**Chapter 3 Amino Acids, Peptides and Proteins**

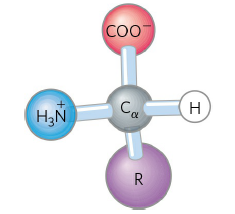
* Cells can produce proteins (enzymes, hormones, antibodies)
* with different properties and activities by joining the same 20 amino acids in many different combinations and sequences.

**3.1 Amino Acids**

* Proteins are polymers of amino acids.

**Amino Acids Share Common Structural Features**

* All 20 amino acids are -amino acids.
* They have a carboxyl group and an amino group bonded to the same carbon atom (the carbon).
* They differ from each other in their side chains (**R groups**),
* vary in structure, size and electric charge.
* The common amino acids of proteins have been assigned three-letter abbreviations and one-letter symbols **(Table 3–1)**.
* The  carbon is bonded to four different groups (except glycine)
* a carboxyl group, an amino group, an R group and a hydrogen atom**.**
* is a chiral center.

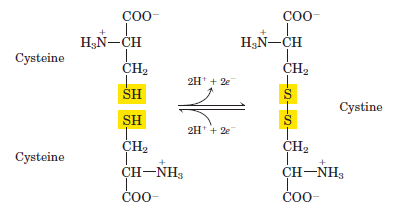
* All molecules with a chiral center are also optically active,
* rotate plane-polarized light.

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

* The term “residue” reflects the loss of the elements of water when one amino acid is joined to another.
* L stereoisomersrotate plane-polarized light to the left.
* D-amino acids residues have been found only in a few.

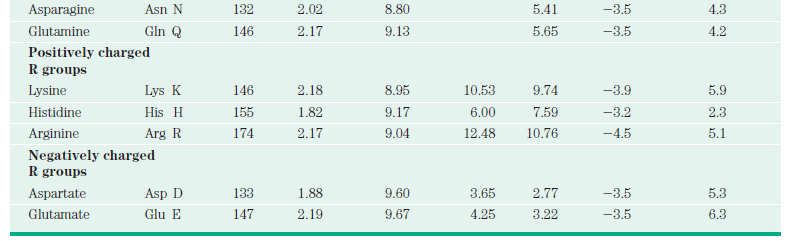
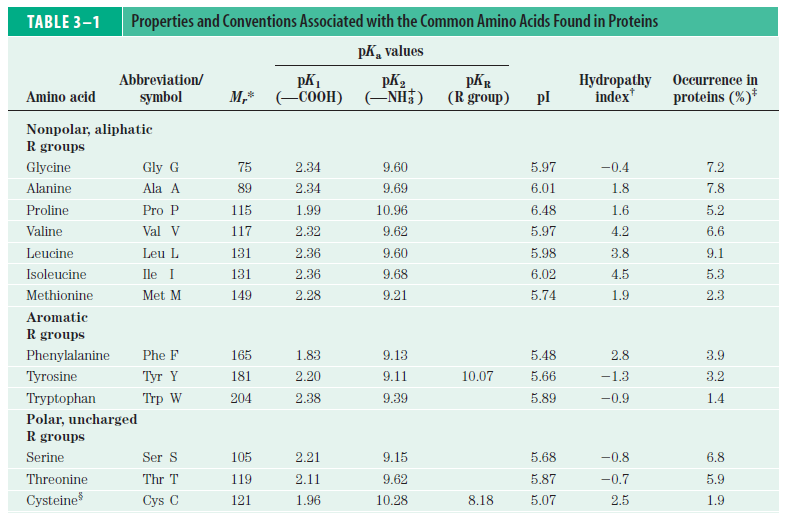
**Amino Acids Can Be Classified by R Group**

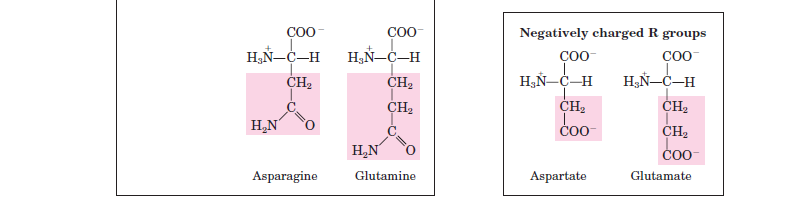
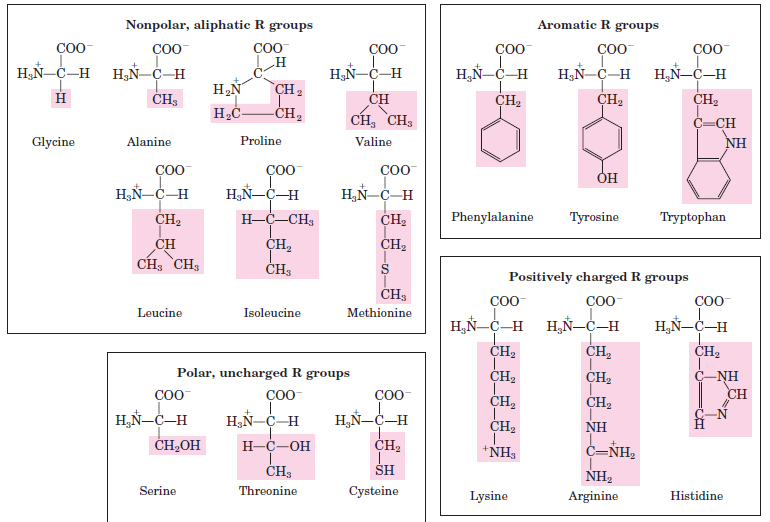
* They differ from each other in their side chains (R groups)
  + vary in structure, polarity, size, electric charge
  + are grouped into five classes **(Fig. 3-5)**.
* **Nonpolar, aliphatic** R groups stabilize protein structure by hydrophobic interactions.
* proline has a distinctive cyclic structure.
* **Aromatic** R groups participate in hydrophobic interactions and hydrogen bonds
* absorb ultraviolet light at 280 nm.
* **Polar, uncharged** R groupsformhydrogen bonds.
* cysteine is oxidized to form cystine by a disulfide bond (**Fig. 3-7)**.





* **Positively charged (basic)** R groups participate in ionic interactions and hydrogen bonds.
* **Negatively charged (asidic)** R groups participate in ionic interactions and hydrogen bonds.





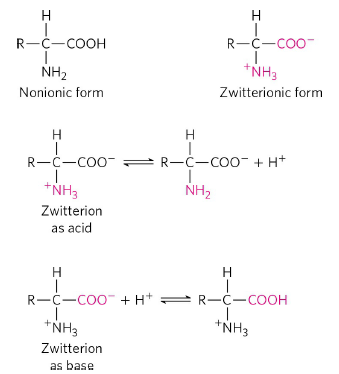


**Uncommon Amino Acids Also Have Important Functions**

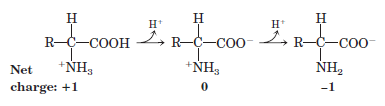
* In addition to the 20 common amino acids, proteins may contain residues created by modification of common residues.
* some of them are 4-hydroxyproline, 5-hydroxylysine, ornithine, citrulline
* 300 additional amino acids have been found in cells.

**Amino Acids Can Act as Acids and Bases**

* When an amino acid lacking an ionizable R group is dissolved in water at neutral pH
* it exists as dipolar ion or **zwitterion**
* a zwitterion can act as either an acid or a base **(Fig. 3-9)**.



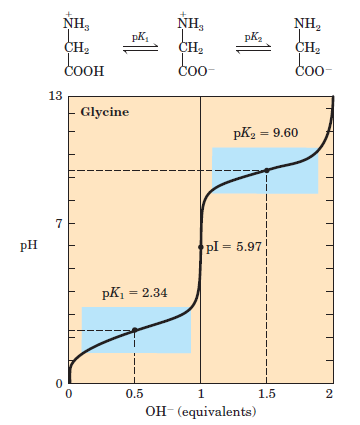
* Alanine, is a diprotic acid when fully protonated
* the —COOH group and the —NH3+ group can yield protons**.**



**Amino Acids Have Characteristic Titration Curves**

**Titration Curves Predict the Electric Charge of Amino Acids**

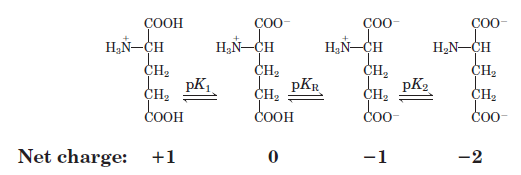
* Amino acids have pKa (pK1, pK2, pKR) values (**Table 3-1).**
* The two ionizable groups of glycine, the carboxyl group and the amino group, are titrated with a strong base such as NaOH.
* Titration curve of glycine has two stages (**Fig. 3-10).**

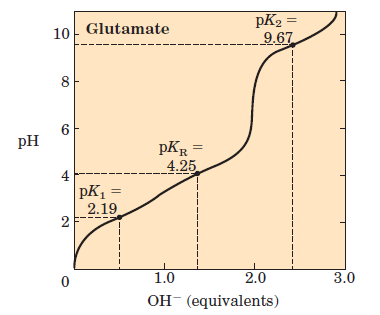


* [0 equilavent OH-], very low pH, fully protonated form (1), net charge = +1
* [0.5 equilavent OH-], midpoint of the first stage, [1] = [2], pH = pK1 = 2.34, first buffering power
* [1 equilavent OH-], zwitterion (2), net charge = 0, **isoelectric point** or **isoelectric pH**, pI = pH = pK1 + pK2 / 2 = 2.34 + 9.60 / 2 = 5.97
* [1.5 equilavent OH-], midpoint of the second stage, [2] = [3], pH = pK2 = 9.60, second buffering power
* [2 equilavent OH-], very high pH, fully unprotonated form (3), net charge = -1

**Amino Acids Differ in Their Acid-Base Properties**

* Amino acids with an ionizable R group have more complex titration curves.
* Titration of glutamate has three stages (**Fig. 3-12).**





**FIGURE 3–12 Titration curve for glutamate**.

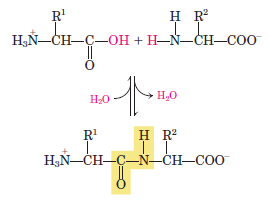
* [0 equilavent OH-], very low pH, fully protonated form (1), net charge = +1
* [0.5 equilavent OH-], midpoint of the first stage, [1] = [2], pH = pK1 = 2.19, first buffering power
* [1 equilavent OH-], zwitterion (2), net charge = 0, isoelectric pointpI = pH = pK1 + pKR / 2 = 2.19 + 4.25 / 2 = 3.22
* [1.5 equilavent OH-], midpoint of the second stage, [2] = [3], pH = pKR = 4.25,second buffering power
* [2 equilavent OH-], (3), net charge = -1, pH = pKR + pK2 / 2 = 4.25 + 9.67 / 2 = 6.96
* [2.5 equilavent OH-], midpoint of the third stage, [3] = [4], pH = pK2 = 9.67,third buffering power
* [3 equilavent OH-], very high pH, fully unprotonated form (4), net charge = -2
* Only histidine has an R group (pKa = 6.0) providing significant buffering power near the neutral pH
* usually found in the intracellular and extracellular fluids of most animals and bacteria.

**3.2 Peptides and Proteins**

* Peptides and proteins are polymers of amino acids.

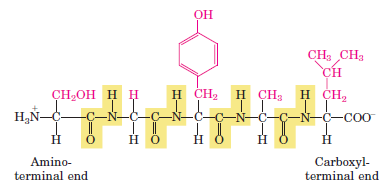
**Peptides Are Chains of Amino Acids**

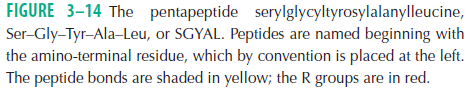
* Two amino acids are covalently joined
* **peptide bond** is formed to yield a dipeptide **(Fig. 3-13)**.





* When a few amino acids are joined, the structure is called an **oligopeptide**.
* When many amino acids are joined, the product is called a **polypeptide**.
* The structure of a pentapeptideis shown in **(Fig. 3-14)**.

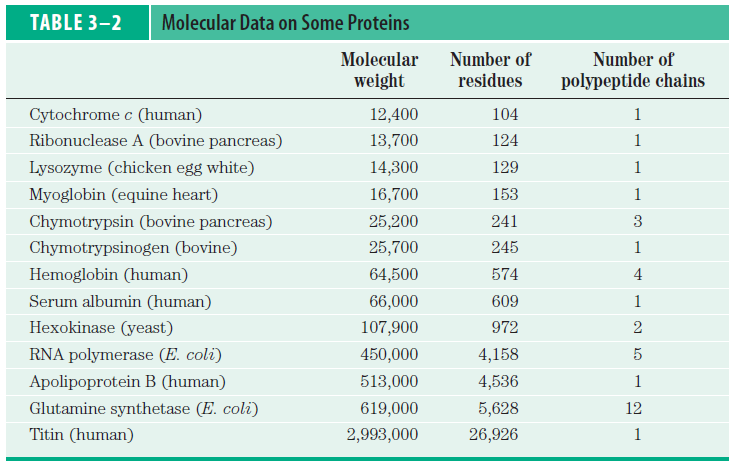




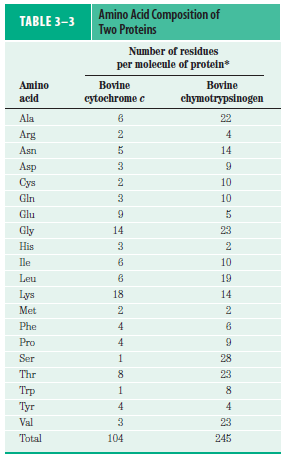
* In a peptide
* the amino acid residue at the end with a free amino group is the **amino-terminal** (or N-terminal) residue
* the residue at the other end, which has a free carboxyl group, is the **carboxyl-terminal** (C-terminal) residue.
* The amino-terminal end is placed on the left, the carboxyl-terminal end on the right.
* The sequence is read left to right, beginning with the amino-terminal end.

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

* Naturally occurring peptides range in length from two to many thousands of amino acid residues **(Table 3-2).**

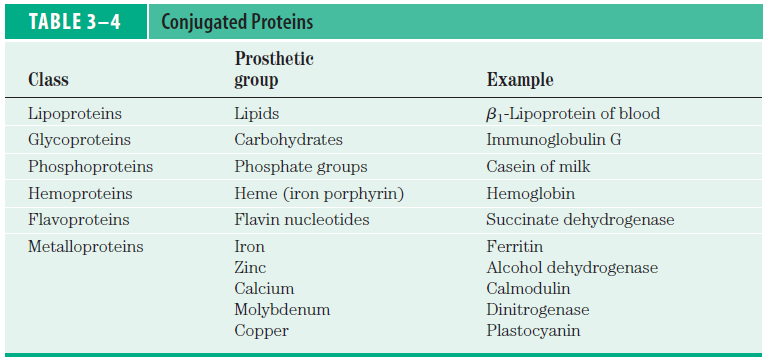


* Even the smallest peptides can have biologically important effects.
* for example, a number of hormones are small peptides (oxytocin -nine amino acid residues).
* Some proteins
* consist of a single polypeptide chain
* have two or more polypeptides (**multisubunit** proteins) **(Table 3-2)**.
* The amino acid composition of proteins is also highly variable **(Table 3-3)**.



**Some Proteins Contain Chemical Groups Other Than Amino Acids**

* These are called **conjugated proteins**.
  + chemical group is called its **prosthetic group**
* Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups **(Table 3-4)**. For example,
  + lipoproteins contain lipids,
  + glycoproteins contain sugar groups,
  + metalloproteins contain a specific metal.
* Some proteins contain more than one prosthetic group.

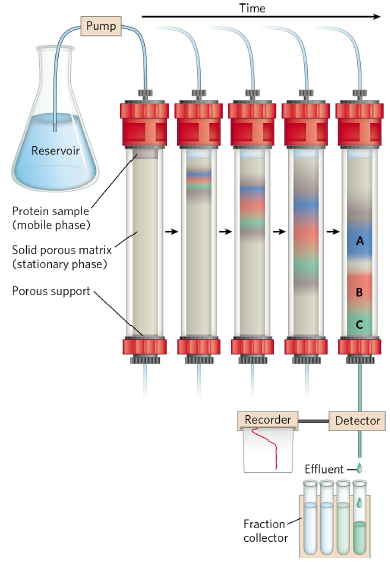


* Usually the prosthetic group plays an important role in the protein’s biological function.

**3.3 Working with Proteins**

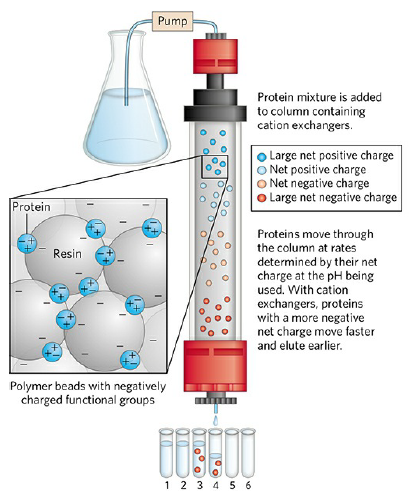
**Proteins Can Be Separated and Purified**

* Cells contain thousands of different kinds of proteins.
* How can one protein be purified?
* Methods for separating proteins take advantage of properties
* vary from one protein to the next
* including size, charge and binding properties.
* The first step is to break open these cells
* releasing their proteins into a solution called a **crude extract**.
* If necessary, differential centrifugation can be used to prepare subcellular fractions or to isolate specific organelles.
* Various methods are available for purifying one or more of the proteins.
* Early steps utilize differences in protein **solubility**.
* The solubility of proteins is lowered at high salt concentrations, (NH4)2SO4.
* an effect called **salting out**.
* addition of (NH4)2SO4 in the right amounts can selectively precipitate some proteins, while others remain in solution.
* A procedure called **dialysis** separates proteins from small solutes by taking advantage of the proteins’ larger size.
* partially purified extract is placed in a bag or tube made of a semipermeable membrane and having buffer solution.
* membrane allows the exchange of salt and buffer but not proteins.
* dialysis retains large proteins within the membranous bag or tube.
* Dialysis might be used, for example, to remove ammonium sulfate from the protein preparation.
* **Column chromatography** separates proteins (**Fig. 3-16)**.
* a porous solid material with appropriate chemical properties (the stationary phase) is held in a column, and a buffered solution (the mobile phase) migrates through it.
* individual proteins migrate faster or more slowly through the column depending on their properties (charge, size, binding affinity).



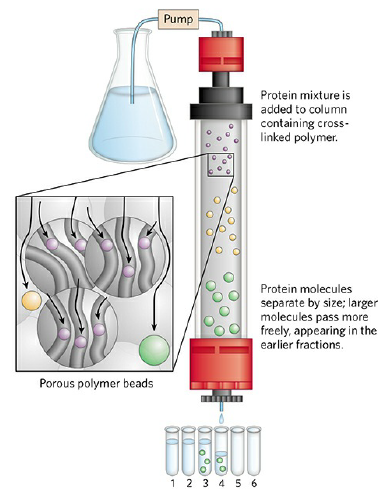


* **Ion-exchange chromatography** exploits differences in the sign and magnitude of the net electric **charge** of proteins at a given pH (**Fig. 3-17a)**.
* The column matrix is a synthetic polymer containing bound charged groups; those with bound anionic groups are called **cation exchangers**, and those with bound cationic groups are called **anion exchangers**.

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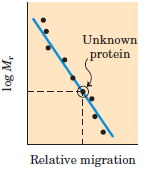
**FIGURE 3-17a Ion-exchange chromatography**

* **Size exclusion chromatography,** also called gel filtration separates proteins according to **size (Fig. 3-17b)**.
* the solid phase consists of polymer beads.



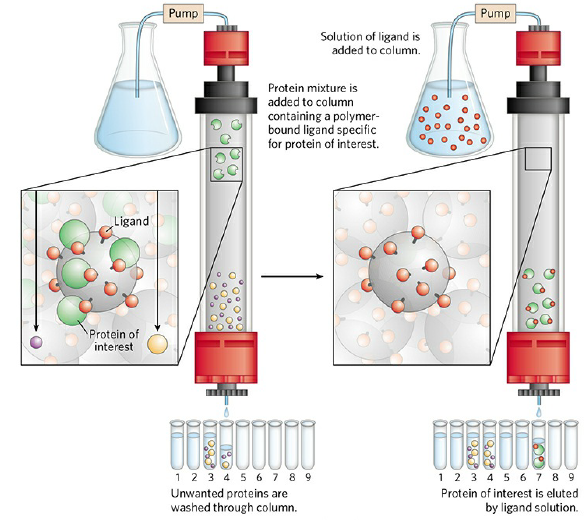
**FIGURE 3-17b Size exclusion chromatography**

* Size exclusion chromatography can also be used to approximate the size of a purified protein.
* standard proteins of known molecular weight are subjected to column.
* these marker proteins can be used to estimate the molecular weight of an unknown protein.
* a plot of molecular weight of the marker proteins versus relative migration (tube number) is linear, which allows the molecular weight of the unknown protein to be read from the graph **(Fig. 3-19)**.



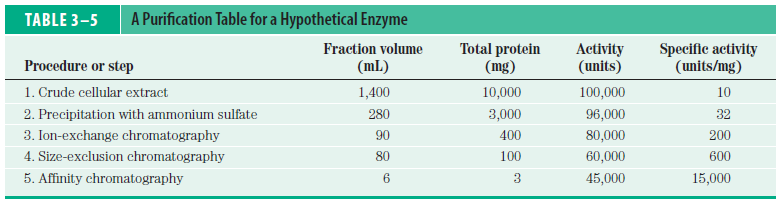


* **Affinity chromatography** is based on **binding affinity** (**Fig. 3-17c)**.
* the beads in the column have a covalently attached chemical group called a **ligand**—a group or molecule that binds to a macromolecule such as a protein.



**FIGURE 3-17c Affinity chromatography**

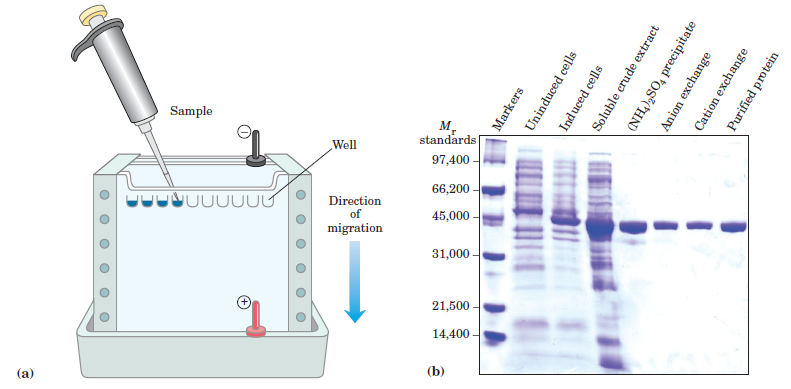
* **High-performance liquid chromatography** (HPLC) use high-pressure pumps.
* pumps speed the movement of the protein molecules down the column.
* HPLC improves resolution
* As each purification step is completed, the sample size becomes smaller **(Table 3–5)**.



* amount of enzymes in a solution can be measured or assayed in terms of the catalytic effect.
* 1 unit of enzyme activity is defined as the amount of enzyme causing transformation of 1 mol of substrate to product per minute.
* the term activity refers to total units of enzyme in a solution.
* the specific activity is the number of enzyme units per milligram of total protein (units / mg).
* The specific activity is a measure of enzyme purity: it increases during purification of an enzyme and becomes maximal and constant when the enzyme is pure.

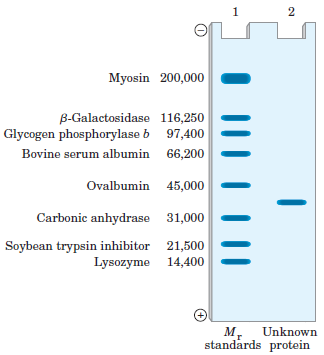
**Proteins Can Be Separated and Characterized by Electrophoresis**

* Another important technique for the separation of proteins is based on the migration of charged proteins in an electric field, a process called **electrophoresis (Fig. 3-18)**.





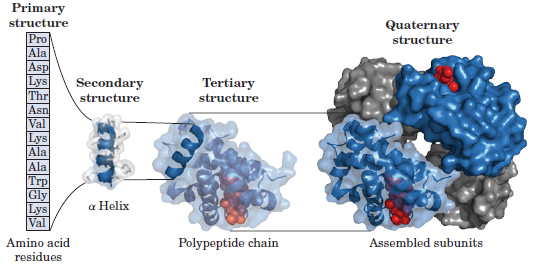
* different samples are loaded in wells at the top of the polyacrylamide gel.
* proteins can be visualized after electrophoresis by treating the gel with a stain such as Coomassie blue,which binds to the proteins.
* each band represents a different protein.
* smaller proteins move through the gel more rapidly than larger proteins.
* Number and molecular weight of different proteins can be estimated **(Fig. 3-19)**.





**3.4 The Structure of Proteins: Primary Structure**

* There are several levels of protein structure (**Fig. 3-23)**.
* **primary structure** consists of a sequence of amino acids by peptide and disulfide bonds (covalent bonds).
* **secondary structure** refers coiled primary structure by H bonds.
* **tertiary structure** describes three-dimensional folding by noncovalent interactions.
* **quaternary structure** refers noncovalent interactions and disulfide bonds of two or more polypeptide subunits having tertiary structure.



**The Amino Acid Sequences of Proteins Have Been Determined by Different Methods**

* Amino-terminal amino acid residue is determined by using 1-fluoro-2,4-dinitrobenzene.
* Amino-terminal amino acid residues are determined by using Edman degradation (40 sequential residues)
* Large proteins must be sequenced in smaller segments.
* Mass spectrometry permits the sequencing of short polypeptides (20 to 30 amino acid residues).
* Sequence of nucleotides in the gene deduces the sequence of a polypeptide.
* Amino acid sequences provide important biochemical information.