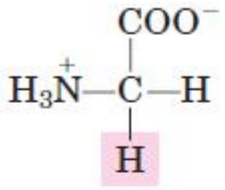
Amino Acids & Properties

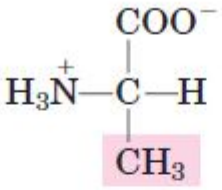
TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r	pK_a values: pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	6.3

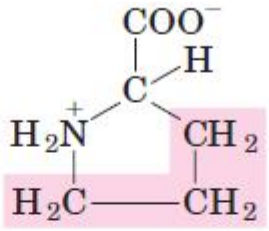
Nonpolar, aliphatic R groups



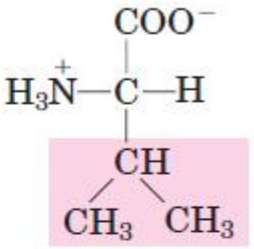
Glycine



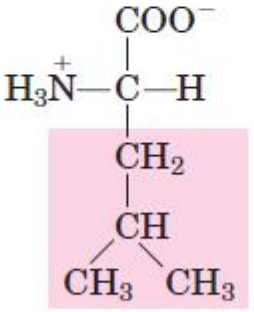
Alanine



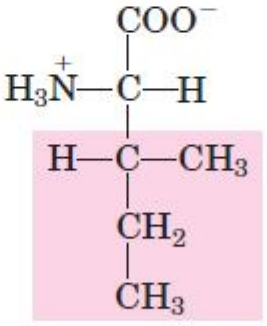
Proline



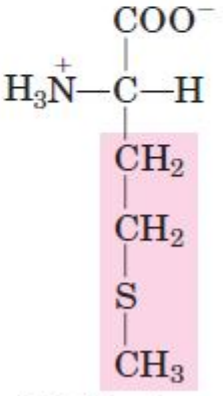
Valine



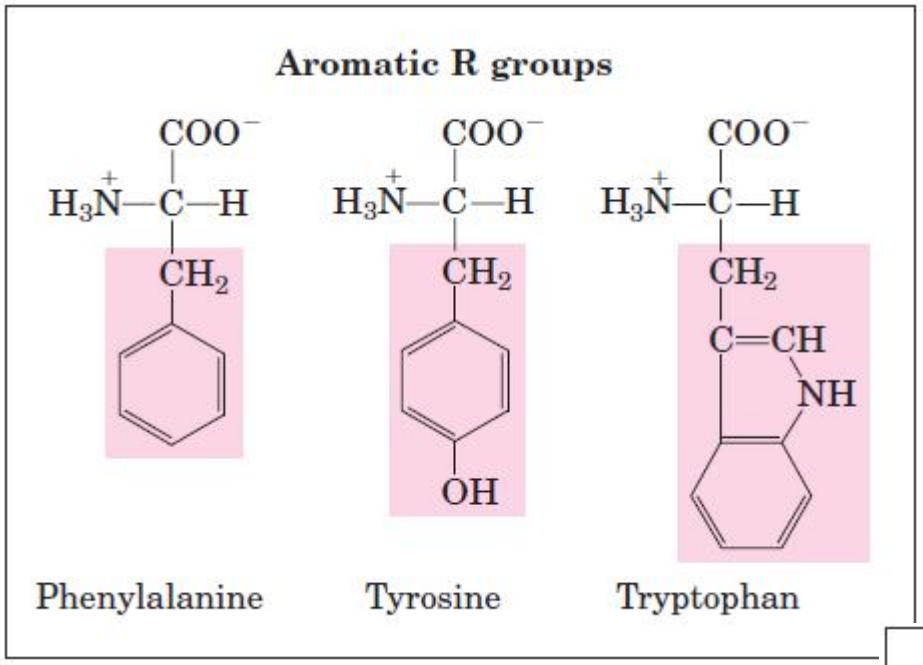
Leucine

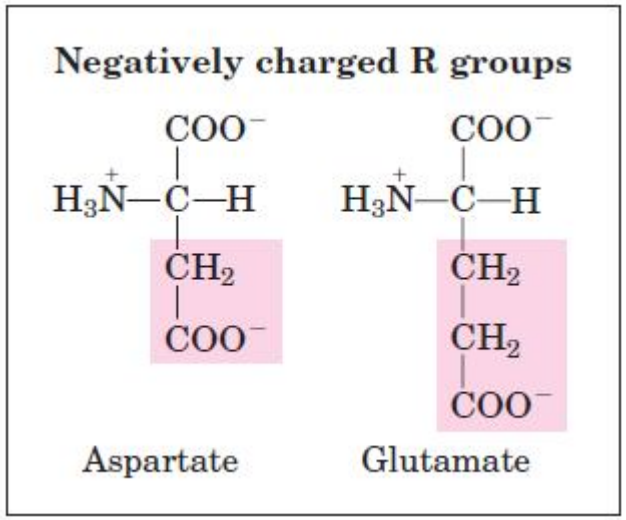


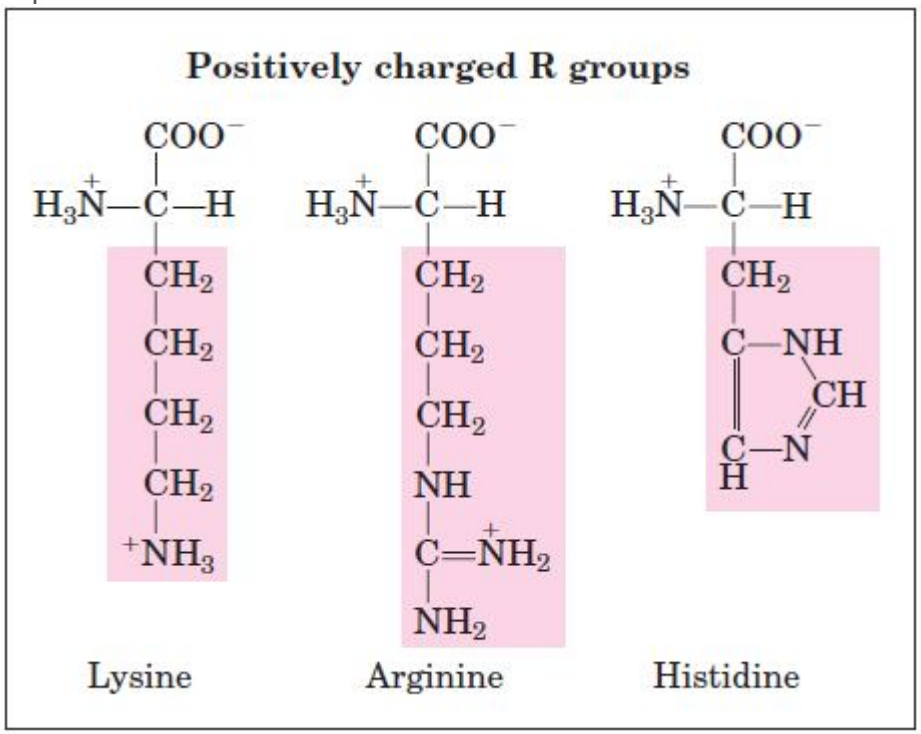
Isoleucine



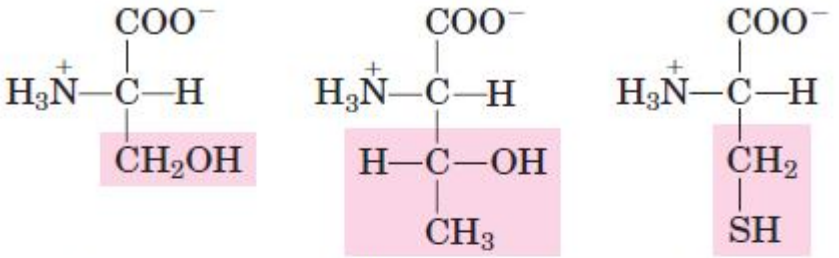
Methionine


Aromatic R groups
Phenylalanine
Tyrosine
Tryptophan


Negatively charged R groups
Aspartate
Glutamate


Positively charged R groups
Lysine
Arginine
Histidine

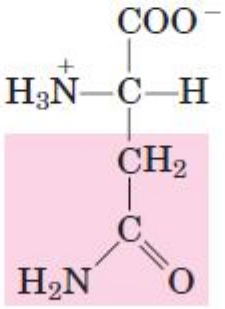
Polar, uncharged R groups



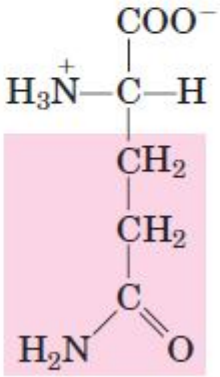
Serine

Threonine

Cysteine



Asparagine



Glutamine

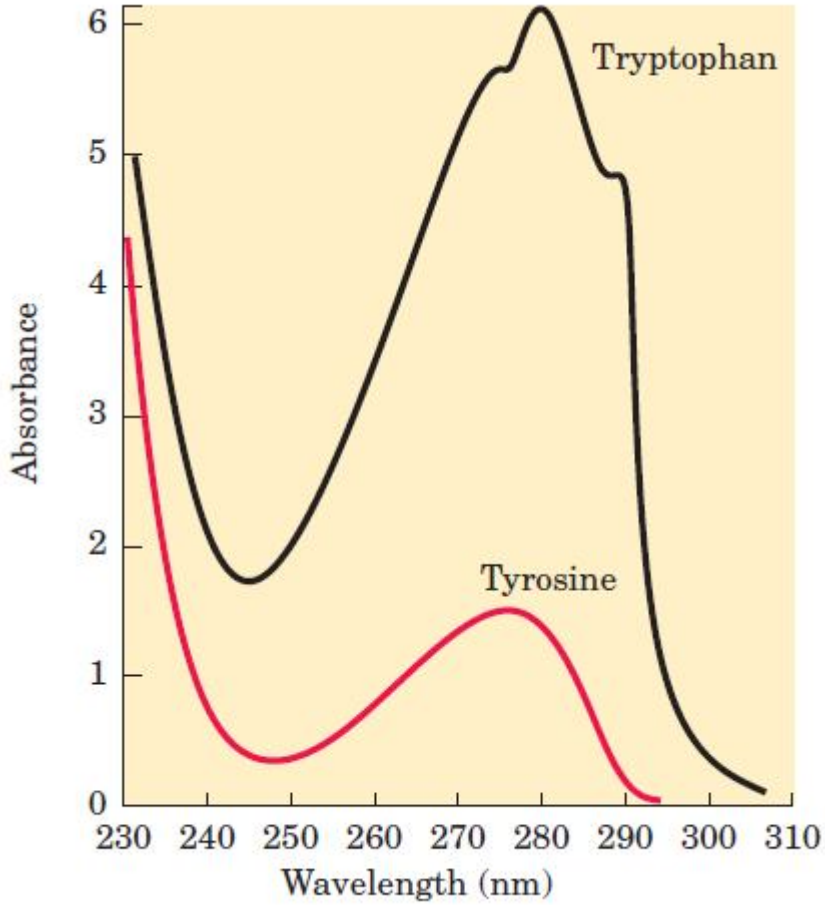
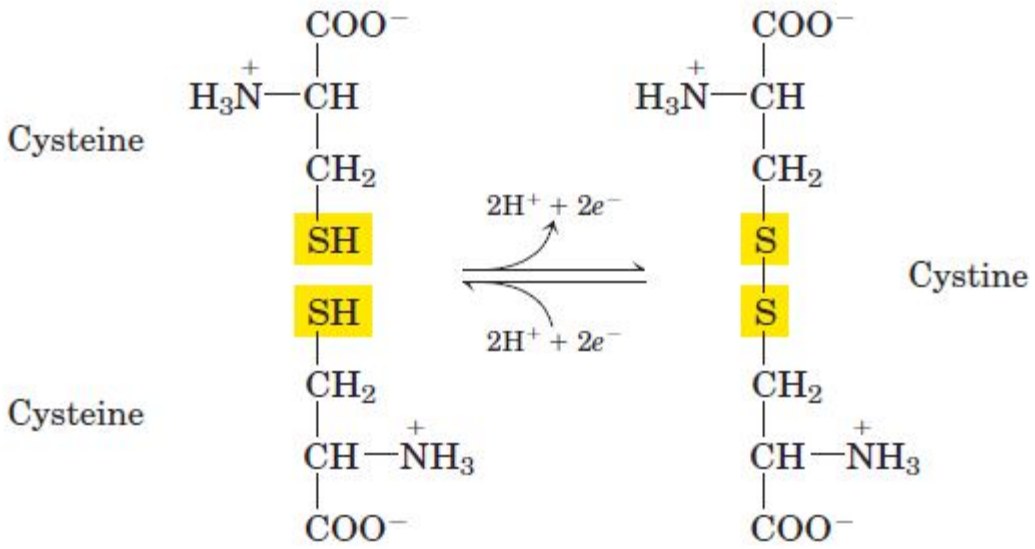
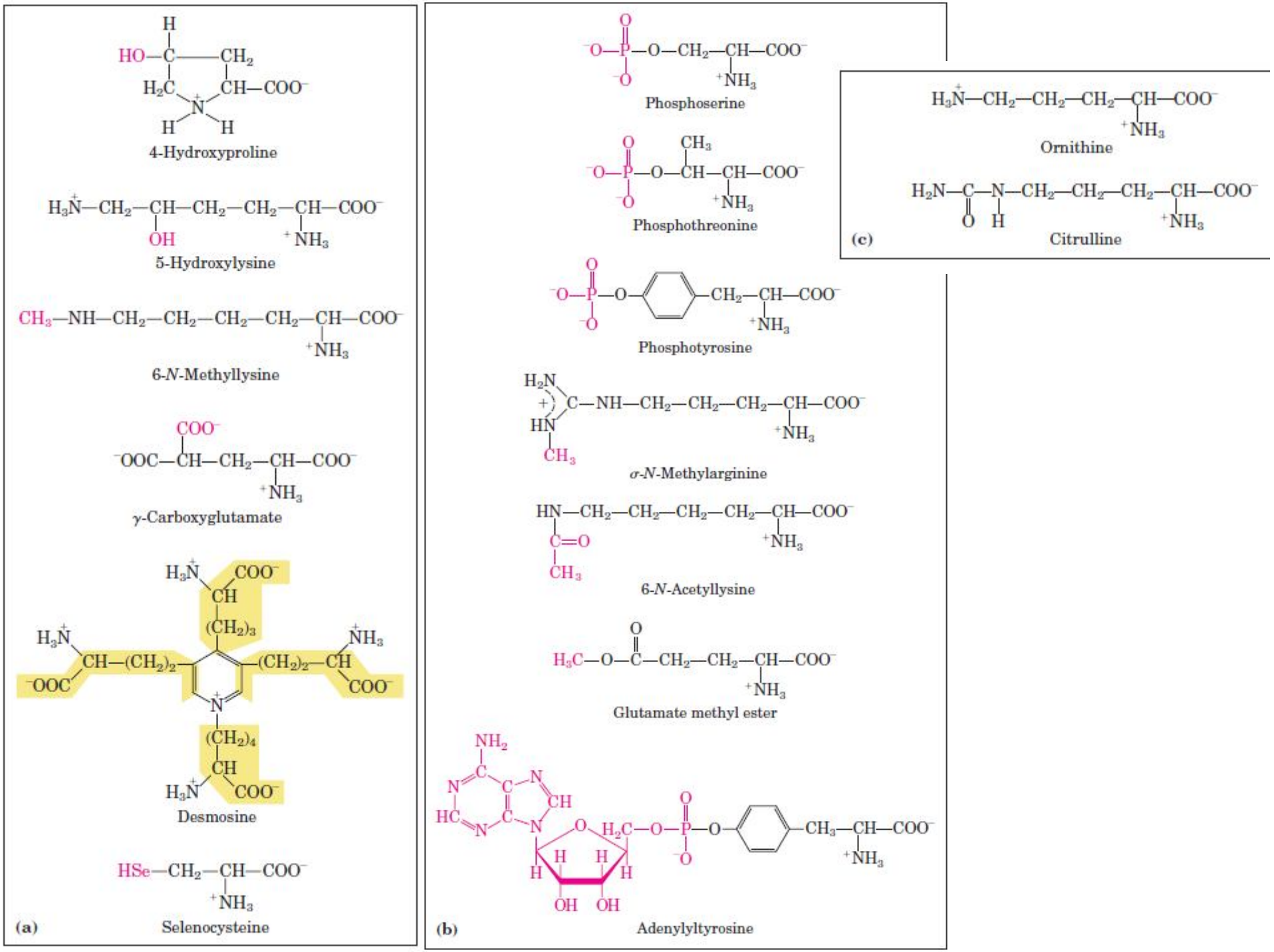


FIGURE 3–6 Absorption of ultraviolet light by aromatic amino acids. Comparison of the light absorption spectra of the aromatic amino acids tryptophan and tyrosine at pH 6.0. The amino acids are present in equimolar amounts (10^{-3} M) under identical conditions. The measured absorbance of tryptophan is as much as four times that of tyrosine. Note that the maximum light absorption for both tryptophan and tyrosine occurs near a wavelength of 280 nm. Light absorption by the third aromatic amino acid, phenylalanine (not shown), generally contributes little to the spectroscopic properties of proteins.


Cysteine
Cysteine
Cystine
2H+ + 2e−
2H+ + 2e−

Uncommon Amino acids

- **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 γ**-carboxyglutamate,** found in the blood clotting protein prothrombin and in certain other proteins that bind 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.


4-Hydroxyproline
5-Hydroxylysine
6-N-Methyllysine
γ-Carboxyglutamate
Desmosine
Selenocysteine
(a)
Phosphoserine
Phosphothreonine
Phosphotyrosine
σ-N-Methylarginine
6-N-Acetyllysine
Glutamate methyl ester
Adenylyltyrosine
(b)
Ornithine
Citrulline
(c)

Absorption of Light by Molecules: The Lambert-Beer Law

A wide range of biomolecules absorb light at characteristic wavelengths, just as tryptophan absorbs light at 280 nm (see Fig. 3–6). Measurement of light absorption by a spectrophotometer is used to detect and identify molecules and to measure their concentration in solution. The fraction of the incident light absorbed by a solution at a given wavelength is related to the thickness of the absorbing layer (path length) and the concentration of the absorbing species (Fig. 1). These two relationships are combined into the Lambert-Beer law,

$$\log \frac{I_0}{I} = \varepsilon c l$$

where I_0 is the intensity of the incident light, I is the intensity of the transmitted light, ε is the molar extinction coefficient (in units of liters per mole-centimeter), c is the concentration of the absorbing species (in moles per liter), and l is the path length of the light-absorbing sample (in centimeters). The Lambert-Beer law assumes that the incident light is parallel and monochromatic (of a single wavelength) and that the solvent and solute molecules are randomly oriented. The expression $\log (I_0/I)$ is called the **absorbance,** designated A.

It is important to note that each successive millimeter of path length of absorbing solution in a 1.0 cm cell absorbs not a constant amount but a constant fraction of the light that is incident upon it. However, with an absorbing layer of fixed path length, *the absorbance,* A, *is directly proportional to the concentration of the absorbing solute.*

The molar extinction coefficient varies with the nature of the absorbing compound, the solvent, and the wavelength, and also with pH if the light-absorbing species is in equilibrium with an ionization state that has different absorbance properties.

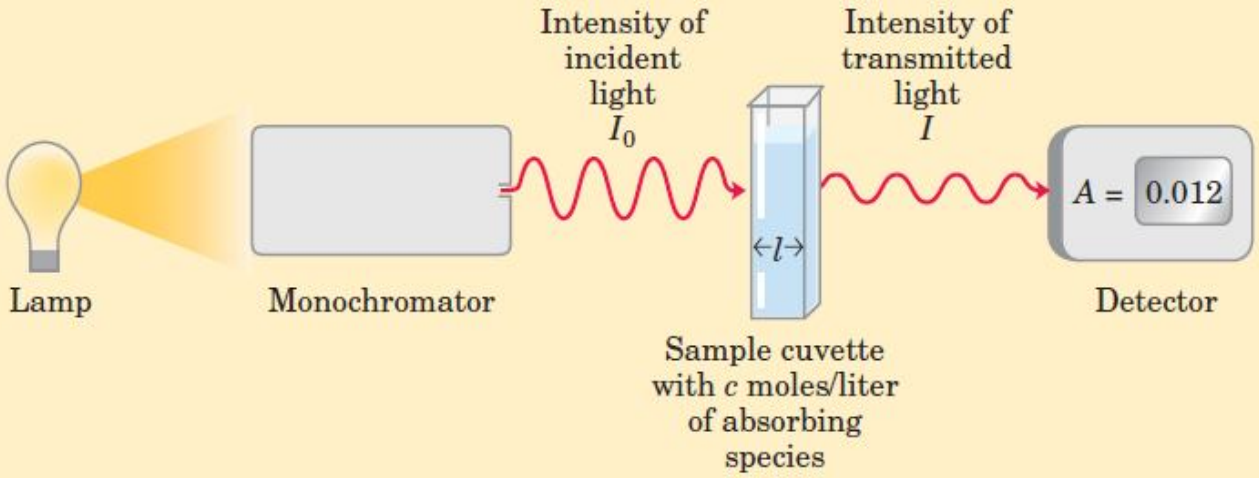


FIGURE 1 The principal components of a spectrophotometer. A light source emits light along a broad spectrum, then the monochromator selects and transmits light of a particular wavelength. The monochromatic light passes through the sample in a cuvette of path length l and is absorbed by the sample in proportion to the concentration of the absorbing species. The transmitted light is measured by a detector.

Ionization of Water, Weak acids, and Weak Bases

- Like all reversible reactions, the ionization of water can be described by an equilibrium constant.

- When weak acids are dissolved in water, they contribute H^+ by ionizing; weak bases consume H^+ by becoming protonated. These processes are also governed by equilibrium constants. The total hydrogen ion concentration from all sources is experimentally measurable and is expressed as the pH of the solution.

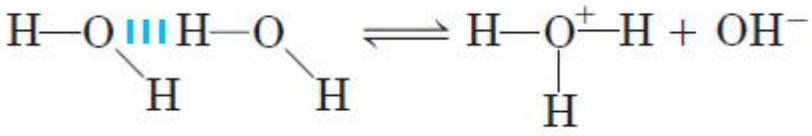
- To predict the state of ionization of solutes in water, we must take into account the relevant equilibrium constants for each ionization reaction.

Pure Water Is Slightly Ionized

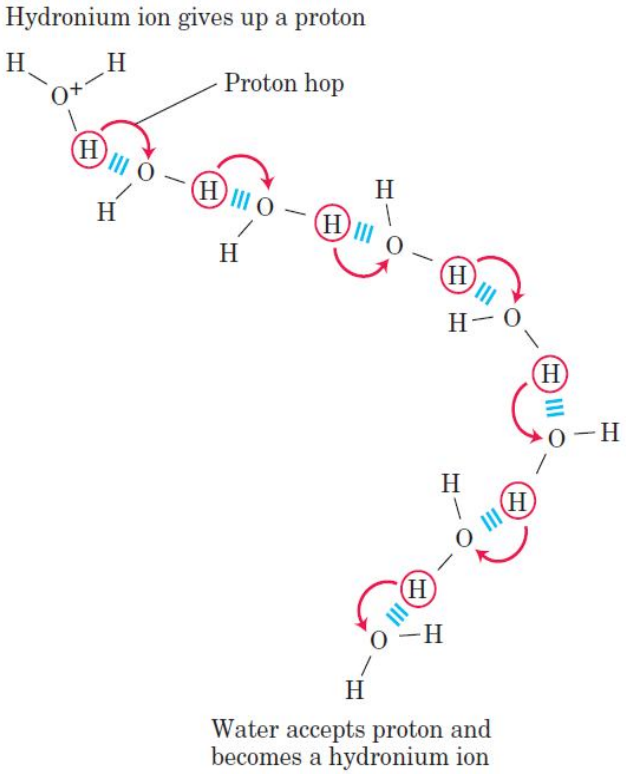
- Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion (a proton) and a hydroxide ion, giving the equilibrium

$$H_2O \rightleftharpoons H^+ + OH^-$$

Dissociation product of water as H^+, free protons do not exist in solution; hydrogen ions formed in water are immediately hydrated to **hydronium ions (H_3O^+).** Hydrogen bonding between water molecules makes the hydration of dissociating protons virtually instantaneous:



The ionization of water can be measured by its electrical conductivity; pure water carries electrical current as H_3O^+ migrates toward the cathode and OH^- toward the anode. The movement of hydronium and hydroxide ions in the electric field is extremely fast compared with that of other ions such as Na^+, K^+, and Cl^+. This high ionic mobility results from the kind of "proton hopping". **No individual proton moves very** far through the bulk solution, but a series of proton hops between hydrogen-bonded water molecules causes the *net movement of a proton over a long distance in a remarkably* short time. As a result of the high ionic mobility of H^+ (and of OH^-, which also moves rapidly by proton hopping, but in the opposite direction), acid–base reactions in aqueous solutions are exceptionally fast.



The position of equilibrium of any chemical reaction is given by its **equilibrium constant, *Keq* *(sometimes*** expressed simply as *K). For the generalized reaction*

$$A + B \rightleftharpoons C + D \qquad (2\text{–}2)$$

an equilibrium constant can be defined in terms of the concentrations of reactants (A and B) and products (C and D) at equilibrium:

$$K_{eq} = \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}}$$

The equilibrium constant is fixed and characteristic for any given chemical reaction at a specified temperature. It defines the composition of the final equilibrium mixture, regardless of the starting amounts of reactants and products. Conversely, we can calculate the equilibrium constant for a given reaction at a given temperature if the equilibrium concentrations of all its reactants and products are known.

The Ionization of Water Is Expressed by an Equilibrium Constant

The degree of ionization of water at equilibrium (Eqn 2–1) is small; at 25° C only about two of every 10^9 molecules in pure water are ionized at any instant. The equilibrium constant for the reversible ionization of water is

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]} \qquad (2–3)$$

In pure water at 25° C, the concentration of water is 55.5 M—grams of H2O in 1 L divided by its gram molecular weight: (1,000 g/L)/(18.015 g/mol)—and is essentially constant in relation to the very low concentrations of H^+ and OH^-, namely, 1 x 10^{-7} M.
Accordingly, we can substitute 55.5 M in the equilibrium constant expression (Eqn 2–3) to yield

$$K_{eq} = \frac{[H^+][OH^-]}{[55.5\ \text{M}]}$$

On rearranging, this becomes

$$(55.5\ \text{M})(K_{eq}) = [H^+]\,[OH^-] = K_w \qquad (2–4)$$

where $\mathbf{K_W}$ designates the product (55.5 M)(Keq), the **ion product of water** at 25° C.

The value for K_{eq}, determined by electrical-conductivity measurements of pure water, is 1.8×10^{-16} M at 25 °C. Substituting this value for K_{eq} in Equation 2–4 gives the value of the ion product of water:

$$K_w = [H^+][OH^-] = (55.5\ \text{M})(1.8 \times 10^{-16}\ \text{M})$$
$$= 1.0 \times 10^{-14}\ \text{M}^2$$

Thus the product $[H^+][OH^-]$ in aqueous solutions at 25 °C always equals 1×10^{-14} M^2. When there are exactly equal concentrations of H^+ and OH^-, as in pure water, the solution is said to be at **neutral pH.** At this pH, the concentration of H^+ and OH^- can be calculated from the ion product of water as follows:

$$K_w = [H^+][OH^-] = [H^+]^2 = [OH^-]^2$$

Solving for $[H^+]$ gives

$$[H^+] = \sqrt{K_w} = \sqrt{1 \times 10^{-14}\ \text{M}^2}$$
$$[H^+] = [OH^-] = 10^{-7}\ \text{M}$$

As the ion product of water is constant, whenever $[H^+]$ is greater than 1×10^{-7} M, $[OH^-]$ must be less than 1×10^{-7} M, and vice versa. When $[H^+]$ is very high, as in a solution of hydrochloric acid, $[OH^-]$ must be very low. From the ion product of water we can calculate $[H^+]$ if we know $[OH^-]$, and vice versa.

The pH Scale Designates the H^+ and OH^- Concentrations

The ion product of water, K_w, is the basis for the **pH scale** (Table 2–6). It is a convenient means of designating the concentration of H^+ (and thus of OH^-) in any aqueous solution in the range between 1.0 M H^+ and 1.0 M OH^-. The term **pH** is defined by the expression

$$pH = \log \frac{1}{[H^+]} = -\log [H^+]$$

The symbol p denotes "negative logarithm of." For a precisely neutral solution at 25 °C, in which the concentration of hydrogen ions is 1.0×10^{-7} M, the pH can be calculated as follows:

$$pH = \log \frac{1}{1.0 \times 10^{-7}} = 7.0$$

Note that the concentration of H^+ must be expressed in molar (M) terms.

TABLE 2–6 The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression $pOH = -\log [OH^-]$, which is analogous to the expression for pH. Note that in all cases, pH + pOH = 14.

What is the concentration of H^+ in a solution of 0.1 M NaOH?

Solution: We begin with the equation for the ion product of water:

$$K_w = [H^+][OH^-]$$

With $[OH^-] = 0.1$ M, solving for $[H^+]$ gives

$$[H^+] = \frac{K_w}{[OH^-]} = \frac{1 \times 10^{-14}\,M^2}{0.1\,M} = \frac{10^{-14}\,M^2}{10^{-1}\,M}$$

$$= 10^{-13}\,M$$

What is the concentration of OH^- in a solution with an H^+ concentration of 1.3×10^{-4} M?

Solution: We begin with the equation for the ion product of water:

$$K_w = [H^+][OH^-]$$

With $[H^+] = 1.3 \times 10^{-4}$ M, solving for $[OH^-]$ gives

$$[OH^-] = \frac{K_w}{[H^+]} = \frac{1 \times 10^{-14}\,M^2}{0.00013\,M} = \frac{10^{-14}\,M^2}{1.3 \times 10^{-4}\,M}$$

$$= 7.7 \times 10^{-11}\,M$$

In all calculations be sure to round your answer to the correct number of significant figures, as here.

Weak Acids and Bases Have Characteristic Acid Dissociation Constants

Acids may be defined as proton donors and bases as proton acceptors. A proton donor and its corresponding proton acceptor make up a **conjugate acid-base pair.**
Acetic acid (CH3COOH), a proton donor, and the acetate anion ($CH3COO^-$), the corresponding proton acceptor, constitute a conjugate acid-base pair, related by the reversible reaction

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$

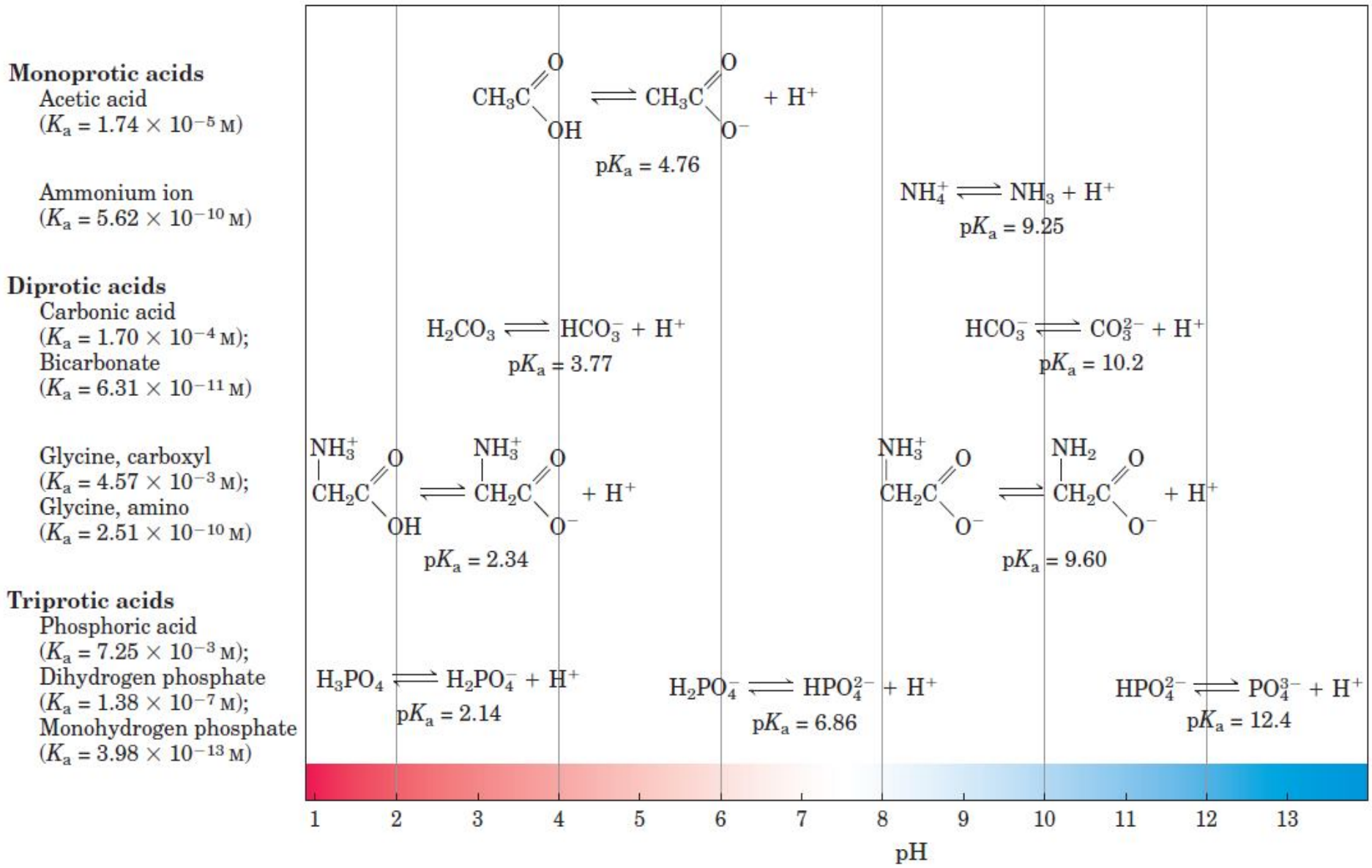
Each acid has a characteristic tendency to lose its proton in an aqueous solution. The stronger the acid, the greater its tendency to lose its proton. The tendency of any acid (HA) to lose a proton and form its conjugate base (A^-) is defined by the equilibrium constant ($\mathbf{K_{eq}}$) for the reversible reaction

$$HA \rightleftharpoons H^+ + A^-,$$

which is

$$K_{eq} = \frac{[H^+][A^-]}{[HA]} = K_a$$

Equilibrium constants for ionization reactions are usually called ionization or **dissociation constants,** often designated *Ka*.
Stronger acids, such as phosphoric and carbonic acids, have larger dissociation constants; weaker acids, such as monohydrogen phosphate (HPO_4^{2-}), have smaller dissociation constants.



Conjugate acid-base pairs consist of a proton donor and a proton acceptor. Some compounds, such as acetic acid and ammonium ion, are monoprotic; they can give up only one proton. Others are diprotic (H_2CO_3 (carbonic acid) and glycine) or triprotic (H_3PO_4 (phosphoric acid)). The dissociation reactions for each pair are shown where they occur along a pH gradient. The equilibrium or dissociation constant (K_a) and its negative logarithm, the pK_a, are shown for each reaction.

pK_a is analogous to pH and is defined by the equation

$$pK_a = \log \frac{1}{K_a} = -\log K_a$$

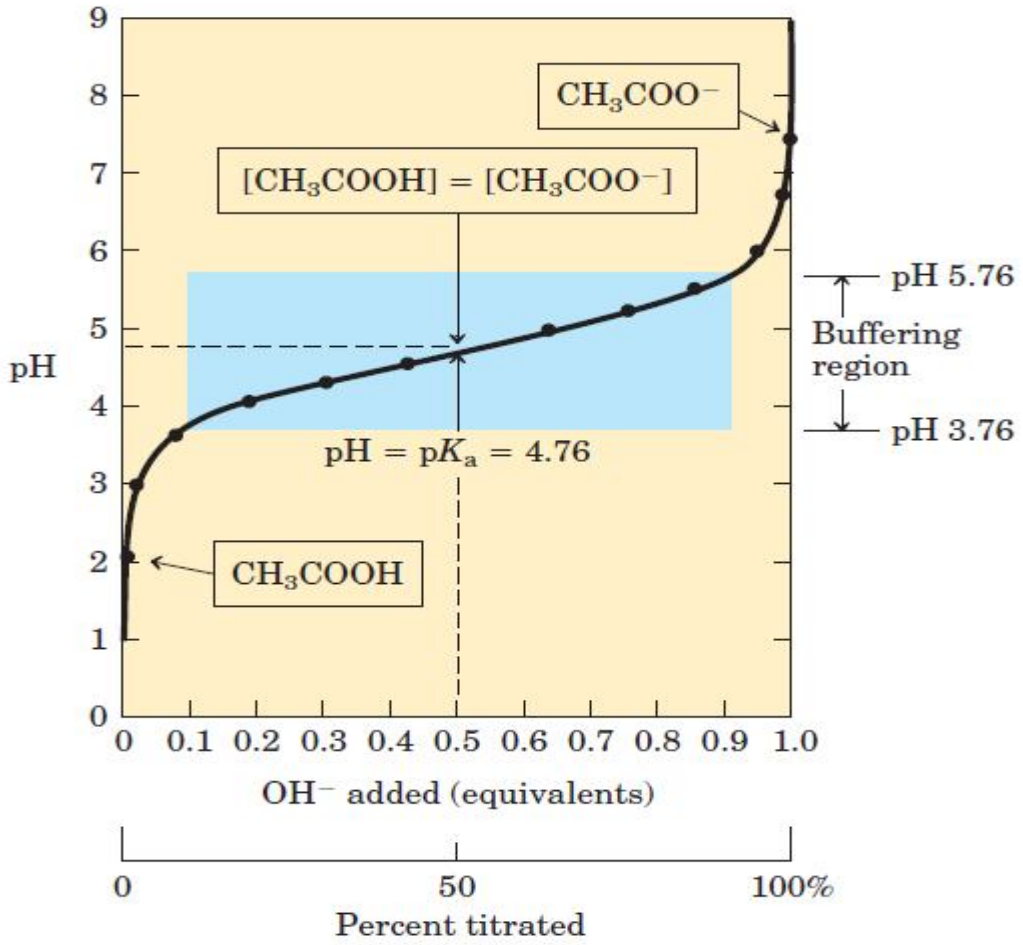
The stronger the tendency to dissociate a proton, the stronger is the acid and the lower its **p*K*$_a$**.

Titration is used to determine the amount of an acid in a given solution. A measured volume of the acid is titrated with a solution of a strong base, usually sodium hydroxide (NaOH), of known concentration. The NaOH is added in small increments until the acid is consumed (neutralized), as determined with an indicator dye or a pH meter. The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added.

A plot of pH against the amount of NaOH added (a **titration curve)** reveals the **p*Ka*** of the weak acid. Consider the titration of a 0.1 M solution of acetic acid (for simplicity denoted as HAc) with 0.1 M NaOH at 25 C°. Two reversible equilibria are involved in the process:

$$H_2O \rightleftharpoons H^+ + OH^- \quad \text{---------- (1)}$$

$$HAc \rightleftharpoons H^+ + Ac^- \quad \text{---------- (2)}$$



The titration curve of acetic acid. After addition of each increment of NaOH to the acetic acid solution, the pH of the mixture is measured. This value is plotted against the amount of NaOH expressed as a fraction of the total NaOH required to convert all the acetic acid to its deprotonated form, acetate. The points so obtained yield the titration curve. Shown in the boxes are the predominant ionic forms at the points designated. At the midpoint of the titration, the concentrations of the proton donor and proton acceptor are equal, and the pH is numerically equal to the p*Ka. The shaded zone is the* useful region of buffering power, generally between 10% and 90% titration of the weak acid.

The equilibria must simultaneously conform to their characteristic equilibrium constants, which are, respectively,

$$K_w = [H^+][OH^-] = 1 \times 10^{-14}\ \text{M}^2 \quad \text{---------- (3)}$$

$$K_a = \frac{[H^+][Ac^-]}{[HAc]} = 1.74 \times 10^{5}\ \text{M} \quad \text{---------- (4)}$$

At the beginning of the titration, before any NaOH is added, the acetic acid is already slightly ionized, to an extent that can be calculated from its dissociation constant (Equ 4).

As NaOH is gradually introduced, the added OH^- combines with the free H^+ in the solution to form H_2O, to an extent that satisfies the equilibrium relationship in Equation 3. As free H^+ is removed, HAc dissociates further to satisfy its own equilibrium constant (Eqn 4). The net result as the titration proceeds is that more and more HAc ionizes, forming Ac^-, as the NaOH is added.

At the midpoint of the titration, at which exactly 0.5 equivalent of NaOH has been added, one-half of the original acetic acid has undergone dissociation, so that the concentration of the proton donor, [HAc], now equals that of the proton acceptor, [Ac^-]. At this midpoint a very important relationship holds: the pH of the equimolar solution of acetic acid and acetate is exactly equal to the p*Ka of acetic acid (pKa 4.76*).

As the titration is continued by adding further increments of NaOH, the remaining nondissociated acetic acid is gradually converted into acetate. The end point of the titration occurs at about pH 7.0: all the acetic acid has lost its protons to OH-, to form H2O and acetate.
Throughout the titration the two equilibria (Eqns 1, 2) coexist, each always conforming to its equilibrium constant.

- Pure water ionizes slightly, forming equal numbers of hydrogen ions (hydronium ions, H_3O^+) and hydroxide ions. The extent of ionization is described by an equilibrium constant, $K_{eq} = \frac{[H^+][OH^-]}{[H_2O]}$, from which the ion product of water, K_w, is derived. At 25 °C, $K_w = [H^+][OH^-] = (55.5\ \text{M})(K_{eq}) = 10^{-14}\ \text{M}^2$.

- The pH of an aqueous solution reflects, on a logarithmic scale, the concentration of hydrogen ions: $\text{pH} = \log \frac{1}{[H^+]} = -\log [H^+]$.

- The greater the acidity of a solution, the lower its pH. Weak acids partially ionize to release a hydrogen ion, thus lowering the pH of the aqueous solution. Weak bases accept a hydrogen ion, increasing the pH. The extent of these processes is characteristic of each particular weak acid or base and is expressed as a dissociation constant, K_a: $K_{eq} = \frac{[H^+][A^-]}{[HA]} = K_a$.

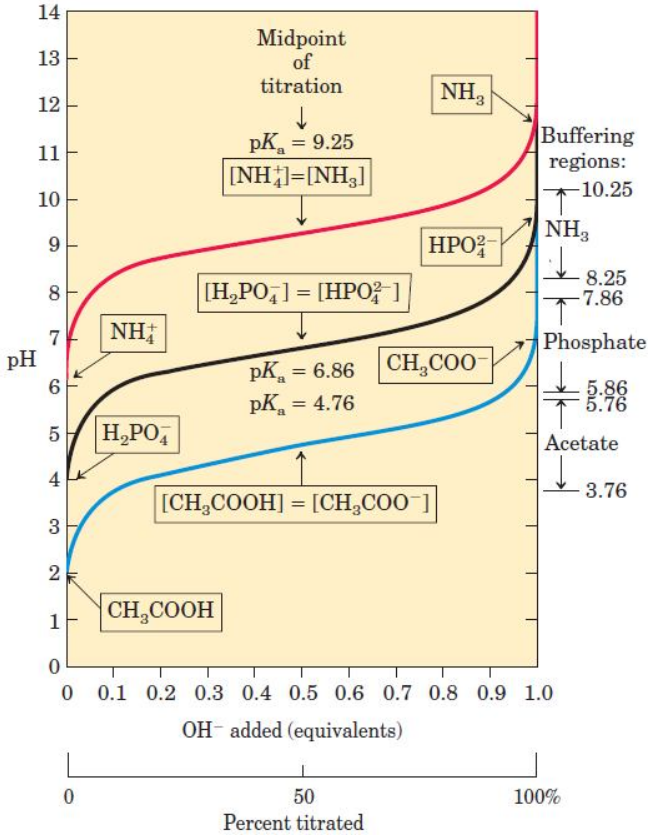
- The pK_a expresses, on a logarithmic scale, the relative strength of a weak acid or base: $pK_a = \log \frac{1}{K_a} = -\log K_a$.

- The stronger the acid, the lower its pK_a; the stronger the base, the higher its pK_a. The pK_a can be determined experimentally; it is the pH at the midpoint of the titration curve for the acid or base.

A Simple Expression Relates pH, pKa, and Buffer Concentration

The titration curves of acetic acid, $H2PO_4^-$, and NH_4^- (Fig) have nearly identical shapes, suggesting that these curves reflect a fundamental law or relationship. The shape of the titration curve of any weak acid is described by the **Henderson- Hasselbalch equation,** which is important for understanding buffer action and acid-base balance in the blood and tissues of vertebrates.

This equation is simply a useful way of restating the expression for the dissociation constant of an acid. For the dissociation of a weak acid HA into H^+ and A^-, the Henderson- Hasselbalch equation can be derived as follows:



$$K_a = \frac{[H^+][A^-]}{[HA]}$$

First solve for $[H^+]$:

$$[H^+] = K_a \frac{[HA]}{[A^-]}$$

Then take the negative logarithm of both sides:

$$-\log [H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

Substitute pH for $-\log [H^+]$ and pK_a for $-\log K_a$:

$$pH = pK_a - \log \frac{[HA]}{[A^-]}$$

Now invert $-\log [HA]/[A^-]$, which involves changing its sign, to obtain the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \qquad (2\text{–}9)$$

Stated more generally,

$$pH = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

This equation fits the titration curve of all weak acids and enables us to deduce a number of important quantitative relationships. For example, it shows why the p*Ka* of a weak acid is equal to the pH of the solution at the midpoint of its titration. At that point, [HA] equals [A^-], and

$$pH = pK_a + \log 1 = pK_a + 0 = pK_a$$

Henderson-Hasselbalch equation also allows us to

(1) calculate p*Ka, given pH and* the molar ratio of proton donor and acceptor;

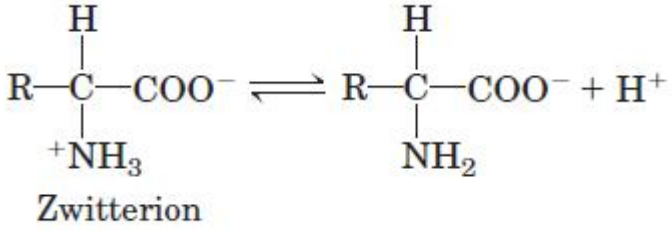
(2) calculate pH, given p*Ka and the molar ratio of proton donor* and acceptor;

(3) calculate the molar ratio of proton donor and acceptor, given pH and p*Ka.*

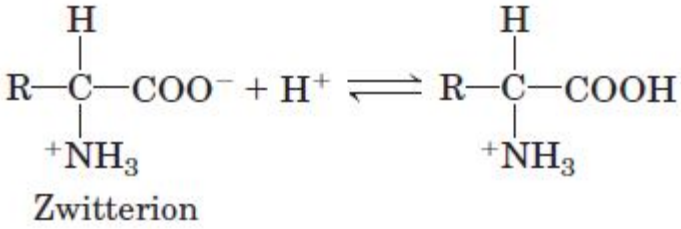
Amino Acids Can Act as Acids and Bases

Nonionic and zwitterionic forms of amino acids. The nonionic form does not occur in significant amounts in aqueous solutions. The zwitterion predominates at neutral pH.

When an amino acid is dissolved in water, it exists in solution as the dipolar ion, or **zwitterion** ("hybrid ion"). A zwitterion can act as either an acid (proton donor):



or a base (proton acceptor):



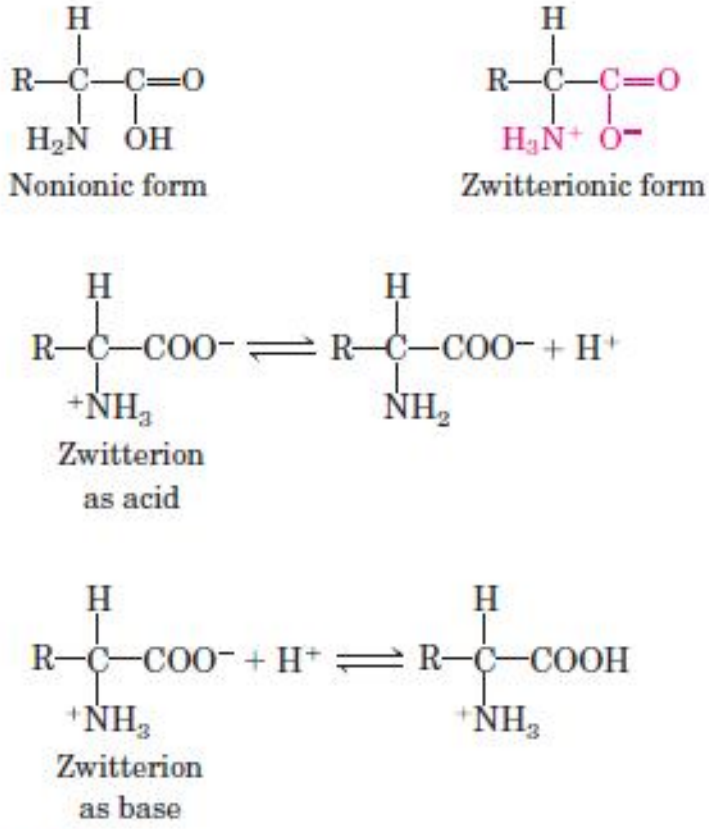


FIGURE 3–9 Nonionic and zwitterionic forms of amino acids. The nonionic form does not occur in significant amounts in aqueous solutions. The zwitterion predominates at neutral pH. A zwitterion can act as either an acid (proton donor) or a base (proton acceptor).

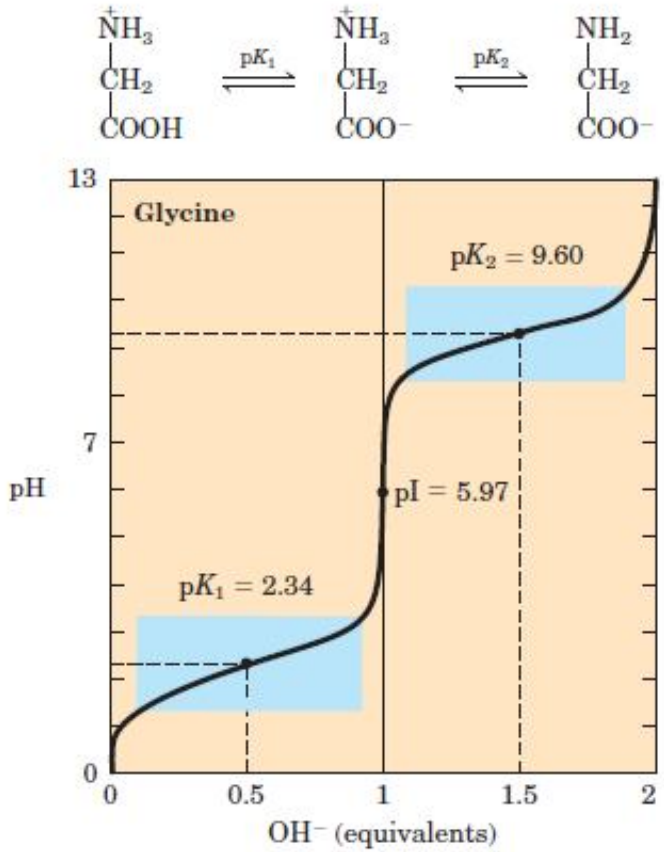
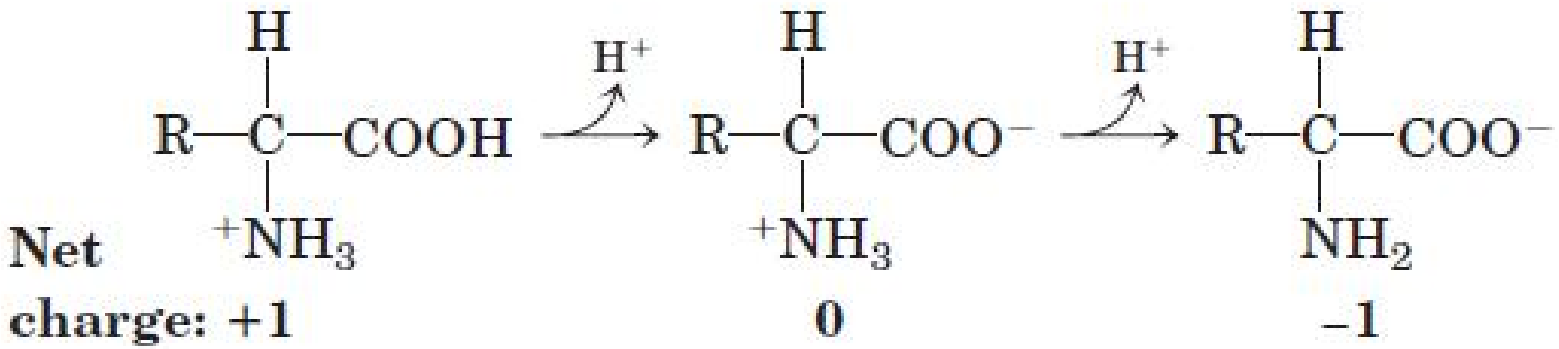


FIGURE 3–10 Titration of an amino acid. Shown here is the titration curve of 0.1 M glycine at 25 °C. The ionic species predominating at key points in the titration are shown above the graph. The shaded boxes, centered at about $pK_1 = 2.34$ and $pK_2 = 9.60$, indicate the regions of greatest buffering power. Note that 1 equivalent of $OH^- = 0.1$ M NaOH added.

Substances having this dual nature are **amphoteric** and are often called **ampholytes (from "amphoteric** electrolytes"). A simple monoamino monocarboxylic - amino acid, such as alanine, is a diprotic acid when fully protonated—it has two groups, the -COOH group and the $-NH^{+3}$ group, that can yield protons:



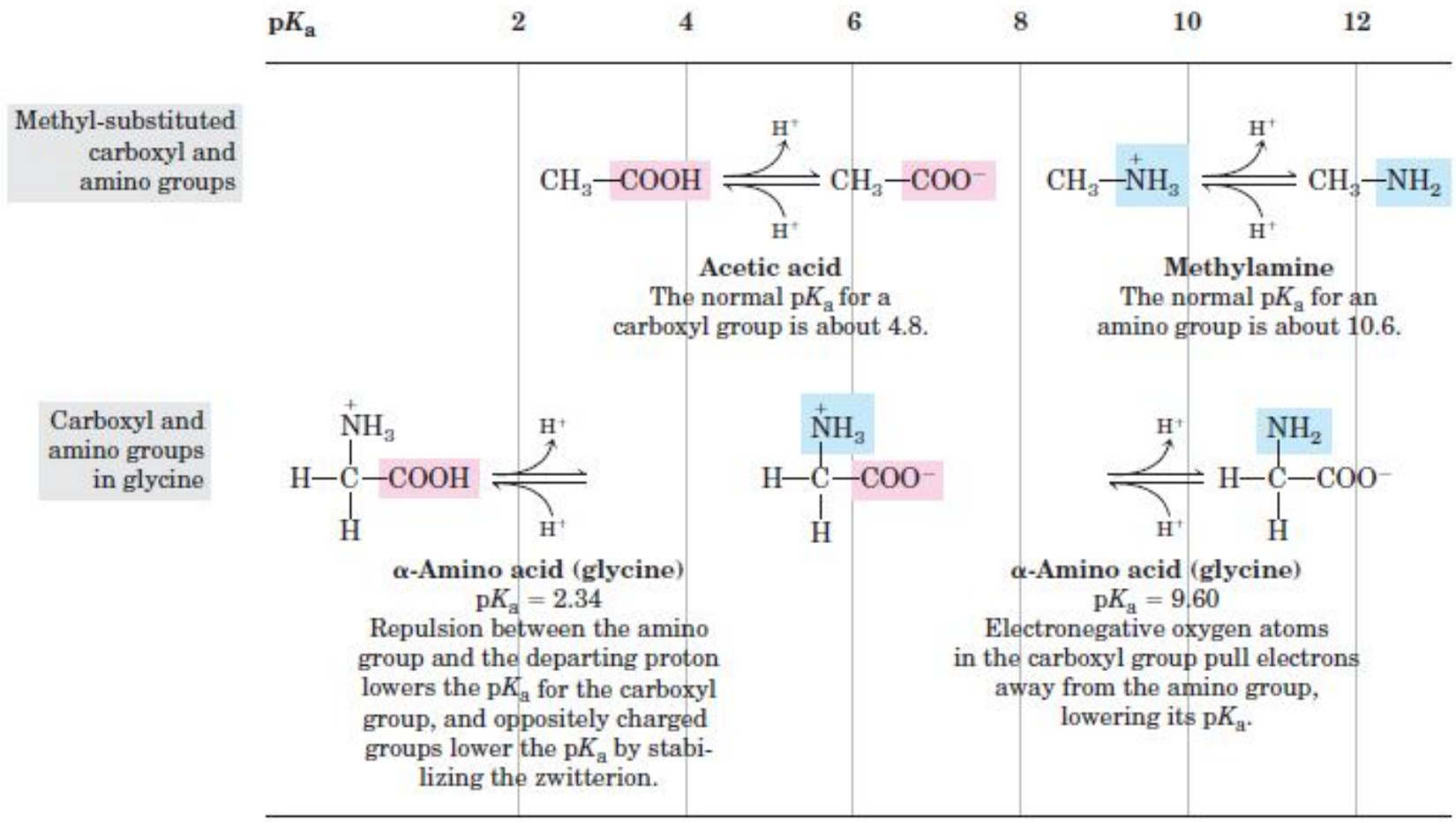


FIGURE 3–11 Effect of the chemical environment on pK_a. The pK_a values for the ionizable groups in glycine are lower than those for simple, methyl-substituted amino and carboxyl groups. These downward perturbations of pK_a are due to intramolecular interactions. Similar effects can be caused by chemical groups that happen to be positioned nearby—for example, in the active site of an enzyme.