Clinical Biochemistry

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DMLT IST YEAR

Aim and scope of Biochemistry
Proteins
Amino Acids
Plasma Proteins

BIOCHEMISTRY

MEANING AND SCOPE

The term Biochemistry was introduced by ***Carl Neuberg*** in ***1903***. Biochemistry is the science concerned with studying the various molecules that occur in living organisms and its environment, with the chemical reactions and processes, they undergo.

In other words; Biochemistry deals with the structure and function of the body at the molecular level.

The Aim of Biochemistry is to describe and explain, in molecular terms, all processes of living organisms and their interactions with their environment both in health and disease conditions.

Scope of Biochemistry:

- The scope of biochemistry is as wide as life itself. Wherever their is life, chemical processes are occurring. Biochemists study the chemical processes that occur in microorganisms, plants, insects, fishes, birds, mammals and human beings.
- The tools for research in all the branches of medical science are mainly biochemical in nature.
- Genetic Engineering (DNA Technology / RDT), a branch of biochemistry, is the most advanced tool of Biotechnology.
- A knowledge of Biochemistry is essential to understand all the branches of Medical Sciences; especially Physiology, Pharmacology, Pathology, Immunology, General Medicine etc.
- Medical Biochemistry deals with the chemical aspects of health and diseases of human beings.
- Biochemistry is useful in the Rational Design of new Drugs.
- Most important use of Medical Biochemistry, however is the biochemical tests done in the Clinical Laboratory.

- The use of Biochemical Tests are in Diagnosis, Monitoring and Screening of Diseases.

BIOMOLECULES

A biomolecule is an organic molecule that is produced by a living organism. Biomolecules act as building blocks of life and perform important functions in living organisms. More than 25 naturally occurring chemicals are found in biomolecules. Biomolecules consists primarily of Carbon, Hydrogen, Nitrogen, Oxygen, Phosphorous and Sulphur. Most biomolecules can be regarded as derivatives of Hydrocarbons.

Sugars, Fatty acids, Amino acids and nucleotides constitute the 4 major families of biomolecules in the cell.

Many of the biomolecules found within the cells are Macromolecules and mostly are Polymers (composed of small, covalently linked monomeric subunits).

SMALL BIOMOLECULES	***MACROMOLECULES***
Fatty Acids	Fats / Lipids
Amino Acids	Proteins
Nucleotides	Nucleic Acids (DNA / RNA)
Sugars	Polysaccharide (carbohydrate)

PROTEINS

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.

Elemental composition of proteins:

Proteins are predominantly constituted by five major elements in the following proportion.

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

Besides the above, proteins may also contain other elements such as P, Fe, Cu, I, Mg, Mn, Zn etc.

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.

Functions of Proteins:

Proteins perform a great variety of specialized and essential functions in the living cells. These functions may be broadly grouped as:

- **Static (Structural) Function**
- **Dynamic Function**

1. STATIC FUNCTION:

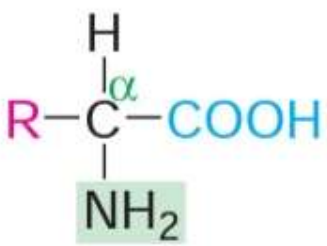
Certain proteins are primarily responsible for structure and strength of body. These include Collagen and Elastin found in bone marrow, vascular system and α - Keratin in epidermal tissues.

2. DYNAMIC FUNCTION:

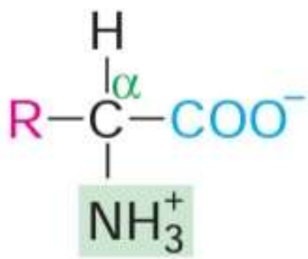
These are diversified in nature. They include proteins acting as Enzymes, Hormones, Blood clotting factors, Immunoglobulins, Membrane receptors, proteins for Muscle contraction, Respiration etc.

AMINO ACIDS:

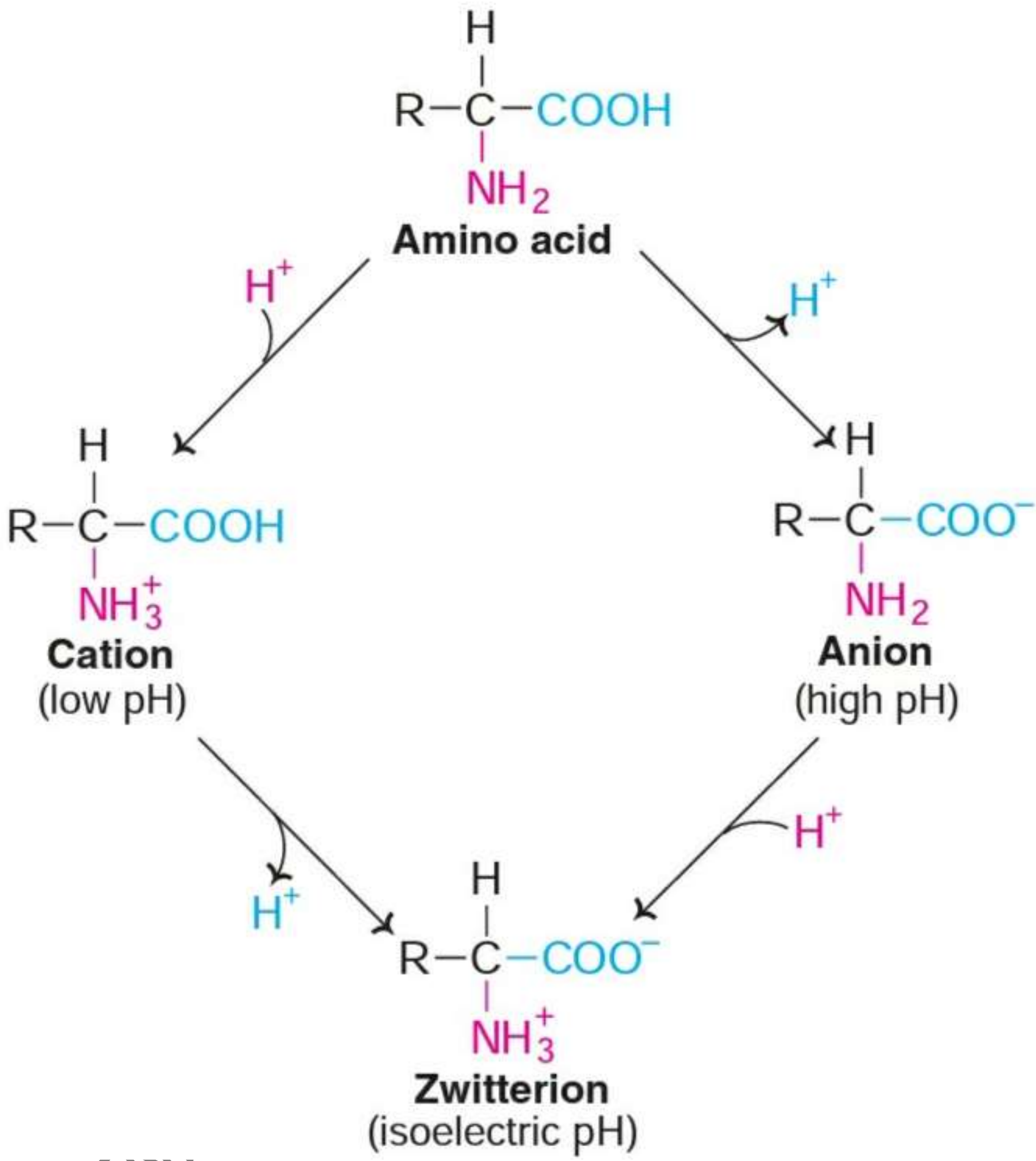
Amino acids are a group of organic compounds containing two functional groups— amino and carboxyl . The amino group (—NH2) is basic while the carboxyl group (—COOH) is acidic in nature. General structure of amino acids The amino acids are termed as D-amino acids, if both the carboxyl and amino groups are attached to the same carbon atom, as depicted below:



General structure



Exists as ion

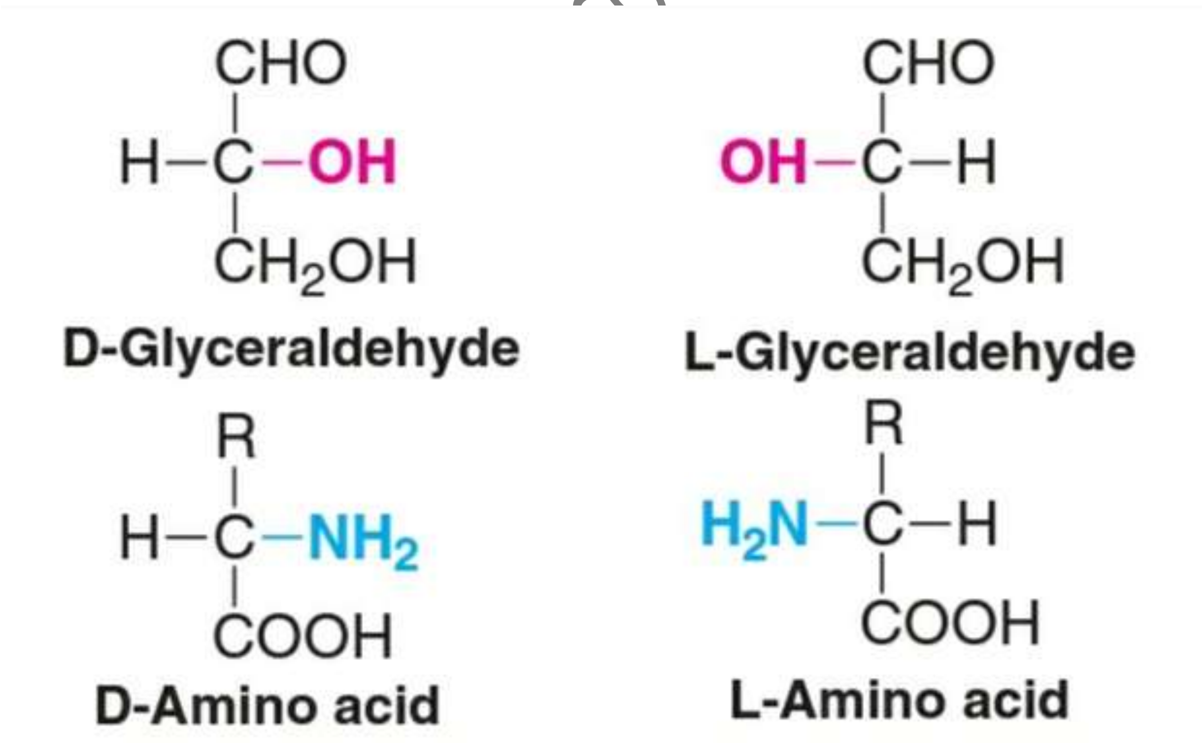


H
R–C–COOH
NH2
Amino acid
H+
H+
H
R–C–COOH
NH3+
Cation
(low pH)
H
R–C–COO−
NH2
Anion
(high pH)
H+
H+
H
R–C–COO−
NH3+
Zwitterion
(isoelectric pH)

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 (shown above). The amino acid composition of a protein determines its physical and chemical properties.

Optical isomers of amino acids:

If a carbon atom is attached to four different groups, it is asymmetric and therefore exhibits optical isomerism . The amino acids (except glycine) possess four distinct groups (R, H, COO–, NH3 +) held by D-carbon. Thus all the amino acids (except glycine where R = H) have optical isomers. The structures of L- and D-amino acids are written based on the configuration of L- and D- glyceraldehyde as shown in Fig.4.1 . The proteins are composed of L-D-amino acids.



❖ ***D - and L - forms of amino acid based on the structure of glyceraldehyde.***

STANDARD AMINO ACIDS

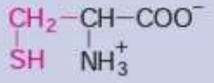
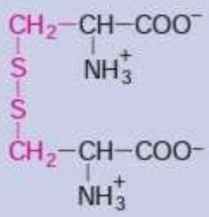
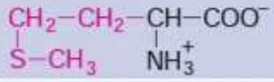
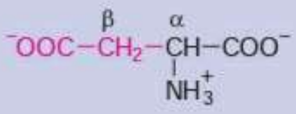
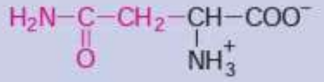
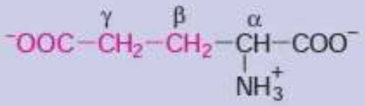
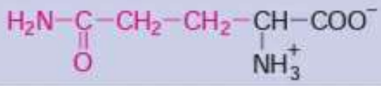
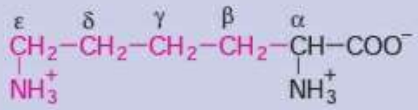
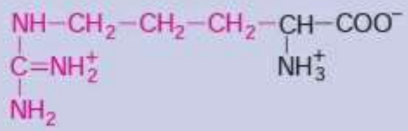
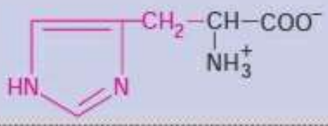
As many as 300 amino acids occur in nature— Of these, only 20 — known as standard amino acids are repeatedly found in the structure of proteins, isolated from different forms of life— animal, plant and microbial. This is because of the universal nature of the genetic code available for the incorporation 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.

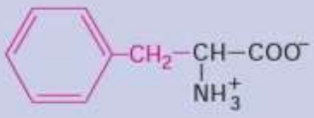
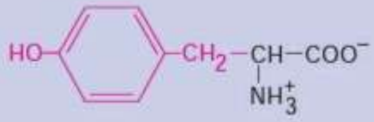
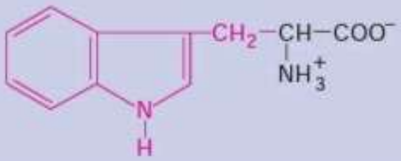
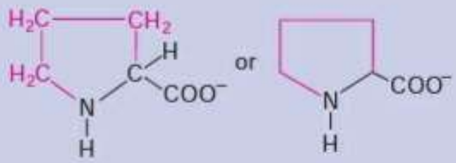
Nutritional classification of amino acids:

The 20 amino acids (Table 4.1) are required for the synthesis of variety proteins, besides other biological functions. However, all these 20 amino acids need not be taken in the diet.

Table 4.1 Structural classification of L-α-amino acids found in proteins

Name	Symbol: 3 letters	Symbol: 1 letter	Structure	Special group present
. Amino acids with aliphatic side chains				
1. Glycine	Gly	G	$H-\underset{NH_3^+}{CH}-COO^-$	
2. Alanine	Ala	A	$CH_3-\underset{NH_3^+}{CH}-COO^-$	
3. Valine	Val	V	$(H_3C)_2CH-\underset{NH_3^+}{CH}-COO^-$	Branched chain
4. Leucine	Leu	L	$(H_3C)_2CH-CH_2-\underset{NH_3^+}{CH}-COO^-$	Branched chain
5. Isoleucine	Ile	I	$CH_3-CH_2-\underset{H_3C}{CH}-\underset{NH_3^+}{CH}-COO^-$	Branched chain
II. Amino acids containing hydroxyl (—OH) groups				
6. Serine	Ser	S	$\underset{OH}{CH_2}-\underset{NH_3^+}{CH}-COO^-$	Hydroxyl
7. Threonine	Thr	T	$H_3C-\underset{OH}{CH}-\underset{NH_3^+}{CH}-COO^-$	Hydroxyl
Tyrosine	Tyr	Y	See under aromatic	Hydroxyl

Name	Symbol: 3 letters	Symbol: 1 letter	Structure	Special group present
III. Sulfur containing amino acids				
8. Cysteine	Cys	C		Sulfhydryl
Cystine	—	—		Disulfide
9. Methionine	Met	M		Thioether
IV. Acidic amino acids and their amides				
10. Aspartic acid	Asp	D		β-Carboxyl
11. Asparagine	Asn	N		Amide
12. Glutamic acid	Glu	E		γ-Carboxyl
13. Glutamine	Gln	Q		Amide
V. Basic amino acids				
14. Lysine	Lys	K		ε-Amino
15. Arginine	Arg	R		Guanidino
16. Histidine	His	H		Imidazole

Name	Symbol: 3 letters	Symbol: 1 letter	Structure	Special group present
VI. Aromatic amino acids				
17. Phenylalanine	Phe	F		Benzene or phenyl
18. Tyrosine	Tyr	Y		Phenol
19. Tryptophan	Trp	W		Indole
VII. Imino acid				
20. Proline	Pro	P		Pyrrolidine

(*Note* : *R group is shown in red*)

Based on the nutritional requirements, amino acids are grouped into two classes—essential and non- essential.

1. Essential or indispensable amino acids:

The amino acids which cannot be synthesized by the body and, therefore, need to be supplied through the diet are called essential amino acids. They are required for proper growth and maintenance of the individual. The ten amino acids listed below are essential for humans (and also rats) : Arginine, Valine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan. [The code A.V. HILL, MP., T. T. (first letter of each amino acid) may be memorized to recall essential amino acids].The two amino acids namely arginine and histidine can be synthesized by adults and not by growing children, hence these are considered as semi–essential amino acids (remember Ah , to recall). Thus, 8 amino acids are absolutely essential while 2 are semi-essential.

2. Non-essential or dispensable amino acids:

The body can synthesize about 10 amino acids to meet the biological needs, hence they need not be consumed in the diet. These are—glycine, alanine, serine, cysteine, aspartate, asparagine, glutamate, glutamine, tyrosine and proline.

❖ *Amino acid classification based on their metabolic fate:*

The carbon skeleton of amino acids can serve as a precursor for the synthesis of glucose (glycogenic) or fat (ketogenic) or both.

From metabolic view point, amino acids are divided into three groups.

1. Glycogenic amino acids:

These amino acids can serve as precursors for the formation of glucose or glycogen. e.g. alanine, aspartate, glycine, methionine etc.

2. Ketogenic amino acids:

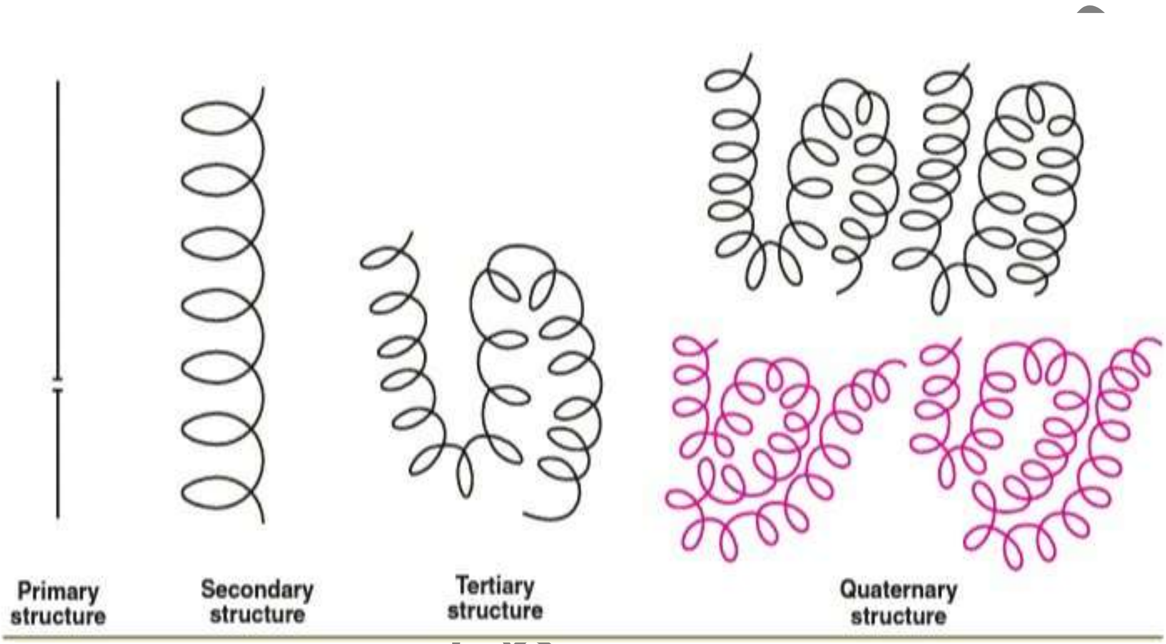
Fat can be synthesized from these amino acids. Two amino acids leucine and lysine are exclusively ketogenic.

3.Glycogenic and ketogenic amino acids:

The four amino acids isoleucine, phenyl- alanine, tryptophan, tyrosine are precursors for synthesis of glucose as well as fat.

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 (Fig.4.4):



Diagrammatic representation of protein structure

(NOTE: The 4 subunits of 2 types in quaternary structure)

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.

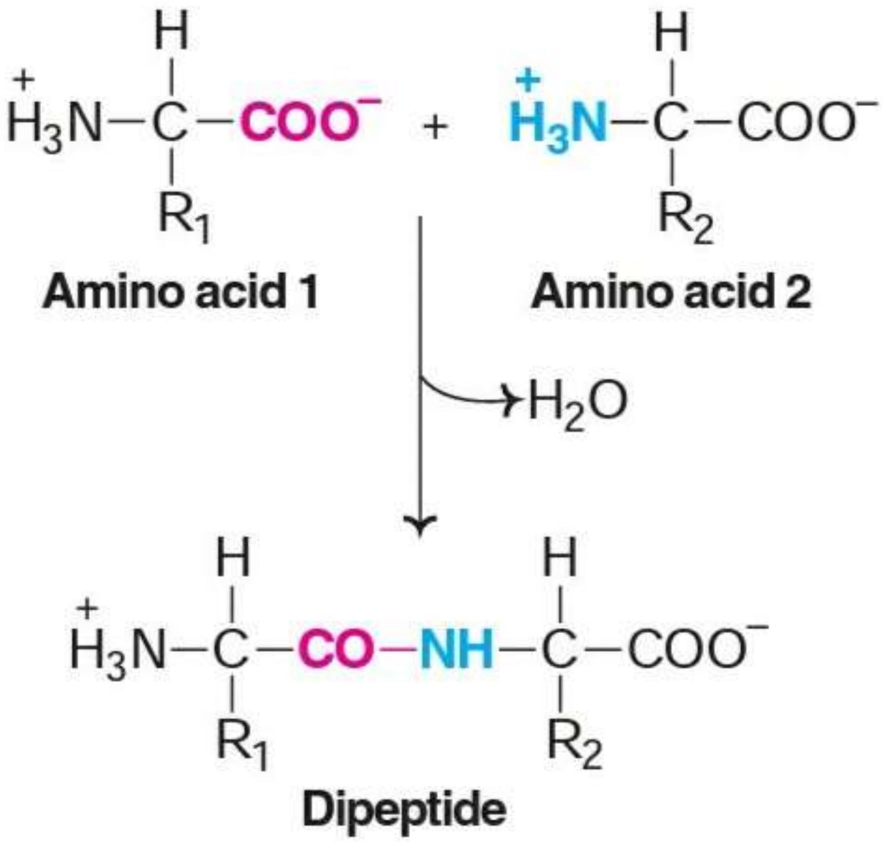
4. Quaternary structure: Some of the proteins are composed of two or more polypeptide chains referred to as subunits. The spatial arrangement of these subunits is known as quaternary structure.

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 (Fig.4.5).



FORMATION OF PEPTIDE BOND

Note that a dipeptide will have two amino acids and one peptide (not two) bond. Peptides containing more than 10 amino acids (decapeptide) are referred to as polypeptides.

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.

Writing of peptide structures :

Conventionally, the peptide chains are written with the free amino end (N-terminal residue) at the left, and the free carboxyl end (C-terminal residue) at the right. The amino acid sequence is read from N-terminal end to C-terminal end. Incidentally, the protein biosynthesis also starts from the N-terminal amino acid.

Shorthand to read peptides :

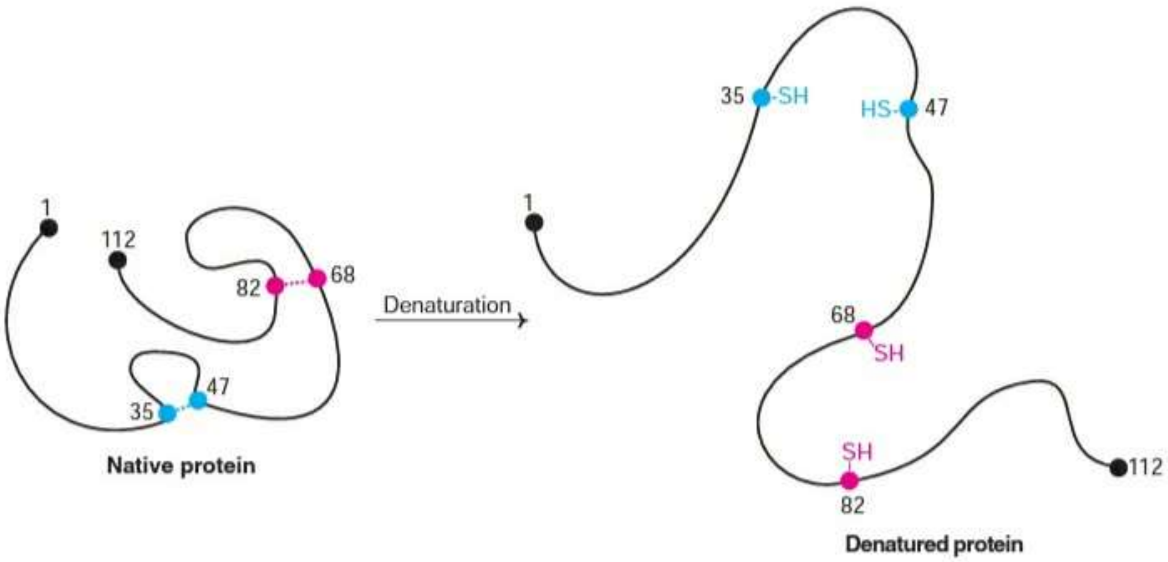
The amino acids in a peptide or protein are represented by the 3-letter or one letter abbreviation. This is the chemical shorthand to write proteins.

Naming of peptides :

For naming peptides, the amino acid suffixes - ine (glycine), - an (tryptophan), - ate (glutamate) are changed to - yl with the exception of C-terminal amino acid. Thus a tripeptide composed of an N-terminal glutamate, a cysteine and a C-terminal glycine is called glutamyl-cysteinyl-glycine. In the Fig.4.6 , the naming and representation of a tripeptide are shown.

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 rever- sible (known as renaturation). Hemoglobin undergoes denaturation in the presence of salicylate. By removal of salicylate, hemoglobin is renatured.

10. Denatured protein cannot be crystallized.

PLASMA PROTEINS:

The plasma is the liquid medium of blood (55-60%), in which the cell components— namely erythrocytes, leukocytes, platelets—are suspended. If blood containing anticoagulants (e.g. heparin, potassium oxalate) is centrifuged, the plasma separates out as a supernatant while the cells remain at the bottom. The packed cell volume or hematocrit is about 45%. The term serum is applied to the liquid medium which separates out after the blood clots (coagulates). Serum does not contain fibrinogen and other clotting factors. Thus, the main difference between plasma and serum is the presence or absence of fibrinogen.

Plasma = Blood - Cells

Serum = Blood - Cells - Fibrinogen

or

Serum = Plasma - Fibrinogen

Separation of plasma proteins:

The total concentration of plasma proteins is about 6-8 g/dl. The plasma is a complex mixture of proteins, and several techniques are employed to separate them. An age-old technique is based on the use of varying concentrations of ammonium sulfate or sodium sulfate. By this method, which is known as ***Salting out process***, the plasma proteins can be separated into three groups—namely:

- Albumin
- Globulins
- Fibrinogen

Electrophoresis:

This is the most commonly employed analytical technique for the separation of plasma (serum) proteins. Paper or agar gel electrophoresis with Vernol buffer (pH-8.6) separates plasma proteins into 5 distinct bands namely albumin, α 1, α 2, β and γ globulins (Fig.9.1). The concentration of each one of these fractions can be estimated by a densitometer.

GENERAL CHARACTERISTICS OF PLASMA PROTEINS:

- They are synthesized in the liver. {Exception: Immunoglobulins}
- Almost all the Plasma proteins are glycoproteins.
- The concentration of certain Plasma proteins increases in disease states such as inflammation and tissue damage. These include CRP (C - Reactive Proteins), Hepatoglobin, Fibrinogen and α_1 antitrypsin.

FUNCTIONS OF PLASMA PROTEINS:

Cummulative functions of plasma proteins are:

- ✓ ***Maintainance of Osmotic Pressure:***

Interstitial fluid is pulled to prevent edema. Albumin is mainly involved in this process.

- ✓ ***Defense:***

Immunoglobulins (Ig G, Ig A, Ig M, Ig E, Ig D) are mainly involved.

- ✓ ***Coagulation:***

It prevents blood loss. When injury occurs:

- Clotting Factors are activated.
- Conversion of Prothrombin to Thrombin.
- Conversion of Fibrinogen to Fibrin.

✓ ***Transport functions:***

- ***Plasma albumin*** binds to several biochemically important compounds and transports them in the circulation. These include ***free fatty acids, bilirubin , steroid hormones, calcium and copper.*** Besides albumin, there are several other plasma transport proteins. Examples are:
- ***Retinol binding protein:*** Transports ***Vitamin A.***
- ***Thyroxine binding protein:*** Transport ***Thyroid Hormomnes***
- ***Transcortin:*** Major transporter of ***steroid hormones*** (e.g. ***cortisol, corticosterone)***

✓ ***Acid - Base balance*** is maintained due to buffering action of Albumin.

✓ ***Vicosity of blood*** (blood pressure) is maintained.

- ✓ ***Erythrocyte Sedimentation Rate*** is maintained. Globulin and Fibrinogen are mainly involved. Rouleaux Formation of RBC's occurs which is important diagnostic and prognostic tool.
- ✓ Production of ***Trephone*** hormone in the tissue culture.