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Chapter four

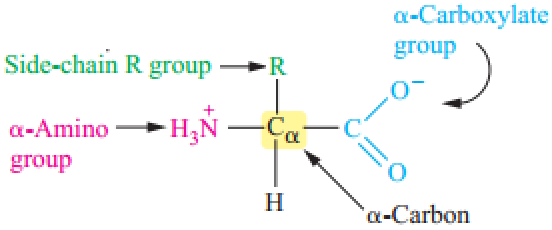
Amino acids and proteins

Amino acids:

Amino acids are organic biomolecules, which are found in small amount (in living systems) in free form, but in the bulk exists as constituent units of protein. Proteins of all species, from bacteria to humans, are made up from the same set of 20 standard amino acids (19 amino acids and 1 imino acid) as building-blocks through peptide bound formation. All the amino acids consist of carbon, hydrogen, oxygen and nitrogen.

Amino acids are molecules having two functional groups (carbonyl group COOH and amino group NH2) at α-carbon atom (the carbon adjacent to the carboxyl group is called the α-carbon). Hence they are named as α-amino acids.

All amino acids have the general formula, but they differ in the side chains or R groups.



The α-carbon of all amino acids is a symmetric except in glycine where the α-carbon is symmetric. Because of this asymmetry, the amino acids (of course, except glycine) exist in two optically active forms: those having — NH_2 group to the right are designated as D-forms and those having — NH_2 group to the left as L-forms.

It is interesting to note that the amino acids found in the proteins belong to the L-configuration. However, D- amino acids are found in some antibiotics produced by microorganism and in bacteria cell walls. Two free amino acids D-serine and Daspartic acid have recently been found to be present in the forebrain and brain periphery respectively.

Many of the naturally occurring L-amino acids rotate the plane of polarized light to the left (i.e., they are levorotatory) while others rotate the plane of polarized light to the right (i.e., they are dextrorotatory).

Classification of amino acids based on their structure:

Amino acids can be classified into three groups depending on their structure(R group):

I- Aliphatic: These are straight or open- chain amino acids which are further subdivided into four groups as:

1- Neutral amino acids: These consist of one amino and one carboxyl groups and hence are neutral to litmus, e.g. glycine, **alanine**, valine, leucine, isoleucine, serine and theronine.

2- Acidic amino acids: These consist of one amino and two carboxyl groups and hence are acidic to litmus, e.g. aspartic acid and glutamic acid.

3. Basic amino acids: These consist of one carboxyl and two amino groups and hence are basic to litmus e.g. lysine, arginine and histidine.

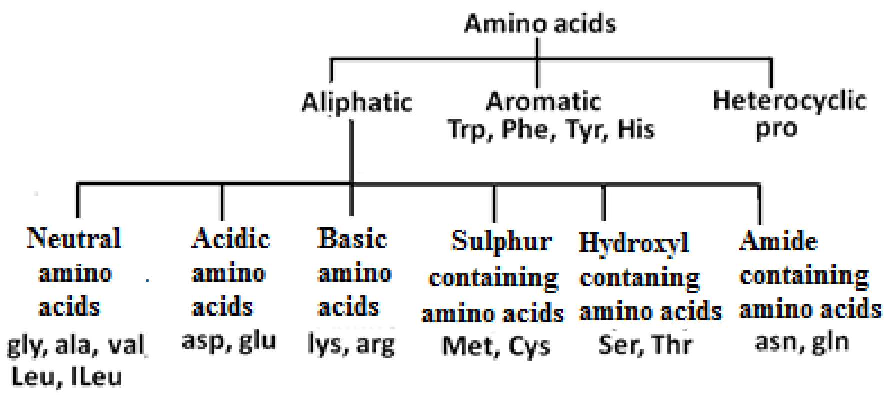
4. Sulphur-containing amino acids: These consist of one or more sulphur atoms, e.g. cysteine, and methionine.

5. Hydroxyl-containing amino acids: These consist of hydroxyl group, e.g. serine, and threonine

6. Amide-containing amino acids: These consist of amide group, e.g. Asparagine, and Glutamine.

II. Aromatic amino acids: These contain an aromatic ring in the molecule, e.g. phenylalanine and tyrosine.

III. Heterocyclic amino acids: These contain an heterocyclic nucleus in the molecule e.g., histidine, tryptophan, proline and hydroxyproline.



Classification of amino acids based on nature of polarity:

According to this type of classification, amino acids are classified into two major classes;

<u>1-Hydrophilic or polar amino acids:</u>

The side chains of the hydrophilic amino acids contain polar groups that may be:

a- Charged Hydrophilic amino acids:

The charged side chains are of two types:

- **Negatively charge side chain** like aspartic acid and the glutamic acid, the side chains of these compounds have the carbonyl groups that are the negatively charged within the physiological PH range.
- **Positively charge side chain** like lysine, arginine and histine, the side chains of these compounds have the amino groups that are the positively charged within the physiological PH range.

b- Uncharged Hydrophilic amino acids:

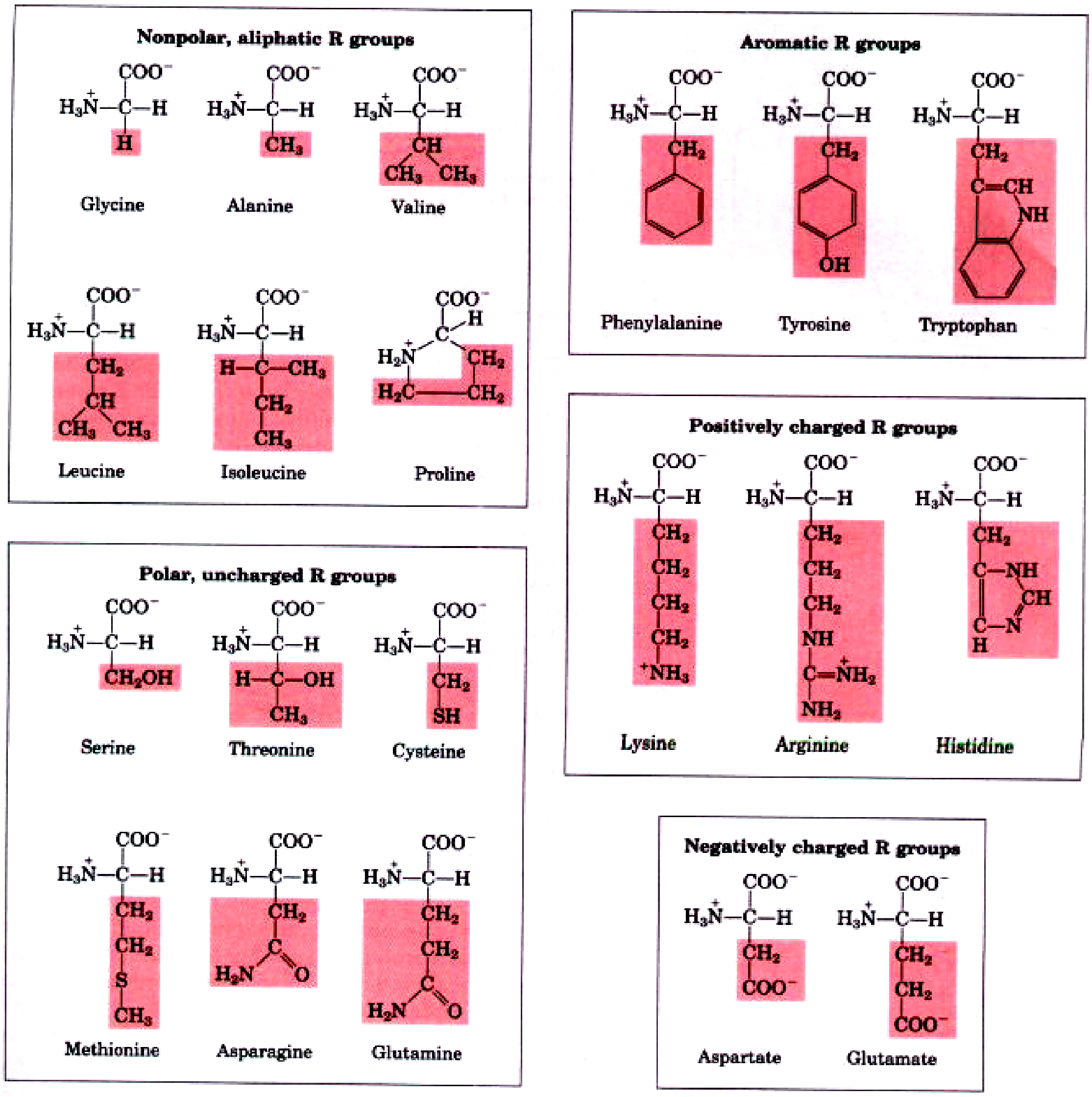
The uncharged side chains of their amino acids have oxygen, sulfur or nitrogen atoms, enabling them to form hydrogen bonds with water. Although they are uncharged, these amino acids are hydrophilic.

- **Theronine and serine,** with hydroxyl group (OH) in the side chain.
- **Aspargine and glutamine** with amide group (CONH2).
- **Cycteine** with thiol group(SH).

<u>2- Hydrophobic or non polar amino acids:</u>

The side chains of hydrophobic amino acids contain non-polar group that may be:

- **Hydrophobic amino acids with aliphatic side chain:** glycine, alanine, valine, leucine, isoleucine, proline and methionine.
- **Hydrophobic amino acids with aromatic side chain:** phenyl alanine, tyrosine and tryptophane.



Physical properties of amino acids:

Amino acids are generally white crystalline substance, soluble in water and quite insoluble in non polar organic solvents such as ether, chloroform and acetone. Aliphatic and aromatic amino acids particularly having several carbon atoms have limited solubility in water but readily soluble in polar organic solvents.

Another property of amino acids that related to their structure is their melting points, they have high melting points from 200-300 or even more, which often result in decomposition. The melting points of the solid carboxylic acids and amines are usually low and sharp.

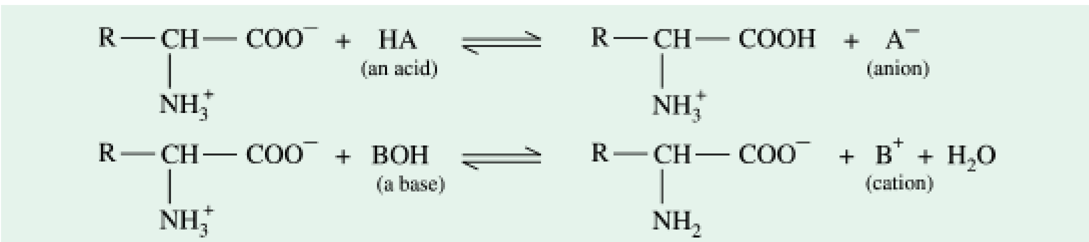
They may be tasteless (tyrosine), sweet (glycine and alanine) or bitter (arginine), and some have good flavor. Sodium glutamate is a valuable flavoring agent and used in the preparation of certain dishes and sauce.

The acid-base properties of amino acids:

All the crystalline standard amino acids have relatively high melting or decomposition points usually above 200°C. The R groups on the standard amino acid confer specific properties of each. The properties may depend on the solution pH.

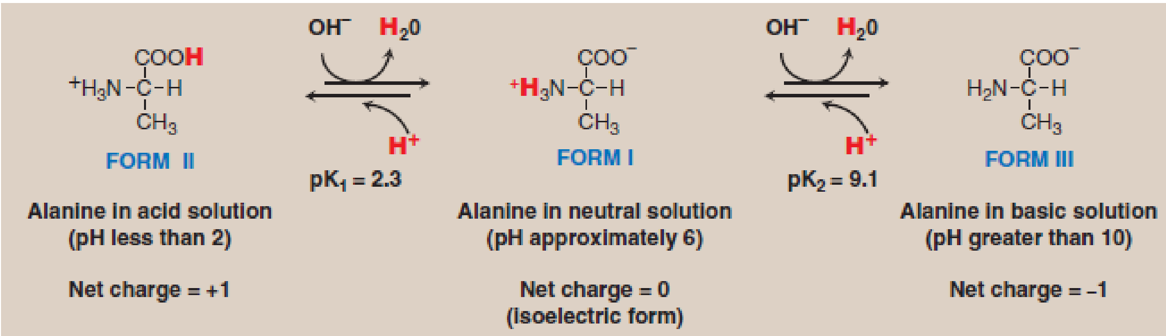
Amino acids in neutral aqueous solution exist as dipolar ion or zwitterions which are electrically neutral in nature. **Zwitterions** are molecules that contain equal number of positive and negative charge and therefore bear no net charge. The characteristic pH at which the net electric charge is zero is called the isoelectric point or isoelectric pH, designated pI.

A zwitterion reacts with both acids and bases. Hence, they are amphoteric and are often called ampholytes in nature.

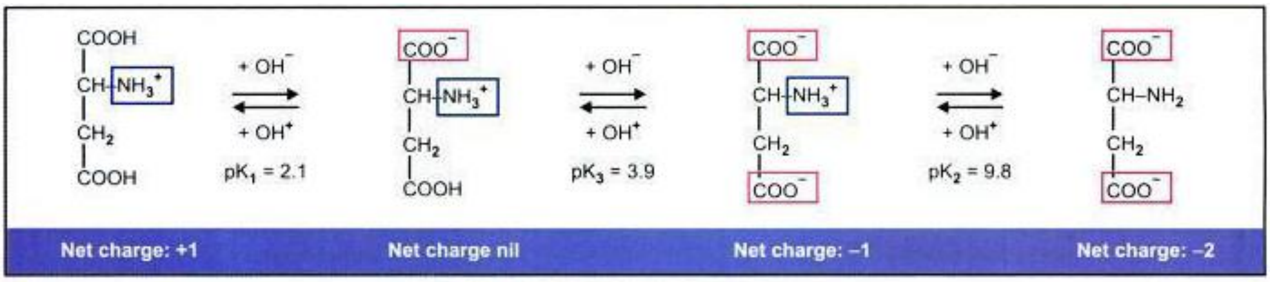


Thus, in acid solution, the COO– ion acquires a proton and the amino acid becomes an ammonium salt of the acid. Conversely, in alkaline solution, the NH_3^+ ion loses a proton and the amino acid becomes the anion of a salt. These reactions are of reversible nature and depend on the pH of the medium

A simple monoamino monocarboxylic-amino acid, such as alanine, is a diprotic acid when fully protonated-it has two groups, the -COOH group and the -NH3 group that can yield protons:



Acidic amino acids such as aspartic acid have a second carboxyl group that can donate and accept protons. The pI for aspartic acid occurs at a pH of 2.8.



Ionization of aspartic acid

Essential amino acids:

Some of the 20 standard amino acids are called essential amino acids because they cannot be synthesized by the body from other compounds through chemical reactions, but instead must be taken in with food. In humans, the essential amino acids are lysine, leucine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine.

Deficiency of one or more of these amino acids leads to impairs protein synthesis and abnormal growth.

Semi-essential amino acids:

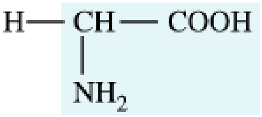
These amino acids are not synthesized in sufficient quantity during growth. They include arginine and hitidine. They become essential in growing children, pregnancy and lacting women. But they are not essential for the adult individual.

Non essential amino acids:

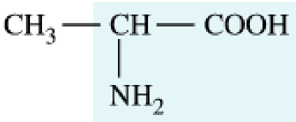
Those are amino acids that can be biosynthesized in adequate amounts in the body and not required in the diet. These amino acids are derived from the metabolism of the lipid or carbohydrates or from the transformation of essential amino acids and include glycine, proline, serine, glutamic, glutamine, alanine, tyrosine, cysteine, and aspartic.

Standard amino acids:

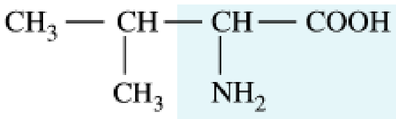
The 20 amino acids found in proteins are referred to as the standard amino acids or protein amino acids:



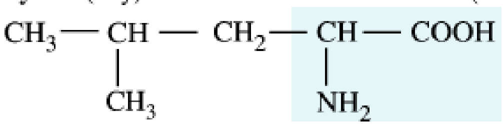
Glycine (Gly)



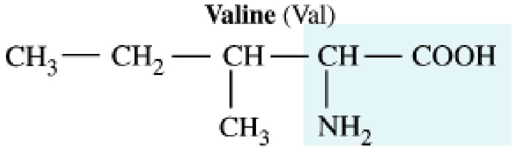
Alanine (Ala)



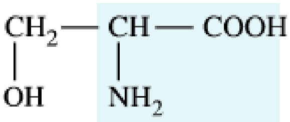
Valine (Val)



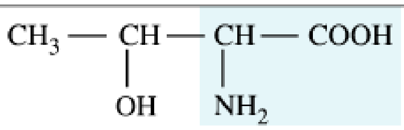
Leucine (Leu)



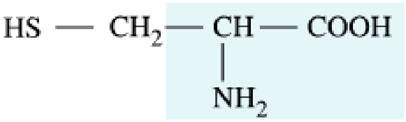
Isoleucine (Ile)



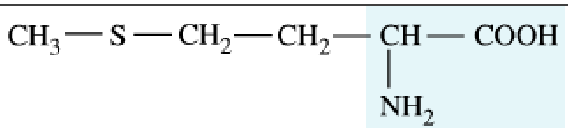
Serine (Ser)



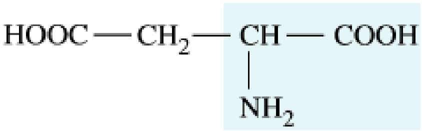
Threonine (Thr)



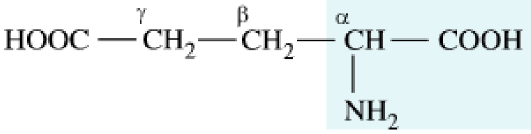
Cysteine (Cys)



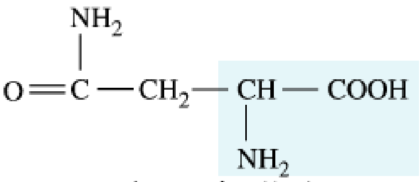
Methionine (Met)



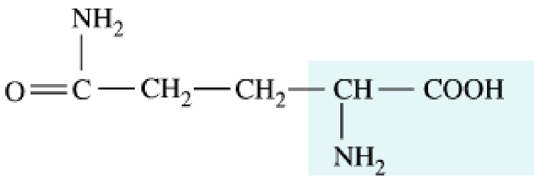
Aspartic acid (Asp)



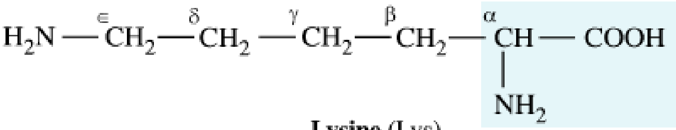
Glutamic acid (Glu)



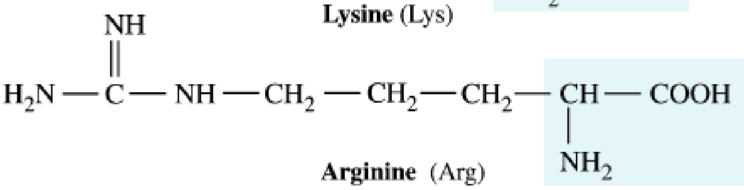
Asparagine (Asn)



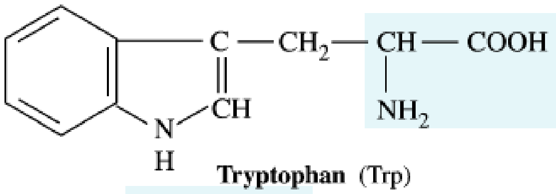
Glutamine (Gln)



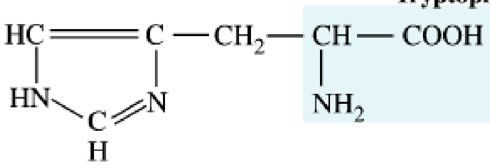
Lysine (Lys)



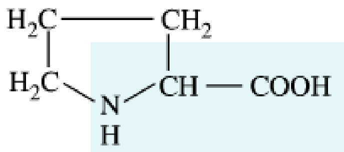
Arginine (Arg)



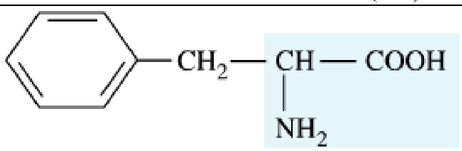
Tryptophan (Trp)



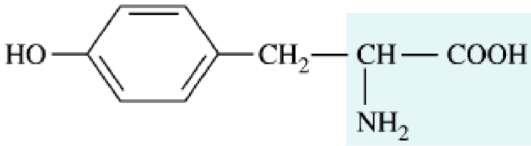
Histidine (His)



Proline (Pro)



Phenylalanine (Phe)

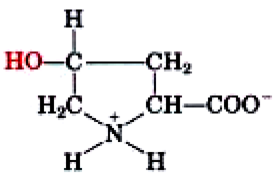


Tyrosine (Tyr)

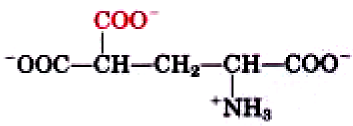
Non Standard or uncommen amino acids of protein (the rare amino acids):

In addition to the 20 common standard amino acids, other amino acids are found in protein in smaller quantities and called **Non standard amino acids or rare amino acids**. For example, 4-hydroxyproline and 5-hydroxylysine both are found in collagen, a fibrous protein of connective tissues.

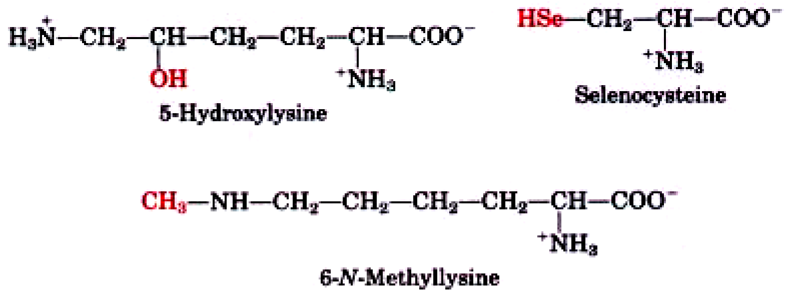
Another important uncommon amino acid is **γ-carboxyglutamate**, found in the blood clotting protein prothrombin and in certain other proteins that bind Ca as part of their biological function. Certain muscle proteins contain methylated amino acids, like **6-N-Methyllysine** is a constituent of myosin, a contractile protein of muscle. **Selenocysteine** is found in glutathione peroxidase and a few other proteins.



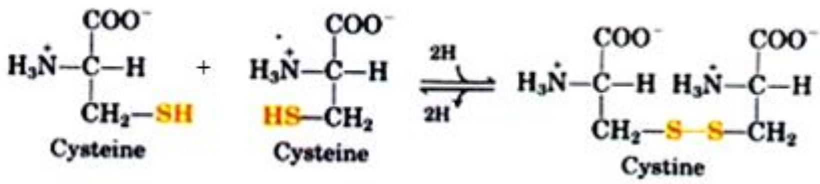
4-Hydroxyproline



γ-Carboxyglutamate

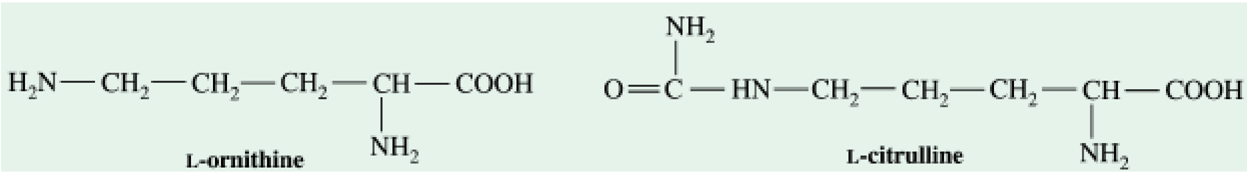


Also, two cysteine molecules are joined by a disulfide bond obtain cystine, this disulfide bonds help to stabilize the structure of some proteins.

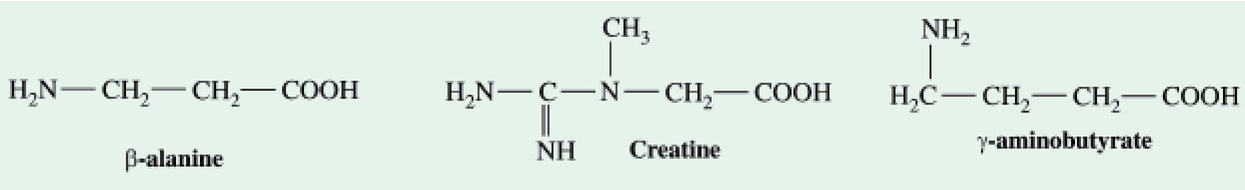


Derivative of amino acids (Non-protein amino acids):

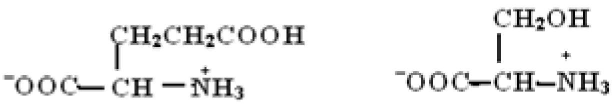
In addition to the 20 common and several rare amino acids of proteins, there are some other amino acids have been found in cells and have a variety of functions but are not substituents of proteins. L-orinthine and L-citrulline, both are metabolic intermediates in the biosynthesis of arginine and in the urea cycle.



As isomer of α-alanine, β-alanine is found free in nature and is a constituent of the vitamin pantothenic acid found in Coenzyme A. D-alanine found in the larvae of some insects. The quaternary amine creatine, a derivative of glycine, plays an important role in the energy storage process in vertebrates where it is phosphorylated and converted to creatine phosphate. Lastly, γ-aminobutyrate is found in free form in the brain.

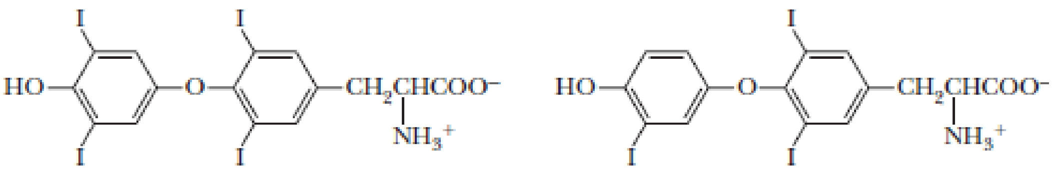


Some of the less common D- enantiomers of amino acids are also found in nature. For example, **D-glutamic acid** is found in the cell walls of many bacteria, and **D-serine** is found in earthworms.



D-glutamic acid D-serine

Thyroxine and triiodothyronine, two of several hormones derived from the amino acid tyrosine, are found in thyroid tissue. Their principal function is to stimulate metabolism in other cells and tissues.



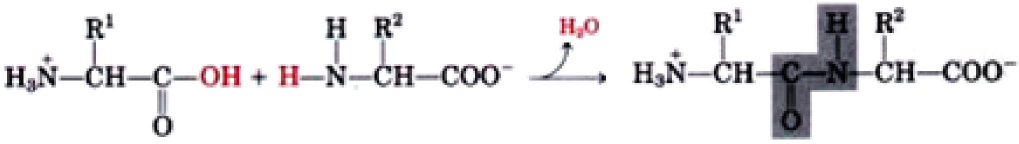
L-Thyroxine, T_4 L-Triiodothyronine, T_3

Peptides:

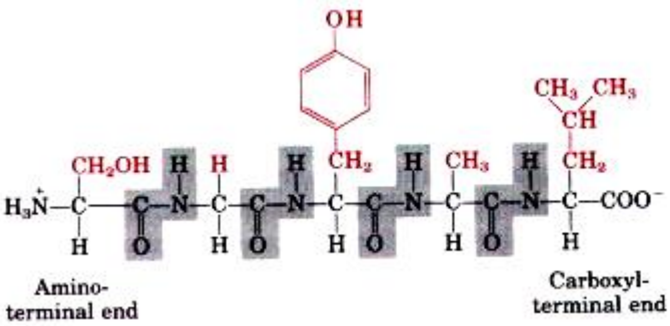
Peptide is a compound composed of amino acids linked together by a peptide bond

Peptide formation:

Peptide is a compound formed by the reaction of the -carboxyl group of one amino acid and the –amino group of another molecule of amino acid by removal of water. These two amino acids are joined together via amide linkage, called a peptide bond which is the backbone of the polypeptide chain. If the peptide contains 2-10 amino acid residues, it is called as an **oligopeptide**. When two amino acids are joined is called a dipeptide, molecule containing 3 amino acids are called **tripeptides**, containing 4 amino acids are called, **tetrapeptides**, containing 5 amino acids are called **pentapeptides**, and so on). Molecules containing more than 10 are called **polypeptides**.

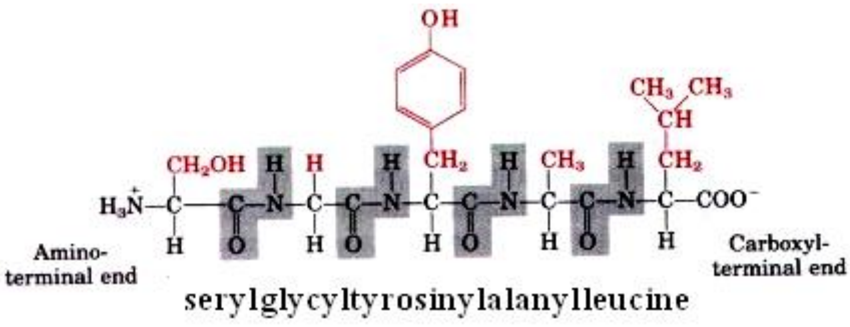


The amino acid residue at that end of a peptide having a free a-amino group is the **amino-terminal (or N-terminal**) residue; and the other, which has a free carboxyl group, is **the carboxyl-terminal (C-terminal).**



Nomenclature of peptides:

Peptides are named beginning from left (the amino-terminal residue) to right. For all amino acids, the ending (-ine) is changed to (-yl) until last amino acid which retains its usual ending. For example, the full and abbreviated name for a tripeptide is given below:



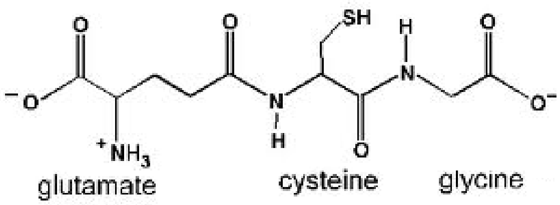
Full name	Abbreviated name
Valyl-aspartyl-lysine	Val-asp-lys

Thus the pentapeptide serylglycyltyrosinylalanylleucine has leucine as its C-terminal amino acid, as indicated by its full name, leucine.

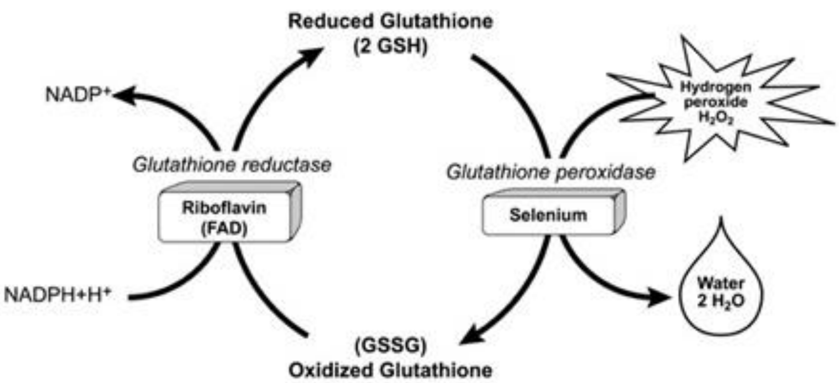
Biologically important peptides:

There are many naturally occurring small polypeptides and oligo peptides, some of which have important biological activities and are called biologically important peptides.

Glutathione is a tripeptide containing glutamate, cysteine, and glycine. Glutathione is found in all mammalian cells except the neurons. Glutathione is an important reducing agent in the tissues because it have (SH) group of the cysteine.

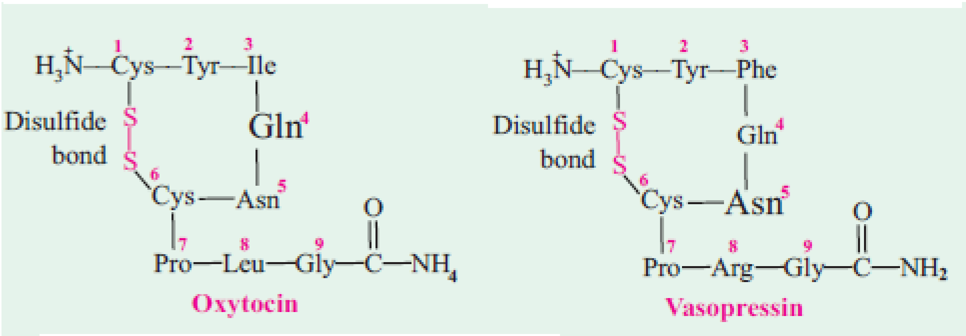


Glutathione protects cells from the destructive effects of oxidative by reacting with a variety substance such as peroxides (byproduct of O_2 metabolism).



Vasopressin and **Oxytocin** are two peptide hormones containing nine amino acids; secreted by the posterior lobe of the pituitary gland. **Oxytocin** stimulates the contraction of smooth muscles, esp., those of uterus, thus facilitating childbirth. In general, it also causes contraction of other smooth muscles like those of intestine, urinary bladder and the ducts of mammary glands resulting in milk ejection.

Vasopressin is a hormone; containing nine amino acids and it increase blood pressure and have an anti-diuretic action. **Oxytocin** also is a hormone, containing nine amino acids, which is secreted by the posterior pituitary and stimulates uterine contractions.



Structure of the two neurohyphyseal hormones

Bradykinin have nine amino acids, which inhibits inflammation of tissues

Arg. Pro. Pro. Gly. Phe. Ser. Pro. Phe. Arg

Bradykinin

Many **antibiotics** are peptides or derivatives of peptides including gramicidin.

Some important naturally occurring peptides

Peptide	**No. of amino acid residues**	**Biological importance**
Glutathione (γ-glutamyl - cysteinyl-glycine)	3	Biological reducing agent
Vasopressin	9	Water balance hormone
Oxytocin	9	Stimulates uterine contraction
Gramicidin A	10	Antibiotic

Proteins:

Proteins are polymers of L-α-amino acids linked by peptide bonds. All the different types of proteins are initially synthesized as polymers of 20 common amino acids. Proteins are macromolecules varying in amino acid composition, structure, shape and properties. All proteins contain C, H, O, and N. Small amounts of S and P are also present. Few proteins contain other elements such as I, Cu, Mn, Zn, Fe…

Classification of proteins:

<u>1- Classification of proteins based on functions of proteins:</u>

1- Biochemical catalysts known as enzymes are proteins and catalyze all the chemical reaction that occurs in living cells.

2- Proteins known as immunoglobulin serve as the first line of defense against bacterial and viral infections.

3- Some proteins present in cell membrane, cytoplasm and nucleus of the cell act as receptor.

4- Transport proteins carry materials from one place to another in the body. Like hemoglobin.

5- Under certain conditions protein can be catabolized to supply energy.

6- Storage proteins bind with specific substances and store them, like ferritin store iron, and casin in the milk, store amino acid. Also the seeds of many plants store proteins.

7- Regulatory proteins control cell function, include hormones such as insulin and glucagon.

8- Some proteins serve as structural materials or as important components of extracellular fluid. Like keratin of skin and hair, collagen of connective tissue.

2- Classification of proteins based on shape and size:

a- Fibrous proteins:

Fibrous proteins are composed of polypeptide chains arranged in parallel along a single axis to yield long fibers or sheets. These proteins often serve structural roles in cells. Typically, they are insoluble in water or in dilute salt solution. They are insoluble in water and physically tough. e.g. **collagen**, the main protein constituent of connective tissue and bone, **elastin**, the main protein constituent of elastin tissue, the **keratins** of hair, skin, feather, horn and nails and **fibroin**, the major constituents of silk is produced by insects and spiders).

b- Globular proteins:

Globular proteins also called spheroproteins are spherical in shape in which the polypeptide chains of these proteins are tightly folded into compact spherical or globular shapes.. Globular proteins are soluble in aqueous solution. This group includes albumin, many enzymes, hormones, protamines, globulin, histones and actin.

3- Classification based on the structure of proteins:

Proteins can be classified according to their structure to:

a- Simple proteins:

These are proteins containing only amino acids. On complete hydrolysis yield only amino acids. Like: Albumin (found in egg white and blood), globulins (found in blood, milk, muscle and in plant seed), glutelins (found in the wheat, rice, and corn), histones (present in cell nuclei in association with nucleic acids), protamine (found in fish) and prolamine (found in wheat and corn plants).

Sub-classification of simple proteins

Name	Example and source	Soluble in	Other special features
Albumins	Ovalbumin (egg white) Serum albumin (blood)	Water and salt solution	Coagulated by heat
Globulins	Serum globulin (blood) Edestin (hemp seed)	Dilute salt solution	Coagulated by heat
Glutelins	Glutenin (wheat) Oryzenin (rice) Glutelin (corn)	Dilute acids and alkalies	Coagulated by heat
Prolamines	Gliadin (wheat) Zein (corn)	60-80% alcohol	Not coagulated by heat; rich in proline and glutamic acid
Protamines	Sturin and salmin (sperm of fish)	Water, dilute acids and ammonia	Not coagulated by heat; strongly basic in reaction due to rich in diamino acids
Histones	Histone (thymus)	Water and dilute acids	Not coagulated by heat; basic in nature due to rich in diamino acids
Scleroprotein (albuminoids)	Elastin- (tendons) Keratin (hair)	Insoluble in common solvents	Hydrolyzed by long boiling with strong acid

b- Conjugated or compound or complex proteins:

These are proteins linked with a non protein part called prosthetic group. The prosthetic group may be a metal or a compound. On hydrolysis with acids, yield the amino acids and prosthetic group. A number of proteins contain more than one prosthetic group. Usually the prosthetic group plays an important role in the protein's biological function.

Holo protein = Protein + non protein part

(Apo protein) (prosthetic group)

Sub-classification of conjugated proteins

Conjugated protein	Prosthetic group	Example
Lipoproteins	Lipid	Plasma β_1-lipoproteins
Glycoproteins	Carbohydrate	Immunoglobulins
Nucleoproteins	Nucleic acid	Ribosomes
Flavoproteins	FAD	Succinate dehydrogenase
Phosphoproteins	Phosphoric acid	Casein (milk)
Chromoproteins	Colored substance	Hemoglobin (contains heme)
Metalloproteins	Metal ion	Ferritin (contains ferric hydroxide)

c- Derived proteins:

These are derivatives of proteins formed from simple and conjugated proteins. These proteins are produced by physical and chemical factors and divided into:

1-primary derived proteins:

These are derived proteins formed from heat, acid, base, etc….which causes slight changes in protein molecules and its properties without hydrolytic cleavage of peptide bond. Like fibrin from fibrinogen.

2- Secondary derived proteins:

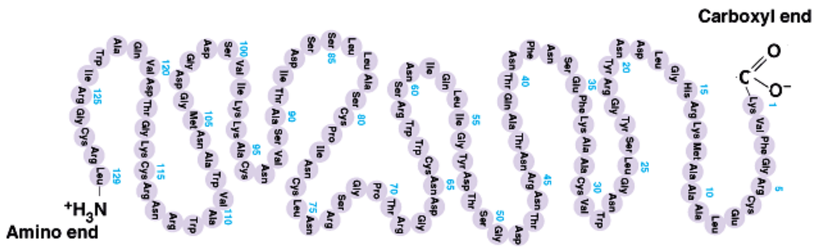
These are derived proteins formed from hydrolytic cleavage of the peptide bond.

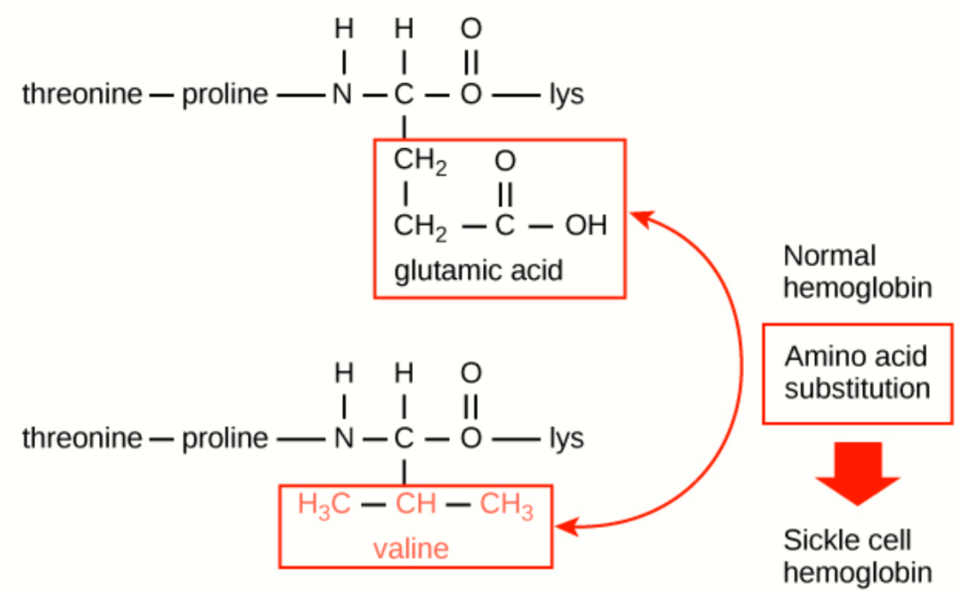
Organization of Protein Structure

Proteins have four levels of structure organization:

1- Primary structure of proteins:

Primary structure of proteins is the linear sequence of amino acids in a protein held together by peptide bonds, one end of the molecule will have a free α- amino group (N-terminal -in the left) and the other end will have a free carboxyl group (C-terminal -in the right). The primary structure of proteins is important because any change in the sequence of amino acids leads to abnormal and may affect the function and properties of proteins. Like sickle cell anemia.

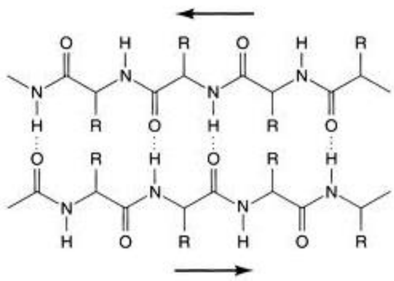




2- Secondary structure of proteins:

It refers to the coiling or folding of a polypeptide chain or a protein. This structure appears to be folded or pleated and is held together by hydrogen bonding between polypeptide units of the folded chain. There are two types of secondary structures in proteins. One type is the alpha (α) helix structure. Right handed and left handed α-helical spiral is stabilized by hydrogen bonds between amino acids in the same polypeptide chain. The second type of secondary structure in proteins is the beta (β) pleated sheet. This structure is stabilized by hydrogen bonds between amino acid in two neighbor polypeptide chains.

α- Helix



β - Pleated sheet

α- Helix: Is the one form of secondary structure in which amino acids in same polypeptide chain is stabilized by hydrogen bonds.

β- Pleated sheet: Is another form of secondary structure in which amino acids in two neibours polypeptide chain is stabilized by hydrogen bonds.

Tertiary structure of proteins:

The polypeptide chain (primary structure), will its secondary structure, may be further folded and coiled a bout itself forming a tertiary structure of protein. Folding occurs from interaction between amino acids relatively for a part in the sequence. The stabilization of this structure is due to the different bonds between groups of amino acids. But some proteins are stabilized by disulfide bonds between cysteine.

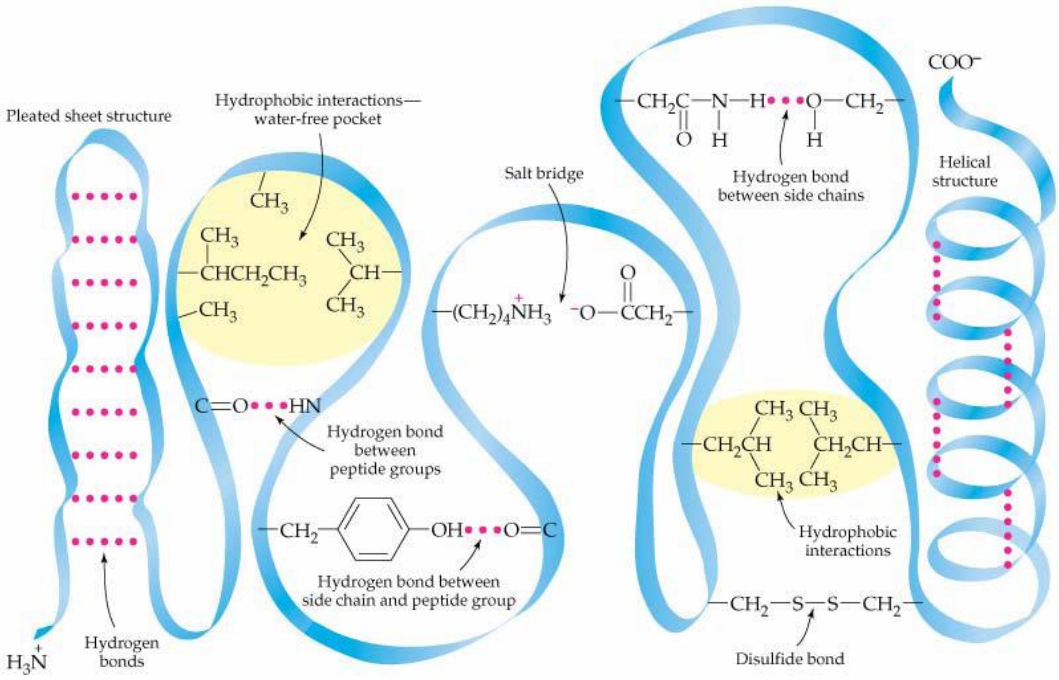
1- Hydrogen bonds: Between the polar R groups of the polar amino acids

2- Ionic bonds (Electrostatic forces, salt linkage): Between R group of positive charge and R group of negative charge.

3-Hydrophobic bonds (Van der Waals forces): Between the R groups of nonpolar amino acids.

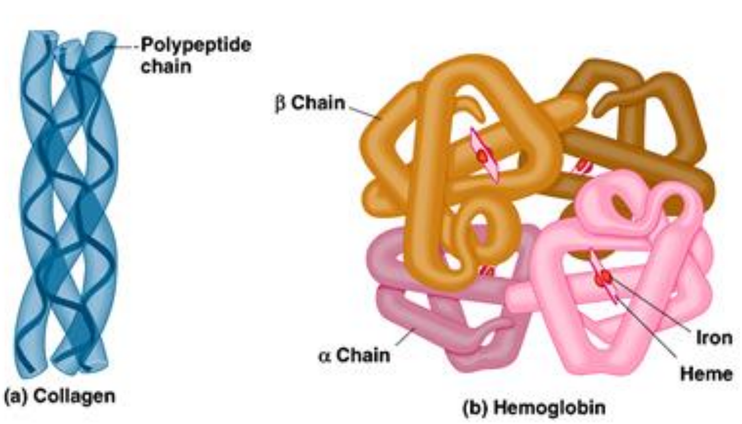
4- Dipole-Dipole interaction: Between polar-unionized side chain.

5-Disulfide bonds (covalent bonds): Between two cysteine groups.

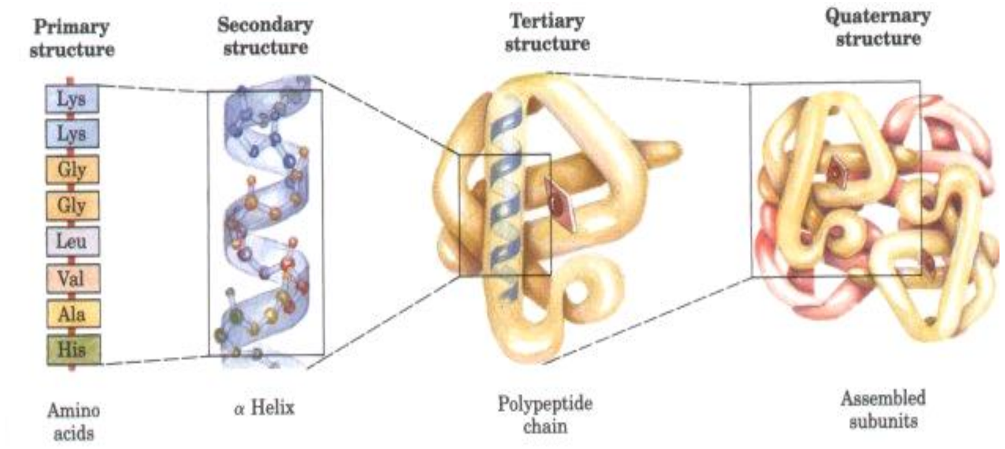


Quaternary structure of proteins:

Many proteins are made up of only one poly peptide chain, and are defined as monomeric proteins. However, others may composed of two or more poly peptide chains (subunits) and the rearrangement of these polypeptide chains (subunits) is called the quaternary structure of protein (Note, if there are two subunits, the protein is called dimeric, if three subunits is called trimeric, if several subunits is called mutimeric. Subunits could be of the same type so will called homogeneous quaternary structure, if the subunits are not similar, a heterogeneous quaternary structure is obtained. For example: hemoglobin is tetramer, consisting of **two identical α-chains and two identical β-chains.**



(a) Collagen (b) Hemoglobin



Denaturation of proteins:

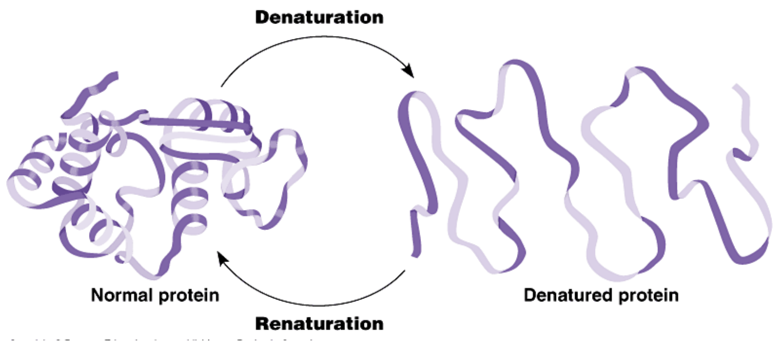
Denaturation of protein is the changes in the properties of a protein. In other words, it is the loss of biologic activity. Denaturation agents include physical and chemical agents or by mechanical action.

Physical agents include heat, pH, and UV light and X-ray.

Chemical agents include acid, alkaline, acid solutions of heavy metals like lead, mercury or organic solvents like acetone and alcohol.

Mechanical actions include shaking or grinding.

Denaturation is decomposition of the secondary, tertiary and quaternary structure of protein, due to cleavage of non covalent bonds. But primary structure which includes peptide bond is not affected. Example of denaturation of proteins is like boiled egg, cooked meat.



Protein denaturants

Agent	**Probable cause of denaturaiton**
Physical agents	
pH	Change in ionization of R groups of amino acids of proteins
Heat	Thermal splitting of salt bridges, ‘melting’ ice crystal bound water
Shaking or stirring	Unfolding of peptide chains (protein monolayer
	forms at surface and in foam)
Ultrasonic waves	Mechnical agitation, thermal effects, release of (O) from water
UV light and X-rays	Absorption of energy, splitting of bonds
Chemical agents	
Mineral acids and alkalies	Cleavage of salt bridges
Urea, acetone and alcohols	Cleavage of hydrogen bonds
Guanidine HCl	Liberation of hydrogen bonds
Tungstic, picric, trichloroacetic acids	Cleavage of salt bridge