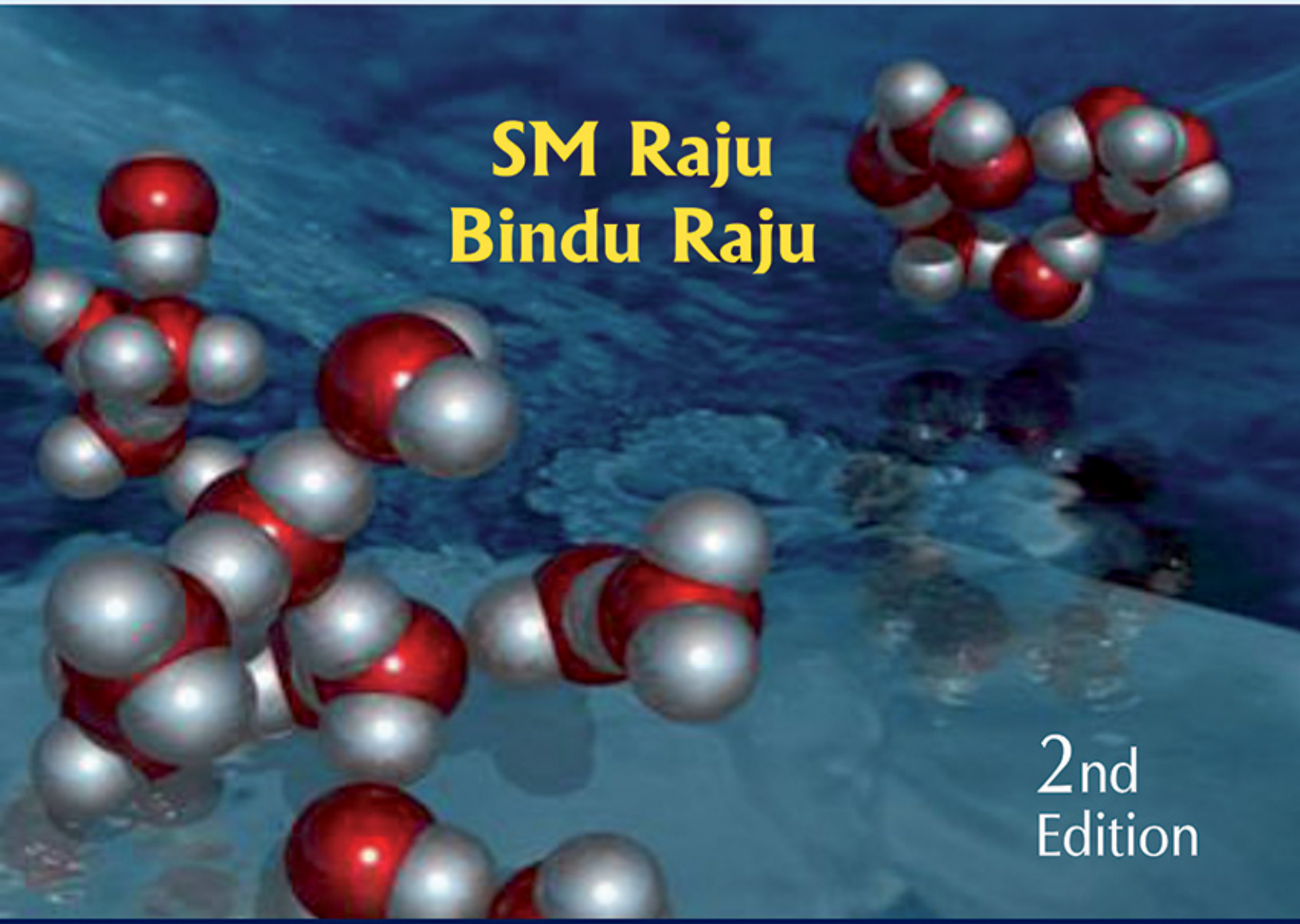
Illustrated Medical Biochemistry



SM Raju
Bindu Raju

2nd Edition

JAYPEE

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4 CHAPTER

Chemistry of Amino Acids and Proteins

AMINO ACIDS

Amino acids are the building blocks of proteins. The general structure of amino acid (Fig. 4.1) has an α-amino group, an α-carboxylate group, and a variable side chain (R group). The α-amino group has a pKa of 9.8, therefore ionized (+vely charged) at physiological pH. The α-carboxylate group has a pKa value of 2.5, therefore ionized (–vely charged) at physiological pH.

Ionization constant (pKa) is the pH at which the group is half deprotonated. It means at any pH less than its pKa the group is fully protonated (+vely charged), and at any pH above its pKa the group is fully deprotonated (–vely charged). Hence, the amino acid backbone at pH 7 is in zwitterion form (Fig. 4.2).

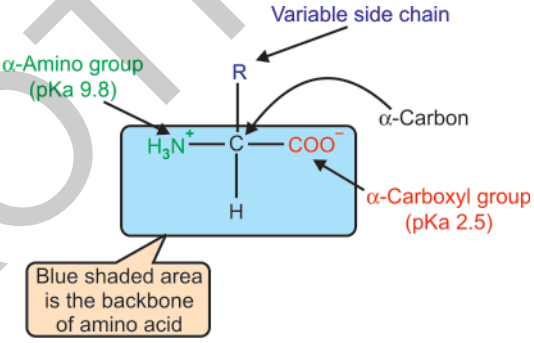

FIGURE 4.1: Structure of an α-amino acid

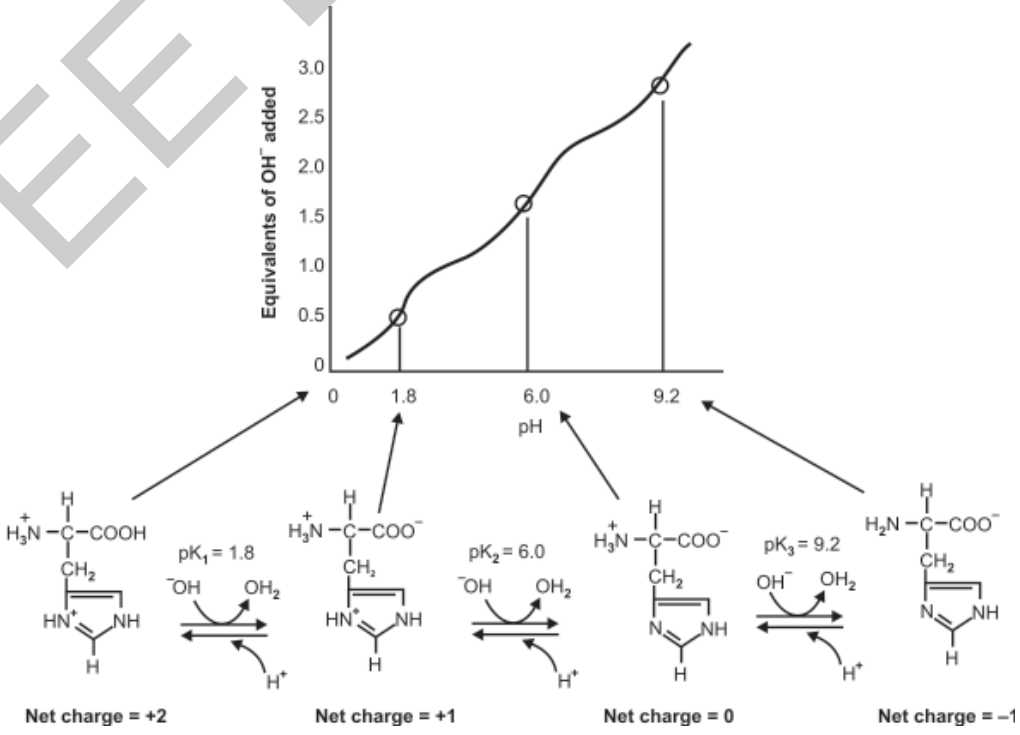

FIGURE 4.2: Titration curve of histidine

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Acid-Base Properties of the Amino Acids

The α-COOH and α-NH_2 groups, as well the acidic and basic R-groups in amino acids are capable of ionizing. As a result of their ionizability the following ionic equilibrium reactions may be written:

$$R\text{-}COOH \rightarrow R\text{-}COO^- + H^+$$
$$R\text{-}NH_3^+ \rightarrow R\text{-}NH_2 + H^+$$

The equilibrium reactions as written, demonstrate that amino acids contain at least two weakly acidic groups. However, the carboxyl group is a far stronger acid than the amino group. At physiological pH (around 7.4) the carboxyl group will be unprotonated and the amino group will be protonated. An amino acid with no ionizable R-group would be electrically neutral at this pH. This species is termed a *zwitterion*.

Like typical organic acids, the acidic strength of the carboxyl, amino, and ionizable R-groups in amino acids can be defined by the association constant K_a or more commonly the negative logarithm of K_a; the pK. The *net charge* (the algebraic sum of all the charged groups present) of any amino acid, peptide or protein, will depend on the pH of the surrounding aqueous environment. As the pH of a solution of an amino acid or protein changes, so too does the net charge on amino acid or protein present in it. This phenomenon can be observed during the titration of any amino acid or protein. The net charge of an amino acid or protein is zero, at a pH equivalent to its isoelectric *point (pI)*.

Optical Properties of the Amino Acids

A tetrahedral carbon atom with four distinct substituted groups is said to be *chiral*. The only amino acid not exhibiting chirality is glycine since its "R-group" is a hydrogen atom. Chirality describes the handedness of a molecule that is observable by its ability in solution to rotate the plane of polarized light either to the right *(dextrorotatory)* or to the left *(laevorotatory)* (Fig. 4.3). All of the amino acids in proteins exhibit the same absolute steric configuration as *L-glyceraldehyde*. Therefore, they are all L-α-amino acids. Although D-amino acids exist in nature, they are never found in proteins, but are often found in, polypeptide antibiotics. The aromatic R-groups in aromatic amino acids have an absorbance maximum in the range of 280 nm. The ability of proteins to absorb ultraviolet light is predominantly due to the presence of the tryptophan, which strongly absorbs ultraviolet light.

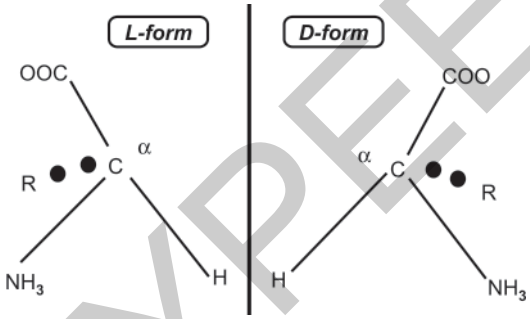

FIGURE 4.3: L- and D-forms of amino acid

Functional Significance of R-groups in Amino Acid

Amino acids differ from each other only in 'R' groups they possess. In solution, it is the nature of the R-groups of amino acids that dictate structure-function relationships of peptides and proteins. The hydrophobic amino acids will generally be encountered in the interior of proteins shielded from direct contact with water. Conversely, the hydrophilic amino acids are generally found on the exterior of proteins

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as well as in the active site of enzymes protein. Indeed, it is the nature of R-groups of certain amino acids that allow enzyme reactions to occur.

The imidazole ring of histidine allows it to act as either a proton donor or acceptor at physiological pH. Hence, it is frequently found in the active site of enzymes. Equally important is the ability of imidazole ring of histidine in hemoglobin to buffer H^+ ions formed during ionization of carbonic acid in red blood cells. It is this property of hemoglobin that allows it to exchange O_2 and CO_2 at the tissues and lungs, respectively.

The primary alcohol of serine and threonine as well as the thiol (-SH) of cysteine allow these amino acids to act as nucleophiles during enzymatic catalysis. Additionally, the thiol of cysteine is able to form a disulfide bond with another cysteine:

Cysteine-SH + HS-Cysteine →Cysteine-S-S-Cysteine

This simple disulfide formed by two cysteine molecules is identified as cystine. The formation of disulfide bonds between cysteines present within proteins is important to the formation of structural domains in a large number of proteins. Disulfide bonding between cysteines in different polypeptide chains of oligomeric proteins plays a crucial role in ordering the structure of complex proteins.

CLASSIFICATION OF AMINO ACIDS

Amino acids are variously classified, but a useful classification in terms of function and chemistry would be based on the solubility and acid-base property of 'R' groups (Figs 4.4 to 4.7).

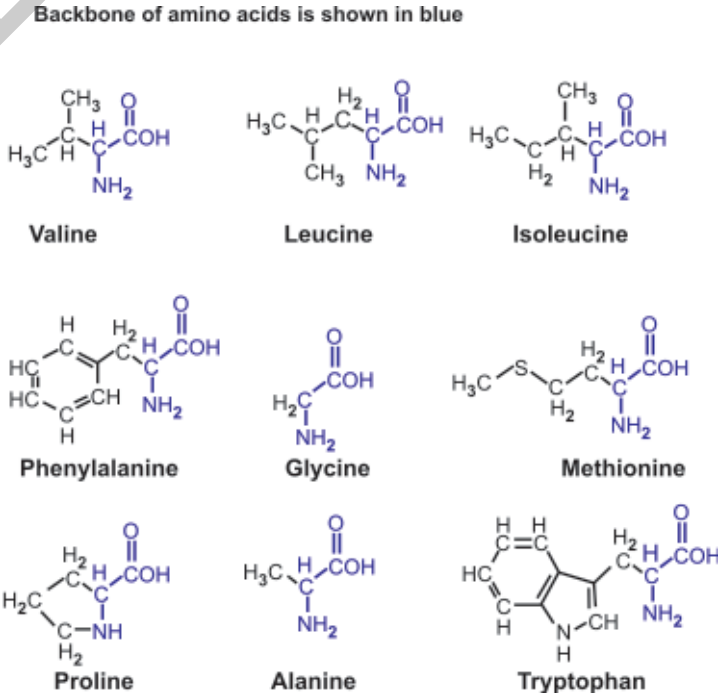

FIGURE 4.4: Hydrophobic amino acids

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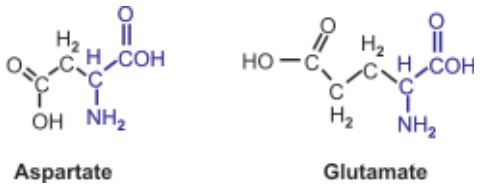

FIGURE 4.5: Polar acidic amino acids

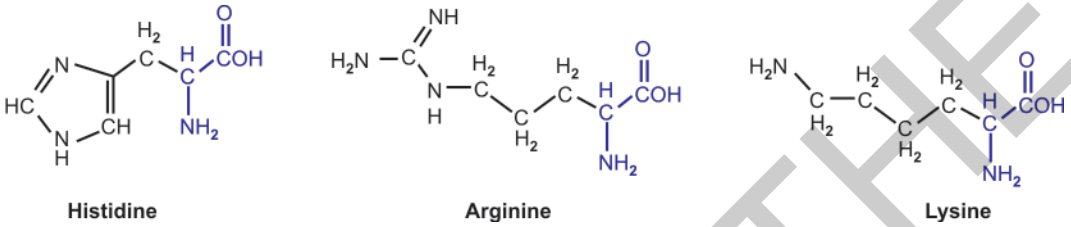

FIGURE 4.6: Polar basic amino acids

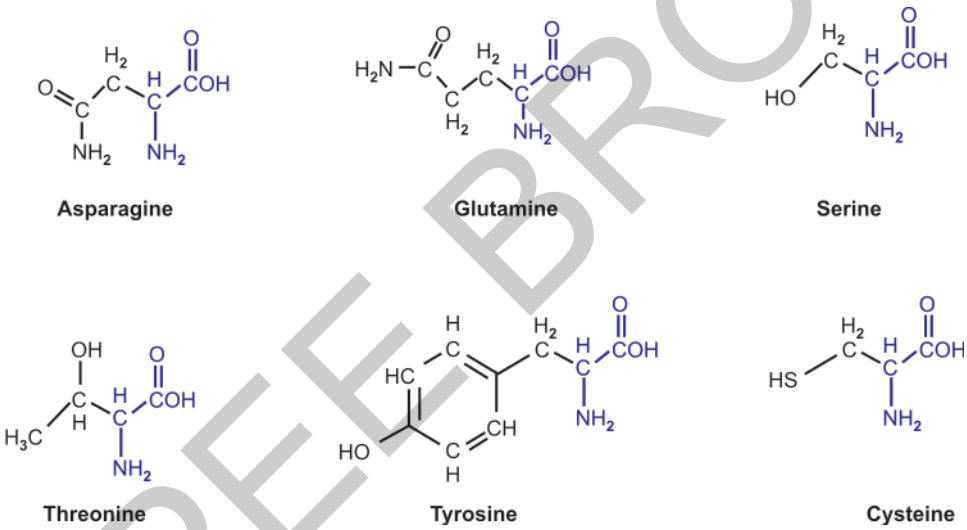

FIGURE 4.7: Polar uncharged amino acids

Classification of Amino Acids based on Structure and Properties of Side Chains (R-groups)

1. **Aliphatic** (R-groups consist of carbons and hydrogens)
   Glycine - R = H, smallest amino acid with no chiral center.
   Alanine - R = CH_3 (methyl group)
   Valine - R = branched and hydrophobic; important in protein folding.
   Leucine - R = 4-carbon branched side chain.
   Isoleucine - R = 4-carbon branched side chain with two chiral centers.
   Proline - R = ring; puts bends or kinks in proteins; amino group is part of the ring.

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2. **Aromatic** (R-groups have phenyl ring). Absorb UV light at 280 nm; this property is made use to quantitate proteins in solution.
   Phenylalanine – R = phenyl ring, which is hydrophobic.
   Tyrosine – R = Phenol ring is hydrophobic, but not as much as phenyl alanine because of the presence of hydroxyl group.
   Tryptophan – R = indole ring.
3. **Sulfur-containing** R-groups
   Methionine – R has sulfur, which is internal (hydrophobic).
   Cysteine – R has sulfur, which is terminal, highly reactive; can form disulfide bonds.
4. **Aliphatic side chains with alcohols**
   Serine – R has β-hydroxyl group that makes it hydrophilic.
   Threonine – R has β-hydroxyl, β-methyl group.
5. **Basic R-groups**
   Histidine – R has imidazole ring, which is hydrophilic– ring nitrogen is positively charged at neutral pH.
   Lysine – R has 4-carbon side chain with an amino group at ε (epsilon) carbon.
   Arginine – R has a guanido group that acts as a strong base.
6. **Acidic** R – groups and amide derivatives
   Aspartate – R has carboxyl group at β-carbon, confer negative charges on proteins.
   Glutamate – R has carboxyl group at γ-carbon, confer negative charge on proteins.
   Asparagine – R has amide of aspartate, side groups uncharged but polar.
   Glutamine – R is amide of glutamate, side group uncharged but polar.
   "Amide groups can form H bonds with atoms of other polar amino acids."

Essential vs Nonessential Amino Acids

Nonessential amino acids	*Essential amino acids*
Alanine, Asparagine, Aspartate, Cysteine, Glutamate, Glutamine, Glycine, Proline, Serine, Tyrosine	Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine

Both essential and nonessential amino acids are equally important for growth and development of an organism. Whenever we refer to an amino acid as essential, it only means it is not synthesized by the organism and need to be supplied in the diet. The amino acids arginine, methionine and phenylalanine are considered essential for reasons not directly related to lack of synthesis; arginine is synthesized by mammalian cells but at a rate that is insufficient to meet the growth needs of the body and the majority that is synthesized is cleaved to form urea, methionine is required in large amounts to synthesize cysteine if the latter amino acid is not adequately supplied in the diet. Similarly, phenylalanine is needed in large amounts to form tyrosine if the latter is not adequately supplied in the diet.

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pKa Values of "R" Groups and Backbone of Amino Acids

Acid/Base Group	*Approximate pKa*
α-amino	9.8
α-carboxylate	2.5
Side chain amino group of lysine and arginine	11.5
Side chain carboxylate of glutamate and aspartate	4.4
Imidazole ring of histidine	6.5
Sulfhydryl group of cysteine	8.5
Alcohol groups of serine and threonine	1 and 14
Phenol group of tyrosine	10

The ionizable groups in R-side chain, change their protonation according to the pH of the surrounding solution.

At any pH more than 1.0 unit above or 1.0 unit below their pKa, the group will be completely in one state. It means these groups can undergo protonation or deprotonation within a pH unit of 1.0 on either side of its pKa value.

It is only the imidazole groups of histidyl residues in proteins that function as buffer at physiological range of pH because of its pKa value of 6.5.

Isoelectric Point

Isoelectric point (pI) is the pH at which an amino acid is electrically neutral (the sum of the negative charges equals the sum of the positive charges).

All those amino acids having only two ionizable groups, the pI is the average of the sum of pK1 and pK2.

In case of those having three ionizable groups, the pI is the average of the sum of two nearest pKs.

Properties of "R" Groups in Amino Acids

- *Optical activity:* all amino acids except glycine are optically active, since they have an asymmetric carbon.
- *Light absorption property:* phenylalanine, tyrosine and tryptophan, which have aromatic ring on their side chain, absorb light at 280 nm.
- Amino acids having –OH group on their side chain forms *esters with phosphoric acid* and *O-glycosidic bond* with carbohydrate.
- Amino acids glutamine and asparagine having amide groups on their side chain form *N-glycosidic* bond with carbohydrates.
- The amino group on the side chain of lysine form *Schiff's base (aldimine)* with aldehyde groups of other molecules.
- Sulfhydryl group of cysteine forms *disulfide bond* with sulfhydryl group of another cysteine molecule.
- The imidazole group of histidine, which is fully deprotonated at physiological pH, *acts as a Base*.
- Cyclic structures of proline side chain causes steric constrain to shape and are *helical breakers* in proteins.

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- Methyl group of active methionine (SAM) participate in *transmethylation reactions.*

REACTIONS OF AMINO ACIDS

All free amino acids excluding proline share similar chemical reactivities due to the presence of α-amino and α-carboxyl groups in their structure. The "R" groups of different amino acid have different chemical reactivities. Therefore, reactivities of different proteins reflect the composition and the unique amino acid sequence in their structure.

The Reactions of Carboxyl and Amino Group

The α-carboxyl and α-amino groups of all amino acids exhibit similar chemical reactivity. The side chains, however, exhibit specific chemical reactivities, depending on the nature of functional groups. Whereas all of these reactivities are important in the study and analysis of isolated amino acids, it is the characteristic behavior of the side chain that governs the reactivity of amino acids incorporated into proteins. There are three reasons to consider these reactivities:

1. Proteins can be chemically modified in very specific ways by taking advantage of the chemical reactivity of certain amino acid side chains.
2. The detection and quantification of amino acids and proteins often depend on reactions that are specific to one or more amino acids and that result in color, radioactivity, or some other quantity that can be easily measured.
3. Finally and most importantly, the biological functions of proteins depend on the behavior and reactivity of specific R groups.

The carboxyl groups of amino acids undergo all the simple reactions common to this functional group (Fig. 4.8). Reaction with

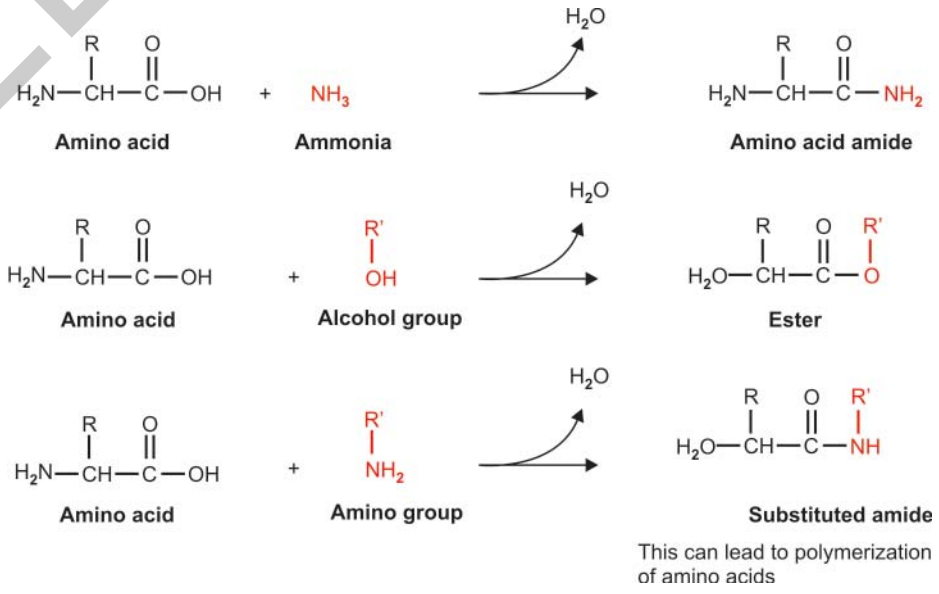

FIGURE 4.8: Typical reactions of the carboxyl groups of amino acids

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ammonia and primary amines yields unsubstituted and substituted amides, respectively. Esterification proceeds in the presence of the appropriate alcohol and a strong acid. Free amino groups may react with aldehydes to form Schiff bases and can be acylated with acid anhydrides and acid halides.

The Ninhydrin Reaction

Amino acids can be readily detected and quantified by reaction with ninhydrin. *Ninhydrin*, or triketohydrindene hydrate, is a strong oxidizing agent and causes the oxidative deamination of the α-amino function. The products of the reaction are the resulting aldehyde, ammonia, carbon dioxide, and hydrindantin, a reduced derivative of ninhydrin. The ammonia produced in this way can react with the hydrindantin and another molecule of ninhydrin to yield a purple product (Ruhemann's Purple) that can be quantified spectrophotometrically at 570 nm. The appearance of CO_2 can also be monitored. Indeed, CO_2 evolution is diagnostic of the presence of α-amino acid. Alpha-imino acids, such as proline and hydroxyproline, give bright yellow ninhydrin products with absorption maxima at 440 nm, allowing these to be distinguished from the α-amino acids. Because amino acids are one of the components of human skin secretions, the ninhydrin reaction was once used extensively by law enforcement and forensic personnel for fingerprint detection. (Fingerprints as old as 15 years can be successfully identified using the ninhydrin reaction.) More sensitive fluorescent reagents are now used routinely for this purpose.

PEPTIDE BOND

The condensation reaction between α-carboxyl carbon of one amino acid and the α-amino nitrogen of the other amino acid with elimination of a water molecule forms a peptide bond. Series of such condensation reactions lead to the polymerization of amino acids into peptides and proteins. Peptides are small amino acid polymers consisting of few amino acids. Proteins are polypeptides of highly divergent length. The simplest peptide, a *dipeptide* has only one peptide bond formed between two amino acids.

The peptide bond is slightly shorter than a standard single bond, reflecting the partial delocalization of *Phi electrons* from the carbonyl group into orbital shared with the *lone pair* electrons of the amide nitrogen. This *partial double-bond character* inhibits rotation around the peptide bond; thus, the four atoms bound to the carbonyl carbon and amide nitrogen form a plane (Fig. 4.9).

A polypeptide chain may be considered as a series of planes with two angles of rotation between each plane (Fig. 4.10); one between C-N bond and the other between C-C bond.

Allowed values of angles Phi (ϕ) and Psi (ψ) are severely restricted by steric interference (Fig. 4.9). Owing to steric interference between consecutive side groups on a polypeptide chain, the *trans*

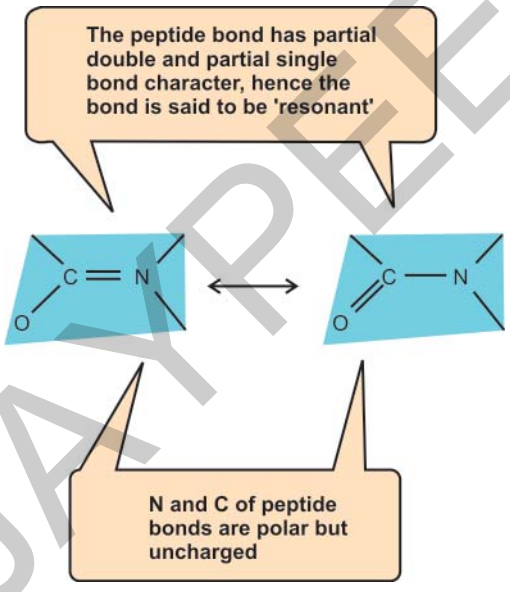

FIGURE 4.9: Partial double-bond character of peptide

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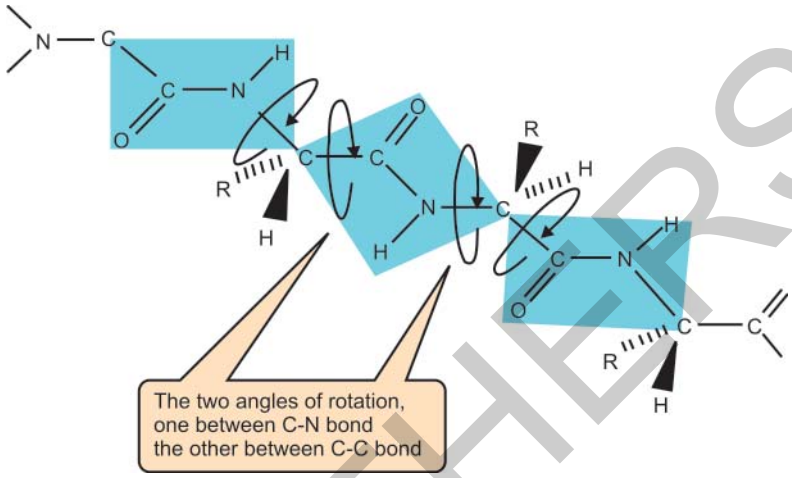

FIGURE 4.10: Angles of rotation in a peptide bond

conformation of the peptide bond is strongly favored over the *cis* conformation (Fig. 4.11).

Peptide bonds are rigid planar structures that confer space and shape limitations. Bonds on either side of peptide bond can rotate, which is important in both α-helix and β-sheet. The peptide bond is in trans conformation. The N and C of peptide bond are polar but uncharged.

Polypeptides have 10 to 2000 amino acyl residues.

Backbone structure is the peptide-linked structure (not the side chain), begins with an α-amino group and ends with an α-carboxylate group.

Proteins contain regions (domains) rich in either hydrophobic or hydrophilic residues.

By convention amino acid sequence is read starting from N-terminal end to C-terminal end.

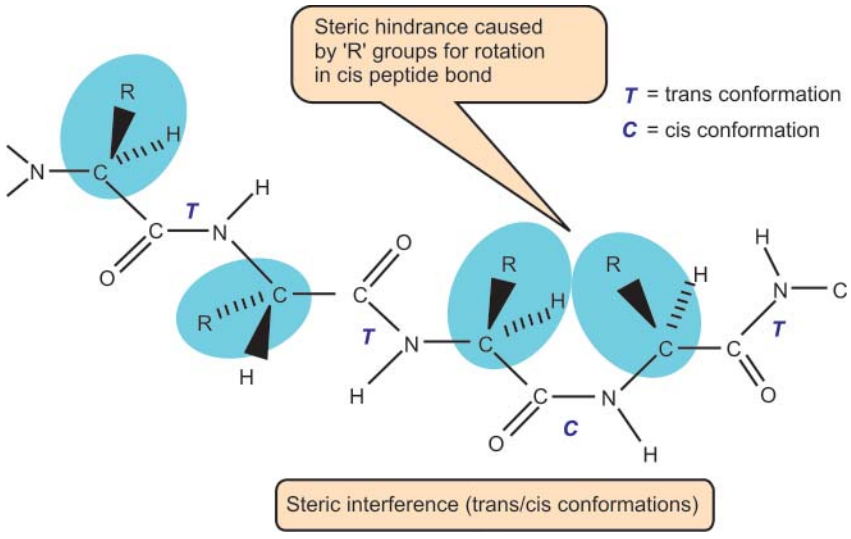

FIGURE 4.11: cis and trans conformation in polypeptide chain

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Examples of Proteins

i. Collagen has 3 polypeptide chains, each with 1052 amino acyl residues and similar sequence.
The unit is described as *homotrimeric*.
ii. Alcohol dehydrogenase has 2 subunits, each with 374 amino acid residues and similar sequence. The unit is described as *homodimeric*.
iii. Insulin has 2 chains with dissimilar amino acid content and sequence hence called *heterodimeric*.
iv. Glucagons has 1 chain, is called monomeric.

Examples of Functional Peptides

The ability of glutathione (γ-glutamylcysteinylglycine) a tripeptide to undergo reversible oxidation-reduction process is important for many biological functions.

Antibiotics such as gramicidin, bacitracin and actinomycin are also peptides.

Hormones such as vasopressin, oxytocin, angiotensin, TRH, bradykinin and kallidin are all peptides.

Proteins have Diversity – How?

Proteins have diversity due to the possible diversity in its amino acid sequence, and the post-translational modifications the proteins undergo.

Diversity in amino acid sequence is possible as a consequence to the statistical potential, where a choice of 20 amino acids are available for each position in protein, and due to the presence of two copies for each gene in eukaryotes (humans have 23 types of chromosomes and one pair of each type – a total of 46 chromosomes). This makes it possible to have 2 slightly different versions of each gene – one on each chromosome, which leads to production of 2 slightly different versions of the same protein.

Example: Sickle cell anemia where the difference between the mutant protein and the normal protein is one amino acid. An individual can have 2 normal copies, or 1 normal and 1 mutant, or 2 mutant copies.

Diversity due to post-synthetic modification is possible because many of the amino acids in a protein chain undergo post-synthetic modifications, to enhance their activity. Such a modification is called post-translational modification of protein. *Examples* are:

i. Hydroxyproline and hydroxylysine found in collagen are formed by hydroxylation of prolyl and lysyl residues of collagen fiber. Inadequate hydroxylation of prolyl and lysyl residues due to vitamin C deficiency causes scurvy.
ii. *gamma-carboxyglutamate* found in prothrombin and some other proteins involved in blood clotting is formed by γ-carboxylation of glutamyl residues. An inadequate carboxylation of glutamyl residues in prothrombin as a result of vitamin K deficiency causes hemorrhage.

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iii. *Addition of carbohydrate groups* on secreted proteins such as hormones and antibodies makes them more hydrophilic (water-soluble).
iv. *Addition of fatty acids* and *farnesyl groups* makes protein more hydrophobic.
v. *Phosphoserine* and *phosphotyrosine* found in intracellular proteins are involved in growth and cell division.
vi. *Selective cleavage* of synthesized proteins that are secreted as inactive precursors makes them active proteins. *Examples*: fibrinogen to fibrin, chymotrypsinogen to chymotrypsin, and HIV proteins arise from selective cleavage.

FUNCTIONS OF PROTEINS

Proteins are the products of expression of genetic information. Chemically they are linear, unbranched polymers of amino acids, wherein the amino acids are linked by peptide bonds formed between α-carboxyl of one amino acid and the α-amino group of another amino acid.

Proteins are involved in diverse activities of the living such as:

1. *Catalysts* in metabolic pathways. *Example*: enzymes.
2. Transporters and *storage*. *Example*: oxygen is transported by hemoglobin. Iron is stored as ferritin.
3. *Structural component*. *Example*: collagen.
4. *Coordinated motion*. *Example*: movement of flagella and cilia, movement of microtubules during mitosis, and muscle contraction.
5. *Decoding of genetic information*. *Example*: protein biosynthesis (translation and gene expression).
6. *Control of growth and differentiation*. *Example*: promoters and repressors.
7. *Immune protection*. *Example*: antibodies.
8. Nerve impulse generation and *transmission*. *Example*: receptors.

PROTEIN STRUCTURE

Protein structure has three-dimensional (3D) shape.

The 3D shape of protein is important for its function. The 3D shape of protein is the result of different levels of structural organization of polypeptide chain. The protein molecules can have four levels of structural organization – *primary, secondary, tertiary* and *quaternary*.

Primary structure is the content and sequence of amino acids held together by peptide bonds in a polypeptide chain.

Secondary structure is the spatial arrangement of amino acids near to each other in the linear sequence of α-helix, β-pleated sheet, or collagen helix, stabilized by mostly noncovalent bonds such as hydrogen bonds.

Tertiary structure is the spatial arrangement of amino acids that are far from each other in the linear sequence of peptide chain, which cause further folding and bending of the chain.

Quaternary structure is the ordered aggregation of two or more subunits by noncovalent bonds to provide a functional unit.

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PRIMARY STRUCTURE

The sequence of amino acids in a polypeptide chain is dictated by nucleotide sequence in the gene that codes for specific protein.

The ordered sequence enables secondary, tertiary and quaternary structures of the protein.

Alterations in nucleotide sequence (mutations) in the gene alter the amino acid sequence that go into protein, which in turn alter the folding and function of protein. *Example*: sickle cell anemia.

Proteins from different species with same function have conserved structure (homology). *Example*: 38 out of 105 residues in cytochrome C from 34 species are invariant and many other residues have conservative substitutes (Glu for Asp). *What conclusion*?

The invariant residues must be vital to the function of this enzyme.

SECONDARY STRUCTURE

The polypeptide chain can fold only in one of the three common ways, i.e. α-helix, β-sheet and collagen helix. The primary sequence of amino acid residues determines which way the peptide chain will fold.

The α-Helix (Fig. 4.12)

- Peptide backbone is coiled in such a way the hydrophilic side chains (R-groups) stick out, whereas hydrophobic R-groups face the interior of helical structure.
- Helix is right handed spiral conformation known as the 'α-helix'.
- The structure repeats itself every 5.4 Angstroms along the helix axis, i.e. we say that the α-helix has a pitch of 5.4 Angstroms. Alpha-helices have 3.6 amino acid residues per turn, i.e. a helix

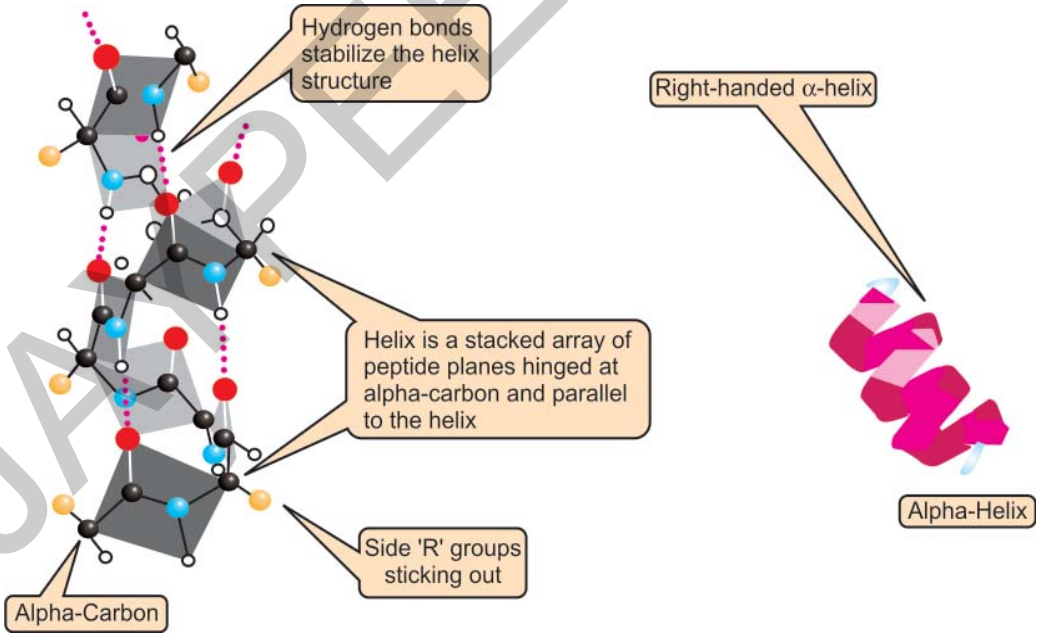

FIGURE 4.12: Alpha-helix

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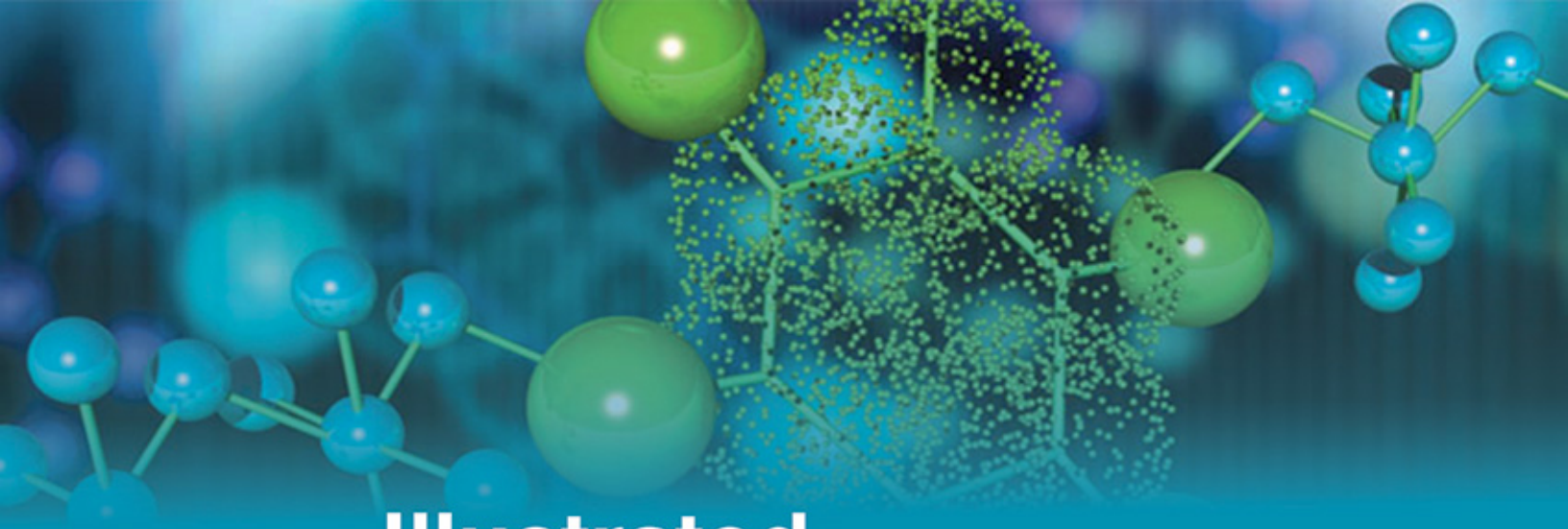


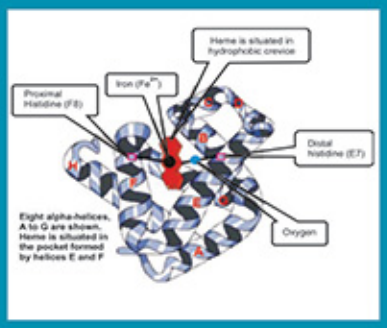
Illustrated Medical Biochemistry

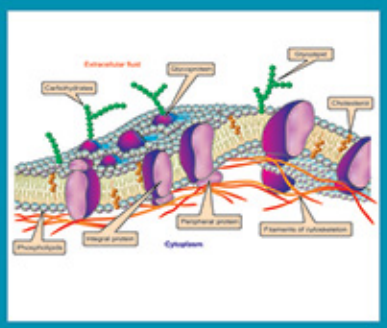
Salient Features

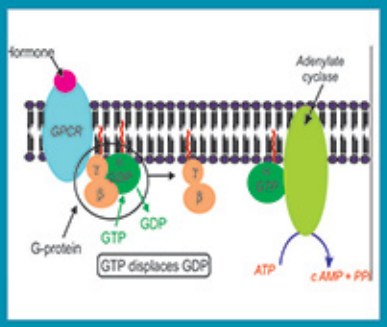
- Correlation to other allied subjects is provided in the form of 'bridges'.
- Biochemistry as related to *human biochemistry* is discussed wherever necessary.
- Text is illustrated with a total of 404 colored figures.
- *74 new figures* with illustrations are included.
- Many illustrations are redrawn.
- Text content is rewritten.

SM Raju at present is professor and heading the department of Biochemistry at Rohilkhand Medical College, Bareilly, UP, India. He is a medical teacher with 35 years of teaching experience at various Medical Colleges in the country. He has provided expertise for biochemistry e-learning launched by MAHE Manipal. This apart he has authored about eight books on different subjects for BSc Nursing students. His first edition of illustrated Medical Biochemistry is also published and marketed by Anshan publishers of Trentbridge, United Kingdom and has been received wide popularity across the world.

Bindu Raju is a medical doctor practicing internal medicine in USA for the past 10 years. Earlier she was an associate professor in internal medicine at Scott and White hospital, Temple, Texas. She is also the co-author for four books authored by SM Raju.







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ISBN 978-81-8448-872-2