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Semester II

Applied BIOCHEMISTRY for BSc NURSING

As Per Revised INC Syllabus

3rd Edition

Manjula Shantaram

Forewords
BM Hegde
Susan Anand

JAYPEE

Contents

CHAPTER

5 Composition and Metabolism of Amino Acids and Proteins

LEARNING OBJECTIVES

At the end of the chapter, the learner will be able to:

- Discuss the structure and function of amino acids.
- Explain the digestion and absorption of proteins.
- Describe amino acid metabolism.
- Discuss about protein synthesis.
- Describe about chromatography and electrophoresis.
- Discuss about chlorophyll, enzymes, and nitrogen fixation.

CHAPTER OUTLINE

- Chemistry of amino acids and proteins
  - General structure
  - Classification
  - Properties
- Digestion and absorption of proteins
  - Stages of protein digestion
  - Absorption
- Amino acid metabolism
  - Nitrogen balance
  - Catabolism of amino acids
  - Urea cycle
  - Disorders of urea cycle
  - Glycine metabolism
  - Tryptophan metabolism
  - Estimation of metabolism
- Protein synthesis
  - Translation
  - Inhibition of protein synthesis
- Techniques
  - Chromatography
  - Electrophoresis
- Protein sequencing
- Nitrogen fixation
- Chlorophyll
- Enzymes

5.1: CHEMISTRY OF AMINO ACIDS AND PROTEINS

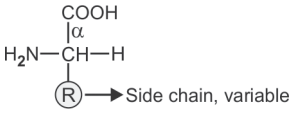
AMINO ACIDS

Amino acids are the basic structural units of proteins. Some exist in free form in human blood. More than 300 amino acids are found in nature. Only 20 amino acids are present in proteins and these 20 amino acids are called the standard amino acids. 21st amino acid is reported recently which is called selenocysteine.

Amino acids are group of organic compounds with two functional groups.

1. Amino - NH_2 (basic)
2. Carboxyl - COOH (acidic)

General Structure



L-α-amino acid

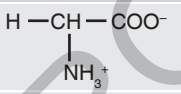
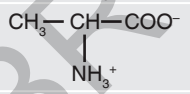
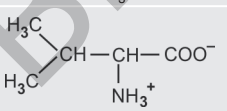
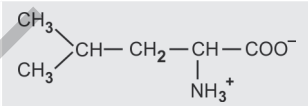
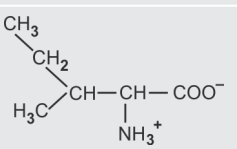
Proteins have only L-α-amino acids in which NH_2 and COOH are attached to the same carbon atom.

Classification of Amino Acids

I. Based on Structure (of the side chain or R - group)

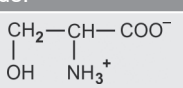
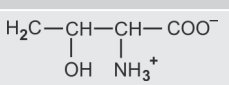
1. **Aliphatic amino acids** are made up of only C or H side chain **(Table 5.1.1)**.
   Examples: Glycine—simplest amino acid

Table 5.1.1: Types of aliphatic amino acids.

	Symbol	*Structure*	*Special group*
Glycine	Gly		
Alanine	Ala		
Valine	Val		Branched chain
Leucine			Branched chain
Isoleucine			Branched chain

2. **Hydroxy amino acids (Table 5.1.2)**
   Examples: Serine, threonine

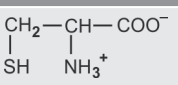
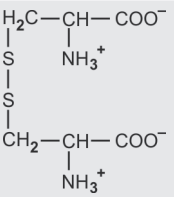
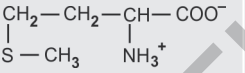
Table 5.1.2: Types of hydroxy amino acids.

Serine	Ser		Hydroxyl
	Symbol	***Structure***	***Special group***
Threonine	Thr		Hydroxyl

3. Sulfur-containing amino acids (Table 5.1.3)

Examples: Cysteine, methionine, cystine

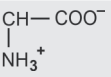
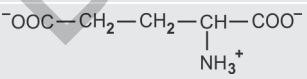
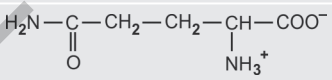
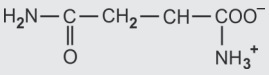
Table 5.1.3: Types of sulfur containing amino acids.

Cysteine	Cys		Sulfydryl
Cystine			Disulfide –S-S-
Methionine	Met		Thioether

4. Acidic amino acids (Table 5.1.4)

Examples: Aspartic acid, glutamic acid and their derivatives.

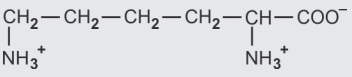
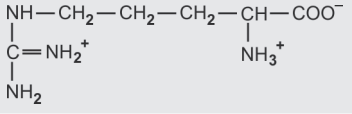
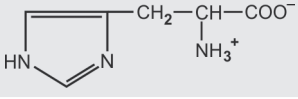
Table 5.1.4: Types of acidic amino acids.

Aspartic acid	Asp		β-carboxyl
Glutamic acid	Glu		γ-carboxyl
Glutamine	Gln		Amide
Asparagine	Asn		Amide

5. Basic amino acids (Table 5.1.5)

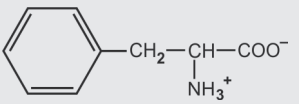
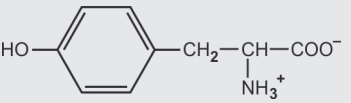
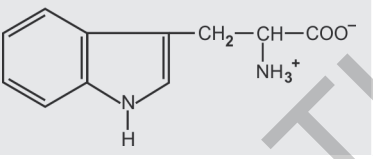
Examples: Arginine, histidine, lysine ε δ γ β α

Table 5.1.5: Types of basic amino acids.

Lysine	Lys		ε-amino
Arginine	Arg		Guanidine
Histidine	His		Imidazole

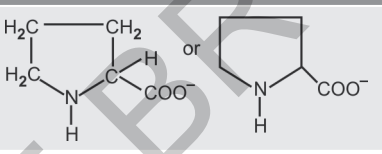
6. Aromatic amino acids (Table 5.1.6)
Examples: Phenylalanine, tyrosine, tryptophan

Table 5.1.6: Types of aromatic amino acids.

	Symbol	*Structure*	*Special group*
Phenylalanine	Phe		Benzene or phenyl
Tyrosine	Tyr		Phenol
Tryptophan	Trp		Indole

7. Imino acid (Table 5.1.7)
Proline (it has imino group)

Table 5.1.7: Types of imino acids.

Proline	Pro		Pyrrolidine

II. Based on Number of –NH_2 and –COOH Group Present

- Monoamino monocarboxylic acid
  Only one NH_2 and one COOH
  Examples: Glycine
- Monoamino dicarboxylic acids
  Examples: Glutamic acid
- Diamino monocarboxylic acids
  Examples: Lysine

III. Based on Net Charge at Neutral pH (7.0)

- Acidic amino acids are negatively charged
  Examples: Aspartic acid, glutamic acid
- Basic amino acids are positively charged
  Examples: Arginine, histidine, lysine
- Neutral amino acids have no net charge
  Examples: Glycine, alanine, valine, leucine (all other 15)

IV. Based on Polarity

- Polar amino acids have hydrophilic (water loving) side chain.
  – Nonionic (uncharged): They have OH group.

Examples: Serine, threonine, cysteine, tyrosine, glutamine, asparagine.
- Ionic (charged): Highly hydrophilic
  - Acidic: *Examples*—aspartic acid, glutamic acid
  - Basic: *Examples*—arginine, histidine, lysine

- Nonpolar amino acids have hydrophobic side chains.
  Examples: Glycine, alanine, valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, proline.

V. Based on Nutritional Requirement

- *Essential amino acids (indispensable):* These are not synthesized in our body and must be provided in the diet. Lack of these amino acids in diet, leads to growth failure. *Examples:* Leucine, isoleucine, threonine, lysine, methionine, phenylalanine, tryptophan and valine.
  Semiessential amino acids: These are synthesized in the body to some extent. They are essential for children. *Examples:* Arginine, histidine.
- *Nonessential amino acids (dispensable):* These are synthesized in our body, so need not be present in the diet. *Examples:* Glycine, serine, tyrosine, glutamic acid, glutamine, aspartic acid, asparagine, cysteine, proline, alanine.

VI. Based on Metabolic Fate

- *Glucogenic amino acids:* Carbon skeleton of amino acid is converted to glucose or intermediate of TCA cycle. Nonessential amino acids are glucogenic. *Examples:* Glycine, alanine, aspartic acid, proline.
- *Ketogenic amino acids:* Carbon skeleton of amino acid converted to ketone body or intermediates of fatty acid metabolism. Only one amino acid is purely ketogenic. *Examples:* Leucine.
- *Glucogenic and ketogenic amino acids:* Carbon skeleton of amino acids are partly glucogenic and partly ketogenic. *Examples:* Phenylalanine, tyrosine, tryptophan, isoleucine, lysine. These amino acids are precursors for the synthesis of glucose as well as fat.

Nonstandard Amino Acids

- *Derived amino acids:* α-amino acids are modified after the protein is synthesized. Such amino acids are present in some proteins.
  Examples:
  1. 4-hydroxyproline and 5-hydroxylysine are found in collagen.
  2. γ-carboxyglutamate is found in prothrombin.
  3. N-methyl lysine is found in myosin.
- *Nonprotein amino acids:* Some amino acids are not present in proteins, but occur freely in body. They play important role in metabolism.
  - α-amino acids: Ornithine, citrulline, arginino succinic acid are the intermediates in biosynthesis of urea.
  - Homocysteine and homoserine are the intermediates in methionine metabolism.
  - Non α-amino acids:

Examples:

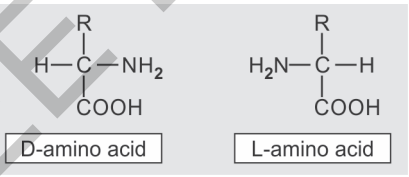
1. β-alanine is a part of coenzyme A
2. γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter
3. γ-aminolevulinic acid is an intermediate in heme synthesis.
4. Taurine is a part of bile acids.

Properties of Amino Acids

Physical Properties

- *Solubility:* All amino acids are soluble in H_2O, alcohol and insoluble in organic solvents (e.g., benzene).
- *Melting point:* Amino acids generally melt at higher temperatures often above 200°C.
- *Taste:* Amino acids may be sweet (Gly, Ala, Val), tasteless (Leu) or bitter (Arg, Ile). Sodium glutamate is a salt of glutamic acid and is used as a flavoring agent in food industry to increase taste and flavor (mono sodium glutamate—Azinamoto).
- *Optical activity:* All standard amino acids except glycine are optically active, i.e., they can rotate plane of polarized light to the right or to the left. They possess asymmetric α carbon atom, i.e., four different groups are bonded to it.

  One asymmetric carbon atom can exhibit two optical isomers. D-amino acid is the mirror image of L-amino acid. Certain D-amino acids are found in antibiotics and also in bacterial cell walls. *Examples:* Gramicidin contains D-amino acid. L-amino acids are found in proteins.



  Isoleucine and threonine have two asymmetric carbons and hence exhibit 4 isomers.
- *Charge (acid–base) properties:* Amino acids are ionized in aqueous solutions. They are amphoteric in nature and can act as acids or bases. Therefore, they contain ionizable groups - COOH, NH_2. Charge of amino acid carried depends on pH of its surrounding medium.
  - *At neutral pH (7.0):* Carboxy group of amino acid exists as COO^- and amino group as NH_3^+, i.e., both groups are ionized to form zwitterion. It is an amino acid molecule with equal number of positive and negative charges.

    It is electrically neutral (net charge is zero) does not move in electrical field. It is the predominant form at pH 7.0.
  - *Isoelectric pH (P^I):* pH at which net charge of an amino acid is zero, i.e., number of positive charges = number of negative charges. At P^I amino

acids exist as zwitterions. So, they do not move in electric field. They have minimum solubility (i.e., maximum tendency to precipitate).

		P^I
Examples:	Glycine	6.0
	Histidine	7.6
	Lysine	9.7

- *At acidic pH (<2):* COOH remains undissociated - amino acids exist in cationic form (positively charged).
- *At alkaline pH (>10):* NH_3^+ dissociates to $-NH_2$ groups. Amino acids exist in anionic form (negatively charged).

Different Forms of Amino Acids (Fig. 5.1.1)

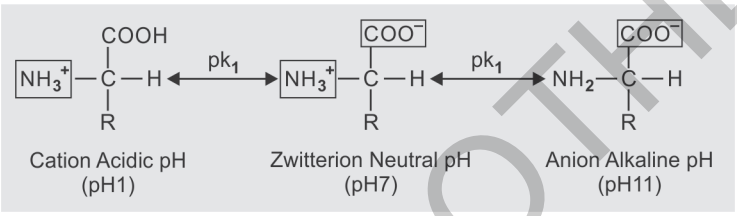


Fig. 5.1.1: Different forms of amino acids.

pk_1 (–COOH) pH at which 50% amino acid molecules are in cationic form and 50% in zwitterion form.

pk_2 ($-NH_3^+$) pH at which 50% amino acid molecules are anionic form and 50% in zwitterion form.

$$P^I = \frac{pk_1 + pk_2}{2}$$

Buffering action is maximum at and around pk_1 or pk_2.

Histidine has extra pk_3 (due to imidazole group) = 6.1 is an effective buffer at pH 7.4. It plays an unique role in enzymatic catalysis.

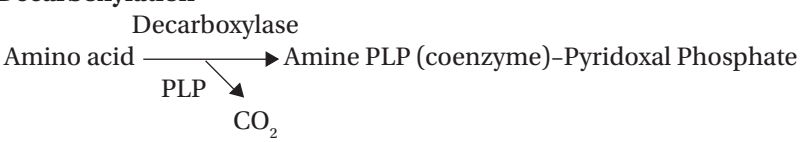
Amino acids have characteristic titration curve. Titration involves gradual addition or removal of protons (H^+).

- *UV absorption:* Aromatic amino acids Phe, Tyr and Trp absorb UV light. Tryptophan absorbs UV light at 280 nm.

Chemical Properties

I. Reactions due to carboxyl groups

- **Decarboxylation**



Decarboxylation produces biologically important amines, such as histamine from histidine, GABA from glutamic acid, tyramine from tyrosine.

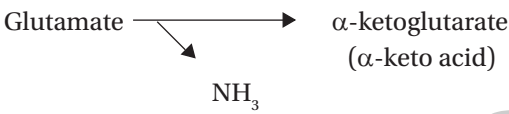
- **Amide formation**
  $-COOH + NH_3 \rightarrow -CO - NH_2$
  (of aspartic acid) (of asparagine)
- **Salt and ester formation:** Amino acids form salts (–COONa) with bases and esters ($-COOR^1$) with alcohols.

II. Reactions due to amino group

- *Transamination:* It is the transfer of α-amino group of an α-amino acid to an α-keto acid.

$$\text{L-α-Glutamate + Pyruvate} \xleftrightarrow[\text{PLP}]{\text{Transaminase}} \text{α-ketoglutarate + Alanine}$$

- *Deamination:* Here removal of α - amino group of amino acid takes place to form an α-ketoacid.



III. Formation of carbamino compounds

$Hb\text{-}NH_2 + CO_2 \rightarrow$ Carbamino Hb

IV. Ninhydrin reaction

α-amino group of amino acid + Ninhydrin $\xrightarrow{\text{heat}}$ Ruhemann's purple (purple/blue colored compound)

This test is done to detect α-amino acids. But proline does not answer this test, therefore it does not have amino group. It is an imino acid. It gives yellow color.

V. Reactions due to side chains

- *Transmethylation:* Transfer of methyl group from active form of methionine [S-adenosyl methionine (SAM)] to noradrenaline to form adrenaline.

$$\text{Noradrenaline} \xrightarrow[(-CH_3)]{\text{SAM}} \text{Adrenaline}$$

- *Formation of disulfide bond:* Here formation of disulfide bond between two cysteine molecules takes place.
  Cys – SH + HS – Cys → – S – S–Disulfide bond of cystine (dimer)
  Disulfide bond links different polypeptide chains.

VI. Peptide bond formation (Fig. 5.1.2)

α-carboxyl group of one amino acid and α-amino group of another amino acid react together to form a peptide bond or CO–NH bond.

Dipeptide contains 2 amino acids and 1 peptide bond. Tripeptide contains 3 amino acids and 2 peptide bonds. Oligopeptide has 10 or less amino acids and polypeptide contains 10–50 (more than 10 up to 50) amino acids.

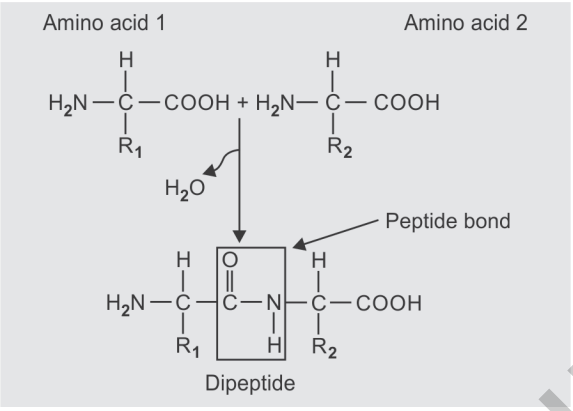


Fig. 5.1.2: Peptide bond formation.

PROTEINS

The term protein is derived from the Greek word *proteios* meaning holding first place in living matter. Proteins make up 75% of total body weight. Proteins contain carbon, hydrogen, oxygen, nitrogen as major components and sulfur, phosphorus as minor components. Average nitrogen content of proteins is 16% by weight. Proteins are high molecular weight compounds. They are polymers of L-α-amino acids linked by peptide bonds. Proteins are structurally and functionally important.

Classification of Proteins

Protein has diverse functions.

A. Based on biological functions proteins are classified into **(Table 5.1.8)**:

Table 5.1.8: Classification of protein based on biological functions.

Function	***Examples***
Structural	Collagen, elastin, keratin
Catalytic (enzymes)	Hexokinase, pepsin, trypsin
Contractile	Actin, myosin
Transport	Hemoglobin, albumin, transferrin
Regulatory (hormones)	Insulin, growth hormone (GH), adrenocorticotropic hormone (ACTH)
Protective (defense)	Immunoglobulins, interferons (antibodies)
Genetic	Histones
Storage	Ferritin (stores iron)
Buffers	Plasma proteins, hemoglobin
Transporters	Na^+ - K^+ ATPase

B. Based on shape proteins are classified into:
   - *Globular proteins:* Polypeptide chain folded into compact, spherical shape, easily soluble. *Example*s: Albumin, hemoglobin, myoglobin.
   - *Fibrous proteins:* Polypeptide chain extended along the axis is insoluble. *Example*s: Collagen, elastin, keratin.

C. Based on composition proteins are classified into:
   - *Simple proteins:* Contain only amino acids. Simple proteins on hydrolysis yield amino acids. *Example*s: Serum albumin, pepsin, trypsin.
     Based on solubility: Simple proteins are subdivided
     - » *Albumins:* Soluble in water and dilute salt solutions and coagulated by heat. *Examples:* Serum albumin, ovalbumin (egg).
     - » *Globulins:* Insoluble in pure H_2O but soluble in dilute salt solutions. *Examples:* Serum globulins.
     - » *Glutelins:* Soluble in dilute acids and alkalies. *Examples:* Glutenin of wheat.
     - » *Protamines:* Soluble in H_2O, dilute acids and alkalies. *Examples:* Salmine of salmon fish.
     - » *Histones:* Soluble in H_2O and dilute acids but insoluble in dilute ammonium hydroxide. Rich in basic amino acids. *Examples:* Thymus histones.
     - » *Prolamines:* Soluble in 70% alcohol; insoluble in water, rich in proline. *Examples:* Zein (of maize).
     - » *Scleroproteins:* Insoluble in water, dilute acids and alkalies. *Examples:* Collagen, elastin, keratin.
   - *Conjugated (compound proteins):* Contain nonprotein part (prosthetic group) attached to protein part. Conjugated protein on hydrolysis yield protein and prosthetic group. **Table 5.1.9** shows types of conjugated proteins.

Table 5.1.9: Types of conjugated proteins.

Subclass	*Prosthetic group*	*Type of linkage*	*Example*
Glycoproteins	Carbohydrates (<10%)	Covalent	• Egg albumen • Immunoglobulins
Lipoproteins	Lipid	Hydrophobic interactions	• Serum lipoproteins (HDL, LDL) • Membrane lipoprotein
Phosphoprotein	Phosphorus	Covalent	• Casein of milk • Vitellin of egg yolk
Nucleoprotein	Nucleic acids (DNA or RNA)	Noncovalent	Nucleohistones of chromatin
Chromoproteins: Colored substance.			
a. Hemoprotein	Heme	Noncovalent	• Hemoglobin • Myoglobin • Cytochromes

Contd...

Contd...

Subclass	*Prosthetic group*	*Type of linkage*	*Example*
b. Flavoproteins	Flavin nucleotides (FMN, FAD)	Covalent	Succinate dehydrogenase
c. Visual pigments	Retinal (vitamin A)	Covalent	Rhodopsin of retina
d. Metallo-proteins	Metals • Fe • Cu • Zn	Noncovalent	 • Ferritin, cytochromes • Tyrosinase • Carbonic anhydrase

(HDL: high-density lipoprotein; LDL: low-density lipoprotein ; DNA: deoxyribonucleic acid; RNA: ribonucleic acid; FMN: flavin mononucleotide ; FAD: flavin adenine dinucleotide)

- *Derived proteins:* Are derivatives of simple or conjugated proteins.
  - » *Primary derived proteins* are denatured or coagulated proteins. Here peptide bonds are not hydrolyzed. *Examples:* Coagulated albumin.
  - » *Secondary derived proteins:* Are partially hydrolyzed (degraded) products of proteins. *Examples:* Proteases, peptones, gelatin, peptides.

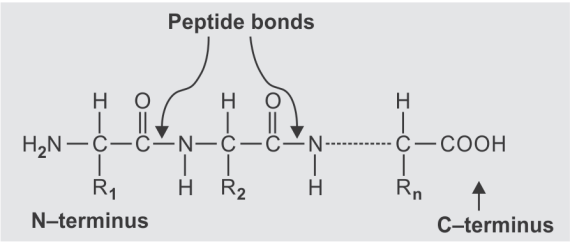
D. Based on nutritional value (based on their essential amino acid content) proteins are classified into:
- *Complete proteins (Class I proteins)* contain all 10 essential amino acids at proportions required by human body. They promote good growth. *Example*s: Egg albumen, casein of milk.
- *Partially incomplete proteins* partially lack one or more essential amino acids. They promote moderate growth. *Example*s: Pulse proteins (deficient in Met), cereal proteins (deficient in Lys).
- *Incomplete proteins (poor proteins)* completely lack one or more essential amino acids. They do not promote growth. *Example*s: Zein from corn (lacks tryptophan, lysine).

Protein Structure (Conformation)

Proteins are polymers of L-α-amino acids. Linear polypeptide chains can be folded to specific 3D shape which is more stable. Function of a protein depends on its 3D structure, which is unique.

There are four levels of structural organization:

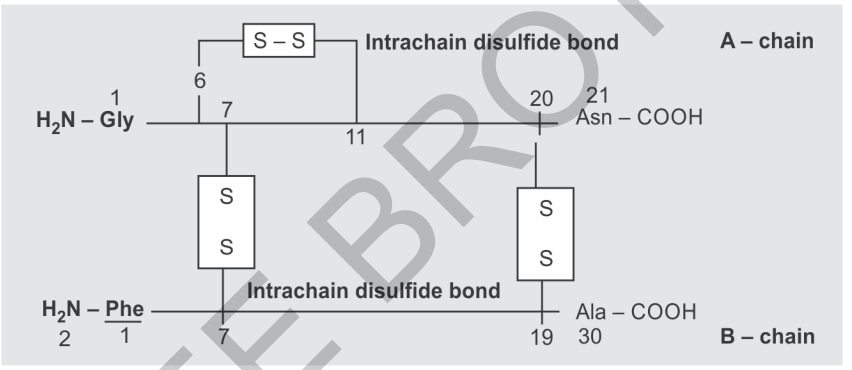
Primary structure: It refers to the linear sequence of amino acids linked by peptide bonds and locations of disulfide bonds (if any). Peptide bonds (CO-NH) and disulfide bonds (S-S) are covalent bonds or (covalent backbone). Primary structure is decided by genes. It influences higher levels of protein conformation.



Peptide bonds: They are covalent bonds. They are rigid, planar and have partial double bond characteristics. C-N is in trans configuration.

Note: Change in a single amino acid of the primary structure can alter the properties of the protein. *Examples:* Normal Hb becomes abnormal sickle cell Hb (HbS) when a single amino acid among 154 in β chain is changed. Sixth amino acid in β chain is glutamic acid in normal Hb and valine in abnormal HbS.

1. Primary Structure (Covalent Structure) of Insulin



Insulin is synthesized as larger precursor

Preproinsulin (109 a. a) → Proinsulin (86 a. a) → Insulin (51 a. a) + C - Precursor

C - Peptide is also called connecting peptide.

C - Peptide estimation is done to measure endogenous insulin.

2. Secondary Structure

- It is the folding of the polypeptide chain along its long axis into regular repeating structure.
- Secondary structure makes protein more compact.
- It adds some new properties to a protein increasing strength and flexibility.
- It is maintained by numerous H– bonds between –NH and –C = O groups of amino acids within the protein.

Two common types of secondary structure are:

1. α-helix
2. β-pleated sheet

α-helix

It is proposed by Pauling and Corney in 1951.

Features

Polypeptide backbone is coiled around a central axis, spontaneously. Numerous hydrogen bonds between –N–H and –C = O groups (that are four residues apart) stabilize the structure. Side chains of amino acids are extended outwards. Distance between two amino acid residues is 1.5Å. Each turn has 3.6 amino acids. Pitch (rise/turn) = 5.4Å. Every –N–H and –C = O participates in hydrogen bonding. Right handed α- helix is more common and more stable **(Fig. 5.1.3)**.

Amino acids which do not allow formation of α-helix (terminators) are for Examples: proline, its derivative hydroxyproline. Amino acids which destabilize α-helix are acidic, basic amino acids. For example, aspartic acid, arginine.

Occurrence: Hemoglobin and myoglobin have abundant α-helical regions.

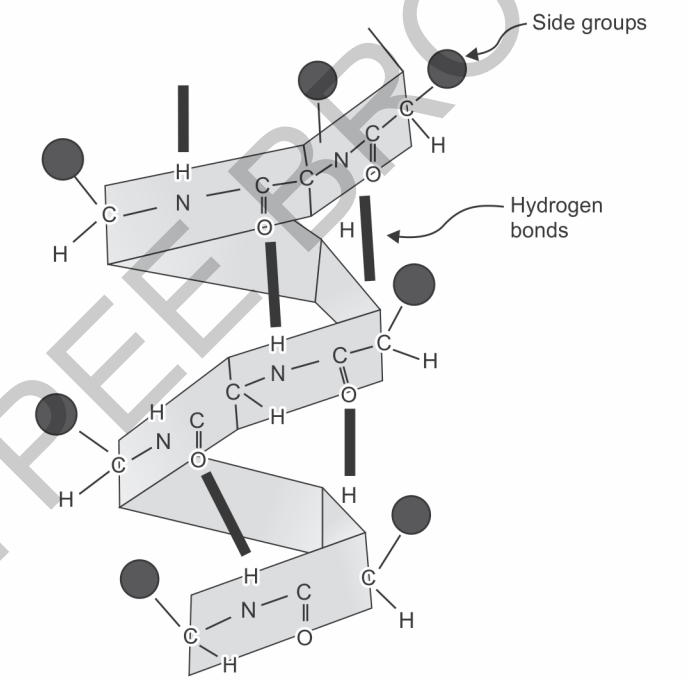


Fig. 5.1.3: The α-helix structure.

H bond: Bond formed by sharing a hydrogen between two electron donors.

H-releasing groups (in proteins)	*H-accepting groups (in proteins)*
–NH (imidazole)	–COO– (Asp, Glu)
–OH (Ser, Thr)	C = O (peptide)

β-pleated Sheet

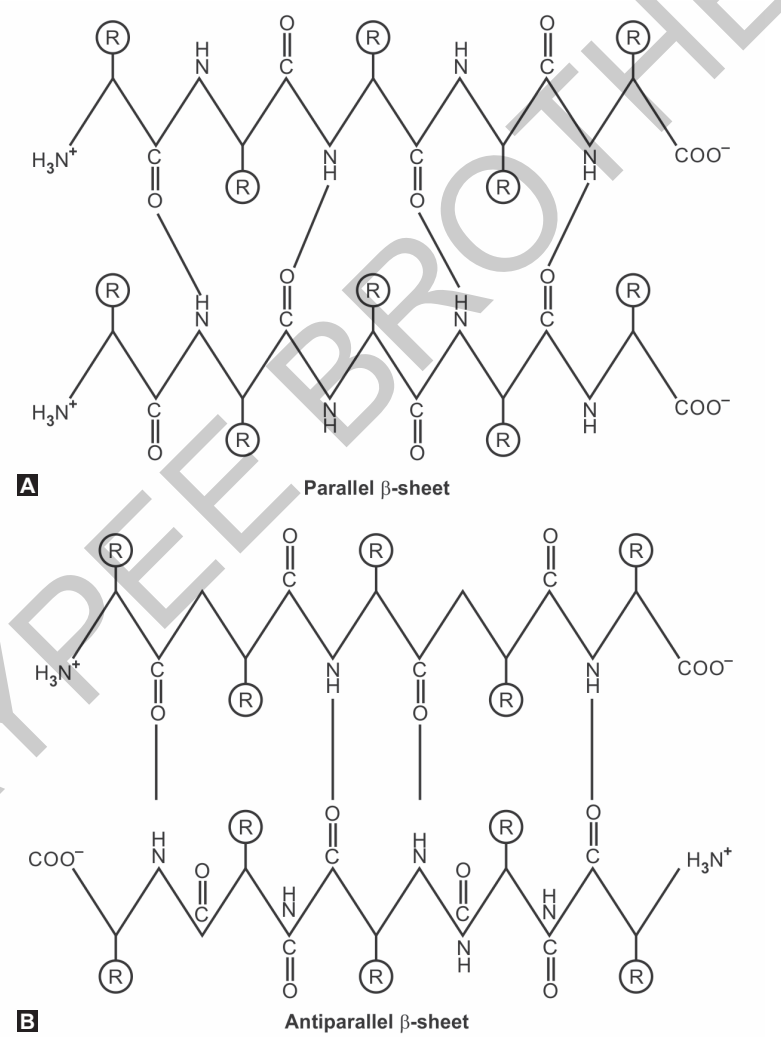
Features

Polypeptide chain is extended into zigzag structures. Two or more adjacent segment of chain line, up side by side to form sheet. The side chains are above or below the plane of the sheet.

Numerous H– bonds between –N–H and –C = O groups of adjacent sheets, stabilize the structure. When adjacent strands run in same direction structure is parallel β-pleated sheets. When adjacent strands run in opposite direction, structure is antiparallel β-pleated sheet **(Figs. 5.1.4A and B)**.

Occurrence: Seen in silk fibroin.

Random coils: These are the regions of proteins which are not organized as helices or parallel sheets.



Figs. 5.1.4A and B: Structure of (A) parallel β-pleated sheet and (B) antiparallel β-pleated sheet.

Bends: β-turn (β-bend or reverse turn) is a hairpin turn (U turn) of polypeptide. And it contains four residues Gly, Ser, Asp, Pro. It changes the direction of chain and connects antiparallel β-pleated sheets which make protein compact **(Fig. 5.1.5)**.

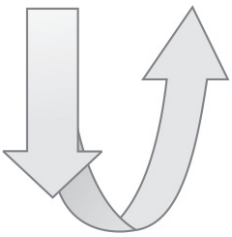


Fig. 5.1.5: Structure of β-bends.

Loops: Loops contain 16 residues. They vary in size and shape. They connect adjacent units of chain and have biologically important functions. For Examples: Site for ligand interactions, antigen-binding site of antibodies.

Triple helix: Three left handed helices wrapped around each other make a triple helix. Collagen contains this structure. Collagen is the most abundant protein in mammals (bones, teeth, tendons, cartilages, skin and blood vessels).

Tertiary structure: It denotes 3D arrangement of proteins and formed by interaction between amino acids far apart in chain. It consists of α-helices, β-pleated sheets, β-turns, motifs and random coils. It is compact and most stable and stabilized by noncovalent bonds.

Bonds Responsible for Protein Structure

Noncovalent Bonds

- *Hydrophobic interaction:* Association between nonpolar side chains of amino acids.
- *Electrostatic bonds (ionic bonds):* Formed between oppositely charged groups of amino acid side chain. For example, between positively charged group of basic amino acids and negatively charged group of acidic amino acids.
- *Hydrogen bonds:* They are formed by sharing of hydrogen atoms between nitrogen and carbonyl oxygen of different peptide bonds. Each hydrogen bond is weak but collectively they are strong.
- *Van der Waals forces:* These are the noncovalent association between electrically neutral molecules. They are formed by the electrostatic interactions due to permanent or induced dipoles.

Covalent Bonds

Peptide and disulfide bonds are the strong bonds in protein structure.

Disulfide bonds: Disulfide bond is formed by the sulfydryl groups of two cysteine residues to produce cystine. Disulfide bonds are formed in a single polypeptide chain or between different polypeptides. These bonds are responsible for the stability of the proteins and their structural conformation.

Quaternary structure ***(Fig. 5.1.6)****:* It is possessed by proteins containing two or more polypeptide chains (oligomeric proteins). Individual polypeptide chains are called monomers or subunits. For Examples: Dimer consists of 2 monomers and tetramer contains 4 monomers. Bonds that keep this structure are noncovalent, such as H bonds, electrostatic bonds, hydrophobic interaction and Van der Waals forces. Protein loses function when subunits are dissociated. Oligomeric proteins play significant role in regulation of metabolism.

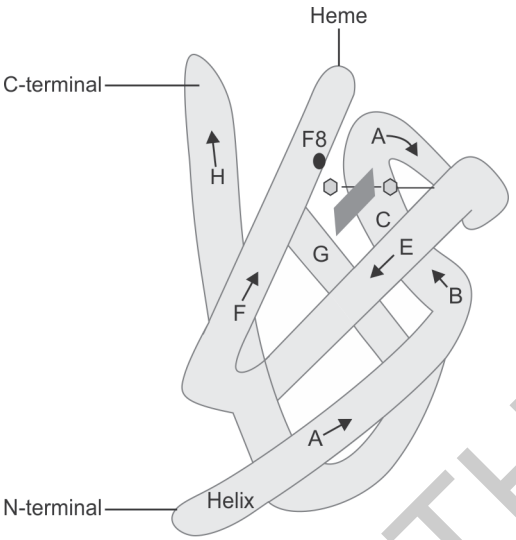


Fig. 5.1.6: Quaternary structure.

Techniques used to study protein structure are:
- X-ray diffraction
- UV light spectroscopy
- Nuclear magnetic resonance (NMR)

Physical Properties of Proteins

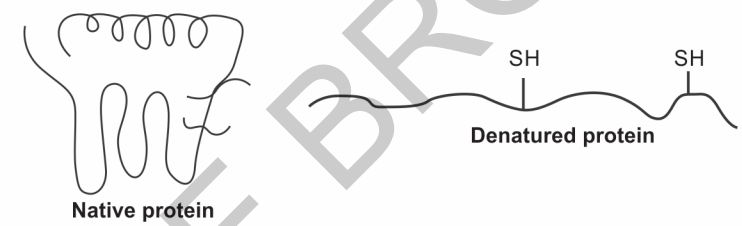
- *Solubility:* Proteins exist as colloids in solution. They scatter light and exert osmotic pressure. *Example*s: Plasma albumin exerts osmotic pressure.
- *Molecular weight:* The proteins vary in their molecular weights depending upon the number of amino acid residues. *Example*s: Insulin—5,700; Albumin—69,000; Hb—6500; Immunoglobulin (Ig)—1,50,000.
- *Shape:* There is a wide variation in the shape of proteins. *Example*s: fibrinogen is elongated or fibrous.
- *Isoelectric pH (P^I):* The nature of the amino acids determines the P^I of a protein. The acidic amino acids and basic amino acids strongly influenced the P^I. The proteins exist as zwitterions or dipolar ions at isoelectric pH. They are electrically neutral with minimum solubility, maximum precipitability and least buffering capacity.

Protein		***P^I***
– Casein	:	4.6
– Human albumin	:	4.7
– Urease	:	5.0
– Human globulin	:	6.4
– Human Hb	:	6.7
– Lysozyme	:	11.0

- *Precipitation of proteins:* Polar groups of proteins attract H_2O molecules around them to form shell of hydration. Proteins precipitate when their charges are neutralized or water of hydration around them is removed.

Precipitation method: By salting out, isoelectric precipitation, by organic solvents where proteins are dehydrated, by heavy metal ions, by anionic/alkaloidal reagents.

- *Denaturation:* Denaturation is due to loss of secondary, tertiary and quaternary structures of native proteins by a variety of agents. Here peptide bonds are not broken while, hydrogen bond, ionic bond, hydrophobic interactions, Van der Waals force are broken. This involves a change in physical, chemical and biological properties of protein molecules. Denaturation is usually irreversible. Denaturing agents are of two types.
  1. *Physical agents:*
     - » Heat
     - » High pressure
     - » Vigorous shaking
     - » X-rays
     - » UV rays
  2. *Chemical agents:*
     - » Urea at high concentration
     - » Salicylates
     - » Strong acids and alkalis
     - » Organic solvents (ether, alcohol, acetone)



Biological importance of denaturation

- Loss of biological activity

  Examples:
  - » Enzyme loses enzymatic activity
  - » Antibodies cannot bind antigens
- Decrease in solubility
- Increase in digestibility (HCl of gastric juice also denatures protein).

- *Renaturation:* Denatured proteins are sometimes regain original structure when the physical agent is removed. *Examples:* Immunoglobulins (Ig) get denatured when exposed to 8 molar (8M) urea. When urea is removed by dialysis, Ig gets its original structure.
- *Heat coagulation:* When heated at P^I, some proteins denature irreversibly to produce thick floating coagulum. *Examples:* Albumin is easily coagulated.

Sanger's Reagent (Fig. 5.1.7)

It was earlier used to identify N-terminal amino acids of peptides. It is fluorodinitrobenzene (FDNB).

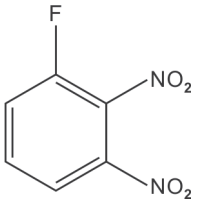


Fig. 5.1.7: Sanger's Reagent.

Principle

FDNB with the N-terminal amino acid gives rise to dinitrophenyl derivative (yellow colored) which is separated by chromatography and identified.

Sanger's reagent has limited use. It can hydrolyze the peptide chain to amino acids.

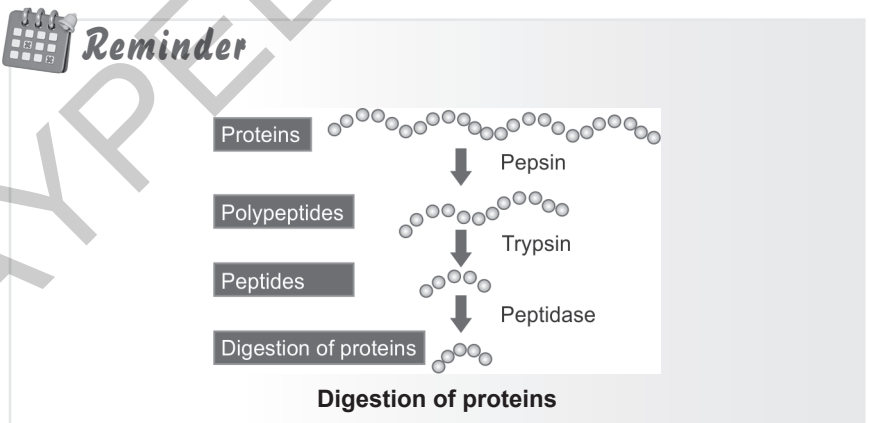
5.2: DIGESTION AND ABSORPTION OF PROTEINS

DIGESTION

Dietary proteins are of plant and animal origin, and average intake is 50–100 g/day. About 30–100 g/day of endogenous protein is derived from the digestive enzymes and worn-out cells of the digestive tract. Cooking denatures dietary proteins and so, it is easily digested. Proteins are degraded by 'hydrolases' which specifically cleave the peptide bonds, hence known as 'Peptidases' or 'Proteases'. These are divided into two groups:

1. *Endopeptidases*: They attack the internal peptide bonds and release peptide fragments, *Examples:* Pepsin, trypsin.
2. *Exopeptidases*: They act on the peptide bonds of terminal amino acids. These are subdivided into (a) Carboxypeptidases (b) Aminopeptidases.

   Based on the amino acids of the active site, proteases can be classified as:
   - Serine proteases containing serine at the active site.
   - Zinc proteases containing Zn^{+2} at the active site
   - Carboxyproteases (acid proteases) have dicarboxylic amino acid at the active site.
   - Thiolproteases contain cysteine at the active site. It is not found in human beings.



Digestion of proteins

PROENZYMES

Some strong proteases are secreted as inactive proenzymes or zymogens. On arrival at the site of activity, one or more specific peptide bonds are hydrolyzed in the proenzyme either enzymatically or by pH changes. This yields the active

enzymes and some inactive peptides called masking substances. *Examples:* Inactive trypsinogen of the pancreatic juice is hydrolyzed to active trypsin in the intestinal lumen by enterokinase of intestinal juice when the intestinal contents have pH around 5.5.

Stages of Protein Digestion (Table 5.2.1)

1. Digestion by gastric juice
2. Digestion by pancreatic juice
3. Digestion by intestinal juice

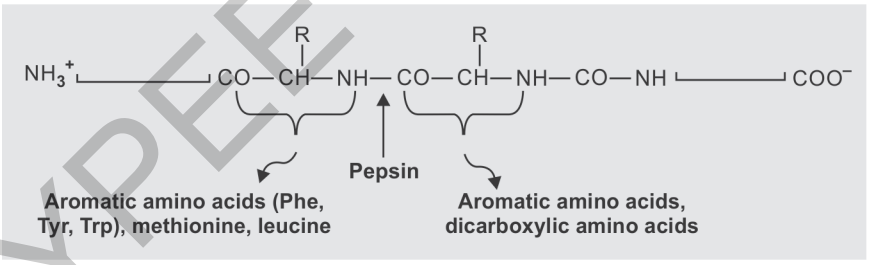
Digestion by Gastric Juice

Protein digestion does not take place in the oral cavity. In stomach, HCl, pepsin and rennin help in digestion of proteins. HCl along with intrinsic factor is secreted from the parietal cells of the gastric gland.

Rennin (chymosin): It is found in infants and absent in stomach of adults. It prevents rapid passage of milk from the stomach. It changes casein of milk irreversibly to a paracasein in presence of calcium. Paracasein is acted upon by pepsin.

Pepsin: Chief cells of gastric gland secrete pepsin. It is secreted in an inactive form known as pepsinogen. This gets activated initially by HCl by the removal of 44 amino acid residues from the N-terminal end. Later, pepsin itself activates remaining pepsinogen (autoactivation). Pepsin is active in the pH range of 1–3. Pepsin is an endopeptidase—carboxy protease (acid protease) with 2 aspartic acid residues in the active site. Proteoses and peptones are produced as a result of action of pepsin on proteins.

Site of action of pepsin



Functions

Functions of HCl

- It activates pepsinogen to pepsin by removal of 44 amino acids from the N-terminal end.
- It provides optimum pH (1–3) for pepsin action.
- It destroys most organisms entering the GI tract due to low pH.
- It causes hydrolysis of sucrose to glucose and fructose.
- It causes denaturation of dietary proteins and acid metaproteins are formed.
- It provides optimum acidic pH for the intrinsic factor—vitamin B_{12} complex absorption.
- HCl releases Fe^{+3} from iron complexes of the diet.

Abnormal conditions of hydrochloric acid secretion

Achlorhydria: It is the absence of HCl in the gastric juice. It is seen in gastric carcinoma, atrophic gastritis, wasting diseases of stomach, chronic gastric ulcer leading to gastric atrophy. Achlorhydria prevents peptic digestion, reduces iron and vitamin absorption, delays emptying of stomach and leads to microbial fermentation in stomach resulting in flatulence and diarrhea.

Achylia gastrica: In this condition, gastric juice lacks in both acid and enzymes. It is observed in pernicious anemia, advanced gastric carcinoma, chronic gastric ulcer and gastritis. Failure of peptic digestion, retention and fermentation of food in the stomach, result in flatulence, diarrhea and megaloblastic anemia.

Hyperchlorhydria: This is an abnormally high secretion of gastric HCl, raising the free acidity of gastric contents and producing postprandial heart burns. It is found in gastric carcinoma, duodenal ulcer.

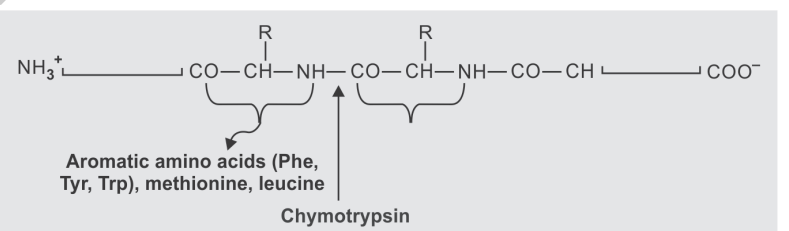
Digestion by Pancreatic Juice

Pancreatic juice contains five proteases. They are trypsin, chymotrypsin, elastase, carboxypeptidase A and B. Pepsin gets inactivated due to increased pH.

Trypsin: Secreted in an inactive trypsinogen form. Along with trypsin, there is trypsin inhibitor protein. At the site of action (i.e., in duodenum and jejunum), there is activation of trypsinogen to trypsin by enterokinase (enteropeptidase) enzyme of the intestinal juice. During activation, 6 amino acids from N - terminal end are removed from trypsinogen to form active trypsin. Trypsin is an endopeptidase. It belongs to serine proteases. It has autoactivation capacity. It is active in pH around 8.0. It helps in the activation of other proenzymes, such as chymotrypsinogen, proelastase, procarboxypeptidase A and B and prophospholipase A_2.

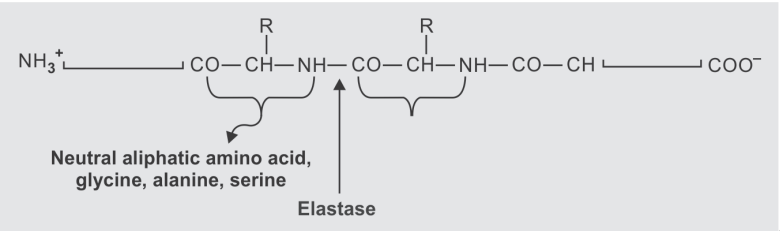
Chymotrypsin: It is secreted as inactive chymotrypsinogen and activated by trypsin. It is an endopeptidase, serine protease, active in the pH range of 7–8. Chymotrypsin action on protein leads to peptide formation.

Site of action



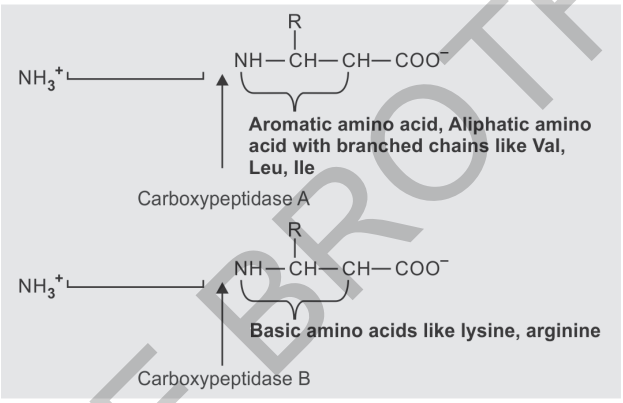
Elastase: It is secreted as inactive proelastase activated by trypsin. Elastase is an endopeptidase and serine protease. It acts mainly on elastin.

Site of action



Carboxypeptidase A and B: They are secreted as inactive procarboxypeptidases and activated by trypsin. Both carboxypeptidases are zinc proteases, exopeptidases, acting on C-terminal peptide bond. They are active in the pH range of 7.5–8.

Site of action



Digestion by Intestinal Juice

Proteases in intestinal juice are, enteropeptidase (enterokinase), aminopeptidases, dipeptidases.

Enteropeptidase: It is an endopeptidase, a glycoprotein and initially anchored to the membrane of the luminal surface of the enterocytes. By the action of bile salts, it gets released into the duodenal lumen.

$$\text{Trypsinogen} \xrightarrow{\text{Enterokinase}} \text{Active trypsin} + \text{Hexapeptide}$$

Aminopeptidases: They are glycoproteins anchored to the intestinal mucosal membrane. They act on amino terminus, release one amino acid at a time from the amino terminal end. Thus, they act as exopeptidases and cannot act on the dipeptides. *Examples:* Leucine aminopeptidase, proline aminopeptidase.

Dipeptidases: Glycylglycine dipeptidase hydrolyzes dipeptide into two molecules of amino acids to complete the digestion of proteins. Dipeptidases are located mainly in the enterocyte cytoplasm and hence digestion is intracellular after the absorption of dipeptides.

Applied BIOCHEMISTRY for BSc NURSING

Salient Features

- The book has been written in simple English with a lucid and easy-to-understand approach.
- It has been divided into nine chapters covering fundamental biochemistry topics such as "Introduction to biochemistry, Detailed coverage of cell structures, membranes, and transport mechanisms, Discussions on acid-base balance and electrolytes, Comprehensive chemistries of carbohydrates, lipids, and proteins with diagrams and reactions, Metabolism of carbohydrates, amino acids, lipids, and hemoglobin, Protein synthesis techniques, sequencing, and enzyme mechanisms, Interpretation of liver, renal, and thyroid function tests, In-depth knowledge of vitamins, minerals, and their roles, Vital chapter on immunochemistry with focus on free radicals, antioxidants, and specialized proteins".
- In this book, there are learning tools that include pictures, diagrams, flowcharts, and chapter-end assessments: Multiple choice questions, Long and short essays.
- This textbook also provides nursing students with accessible biochemistry essentials, reinforced by visual aids and thorough assessment resources for effective learning.

Manjula Shantaram is presently serving as the Chief of Research Centre at AJ Institute of Medical Sciences and Research Centre (affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru), Mangalore, Karnataka, India since July 2023. She was the Professor of Biochemistry at Mangalore University, Jnana Kaveri Postgraduate Centre, Kushalnagar, Kodagu, Karnataka. She served as the Co-ordinator, Chairperson of the Department, Director of the PG Centre, and Director of Student Welfare at Mangalagangothri till 2021.

She obtained her MSc in 1988 from the PG Department of Biosciences, Mangalagangothri and pursued her research work at Biochemistry Department in Kasturba Medical College, Manipal and obtained her PhD in Biochemistry in 1993, from Mangalore University. She is a Fellow of Academy of General Education, Manipal.

She worked as a part-time lecturer in Karnataka College of Physiotherapy, SCS College of Nursing, Mangalore, Guest Faculty at the PG Department of Biosciences, Mangalagangothri, Department of Biochemistry, Kasturba Medical College, Mangalore. She joined Yenepoya Medical and Dental College, Mangalore as an Assistant Professor in 1999 and rose to the level of Professor after serving at various capacities in June 2013.

She has got 30 years of total teaching experience for undergraduates and postgraduates. She has also contributed "Biology and Biochemistry" core tutorials for e-learning in California, USA. More than 200 research and review papers were published in various national and international indexed, peer reviewed journals as on date, with 1,338 citations. She has reviewed many scientific papers and contributed four book chapters too. She was the Editor-in-Chief, Editor, Associate Editor, and Chief Advisor of three indexed journals such as Biomedicine, IJHRS, and JARBS, respectively. Currently, she is the Editor-in-Chief of AJ Journal of Medical Sciences (https://ajjms.com). She is a life member of ACBI, IABMS, SOBSI, and SBC (India). She has been awarded fellowship title FABMS of IABMS at Chennai. As on date, she has successfully guided 15 PhD scholars at Vinayaka Missions University, Yenepoya Deemed to be University and Mangalore University. She was the co-guide for five PhD candidates. Eleven PhD scholars are still working under her supervision in other private universities.

She has worked as an examiner and question paper setter for MBBS, BDS, MSc (Biosciences, Environmental Science, Applied Zoology, Biotechnology, Bioinformatics, and Biochemistry), BNYS, BSc Nursing, BSc Biotechnology, and BPT of various universities since 1999. She has successfully organized international and national conferences, Global Initiative for Academic Network (GIAN) and other scientific programs so far.

She published a textbook on *Biochemistry and Nutrition for BSc Nursing* through Jaypee Brothers Medical Publishers (P) Ltd., in 2011, which was widely circulated all over the world. Its second edition was brought out in 2022 with the title *Applied Biochemistry for BSc Nursing.*

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