JAUNDICE

Jaundice or hyperbilirubinemia also called icterus is the accumulation of bilirubin or bile pigments above normal levels in the plasma leading to the yellowish discoloration of skin, mucous membrane and tissues. Bilirubin has been shown to inhibit DNA synthesis, uncouple oxidative phosphorylation, and inhibit ATPase activity in brain and mitochondria. Bilirubin also inhibits a variety of different classes of enzymes including dehydrogenases, electron transport proteins, hydrolyases, and enzymes of RNA synthesis, protein synthesis and carbohydrate metabolism, hence very toxic in the system.

There are three major types of jaundice;

1. Prehepatic jaundice this occurs as a result of increased production of bilirubin as a result of more rapid breakdown of RBCs than normal, more bilirubin is conjugated and excreted than normally, but the conjugation mechanism is overwhelmed, and an abnormally large amount of unconjugated bilirubin is found in the blood. This may occur as a consequence of a hemolytic disease causing massive destruction of RBCs.
2. Hepatic jaundice occurs because of an inability of the hepatocytes to adequately conjugate bilirubin either as a result of inability to take up bilirubin from the blood (As a result, unconjugated bilirubin accumulates), or an impairment of the conjugation pathway (also unconjugated bilirubin accumulates) or inability of the hepatocytes to secrete the already conjugated bilirubin after it is formed hence conjugated bilirubin returns to the blood.
3. Post hepatic jaundice is caused by an obstruction distal to the liver e.g. biliary obstruction like a calculi that interferes with secretion or passage of the conjugated bilirubin into the intestine hence the is reabsorbtion of conjugated bilirubin back into the system (a proportion normally exchanges back into the blood plasma but in health this is very small).

PORPHYRIAS

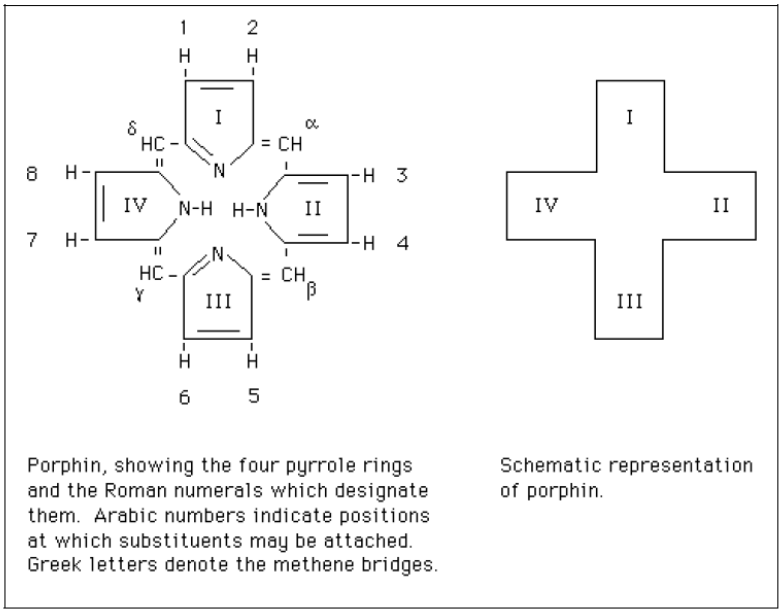
These are disorders that arise in heme biosynthesis as a result of defects in enzymes that catalyze the various reactions. Typically there is an increase in levels of intermediates of heme synthesis within the blood, urine and other body tissues and fluids and these can cause toxic effects.

Porphyrias maybe either acquired (as a results of poisonous or drug effects on enzymes) or hereditary (caused by a gene defect). Porphyrias may also be classified as erythroid or hepatic depending on site of enzyme defect.

The most common porphyrias known is that caused by a defect in the enzyme porphobilinogen deaminase (PBG deaminase) called acute intermittent porphyrias.

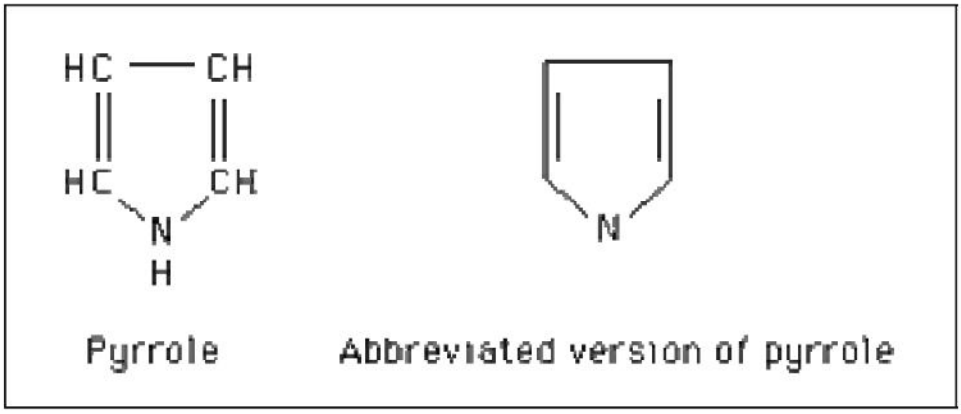
Porphyrias generally lead to excretion of deposits in urine that color it red or reddish brown; they may also be deposited in teeth. Ulcerative and photosensitive systems on the skin may also

results when the porphobilinogens are oxidized to porphins. There may also be neurological symptoms, which cannot be explained.



Porphin, showing the four pyrrole rings and the Roman numerals which designate them. Arabic numbers indicate positions at which substituents may be attached. Greek letters denote the methene bridges.

Schematic representation of porphin.



AMINO ACIDS AND PEPTIDES

OCCURRENCE

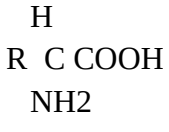
Amino acids and peptides are present in humans, animals, tissues, blood, microorganisms and plants.

MEDICAL AND BIOLOGICAL IMPORTANCE

1. Amino acids serve as building blocks of proteins. Some amino acids are found in free form in human blood.
2. They also serve as precursors of hormones, purines, pyrimidines, porphyrins, vitamins and biologically important amines like histamine.
3. Peptides have many important biological functions. Some of them are hormones. They are used as anti-biotics and antitumor agents.
4. Some peptides are required for detoxification reactions. Some peptides serve as neurotransmitters.
5. Amino acid proline protects living organisms against free radical induced damage.
6. Some peptides are involved in regulation of cell cycle and apoptosis.
7. Peptides of vertebrates and invertebrates act as antimicrobial agents. They are part of innate immunity. Bacterial infections at epithelial surface induce production of antimicrobial peptides, which cause lysis of microbes.
8. Peptides are enzyme inhibitors. Natural and synthetic peptide inhibitors of angiotensin converting enzyme (ACE) act as a anti hypertensives. Peptide inhibitors of ACE present in physiological foods, lowers blood pressure after they are absorbed from intestine. Lisinopril, Enalapril etc. are synthetic peptide inhibitors of ACE that are used as drugs in the treatment of hypertension.
9. Some synthetic peptides are used as enzyme substrates.

CHEMICAL NATURE OF AMINO ACIDS

Amino acids are carboxylic acids containing an amino group. In most of the amino acids, an amino group is attached to α-carbon atom next to the carboxyl group hence they are α-amino acids. The general formula is shown in Figure 2.1.



α - Carbon atom

Where ‘R’ is called as side chain and it represents variety of structures

COMMON AMINO ACIDS

Though more than 200 amino acids are identified in nature, only 20 amino acids serve as building blocks of body proteins. They are known as common amino acids. In addition to the common amino acids, derived amino acids are also found in proteins.

CLASSIFICATION OF AMINO ACIDS

Amino acids have been classified in various ways.

I. Based on side chain and ring structure present, amino acids are classified into 7 major classes.

1. Amino acids with aliphatic side chain. They are also called as *aliphatic amino acids.* They are glycine, alanine, valine, leucine and isoleucine . Valine, leucine and isoleucine are called as branched chain amino acids.

Aliphatic amino acids

2. Amino acids with side chain containing hydroxyl groups. They are also called as hydroxy amino acids. They are serine and threonine.
3. Amino acids with side chain containing sulfur atoms. They are also called as sulfur containing amino acids. They are cysteine, methionine and cystine.
4. Amino acids with side chain containing acidic groups or their amides. They are also called as *acidic amino acids*. They are aspartic acid, aspargine, glutamic acid and glutamine.
5. Amino acids with side chain containing basic groups. They are also called as *basic amino acids*. They are arginine, lysine, hydroxy lysine and histidine
6. Amino acids containing aromatic rings. They are also called as *aromatic amino acids*. They are phenylalanine, tyrosine and tryptophan.
7. Imino acids. They are proline and hydroxy proline.

II. Amino acids are also classified according to the reaction in solution or charge. They are categorized in 3 classes, acidic, basic and neutral amino acids. Acidic amino acids are aspartic acid, glutamic acid. Basic amino acids are arginine, lysine and histidine. Rest of the amino acids are neutral amino acids.

III. Another classification of amino acids is based on the number of amino and carboxyl groups present in the molecule.

Example. Mono-amino mono-carboxylic acid (Glycine), Mono-amino dicarboxylic acid (Glutamate).

IV. Amino acids are also classified according to their nutritional importance. Nutritionally amino acids are classified into

(*a*) *Essential amino acids:* These amino acids are not synthesized in the body and hence they have to be obtained from the diet. They are also referred as indispensable amino acids. They are methionine (M), arginine (A), tryptophan (T), threonine (T), valine (V), isoleucine (IL), leucine (L), phenyl alanine (P), histidine (H) and Lysine (L). Together they are remembered as (MATTVILLPHLY). Sometimes histidine and arginine are referred as semi-essential because body synthesizes these amino acids to some extent. Lack of essential amino acids in the diet gives rise to growth failure.

(*b*) *Non-essential amino acids*: These amino acids are synthesized in the body. They are alanine, glycine, serine, tyrosine, glutamate, glutamine, aspartate, aspargine, cysteine and proline. They need not be present in the diet.

Rare Amino Acids or Unusual Amino Acids

These are the amino acids that are not found in proteins but play important roles in metabolism.

Examples

1. Ornithine, citrulline and arginino succinic acid of urea cycle.
2. β-alanine is part of co-enzyme A
3. Taurine is part of bile acids.
4. γ-aminobutyric acid is a neurotransmitter.
5. Mono- and di-iodotyrosine are precursors of thyroxine.
6. Pantothenic acid is a water-soluble vitamin.
7. Homoserine is an intermediate of methionine catabolis
8. **Homocysteine.** It is also an intermediate of methionine catabolism. It is a atherothrombogenic agent. It triggers platelet adhesion. Hence, it is considered as a risk factor for development of coronary artery disease (CAD).
9. **S-allylcysteine sulfoxide.** It is an amino acid obtained from garlic. It has many therapeutic effects. It is commonly called as alli

PROPERTIES OF AMINO ACIDS

1. *Optical isomerism:* All the amino acids except glycine have at least one asymmetric carbon atom because of this they exhibit optical isomerism. Presence of single asymmetric carbon atom gives rise to two optical isomers. One isomer is the mirror image of the other isomer. If a carbon atom is linked to four different groups through covalent bonds then it is called as *asymmetric carbon*. The two mirror images of amino acid serine are L-serine and D-serine.Further, the optical isomers of amino acids are optically active. They are capable of rotating plane polarized light. Some amino acids rotate plane polarized to left and some rotate the plane polarized light to right. All the amino acids present in human proteins are L-isomers. D-isomers are usually absent but they are found in some peptide antibiotics.

Optical isomers of serine (*b*) Asymmetric carbon atom

2. *Acid-base or charge properties of amino acids:* Amino acids act as acids and bases. So they are called as *ampholytes* or *amphoteric substances*. Acids are those compounds that give protons on dissociation. Bases are those compounds that combine with protons. Bases are also called as alkalies. Proton concentration is quantitatively expressed as pH. It is defined as negative logerithem of proton or H+ or hydrogen ion concentration.

pH = – log [H+]

The pH scale extends from 1 to 14, which corresponds to hydrogen ion concentration of 1M to 1 × 10–14 M. The pH 7.0 represents neutrality pH values less than 7 represents acidity or acids and pH values above 7 refers to bases or alkalinity. Some common acids are hydrochloric acid (HCl), sulphuric acid (H_2SO4) and bases are sodium hydroxide (NaOH) and potassium hydroxide (KOH). Further acid is neutralized by base and vice versa.

Function of an amino acid as acid:

As base: R COO Addition of acid R COOH

So, amino acids have two ionizable groups (–COOH, NH_3^+). The –COOH is several times more easily dissociates than - NH_3^+).

At neutral pH both groups are ionized, i.e., the carboxyl group exist in dissociated form where as amino group exist as associated form.

This doubly charged molecule of amino acid containing positive and negative charges is called as zwitter ion. It is electrically neutral so it does not move in an electrical field.

The charge of an amino acid always depends on the pH of its surroundings. In other words, the charge of amino acid is altered by changing pH of its surroundings. This property is exploited for the separation of amino acids. In strong acidic conditions (pH < 2) the –COOH remains undissociated. When the pH is raised at pH of about 3 the proton from the –COOH is lost –COO– is generated. This is called pK of acid group because at this pH dissociated (–COO–) and undissociated (–COOH) species are found in equal amounts.

Similarly, if the pH is increased to 10, the amino group ($–NH_3^+$) dissociates to $–NH_2$ group. This pH is called the pK of amino group of amino acid because at this pH associated ($–NH_3^+$) and dissociated ($–NH_2$) species are present in equal amounts.

Therefore, an amino acid has two pK values corresponding to the two ionizable groups. pK values indicates strength of each group. Further an amino acid exist as zwitter ion at neutral pH and as cation at acidic pH and as anion at basic pH.

Example: For alanine, pKa is 2.4 and pKam is 9.7 (K is dissociation constant), the low pK value of –COOH indicates more ionizing power.

Isoelectric pH: It is the pH at which the net charge of an amino acid is zero or when the number of positive charges are equal to number of negative charges. At isoelectric pH amino acids have minimum solubility. The isoelectric pH of an amino acid having one amino group and one carboxyl group is equal to the arithamatic mean of pKa and pKam values.

For most amino acids pI is close to 6.0. The situation differs for amino acids having more than two ionizable groups. For example, glutamate is dicarboxylic acid so it can have three pK values (two for carboxyl groups and one for amino group). Similarly, the basic amino acid lysine can have three pK values (two for amino groups and one for carboxyl group). In these cases, a different formula is used to obtain isoelectric pH. For acidic amino acid like glutamate the isoelectric pH is equal to the half of sum of two pK values of acidic groups.

For basic amino acid like lysine the isoelectric pH is equal to the half of sum of two pK values of amino groups.

3. *Buffering action of amino acids:* Buffers are salts of weak acids and they resist change in pH when acid or alkali is added. Since amino acids are ampholytes they act as buffers. However, the buffering action of amino acids in the blood is insignificant because of their low concentration.

4. *Ultra violet light (UV) absorption of amino acids.* Amino acids do not absorb visible light. Aromatic amino acids absorb ultraviolet light. Tryptophan absorb ultra violet light at 280 nm. The ultra violet light absorption is also exhibited by proteins containing tryptophan. Hence, it is used for quantitative estimation of proteins and to study folding

of protein molecules. Phenylalanine and tyrosine also absorb light in ultra violet region.

PEPTIDES

1. Peptides consist of 2 or more amino acid residues linked by peptide bond.
2. A peptide bond is formed when carboxyl group of an amino acid react with α-amino group of another amino acid. Peptide bond formation between two amino acids is always accompanied by loss of one water molecule. Further, peptide and proteins contain an amino (N–) terminus and carboxy (C–) terminus.
3. A peptide or protein is named starting with N-terminal amino acid and usually the N-terminal is located on the left hand side.
4. Animal, plant and bacterial cells contain wide variety of low molecular weight peptides (2-10 amino acids residues) having profound biological functions.

DIPEPTIDES

A dipeptide consist of two amino acid residues and one peptide bond.

Carnosine and Anserine

Are two peptides present in muscle and brain.

Carnosine consist of β-alanine and histidine (β-alanyl histidine). Anserine consist of β-alanine and N-methyl histidine (β-alanyl N-methyl histidine). Short hand formula for carnosine is β-ala-His.

Function

Remains unknown.

Aspartame

It consist of aspartate and phenylalanine (Aspartyl phenylalanine, Asp-Phe). It is present in African berry.

Function

It is a sweetening agent.

Tripeptides

A tripeptide consist of three amino acid residues and two peptide bonds.

Glutathione

Structure

It consists of glutamate. Cysteine and glycine. In glutathione, γ-carboxyl group of glutamate is involved in peptide linkage with cysteine hence it is named as γ-glutamyl cysteinyl glycine (Glu-Cys-Gly, G-SH,).

Functions

1. It acts as reducing agent in all cells. It assumes dimeric form on oxidation.
It is responsible for the maintenance of –SH groups of proteins in reduced form
2. It participates in the removal of H_2O_2 in erythrocytes.
3. It is required for removal of toxins from body.
4. It is involved in release of hormones.

5. It protects body proteins from radiation effects.
6. It is involved in cellular resistance to anticancer agents.
7. Glutathione regulates telomerase activity and of the cell cycle.
8. Glutathione is involved in modulation of apoptosis.

Thyrotropin Releasing Harmone (TRH)

Structure

It consists of glutamate, histidine and proline. It is an unusual tripeptide with blocked N and C terminals.

Function

It is a hormone secreted by hypothalamus.

Chemotactic Peptide

Structure

It consists of N-Formyl methionine, leucine and phenylalanine (f met-leu-phe). Its N-terminal contains formyl (–CHO) group.

Function

It is present in leukocytes. It plays an important role in chemotaxis.

Penta Peptides

They consist of five amino acids and four peptide bonds.

Enkaphalin

Structure

It consist of tyrosine, glycine, glycine, phenylalanine and methionine (Tyr-gly-gly-phe-met).

Function

It is present in brain. It binds to opiate receptors present in brain. So, it is body own opiate or analgesic. Enkaphalins containing six amino acid residues (hexa peptide), seven amino acid residues (hepta peptide) and eight amino acid residues (octa peptide) are also found in brain.

Other noteworthy peptides are

Angiotensin II. It is an octa peptide, found in lungs and other cells. It is a powerful vaso constrictor and raises blood pressure.

Bradykinin. It consist of nine amino acid residues (Nona peptide). It is a powerful vasodilator and anti inflammatory.

Oxytocin I. It is also a nona peptide. It stimulates uterus contraction.

Vasopressin. A nona peptide produced by pituitary gland. It has a disulfide bridge. It is also known as antidiuretic harmone (ADH).

Angiotensin I and Kallidin are examples for decapeptides containing ten amino acid residues.

CYCLIC PEPTIDES

1. They differ from normal peptides.

2. In these peptides N-terminus and C-teminus are linked by peptide bond resulting in cyclization of peptide.
3. An antibiotic gramicidin-S is a cyclic peptide. It consist of ten amino acids. So gramicidin-S is a cyclic decapeptide. Further it contains D-Phenyl alanine which is usually absent in life forms.
4. Tyrocidin is another cyclic decapeptide.

TOXIC PEPTIDES

1. Some peptides act as toxins.
2. α-amanitin is a bicyclic octapeptide present in a particular variety of mushrooms. It is extremely toxic to humans.
3. It is responsible for mushroom poisoning cases around the world.
4. When the mushrooms are consumed it causes pain in the gastrointestinal tract, vomitting, diarrhoea and nausae.
5. Death occurs within a week due of impairment of liver and kidney functions.

CYCLOTIDES (CYCLIC PEPTIDES)

In some peptides disulfide bonds are more. These disulfide bonds create knot with in the molecule. Two disulfide bonds and their connecting back bone segment form ring. They are known as cyclotides. These cyclic peptides show diverse actions. Some are anti HIV, anti bacterial and insecticidal agents. Some examples are given below:

1. ***Sunflower trypsin inhibitor (SFTI).*** It is smallest circular peptide with just 14 amino acids. It is an enzyme inhibitor.
2. ***RTD-1.*** It is a circular peptide present in leucocytes. It is a defensin. It consists of only 18 amino acids.
3. ***Microsin.*** It is 21 residue cyclic peptide. It is produced by E. coli. It is an antibiotic.

EXERCISES

ESSAY QUESTIONS

1. Classify amino acids. Give examples for each class.
2. Name five biologically important peptides. Write one function for each of them.
3. Write an essay on properties of amino acids.

SHORT QUESTIONS

1. Define amino acid and isoelectric pH. Write two properties of an amino acid at isoelectric pH.
2. Write composition of glutathione. How it differs from other peptides? Write two of its functions.
3. Explain acid-base properties of amino acids.
4. Define essential amino acids. Give examples.
5. Write structures of tyrosine, methionine and valine.
6. What are unusual amino acids? Give examples.
7. Define cyclic peptide. How it differs from other peptides? Write 2 examples with functions.
8. Write a note on semi essential amino acids.

9. Calculate isoelectric point of glutamic acid. How it differs from the isoelectric point of glycine?
10. What are the functions of amino acids?
11. Draw structure of peptide. Label its various parts.

Amino Acids and Peptides 25

MULTIPLE CHOICE QUESTIONS

1. Most of the amino acids found in human body are
(*a*) L-isomers (*b*) D-isomers
(*c*) D and L-isomers (*d*) Optical isomers
2. Which of the following amino acids has more pK values.
(*a*) Glycine (*b*) Alanine
(*c*) Glutamate (*d*) Glutamine
3. The isoelectric pH of lysine is equal to
(*a*) Arithamatic mean of amino groups pK values.
(*b*) Half of sum of amino group and carboxyl group pK values.
(*c*) Arithamatic mean of amino groups and carboxyl groups pK values.
(*d*) None of the above.
4. An example for unusual amino acid is
(*a*) Aspargine (*b*) Taurine
(*c*) Cystine (*d*) Anserine
5. All of the following statements are correct regarding peptide except
(*a*) It contains amino terminus (*b*) It contains carboxy terminus
(*c*) It contains peptide bonds (*d*) It contains only basic amino acids

FILL IN THE BLANKS

1. ----------absorbs light in ultraviolet region.
2. ----------is a dipeptide having sweet taste.
3. In a cyclic peptide N-terminus and C-terminus are linked by ---------- bond.
4. An unusual amino acid that function as neurotransmitter is ----------

PROTEIN

OCCURRENCE

Proteins are present in every cell of humans, animals, plant tissues, tissue fluids and in micro organisms. They account for about 50% of the dry weight of a cell. The term protein is derived from the Greek word *proteios* meaning holding first place or rank in living matter.

MEDICAL AND BIOLOGICAL IMPORTANCE

Proteins perform wide range of essential functions in mammalians.
1. Proteins are involved in the transport of substances in the body.
Example: Haemoglobin transports oxygen.
2. Enzymes which catalyze chemical reactions in the body are proteins.
3. Proteins are involved in defence function. They act against bacterial or viral infection.
Example: Immunoglobulins.

4. Hormones are proteins. They control many biochemical events.
Example: Insulin.
5. Some proteins have role in contraction of muscles.
Example: Muscle proteins.
6. Proteins are involved in the gene expression. They control gene expression and translation.
Example: Histones.
7. Proteins serve as nutrients Proteins are also involved in storage function.
Examples: Casein of milk, Ferritin that stores iron.
8. Proteins act as buffers.
Example: Plasma proteins.
9. Proteins function as anti-vitamins.
Example: Avidin of egg.
10. Proteins are infective agents.
Example: Prions which cause mad cow disease are proteins.
11. Some toxins are proteins.
Example: Enterotoxin of cholera microorganism.
12. Some proteins provide structural strength and elasticity to the organs and vascular system.
Example: Collagen and elastin of bone matrix and ligaments.
13. Some proteins are components of structures of tissues.
Example: α-keratin is present in hair and epidermis.
In order to understand how these substances though they are all proteins play such diverse functions their structures, and composition must be explored.

CHEMICAL NATURE OF PROTEINS

All proteins are polymers of aminoacids. The aminoacids in proteins are united through "Peptide" linkage. Sometimes proteins are also called as polypeptides because they contain many peptide bonds.

PROPERTIES OF PROTEINS

1. Proteins have high molecular weight, *e.g.*, the lactalbumin of milk molecular weight is 17000 and pyruvate dehydrogenase molecular weight is 7 × 106.
2. Proteins are colloidal in nature.
3. Proteins have large particle size.
4. Different kinds of proteins are soluble in different solvents.
5. Proteins differ in their shape.
6. Some proteins yield amino acids only on hydrolysis where as others produce amino acids plus other types of molecules.
7. **Charge properties:** Charge of a protein depends on the surroundings like amino acids. So, by changing the pH of surroundings the charge of protein can be altered. This property is used for separation of proteins.

Isoelectric point: Proteins have characteristic isoelectric points. At the isoelectric

point its net charge is zero because the number of positive charges are equal to number of negative charges. So proteins are insoluble or have minimum solubility at isoelectric point. This property is used for the isolation of casein from milk. The isoelectric point for casein is 4.6. If the pH of the surrounding is raised above the isoelectric point, the protein is negatively charged *i.e.*, it exists as anion. Likewise, if the pH of the surrounding is lowered, the protein is positively charged *i.e.*, it exist as cation. Further, proteins do not move in an electrical field at isoelectric point like amino acids. However, if the pH of the medium is raised or lowered protein moves towards anode or cathode respectively. This property is exploited for the separation of proteins.

8. **Proteins act as buffers:** Since proteins are amphoteric substances, they act as buffers. Hemoglobin (Hb) of erythrocytes and plasma proteins are important buffers. Hb accounts for 60% of buffering action within erythrocytes and plasma proteins contributes to 20% of buffering action of blood

CLASSIFICATION OF PROTEINS

There is no single universally satisfactory system of protein classification so far.

1. One system classifies proteins according to their composition or structure.
2. One system classifies them according to solubility.
3. One system classifies them according to their shape.
4. Classification of proteins based on their function also found in literature.

Classification of proteins based on their composition

Proteins are divided into three major classes according to their structure.

1. **Simple proteins:** Simple proteins are made up of amino acids only. On hydrolysis, they yield only amino acids.

Examples: Human plasma albumin, Trypsin, Chymotrypsin, pepsin, insulin, soyabean trypsin inhibitor and ribonuclease.

2. **Conjugated proteins:** They are proteins containing non-protein part attached to the protein part. The non-protein part is linked to protein through covalent bond, non-covalent bond and hydrophobic interaction. The non-protein part is loosely called as prosthetic group. On hydrolysis, these proteins yield non-protein compounds and amino acids.

Conjugated protein → Protein + Prosthetic group

The conjugated proteins are further classified into subclasses based on prosthetic groups.

Different classes of conjugated proteins

Subclass Prosthetic group Examples Type of linkage

	SUBCLASS	**PR0STHETIC GROUP**	**EXAMPLES**	**TYPES OF LINKAGE**
1	**Lipoproteins**	**Lipids**	**Various classes of lipoproteins. Lipovitellin of eggs**	**Hydrophobic interaction**

2	**Glycoproteins**	**Carbohydrates**	**Immunoglobulin of blood, Egg albumin**	**covalent**
3	**Phosphoproteins**	**Phosphorus**	**Caesin of milk,Vitellin of egg yolk**	**Colvalent**
4	**Nucleoproteins**	**Nucleic acids**	**Chromatins, Ribosomes**	**Non covalent**
5	**Haemoproteins/Chromoproteins**	**Haem**	**Haemoglobin, myoglobin, chytochromes**	**Non covalent**
6	**Flavoproteins**	**Flavin nucleotides, FMN, FAD**	Succinate dehydrogenase	**Covalent**
7	**Metaloproteins**	**Iron**	**Ferritin ,chytochrome**	**Non covalent**
8	**Visualproteins**	**Retinal**	**Rhodopsin**	**Colvent**

3. **Derived proteins:** As the name implies this class of proteins are formed from simple and conjugated proteins. There are two classes of derived proteins.
(*i*) *Primary derived proteins:* They are formed from natural proteins by the action of heat or alcohol etc. The peptide bonds are not hydrolysed. They are synonymous with denatured proteins.
Example: Coagulated proteins like cooked-egg albumin.
(*ii*) *Secondary derived proteins:* They are formed from partial hydrolysis of proteins.
Examples: Proteoses, peptone, gelatin, and peptides.

Protein classification according to their solubility

1. **Albumins:** Soluble in water and salt solutions.
Examples: Albumin of plasma, egg albumin and lactalbumin of milk.
2. **Globulins:** Sparingly soluble in water but soluble in salt solutions.
Examples: Globulins of plasma, ovoglobulins of egg, lactoglobulin of milk.
3. **Glutelins:** Soluble in dilute acids and alkalies.
Examples: Glutenin of wheat, oryzenin of rice, zein of maize.
4. **Protamins:** Soluble in ammonia and water.
Examples: Salmine from salmon fish, sturine of sturgeon.
5. **Histones:** Soluble in water and dilute acids.
Example: Histones present in chromatin.
6. **Prolamines:** Soluble in dilute alcohol and insoluble in water and alcohol.
Examples: Gliadin of wheat, zein of corn.
7. **Sclero proteins:** Insoluble in water and dilute acids and alkalies.

Examples: Collagen, elastin and keratin.

Classification of proteins based on shape

Proteins are divided into two classes based on their shape.

1. **Globular proteins:** Polypeptide chain(s) of these proteins are folded into compact globular (Spherical) shape.

Examples: Haemoglobin, myoglobin, albumin, lysozyme, chymotrypsin.

2. **Fibrous proteins:** Poly peptide chains are extended along one axis.

Examples: α-keratin, β-keratin, collagen and elastin.

PROTEIN STRUCTURE

Since proteins are built from amino acids by linking them in linear fashion, it may be viewed as proteins having long chain like structures. However, such arrangement is unstable and polypeptide or protein folds to specific shape known as *conformation,* which is more stable. Various stages involved in the formation of final conformation from linear chain are divided into four levels or orders of protein structure. They are

1. Primary Structure

The linear sequence of amino acid residues in a polypeptide chain is called as primary structure. Generally disulfide bonds if any are also included in the primary structure. *Bonds responsible for the maintenance of primary structure* are mainly peptide bonds and *disulfide* bonds. Both of them are covalent bonds .

Primary Structure of Insulin

This protein consist of two polypeptide chains A and B. The two chains are covalently linked by disulfide bonds. The A chain has N-terminal glycine and C-terminal aspargine. The B chain has phenylalanine and alanine as N-and C-terminal residues, respectively. Insulin is a hormone and its molecular weight is 5,700.

2. Secondary Structure

Folding of polypeptide chain along its long axis is called as secondary structure of protein. Folding of polypeptide chain can be *ordered, disordered* or *random.* Secondary structure is often referred as *conformation.* So, proteins has *ordered secondary structure* or *conformation* and *random* or *disordered secondary structure* or *conformation.*

Ordered Conformation of Polypeptides

The polypeptide chain of some proteins may exist in highly ordered conformation. The conformation is maintained by *hydrogen bonds* formed between peptide residues.

Hydrogen bond

It is a weak ionic interaction between positively charged hydrogen atom and negatively charged atoms like oxygen, nitrogen, sulfur etc. It is indicated with broken lines (---).

There are two types of ordered secondary structure observed in proteins.

1. The polypeptide chain of α-keratin, which is present in hair, nails, epidermis of the skin is arranged as α-*Helix*. α-letter is given to this type of structure because it was first ordered structure noticed in proteins.

2. Polypeptide chain of β-keratin, which is present in silk fibroin and spider web is arranged

in β-*pleated* sheet. The β-letter is given because it was observed later.

Main Features of α-Helix

1. In α-helix polypeptide, backbone is tightly wound round (coiled) long axis of the molecule.
2. The distance between two amino acid residues is 1.5 Å.
3. α-helix contain 3.6 amino acid residues per turn. The R-group of amino acids project outwards of the helix.
4. The pitch of the α-helix is 5.4 Å long and width is 5.0 Å .
5. The α-helix is stabilized by intra chain hydrogen bonds formed between –N–H groups and –C=O groups that are four residues back, *i.e.,* –N–H group of a 6th peptide bond is hydrogen bonded to –C=O group of 2nd peptide bond .
6. Each peptide bond participates in the hydrogen bonding. This gives maximum stability to α-helix.
7. α-helix present in most fibrous proteins is right handed. The right handed α-helix is more stable than the left handed helix.
8. α-helix is hydrophobic in nature because of intra chain hydrogen bonds.
9. An α-helix forms spontaneously since it is the most stable conformation of polypeptide chain.
10. Some amino acids act as terminators for α-helix.

Example: Proline.

11. Aromatic amino acids stabilizes α-helix.
12. Charged and hydrophobic amino acids destabilize α-helix.
13. Content of α-helix varies from protein to protein.

β-Pleated Sheet Features

1. In β-pleated sheet, the polypeptide chain is fully extended.
2. In β-pleated sheet, polypeptide chains line up side by side to form sheet . The side chains are above or below the plane of the sheet.
3. From 2 to 5, adjacent strands of polypeptides may combine and form these structure.
4. When the adjacent polypeptide chains run in same direction (N to C terminus) the structure is termed as parallel β-pleated sheet.
5. When the adjacent polypeptide chains run in opposite direction the structure is termed as anti-parallel β-pleated sheet.
6. The β-pleated sheet is stabilized by inter chain hydrogen bonds .
7. β-keratin contains anti parallel β-pleated sheet.
8. Both parallel and anti-parallel β-pleated sheet occur in other proteins. Amyloid protein present in Alzheimer's disease has anti parallel β-pleated sheet. It accumulates in the CNS.

Random Coil (Disordered) Conformation

Regions of proteins that are not organized as helices and pleated sheet are said to be present in random coil conformation. These are also equally important for biological function of proteins as those of helices and β-pleated sheet.

β-turn or β-bends (Reverse Turn)

Hair pin turn of a polypeptide chain is called as β-turn. The change in the direction of a polypeptide chain is achieved by β-turn. β-turn connects anti parallel β-sheets. Usually four aminoacids make up β-turn. Gly, Ser, Asp, proline are involved in β-turns.

Super Secondary Structure

In some globular proteins regions of α-helix and β-pleated sheet join to form super secondary structure or motifs. They are very important for biological function.

Super Helix

α-keratin consist of right handed α-helix as basic unit. Three such α-helices get cross linked by disulfide bonds and form super secondary structure.

Triple Helix

Collagen present in skin, cartilage, bone and tendons consists of left handed helix as basic unit. Three left handed helices are wrapped around each other to right handed super secondary structure triple helix.

3. Tertiary Structure

Three-dimensional folding of polypeptide chain is called as tertiary structure. It consists of regions of α-helices, β-pleated sheet, β-turns, motifs and random coil conformations. Interrelationships between these structures are also a part of tertiary structure.

Tertiary structure of a protein is mainly stabilized by non-covalent bonds. Non-covalent bonds present in tertiary structure

(*a*) Hydrophobic interaction (*b*) Electrostatic bonds

(*c*) Internal hydrogen bonds (*d*) vander waal's interactions

A. Hydrophobic interactions

The non-polar side chains of neutral amino acids tend to associate in proteins. These are called as hydrophobic interactions. They play significant role in maintaining tertiary structure.

B. Electrostatic bonds

These bonds are formed between oppositely charged groups of amino acid side chains. The ε-amino groups of lysine is positively charged and second (non-α-) carboxyl group of aspartic acid is negatively charged at physiological or body pH. These interact electrostatically to stabilize tertiary structure of protein. They are also called as salt bridges.

C. Internal hydrogen bonds

Amino acid side chains are involved in the hydrogen bond formation. Hydroxyl group of serine, threonine, the amino groups and carbonyl oxygen of glutamine and aspargine, the ring nitrogen of histidine participates in internal hydrogen bond formation.

D. Vander waals interactions

These are the weak interactions between uncharged groups of protein molecule. They also contribute to the stability of proteins.

4. Quaternary Structure

Proteins containing two or more polypeptide chains possess quaternary structure. These proteins are called as *oligomers*. The individual polypeptide chains are called as protomer, *monomers* or *subunits*. The protomers are united by forces other than covalent bonds.

Occasionally, they may be joined by disulfide bonds.
The most common oligomeric proteins contain 2 or 4 protomers and are termed dimers and tetramers.

Forces that stabilize these aggregates (assembles of monomers) are:

1. Hydrogen bonding
2. Electrostatic interactions
3. Hydrophobic interactions
4. Vander waals interactions
5. Disulfide bridges (in some proteins)

Examples: 1. Haemoglobin consist of 4 polypeptide chains.
2. Hexokinase contains 2 subunits.
3. Pryuvate dehydrogenase contains 72 subunits.

Determination of Protein Structure

The primary structure of protein directs specific folding (secondary structure) and its tertiary structure. If there is a change in one of the amino acids of protein, then conformation of polypeptide chain alters, which results change in biological function. Further, the sequence of amino acids in proteins that gives them their striking specific biological actions. Therefore knowledge of primary structure of a protein is required for the understanding of relationship of a protein's structure to its function at molecular level.

Determination of Primary Structure of Protein

1. Sanger's reagent

Sanger used FDNB (1-Fluoro-2, 4-Dinitrobenzene) to determine the amino acid sequence of a polypeptide chain from N-terminus. Sanger's reagent can be used to determine only one amino acid at a time because FDNB reacts with other amino acids. FDNB arylates free amino acid group and produces intense yellow 2, 4-dinitrophenyl residues of amino acids. These derivatives are separated by chromatography and identified

2. Edman's reagent

Edman used phenylisothiocyanate (Edman's reagent) for the determination of amino acid sequence of a protein from the N-terminus. Edmans reagent not only identifies N-terminus but also when used repeatedly provides complete sequence of the polypeptide chain. In Edman's reaction, the polypeptide chain is shortened by only one residue and rest of the polypeptide remains intact. The reaction is repeated and second residue is determined. By continued repetition, complete sequence of protein is determined starting from N-terminus

Edman's reaction for sequence determination of protein from N-terminus
Edman's reagent react with amino group and produces phenylthiocarbamyl derivatives on treatment with acid. Phenylthiocarbamyl derivative cyclizes to phenylthiohydantoins. They are estimated using chromatography.

Protein Folding

Let us examine how polypeptide chain attains native conformation as soon as it comes out

of protein synthesizing machinery. Though exact mechanisms involved in protein folding are not known due to extensive investigations carried out some information on protein folding mechanisms is available.

Stages of Protein Folding

Protein folding occurs by stages:

(a) Domains formation

α-helical, β-pleated sheet, β-bend containing domains are formed in the initial step of folding of polypeptide chain. This self assembling process mostly depends on primary structure. It involves extensive interaction among amino acids residues side chains of polypeptide chain. It is governed by thermodynamic principles like free energy etc.

(b) Molten globule

In the next step domains from molten globule state in which secondary structure predominates and tertiary structure is highly disordered.

(c) Native conformation

Finally native conformation develops from molten globule state after several minor conformational changes and rearrangements.

(d) Oligomer formation

In the case of multimeric or oligomeric proteins after attaining specific conformation protomers or sub-units may assemble into native like structure initially. After some realignments it ultimately gives rise to final conformation of oligomer.

Additional Protein Folding Factors

Though self association of polypeptide chain into ordered conformation is largely determined by amino acid sequence (primary structure) recent research has shown that in some cases folding of protein requires additional factors. Some of them are enzymes and some are protein factors.

Protein Folding Enzymes

Two protein folding enzymes are known:

(a) Disulfide isomerase

In the newly formed protein molecules –SH groups of cysteine residues may form several intra or inter disulfide linkages. However, only few disulfide linkages may be essential for proper protein folding. The disulfide isomerase favours formation of such disulfide linkages by breaking unwanted linkages formed.

(b) Cis-trans prolyl isomerase

It aids folding process by catalyzing inter conversion of *cis-trans* peptide bonds of proline residues of folding protein.

Protein Factors

Chaperons (Chaperonins)

These proteins aid protein folding process by preventing formation of aggregates. Usually aggregate formation slows down protein folding process. Chaperons accelerate protein folding by blocking protein folding pathways of unproductive nature. They bind to hydrophobic parts of protein molecules and prevent formation of aggregates. They are also involved

in protein refolding that occurs when proteins cross membrane structures.

Denaturation of Proteins

Denaturation is loss of native conformation. On denaturation, physical chemical and biological properties of a protein are altered.

Some of the changes in properties are:

1. Decreased solubility
2. Unfolding of polypeptide chain
3. Loss of helical structure
4. Decreased or loss of biological activity
5. More susceptible to action of enzymes
6. Increased chemical reactivity
7. Dissociation of subunits in case of oligomeric proteins.

Causes of Denaturation

1. High temperature
2. Extreme alkaline or acidic pH
3. Use of urea and guanidine at high concentration
4. UV radiation
5. Sonication
6. Vigorous shaking
7. Detergent like sodium dodecylsulfate also denatures protein
8. Treatment with organic solvents like ethanol, acetone etc.
9. Treatment with strong acids like trichloro acetic acid, picric acid and tungstic acid
10. Exposure to heavy metals like Pb^{2+}, Ag^{2+} and Cu^{2+}

Biomedical Importance

1. These properties are exploited for the separation of serum proteins from the other compounds of clinical importanc
2. Denaturation knowledge is required when activities of enzymes in biological fluids like blood are measured for diagnosis.
3. Purification of protein from mixture of proteins also needs denaturation properties.
4. Lead poisoning cases are treated with egg white to decrease toxicity of lead in the body.

Many cases of the process of denaturation is irreversible.

Examples of Denaturation

1. When egg white is exposed to high temperature coagulum is formed because heat denatures egg albumin. The solubility of denatured protein is decreased.
2. Formation of coagulum when albumin is exposed to high temperature.
3. Heat treatment of trypsin results in loss of biological activity.
4. Monellin is a dimeric protein has sweet taste. On denaturation the sweet taste is lost.

Renaturation

Though denaturation is irreversible in majority of the cases, in few cases, renaturation is observed.

Example: Ribonuclease denatures on exposure to heat but come back to its native conformation when temperature is lowered.