Subject Name: Microbiology

Semester: II

Name of the Teacher:

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Name of Topic:

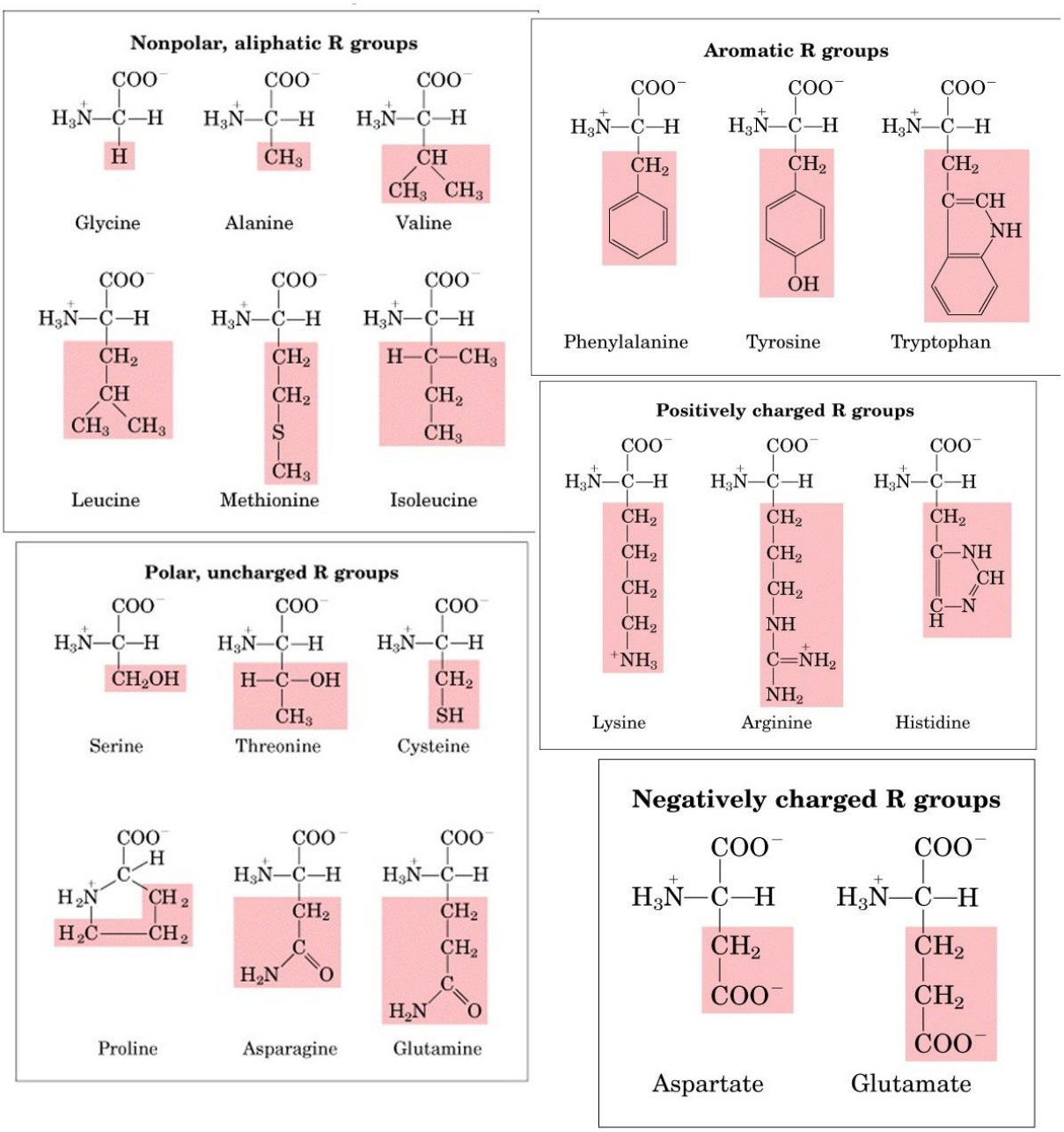
PROTEINS (CC3; Unit 4)

B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)

CC-3: PROTEINS (THEORY)

SEMESTER -2

Structure and Classification of 20 Standard Amino Acids:



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

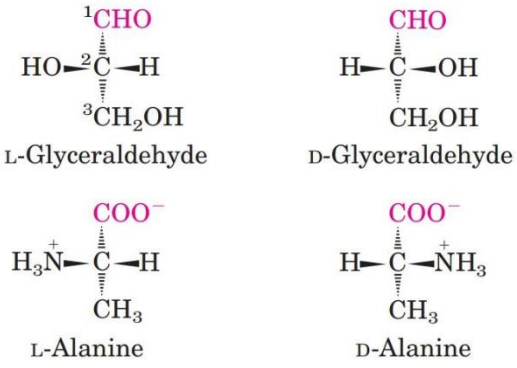
[* **Tryptophan and tyrosine, and to a much lesser extent phenylalanine, absorb ultraviolet light. This accounts for the characteristic strong absorbance of light by most proteins at a wavelength of 280 nm, a property exploited by researchers in the characterization of proteins.]**

Chirality and Absolute Configuration of Amino acids:

For all the common amino acids except glycine, the α carbon is bonded to four different groups: a carboxyl group, an amino group, an R group, and a hydrogen atom (In glycine, the R group is another hydrogen atom; hence glycine is achiral). The α -carbon atom is thus a chiral center. Because of the tetrahedral arrangement of the bonding orbitals around the α -carbon atom, the

four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers. Since they are nonsuperimposable mirror images of each other, the two forms represent a class of stereoisomers called enantiomers. All molecules with a chiral center are also optically active—that is, they rotate plane-polarized light.

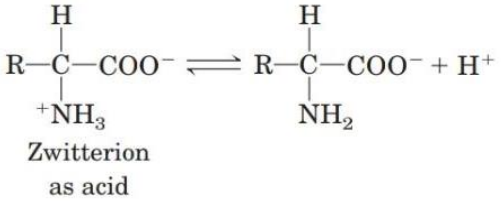
Special nomenclature has been developed to specify the absolute configuration of the four substituents of asymmetric carbon atoms. The absolute configurations of simple sugars and amino acids are specified by the D, L system, based on the absolute configuration of the three-carbon sugar glyceraldehyde, a convention proposed by Emil Fischer in 1891. (Fischer knew what groups surrounded the asymmetric carbon of glyceraldehyde but had to guess at their absolute configuration; his guess was later confirmed by x-ray diffraction analysis.) For all chiral compounds, stereoisomers having a configuration related to that of L-glyceraldehyde are designated as L, and stereoisomers related to D-glyceraldehyde are designated as D. The functional groups of L-alanine are matched with those of L-glyceraldehyde by aligning those that can be interconverted by simple, one-step chemical reaction. Thus the carboxyl group of L-alanine occupies the same position about the chiral carbon as does the aldehyde group of L-glyceraldehyde, because an aldehyde is readily converted to a carboxyl group via a one-step oxidation. Historically, the similar L and D designations were used for levorotatory (rotating plane polarized light to the left) and dextrorotatory (rotating light to the right). However, not all L-amino acids are levorotatory, and the convention shown was needed to avoid potential ambiguities about absolute configuration. By Fischer's convention, L and D refer only to the absolute configuration of the four substituents around the chiral carbon, not to optical properties of the molecule.

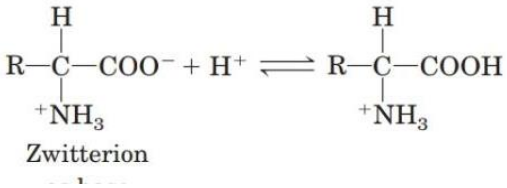


Another system of specifying configuration around a chiral center is the RS system, which is used in the systematic nomenclature of organic chemistry and describes more precisely the configuration of molecules with more than one chiral center.

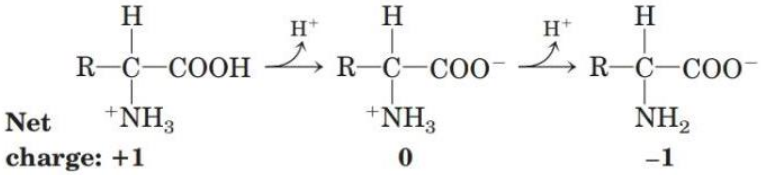
Zwitterionic form of Amino Acids:

The amino and carboxyl groups of amino acids, along with the ionizable R groups of some amino acids, function as weak acids and bases. When an amino acid lacking an ionizable R group is dissolved in water at neutral pH, it exists in solution as the **dipolar ion, or zwitterion** (German for "hybrid ion"), which can act as either an acid or a base. Substances having this dual (acid-base) nature are amphoteric and are often called ampholytes (from "amphoteric electrolytes").





A simple monoamino monocarboxylic α -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:



Titration Curve of Amino Acids and its Significance:

Acid-base titration involves the gradual addition or removal of protons. We will focus on titration curve of the diprotic form of glycine as an example. The two ionizable groups of glycine, the carboxyl group and the amino group, are titrated with a strong base such as NaOH. The plot has two distinct stages, corresponding to deprotonation of two different groups on glycine. At very low pH, the predominant ionic species of glycine is the fully protonated form, $^{+}H_3N—CH_2—COOH$. At the midpoint in the first stage of the titration, in which the —COOH group of glycine loses its proton, equimolar concentrations of the proton-donor ($^{+}H_3N—CH_2—COOH$) and proton-acceptor ($^{+}H_3N—CH_2—COO^{-}$) species are present. At the midpoint of any titration, a point of inflection is reached where the pH is equal to the pKa of the protonated group being titrated. For glycine, the pH at the midpoint is 2.34, thus its —COOH group has a pKa (labeled pK1 in **Figure. 1**) of 2.34. (pH and pKa are simply convenient notations for proton concentration and the equilibrium constant for ionization, respectively. The pKa is a measure of the tendency of a group to give up a proton, with that tendency decreasing tenfold as the pKa increases by one unit.) As the titration proceeds, another important point is reached at pH 5.97. Here there is another point of inflection, at which removal of the first proton is essentially complete and removal of the second has just begun. At this pH glycine is present largely as the dipolar ion (zwitterion) $^{+}H_3N—CH_2—COO^{-}$. (labeled pI in **Figure. 1**) .

The second stage of the titration corresponds to the removal of a proton from the $—NH_3^{+}$ group of glycine. The pH at the midpoint of this stage is 9.60, equal to the pKa (labeled pK2 in **Figure.1**) for the $— NH_3^{+}$ group. The titration is essentially complete at a pH of about 12, at which point the predominant form of glycine is $H_2N—CH_2—COO^{-}$.

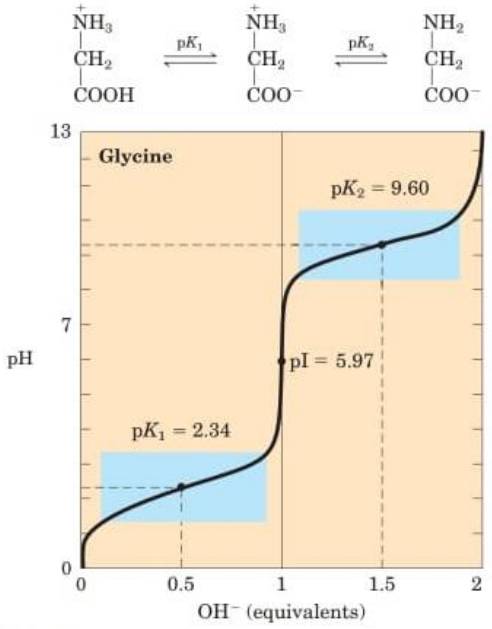


Figure 1: Titration of an amino acid: Shown here is the titration curve of 0.1 M glycine at 25^0C. The ionic species predominating at key points in the titration are shown above the graph. The shaded boxes, centered at about pK1 = 2.34 and pK2 = 9.60, indicate the regions of greatest buffering power. Note that 1 equivalent of OH^- = 0.1 M NaOH added.

From the titration curve of glycine, we can derive several important pieces of information. First, it gives a quantitative measure of the pKa of each of the two ionizing groups: 2.34 for the —COOH group and 9.60 for the— NH_3^+ group. Note that the carboxyl group of glycine is over 100 times more acidic (more easily ionized) than the carboxyl group of acetic acid having a pKa of 4.76—about an average for a carboxyl group attached to an otherwise unsubstituted aliphatic hydrocarbon. The perturbed pKa of glycine is caused by repulsion between the departing proton and the nearby positively charged amino group on the α-carbon atom, as described in **Figure 2**. The opposite charges on the resulting zwitterion are stabilizing. Similarly, the pKa of the amino group in glycine is perturbed downward relative to the average pKa of an amino group. This effect is due partly to the electronegative oxygen atoms in the carboxyl groups, which tend to pull electrons toward them, increasing the tendency of the amino group to give up a proton. Hence, the α-amino group has a pKa that is lower than that of an aliphatic amine such as methylamine (**Figure . 2**). In short, the pKa of any functional group is greatly affected by its chemical environment, a phenomenon sometimes exploited in the active sites of enzymes to promote exquisitely adapted reaction mechanisms that depend on the perturbed pKa values of proton donor/acceptor groups of specific residues.

The second piece of information provided by the titration curve of glycine is that this amino acid has *two* regions of buffering power. One of these is the relatively flat portion of the curve, extending for approximately 1 pH unit on either side of the first pKa of 2.34, indicating that glycine is a good buffer near this pH. The other buffering zone is centered around pH 9.60. (Note that glycine is not a good buffer at the pH of intracellular fluid or blood, about 7.4.)

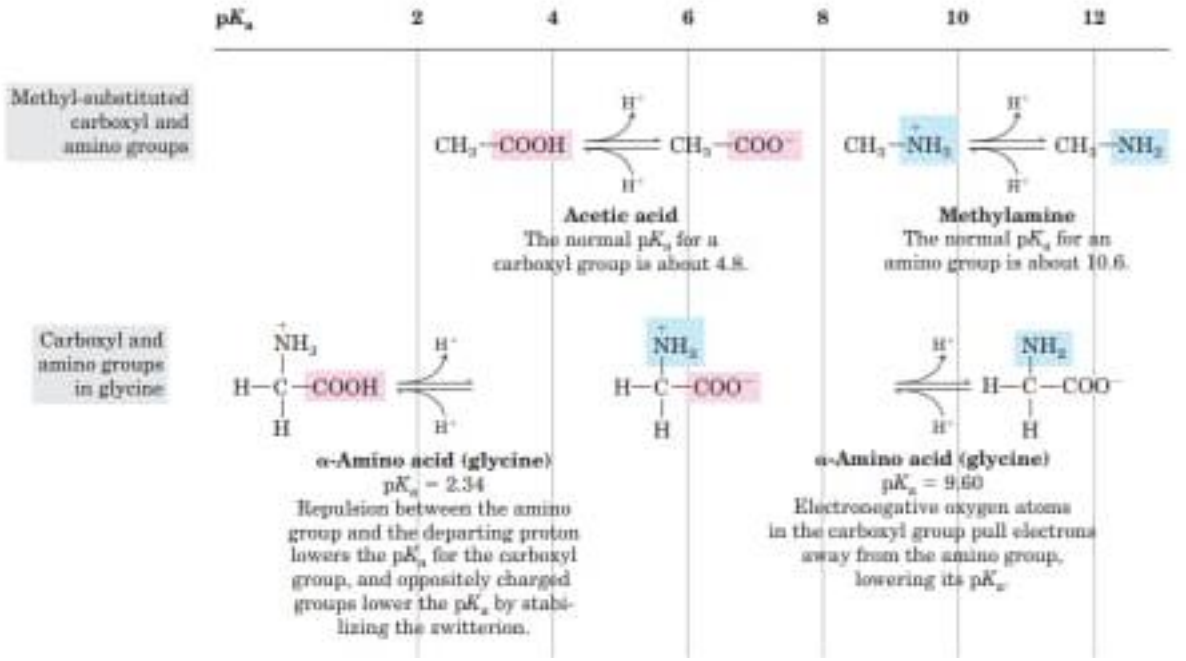


Figure 2: Effect of the chemical environment on pKa. The pKa values for the ionizable groups in glycine are lower than those for simple, methyl-substituted amino and carboxyl groups. These downward perturbations of pKa are due to intramolecular interactions. Similar effects can be caused by chemical groups that happen to be positioned nearby—for example, in the active site of an enzyme.

Titration Curves Predict the Electric Charge of Amino Acids

Another important piece of information derived from the titration curve of an amino acid is the relationship between its net charge and the pH of the solution. At pH 5.97, the point of inflection between the two stages in its titration curve, glycine is present predominantly as its dipolar form, fully ionized but with no net electric charge (**Figure. 1**). The characteristic pH at which the net electric charge is zero is called the isoelectric point or isoelectric pH, designated pI. For glycine, which has no ionizable group in its side chain, the isoelectric point is simply the arithmetic mean of the two pKa values:

$$pI = \tfrac{1}{2}(pK_1 + pK_2) = \tfrac{1}{2}(2.34+9.60) = 5.97$$

As is evident in Figure 1, glycine has a net negative charge at any pH above its pI and will thus move toward the positive electrode (the anode) when placed in an electric field. At any pH below its pI, glycine has a net positive charge and will move toward the negative electrode (the cathode). The further the pH of a glycine solution is from its isoelectric point, the greater the net electric charge of the population of glycine molecules. At pH 1.0, for example, glycine exists almost entirely as the form with a net positive charge of 1.0. At pH 2.34, where there is an equal mixture of $^{+}H_3N—CH_2—COOH$ and $^{+}H_3N—CH_2—COO^{-}$ the average or net positive charge is 0.5. The sign and the magnitude of the net charge of any amino acid at any pH can be predicted in the same way.

Amino Acids Differ in Their Acid-Base Properties

The shared properties of many amino acids permit some simplifying generalizations about their acid-base behaviors. First, all amino acids with a single α-amino group, a single α-carboxyl group, and an R group that does not ionize have titration curves resembling that of glycine **(Figure. 1).** These amino acids have very similar, although not identical, pKa values: pKa of the —COOH group in the range of 1.8 to 2.4, and pKa of the - $^{+}H_3N$ group in the range of 8.8 to 11.0. The differences in these pKa values reflect the effects of the R groups.

Second, amino acids with an ionizable R group have more complex titration curves, with three stages corresponding to the three possible ionization steps; thus they have three pKa values. The additional stage for the titration of the ionizable R group merges to some extent with the other two. The titration curves for two amino acids of this type, glutamate and histidine, are shown in **Figure 3.** The isoelectric points reflect the nature of the ionizing R groups present. For example, glutamate has a pI of 3.22, considerably lower than that of glycine. This is due to the presence of two carboxyl groups, which, on average of their pKa values (3.22), contribute a net charge of -1 that balances the +1 contributed by the amino group. Similarly, the pI of histidine, with two groups that are positively charged when protonated, is 7.59 (the average of the pKa values of the amino and imidazole groups), is much higher than that of glycine.

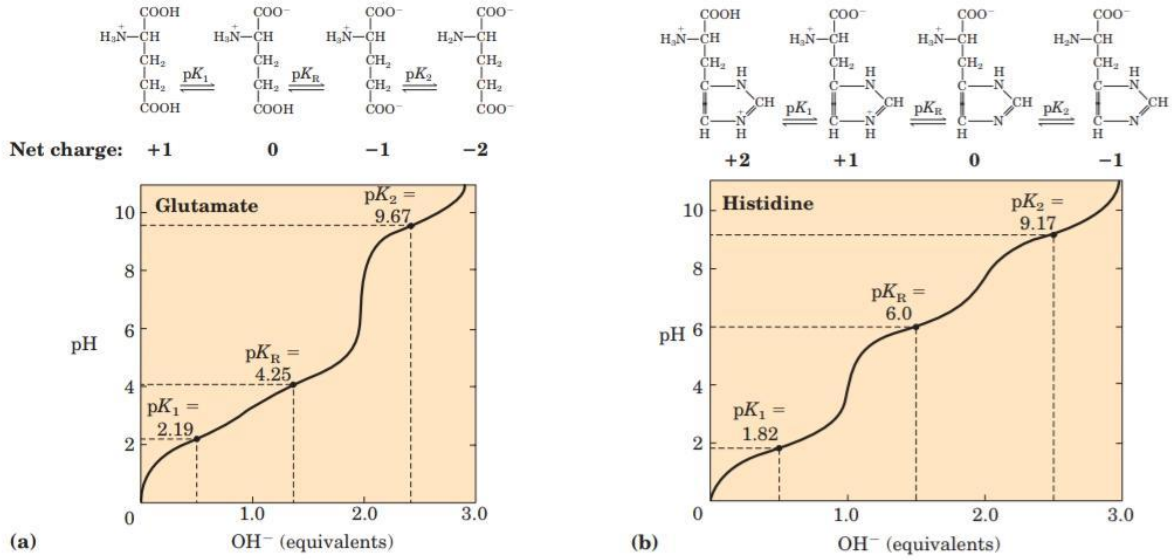
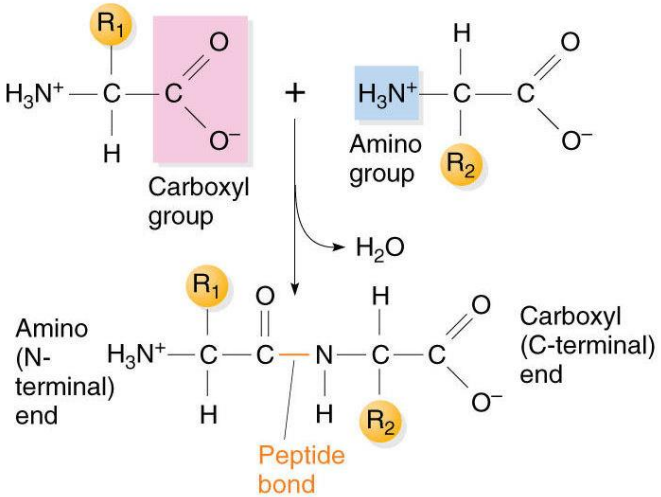


Figure 3: Titration curve for a) Glutamate and b) Histidine

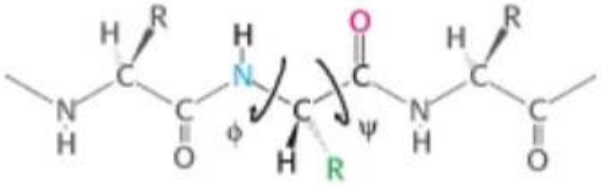
The Peptide Bond:

Proteins are linear polymers formed by linking the α -carboxyl group of one amino acid to the α -amino group of another amino acid with a peptide bond (also called an amide bond). The formation of a dipeptide from two amino acids is accompanied by the loss of a water molecule. The equilibrium of this reaction lies on the side of hydrolysis rather than synthesis. Hence, the biosynthesis of peptide bonds requires an input of free energy.



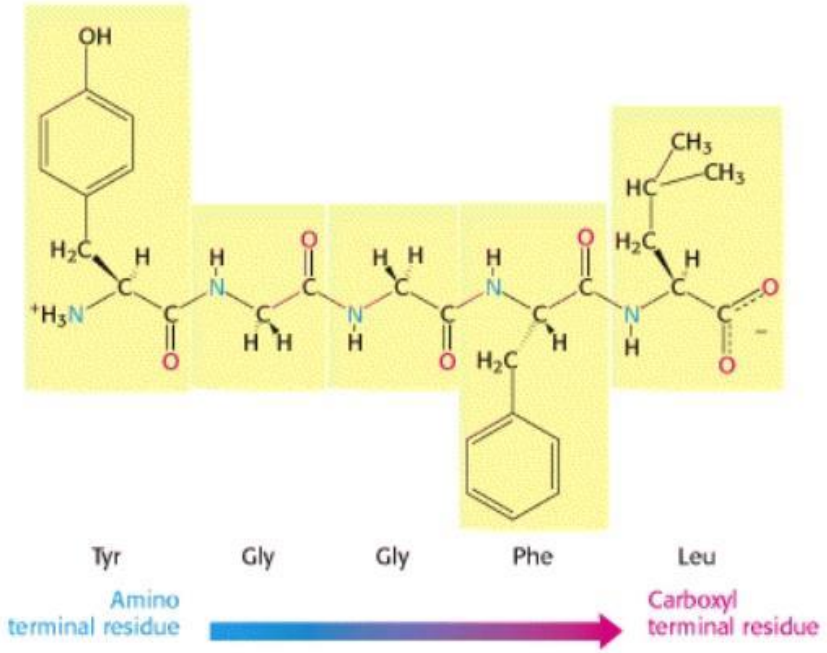
The peptide bond is essentially planar and has considerable double-bond character, which prevents rotation about this bond.

The structure of each amino acid in a polypeptide can be adjusted by rotation about two single bonds. Phi (φ) is the angle of rotation about the bond between the nitrogen and the α-carbon atoms, whereas psi (ψ) is the angle of rotation about the bond between the α-carbon and the carbonyl carbon atoms.



Primary Structure:

A series of amino acids joined by peptide bonds form a polypeptide chain, and each amino acid unit in a polypeptide is called a residue. A polypeptide chain has polarity because its ends are different, with an α -amino group at one end and an α -carboxyl group at the other. By convention, the amino end is taken to be the beginning of a polypeptide chain, and so the sequence of amino acids in a polypeptide chain is written starting with the amino terminal residue. Thus, in the pentapeptide Tyr-Gly-Gly-Phe-Leu (YGGFL), phenylalanine is the amino-terminal (N-terminal) residue and leucine is the carboxyl-terminal (C-terminal) residue).



Peptides made of small numbers of amino acids are called oligopeptides or simply peptides.

A polypeptide chain consists of a regularly repeating part, called the main chain or backbone, and a variable part, comprising the distinctive side chains. The polypeptide backbone is rich in hydrogen-bonding potential. Each residue contains a carbonyl group, which is a good hydrogen-bond acceptor and, with the exception of proline, an NH group, which is a good hydrogen-bond donor. These groups interact with each other and with functional groups from side chains to stabilize particular structures, as will be discussed in detail.

In some proteins, the linear polypeptide chain is cross-linked. The most common cross-links are disulfide bonds, formed by the oxidation of a pair of cysteine residues. The resulting unit of linked cysteines is called cystine. Extracellular proteins often have several disulfide bonds, whereas intracellular proteins usually lack them.

Secondary Structure:

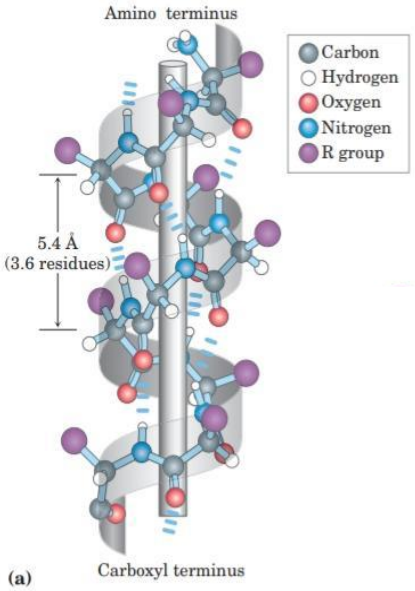
The term secondary structure refers to any chosen segment of a polypeptide chain and describes the local spatial arrangement of its main-chain atoms, without regard to the conformation of its side chains or its relationship to other segments. A regular secondary structure occurs when each dihedral angle, Φ and Ψ, remains the same or nearly the same throughout the segment. There are a few types of secondary structure that are particularly stable and occur widely in proteins. The most prominent are the α helix and β conformations; an- other common type is the β turn. Where a regular pattern is not found, the secondary structure is sometimes referred to as undefined or as a random coil.

The Alpha Helix:

In evaluating potential structures, Pauling and Corey considered the conformations of peptides which were sterically allowed and those that most fully exploited the hydrogen-bonding capacity of the backbone NH and CO groups. The first of their proposed structures, the α helix, is a rodlike structure. A tightly coiled backbone forms the inner part of the rod and the side chains extend outward in a helical array. The α helix is stabilized by hydrogen bonds between the NH and CO groups of the main chain. In particular, the CO group of each amino acid forms a hydrogen bond with the NH group of the amino acid that is situated four residues ahead in the sequence. Thus, except for amino acids near the ends of an α helix, all the main-chain CO and NH groups are hydrogen bonded. Each residue is related to the next one by a rise of 1.5 Å

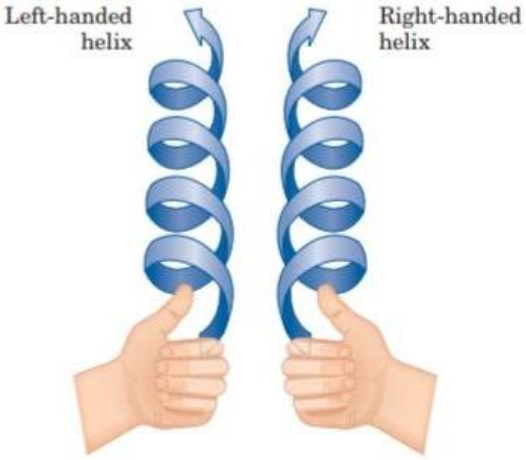
along the helix axis and a rotation of 100 degrees, which gives 3.6 amino acid residues per turn of helix. Thus, amino acids spaced three and four apart in the sequence are spatially quite close to one another in an α helix. In contrast, amino acids two apart in the sequence are situated on opposite sides of the helix and so are unlikely to make contact. The pitch of the α helix, which is equal to the product of the translation (1.5 Å) and the number of residues per turn (3.6), is 5.4 Å. The screw sense of a helix can be right-handed (clockwise) or left-handed (counterclockwise). The Ramachandran diagram reveals that both the right-handed and the left-handed helices are among the allowed conformations. However, right-handed helices are energetically more favourable because there is less steric clash between the side chains and the backbone. Essentially all α helices found in proteins are right handed.

The α-helical content of proteins ranges widely, from nearly none to almost 100%. For example, about 75% of the residues in ferritin, a protein that helps store iron, are in α helices. Single α helices are usually less than 45 Å long. However, two or more α helices can entwine to form a very stable structure, which can have a length of 1000 Å (100 nm, or 0.1 μ m) or more. Such α-helical coiled coils are found in myosin and tropomyosin in muscle, in fibrin in blood clots, and in keratin of hair. The helical cables in these proteins serve a mechanical role in forming stiff bundles of fibers, as in porcupine quills. The cytoskeleton (internal scaffolding) of cells is rich in so-called intermediate filaments, which also are two-stranded α-helical coiled coils. Many proteins that span biological membranes also contain α helices.



(a) Ball-and-stick model showing the intrachain hydrogen bonds. The repeat unit is a single turn of the helix, 3.6 residues

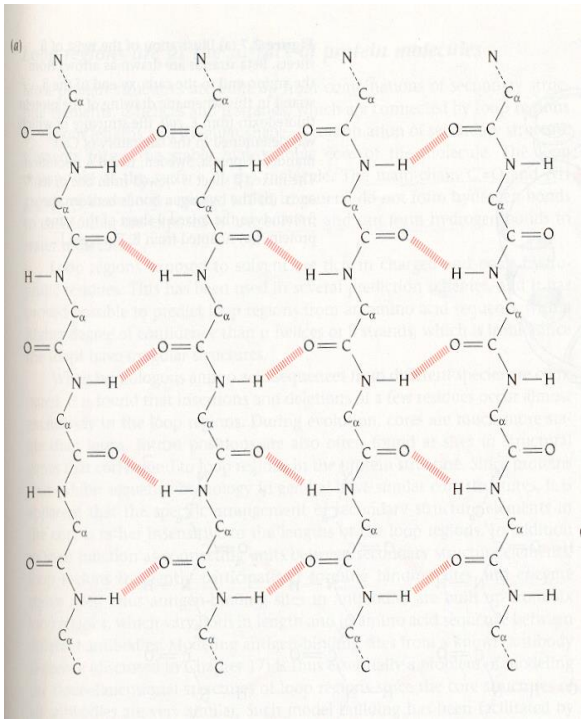
There is a simple method for determining whether a helical structure is right-handed or left-handed. Make fists of your two hands with thumbs outstretched and pointing away from you. Looking at your right hand, think of a helix spiraling up your right thumb in the direction in which the other four fingers are curled as shown (clockwise). The resulting helix is right-handed. Your left hand will demonstrate a left-handed helix, which rotates in the counterclockwise direction as it spirals up your thumb.



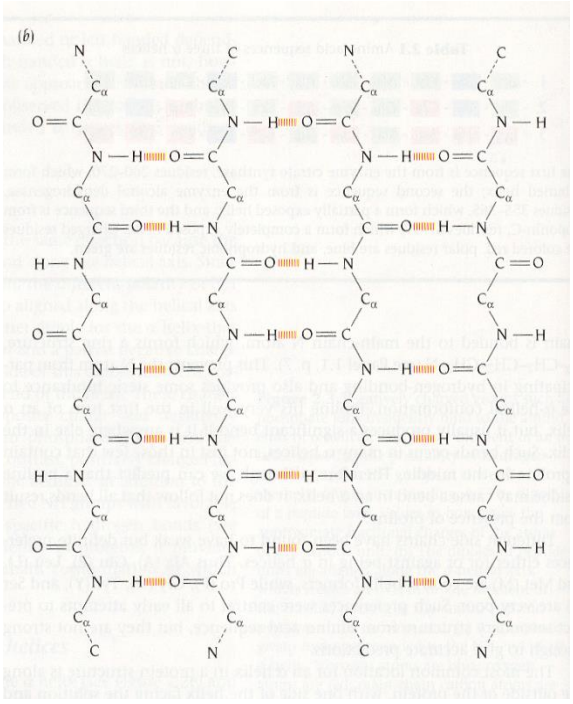
Beta Sheets:

Pauling and Corey discovered another periodic structural motif, which they named the β pleated sheet (β because it was the second structure that they elucidated, the α helix having been the first). The β pleated sheet (or, more simply, the β sheet) differs markedly from the rod-like α helix. A polypeptide chain, called a β strand, in a β sheet is almost fully extended rather than being tightly coiled as in the α helix. A range of extended structures are sterically allowed. The distance between adjacent amino acids along a β strand is approximately 3.5 Å, in contrast with a distance of 1.5 Å along an α helix. The side chains of adjacent amino acids point in opposite directions. A β sheet is formed by linking two or more β strands by hydrogen bonds. Adjacent chains in a β sheet can run in opposite directions (antiparallel β sheet) or in the same direction (parallel β sheet). In the antiparallel arrangement, the NH group and the CO group of each amino acid are respectively hydrogen bonded to the CO group and the NH group of a partner on the adjacent chain. In the parallel arrangement, the hydrogen-bonding scheme is slightly more complicated. For each amino acid, the NH group is hydrogen bonded to the CO group of one amino acid on the adjacent strand, whereas the CO group is hydrogen bonded to the NH group on the amino acid two residues farther along the chain. Many strands, typically 4 or 5 but as many as 10 or more, can come together in β sheets. Such β sheets can be purely antiparallel, purely parallel, or mixed.

The β sheet is an important structural element in many proteins. For example, fatty acid-binding proteins that are important for lipid metabolism, are built almost entirely from β sheets.



Parallel β sheet



Antiparallel β sheet

β Turn:

Turn in globular proteins, which have a compact folded structure, nearly one-third of the amino acid residues are in turns or loops where the polypeptide chain reverses direction. These are the connecting elements that link successive runs of α helix or β conformation. Particularly common are **β turns** that connect the ends of two adjacent segments of an antiparallel β sheet. The structure is a 180^{o} turn involving four amino acid residues, with the carbonyl oxygen of the first residue forming a hydrogen bond with the amino-group hydrogen of the fourth. The peptide groups of the central two residues do not participate in any inter-residue hydrogen bonding. Gly and Pro residues often occur in β turns, the former being small and flexible and the latter because of the peptide bonds involving the imino nitrogen of proline that readily assume the cis configuration; a form that is particularly amenable to a tight turn.

Tertiary Structures of Protein :

The way in which the different sections of α-helix, β-sheet, other minor secondary structures and connecting loops fold in three dimensions is the tertiary structure of the polypeptide. The nature of the tertiary structure is inherent in the primary structure and, given the right

conditions, most polypeptides will fold spontaneously into the correct tertiary structure as it is generally the lowest energy conformation for that sequence. However, in vivo, correct folding is often assisted by proteins called chaperones which help prevent misfolding of new polypeptides before their synthesis (and primary structure) is complete. Folding is such that amino acids with hydrophilic side chains locate mainly on the exterior of the protein where they can interact with water or solvent ions, while the hydrophobic amino acids become buried in the interior from which water is excluded. This gives overall stability to the structure.

Various types of noncovalent interaction between side chains hold the tertiary structure together: van der Waals forces, hydrogen bonds, electrostatic salt bridges between oppositely charged groups (e.g. the ε-NH_3^+ group of lysine and the side chain COO^- groups of aspartate or glutamate) and hydrophobic interactions between the nonpolar side chains of the aliphatic and aromatic amino acids. In addition, covalent disulfide bonds can form between two cysteine residues which may be far apart in the primary structure but close together in the folded tertiary structure.

Quaternary Structures of Protein:

Many proteins are composed of two or more polypeptide chains (subunits). These may be identical or different. Hemoglobin has two α-globin and two β-globin chains (α2β2). The same forces which stabilize tertiary structure hold these subunits together, including disulfide bonds between cysteines on separate polypeptides. This level of organization is known as the quaternary structure and has certain consequences. First, it allows very large protein molecules to be made. Tubulin is a dimeric protein made up of two small, nonidentical α and β subunits. Upon hydrolysis of tubulin-bound GTP, these dimers can polymerize into structures containing many hundreds of α and β subunits. These are the microtubules of the cytoskeleton. Secondly, it can provide greater functionality to a protein by combining different activities into a single entity, as in the fatty acid synthase complex. Often, the interactions between the subunits are modified by the binding of small molecules and this can lead to the allosteric effects seen in enzyme regulation.

Forces holding the polypeptide together:

A multisubunit protein may consist of identical or nonidentical polypeptide chains. We shall refer to proteins with identical subunits as oligomers and to these identical subunits as protomers. A protomer may therefore consist of one polypeptide chain or several unlike polypeptide chains. In this sense, hemoglobin is a dimer (oligomer of two protomers) of α and β protomers .

The association of two subunits typically buries 1000 to 2000 $Å^2$ of surface area that would otherwise be exposed to solvent. The resulting contact regions superficially resemble the interiors of single subunit proteins: They contain closely packed nonpolar side chains, hydrogen bonds, and in some cases, interchain disulphide bonds. However, protein–protein interfaces differ from subunit interiors in several aspects:

1. They tend to have hydrophobicities between those of protein interiors and exteriors. In particular, the subunit interfaces of proteins that dissociate in vivo have lesser hydrophobicities than do permanent interfaces.

2. An average of approximately 77% of intersubunit hydrogen bonds are between side chains. In contrast, an average of approximately 68% of the hydrogen bonds within subunits are between backbone atoms. This is mainly because secondary structural elements are not continued across subunit boundaries (with the occasional exception of sheets).

3. Around 56% of protein–protein interfaces contain salt bridges. These contribute to the specificity as well as to the stability of subunit associations.

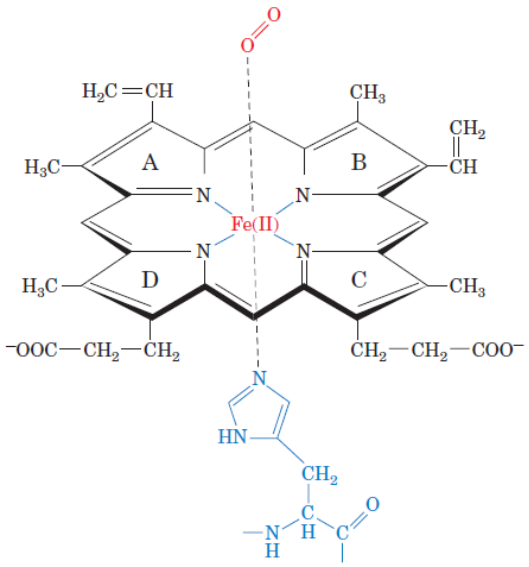
   In addition, there are negligibly few hydrogen bonds and salt bridges at the edges of the contact regions. Not surprisingly, the residues at protein–protein interfaces are evolutionarily well conserved compared to other surface residues.

Human Hemoglobin Structure:

Hemoglobin (Hb) is a 65-kD heterotetramer, α2β2 (alternatively, a dimer of αβ protomers).The α and β subunits are structurally and evolutionarily related to each other and to myoglobin (Mb), the 18-kD monomeric oxygen-binding protein of skeletal and heart muscle. Hemoglobin transports oxygen from the lungs, gills, or skin of an animal to its capillaries for use in respiration.

Heme

Myoglobin and each of the four subunits of haemoglobin noncovalently bind a single heme group . This is the same group that occurs in the cytochromes (Section 9-6A) and in certain redox enzymes such as catalase. Heme is responsible for the characteristic red color of blood and is the site at which each globin monomer binds one molecule of O2 (globins are the heme-free proteins of Hb and Mb). The heterocyclic ring system of heme is a porphyrin derivative; it consists of four pyrrole rings linked by methene bridges.The porphyrin in heme, with its particular arrangement of four methyl, two propionate, and two vinyl substituents, is known as protoporphyrin IX. Heme, then, is protoporphyrn IX with a centrally bound iron atom. In Hb and Mb, the iron atom normally remains in the Fe(II) (ferrous) oxidation state whether or not the heme is oxygenated (binds O2).



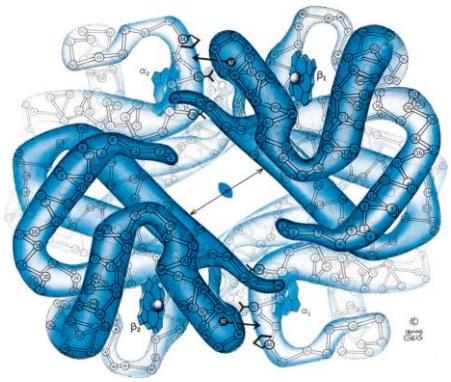
The heme group: Fe(II)–heme (ferroprotoporphyrin IX) is shown liganded to His and O_2 as it is in oxygenated myoglobin and oxygenated hemoglobin.

The hemoglobin tetramer is a spheroidal molecule of dimensions 64 x 55 x 50 Å. Its two αβ protomers are symmetrically related by a twofold rotation. The tertiary structures of the α and β subunits are remarkably similar.

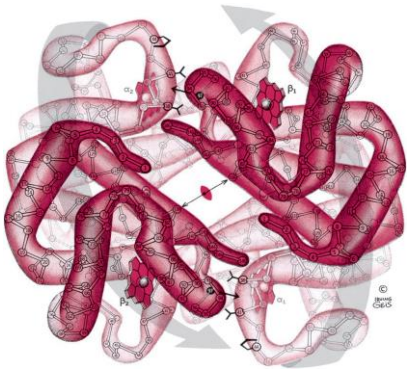
The polypeptide chains of Hb are arranged such that there are extensive interactions between unlike subunits. The α 1–β1 interface (and its α 2–β2 symmetry equivalent) involves35 residues, whereas the α 1–β2 (and α 2–β1) interface involves 19 residues. These associations are predominantly hydrophobic in character, although numerous hydrogen bonds and several ion pairs are also involved. In contrast, contacts between like subunits, α 1–α 2 and β 1– β 2, are few and largely polar in character. This is because like subunits face each other across an approximately 20-Å-diameter solvent-filled channel that parallels the 50-Å length of the exact 2-fold axis.

Oxy- and Deoxyhemoglobins Have Different Quaternary Structures:

Oxygenation causes such extensive quaternary structural changes to Hb that oxy- and deoxyHb have different crystalline forms; indeed, crystals of deoxyHb shatter on exposure to O2. The crystal structures of hemoglobin's oxy and deoxy forms therefore had to be determined independently. The quaternary structural change preserves hemoglobin's exact 2-fold symmetry and takes place entirely across its α1–β2 (and α2–β1) interface. The α 1– β 1 (and α2– β 2) contact is unchanged, presumably as a result of its more extensive close associations. This contact provides a convenient frame of reference from which the oxy and deoxy conformations may be compared. Viewed in this way, oxygenation rotates the α1β1 dimer about 15° with respect to the α2β2 dimer, so that some atoms at the α1β2 interface shift by as much as 6 Å relative to each other.



(a) **deoxyHb**



(*b*) oxyHb

The quaternary conformation of deoxyHb is denoted as the T state (T for "tense") while that of oxyHb, which is essentially independent of the ligand used to induce it (e.g., O_2, CO, CN^-, and NO hemoglobins all have the same quaternary structure), is called the R state (R for "relaxed"). Similarly, the tertiary conformational states for the deoxy and liganded subunits are designated as the t and r states, respectively.

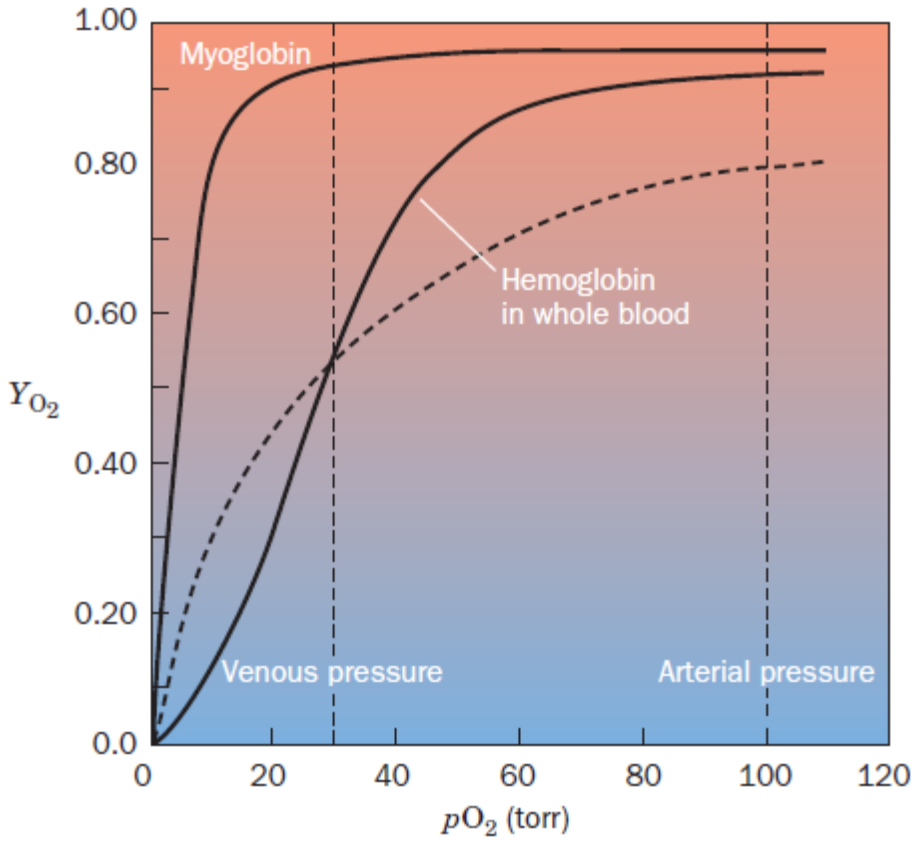


Fig : Oxygen dissociation curve for Mb and Hb in whole blood:

pO_2 = Fractional saturation

Y_{O2} = Partial Pressure or Oxygen Tension