



## 4

# PROTEINS

## 4.1 INTRODUCTION

Proteins are the most abundant biological macromolecules, occurring in all cells and all parts of cells. Amino acids are the building blocks of proteins. All proteins, whether from the most ancient lines of bacteria or from the most complex forms of life, are constructed from the same set of 20 amino acids. What is most remarkable is that cells can produce proteins with strikingly different properties and activities by joining the same 20 amino acids in many different combinations and sequences. From these building blocks different organisms can make such widely diverse products as enzymes, hormones, antibodies, transporters, muscle fibers, the lens protein of the eye, feathers, spider webs, rhinoceros horn, milk proteins, antibiotics, and mushroom poisons and other substances having distinct biological activities. While proteins contain only L- $\alpha$ -amino acids, microorganisms elaborate peptides that contain both D- and L- $\alpha$ -amino acids.



## OBJECTIVES

After reading this lesson, you will be able to

- describe amino acids
- explain the structure of amino acids
- classify amino acids
- describe proteins
- describe the structure of protein
- explain the function of proteins
- explain the digestion and absorption of proteins
- describe products of amino acids
- explain transamination, Deamination, Urea Cycle

## 4.2 AMINO ACIDS



## Notes

Proteins are the essential agents of biological function, and amino acids are the building blocks of proteins. The diversity of the thousands of proteins found in nature arises from the commonly occurring 20 amino acids. Proteins are polymers of amino acids, with each amino acid residue joined to its neighbor by a specific type of covalent bond. Proteins can be broken down (hydrolyzed) to their constituent amino acids the free amino acids derived from them. Of the over 300 naturally occurring amino acids, 20 constitute the monomer units of proteins. All 20 amino acids (Table 4.1) are biologically essential. Humans can synthesize 12 (nutritionally nonessential) of the 20 common amino acids from the amphibolic intermediates of glycolysis and of the citric acid cycle. Of the 12 nutritionally nonessential amino acids, nine are formed from amphibolic intermediates and three (cysteine, tyrosine and hydroxylysine) from nutritionally essential amino acids.

**Table 4.1** List of essential and nonessential amino acids

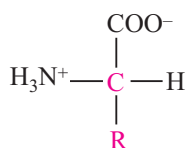
Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Aspartic acid
Lysine	Cysteine
Methionine	Glutamic acid
Phenylalanine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
	Tyrosine
	Asparagine
	Selenocysteine
	Pyrrolysine

Essential amino acids are "essential" not because they are more important to life than the others, but because the body does not synthesize them. They must be present in the diet or they will not be present in the body. In addition, the amino acids arginine, cysteine, glycine, glutamine, histidine, proline, serine and tyrosine are considered conditionally essential, meaning they are not normally required in the diet, but must be supplied exogenously to specific populations that do not synthesize them in adequate amounts.

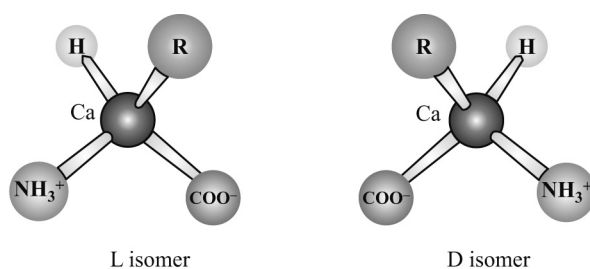
Selenocysteine, while not normally considered an amino acid present in proteins, selenocysteine occurs at the active sites of several enzymes. Examples include the human enzymes thioredoxin reductase, glutathione peroxidase, and the deiodinase that converts thyroxine to triiodothyronine. Pyrrolysine sometimes considered “the 22nd amino acid”, is not listed here as it is not used by humans.

#### 4.2.1 Amino Acids are Chiral Molecules

An  $\alpha$ -amino acid consists of a central carbon atom, called the  $\alpha$  carbon, linked to an amino group, a carboxylic acid group, a hydrogen atom, and a distinctive R group. For all the common amino acids except glycine, the  $\alpha$  carbon is bonded to four different groups: a carboxyl group, an amino group, an R group, and a hydrogen atom (Fig. 4.1; in glycine, the R group is another hydrogen atom). The  $\alpha$ -carbon atom is thus a **chiral center**. Because of the tetrahedral arrangement of the bonding orbitals around the  $\alpha$ -carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers. Since they are nonsuperimposable mirror images of each other (Fig. 4.2), the two forms represent a class of stereoisomers called **enantiomers** (Fig. 4.3). The R group is often referred to as the side chain. Enantiomeric molecules display a special property called **optical activity** – the ability to rotate the plane of polarization of plane-polarized light. Clockwise rotation of incident light is referred to as **dextrorotatory** (D) behavior, and counterclockwise rotation is called **levorotatory** (L) behavior. Only L amino acids are constituents of proteins. The magnitude and direction of the optical rotation depend on the nature of the amino acid side chain.



**Fig. 4.1:** General structure of an amino acid. This structure is common to all but one of the  $\alpha$ -amino acids. (Proline, a cyclic amino acid, is the exception.) The R group or side chain (red) attached to the  $\alpha$  carbon (blue) is different in each amino acid.



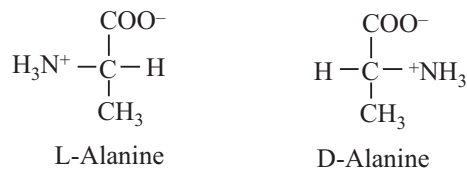
**Fig. 4.2:** The L and D Isomers of Amino Acids. R refers to the side chain. The L and D isomers are mirror images of each other.



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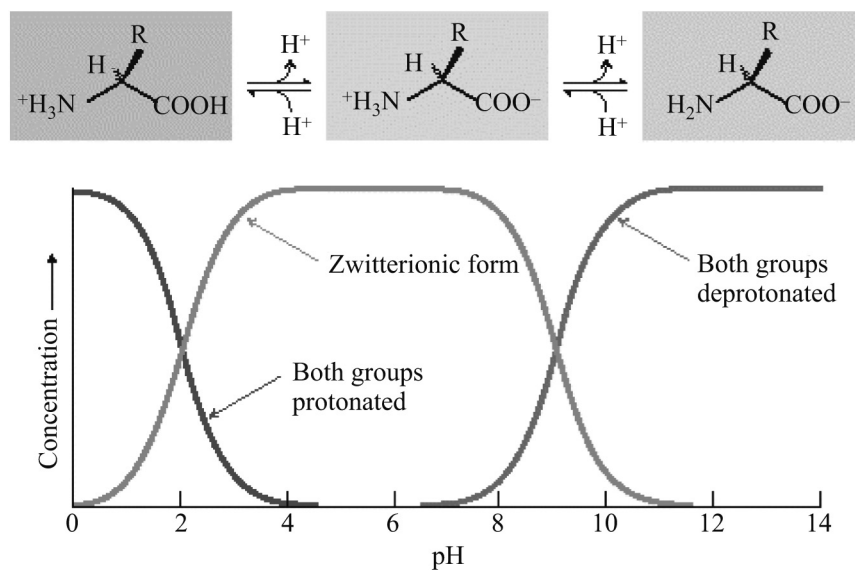
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**Fig. 4.3:** Stereoisomerism in  $\alpha$ -amino acids. The two stereoisomers of alanine, L- and D-alanine, are nonsuperimposable mirror images of each other (enantiomers).

### 4.2.2 Structure of a Typical Amino Acid

Amino acids in solution at neutral pH exist predominantly as dipolar ions (also called zwitterions). Amino acids can exist as zwitterions - substances containing equal numbers of positive and negative charge due to their carboxyl and amine groups, which can be negatively and positively charged, respectively. In the dipolar form, the amino group is protonated ( $\text{NH}_3^+$ ) and the carboxyl group is deprotonated ( $\text{COO}^-$ ). The ionization state of an amino acid varies with pH (Figure 4.4). They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water.



**Fig. 4.4:** Ionization State as a Function of pH. The ionization state of amino acids is altered by a change in pH. The zwitterionic form predominates near physiological pH.

### 4.2.3 Amino Acids can join via Peptide Bonds

The crucial feature of amino acids that allows them to polymerize to form peptides and proteins is the existence of their two identifying chemical groups:

the amino ( $\text{NH}_3^+$ ) and carboxyl ( $\text{COO}^-$ ) groups. The amino and carboxyl groups of amino acids can react in a head-to-tail fashion, eliminating a water molecule and forming a covalent amide linkage, which, in the case of peptides and proteins, is typically referred to as a peptide bond.

### 4.3 CLASSIFICATION

The structures and abbreviations for the 20 amino acids commonly found in proteins are shown in Figure 4.5. All the amino acids except proline have both free amino and free carboxyl groups. The classifications of amino acids is based on the polarity of the side chains. Thus, the structures shown in Figure 4.5 are grouped into the following categories: (1) nonpolar or hydrophobic amino acids, (2) neutral (uncharged) but polar amino acids, (3) acidic amino acids (which have a net negative charge at pH 7.0), and (4) basic amino acids (which have a net positive charge at neutral pH).

#### 4.3.1 Nonpolar Amino Acids

The nonpolar amino acids include all those with alkyl chain R groups (alanine, valine, leucine, and isoleucine), as well as proline (with its unusual cyclic structure), methionine (one of the two sulfur-containing amino acids), and two aromatic amino acids, phenylalanine and tryptophan. Tryptophan is sometimes considered a borderline member of this group because it can interact favorably with water via the N–H moiety of the indole ring. Proline, strictly speaking, is not an amino acid but rather an  $\alpha$ -imino acid.

#### 4.3.2 Polar, Uncharged Amino Acids

The polar, uncharged amino acids except for glycine contain R groups that can form hydrogen bonds with water. Thus, these amino acids are usually more soluble in water than the nonpolar amino acids. Tyrosine displays the lowest solubility in water of the 20 common amino acids. Glycine, the simplest amino acid, has only a single hydrogen for an R group, and this hydrogen is not a good hydrogen bond former. Glycine's solubility properties are mainly influenced by its polar amino and carboxyl groups, and thus glycine is best considered a member of the polar, uncharged group. It should be noted that tyrosine has significant nonpolar characteristics due to its aromatic ring and could arguably be placed in the nonpolar group.

#### 4.3.3 Acidic Amino Acids

There are two acidic amino acids – aspartic acid and glutamic acid – whose R groups contain a carboxyl group. Aspartic acid and glutamic acid thus have a net negative charge at pH 7. Many proteins that bind metal ions for structural





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or functional purposes possess metal binding sites containing one or more aspartate and glutamate side chains.

### 4.3.4 Basic Amino Acids

Three of the common amino acids have side chains with net positive charges at neutral pH: histidine, arginine, and lysine. The ionized group of histidine is an imidazolium, that of arginine is a guanidinium, and lysine contains a protonated alkyl amino group. Arginine and lysine side chains, which are protonated under physiological conditions, participate in electrostatic interactions in proteins.

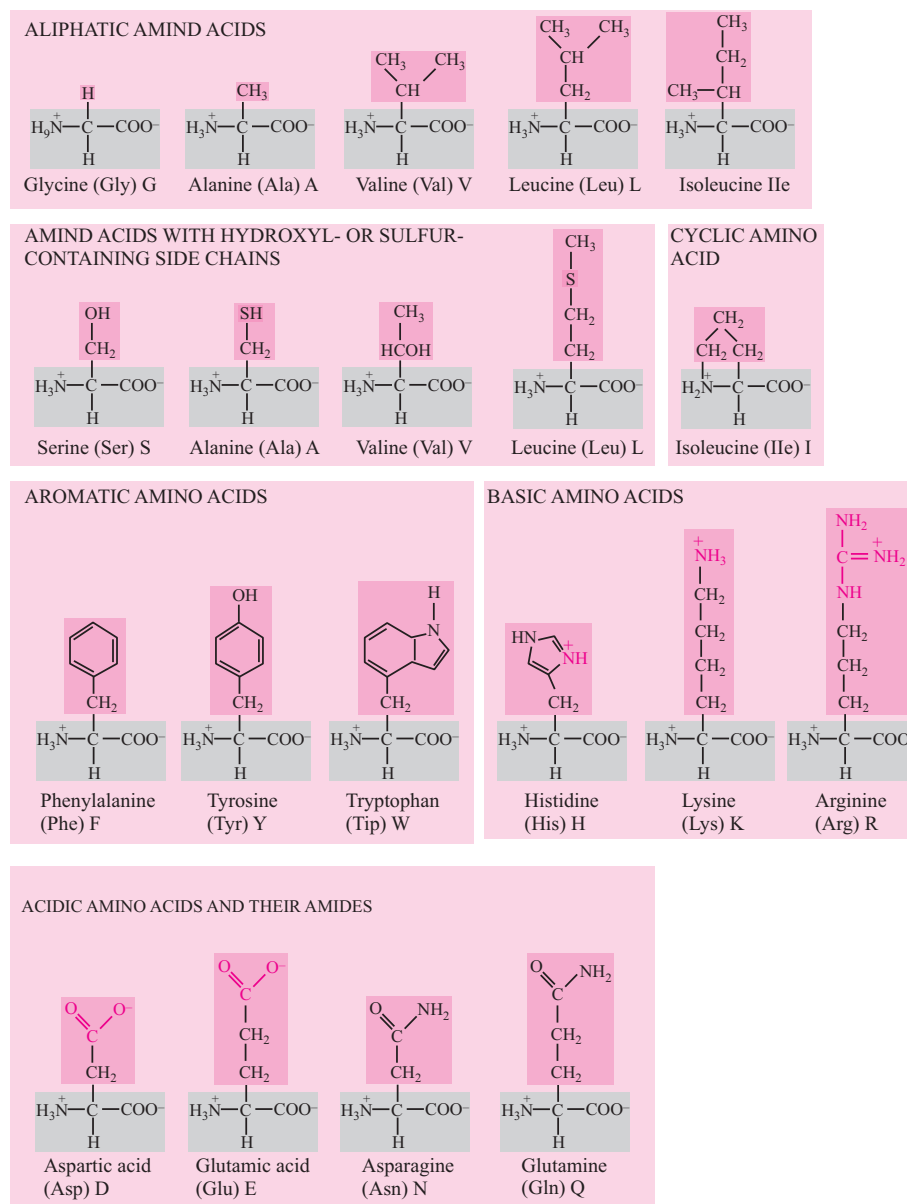


Fig. 4.5: Classification of amino acids.

## 4.4 PROTEIN

Proteins are a diverse and abundant class of biomolecules, constituting more than 50% of the dry weight of cells. This diversity and abundance reflect the central role of proteins in virtually all aspects of cell structure and function. Biologically occurring polypeptides range in size from small to very large, consisting of two or three to thousands of linked amino acid residues. Peptides are chains of amino acids, two amino acid molecules can be covalently joined through a substituted amide linkage, termed a peptide bond (Figure 4.6), to yield a dipeptide. Such a linkage is formed by removal of the elements of water (dehydration) from the  $\alpha$ -carboxyl group of one amino acid and the  $\alpha$ -amino group of another. Peptide bond formation is an example of a condensation reaction, a common class of reactions in living cells. Three amino acids can be joined by two peptide bonds to form a tripeptide; similarly, amino acids can be linked to form tetrapeptides, pentapeptides, and so forth. When a few amino acids are joined in this fashion, the structure is called an oligopeptide. When many amino acids are joined, the product is called a polypeptide. Proteins may have thousands of amino acid residues. Although the terms “protein” and “polypeptide” are sometimes used interchangeably, molecules referred to as polypeptides generally have molecular weights below 10,000, and those called proteins have higher molecular weights. Proteins can be assigned to one of three global classes on the basis of shape and solubility: fibrous, globular, or membrane.



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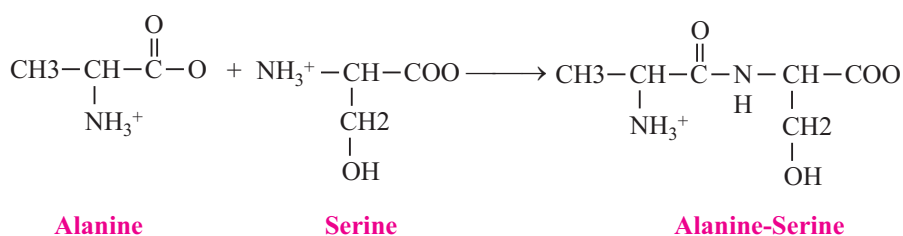


Fig. 4.6: Peptide bond formation between two amino acids Alanine and Serine.

**Fibrous proteins** tend to have relatively simple, regular linear structures. These proteins often serve structural roles in cells. Typically, they are insoluble in water or in dilute salt solutions. In contrast, **globular proteins** are roughly spherical in shape. The polypeptide chain is compactly folded so that hydrophobic amino acid side chains are in the interior of the molecule and the hydrophilic side chains are on the outside exposed to the solvent, water. **Membrane proteins** are found in association with the various membrane systems of cells. For interaction with the nonpolar phase within membranes, membrane proteins have hydrophobic amino acid side chains oriented outward. As such, membrane proteins are insoluble in aqueous solutions but can be solubilized in solutions of detergents.

## 4.5 THE LEVELS OF PROTEIN STRUCTURE

The various levels of protein structural organization are defined as follows.

### 4.5.1 Primary Structure

The amino acid sequence is the primary ( $1^\circ$ ) structure of a protein, such as that shown in Figure 4.7.

### 4.5.2 Secondary Structure

Through hydrogen bonding interactions between adjacent amino acid residues the polypeptide chain can arrange itself into characteristic helical or pleated segments. These segments constitute structural conformities, so-called regular structures that extend along one dimension, like the coils of a spring. Such architectural features of a protein are designated secondary ( $2^\circ$ ) structures (Figure 4.7). Secondary structures are just one of the higher levels of structure that represent the three-dimensional arrangement of the polypeptide in space.

### 4.5.3 Tertiary Structure

When the polypeptide chains of protein molecules bend and fold in order to assume a more compact three-dimensional shape, a tertiary ( $3^\circ$ ) level of structure is generated (Figure 4.7). It is by virtue of their tertiary structure that proteins adopt a globular shape. A globular conformation gives the lowest surface to-volume ratio, minimizing interaction of the protein with the surrounding environment.

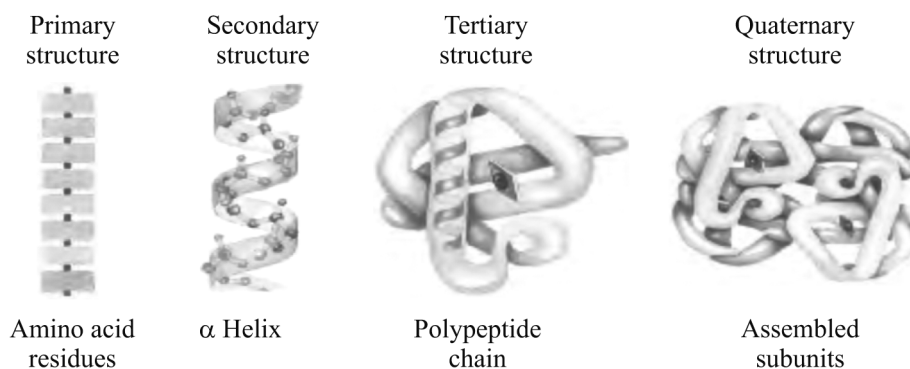


Fig. 4.7: Structures of protein.

### 4.5.4 Quaternary Structure

Many proteins consist of two or more interacting polypeptide chains of characteristic tertiary structure, each of which is commonly referred to as a subunit of the protein. Subunit organization constitutes another level in the



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hierarchy of protein structure, defined as the protein's quaternary (4°) structure (Figure 4.7). Whereas the primary structure of a protein is determined by the covalently linked amino acid residues in the polypeptide backbone, secondary and higher orders of structure are determined principally by noncovalent forces such as hydrogen bonds and ionic, van der Waals, and hydrophobic interactions.

## 4.6 FUNCTIONS OF PROTEINS

Proteins are the agents of biological function. Virtually every cellular activity is dependent on one or more particular proteins. Thus, a convenient way to classify the enormous number of proteins is by the biological roles they fill. The various functions of proteins are as follows.

### 4.6.1 Enzymes

By far the largest class of proteins is enzymes. More than 3000 different enzymes are listed in *Enzyme Nomenclature*, the standard reference volume on enzyme classification. **Enzymes** are catalysts that accelerate the rates of biological reactions. Each enzyme is very specific in its function and acts only in a particular metabolic reaction. Virtually every step in metabolism is catalyzed by an enzyme. Enzymes are systematically classified according to the nature of the reaction that they catalyze, such as the transfer of a phosphate group (phosphotransferase) or an oxidation–reduction (oxidoreductase). The formal names of enzymes come from the particular reaction within the class that they catalyze, as in ATP: D-fructose-6-phosphate 1-phosphotransferase. Often, enzymes have common names in addition to their formal names. ATP: D-fructose-6-phosphate 1-phosphotransferase is more commonly known as phosphofructokinase (kinase is a common name given to ATP-dependent phosphotransferases).

### 4.6.2 Regulatory Proteins

A number of proteins do not perform any obvious chemical transformation but nevertheless can regulate the ability of other proteins to carry out their physiological functions. Such proteins are referred to as regulatory proteins. A well-known example is insulin, the hormone regulating glucose metabolism in animals. Insulin is a relatively small protein and consists of two polypeptide chains held together by disulfide cross-bridges. Other hormones that are also proteins include pituitary somatotropin and thyrotropin, which stimulates the thyroid gland.

### 4.6.3 Transport Proteins

A third class of proteins is the transport proteins. These proteins function to transport specific substances from one place to another. One type of transport



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is exemplified by the transport of oxygen from the lungs to the tissues by haemoglobin or by the transport of fatty acids from adipose tissue to various organs by the blood protein serum albumin. Membrane transport proteins take up metabolite molecules on one side of a membrane, transport them across the membrane, and release them on the other side. Examples include the transport proteins responsible for the uptake of essential nutrients into the cell, such as glucose or amino acids.

#### 4.6.4 Storage Proteins

Proteins whose biological function is to provide a reservoir of an essential nutrient are called storage proteins. Because proteins are amino acid polymers and because nitrogen is commonly a limiting nutrient for growth, organisms have exploited proteins as a means to provide sufficient nitrogen in times of need. For example, ovalbumin, the protein of egg white, provides the developing bird embryo with a source of nitrogen during its isolation within the egg. Casein is the most abundant protein of milk and thus the major nitrogen source for mammalian infants. The seeds of higher plants often contain as much as 60% storage protein to make the germinating seed nitrogen-sufficient during this crucial period of plant development. In corn (*Zea mays* or maize), a family of low molecular weight proteins in the kernel called zeins serve this purpose. Ferritin is a protein found in animal tissues that binds iron, retaining this essential metal so that it is available for the synthesis of important iron-containing proteins such as hemoglobin.

#### 4.6.5 Contractile and Motile Proteins

Certain proteins endow cells with unique capabilities for movement. Cell division, muscle contraction, and cell motility represent some of the ways in which cells execute motion. Examples include actin and myosin, the filamentous proteins forming the contractile systems of cells, and tubulin, the major component of microtubules.

#### 4.6.6 Structural Proteins

An apparently passive but very important role of proteins is their function in creating and maintaining biological structures. Structural proteins provide strength and protection to cells and tissues. Monomeric units of structural proteins typically polymerize to generate long fibers (as in hair).  $\alpha$ -Keratins are insoluble fibrous proteins making up hair, horns, and fingernails. Collagen, another insoluble fibrous protein, is found in bone, connective tissue, tendons, and cartilage, where it forms inelastic fibrils of great strength. One-third of the total protein in a vertebrate animal is collagen. A structural protein having elastic properties is, appropriately, elastin, an important component of ligaments.

Certain insects make a structurally useful protein, fibroin (a  $\alpha$ -keratin), the major constituent of cocoons (silk) and spider webs.

#### 4.6.7 Scaffold Proteins (Adapter Proteins)

Some proteins play a recently discovered role in the complex pathways of cellular response to hormones and growth factors. These proteins, the scaffold or adapter proteins, have a modular organization in which specific parts (modules) of the protein's structure recognize and bind certain structural elements in other proteins through protein-protein interactions.

#### 4.6.8 Protective and Exploitive Proteins

In contrast to the passive protective nature of some structural proteins, another group can be more aptly classified as protective or exploitive proteins because of their biologically active role in cell defense, protection, or exploitation. Prominent among the protective proteins are the immunoglobulins or antibodies produced by the lymphocytes of vertebrates. Antibodies have the remarkable ability to specifically recognize and neutralize "foreign" molecules resulting from the invasion of the organism by bacteria, viruses, or other infectious agents. Another group of protective proteins is the blood-clotting proteins, thrombin and fibrinogen, which prevent the loss of blood when the circulatory system is damaged. Arctic and Antarctic fishes have antifreeze proteins to protect their blood against freezing in the below-zero temperatures of high-latitude seas. Another class of exploitive proteins includes the toxins produced by bacteria, such as diphtheria toxin and cholera toxin. It is worth repeating that the great diversity of function in proteins, as reflected is attained using just 20 amino acids.

### 4.7 DIGESTION AND ABSORPTION OF PROTEINS

Several groups of enzymes catalyze the digestion of proteins. There are two main classes of proteolytic digestive enzymes (**proteases**), with different specificities for the amino acids forming the peptide bond to be hydrolyzed. **Endopeptidases** hydrolyze peptide bonds between specific amino acids throughout the molecule. They are the first enzymes to act, yielding a larger number of smaller fragments, eg, **pepsin** in the gastric juice and **trypsin**, **chymotrypsin**, and **elastase** secreted into the small intestine by the pancreas. **Exopeptidases** catalyze the hydrolysis of peptide bonds, one at a time, from the ends of polypeptides. **Carboxypeptidases**, secreted in the pancreatic juice, release amino acids from the free carboxyl terminal, and **aminopeptidases**, secreted by the intestinal mucosal cells, release amino acids from the amino terminal. Dipeptides, which are not substrates for exopeptidases, are hydrolyzed in the brush border of intestinal mucosal cells by **dipeptidases**. The proteases are secreted as inactive



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**zymogens**; the active site of the enzyme is masked by a small region of its peptide chain, which is removed by hydrolysis of a specific peptide bond. **Pepsinogen** is activated to pepsin by gastric acid and by activated pepsin (autocatalysis). In the small intestine, **trypsinogen**, the precursor of trypsin, is activated by **enteropeptidase**, which is secreted by the duodenal epithelial cells; trypsin can then activate **chymotrypsinogen** to chymotrypsin, **proelastase** to elastase, **procarboxypeptidase** to carboxypeptidase, and **proaminopeptidase** to aminopeptidase.

#### 4.7.1 Free amino acids and small peptides are absorbed by different mechanisms

The end product of the action of endopeptidases and exopeptidases is a mixture of free amino acids, di- and tripeptides, and oligopeptides, all of which are absorbed. Free amino acids are absorbed across the intestinal mucosa by sodium-dependent active transport. There are several different amino acid transporters, with specificity for the nature of the amino acid side chain (large or small; neutral, acidic, or basic). Dipeptides and tripeptides enter the brush border of the intestinal mucosal cells, where they are hydrolyzed to free amino acids, which are then transported into the hepatic portal vein.

### 4.8 SPECIALIZED PRODUCTS OF AMINO ACIDS PHENYLALANINE, TYROSINE

#### 4.8.1 Phenylalanine

Phenylalanine is first converted to tyrosine. **Hyperphenylalaninemias** arise from defects in phenylalanine hydroxylase itself (**type I, classic phenylketonuria** or **PKU**), in dihydrobiopterin reductase (**types II and III**), or in dihydrobiopterin biosynthesis (**types IV and V**). DNA probes facilitate prenatal diagnosis of defects in phenylalanine hydroxylase or dihydrobiopterin reductase. A diet low in phenylalanine can prevent the mental retardation of PKU (frequency 1:10,000 births). Elevated blood phenylalanine may be detectable by a less reliable screening test that employs  $\text{FeCl}_3$  to detect urinary phenylpyruvate.  $\text{FeCl}_3$  screening for PKU of the urine of newborn infants is compulsory in the United States and many other countries.

#### 4.8.2 Tyrosine

The probable metabolic defect in **type I tyrosinemia (tyrosinosis)** is at **fumarylacetoacetate hydrolase**. Therapy employs a diet low in tyrosine and phenylalanine. Untreated acute and chronic tyrosinosis leads to death from liver failure. Alternate metabolites of tyrosine are also excreted in **type II tyrosinemia**

(**Richner-Hanhart syndrome**), a defect in **tyrosine aminotransferase**, and in **neonatal tyrosinemia**, due to lowered *p*-hydroxyphenylpyruvate hydroxylase activity. Therapy employs a diet low in protein.

#### 4.9 TRANSAMINATION (IMPORTANCE OF TRANSAMINASES)

The first step in the catabolism of most L-amino acids, once they have reached the liver, is removal of the  $\alpha$ -amino groups, promoted by enzymes called **aminotransferases** or **transaminases**. The effect of transamination reactions is to collect the amino groups from many different amino acids in the form of L-glutamate. The glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous waste products. Cells contain different types of aminotransferases. All aminotransferases have the same prosthetic group and the same reaction mechanism. The prosthetic group is **pyridoxal phosphate (PLP)**, the coenzyme form of pyridoxine, or vitamin B6. Its primary role in cells is in the metabolism of molecules with amino groups. Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of aminotransferases.

#### 4.10 DEAMINATION

While ammonia, derived mainly from the  $\alpha$ -amino nitrogen of amino acids, is highly toxic, tissues convert ammonia to the amide nitrogen of nontoxic glutamine. Subsequent deamination of glutamine in the liver releases ammonia, which is then converted to nontoxic urea. The deamination of amino acids leaves  $\alpha$ -keto acid carbon skeletons. Several of these  $\alpha$ -keto acids are citric acid cycle intermediates. Amino acids are used to synthesize liver and plasma proteins, or their carbon skeletons are converted to glucose and glycogen by gluconeogenesis; the ammonia formed by deamination is converted to urea. The  $\alpha$ -amino acids collected in the liver in the form of the amino group of L-glutamate molecules must be removed from glutamate to prepare them for excretion. In hepatocytes, glutamate is transported from the cytosol into mitochondria, where it undergoes **oxidative deamination** catalyzed by **L-glutamate dehydrogenase**. In mammals, this enzyme is present in the mitochondrial matrix.

#### 4.11 UREA CYCLE

Urea is the major end product of nitrogen catabolism in humans. Urea synthesis is a cyclic process. Synthesis of 1 mol of urea requires 3 mol of ATP plus 1 mol each of ammonium ion and of the  $\alpha$ -amino nitrogen of aspartate. Five enzymes





catalyze the numbered reactions of Figure 3.8. Of the six participating amino acids, N-acetylglutamate functions solely as an enzyme activator. The others serve as carriers of the atoms that ultimately become urea. The major metabolic role of **ornithine**, **citrulline**, and **argininosuccinate** in mammals is urea synthesis. Since the ornithine consumed in reaction 2 is regenerated in reaction 5, there is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine. Ammonium ion,  $\text{CO}_2$ , ATP, and aspartate are, however, consumed. Some reactions of urea synthesis occur in the matrix of the mitochondrion, other reactions in the cytosol.

#### 4.11.1 Carbamoyl phosphate synthase I initiates Urea biosynthesis

Condensation of  $\text{CO}_2$ , ammonia, and ATP to form **carbamoyl phosphate** is catalyzed by mitochondrial **carbamoyl phosphate synthase I**. Carbamoyl phosphate synthase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of its allosteric activator **N-acetylglutamate**, which enhances the affinity of the synthase for ATP.

#### 4.11.2 Carbamoyl phosphate plus Ornithine forms Citrulline

**L-Ornithine transcarbamoylase** catalyzes transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate. While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol.

#### 4.11.3 Citrulline plus Aspartate forms Argininosuccinate

**Argininosuccinate synthase** links aspartate and citrulline via the amino group of aspartate and provides the second nitrogen of urea. The reaction requires ATP and involves intermediate formation of citrullyl-AMP. Subsequent displacement of AMP by aspartate then forms citrulline.

#### 4.11.4 Cleavage of Argininosuccinate forms Arginine and Fumarate

Cleavage of argininosuccinate, catalyzed by **argininosuccinase**, proceeds with retention of nitrogen in arginine and release of the aspartate skeleton as fumarate.

#### 4.11.5 Cleavage of Arginine releases Urea and re-forms Ornithine

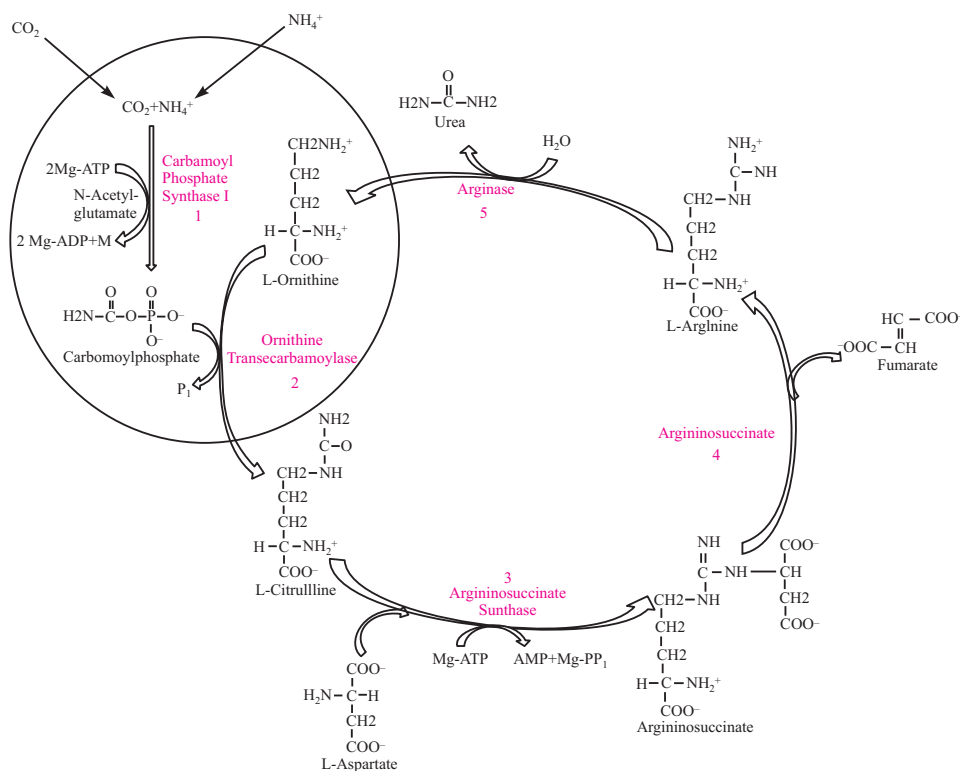
Hydrolytic cleavage of the guanidino group of arginine, catalyzed by liver arginase, releases urea. The other product, ornithine, reenters liver mitochondria for additional rounds of urea synthesis.

### 4.11.6 Carbamoyl phosphate synthase I is the pacemaker enzyme of the Urea cycle

The activity of carbamoyl phosphate synthase I is determined by N-acetylglutamate, whose steady-state level is dictated by its rate of synthesis from acetyl-CoA and glutamate and its rate of hydrolysis to acetate and glutamate. These reactions are catalyzed by N-acetylglutamate synthase and N-acetylglutamate hydrolase, respectively. Major changes in diet can increase the concentrations of individual urea cycle enzymes 10-fold to 20-fold. Starvation, for example, elevates enzyme levels, presumably to cope with the increased production of ammonia that accompanies enhanced protein degradation.



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**Fig. 4.8:** Reactions and intermediates of urea biosynthesis. Reactions 1 and 2 occur in the matrix of liver mitochondria and reactions 3, 4, and 5 in liver cytosol. CO<sub>2</sub> (as bicarbonate), ammonium ion, ornithine, and citrulline enter the mitochondrial matrix via specific carriers present in the inner membrane of liver mitochondria.





## Proteins

- |                           |                               |
|---------------------------|-------------------------------|
| 13. Storage protein       | (c) L-glutamate dehydrogenase |
| 14. Oxidative deamination | (d) proteases                 |
| 15. Proteolytic enzymes   | (e) Alanine                   |



## WHAT HAVE YOU LEARNT

- Both D-amino acids and non- $\alpha$ -amino acids occur in nature, but only L- $\alpha$ -amino acids are present in proteins.
- The 20 amino acids commonly found as residues in proteins contain an  $\alpha$ -carboxyl group, an  $\alpha$ -amino group, and a distinctive R group substituted on the  $\alpha$ -carbon atom. The  $\alpha$ -carbon atom of all amino acids except glycine is asymmetric, and thus amino acids can exist in at least two stereoisomeric forms.
- Amino acids can be joined covalently through peptide bonds to form peptides and proteins.
- Cells generally contain thousands of different proteins, each with a different biological activity.
- Differences in protein function result from differences in amino acid composition and sequence.
- Every protein has a three-dimensional structure that reflects its function. Tertiary structure is the complete three dimensional structure of a polypeptide chain. There are two general classes of proteins based on tertiary structure: fibrous and globular.
- Proteins are the agents of biological functions, virtually occurring in every cellular activity in the form of enzymes, regulatory proteins, transport proteins, storage proteins, contractile and motile proteins, structural proteins, adapter proteins, and protective and exploitive proteins.
- Proteins are digested at the intestinal region by several groups of proteolytic digestive enzymes (proteases), with different specificities for the amino acids forming the peptide bond to be hydrolyzed.
- Phenylalanine is first converted to tyrosine. The defects in phenylalanine hydroxylase leads to Hyperphenylalaninemia (type I, classic phenylketonuria or PKU). The metabolic defect in fumarylacetoacetate hydrolase leads to type I tyrosinemia (tyrosinosis). Alternate metabolites of tyrosine are also excreted in type II tyrosinemia (Richner-Hanhart syndrome), a defect in tyrosine aminotransferase.

## MODULE

Biochemistry



Notes

## MODULE

Biochemistry



Notes

## Proteins

- The catabolism of most L-amino acids in the liver is promoted by transamination and deaminations reactions. Glutamate functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous waste products.
- Urea is the major end product of nitrogen catabolism in humans. Urea synthesis is a cyclic process. Some reactions of urea synthesis occur in the matrix of the mitochondrion, other reactions in the cytosol.



## ANSWERS TO INTEXT QUESTIONS

### 4.1

- I.** 1. (b)      2. (a)      3. (c)      4. (a)      5. (c)
- II.** 6. Enteropeptidase  
7. Pyridoxine  
8. Mitochondrial matrix  
9. Carbamoyl phosphate synthase I  
10. Cytosol
- III.** 11. (b)      12. (e)      13. (a)      14. (c)      15. (d)