sequence of amino acids. Presence of a fixed amino acid at fixed position is very important for a particular protein. Any deviation in the sequence of amino acid is abnormal and it may alter the properties and function of proteins.



2. Secondary structure (Formation of Helix):

Secondary structure deals with the coiling of the polypeptide chain into a helicoid structure. This structure involves the hydrogen bonding between adjacent amino acids which occurs at the folding site of polypeptide to form a helix. Hydrogen bonding form between carbonyl oxygen

and amide nitrogen. i.e. O of CO is bind with H to N of NH. A helicoid rigid structure is formed due to hydrogen bonding.



α- Helical structure of protein

Three type of secondary structure are possible:

I. A- helical structure
II. Reverse turn
III. Pleated structure

I. α- helical structure:

A peptide chain creates a regular coil known as α- helix. These coils are held together by hydrogen bond between the carbonyl 0 of the first amino acid amide N of the forth amino acid. The α-helix may be right handed and left handed. α- helix at left handed side is less stable then right handed. The protein rich in α- helical structure is keratin of skin, hair and nail.

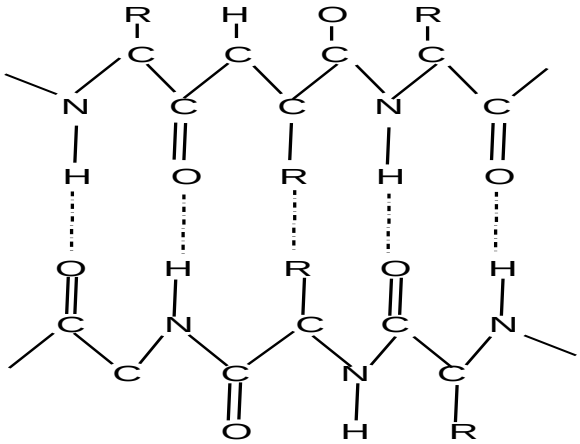
II. Reverse turns:

During folding polypeptide chain may change the direction. Some time, the direction of chain can be reverse this is recognized as reverse turn or β-bands. Reverse turns commonly occurs at spots where the amino acids Glycin and Proline are present. These two amino acids don't have bulky side chains and facilitate folding.

III. Pleated sheet structure:

In this type of structure, a number of polypeptide chains arise side by side. The polypeptides chain are connected by hydrogen bond which followed between C=O and N-H group of adjacent polypeptide chain.

The pleated structure may be parallel if the polypeptide chain goes in the same direction or antiparallel if they run in the opposite direction.



β- Pleated structure of protein

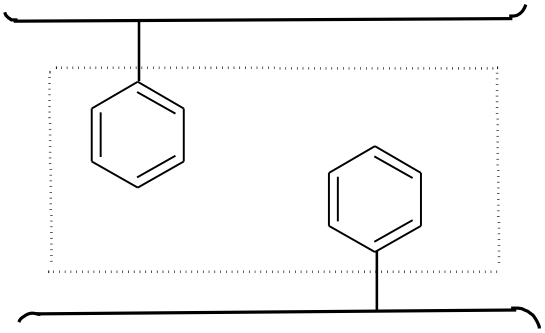
3 Tertiary structure of protein (chain folding):

Tertiary structure deals with the protein chain folding to form a fitted and dense three dimensional structure. It is only conformation which is biologically active. Protein in such a conformation is called *Native protein*. Such a complex folding on the tertiary structure brings, closer the amino acids which are far away. This structure is bound by the following forces:

I. Hydrophobic interaction
II. Hydrogen bond
III. Ionic bond
IV. Disulphide bond

I. Hydrophobic interaction:

Hydrophobic force occurs between non-polar side chains of amino acids. They form major stabilizing forces which make the tertiary structure into a compact three dimensional structure.



Hydrophobic interaction

II. ***Ionic bonds:***

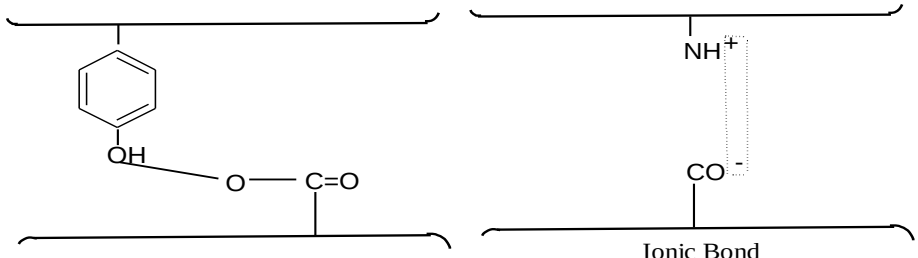
Ionic bonds are created between oppositely charged polar side chains of amino acids like acidic and basic amino acids.

III. ***Hydrogen bond:***

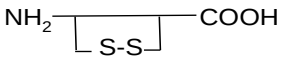
They occur between polar side chains of different amino acids.

IV. ***Disulphide bonds:***

They are S-S bonds between the SH groups of two cysteine molecules.



Hydrogen bond Ionic Bond



Disulphide Bond

4 ***Quaternary structure of protein (Protein-protein interaction):***

It is the fourth level of organization in protein structure. It is represented by protein containing more than one polypeptide chain. These peptide chains are further supported by ionic bonds, hydrogen bonds and hydrophobic interaction. This assembly as whole is called oligomer. Each integral peptide chain is called as monomer or sub unit. These subunits may be similar and different in their structure.

1.5.9 Protein denaturation:

Denaturation of proteins may be defined as the disruption and destruction of both the secondary and tertiary structures. Since denaturation reactions are not strong to break the peptide bonds, the primary structure remains the same after a denaturation process. Denaturation disturbs the normal α-helix and β sheets in a protein and uncoils it into a random shape.

Denaturation occurs because the bonding interactions responsible for the secondary structure (hydrogen bonds to amides) and tertiary structure are disrupted. In tertiary structure involve four types of bonding interactions between "side chains" including: hydrogen bonding, salt bridges, disulfide bonds, and non-polar hydrophobic interactions that may be disrupted. So, a variety of reagents and conditions can leads to denaturation. The most common observation in denaturation process is the precipitation or coagulation of the protein.

Agents that produce denaturation:

Heat:

Heat may be used to disrupt hydrogen bonds and non-polar hydrophobic interactions. This happens because heat increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disturbed. The proteins in eggs denature and coagulate during cooking. Other foods are cooked to denature the proteins to make it easier for enzymes to digest them.

Alcohol:

Hydrogen bonding involves between amide groups in the secondary protein structure. Hydrogen bonding between "side chains" occurs in tertiary protein structure in a variety of amino acid combinations. These are disrupted by the addition of alcohol.

Acids and Bases:

Salt bridges are the effect of neutralization of an acid and amine on side chains. The final interaction is ionic between the positive ammonium group and the negative acid group. Any combination of the acidic or amine amino acid side chains will have this effect.

As may be expected, acids and bases disrupt salt bridges joined by ionic charges. A type of double replacement reaction occurs where the positive and negative ions in the salt change associates with the positive and negative ions in the new acid or base added. This reaction occurs in the digestive system, when the acidic gastric juices cause the curdling (coagulating) of milk.

Heavy Metal Salts:

Heavy metal salts act to denature proteins in much the same manner as acids and bases. **Heavy metal salts** usually contain Hg^{+2}, Pb^{+2}, Ag^{+1} Tl^{+1}, Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

Heavy Metal Salts Disrupt Disulfide Bonds:

Heavy metals may also disrupt disulfide bonds because of their high affinity and attraction for sulfur and will also lead to the denaturation of proteins.

Reducing Agents Disrupt Disulfide Bonds:

Disulfide bonds are formed by oxidation of the sulfhydryl groups on cysteine. Different protein chains or loops within a single chain are held together by the strong covalent disulfide bonds. Both of these examples are exhibited by the insulin in the graphic on the left.

If oxidizing agents cause the formation of a disulfide bond, then reducing agents, of course, act on any disulfide bonds to split it apart. Reducing agents add hydrogen atoms to make the thiol group, -SH.

Denaturation may lead to following:

Physical changes:

- Decrease in solubility
- Increase viscosity that affects the rate of diffusion.
- Surface tension is altered.

- Denaturation also inhibit the crystallization of protein

Chemical changes:

- Denaturation causes the splitting of bonding like hydrogen bond and Disulphide bond in the protein molecules which cause unfolding and uncoiling of the peptide chain. Chemical groups which are exposed lose their activity.

Biological changes:

- Denaturation facilitates the digestion of protein by photolytic enzymes.
- Denaturation also decreases the biological activity of enzymes and hormones.
- Denaturation also affects the antibody role of proteins.

Coagulation of proteins:

Coagulation may be defined as flocculation and separation of protein particle from an aqueous solution. Following steps are involved in coagulation:

1. First denaturation which convert the protein into metaprotein.
2. Addition of acid, alkali or neutral salt solution which gets these reaction to isoelectric point. This leads to precipitation of metaprotein.

When heat is applied to precipitate metaprotein, the precipitate is tangled together to form an insoluble mass. It is an irreversible reaction since coagulated protein does not dissolve in acid or alkali.

Precipitation of protein:

It is also known as flocculation. Precipitation is carried by neutralizing the charges of the metaprotein and carrying it to the isoelectric point. This can be achieved by the action of heat, acid, alkali or enzyme. So precipitation can be defined as clumping together of the denatured metaprotein by neutralization of the charges. The precipitate can be dissolved by sufficient acid or alkali to alter the pH to a value above or below the isoelectric point. So precipitation is a reversible change.

Agents or method that causes precipitation:

I. Heavy metals- lead acetate, mercuric chloride, mercuric nitrate.
II. Alkaloidal solution (acidic agents) such as a Trchlorocetic acid, Sulphosalicylic acid, Tannic acid.
III. Organic solvents such as ethanol or acetone.
IV. Concentrated salt solutions such as ammonium sulphate.
V. Bringing to isoelectric point.
VI. Heat.

Protein and nutrition:

Function of protein:

- Proteins are required for growth of cells or tissues within the body hence considered as essential constituent for body.
- Proteins help in repairmen and maintenance of cells and tissues.
- Proteins are required for formation of hormones, antibodies, haemoglobin and enzymes.
- Proteins helps in cell mediated immunity and bactericidal action of leukocytes.
- Proteins are also act as source of energy.

Sources of proteins:

Main source of proteins are as follow:

I. **Vegetable sources:** cereals, pulses, nuts, beans oil seeds etc. but these sources are poor in essential amino acids.

II. **Animal sources**: cheese, milk, fish, meat, egg etc. animal source contain all essential amino acids.

1.6 Protein deficiency disease:

Protein deficiency disease is caused due to the deficiency of protein in dietary sources and some may cause due to abnormal metabolism of proteins within body.

- Kawashiorkar
- Marasmus

Kawashiorkar:

It is also known as "edematous malnutrition" because of its link with edema (fluid retention), is a nutritional disorder mostly seen in regions facing famine. It is a type of malnutrition caused by a deficiency of protein in the diet. People who are suffering from kwashiorkor normally have an extremely shrunken look in all body parts except ankles, feet, and belly, which swell with fluid.

It is common in sub-Saharan Africa and other countries where people regularly have a restricted supply of food.

Most people who are suffered from kwashiorkor recover fully if they are treated early. Treatment involves administration of extra calories and protein into the diet. Children who grow kwashiorkor may not grow or develop properly and may remain stunted for the rest of their lives. There can be serious complications when treatment is delayed, including coma, shock, and permanent mental and physical disabilities. Kwashiorkor may be life-threatening if it's not treated. It may cause major organ failure and finally death.

Causes kwashiorkor

Kwashiorkor is developed due to lack of protein in the diet. Every cell in our body contains protein. We need protein in our diet for our body to repair cells and make new cells. A healthy human body redevelops cells in this way continuously. Protein is also especially important

for growth during childhood and pregnancy. If the body lacks protein, growth and normal body functions may be alter and kwashiorkor may develop.

The symptoms of kwashiorkor:

- Change in skin and hair color (to a rust color) and texture
- Fatigue
- Diarrhea
- Loss Of Muscle Mass
- Failure to grow or gain weight
- Edema (swelling) of the ankles, feet, and belly
- Damaged immune system, which can lead to more frequent and severe infections
- Irritability
- Flaky rash
- Shock

Treatment involves the intake of diet rich in milk, egg, soyabeen and these sources are helpful in controlling Kawashiorkar.

Marasmus:

Marasmus is also a form of malnutrition. It occurs when the consumption of nutrients and energy is very low for a person's needs. It may cause wasting, or the loss of body fat and muscle. A child with marasmus may not grow normally as other childe.

Malnutrition happens when a lack of nutrients, diet does not contain all the vitamins and nutrients that the body required to function properly.

In many countries of the world, marasmus occurs because people do not eat sufficient food. In developed countries, it may occur due to eating disorder anorexia nervosa.

It is severe form of protein-energy malnutrition (PEM) that cause when a person does not gain enough protein and calories. Lacking these vital nutrients, energy levels become dangerously low and important functions begin to stop.

Both adults and children may have marasmus, but it generally affects young children in developing countries.

UNICEF estimate that nearly half of all deaths in children occur in the age of 5 years.

Causes and risk factors

Causes of marasmus include:

- not having enough nutrition or having too little food
- consuming the wrong nutrients or too much of one and not enough of another
- having a health condition that makes it difficult to absorb or process nutrients correctly

The primary symptom of marasmus:

- Wasting
- Loss of body fat and muscle tissues, leads to low body mass index (BMI).
- Failure to grow (stunted growth).
- Anorexia
- Persistent dizziness
- Lack of energy
- Dry skin
- Brittle hair
- Weight loss

Diarrhea, measles, or a respiratory infection is serious complications that can be fatal in a child with marasmus. Diarrhea may be a causative element of marasmus. Other complications include Bradycardia, Hypotension, and Hypothermia.

Disease related to abnormal metabolism of amino acids

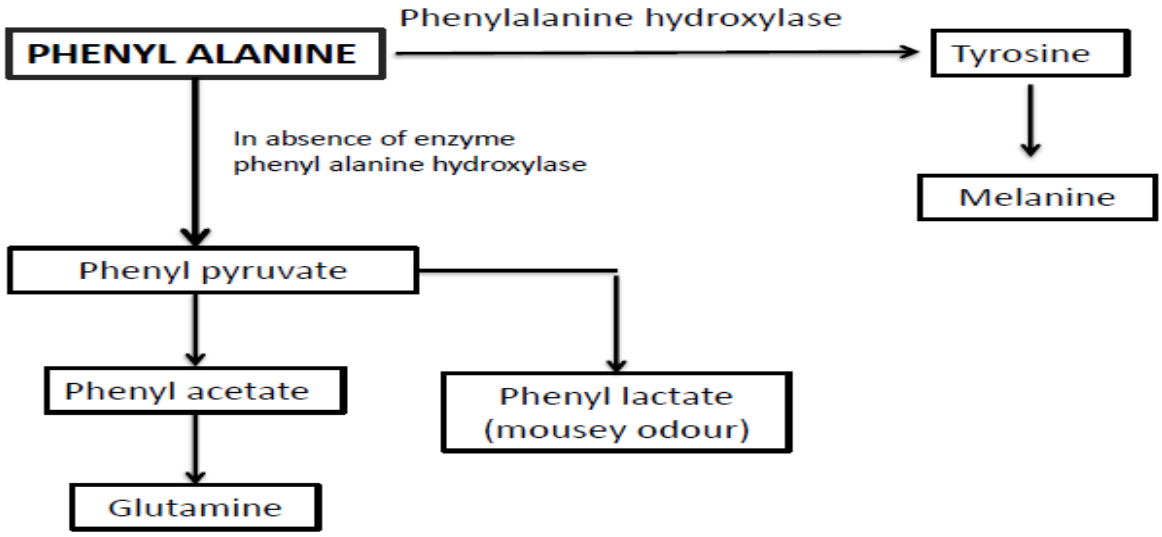
1. ***Phenylketonuria (PKU)***

- ✓ Homozygous defect of phenylalanine hydroxylase
- ✓ Excess of Phenylalanine causes symptoms only after birth; intrauterine development normal cognitive and neurological shortages, possibly due to cerebral serotonin insufficiency treatment with phenylalanine-restricted diet some cases are due to reduced affinity of enzyme for cofactor THB, can be treated with high dosages of THB.

As with most genetic enzyme defects, the clinical disease is manifest only in homozygous individuals. Dietary phenylalanine that is not used for protein synthesis accumulates and causes toxicity.

It appears that the excess phenylalanine crowds out tryptophan at the L-aromatic amino acid transporter in brain capillaries. This transporter keeps the brain supplied with all aromatic amino acids. Since trypto-phan is the precursor of the neurotransmitter serotonin, the competitive inhibition of its transport to the brain results in a lack of cerebral serotonin which is believed to cause the observed deficits in brain function and development.

In addition to phenylalanine itself, some aberrant metabolites derived from it also occur at increased levels, and the appearance of ketone derivatives such as phenylpyruvic acid in the urine has given the disease its name. These metabolites have no proven connection to the pathogenesis of the disease.



Clinical /Biological manifestation of PKU

- Increasing concentration of phenylalanine metabolite in our body produce effect on CNS and on Pigmentation.

Effect on CNS

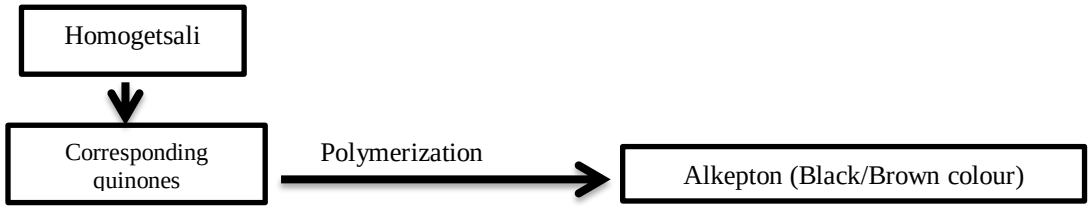
- Mental retardness
- Failure in walk and talk
- Failure of growth, seizures and tremors
- If untreated patients show vary low intellectual quotient below 50.
  Accumulation of phenylalanine in brain impairs the transport and metabolism of other amino acids such as tryptophan and tyrosine.

Effect on pigmentation

- Melanin is the pigment synthesize from tyrosine.
- Accumulation of phenylalanine competitively inhibits the tyrosinase and impairs melanin formation, therefore result in hypopigmentation. That causes light skin color, fair hairs and blue eyes etc.

2. *Alkeptonuria (Black Urine disease):*

It is an autosomal recessive disorder having incidence of 1 in 25000. It is occur due to defective enzyme *Homogentisic Dioxygenase* (*HGD*). Therefore accumulate in tissues and in blood. Urine of patient resembles *Coke* color.



Biochemical manifestation:

1. Alkeptons deposition occurs in connective tissue, bone, various organs & therefore patient suffers from Arthritis.
2. Albinism: it is a born error. It may occur due to lack of pigment melanin.

Possible cause

I. Deficiency or lack of enzyme tyrosinase.
II. Impairment of melanin polymerization.
III. Limitation of substrate i.e. tyrosine.
IV. Absence or inhibition of tyrosinase.

Clinical manifestation

1. Important function of melanin is protection of body from sun radiation.
2. Lack of melanin in Albinose (white person) makes them sensitive to the sunlight.
3. Increase the susceptibility to the skin cancer
4. Photophobia (intolerance of sunlight) due to lack of pigmentation to the eye.