

<b>COURSE CODE:</b>	BCH 305
<b>COURSE TITLE:</b>	Metabolism of amino acids and proteins
<b>NUMBER OF UNITS:</b>	2 Units
<b>COURSE DURATION:</b>	<i>Two hours per week</i>

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### **COURSE DETAILS:**

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<b>Office Location:</b>	Room B 307, College of Natural Science

### **COURSE CONTENT:**

Amino acids as building blocks of proteins; covalent backbones of proteins. Primary structure of proteins. Reactions of amino acid side chains. Secondary, tertiary and quaternary structure of proteins. Isolation, fractionation, purification and characterization of proteins. Protein-protein interaction. Biological functions of protein-correlation of structure with function in a few specific proteins. Oxidative degradation of amino acids, Ammonia (NH<sub>4</sub>) transport. Biosynthesis of proteins.

### **COURSE REQUIREMENTS:**

This is a compulsory course for all 300 level Biochemistry students in the university who are expected to have passed BCH 201 and BCH 202. Students are expected to participate in all course activities and have minimum of 75% attendance to be able to write the final examination.

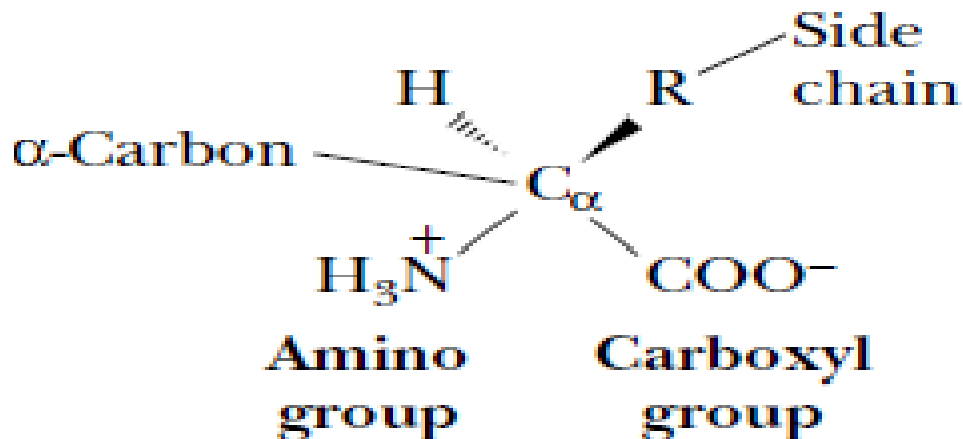
### **READING LIST:**

1. Lehninger, A. L. Principles of Biochemistry. 2nd ed. Worth, 1982
2. Nelson D.L. and Cox M. M. Lehninger Principles of Biochemistry. 4<sup>th</sup> ed. 2004
3. [www.whfreeman.com/lehninger](http://www.whfreeman.com/lehninger)
4. Garrett, R. H. and Grisham, C. M. Biochemistry 3<sup>rd</sup> ed. Brooks/Cole 2005

## LECTURE NOTES

### Amino Acids Are The Building Blocks of Proteins

Central to the structure of amino acids is the tetrahedral alpha ( $\alpha$ ) carbon ( $C_\alpha$ ), which is covalently linked to both the amino group and the carboxyl group. Also bonded to this  $\alpha$ -carbon is hydrogen and a variable side chain. It is the side chain, the so called R group that gives each amino acid its identity.

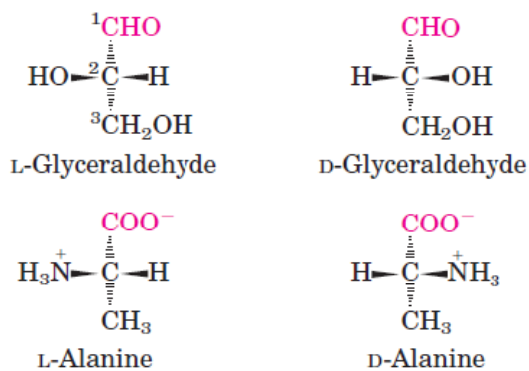


In neutral solution (pH 7), the carboxyl group exists as  $-COO^-$  and the amino group as  $-NH_3^+$ . Because the resulting amino acid contains one positive and one negative charge, it is a neutral molecule called a **zwitterion**. Amino acids are also *chiral* molecules. With four different groups attached to it, the  $\alpha$ -carbon is said to be *asymmetric*.

### The Amino Acid Residues in Proteins Are L Stereoisomers

Nearly all biological compounds with a chiral center occur naturally in only one stereoisomeric form, either D or L. The amino acid residues in protein molecules are exclusively L stereoisomers. D-Amino acid residues have been found only in a few, generally small peptides, including some peptides of bacterial cell walls and certain peptide antibiotics.

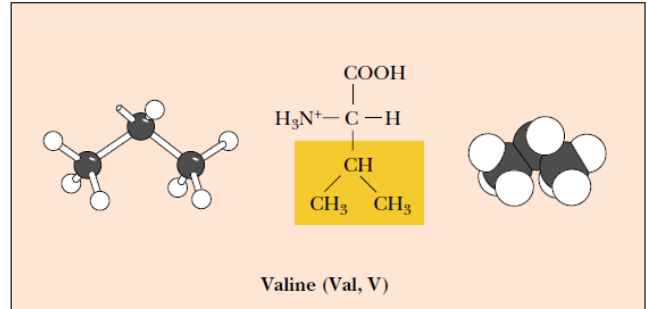
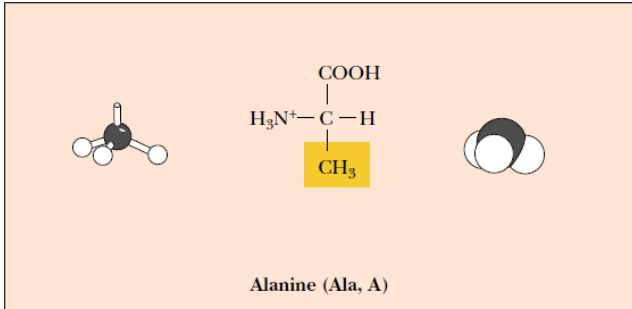
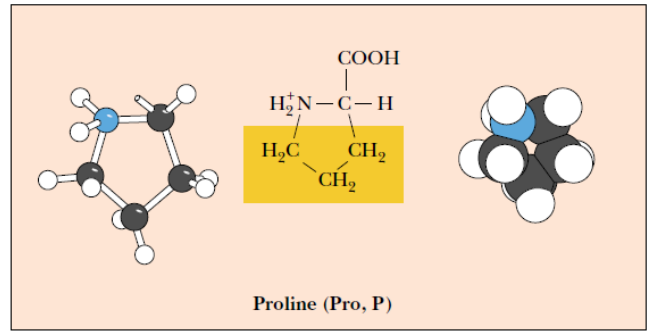
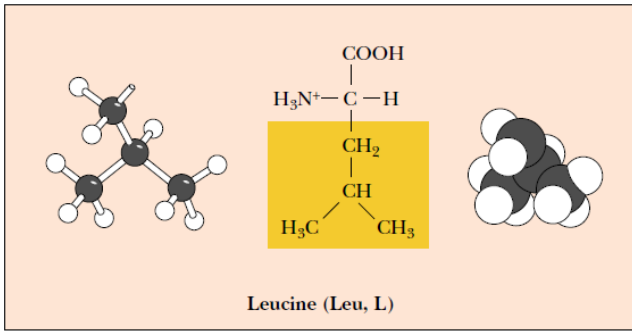
To a living system, D and L isomers are as different as the right hand and the left. The formation of stable, repeating substructures in proteins generally requires that their constituent amino acids be of one stereochemical series. Cells are able to specifically synthesize the L isomers of amino acids because the active sites of enzymes are asymmetric, causing the reactions they catalyze to be stereospecific.



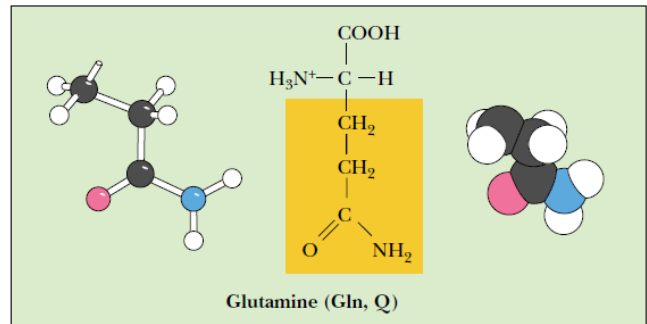
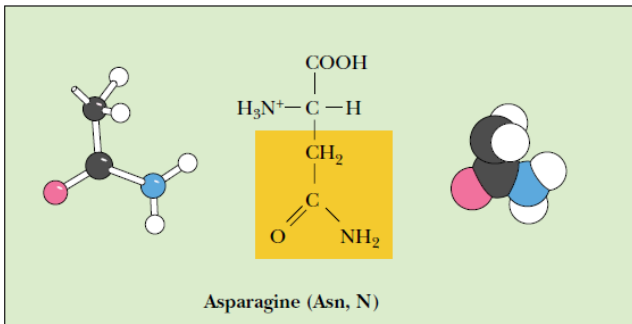
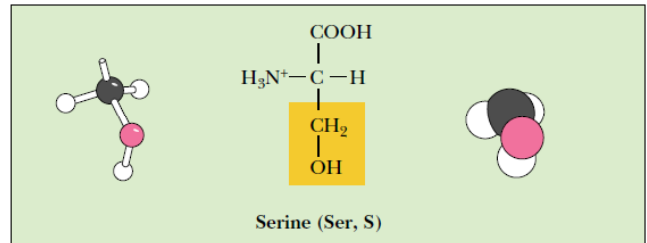
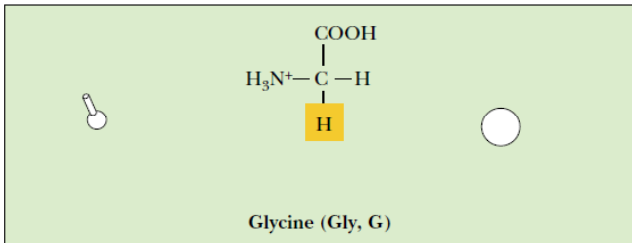
**Steric relationship of the stereoisomers of alanine to the absolute configuration of L- and D-glyceraldehyde.**

In these perspective formulas, the carbons are lined up vertically, with the chiral atom in the center. The carbons in these molecules are numbered beginning with the terminal aldehyde or carboxyl carbon (red), 1 to 3 from top to bottom as shown. When presented in this way, the R group of the amino acid (in this case the methyl group of alanine) is always below the  $\alpha$  carbon. L-Amino acids are those with the  $\alpha$ -amino group on the left, and D-amino acids have the  $\alpha$ -amino group on the right.

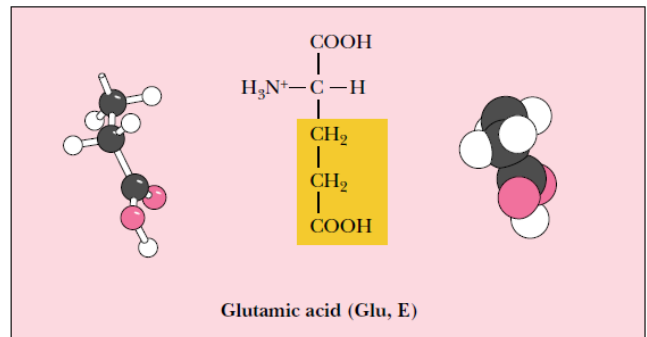
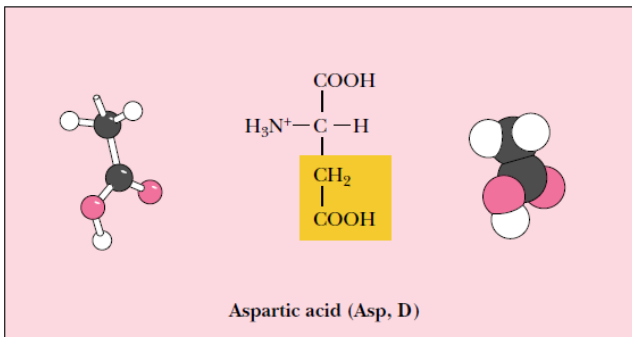
(a) Nonpolar (hydrophobic)

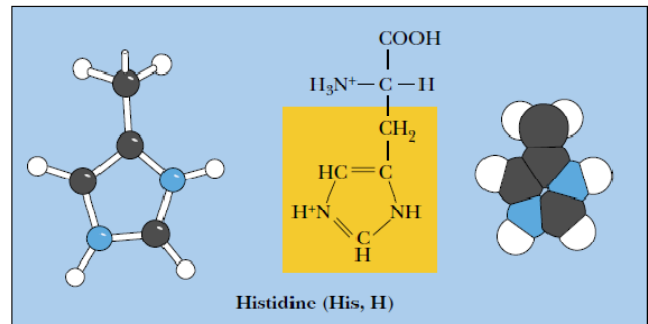
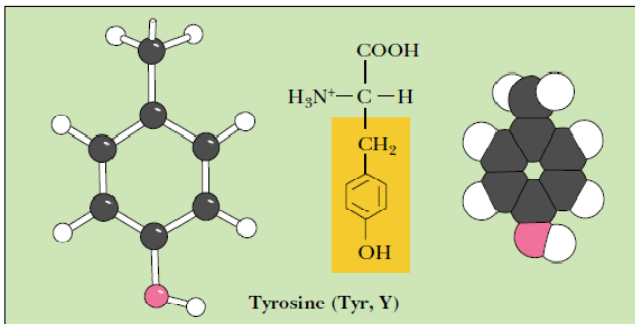
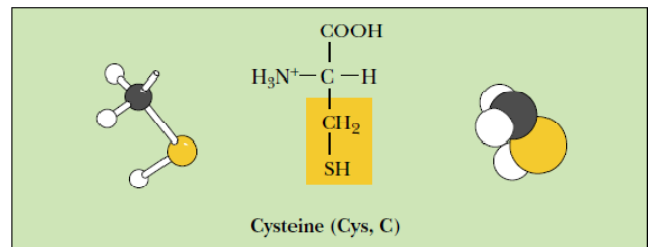
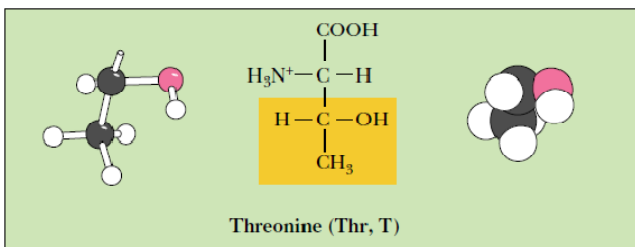
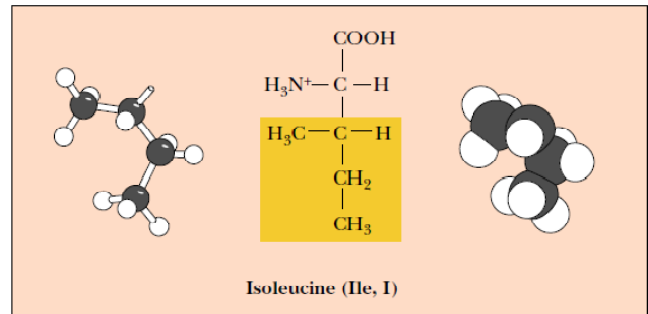
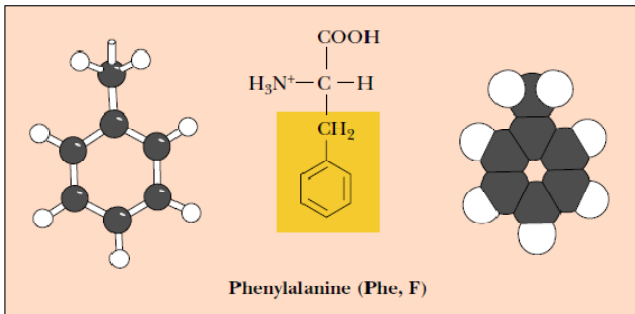
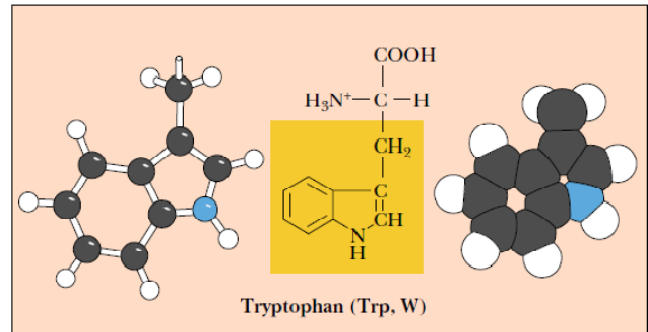
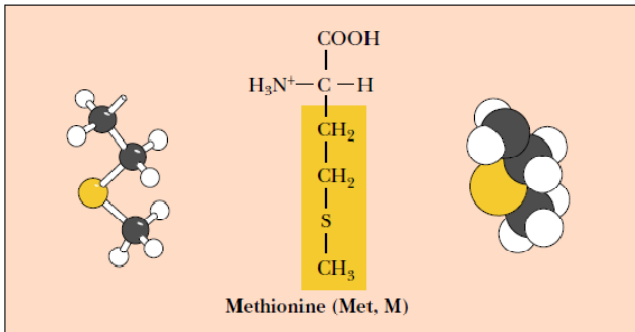


(b) Polar, uncharged

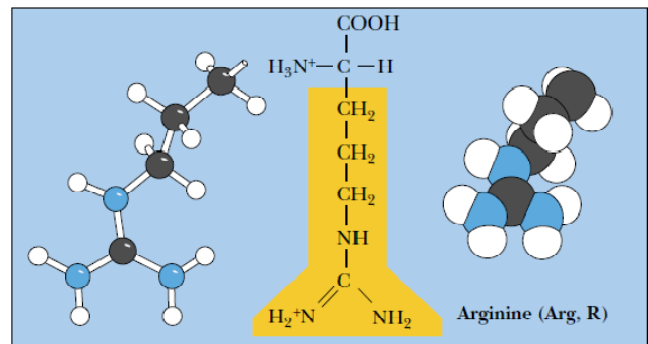
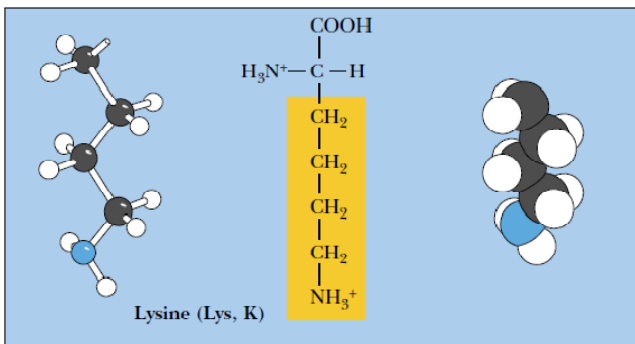


(c) Acidic





**(d) Basic**



\*The 20 amino acids that are building blocks of most proteins can be classified as (a) Nonpolar (Hydrophobic), (b) Polar, Neutral, (c) Acidic, or (d) Basic

### ***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.

### ***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. Several exceptions should be noted. Tyrosine displays the lowest solubility in water of the 20 common amino acids (0.453 g/L at 25°C). Also, proline is very soluble in water, and alanine and valine are about as soluble as arginine and serine. The amide groups of asparagine and glutamine; the hydroxyl groups of tyrosine, threonine, and serine; and the sulfhydryl group of cysteine are all good hydrogen bond-forming moieties. 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. However, with a  $pK_a$  of 10.1, tyrosine's phenolic hydroxyl is a charged, polar entity at high pH.

### ***Acidic Amino Acids***

There are two acidic amino acids—aspartic acid and glutamic acid—whose R groups contain a carboxyl group. These side chain carboxyl groups are weaker acids than the  $\alpha$ -COOH group, but are sufficiently acidic to exist as  $-\text{COO}^-$  at neutral pH. Aspartic acid and glutamic acid thus have a net negative charge at pH 7. These negatively charged amino acids play several important roles in proteins. Many proteins that bind metal ions for structural or functional purposes possess metal binding sites containing one or more aspartate and glutamate side chains. Carboxyl groups may also act as nucleophiles in certain enzyme reactions and may participate in a variety of electrostatic bonding interactions.

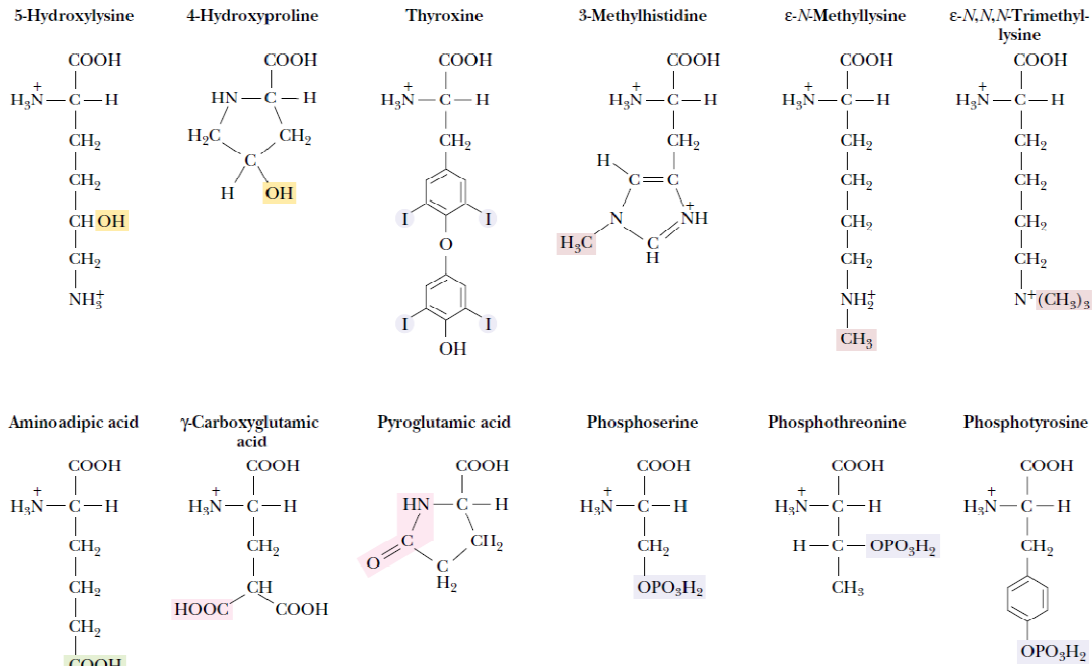
### ***Basic Amino Acids***

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. The side chains of the latter two amino acids are fully protonated at pH 7, but histidine, with a side chain  $pK_a$  of 6.0, is only 10% protonated at pH 7. With a  $pK_a$  near neutrality, histidine side chains play important roles as proton donors and acceptors in many enzyme reactions. Histidine-containing peptides are important biological buffers, Arginine and lysine side chains, which are protonated under physiological conditions, participate in electrostatic interactions in proteins.

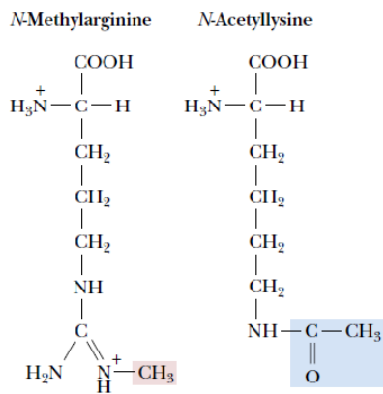
### ***Uncommon Amino Acids***

Several amino acids occur only rarely in proteins. These include **hydroxylysine** and **hydroxyproline**, which are found mainly in the collagen and gelatin proteins, and **thyroxine** and **3,3,5-triiodothyronine**, iodinated amino acids that are found only in thyroglobulin, a protein produced by the thyroid gland. (Thyroxine and 3,3,5-triiodothyronine are produced by iodination of tyrosine residues in thyroglobulin in the thyroid gland. Degradation of thyroglobulin releases these two iodinated amino acids, which act as hormones to regulate growth and development.) Certain muscle proteins contain methylated amino acids, including **methylhistidine**,  $\epsilon$ -**N-methyllysine**, and  $\epsilon$ -**N,N,N-trimethyllysine**.  $\gamma$ -**Carboxyglutamic acid** is found in several proteins involved in blood clotting, and **pyroglutamic acid** is found in a unique light-driven proton-pumping protein called bacteriorhodopsin. Certain proteins involved in cell growth and regulation are reversibly phosphorylated on the -OH groups of serine, threonine, and tyrosine residues. **Amino adipic acid** is found in

proteins isolated from corn. Finally, **N-methylarginine** and **N-acetyllysine** are found in histone proteins associated with chromosomes.



The structures of several amino acids that are less common but nevertheless found in certain proteins. Hydroxylysine and hydroxyproline are found in connective tissue proteins, pyroglutamic acid is found in bacteriorhodopsin (a protein in *Halobacterium halobium*), and amino adipic acid is found in proteins isolated from corn.

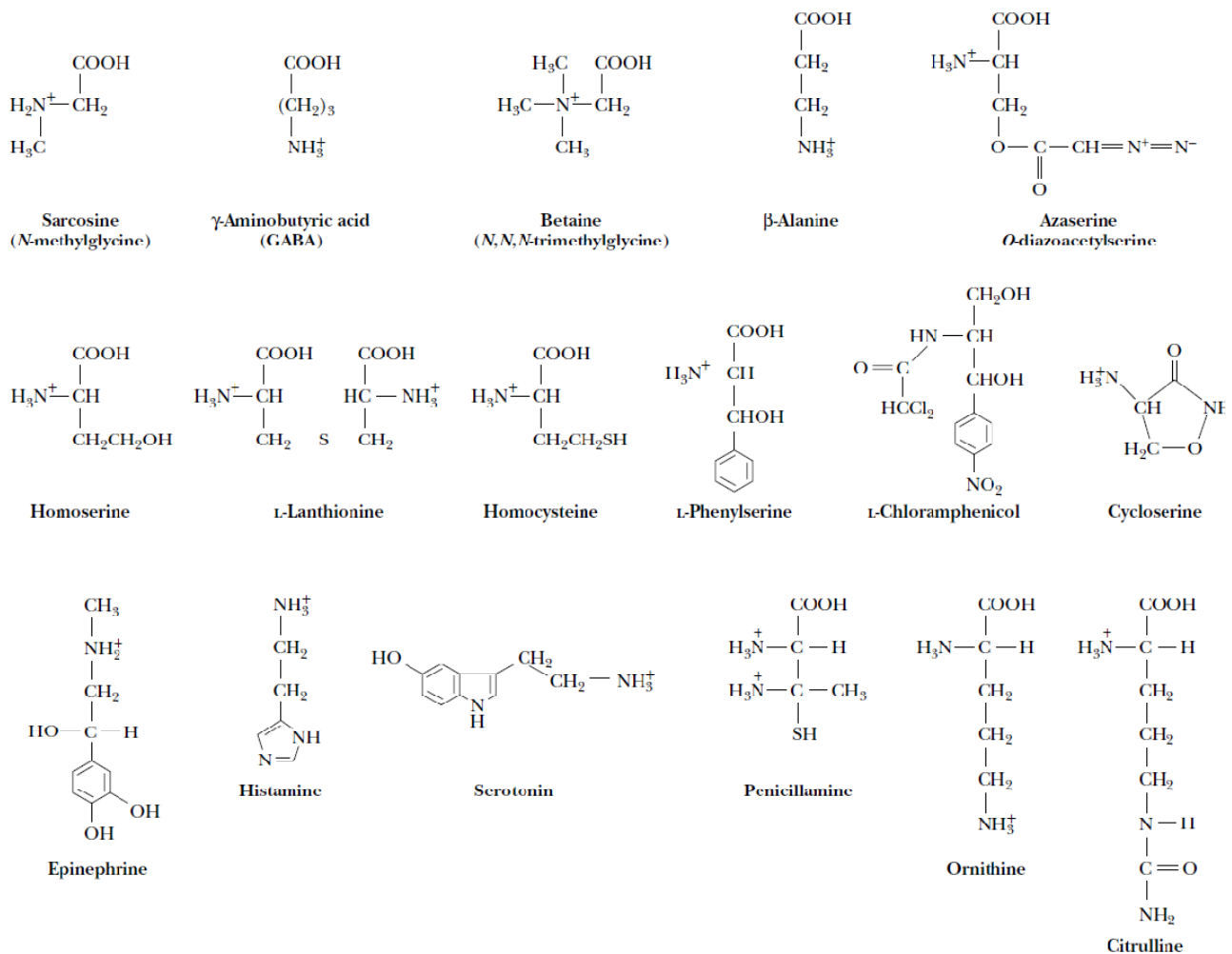


### Amino Acids Not Found in Proteins

In addition to the 20 common amino acids, proteins may contain residues created by modification of common residues already incorporated into a polypeptide. These amino acids and their derivatives, although not found in proteins, nonetheless are biochemically important. Among these uncommon amino acids are **4-hydroxyproline**, a derivative of proline, and **5-hydroxylysine**, derived from lysine. The former is found in plant cell wall proteins, and both are found in collagen, a fibrous protein of connective tissues. **6-N-Methyllysine** is a constituent of myosin, a contractile protein of muscle. Another important uncommon amino acid is  **$\gamma$ -carboxyglutamate**, found in the bloodclotting protein prothrombin and in certain other proteins that bind  $\text{Ca}^{2+}$  as part of their biological function. More complex is **desmosine**, a derivative of four Lys residues, which is found in the fibrous protein elastin. **Selenocysteine** is a special case. This rare amino

acid residue is introduced during protein synthesis rather than created through a postsynthetic modification. It contains selenium rather than the sulfur of cysteine. Actually derived from serine, selenocysteine is a constituent of just a few known proteins. Some 300 additional amino acids have been found in cells. They have a variety of functions but are not constituents of proteins. **Ornithine** and **citrulline** deserve special note because they are key intermediates (metabolites) in the biosynthesis of arginine (precursors of arginine) and in the urea cycle

Other more notable examples are shown below  $\gamma$ -**Aminobutyric acid**, or **GABA**, is produced by the decarboxylation of glutamic acid and is a potent neurotransmitter. **Histamine**, which is synthesized by decarboxylation of histidine, and **serotonin**, which is derived from tryptophan, similarly function as neurotransmitters and regulators.  $\beta$ -**Alanine** is found in nature in the peptides carnosine and anserine and is a component of pantothenic acid (a vitamin), which is a part of coenzyme A. **Epinephrine** (also known as **adrenaline**), derived from tyrosine, is an important hormone. **Penicillamine** is a constituent of the penicillin antibiotics. **Ornithine**, **betaine**, **homocysteine**, and **homoserine** are important metabolic intermediates.



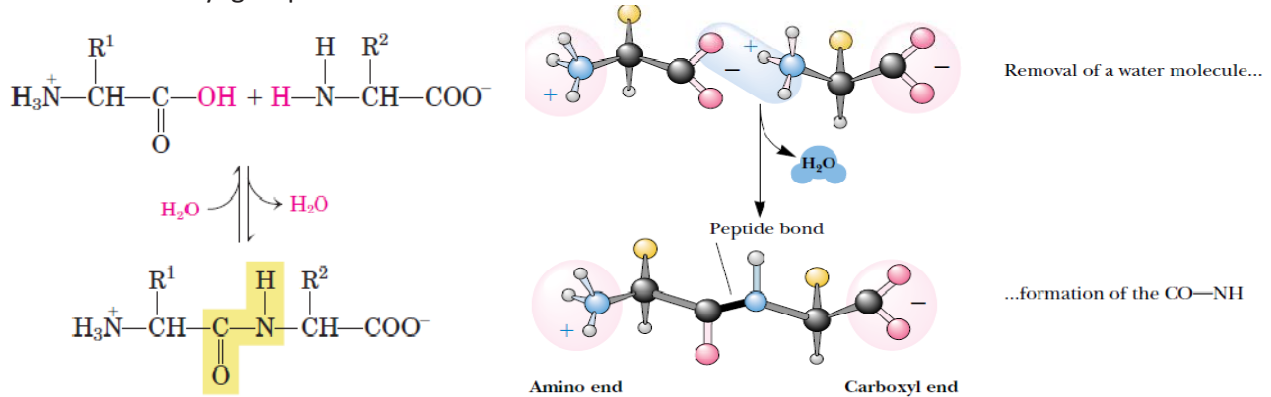
### Peptides Are Chains of Amino Acids

Two amino acid molecules can be covalently joined through a substituted covalent amide linkage, termed a **peptide bond**, to yield a dipeptide. Such a linkage is formed by removal of the elements of water (dehydration) from

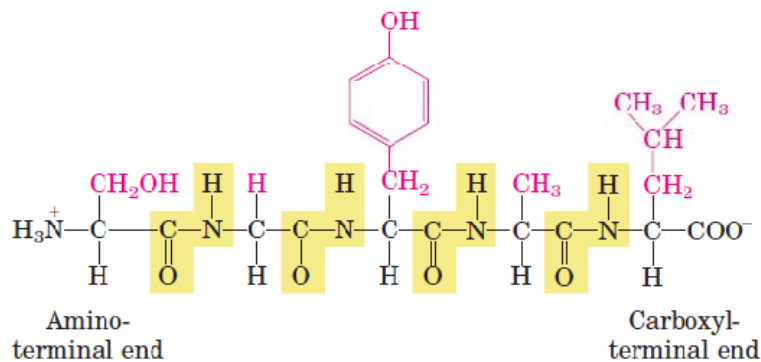


the  $\alpha$ -carboxyl group of one amino acid and the  $\alpha$ -amino group of another. Peptide bond formation is an example of a condensation reaction, a common class of reactions in living cells.

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



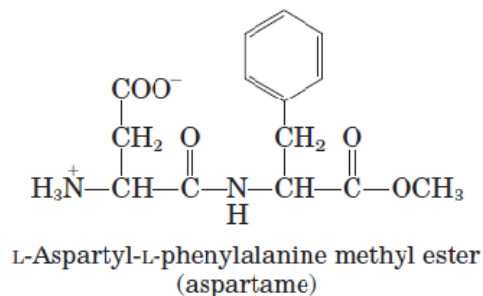
\*The  $\alpha$ -COOH and  $\text{NH}_3^+$  groups of two amino acids can react with the resulting loss of water to form a covalent amide bond



The pentapeptide serylglycyltyrosylalanylleucine, or Ser–Gly–Tyr–Ala–Leu. Peptides are named beginning with the aminoterminal residue, which by convention is placed at the left. The peptide bonds are shaded in yellow; the R groups are in red.

### Biologically Active Peptides and Polypeptides Occur in a Vast Range of Sizes

No generalizations can be made about the molecular weights of biologically active peptides and proteins in relation to their functions. Naturally occurring peptides range in length from two to many thousands of amino acid residues. Even the smallest peptides can have biologically important effects. Consider the commercially synthesized dipeptide L-aspartyl-L-phenylalanine methyl ester, the artificial sweetener better known as aspartame or NutraSweet.



Many small peptides exert their effects at very low concentrations. For example, a number of vertebrate hormones are small peptides. These include oxytocin (nine amino acid residues), which is secreted by the posterior pituitary and stimulates uterine contractions; bradykinin (nine residues), which inhibits inflammation of tissues; and thyrotropin-releasing factor (three residues), which is formed in the hypothalamus and stimulates the release of another hormone, thyrotropin, from the anterior pituitary gland. Some extremely toxic mushroom poisons, such as amanitin, are also small peptides, as are many antibiotics. Slightly larger are small polypeptides and oligopeptides such as the pancreatic hormone insulin, which contains two polypeptide chains, one having 30 amino acid residues and the other 21. Glucagon, another pancreatic hormone, has 29 residues; it opposes the action of insulin. Corticotropin is a 39-residue hormone of the anterior pituitary gland that stimulates the adrenal cortex.

### Reactions of Amino Acids Side Chains

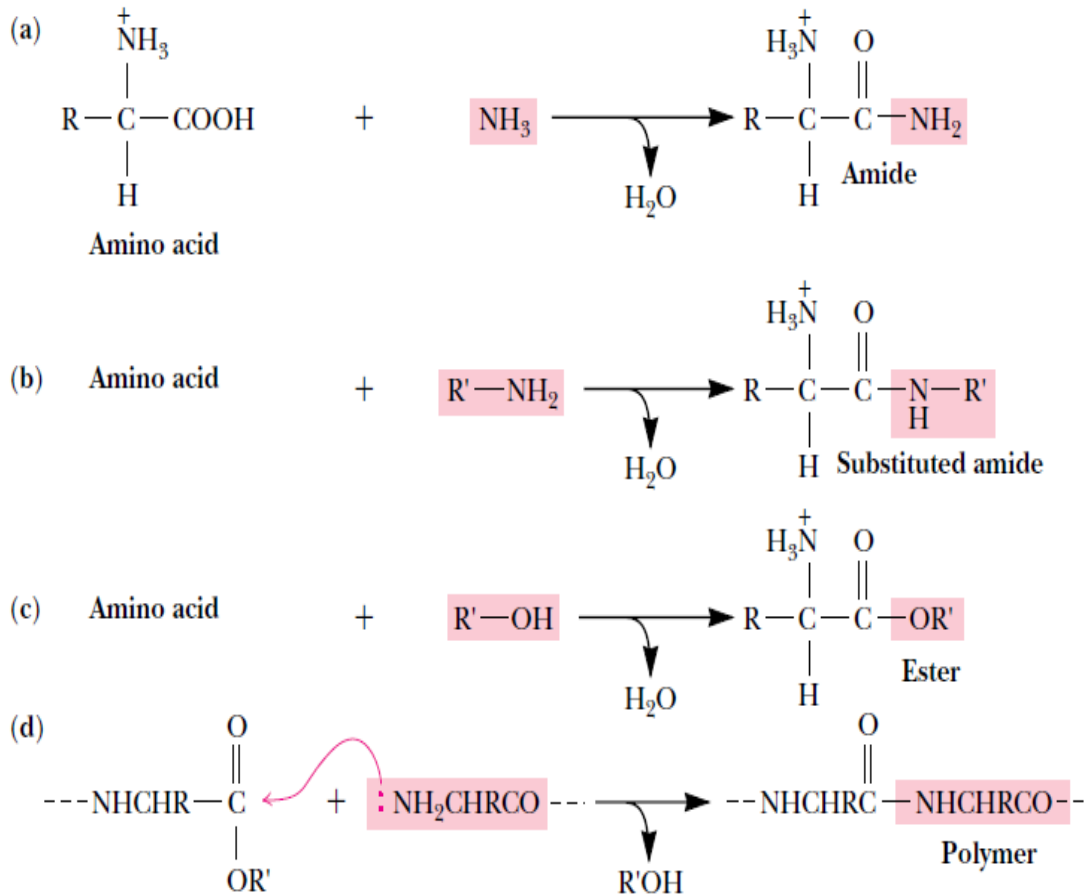
#### Carboxyl and Amino Group Reactions

The  $\alpha$ -carboxyl and  $\alpha$ -amino groups of all amino acids exhibit similar chemical reactivity. The side chains, however, exhibit specific chemical reactivities, depending on the nature of the 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. Proteins can be chemically modified in very specific ways by taking advantage of the chemical reactivity of certain amino acid side chains. 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. 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. Reaction with ammonia and primary amines yields unsubstituted and substituted amides, respectively. Esters and acid chlorides are also readily formed. Esterification proceeds in the presence of the appropriate alcohol and a strong acid. Polymerization can occur by repetition of the reaction shown in Free amino groups may react with aldehydes to form Schiff bases and can be acylated with acid anhydrides and acid halides.

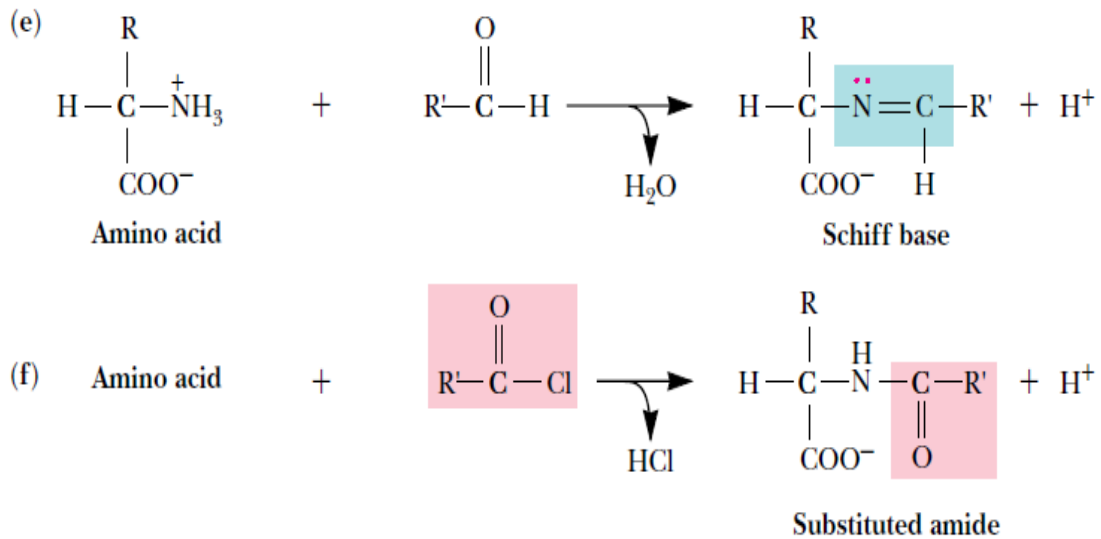
#### The Ninhydrin Reaction

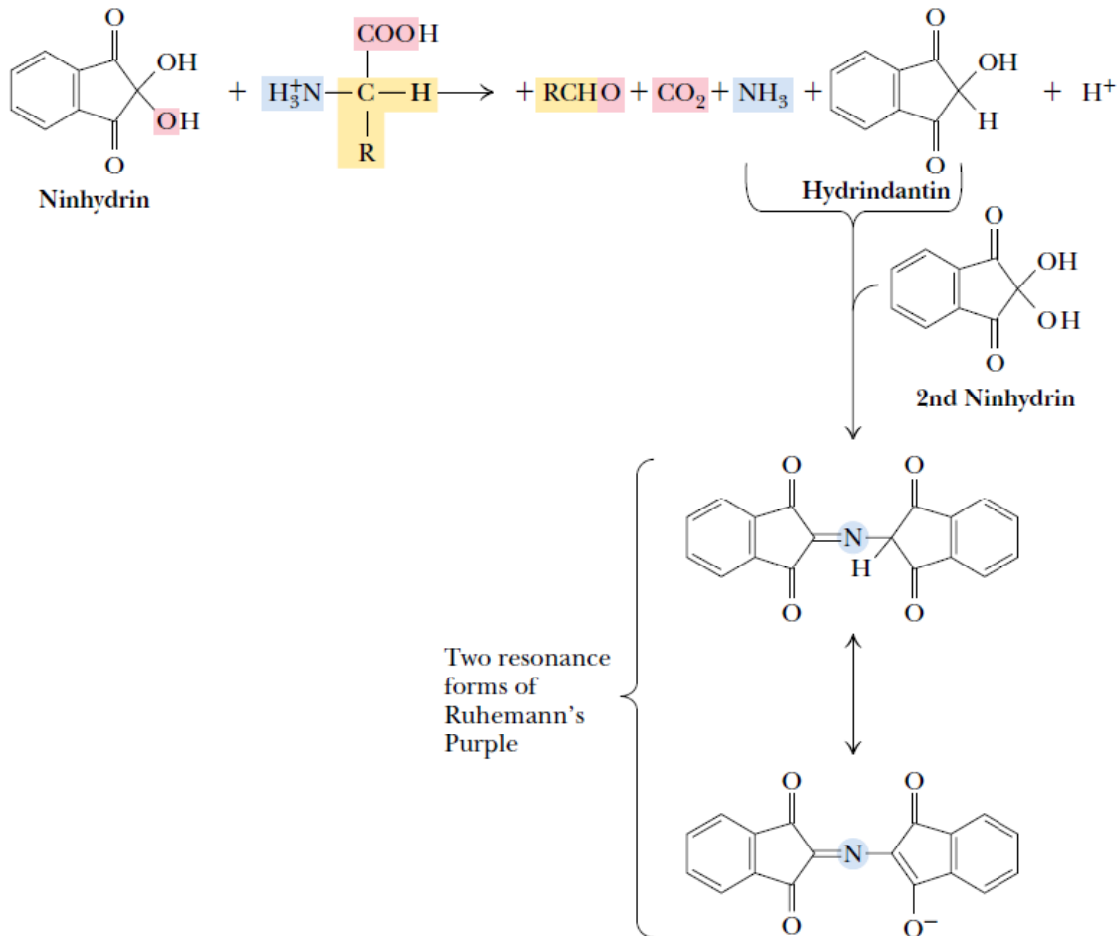
Amino acids can be readily detected and quantified by reaction with ninhydrin. *Ninhydrin*, or triketohydrindene hydrate, is a strong oxidizing agent and causes the oxidative deamination of the  $\alpha$ -amino function. The products of the reaction are the resulting aldehyde, ammonia, carbon dioxide, 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  $\text{CO}_2$  can also be monitored. Indeed,  $\text{CO}_2$  evolution is diagnostic of the presence of an  $\alpha$ -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  $\alpha$ -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.

### CARBOXYL GROUP REACTIONS



### AMINO GROUP REACTIONS

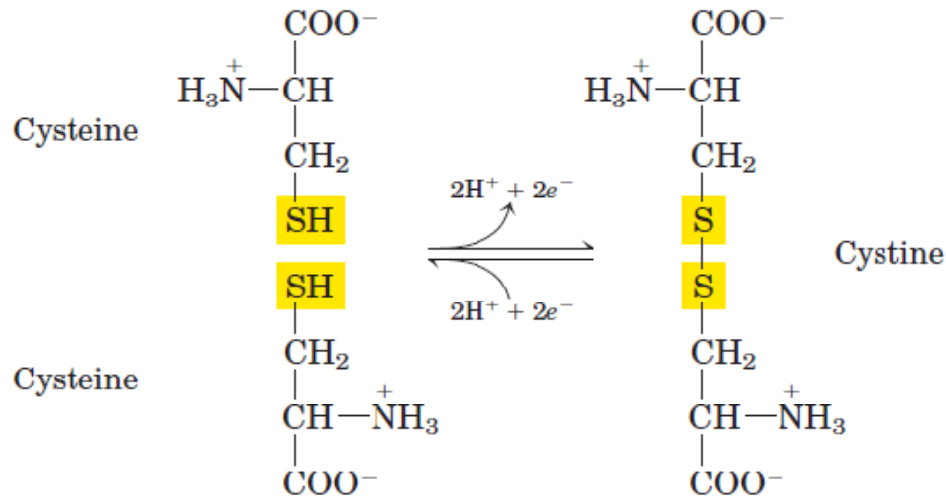




\*The pathway of the ninhydrin reaction, which produces a colored product called “Ruhemann’s Purple” that absorbs light at 570 nm. Note that the reaction involves and consumes two molecules of ninhydrin

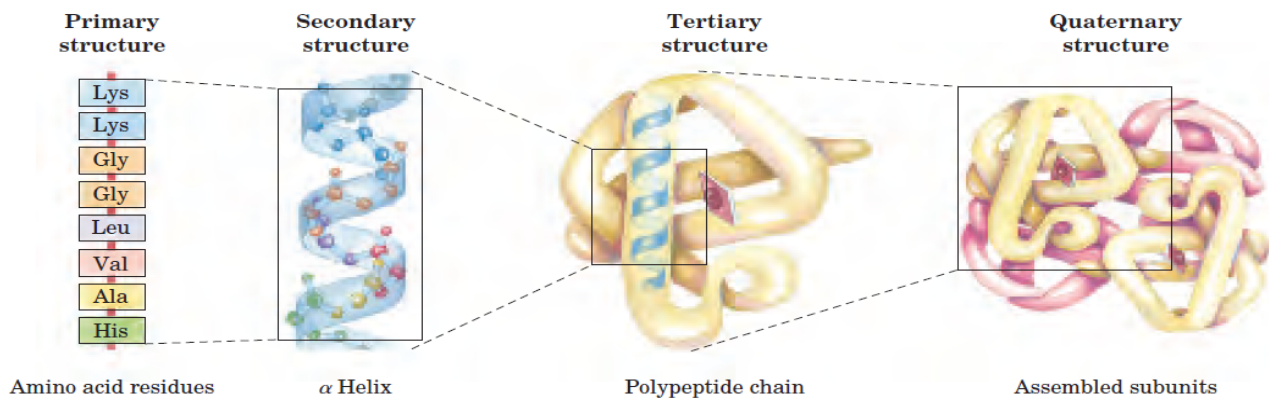
### Specific Reactions of Amino Acid Side Chains

A number of reactions of amino acids have become important in recent years because they are essential to the degradation, sequencing, and chemical synthesis of peptides and proteins. In recent years, biochemists have developed an arsenal of reactions that are relatively specific to the side chains of particular amino acids. These reactions can be used to identify functional amino acids at the active sites of enzymes or to label proteins with appropriate reagents for further study. Cysteine residues in proteins, for example, react with one another to form disulfide species and also react with a number of reagents, including maleimides (typically *N*-ethylmaleimide), Cysteines also react effectively with iodoacetic acid to yield *S*-carboxymethyl cysteine derivatives. There are numerous other reactions involving specialized reagents specific for particular side chain functional groups. It is important to realize that few if any of these reactions are truly specific for one functional group; consequently, care must be exercised in their use.



### Levels of Protein Structure

For large macromolecules such as proteins, the tasks of describing and understanding structure are approached at several levels of complexity, arranged in a kind of conceptual hierarchy. Four levels of protein structure are commonly defined. A description of all covalent bonds (mainly peptide bonds and disulfide bonds) linking amino acid residues in a polypeptide chain is its **primary structure**. The most important element of primary structure is the *sequence* of amino acid residues. **Secondary structure** refers to particularly stable arrangements of amino acid residues giving rise to recurring structural patterns. **Tertiary structure** describes all aspects of the three-dimensional folding of a polypeptide. When a protein has two or more polypeptide subunits, their arrangement in space is referred to as **quaternary structure**.



**\*Levels of structure in proteins.** The **primary structure** consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be coiled into units of **secondary structure**, such as an  $\alpha$  helix. The helix is a part of the **tertiary structure** of the folded polypeptide, which is itself one of the subunits that make up the **quaternary structure** of the multisubunit protein, in this case hemoglobin.

### Biological functions of proteins

The name Protein is derived from the Greek PROTEIOS meaning 'of primary importance or first or foremost' are the most abundant macromolecules in cells. Constitute over half the dry weight of most organisms. Proteins are the instrument by which genetic information is expressed. There are thousands of different kinds

of proteins in the cell ,each carrying out a specific function determined by its gene. Proteins thus are not only the most abundant macromolecules but are extremely versatile in their functions.

All Proteins in all in all species regardless of the functions or biological activity, are built from the same basic set of 20 standard amino acids, which are by themselves have no intrinsic biological activity. Proteins differ from each other because each has a distinctive sequence of its amino acid units. The amino acids are the alphabet of protein structure, since they can be arranged in almost infinite number of sequence to make an almost infinite number of different proteins

## **CLASSIFICATION OF PROTEINS ACCORDING TO BIOLOGICAL FUNCTION**

We can identify several major classes of proteins according

1.Enzymes: These are the most varied, most highly specialized proteins with catalytic activity. Nearly all the chemical reaction of biomolecules in cells are catalyzed by enzymes. To date over 2000 different enzymes, each capable of catalyzing a different kind of chemical reaction have been discovered in different forms of lives are Ribonuclease ,Trypsin ,Hexokinase etc.

2.Transport Proteins: Transport proteins in blood plasma binds and carry specific molecules or ions from one organ to another. Hemoglobin of red blood cell binds oxygen as the blood passes through the lungs and carries it to the peripheral tissues, where the oxygen is released to carry out the energy yielding oxidation of nutrients. The blood plasma contains Lipoproteins, which carry lipids from the liver to other organs. Other kinds of transport proteins are present in cell membranes are adapted to bind and transport glucose,amino acid and other nutrients across the membrane into cells e.g transport proteins are Hemoglobin Myoglobin, Serum albumin Phosphoprotein

3.NUTRIENT AND STORAGE PROTEINS: The seed of many plants store nutrient proteins required for the growth of the embryonic plant.Particularly familiar examples are seed proteins of wheat (Gliadin), corn and rice. Ovalbumin, the major protein of egg white and casein, the major protein of milk, are other example of nutrient proteins. Ferritin of animal tissue stores iron.

4.CONTACTILE OR MOTILE PROTEINS:. Some proteins endow cells and organism with the ability to contract,to change shape or to move about. Actin and Myosin are filamentous proteins functioning in the contractile system of skeletal muscle and also in many nonmuscle cells. Tubulin is another example the protein from which microtubules are built. Microtubules are important component of flagella and cilia which can propel cells. Dynein is also in this class.

5. STRUCTURAL PROTEINS: Many proteins serve as supporting filaments,cables or sheet to give biological structures strength or protection. The major component of tendon and cartilage is the fibrous protein COLLAGEN, which has very high tensile strength. Leather is almost pure collagen. Ligament contain ELASTIN, a structural protein capable of stretching in two dimensions. Hair, fingernails, and feathers consist Largely of tough, insoluble protein keratin. The major component of silk fibers . Spider web is the protein FIBROIN another e.g is PROTEOGLYCANS which are a family of hybrid molecules consisting of proteins covalently formed to Polysaccharides.

6. DEFENSE PROTEINS: Many proteins defend or famifms against invasion by other species or protect them from injury. The immunoglobulins or antibodies of vertebrates are specialized proteins made by lymphocytes which can recognize and participate or neutralize invading bacteria , viruses or foreign protein from another species, Fibrinogen and thrombin are blood clotting proteins that prevent loss of blood when the vascular system is injured. Snake venoms, bacterial such as botulinus and diphtheria toxin. Toxic plant protein, such as ricin, also appear to function in defense.

7. REGULATORY PROTEINS: Some proteins help regulate cellular or physiological activity. Among them are many hormones, such as insulin, which regulates sugar metabolism and whose deficiency is a cause of dibetes, growth hormone of the pituitary, and parathyroid hormone, which regulate Ca<sup>2</sup>. Phosphate transport, other regulatory proteins called repressor regulate the biosynthesis of enzymes by bacterial cells. Corticotropin is another example.

8. OTHER PROTEINS: There are numerous other proteins whose functions are rather exotic and not easily classified. Monellin a protein of an African plant has an intensely sweet taste. It being studied as a non-fattening , non toxic food sweetener for human use. The blood plasma of some Antarctic fish contains ANTIFREEZE PROTEINS which protect their blood from freezing. The wing hinges of some insect are made of the protein RESILIN which has nearly perfect elastic properties.

#### **CLASSIFICATION OF PROTEINS ACCORDING TO SHAPE.**

Proteins can also be divided into 2 great classes on the basis of their shape and certain physical charteristic : GLOBULAR AND FIBRONS PROTEINS.

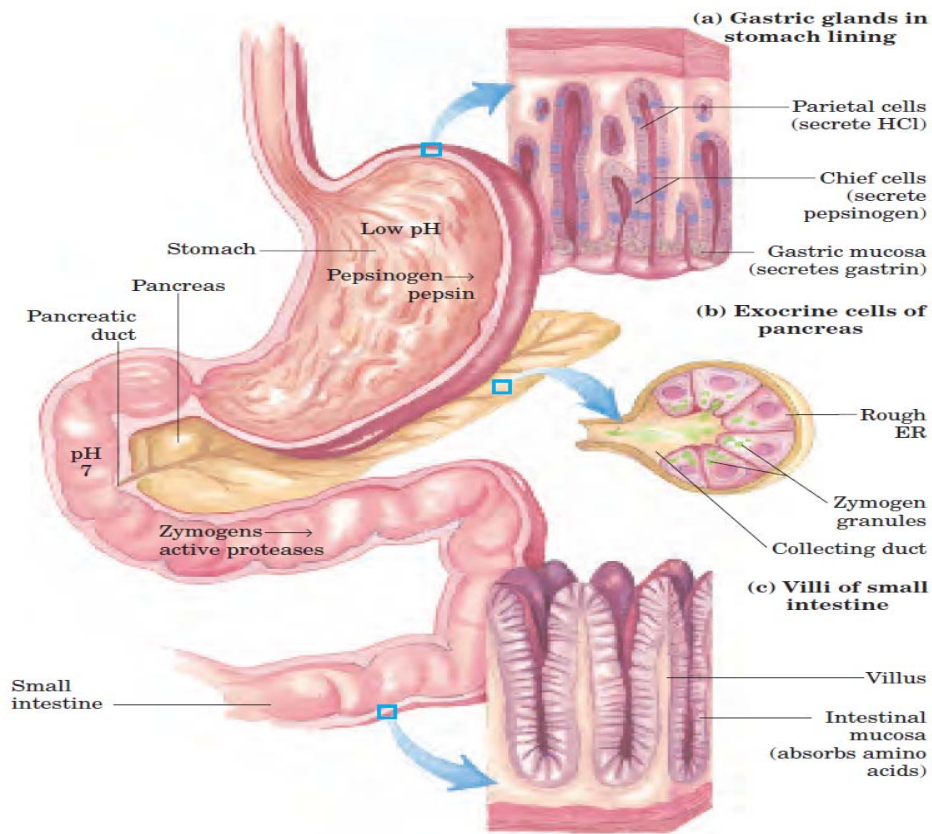
1. Globular Proteins: in globular proteins the polypeptide chain chains are tightly folded into compact spherical or globular shapes. Globular proteins are usually soluble in aqueous systems and diffuse readily, most have mobile or dynamic functions. Nearly all enzymes are globular protein as are blood transport proteins antibodies, and nutrient storage proteins.

2. FIBROUS PROTEINS: are water insoluble, long, stringy molecules with the polypeptide chains extended along one axis rather than folded into a globular shape. Most fibrous proteins serve in a structural or protective role. Typical Fibrous proteins are KERATIN of the hair and wool Fibroin of silk and COLLAGEN of tendons.

We can also include in this class filamentous proteins that participate in contractile events in both muscle and non muscle cells such as Act in and Myosin as well as the protofilament from which microtubules are constructed.

The functioning of keratin, Collagen, Myoglobin, Hemoglobin relate to their structure.

## DIGESTION OF PROTEINS AND ABSORPTION OF AMINO ACIDS.



Ingested proteins are enzymatically hydrolyzed into their constituent amino acids in the gastrointestinal tract when proteins enter the stomach, it stimulates the secretion of hydrochloric acid by parietal cells of the gastric glands and pepsinogen by the chief cells. The gastric juice has pH between 1.5 and 2.5. The acidity of gastric juice acts as an antiseptic and kills most bacteria and other cells. In addition, it causes globular proteins to undergo denaturation or unfolding at this low pH, rendering their internal peptide bonds more accessible to enzymatic hydrolysis. Pepsinogen an inactive precursor or Zymogen, is converted to active pepsin in the gastric juice by the enzymatic action of pepsin itself, an example of Autocatalysis. In this process, 42 amino acids residues are removed from the amino terminal end of the polypeptide chain of pepsinogen as a mixture of small peptide. The rest of the pepsinogen molecules which remains intact is enzymatically active pepsin. In the stomach, pepsin hydrolyzes those peptide bonds of ingested proteins involving the aromatic amino acids –TYROSINE, PHEYLALANINE AND TRYPTOPHAN among others, this cleaving long polypeptide chains into a mixture of smaller peptides.

As the acid stomach contents pass into the small intestine, the low pH triggers the secretion of the hormone SECRETION into the blood. Secretion stimulates the pancreas to secrete bicarbonate into the small intestine to neutralize the gastric HCl. The pH then rises abruptly from between pH 1.5 and 2.5 to about pH 7. In the small intestine the digestion of proteins continues. The entry of amino acids into the duodenum releases the hormone CHOLECYSTOKININ which stimulates secretion of several pancreatic enzymes, whose optimum pH are near 7. Three of these, TRYPSIN, CHYMOTRYPSIN AND CARBOXYPEPTIDASE are made by the exocrine cells of the pancreas as their respective enzymatically inactive zymogens, Trypsinogen, Chymotrypsinogen and Procarboxypeptidase synthesis of these enzymes as inactive precursor protect the exocrine cells from



destructive proteolytic attack. After trypsinogen enters the small intestine, it is converted into its active form TRYPsin by ENTEROKINASE, specialized proteolytic enzyme secreted by intestinal cells. Once some free trypsin has been formed. It also can catalyze the conversion of trypsinogen into trypsin. The formation of free trypsin is brought about by removal of a hexapeptide from the amino terminal end of the trypsinogen chain. Trypsin hydrolyses those peptide bonds whose carbonyl groups are contributed by lysine and Arginine residues.

CHYMOTRYPSINOGEN has a single polypeptide chain with a number of interchain disulfide bond when it reaches the small intestine, it is converted into CHYMOTRYPSIN BY TRYPsin which cleaves the single long polypeptide chain of chymotrypsinogen at 2 points by excision of dipeptides. Chymotrypsin hydrolyses those peptide bonds involving PHENYLALANINE, TYROSINE and TRYPTOPHAN residues. Trypsin and chymotrypsin thus hydrolyze into smaller peptides the polypeptides resulting from the action of pepsin in the stomach. This stage of protein digestion is accomplished very efficiently because pepsin, trypsin, and chymotrypsin have different amino acid specificities in hydrolyzing polypeptide chains.

Degradation of the short peptides in small intestine is now completed by other peptidases. The first is CARBOXYPEPTIDASES, a zinc containing enzyme, which the pancreas makes as its inactive zymogen PROCARBOXYPEPTIDASE. Carboxypeptidase removes successive carboxyl terminal residues from peptides. The small intestine also secretes an AMINOPEPTIDASES from short peptides. By the sequential action of these proteolytic enzymes and peptidases, ingested proteins are ultimately hydrolyzed to yield a mixture of free amino acids. Which are then transported across the epithelial cells lining the small intestine. The free amino acids enter the blood capillaries in the villi and are transported to the liver. Not all proteins are completely digested by human beings. Most animal proteins are almost completely hydrolysed into amino acids, but some fibrous proteins, such as KERATIN are only partially digested. Many proteins of plant foods, such as cereals, grains, are incompletely digested because the protein part of grains or seeds is surrounded by non-digestible cellulose husks.

**CELIAC DISEASE:** is a rare condition in which the intestinal enzymes are unable to digest certain water, insoluble proteins of wheat, particularly GLIADIN, which is injurious to the cells lining the small intestine. Wheat product must therefore be avoided by such patients.

**ACUTE PANCREATITIS:** is caused by obstruction of the normal pathway of secretion of pancreatic juice into the intestine, the zymogene of the proteolytic enzymes are converted into their catalytically active forms prematurely, inside the pancreatic cells. As a result, these powerful enzymes attack the pancreatic tissue itself causing a painful and serious destruction of the organ, which can be fatal. Normally the pancreatic zymogen are not activated until they reach the small intestine. The pancreas protects itself against self digestion in another way. It makes a specific TRYPsin INHIBITOR, itself a protein. Since free trypsin can activate not only trypsinogen and chymotrypsinogen but also two (2) other digestive zymogens, PROCARBOXYPEPTIDASE and PROELASTASE. Trypsin inhibitor effectively prevents premature production of free proteolytic enzymes in pancreatic cells.

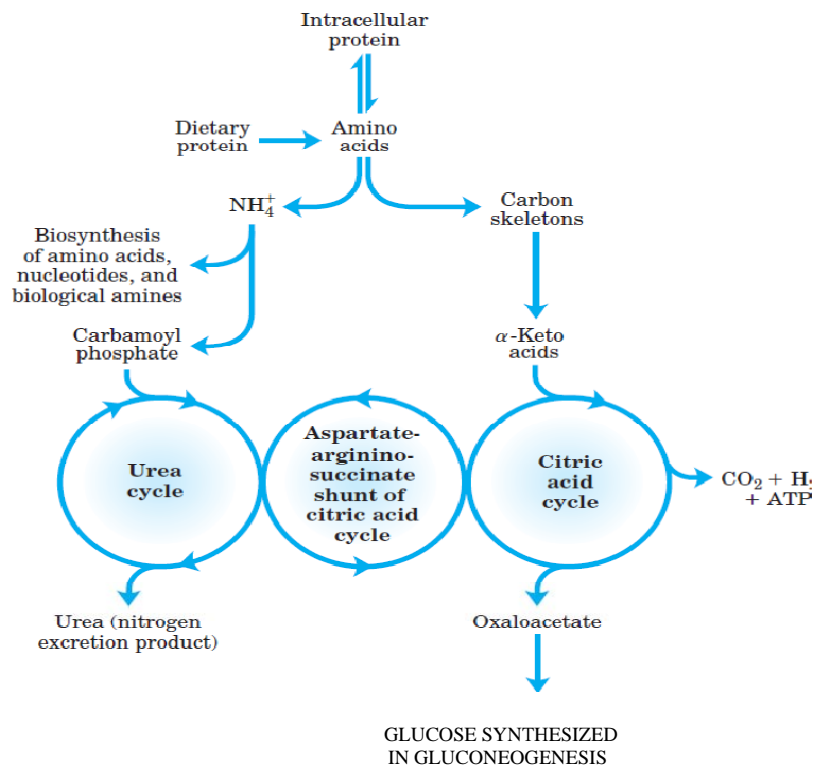
## DEGRADATION OF AMINO ACIDS: OXIDATIVE, NON-OXIDATIVE DEAMINATION, TRANSAMINATION REACTION

Most of the metabolic energy generated in the tissues comes from the oxidation of carbohydrate and triacylglycerols, which together furnish up to 90% of the energy requirement of the adult human male. The remainder from 10 to 15%, depending on the diet, is furnished by the oxidation of amino acids.

Although amino acids function primarily as building blocks for the biosynthesis of protein, they can undergo oxidative deamination in 3 different metabolic circumstances

1. During normal dynamic turnover of body proteins the amino acids released, if not needed for synthesis of new body proteins may undergo oxidative degradation
2. When amino acids are ingested in excess of the body's needs for protein synthesis, the surplus may be catabolised, since amino acid cannot be stored
3. During fasting or in diabetes mellitus, when carbohydrate are either unavailable or not properly utilised, body proteins are called upon as fuel. Under these different circumstances, amino acids undergo loss of their amino groups, and the keto acids so formed may undergo oxidation to carbon dioxide and water, in part via the citric acid cycle or often more importantly, provide three and four carbon units that can be converted by gluconeogenesis into glucose, the fuel for brain, skeletal muscle and other tissues. The pathways of amino acid catabolism are quite similar in most organisms. The process of amino acid degradation converges on the central catabolic pathways with the carbon skeletons of most amino acids finding their way to the citric acid cycle.

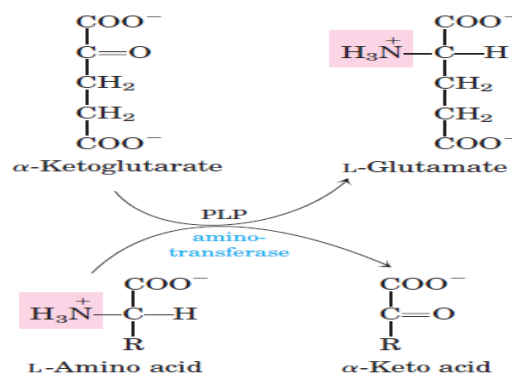
One important feature distinguishes amino acid degradation from other catabolic processes: every amino acid contains an amino group and the pathways for amino acid degradation therefore include a key step in which the amino group is separated from the carbon skeleton and shunted into the pathways of amino group metabolism.



### TRANSFER OF $\alpha$ -AMINO GROUP IS CATALYZED BY TRANSAMINASES ( TRANSAMINATION).

The  $\alpha$ - amino group of the 20 L-amino acids commonly found in proteins are ultimately removed at some stage in their oxidative degradation. If not reused for synthesis of new amino acids or other nitrogenous products, these amino groups are collected and ultimately converted into a single excretory end product which in human beings and most other terrestrial vertebrates is UREA. The removal of the  $\alpha$ - amino groups of most of the L –amino acids is promoted by enzymes called TRANSAMINASES or AMINOTRANSFERASES. In these reactions called TRANSAMINATION, the  $\alpha$ -amino group is enzymatically transferred from the amino acid to the  $\alpha$ -carbon of  $\alpha$ - ketoglutarate, leaving behind the corresponding  $\alpha$ - keto acid analog of the incoming amino acid and causing the amination of the  $\alpha$ - ketoglutarate to form L- GLUTAMATE .

L- $\alpha$ - Amino Acid +  $\alpha$ -ketoglutarate  $\rightarrow$   $\alpha$ -ketoacid +L-Glutamate.



It will be noted that there is no deamination, i.e. loss of amino groups, in such a reaction since the  $\alpha$ -ketoglutarate becomes aminated as the  $\alpha$ -amino acid is deaminated. The whole point of transamination reactions is to collect the amino groups from many different amino acids in the form of only one, namely L-GLUTAMATE. This amino group catabolism converges into a single product.

Most transaminases are specific for  $\alpha$ -ketoglutarate as the amino group acceptor in the reaction as written above. However, they are less specific for the other substrate, the L-amino acid that denotes the amino group. Some of the most important transaminases which are designated by the following equation:

L-ALANINE +  $\alpha$ -KETOGLUTARATE  $\xrightarrow{\text{Alanine transaminase}}$  PYRUVATE + L-GLUTAMATE.

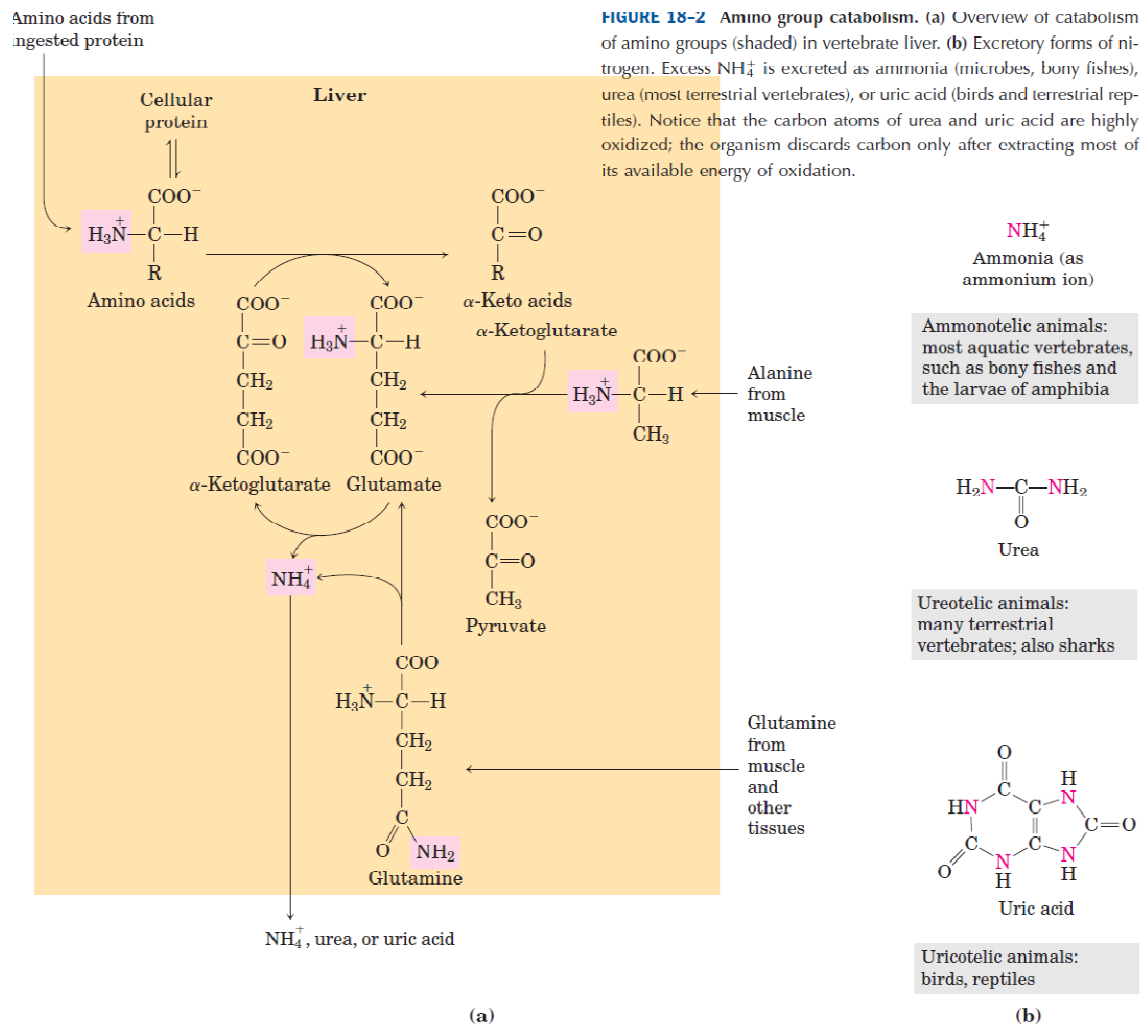
L-ASPARTATE +  $\alpha$ -KETOGLUTARATE  $\xrightarrow{\text{aspartate transaminase}}$  OXALOACETATE + L-GLUTAMATE.

L-LEUCINE +  $\alpha$ -KETOGLUTARATE  $\xrightarrow{\text{leucine transaminase}}$   $\alpha$ -KETOISOCAPROATE.

L-TYROSINE +  $\alpha$ -KETOGLUTARATE  $\xrightarrow{\text{tyrosine transaminase}}$  P-HYDROXYPHENYLPYRUVATE.

Thus  $\alpha$ -ketoglutarate is the common acceptor of amino groups from most of the other amino acids. The L-glutamate so formed serves to channel amino groups into certain biosynthetic pathways or into a final sequence of reactions by which nitrogenous waste products are formed and then excreted. The reactions catalysed by the transaminases are freely reversible.

All transaminases have a tightly bound prosthetic group share a common reaction mechanism. The prosthetic group is PYRIDOXAL PHOSPHATE, a derivative of pyridoxine or vitamin B6. PYRIDOXAL PHOSPHATE functions as an intermediate carrier of amino group on the active site of TRANSAMINASES. During the catalytic cycle it undergoes reversible transitions between its aldehyde form, PYRIDOXAL PHOSPHATE which can accept amino group and its aminated form, PYRIDOXAMINE PHOSPHATE which can donate its amino group to  $\alpha$ -ketoglutarate. In this way the prosthetic group acts as a reversible transient carrier of amino groups from an  $\alpha$ -amino acid to  $\alpha$ -ketoglutarate. The measurement of ALANINE and ASPARTATE TRANSAMINASES in blood serum is an important diagnostic procedure in medicine, used to determine the severity of heart attacks and to monitor recovery. It is also used to detect the toxic effect of some industrial chemicals.



### AMMONIA IS FORMED FROM GLUTAMATE.

$\alpha$ -Amino groups are removed from nearly all the  $\alpha$ -amino acids by transamination to  $\alpha$ -ketoglutarate to form L-glutamate. In hepatocytes, glutamate is transported from cytosol into the mitochondria where it undergoes OXIDATIVE DEAMINATION BY the action of L-GLUTAMATE DEHYDROGENASE which requires  $\text{NAD}^+$  or  $\text{NADP}^+$  as the acceptor of the reducing equivalent. (The combined action of an amino transferase and glutamate dehydrogenase is referred to as **transdeamination**)

L-Glutamate dehydrogenase is present only in the mitochondria in mammals where it is localised in the matrix. Glutamate dehydrogenase is responsible for most of the ammonia formed in animal tissues, since glutamate is the only amino acid whose  $\alpha$ -amino group can be directly removed at a high rate in this manner. Glutamate dehydrogenase is strongly activated by the positive modulator – ADP but inhibited by GTP – the product of the succinyl-coA synthetase reaction in the Citric Acid cycle.

Whenever the liver cells need fuel for the citric acid cycle to form more ATP, glutamate dehydrogenase activity is increased making  $\alpha$ -ketoglutarate available for the citric acid cycle to form more ATP, and releasing  $\text{NH}_3$  to be excreted. On the other hand whenever GTP accumulates in the mitochondria as a result of high citric acid cycle activity, oxidative deamination of glutamate is inhibited.

Ammonia can be salvaged (saved) and released in the synthesis of amino acids. In this case glutamate dehydrogenase acts in reverse, reducing ammonia and  $\alpha$ -ketoglutarate to form glutamate. Instead of being the simple reverse of the NAD-linked reaction written above, however, this reaction is promoted by the NADP<sup>+</sup> linked reaction.



#### L – Amino Acid

Amino acid is converted to glutamate by transaminase to yield keto acid, glutamate is converted to  $\alpha$ -ketoglutarate by glutamate dehydrogenase to give off  $\text{NH}_3$  using  $\text{NAD}^+$ .

The use of two different coenzymes by glutamate dehydrogenase for the release and uptake of  $\text{NH}_3$  makes possible independent regulation of the deamination of glutamate and the amination of  $\alpha$ -ketoglutarate, even though both are catalyzed by the enzyme.

Ammonia resulting from the oxidative deamination of glutamate by glutamate dehydrogenase, a process that occurs in nearly all tissues is a very toxic substance, particularly to the brain. Ammonia is so toxic that injection of even very diluted solutions into the blood stream can render animals comatose. Speculations centre on a potential depletion of ATP in brain cells.

The toxicity of ammonia to the brain is not completely understood but two major factors can be identified:

The  $\text{pK}_a$  of ammonia is quite high, so that at the pH of the blood it occurs almost entirely as ammonium ion ( $\text{NH}_4^+$ ).  $\text{NH}_4^+$  ions are not readily permeant through the plasma membrane or mitochondrial membranes. However, free ammonia ( $\text{NH}_3$ ), a neutral molecule is freely permeant.

Although only about 1% of the total ammonia in the blood occurs in the form of free  $\text{NH}_3$  at pH 7.4, this small amount can penetrate membranes and gain entry into the brain cells and their mitochondria. Riding the cytosol of excess ammonia requires reductive amination of  $\alpha$ -ketoglutarate to glutamate dehydrogenase and conversion of glutamate to glutamine by glutamine synthetase. Both enzymes are present at high levels in the brain although the glutamine synthetase reaction is probably the more important pathway for removal of ammonia.

A shift in the equilibrium of the glutamate dehydrogenase reaction towards glutamate production could limit the availability of  $\alpha$ -ketoglutarate for citric acid cycle,



and the glutamine synthetase reaction depletes ATP. Overall, toxic concentration of  $\text{NH}_4^+$  may interfere with the very high levels of ATP production required to maintain brain function.

Depletion of glutamate in the glutamine synthetase reaction may have additional effect on the brain. Glutamate and its derivative  $\alpha$ -aminobutyrate (GABA) are important neurotransmitters as well as change in cellular ATP metabolism.

The entry of ammonia into brain mitochondria leads to the formation of glutamate from ammonia and  $\alpha$ -ketoglutarate, through the reverse action of glutamate dehydrogenase.

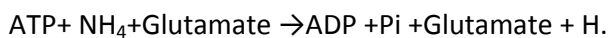


The net result is that  $\alpha$ -ketoglutarate is withdrawn from the pool of citric acid cycle intermediates in brain mitochondria, lowering the rate of oxidation of glucose, the major fuel of the brain. Although these two factors are highly significant, other aspects of the sensitivity of the brain to ammonia are not fully understood.

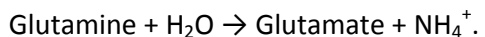
## **AMMONIA DETOXIFICATION.**

### **1. THROUGH GLUTAMINE;**

It is important that toxic ammonia get from the peripheral tissues to the organs that detoxify or excrete it without putting the brain at risk. The way taken to transport ammonia from peripheral tissues to the liver or kidney in most animals is to convert it to a non-toxic compound before exporting it via the blood. In many tissues, including the brain, ammonia is enzymatically combined with glutamate to yield glutamine by the action of glutamine synthetase, which promotes the reaction:



In this reaction, glutamyl-5-phosphate is an enzyme-bound high energy intermediate, an acyl phosphate resulting from the phosphorylation of the 5-carboxyl group of glutamate by ATP. The bound glutamyl 5-phosphate combines with ammonia on the active site to form glutamine and release phosphate. The glutamine is carried via the blood to the liver in most land animals, where it can be acted upon by glutaminase to yield glutamate and ammonia.

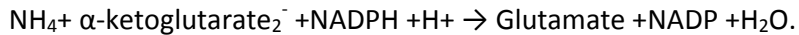


The ammonia so formed is converted by the liver into urea. Glutamine is a major transport form of ammonia, it is present in normal blood in much higher concentrations than other amino acids.

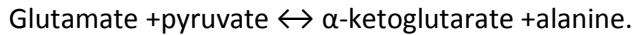
### **2. THROUGH ALANINE:**

Alanine also plays a special role in transporting ammonia to the liver in a non-toxic form. Muscles like other tissues produce ammonia during the detoxification of amino acids. In addition, ammonia also arises from the deamination of adenylate (AMP), a rather prominent process in very active skeletal muscles. Ammonia formed from these two sources is carried from the muscle to the liver by the amino acid, Alanine, through the

action of glucose – alanine cycle. In this cycle ammonia is converted into the amino group of glutamate by the action of glutamate dehydrogenase.



The glutamate so formed now transfers its  $\alpha$ -amino group to pyruvate, a readily available product of muscle glycolysis, by the action of alanine transaminase.



The alanine, a neutral amino acid with no net charge at pH near 7, escapes into the blood and is carried to the liver. Here the alanine transfers its amino group to  $\alpha$ -ketoglutarate by action of alanine transaminase to yield glutamate, which then undergoes deamination to yield  $\alpha$ -ketoglutarate and ammonia through the action of glutamate dehydrogenase. The ammonia so yielded is converted by the liver into urea.

### **AMINO GROUP NITROGEN EXCRETION.**

For the excess amino nitrogen excretion from the body finally, from comparative biochemical studies of different animal species, it has been found that amino nitrogen is excreted in one of 3 major forms namely: as Ammonia, as urea or as uric acid. Bacteria and free living protozoa also most aquatic species of the teleost or bony fishes, excrete amino nitrogen as ammonia and are called AMMONOTELIC animals: most terrestrial animals excrete amino nitrogen in form of UREA and are thus UREOTELIC; and uric acid and are called URICOTELIC:

The basis for these differences lies in the anatomy and physiology of different organisms in relationship to their habitat.

Bacteria and free living protozoa simply release ammonia to their aqueous environment, where it is diluted and thus made harmless.

The bony fishes transport amino nitrogen in the blood as glutamine but excrete it in form ammonia through their gills, which contain glutaminase and thus allow glutamine to be hydrolysed to glutamate and ammonia. Since ammonia is freely soluble in water, it is quickly swept away and diluted by the large volume of water that passes through the gills. These bony fishes do not require a complex urinary system to excrete ammonia.

In birds weight is an important consideration. Since excretion of urea into urine requires that a rather large volume of water be excreted as well. Birds in the evolution learned to excrete amino nitrogen in a form that does not require a large volume of water to be carried around. They convert amino nitrogen into uric acid crystals containing very little water.

Terrestrial animals need kidneys and a urinary bladder to excrete water-soluble nitrogenous waste products, but since free  $\text{NH}_3$  can penetrate membranes readily, excretion of large amounts of ammonia directly into the urine could result in its reabsorption into the blood. Another disadvantage is that since ammonia occurs in the blood largely as  $\text{NH}_4^+$  ion. Its excretion would require the excretion of an equivalent number of anions, such as chloride or phosphate: To avoid such complication most terrestrial animals acquired the ability to excrete amino nitrogen as UREA, a neutral, highly soluble non-toxic molecule. The capacity to make and excrete urea requires considerable ATP energy.

### **UREA CYCLE**

In ureotelic animals, the ammonia resulting from the deamination of amino acids is converted into urea in the liver by a cycle mechanism, the urea cycle.

The first amino group to enter the urea cycle arises in the form of free ammonia by the oxidative deamination of glutamate inside the mitochondria of the liver cell catalysed by glutamate dehydrogenase, which require  $\text{NAD}^+$ .



The free ammonia so formed is immediately used together with carbon dioxide generated in mitochondria by respiration, to form carbamoyl phosphate in the matrix in an ATP dependent reaction catalysed by the enzyme carbamoyl phosphate synthetase 1 ( the roman designates the mitochondrial form of this enzyme to distinguish it from the cytosolic form 2 which has a different function being required in nucleotide biosynthesis ). The mitochondrial reaction is:  $\text{HCO}_3 + \text{NH}_4 + 2\text{ATP} \rightarrow$

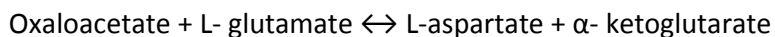
Carbamoyl phosphate synthetase 1 is a regulatory enzyme. It requires N-acetylglutamate as a positive or stimulating modulator. Carbamoyl phosphate is a high-energy compound. It may be regarded as an activated carbamoyl group donor. Note that the terminal phosphate groups of 2ATP molecules are used to form one molecule of carbamoyl phosphate.

In the next step of the urea cycle carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline and release phosphate in a reaction catalysed by ornithine transcarbamoylase a  $\text{Mg}^{2+}$  requiring mitochondrial enzyme.

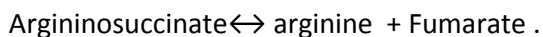


The citrulline so formed now leaves the mitochondrial and passes into the cytosol of the liver cell.

The second amino group now is introduced in the form of L- Aspartate, which in turn acquires it ( amino group ) from L- glutamate by the action of aspartate transamination

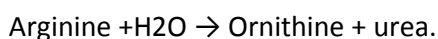


L- glutamate receives its amino group from most of the other common amino acids by transamination to  $\alpha$ -ketoglutarate . The transfer of the second amino group to citrulline occurs by a condensation reaction between the amino group of aspartate and the carbonyl carbon of citrulline in the presence of ATP to form Argininosuccinate synthase of the liver cytosol, a  $\text{Mg}^{2+}$  dependent enzyme



The fumarate so formed returns to the pool of citric acid cycle intermediates. ( Note that we have a link between the urea cycle and the citric acid cycle. ( Indeed, the two krebs' cycles together have been referred to as the krebs' bi-cycle i.e TCA and urea cycles which are both discovered by sir Hans krebs ).

In the last reaction of urea cycle the liver enzyme arginase cleaves arginine to yield urea and ornithine.



Ornithine is thus regenerated and can enter the mitochondrial again to initiate another round of the urea cycle.



Note that arginine is one of the standard amino acids found in proteins. Although ornithine and citrulline are also  $\alpha$ -amino acids, they do not occur as building blocks of protein molecules so are not among the standard amino acids.

