BIOL 157: BIOLOGICAL CHEMISTRY

Lecture 10

Chemistry of Amino Acids

Lecturer:

Christopher Larbie, *PhD*

Introduction

- *Proteins* are the most versatile macromolecules in living systems and serve crucial functions in essentially all biological processes.

- They function as **catalysts**, they **transport** and **store** other molecules such as oxygen, provide **mechanical support** and **immune protection**

- They generate **movement**, **transmit nerve impulses**, and they **control growth** and **differentiation**.

- Indeed, much of this text will focus on understanding what proteins do and how they perform these functions.

- Several key properties enable proteins to participate in such a wide range of functions;

1. *Proteins are linear polymers built of monomer units called amino acids*.
   - The construction of a vast array of macromolecules from a limited number of monomer building blocks is a recurring theme in biochemistry. Does protein function depend on the linear sequence of amino acids?
   - The function of a protein is directly dependent on its three-dimensional structure. Remarkably, proteins spontaneously fold up into three-dimensional structures that are determined by the sequence of amino acids in the protein polymer.
   - Thus, *proteins are the embodiment of the transition from the one-dimensional world of sequences to the three-dimensional world of molecules capable of diverse activities*.

2. ***Proteins contain a wide range of functional groups*.**

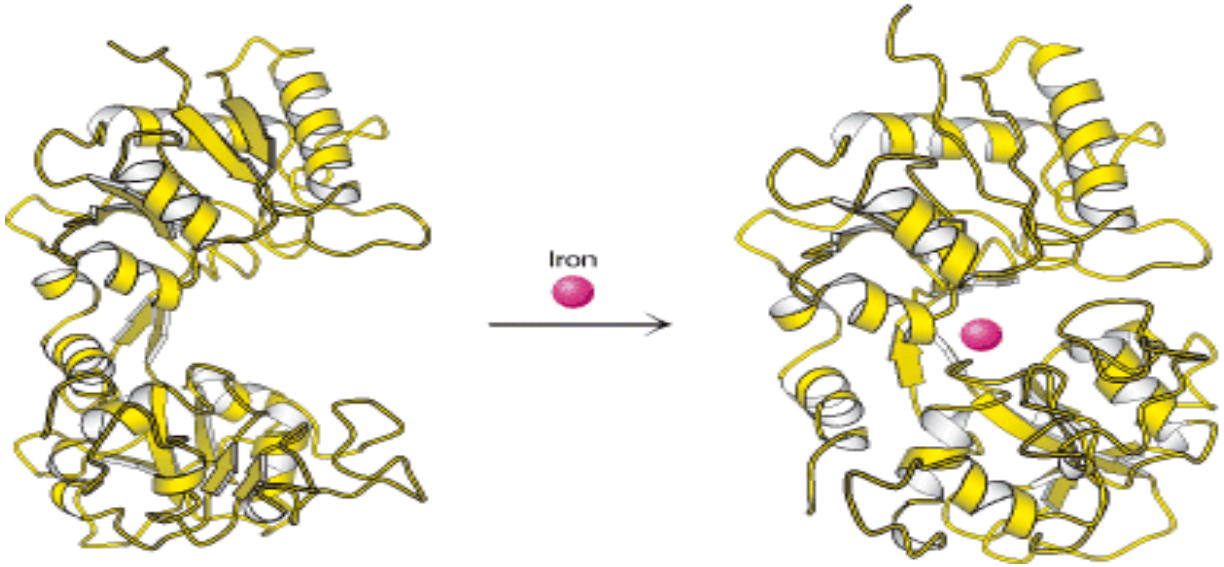
- These functional groups include alcohols, thiols, thioethers, carboxylic acids, carboxamides, and a variety of basic groups.
- When combined in various sequences, this array of functional groups accounts for the broad spectrum of protein function.
- For instance, the chemical reactivity associated with these groups is essential to the function of *enzymes,* the proteins that catalyse specific chemical reactions in biological systems.

3. ***Proteins can interact with one another and with other biological macromolecules to form complex assemblies*.**

- The proteins within these assemblies can act synergistically to generate capabilities not afforded by the individual component proteins.
- These assemblies include macro-molecular machines that carry out the accurate replication of DNA, the transmission of signals within cells, and many other essential processes.

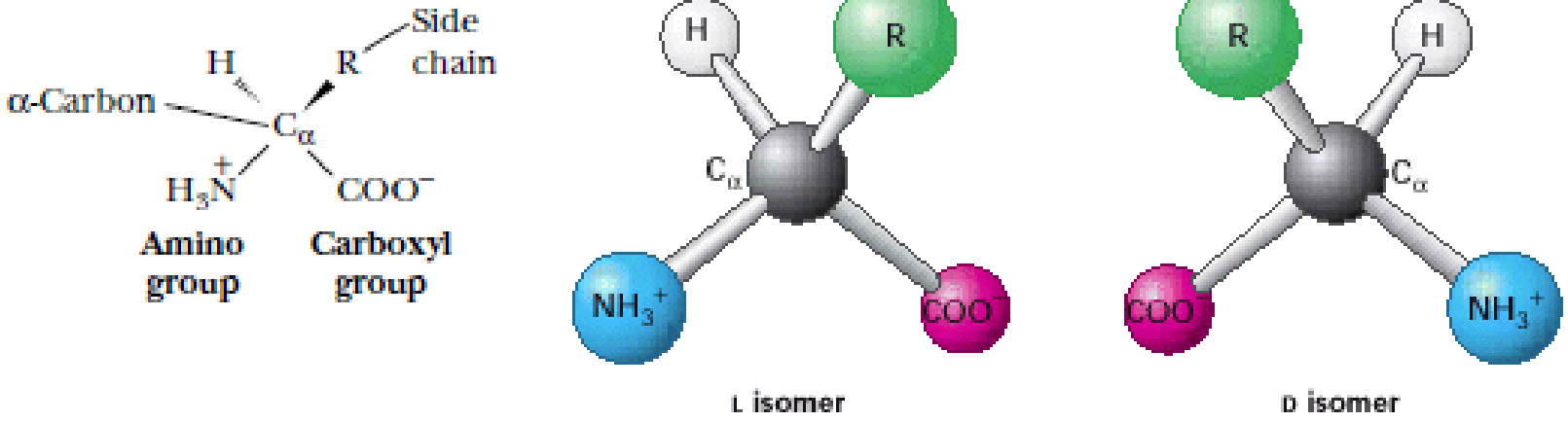
4. **Some proteins are quite rigid, whereas others display limited flexibility.**

- Rigid units can function as structural elements in the cytoskeleton (the internal scaffolding within cells) or in connective tissue.
- Parts of proteins with limited flexibility may act as hinges, springs, and levers that are crucial to protein function, to the assembly of proteins with one another and with other molecules into complex units, and to the transmission of information within and between cells.



Structure of a Typical Amino Acid

- The structure of a single typical amino acid is shown



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

- It is sufficient for now to realize that, 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 α-carbon is said to be *asymmetric.* The two possible configurations for the α-carbon constitute nonidentical mirror image isomers or *enantiomers.*

Common Amino Acids

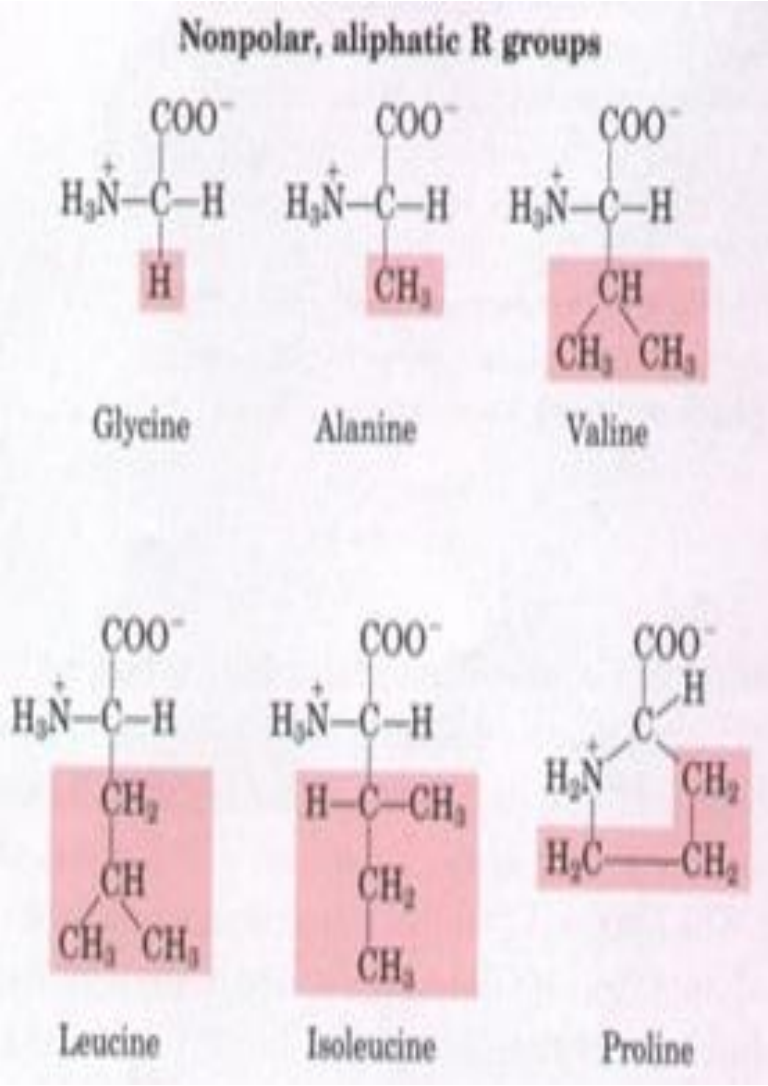
- There are 20 amino acids commonly found in proteins.
  - All the amino acids except ***proline*** have both free α-amino and free α-carboxyl groups. There are several ways to classify the common amino acids.

- The most useful of these classifications is based on the polarity of the side chains. This gives us the following categories:
  - **nonpolar or hydrophobic** amino acids
  - **neutral** (uncharged) but **polar** amino acids
  - **acidic** amino acids (which have a net negative charge at pH 7.0)
  - **basic** amino acids (which have a net positive charge at neutral pH).

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 sulphur-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 favourably with water via the N–H moiety of the indole ring. Proline, strictly speaking, is not an amino acid but rather an **α-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.

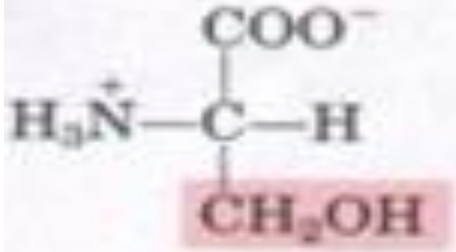
- Several **exceptions** should be noted. Tyrosine displays the lowest solubility in water of the 20 common amino acids (0.453 g/L at 25°C). Also, proline is very soluble in water, and alanine and valine are about as soluble as arginine and serine.

- The amide groups of asparagine and glutamine; the hydroxyl groups of tyrosine, threonine, and serine; and the sulphydryl group of cysteine are all good hydrogen bond–forming moieties.

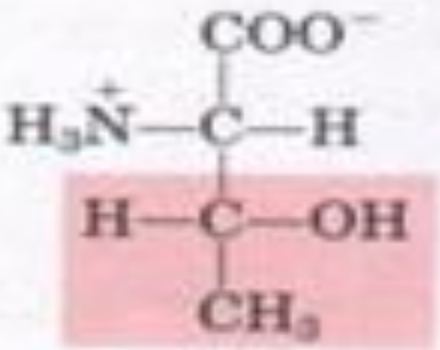
- **Glycine**, the simplest amino acid, has only a single hydrogen for an R group, and this hydrogen is not a good hydrogen bond former. Glycine's solubility properties are mainly influenced by its polar amino and carboxyl groups, and thus glycine is best considered a member of the polar, uncharged group.

- 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 pKa of 10.1, tyrosine's phenolic hydroxyl is a charged, polar entity at high pH.

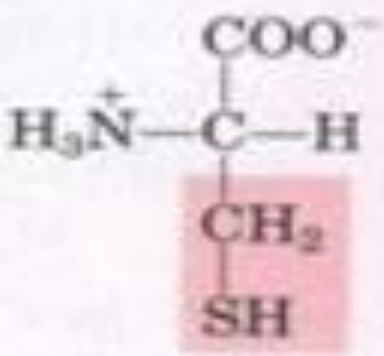
Polar, uncharged R groups



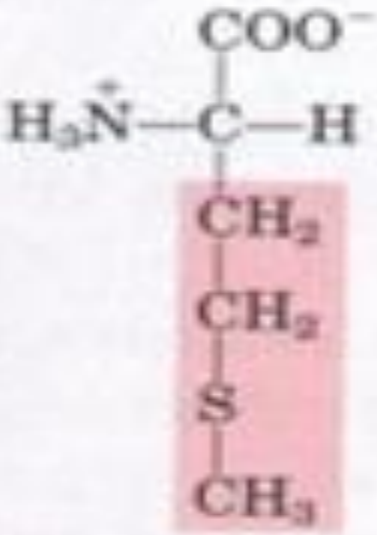
Serine



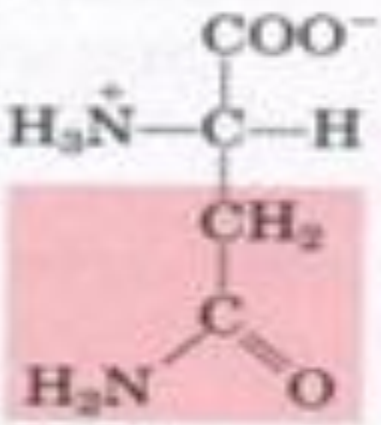
Threonine



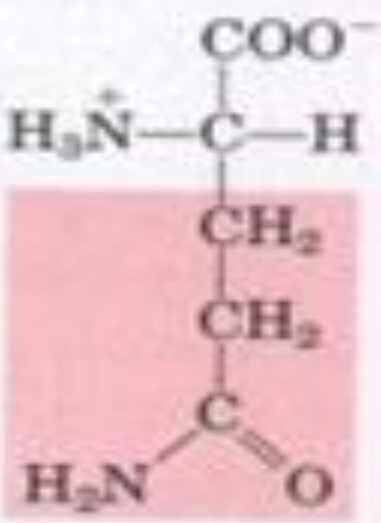
Cysteine



Methionine



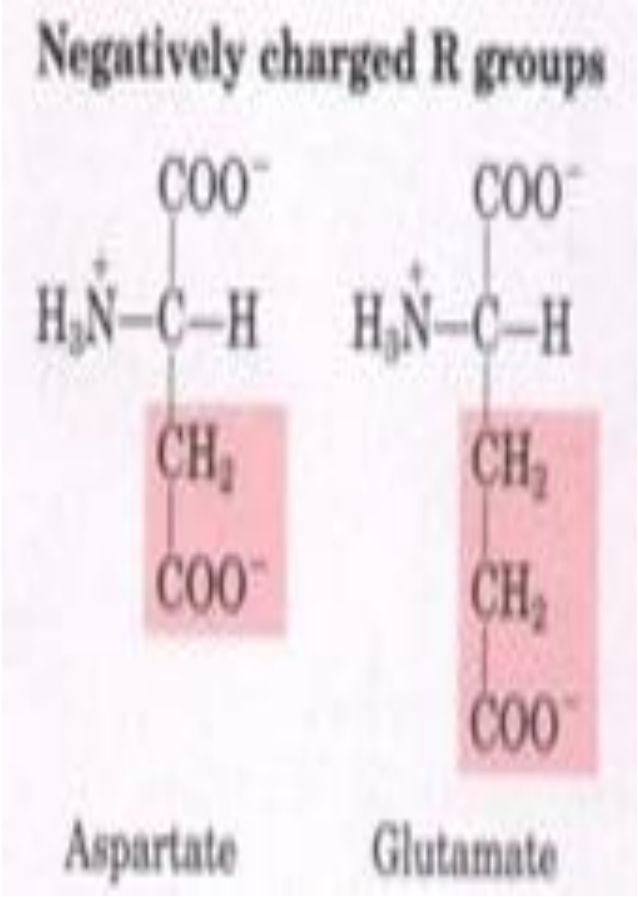
Asparagine



Glutamine

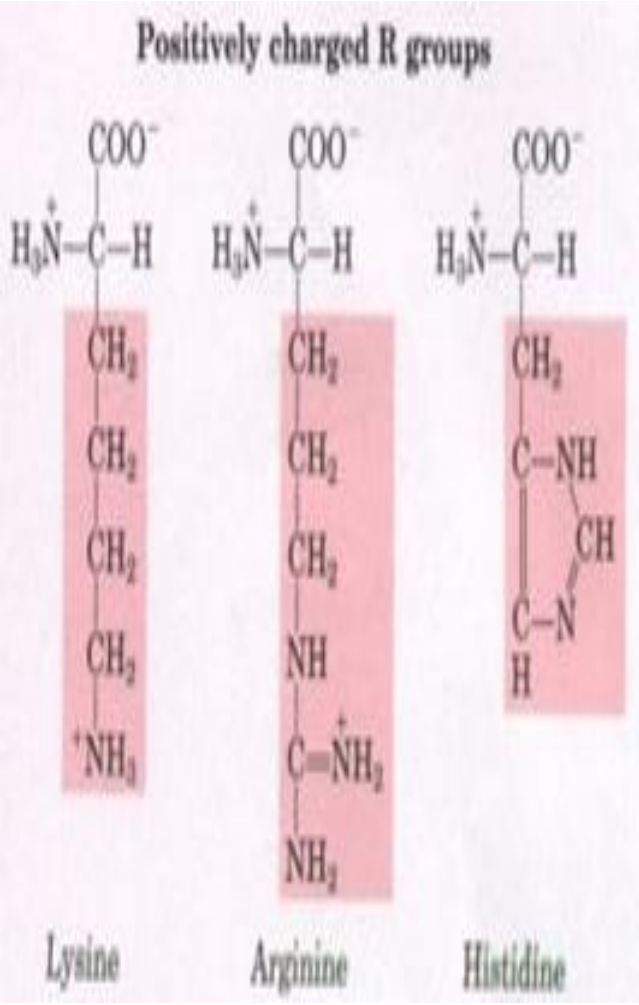
Acidic Amino Acids

- 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 α-COOH group, but are sufficiently acidic to exist as $–COO^-$ 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 pKa of 6.0, is only 10% protonated at pH 7. With a pKa 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.

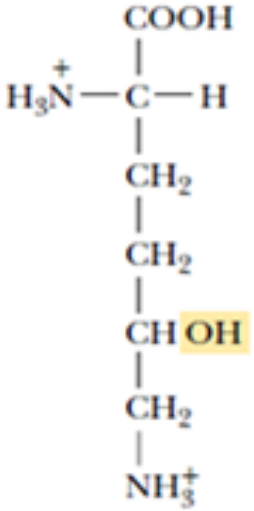
- Thyroxine and 3,3',5-triiodothyronine are produced by iodination of tyrosine residues in thyroglobulin in the thyroid gland. Degradation of thyroglobulin releases these two iodinated amino acids, which act as hormones to regulate growth and development.

- Certain muscle proteins contain **methylated amino acids**, including methylhistidine, ε-N-methyllysine, and ε-N,N,N-trimethyllysine. γ-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.

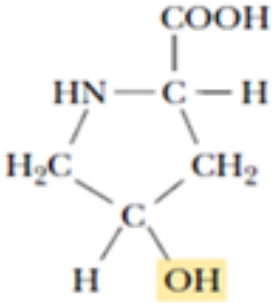
- Certain proteins involved in cell growth and regulation are reversibly phosphorylated on the -OH groups of serine, threonine, and tyrosine residues.

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

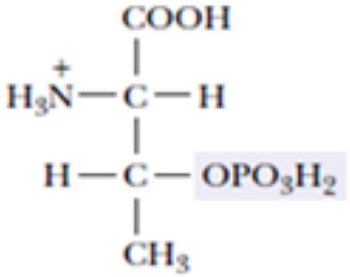
5-Hydroxylysine



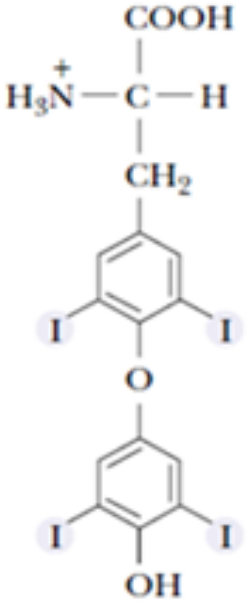
4-Hydroxyproline



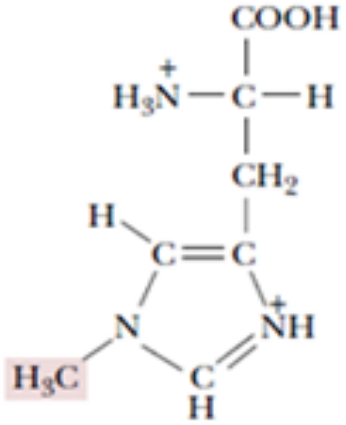
Phosphothreonine



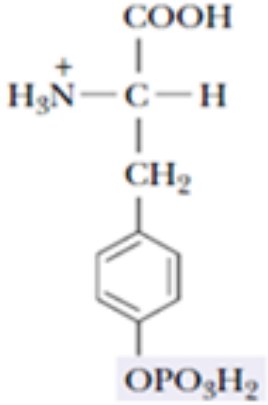
Thyroxine



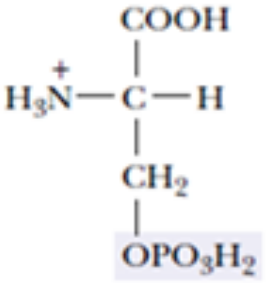
3-Methylhistidine



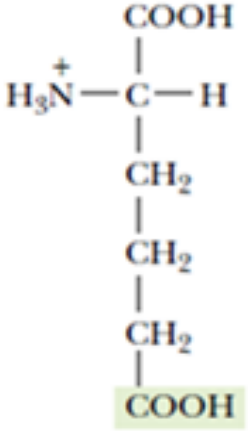
Phosphotyrosine



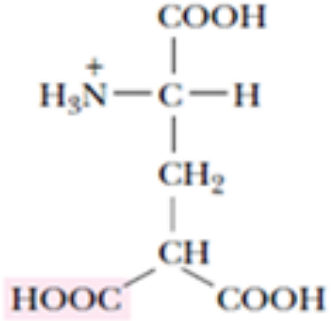
Phosphoserine



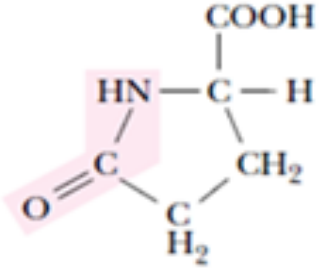
Aminoadipic acid



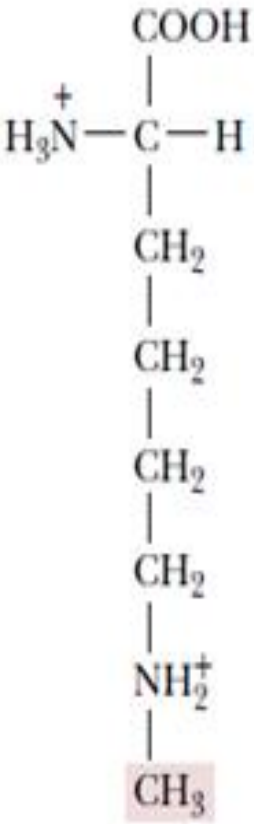
γ-Carboxyglutamic acid



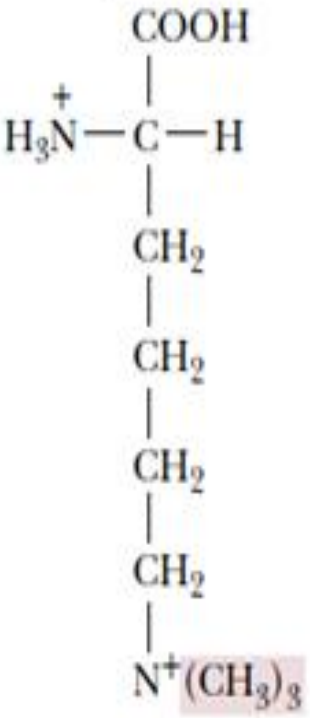
Pyroglutamic acid



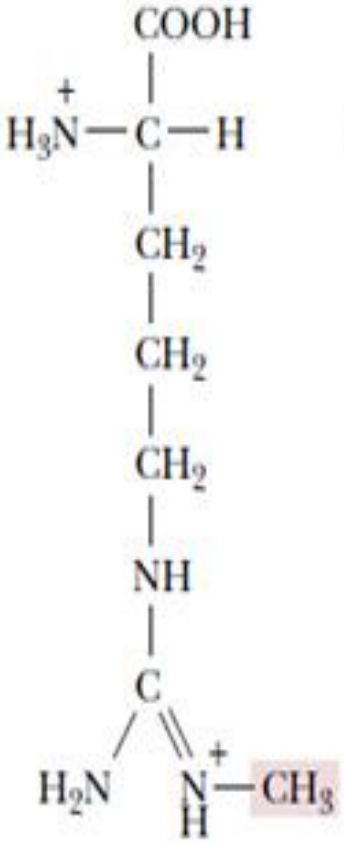
ε-*N*-Methyllysine



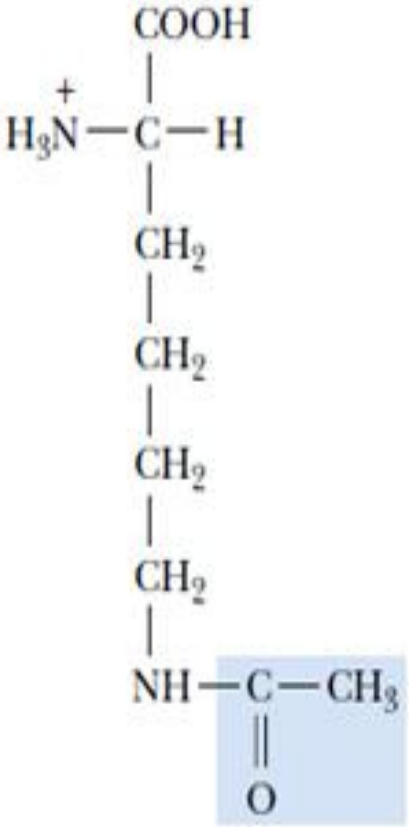
ε-*N*,*N*,*N*-Trimethyllysine



N-Methylarginine



N-Acetyllysine



- **Amino Acids Not Found in Proteins**

- Certain amino acids and their derivatives, although not found in proteins, nonetheless are biochemically important.

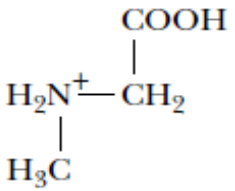
- γ-Aminobutyric acid, or GABA, is produced by the decarboxylation of glutamic acid and is a potent neurotransmitter.

- Histamine, which is synthesized by decarboxylation of histidine, and serotonin, which is derived from tryptophan, similarly function as neurotransmitters and regulators.

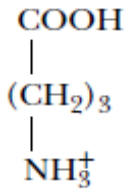
- β-Alanine is found in nature in the peptides carnosine and anserine and is a component of pantothenic acid (a vitamin), which is a part of coenzyme A.

- Epinephrine (also known as adrenaline), derived from tyrosine, is an important hormone. Penicillamine is a constituent of the penicillin antibiotics.

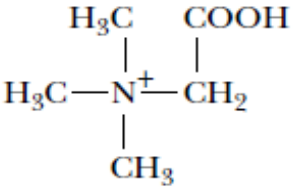
- Ornithine, betaine, homocysteine, and homoserine are important metabolic intermediates. Citrulline is the immediate precursor of arginine.



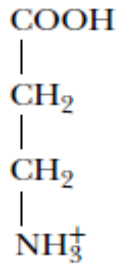
Sarcosine
(*N*-methylglycine)



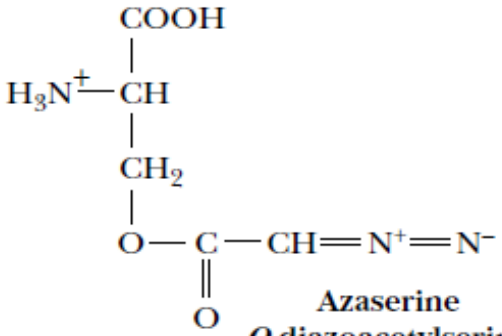
γ-Aminobutyric acid
(GABA)



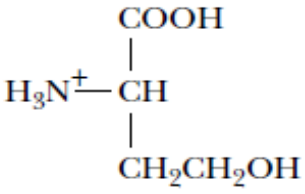
Betaine
(*N*,*N*,*N*-trimethylglycine)



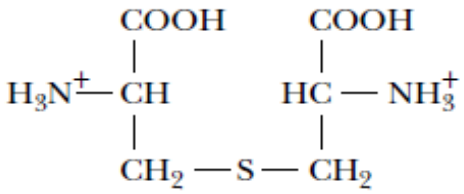
β-Alanine



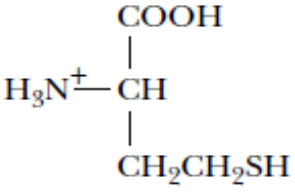
Azaserine
O-diazoacetylserine



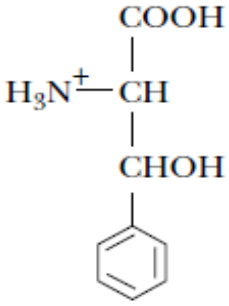
Homoserine



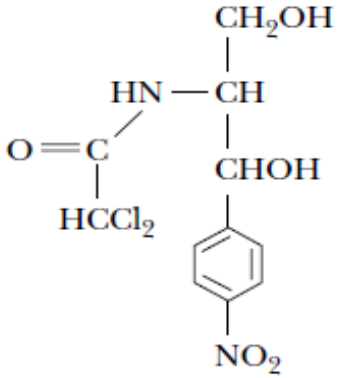
L-Lanthionine



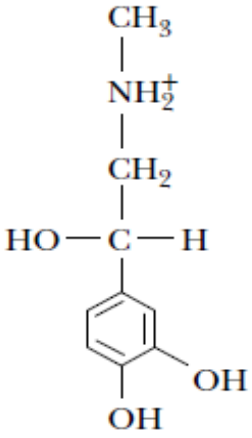
Homocysteine



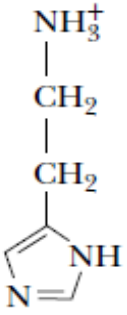
L-Phenylserine



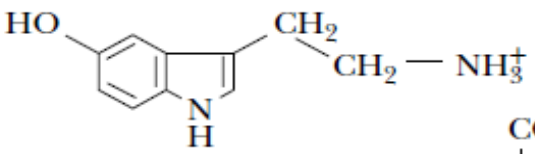
L-Chloramphenicol



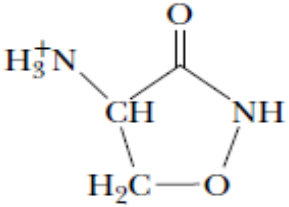
Epinephrine



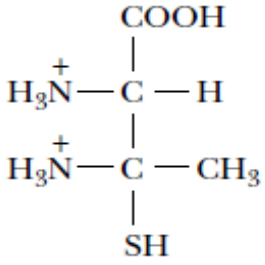
Histamine



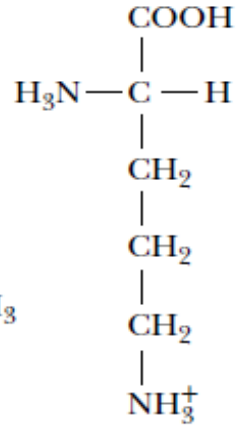
Serotonin



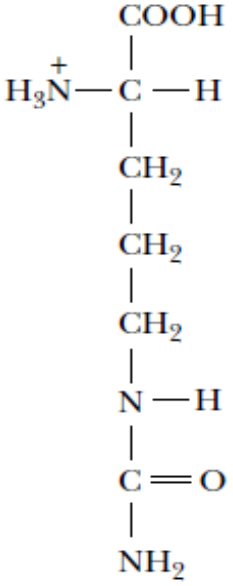
Cycloserine



Penicillamine



Ornithine



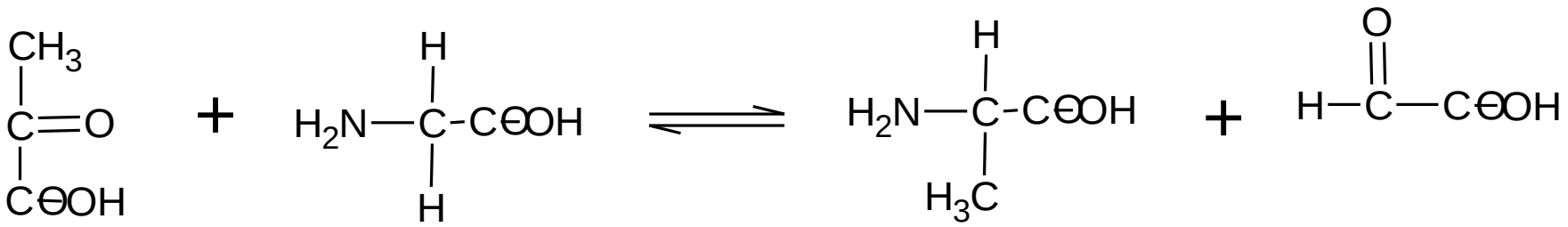
Citrulline

Essential amino acids

- These cannot be synthesized in the body of higher animals and have to be provided in the diet. The inability to synthesize them may be due to the absence of

1. The corresponding α-keto acid of the amino acid.

2. The enzyme involved in transamination i.e. a specific transaminase. The process is called transamination.
Example;



Glycine Alanine

- Since most proteins in the body contain the full complement of amino acid, young animals fail to grow on a diet deficient in even one essential amino acid since without it they are unable to synthesize adequate proteins.

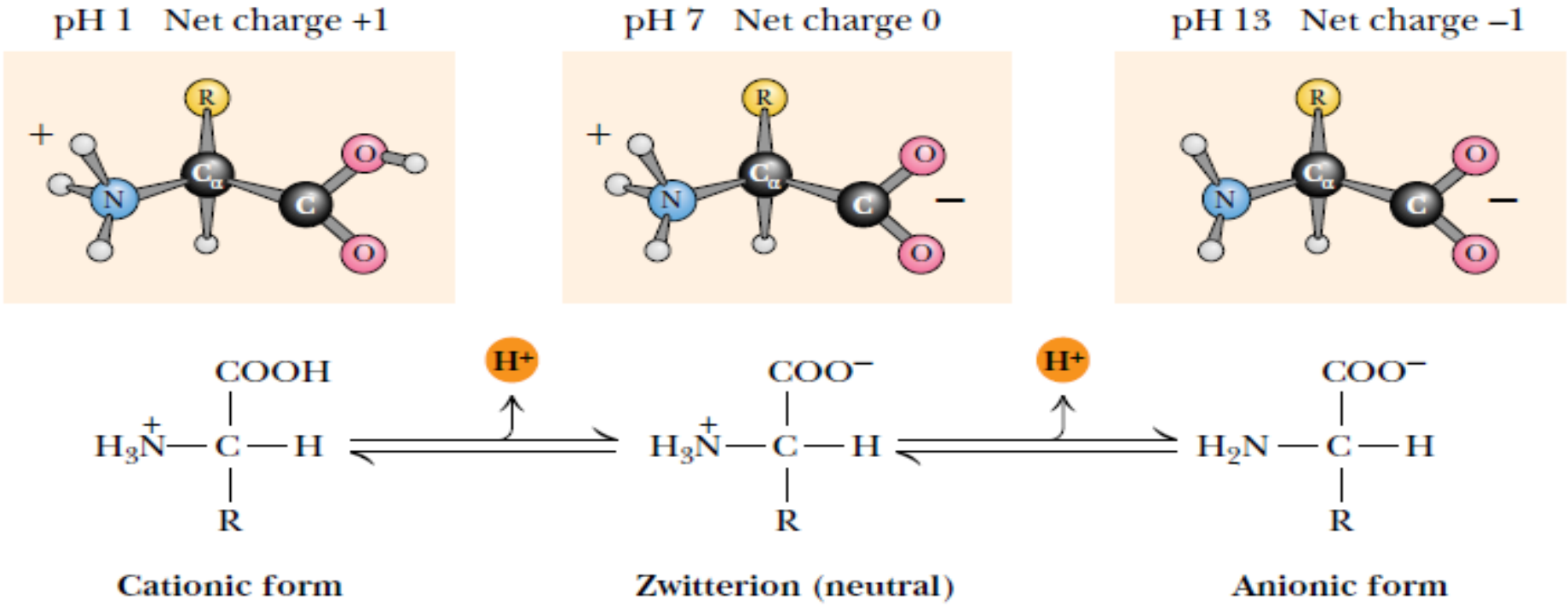
- Sufficient quantities are needed to maintain the proper nitrogen balance in the body. Prolonged deficiency leads to the disease kwashiorkor in children. Other deficiencies are
- 1. fall in plasma protein level, and
- 2. low haemoglobin levels in adults.

Essential amino acids	**Nonessential amino acids**
Histidine	Alanine
Leucine	Glycine
Isoleucine	Asparagine
Methionine	Praline
Phenylalanine	Serine
Threonine	Tyrosine
Tryptophan	Cysteine
Valine	Glutamine
Arginine	Aspartic acid
Lysine	Glutamic acid

Ideally, **lysine** and **arginine** are called **semi essential** because they can be synthesized by the body but not in adequate quantities. Proteins from cereals are poor in lysine and those from legumes low in methionine

Acid-Base properties of amino acids

- From a chemical point of view, the common amino acids are all weak polyprotic acids.
- The ionizable groups are not strongly dissociating ones, and the degree of dissociation thus depends on the pH of the medium. All the amino acids contain at least two dissociable hydrogens



- Consider the acid–base behaviour of glycine, the simplest amino acid.
- At low pH, both the amino and carboxyl groups are protonated and the molecule has a net positive charge. If the counterion in solution is a chloride ion, this form is referred to as glycine hydrochloride.

- If the pH is increased, the carboxyl group is the first to dissociate, yielding the neutral zwitterionic species Gly^0. Further increase in pH eventually results in dissociation of the amino group to yield the negatively charged glycinate. If we denote these three forms as Gly^+, Gly^o, and Gly^-, we can write the first dissociation of Gly^+ as

$$Gly^+ + H_2O \rightleftharpoons Gly^0 + H_3O^+$$

- The dissociation constant K_1 written as

$$K_1 = \frac{[\mathrm{Gly}^0]\,[\mathrm{H_3O^+}]}{[\mathrm{Gly}^+]}$$

- Values for K_1 for the common amino acids are typically 0.4 to 1.0 x 10^{-2} *M,* so that typical values of pK_1 centre on values of 2.0 to 2.4. In a similar manner, we can write the second dissociation reaction as

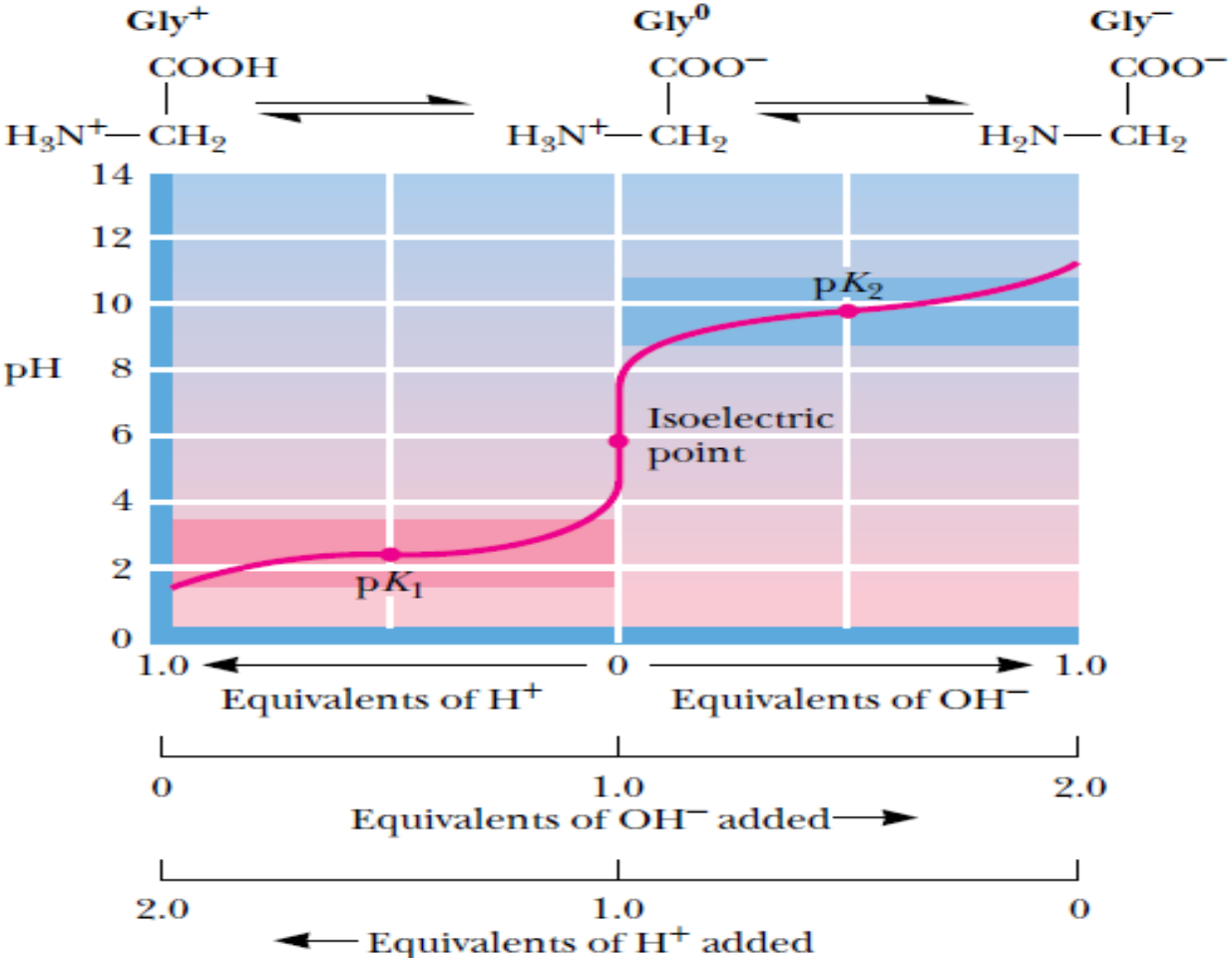
$$\mathrm{Gly}^0 + \mathrm{H_2O} \rightleftharpoons \mathrm{Gly}^- + \mathrm{H_3O}^+$$

- and the dissociation constant K_2 as

$$K_2 = \frac{[\mathrm{Gly}^-]\,[\mathrm{H_3O^+}]}{[\mathrm{Gly}^0]}$$

Typical values for pK_2 are in the range of 9.0 to 9.8. At physiological pH, the α-carboxyl group of a simple amino acid (with no ionizable side chains) is completely dissociated, whereas the α-amino group has not really begun its dissociation.

- The titration curve for such an amino acid is shown below



- Note that the dissociation constants of both the α-carboxyl and α-amino groups are affected by the presence of the other group.

- The adjacent α-amino group makes the α-COOH group more acidic (that is, it lowers the p*K*a) so that it gives up a proton more readily than simple alkyl carboxylic acids.
- Thus, the pK_1 of 2.0 to 2.1 for α-carboxyl groups of amino acids is substantially lower than that of acetic acid (p*K*a = 4.76)

- for example. The α-NH_3 (ammonium) group is strongly electron-withdrawing, and the positive charge of the amino group exerts a strong field effect and stabilizes the carboxylate anion.

Ionization of Side Chains

- As we have seen, the side chains of several of the amino acids also contain dissociable groups.

- Thus, aspartic and glutamic acids contain an additional carboxyl function, and lysine possesses an aliphatic amino function. Histidine contains an ionizable imidazolium proton, and arginine carries a guanidinium function.

- Typical p*K*a values of these groups are shown in the table below. The β-carboxyl group of aspartic acid and the γ-carboxyl side chain of glutamic acid exhibit p*K*a values intermediate to the α-COOH on the one hand and typical aliphatic carboxyl groups on the other hand.

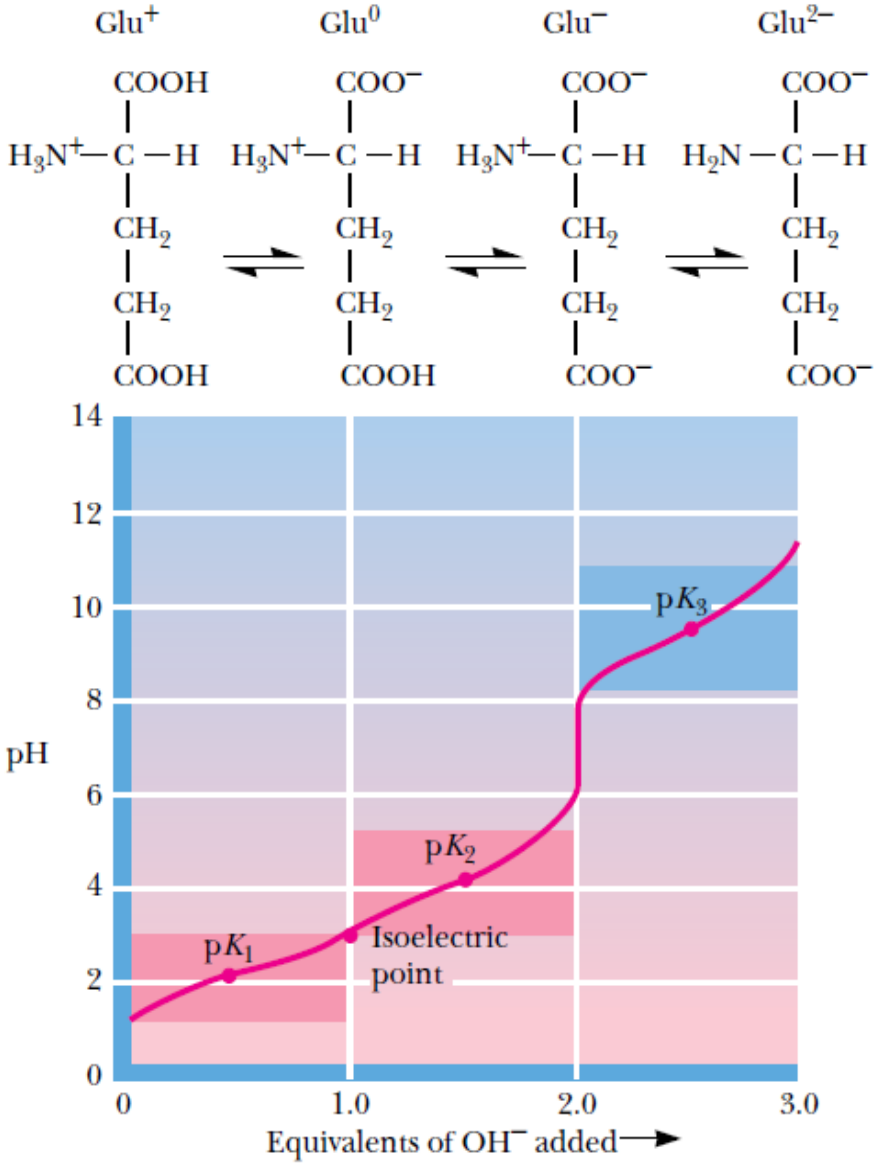
- In a similar fashion, the ε-amino group of lysine exhibits a p*K*a that is higher than the α-amino group but similar to that for a typical aliphatic amino group. These intermediate values for side-chain p*K*a values reflect the slightly diminished effect of the α-carbon dissociable groups that lie several carbons removed from the side-chain functional groups.

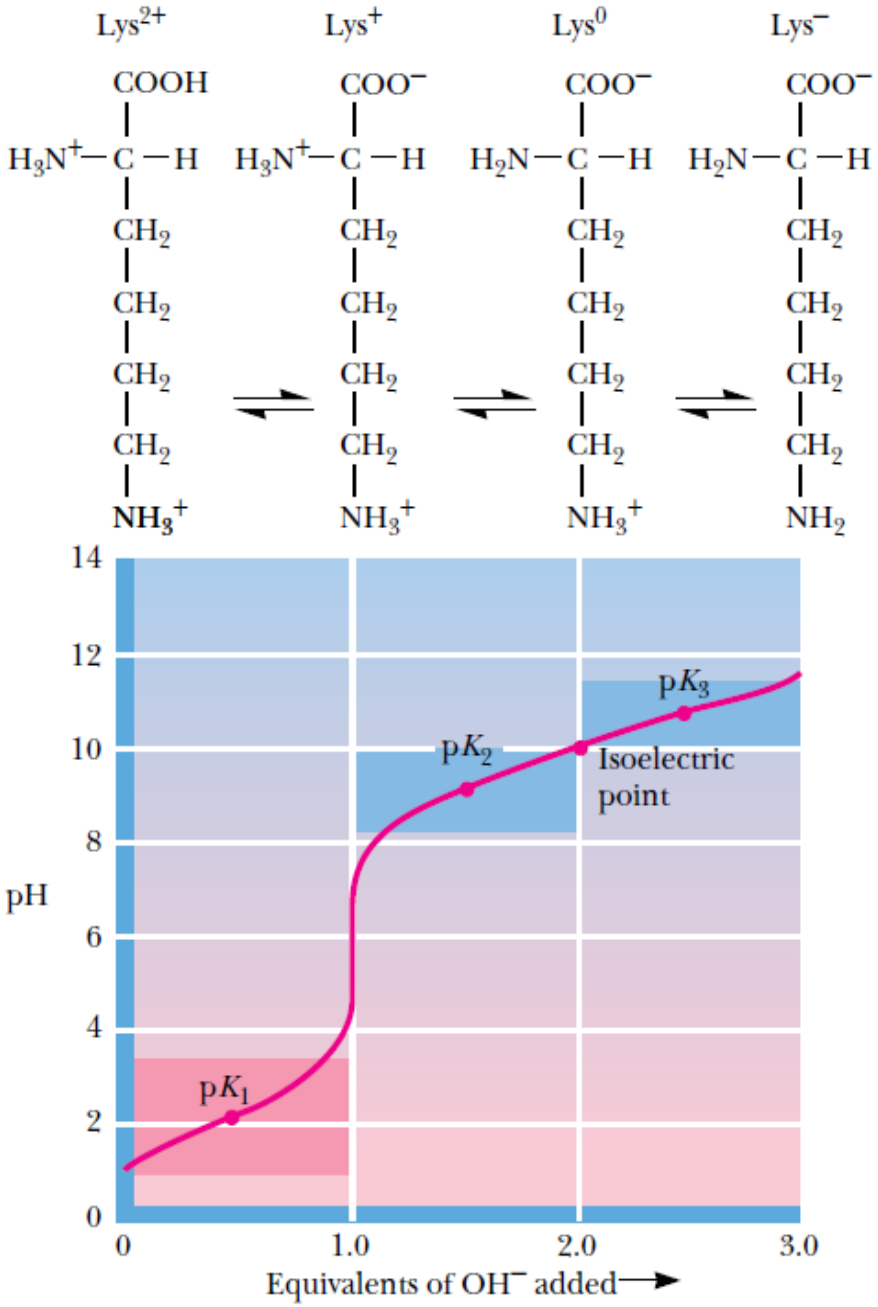
- The only other side-chain groups that exhibit any significant degree of dissociation are the *para*-OH group of tyrosine and the -SH group of cysteine

- The p*K*a of the cysteine sulphydryl is 8.32, so that it is about 12% dissociated at pH 7. The tyrosine *para*-OH group is a very weakly acidic group, with a p*K*a of about 10.1. This group is essentially fully protonated and uncharged at pH 7.

pK_a Values of Common Amino Acids

Amino Acid	α-COOH pK_a	α-NH_3^+ pK_a	R group pK_a
Alanine	2.4	9.7	
Arginine	2.2	9.0	12.5
Asparagine	2.0	8.8	
Aspartic acid	2.1	9.8	3.9
Cysteine	1.7	10.8	8.3
Glutamic acid	2.2	9.7	4.3
Glutamine	2.2	9.1	
Glycine	2.3	9.6	
Histidine	1.8	9.2	6.0
Isoleucine	2.4	9.7	
Leucine	2.4	9.6	
Lysine	2.2	9.0	10.5
Methionine	2.3	9.2	
Phenylalanine	1.8	9.1	
Proline	2.1	10.6	
Serine	2.2	9.2	~13
Threonine	2.6	10.4	~13
Tryptophan	2.4	9.4	
Tyrosine	2.2	9.1	10.1
Valine	2.3	9.6	


Glu+
Glu0
Glu−
Glu2−
COOH
COO−
H3N+—C—H
H2N—C—H
CH2
pK1
pK2
pK3
Isoelectric point
pH
Equivalents of OH− added
0
1.0
2.0
3.0
0
2
4
6
8
10
12
14


Lys2+
Lys+
Lys0
Lys−
COOH
COO−
H3N+—C—H
H2N—C—H
CH2
NH3+
NH2
pK1
pK2
pK3
Isoelectric point
pH
Equivalents of OH− added
0
1.0
2.0
3.0
0
2
4
6
8
10
12
14

Reactions of Amino Acids

- The α-carboxyl and α-amino groups of all amino acids exhibit similar chemical reactivity. The side chains, however, exhibit specific chemical reactivities, depending on the nature of the functional groups.

- Whereas all of these reactivities are important in the study and analysis of isolated amino acids, it is the characteristic behaviour of the side chain that governs the reactivity of amino acids incorporated into proteins.

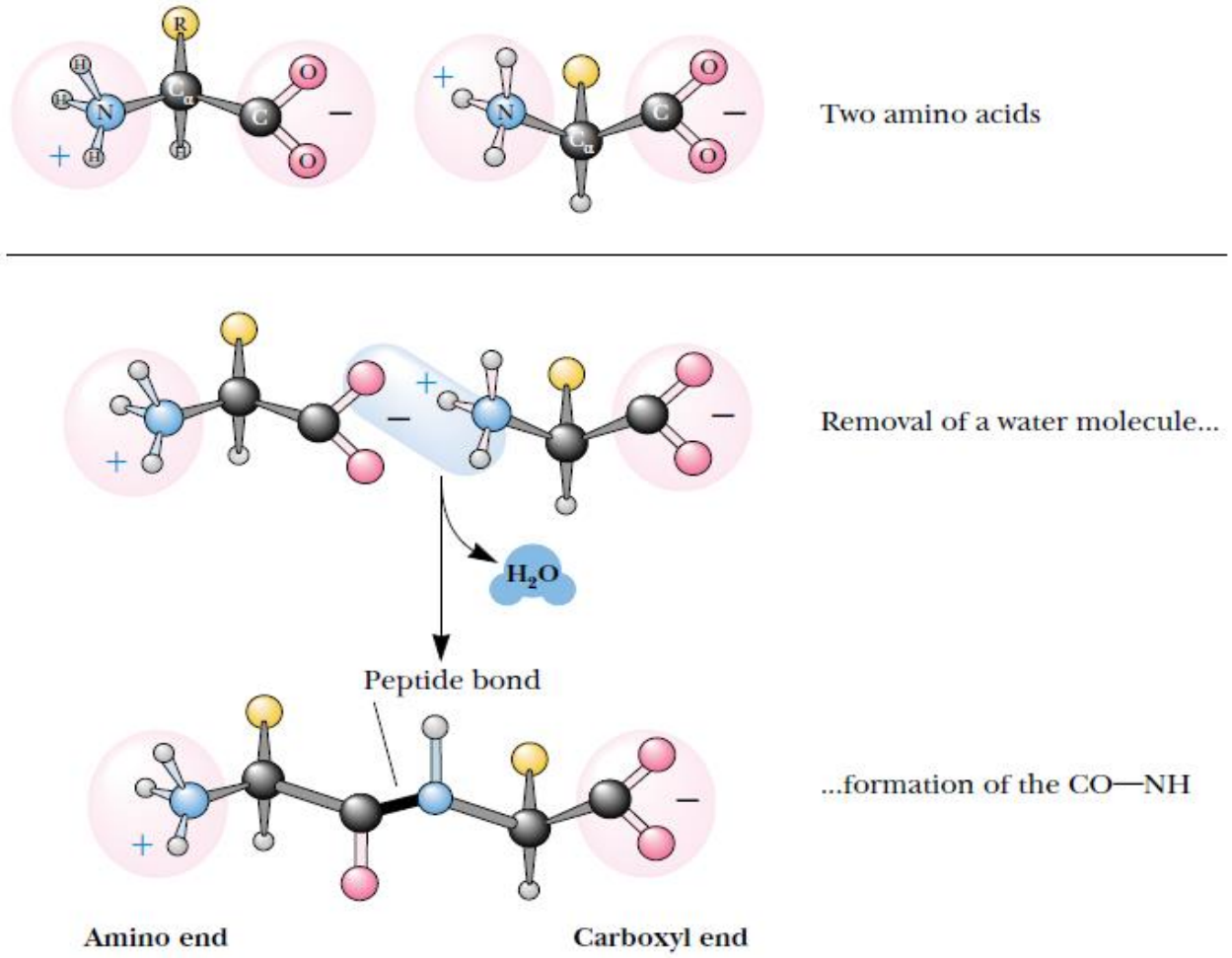
- There are three reasons to consider these reactivities. Proteins can be chemically modified in very specific ways by taking advantage of the chemical reactivity of certain amino acid side chains.

- 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 colour, radioactivity, or some other quantity that can be easily measured.

- Finally and most importantly, the biological functions of proteins depend on the behaviour and reactivity of specific R groups.

Formation of Peptide Bond

- 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 ($-NH_3^+$) and carboxyl ($-COO^-$) groups.



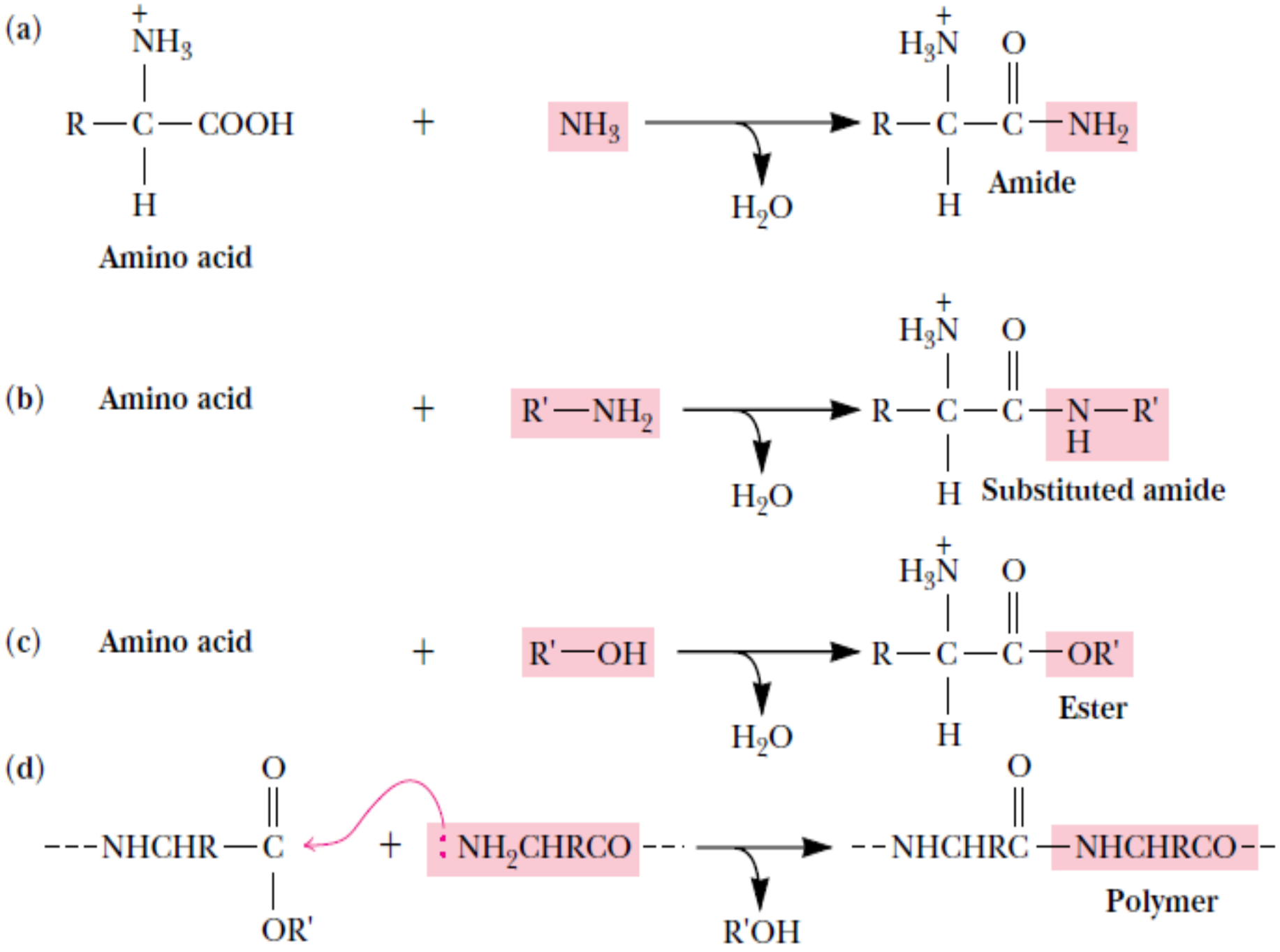
- The amino and carboxyl groups of amino acids can react in a head-to-tail fashion, eliminating a water molecule and forming a covalent amide linkage, which, in the case of peptides and proteins, is typically referred to as a peptide bond.

- The equilibrium for this reaction in aqueous solution favours peptide bond hydrolysis.
  - For this reason, biological systems as well as peptide chemists in the laboratory must carry out peptide bond formation in an indirect manner or with energy input.

Reactions of the Carboxyl group

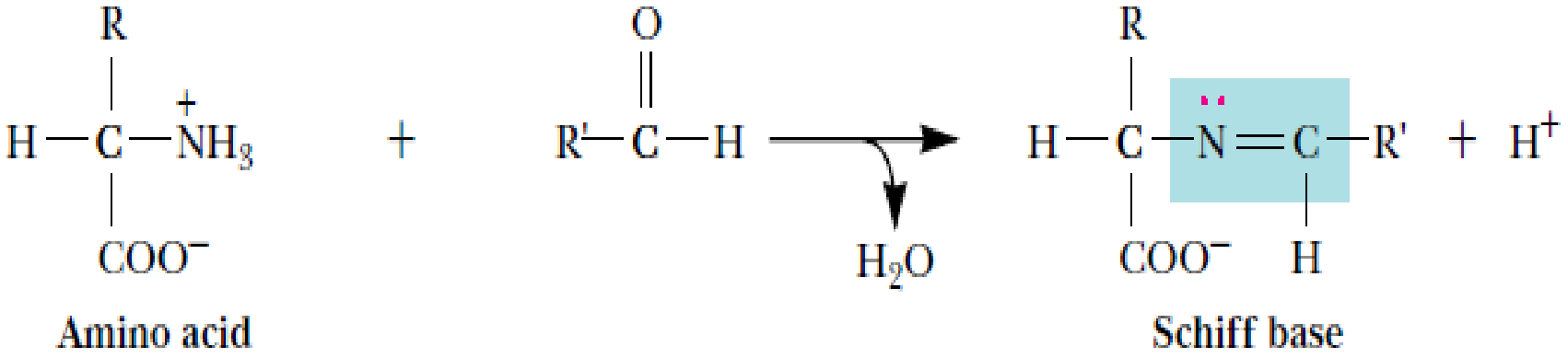
- The carboxyl groups of amino acids undergo all the simple reactions common to this functional group. Reaction with ammonia and primary amines yields unsubstituted and substituted amides, respectively.

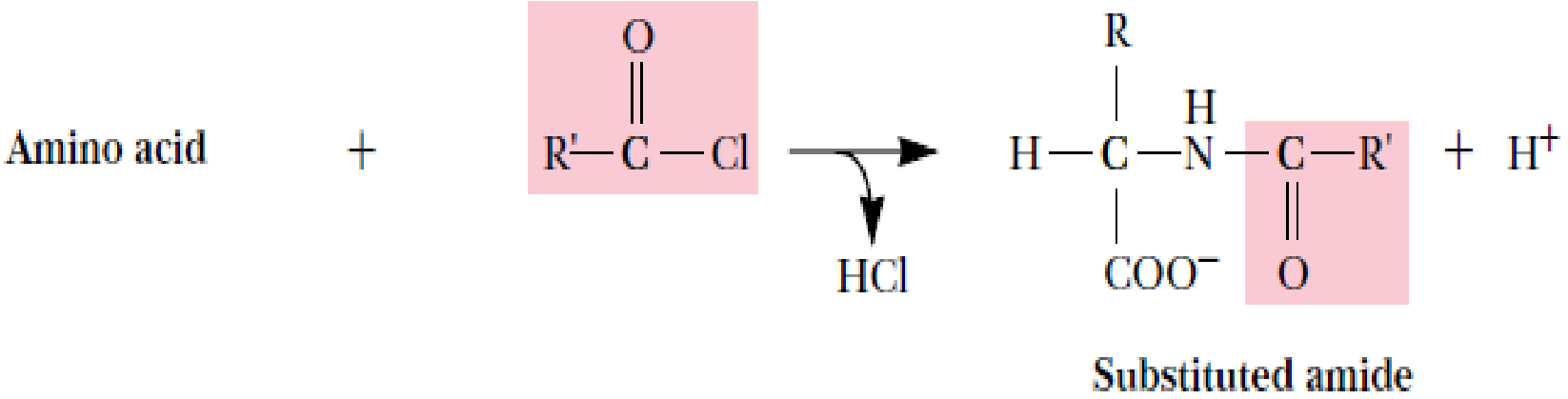
- Esters and acid chlorides are also readily formed. Esterification proceeds in the presence of the appropriate alcohol and a strong acid. Polymerization can occur by repetition of the reaction


(a)
Amino acid
Amide
(b)
Amino acid
Substituted amide
(c)
Amino acid
Ester
(d)
Polymer

Reactions of the Amino group

- Free amino groups may react with aldehydes to form Schiff bases and can be acylated with acid anhydrides and acid halides.



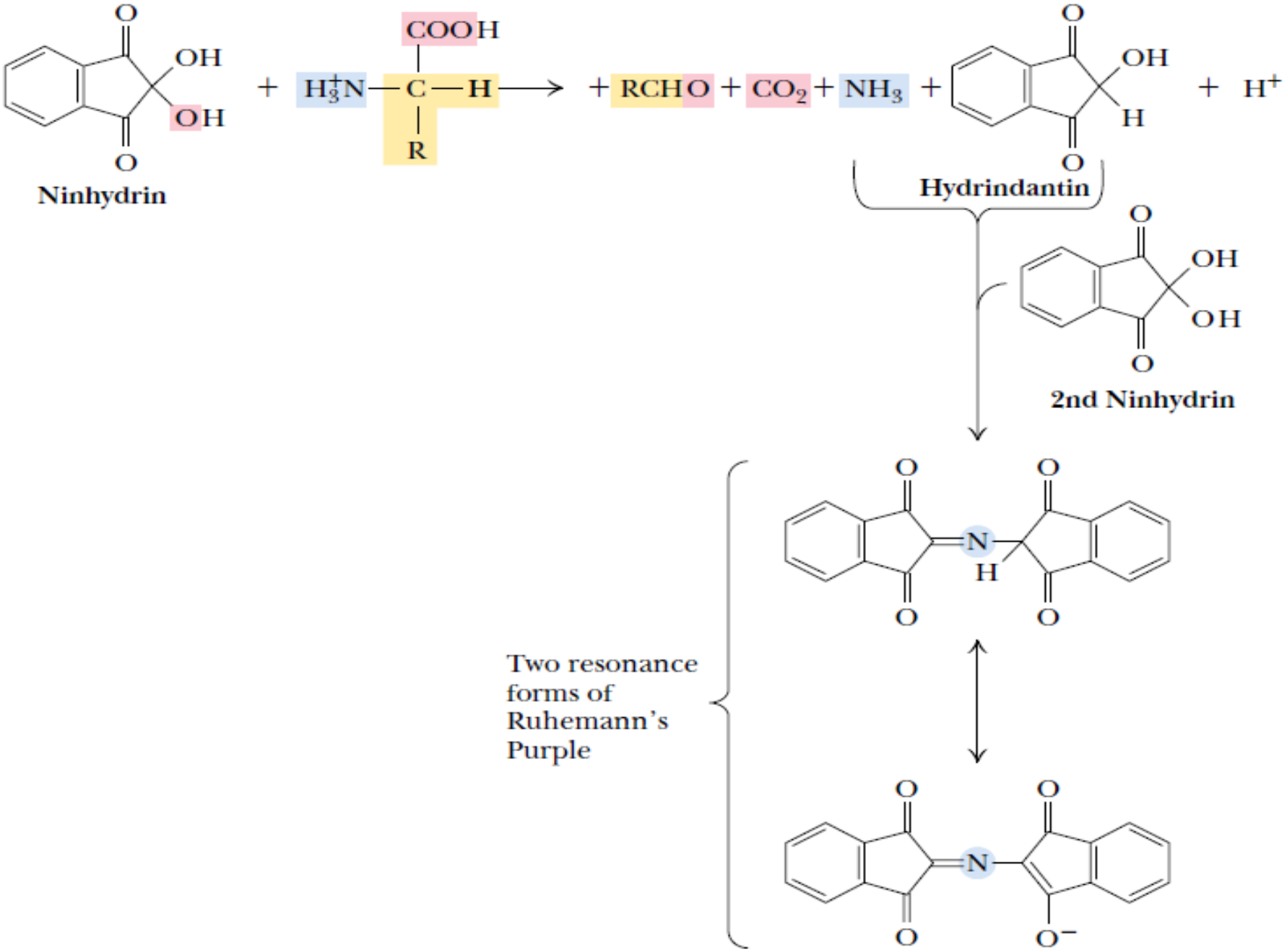


The Ninhydrin Reaction

- Amino acids can be readily detected and quantified by reaction with ninhydrin. *Ninhydrin,* or triketohydrindene hydrate, is a strong oxidizing agent and causes the oxidative deamination of the α-amino function.

- The products of the reaction are the resulting aldehyde, ammonia, carbon dioxide, and hydrindantin, a reduced derivative of ninhydrin.

- The ammonia produced in this way can react with the hydrindantin and another molecule of ninhydrin to yield a purple product (Ruhemann's Purple) that can be quantified spectrophotometrically at 570 nm. The appearance of CO_2 can also be monitored.


Ninhydrin
COOH
H3N
C
H
R
RCHO
CO2
NH3
H+
Hydrindantin
2nd Ninhydrin
Two resonance forms of Ruhemann's Purple

- Indeed, CO_2 evolution is diagnostic of the presence of an α-amino acid. α-Imino acids, such as proline and hydroxyproline, give bright yellow ninhydrin products with absorption maxima at 440 nm, allowing these to be distinguished from the α-amino acids.

- Because amino acids are one of the components of human skin secretions, the ninhydrin reaction was once used extensively by law enforcement and forensic personnel for fingerprint detection.
  - More sensitive fluorescent reagents are now used routinely for this purpose.

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.

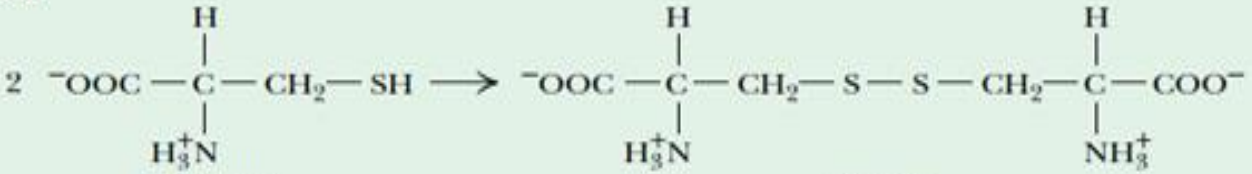
- In recent years, biochemists have developed an arsenal of reactions that are relatively specific to the side chains of particular amino acids. These reactions can be used to identify functional amino acids at the active sites of enzymes or to label proteins with appropriate reagents for further study.

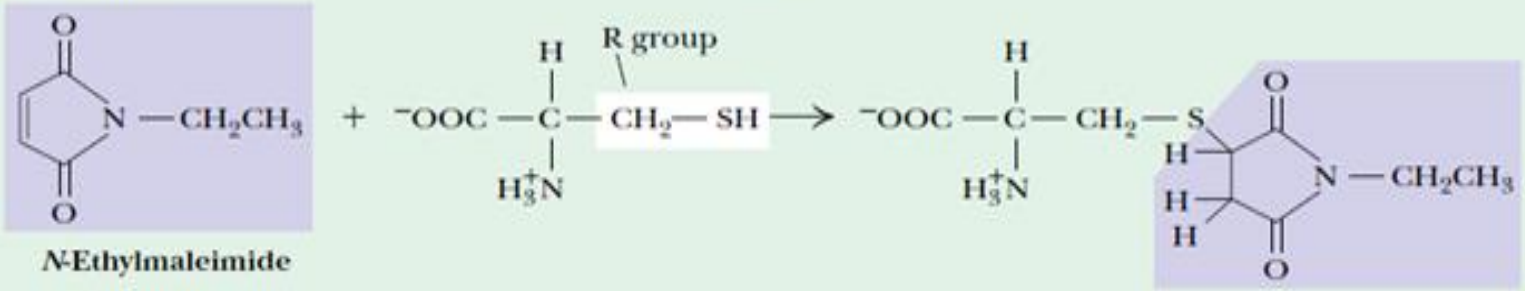
- Cysteine residues in proteins, for example, react with one another to form disulphide species and also react with a number of reagents, including maleimides (typically *N*-ethylmaleimide).

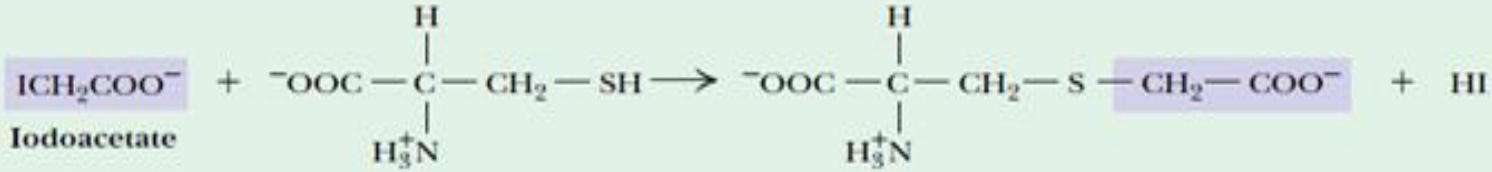
- Cysteines also react effectively with iodoacetic acid to yield *S*-carboxymethyl cysteine derivatives.

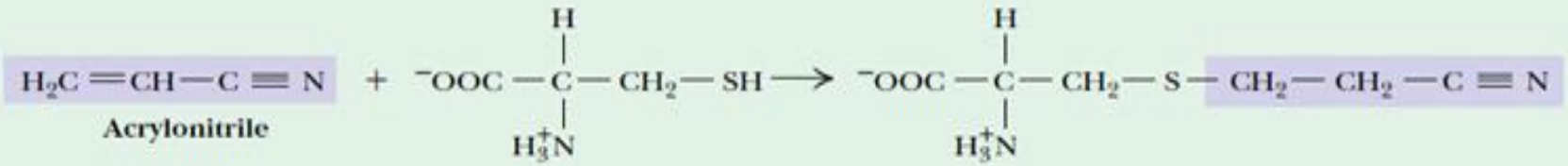
- There are numerous other reactions involving specialized reagents specific for particular side chain functional groups

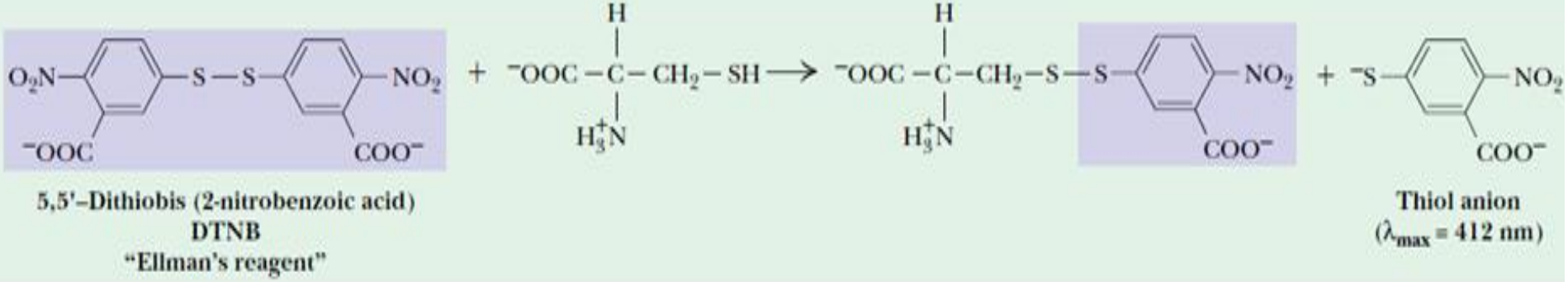
CYSTEINE

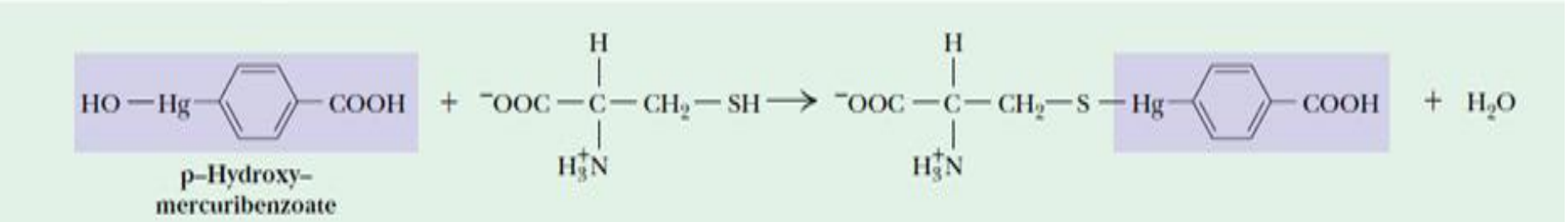

2
Cysteine
Cystine

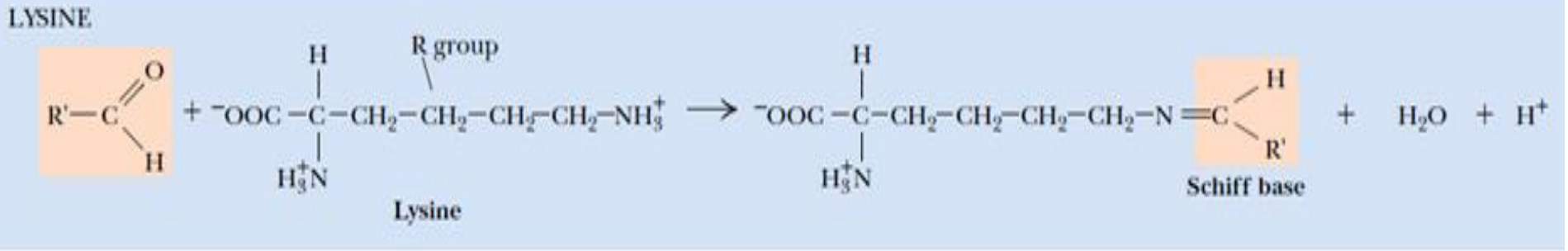

R group
N-Ethylmaleimide


Iodoacetate


Acrylonitrile


5,5'–Dithiobis (2-nitrobenzoic acid)
DTNB
"Ellman's reagent"
Thiol anion
(λ_{max} = 412 nm)

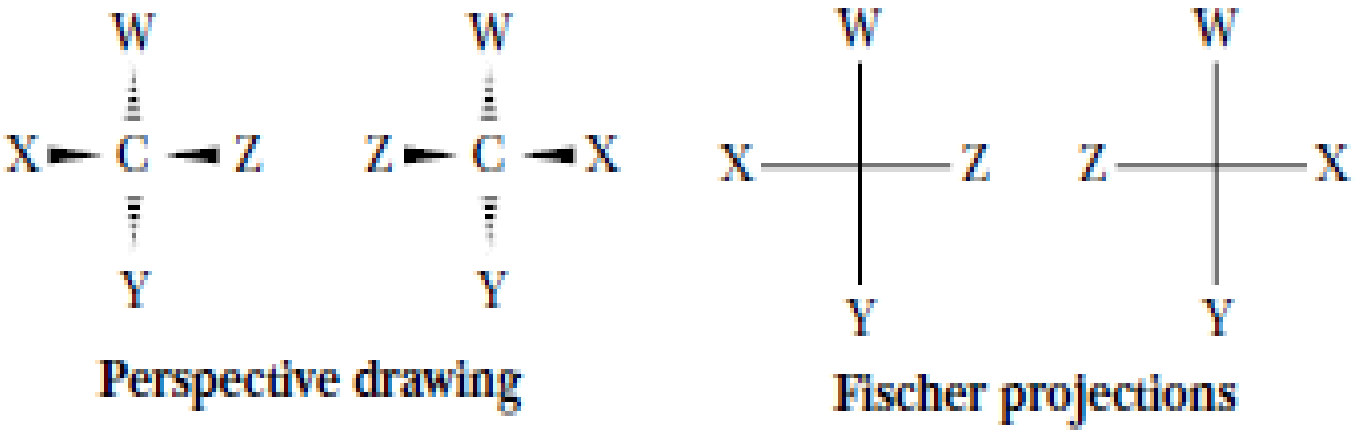

p–Hydroxy–
mercuribenzoate


LYSINE
R group
Lysine
Schiff base

Optical Activity and Stereochemistry of Amino Acids

Chirality Amino Acids

- Except for glycine, all of the amino acids isolated from proteins have four different groups attached to the α-carbon atom.
- In such a case, the α-carbon is said to be **asymmetric** or **chiral**, and the two possible configurations for the α-carbon constitute non-superimposable mirror image isomers, or **enantiomers**



Perspective drawing

Fischer projections

• 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** behaviour, and counter-clockwise rotation is called **levorotatory** behaviour.

- The magnitude and direction of the optical rotation depend on the *nature of the amino acid side chain*. The temperature, the wavelength of the light used in the measurement, the ionization state of the amino acid, and therefore the pH of the solution, can also affect optical rotation behaviour.
- The direction of optical rotation can be specified in the name by using a (+) for dextrorotatory compounds and a (-) for levorotatory compounds, as in L(+)-leucine

Specific Rotations for Some Amino Acids

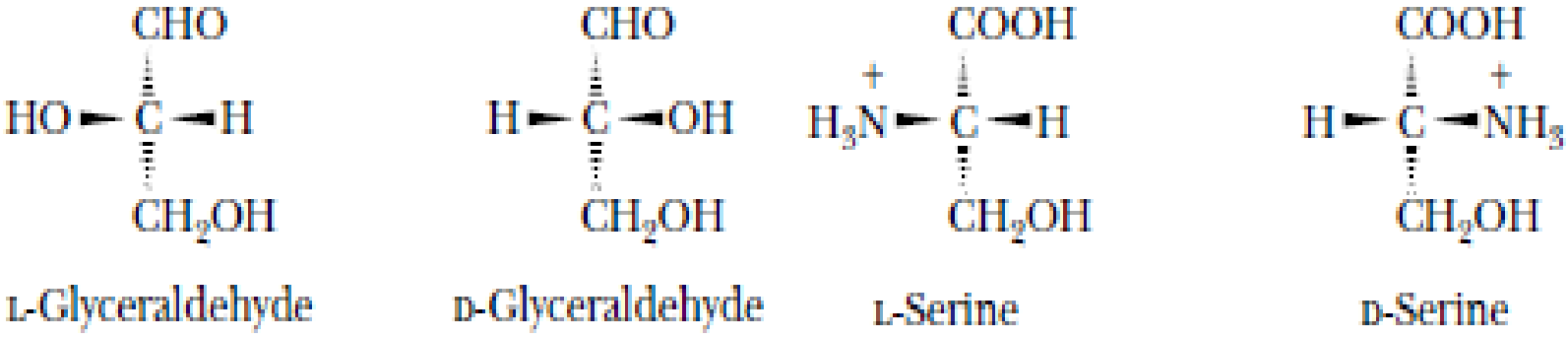
Amino Acid	Specific Rotation $[\alpha]_D^{25}$, Degrees
L-Alanine	+1.8
L-Arginine	+12.5
L-Aspartic acid	+5.0
L-Glutamic acid	+12.0
L-Histidine	−38.5
L-Isoleucine	+12.4
L-Leucine	−11.0
L-Lysine	+13.5
L-Methionine	−10.0
L-Phenylalanine	−34.5
L-Proline	−86.2
L-Serine	−7.5
L-Threonine	−28.5
L-Tryptophan	−33.7
L-Valine	+5.6

Nomenclature for Chiral Molecules

- The discoveries of optical activity and enantiomeric structure made it important to develop suitable nomenclature for chiral molecules.

- Two systems are in common use today: the so-called D,L system and the (*R,S*) system. In the **D,L system** of nomenclature, the (+) and (-) isomers of glyceraldehyde are denoted as **D-glyceraldehyde** and **L-glyceraldehyde,** respectively.

- Absolute configurations of all other carbon-based molecules are referenced to D- and L-glyceraldehyde. When sufficient care is taken to avoid racemization of the amino acids during hydrolysis of proteins, it is found that all of the amino acids derived from natural proteins are of the L configuration.



- Amino acids of the D configuration are nonetheless found in nature, especially as components of certain peptide antibiotics, such as valinomycin, gramicidin, and actinomycin D, and in the cell walls of certain microorganisms.

- In spite of its widespread acceptance, problems exist with the D,L system of nomenclature. For example, this system can be ambiguous for molecules with two or more chiral centres.

- To address such problems, the (***R,S***) **system** of nomenclature for chiral molecules was proposed in 1956 by Robert Cahn, Sir Christopher Ingold, and Vladimir Prelog.

- In this more versatile system, priorities are assigned to each of the groups attached to a chiral centre on the basis of atomic number, atoms with higher atomic numbers having higher priorities.

- The newer (*R,S*) system of nomenclature is superior to the older D,L system in one important way.

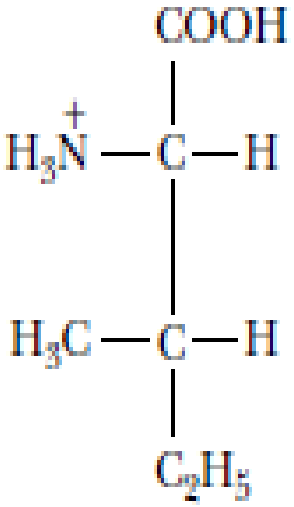
- The configuration of molecules with more than one chiral centre can be more easily, completely, and unambiguously described with (*R,S*) notation.

- Several amino acids, including isoleucine, threonine, hydroxyproline, and hydroxylysine, have two chiral centers. In the (*R,S*) system, L-threonine is (2*S,*3*R*)-threonine.

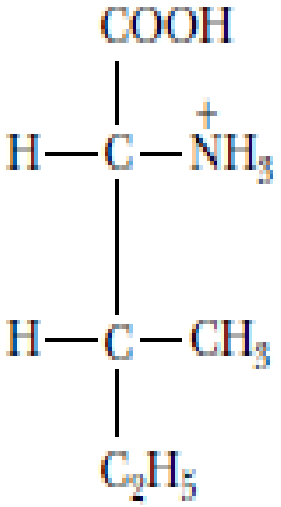
- A chemical compound with *n* chiral centres can exist in 2*n*-isomeric structures, and the four amino acids just listed can thus each take on four different isomeric configurations.

- This amounts to two pairs of enantiomers. Isomers that differ in configuration at only one of the asymmetric centres are non–mirror image isomers or **diastereomers.**

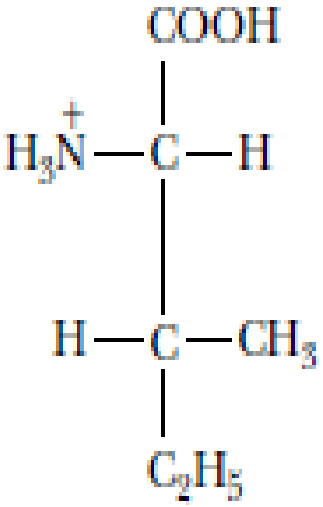
- In the (*R,S*) system, L-isoleucine is (2*S,*3*S*)-isoleucine. Its diastereomer is referred to as L-alloisoleucine. The D-enantiomeric pair of isomers is named in a similar manner.



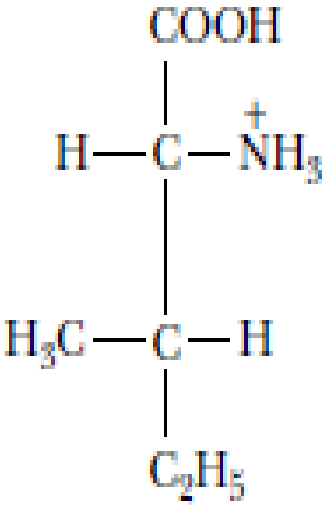
L-Isoleucine
(2*S*,3*S*)-Isoleucine



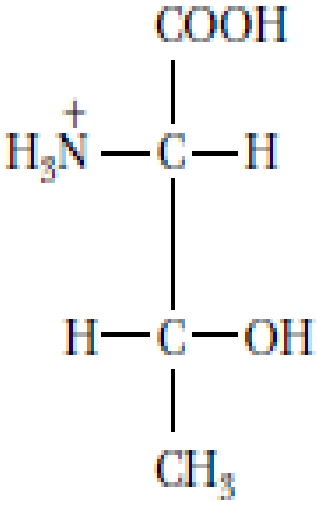
D-Isoleucine
(2*R*,3*R*)-Isoleucine



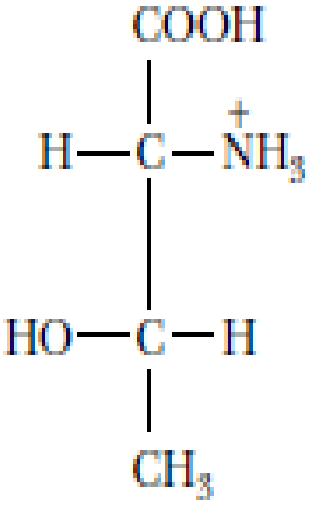
L-Alloisoleucine
(2*S*,3*R*)-Isoleucine



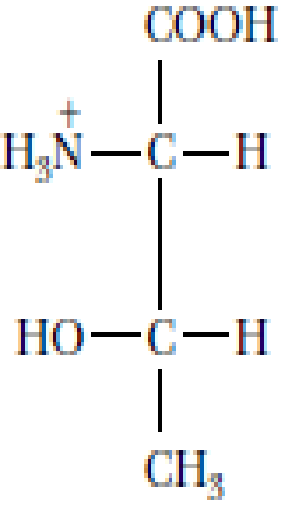
D-Alloisoleucine
(2*R*,3*S*)-Isoleucine



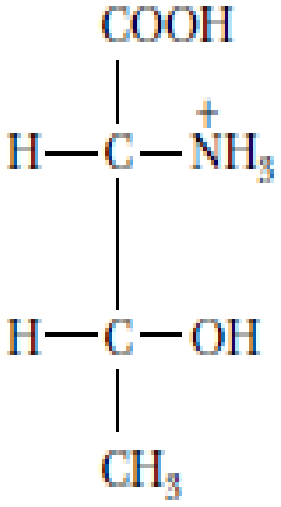
L-Threonine



D-Threonine



L-Allothreonine



D-Allothreonine

Spectroscopic Properties of Amino Acids

- One of the most important and exciting advances in modern biochemistry has been the application of **spectroscopic methods,** which measure the absorption and emission of energy of different frequencies by molecules and atoms.

- Spectroscopic studies of proteins, nucleic acids, and other biomolecules are providing many new insights into the structure and dynamic processes in these molecules.

Ultraviolet Spectra

- Many details of the structure and chemistry of the amino acids have been elucidated or at least confirmed by spectroscopic measurements. None of the amino acids absorbs light in the visible region of the electromagnetic spectrum

- Several of the amino acids, however, do absorb **ultraviolet** radiation, and all absorb in the **infrared** region.

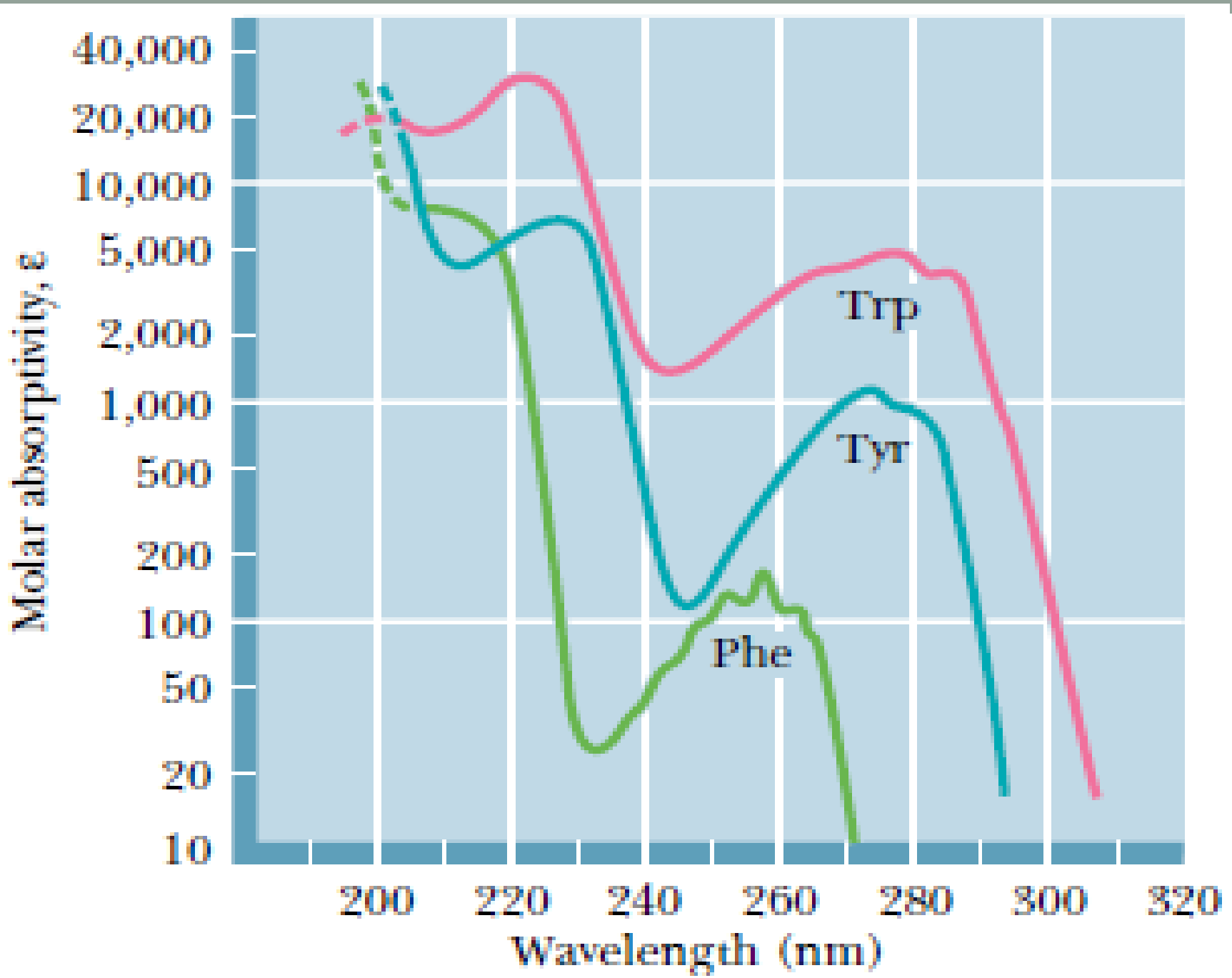
- The absorption of energy by electrons as they rise to higher energy states occurs in the ultraviolet/visible region of the energy spectrum.

- Only the aromatic amino acids phenylalanine, tyrosine, and tryptophan exhibit significant ultraviolet absorption above 250 nm.

- These strong absorptions can be used for spectroscopic determinations of protein concentration.

- The aromatic amino acids also exhibit relatively weak fluorescence, and it has recently been shown that tryptophan can exhibit *phosphorescence*—a relatively long-lived emission of light.

- These fluorescence and phosphorescence properties are especially useful in the study of protein structure and dynamics


Molar absorptivity, ε
40,000
20,000
10,000
5,000
2,000
1,000
500
200
100
50
20
10
Trp
Tyr
Phe
200
220
240
260
280
300
320
Wavelength (nm)

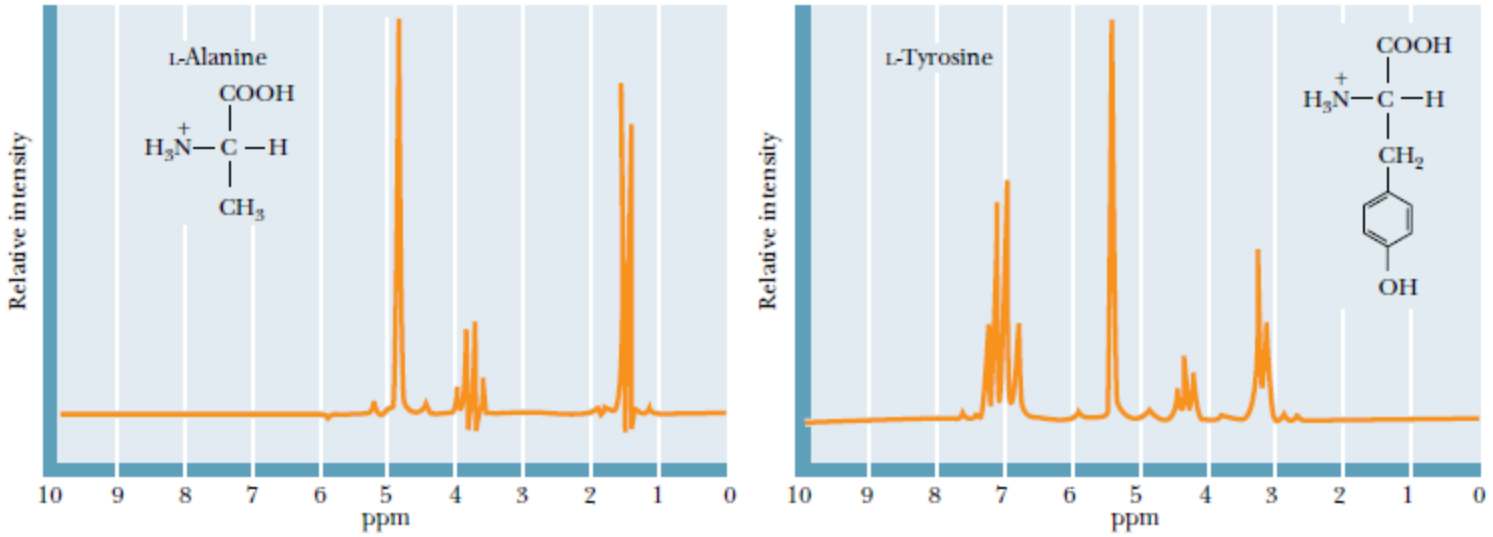
Nuclear Magnetic Resonance Spectra

- The development in the 1950s of **nuclear magnetic resonance** (NMR), a spectroscopic technique that involves the absorption of radio frequency energy by certain nuclei in the presence of a magnetic field, played an important part in the chemical characterization of amino acids and proteins.

- Several important principles rapidly emerged from these studies. First, the **chemical shift** of amino acid protons depends on their particular chemical environment and thus on the state of ionization of the amino acid.

- Second, the change in electron density during a titration is transmitted throughout the carbon chain in the aliphatic amino acids and the aliphatic portions of aromatic amino acids, as evidenced by changes in the chemical shifts of relevant protons.

Finally, the magnitude of the **coupling constants** between protons on adjacent carbons depends in some cases on the ionization state of the amino acid. This apparently reflects differences in the preferred conformations in different ionization states.



Separation and Analysis of Amino Acid Mixtures

Chromatographic Methods

- The purification and analysis of individual amino acids from complex mixtures was once a very difficult process.

- Today, however, the biochemist has a wide variety of methods available for the separation and analysis of amino acids, or for that matter, any of the other biological molecules and macromolecules we encounter.

- All of these methods take advantage of the relative differences in the physical and chemical characteristics of amino acids, particularly ionization behaviour and solubility characteristics.

- The methods important for amino acids include separations based on **partition** properties (the tendency to associate with one solvent or phase over another) and separations based on **electrical charge.**

- In all of the partition methods discussed here, the molecules of interest are allowed (or forced) to flow through a medium consisting of two phases—solid–liquid, liquid–liquid, or gas–liquid.

- In all of these methods, the molecules must show a preference for associating with one or the other phase.
- In this manner, the molecules partition, or distribute themselves, between the two phases in a manner based on their particular properties.
- The ratio of the concentrations of the amino acid (or other species) in the two phases is designated the *partition coefficient.*

- **Chromatography** is term is now applied to a wide variety of separation methods, and the success of all chromatography techniques depends on the repeated microscopic partitioning of a solute mixture between the available phases.

- Chromatographic methods have advanced rapidly in recent years, due in part to the development of sophisticated new solid-phase materials.

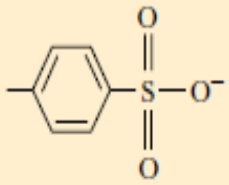
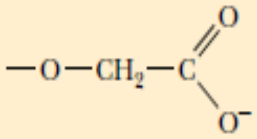
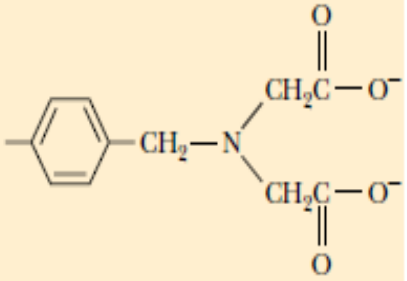
- Methods important for amino acid separations include ion exchange chromatography, gas chromatography (GC), and high-performance liquid chromatography (HPLC)

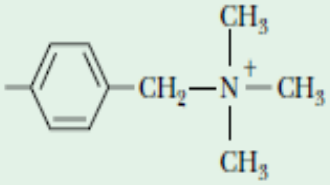
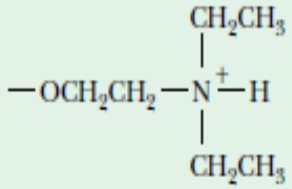
Ion Exchange Chromatography

- The separation of amino acids and other solutes is often achieved by means of **ion exchange chromatography,** in which the molecule of interest is *exchanged* for another ion onto and off of a charged solid support.

- In a typical procedure, solutes in a liquid phase, usually water, are passed through columns filled with a porous solid phase, usually a bed of synthetic resin particles, containing charged groups. Resins containing positive charges attract negatively charged solutes and are referred to as *anion exchangers.*

- Solid supports possessing negative charges attract positively charged species and are referred to as *cation exchangers.* Several typical cation and anion exchange resins with different types of charged groups

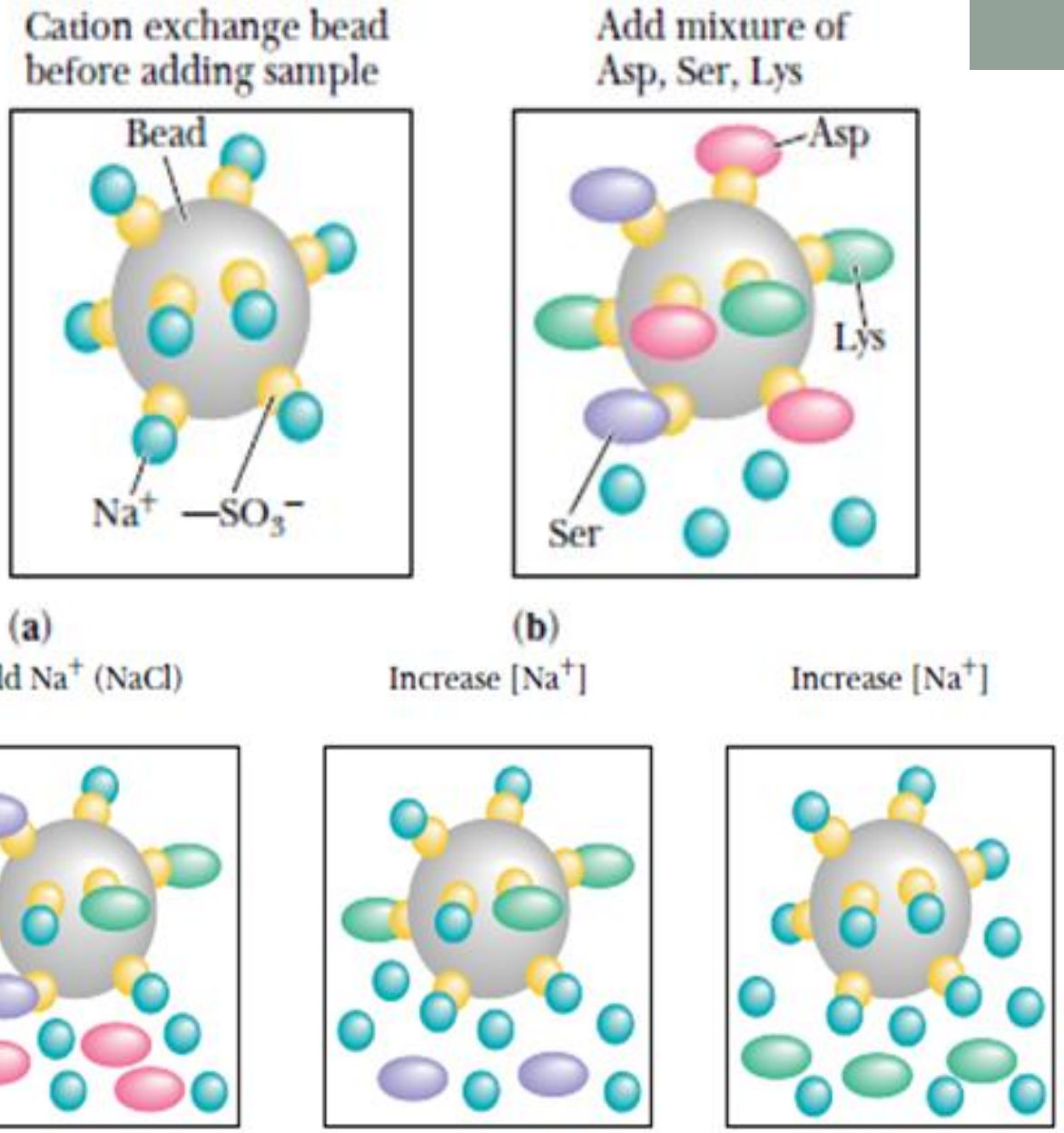
(a) Cation Exchange Media	Structure
Strongly acidic, polystyrene resin (Dowex–50)	
Weakly acidic, carboxymethyl (CM) cellulose	
Weakly acidic, chelating, polystyrene resin (Chelex–100)	

(b) Anion Exchange Media	Structure
Strongly basic, polystyrene resin (Dowex–1)	
Weakly basic, diethylaminoethyl (DEAE) cellulose	

- Strength of the acidity or basicity of these groups and their number per unit volume of resin determine the type and strength of binding of an exchanger.

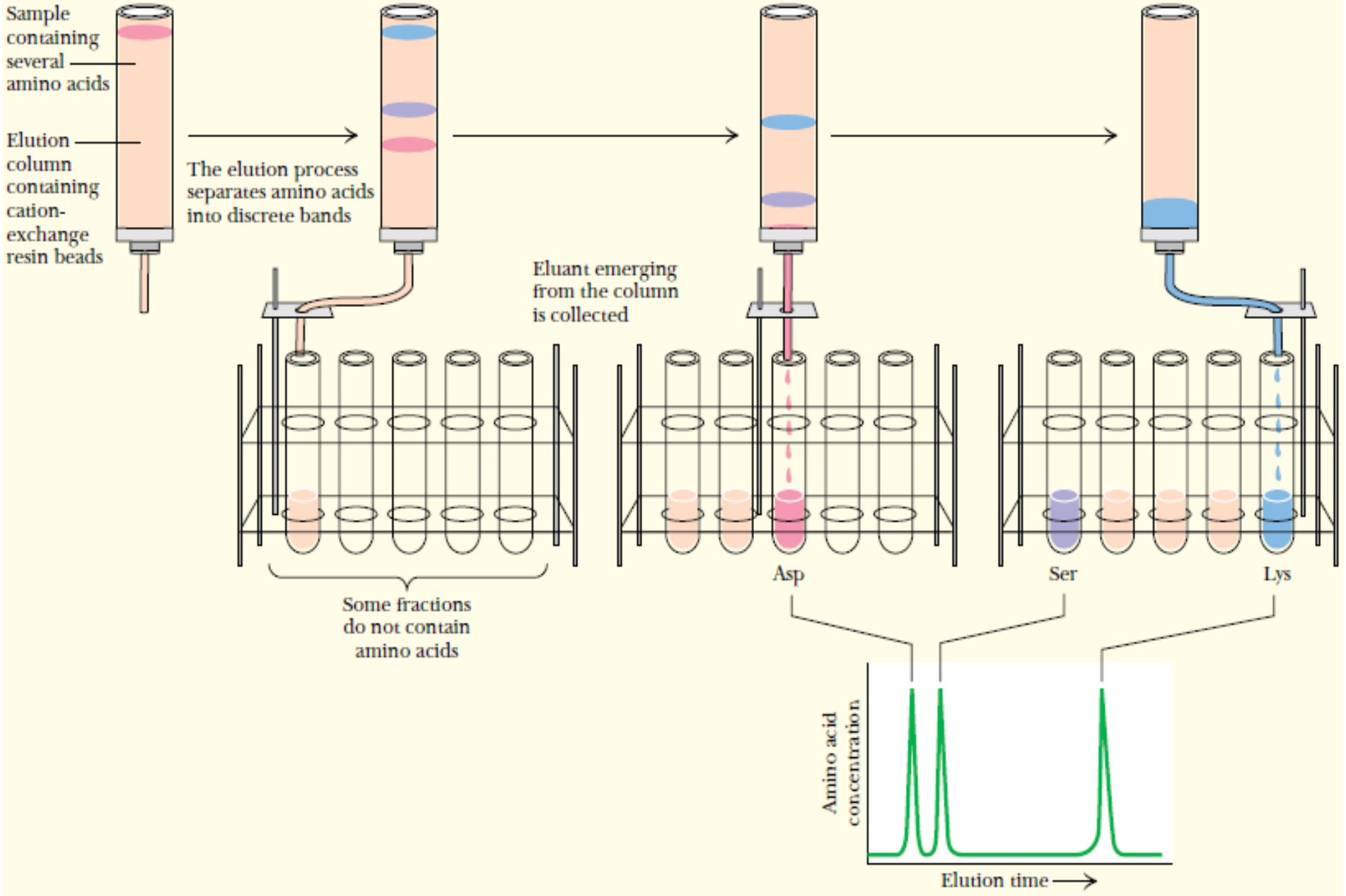
- Fully ionized acidic groups such as sulphonic acids result in an exchanger with a negative charge which binds cations very strongly. Weakly acidic or basic groups yield resins whose charge (and binding capacity) depends on the pH of the eluting solvent. The choice of the appropriate resin depends on the strength of binding desired.

- The bare charges on such solid phases must be counterbalanced by oppositely charged ions in solution ("counterions"). Washing a cation exchange resin, such as Dowex-50, which has strongly acidic phenyl-SO_3^- groups, with a NaCl solution results in the formation of the so-called sodium form of the resin


Cation exchange bead before adding sample
Bead
Na^+
$-SO_3^-$
(a)
Add Na^+ (NaCl)
Add mixture of Asp, Ser, Lys
Asp
Lys
Ser
(b)
Increase $[Na^+]$
Increase $[Na^+]$
(c) Asp, the least positively charged amino acid, is eluted first
(d) Serine is eluted next
(e) Lysine, the most positively charged amino acid, is eluted last

- When the mixture whose separation is desired is added to the column, the positively charged solute molecules displace the Na^+ ions and bind to the resin.

- A gradient of an appropriate salt is then applied to the column, and the solute molecules are competitively (and sequentially) displaced (eluted) from the column by the rising concentration of cations in the gradient, in an order that is inversely related to their affinities for the column.


Sample containing several amino acids
Elution column containing cation-exchange resin beads
The elution process separates amino acids into discrete bands
Eluant emerging from the column is collected
Some fractions do not contain amino acids
Asp
Ser
Lys
Amino acid concentration
Elution time