**COURSE BOOK OF**

**CHEMISTRY 2**

**(BIOCHEMISTRY)**

**Desert land and reclamation program**

**Level 1**

**Department of Biochemistry**

**Benha University, Agriculture College**

**Theoretical**

**Theoretical of Chemistry 2**

**BIOCHEMISTRY**

**AC 0102**

**Amino Acids Have Characteristic Titration Curves**

Acid-base titration involves the gradual addition or removal of protons. The plot has two distinct stages, corresponding to deprotonation of two different groups on glycine. Each of the two stages resembles in shape the titration curve of a monoprotic acid, such as acetic acid and can be analyzed in the same way. At very low pH, the predominant ionic species of glycine is the fully protonated form, +H3N - CH2 - 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 (+H3N - CH2 - COOH) and proton acceptor (+H3N - CH2 – 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 it’s -COOH group has a pKa of 2.34.

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 +H3N - CH2 – COO-. We shall return to the significance of this inflection point in the titration curve shortly. The second stage of the titration corresponds to the removal of a proton from the -NH3+ group of glycine. The pH at the midpoint of this stage is 9.60, equal to the pKa for the -NH3+ group. The titration is essentially complete at a pH of about 12, at which point the predominant form of glycine is H2N - CH2 – COO-.

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 -NH3+ group. Note that the carboxyl group of glycine is over 100 times more acidic (more easily ionized) than the carboxyl group of acetic acid, a pKa of 4.76 about 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 carbon atom. The opposite charges on the resulting zwitterion are stabilizing, nudging the equilibrium farther to the right. 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 amino group has a pKa that is lower than that of an aliphatic amine such as methylamine. 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.) Within the buffering ranges of glycine, the Henderson-Hasselbalch equation can be used to calculate the proportions of proton-donor and proton-acceptor species of glycine required to make a buffer at a given pH.

**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 electric 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. 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:



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 farther 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 +H3N - CH2 - COOH, with a net positive charge of 1.0. At pH 2.34, where there is an equal mixture of +H3N - CH2 - COOH and +H3N - CH2 – 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 amino group, a single carboxyl group, and an R group that does not ionize have titration curves resembling that of glycine. 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 -NH3+ group in the range of 8.8 to 11.0.

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. 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, at the 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), much higher than that of glycine.

Finally, as pointed out earlier, under the general condition of free and open exposure to the aqueous environment, 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.





**Peptides and Proteins**

We now turn to polymers of amino acids, the peptides and proteins. Biologically occurring polypeptides range in size from small to very large, consisting of two or three to thousands of linked amino acid residues. Our focus is on the fundamental chemical properties of these polymers.

**Peptides Are Chains of Amino Acids**

Two amino acid molecules can be covalently joined through a substituted amide linkage, termed a peptide bond, to yield a dipeptide. Such a linkage is formed by removal of the elements of water (dehydration) from the carboxyl group of one amino acid and the amino group of another. Peptide bond formation is an example of a condensation reaction, a common class of reactions in living cells. Under standard biochemical conditions, favors the amino acids over the dipeptide. To make the reaction thermodynamically more favorable, the carboxyl group must be chemically modified or activated so that the hydroxyl group can be more readily eliminated.

Three amino acids can be joined by two peptide bonds to form a tripeptide; similarly, amino acids can be linked to form tetrapeptides, pentapeptides, and so forth. When a few amino acids are joined in this fashion, the structure is called an oligopeptide. When many amino acids are joined, the product is called a polypeptide. Proteins may have thousands of amino acid residues. Although the terms “protein” and “polypeptide” are sometimes used interchangeably, molecules referred to as polypeptides generally have molecular weights below 10,000, and those called proteins have higher molecular weights.

As already noted, an amino acid unit in a peptide is often called a residue (the part left over after losing a hydrogen atom from its amino group and the hydroxyl moiety from its carboxyl group). 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. Although hydrolysis of a peptide bond is an exergonic reaction, it occurs slowly because of its high activation energy. As a result, the peptide bonds in proteins are quite stable, with an average half-life (*t*1/2) of about 7 years under most intracellular conditions.

**Peptides Can Be Distinguished by Their Ionization Behavior**

Peptides contain only one free amino group and one free carboxyl group, at opposite ends of the chain. These groups ionize as they do in free amino acids, although the ionization constants are different because an oppositely charged group is no longer linked to the carbon. The amino and carboxyl groups of all nonterminal amino acids are covalently joined in the peptide bonds, which do not ionize and thus do not contribute to the total acid-base behavior of peptides. However, the R groups of some amino acids can ionize and in a peptide these contribute to the overall acid-base properties of the molecule. Thus the acid-base behavior of a peptide can be predicted from its free amino and carboxyl groups as well as the nature and number of its ionizable R groups. Like free amino acids, peptides have characteristic titration curves and a characteristic isoelectric pH (pI) at which they do not move in an electric field. These properties are exploited in some of the techniques used to separate peptides and proteins, as we shall see later in the chapter. It should be emphasized that the pKa value for an ionizable R group can change somewhat when an amino acid becomes a residue in a peptide. The loss of charge in the carboxyl and amino groups, the interactions with other peptide R groups, and other environmental factors can affect the pKa.

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

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



Many small peptides exert their effects at very low concentrations. For example, a number of vertebrate hormones are small peptides. These include oxytocin (nine amino acid residues), which is secreted by the posterior pituitary and stimulates uterine contractions; bradykinin (nine residues), which inhibits inflammation of tissues; and thyrotropin-releasing factor (three residues), which is formed in the hypothalamus and stimulates the release of another hormone, thyrotropin, from the anterior pituitary gland. Some extremely toxic mushroom poisons, such as amanitin, are also small peptides, as are many antibiotics.

Slightly larger are small polypeptides and oligopeptides such as the pancreatic hormone insulin, which contains two polypeptide chains, one having 30 amino acid residues and the other 21. Glucagon, another pancreatic hormone, has 29 residues; it opposes the action of insulin. Corticotropin is a 39-residue hormone of the anterior pituitary gland that stimulates the adrenal cortex.

How long are the polypeptide chains in proteins? As lengths vary considerably. Human cytochrome c has 104 amino acid residues linked in a single chain; bovine chymotrypsinogen has 245 residues. At the extreme is titin, a constituent of vertebrate muscle, which has nearly 27,000 amino acid residues and a molecular weight of about 3,000,000. The vast majority of naturally occurring proteins are much smaller than this, containing fewer than 2,000 amino acid residues.

Some proteins consist of a single polypeptide chain, but others, called multisubunit proteins, have two or more polypeptides associated noncovalently. The individual polypeptide chains in a multisubunit protein may be identical or different. If at least two are identical the protein is said to be oligomeric, and the identical units (consisting of one or more polypeptide chains) are referred to as protomers. Hemoglobin, for example, has four polypeptide subunits: two identical chains and two identical chains, all four held together by noncovalent interactions. Each subunit is paired in an identical way with a subunit within the structure of this multisubunit protein, so that hemoglobin can be considered either a tetramer of four polypeptide subunits or a dimer of protomers.

A few proteins contain two or more polypeptide chains linked covalently. For example, the two polypeptide chains of insulin are linked by disulfide bonds. In such cases, the individual polypeptides are not considered subunits but are commonly referred to simply as chains.

We can calculate the approximate number of amino acid residues in a simple protein containing no other chemical constituents by dividing its molecular weight by 110. Although the average molecular weight of the 20 common amino acids is about 138, the smaller amino acids predominate in most proteins. If we take into account the proportions in which the various amino acids occur in proteins the average molecular weight of protein amino acids is nearer to 128. Because a molecule of water (*M*r 18) is removed to create each peptide bond, the average molecular weight of an amino acid residue in a protein is about 128 - 18 = 110.

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

Many proteins, for example the enzymes ribonuclease A and chymotrypsinogen, contain only amino acid residues and no other chemical constituents; these are considered simple proteins. However, some proteins contain permanently associated chemical components in addition to amino acids; these are called conjugated proteins. The non-amino acid part of a conjugated protein is usually called its prosthetic group. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups for example, lipoproteins contain lipids, glycoproteins contain sugar groups, and metalloproteins contain a specificmetal. A number of proteins contain more than one prosthetic group. Usually the prosthetic group plays an important role in the protein’s biological function.



**References:**

* Lehninger Principles of Biochemistry (Nelson W. H. Freeman. 4th Ed, 2004).