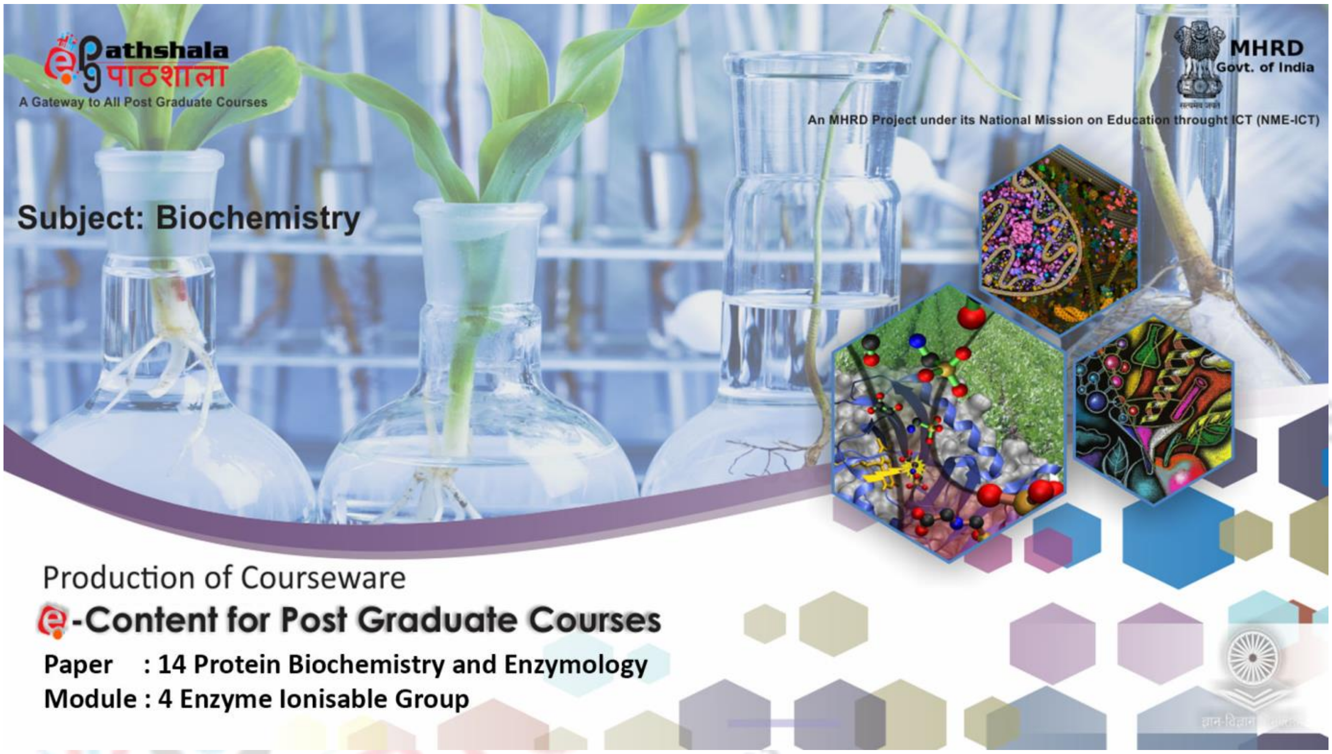

e-Pathshala पाठशाला

MHRD Govt. of India

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Description of Module	
Subject Name	Biochemstry
Paper Name	14
Module Name/Title	4 Enzyme ionizable groups

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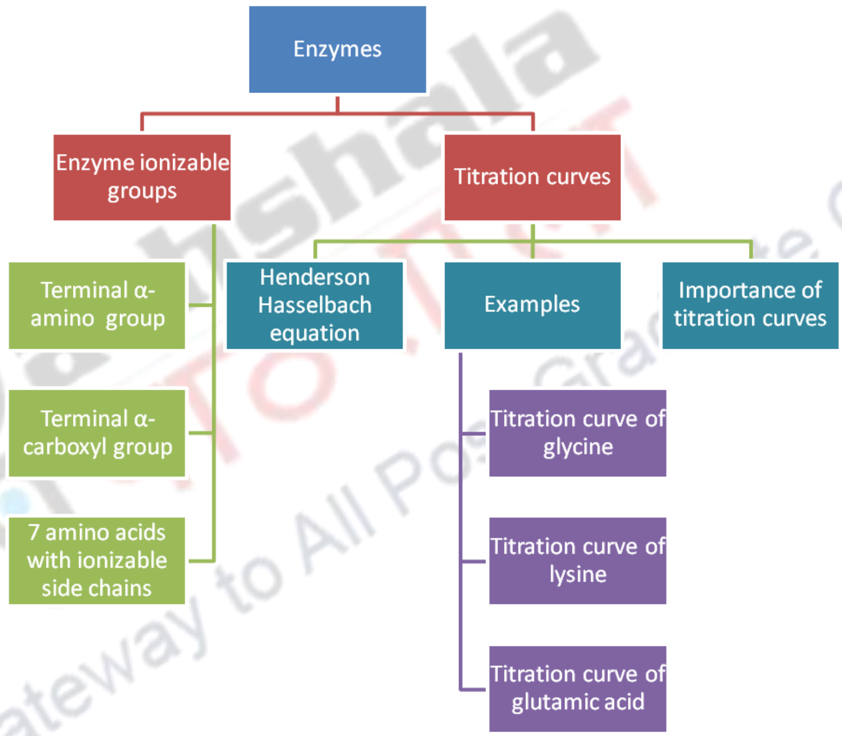
1. Objectives

- Understand what are ionizable groups in enzymes
- Look at the various ionizable groups in enzymes
- Understanding the titration curves of some amino acids

2. Concept Map



3. Description

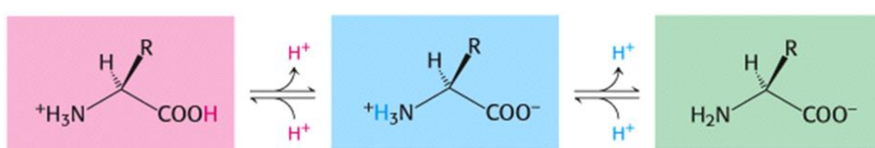
3.1 Introduction

Enzymes are protein molecules which contain a large number of acid and basic groups. Depending on the type of amino acid present within the enzyme and their ionization with the pH of their environment, the charges on these groups vary. The overall charge on the enzyme, along with its activity, stability and solubility is therefore affected.

3.2 Existence of amino acids in solution

The functional groups in amino acids in the enzyme have the ability to readily ionize. Each amino acid has at least two acid-base groups (an amino group and a carboxyl group). Certain amino acids within enzyme may also have ionizable side chains. At physiological pH (pH = ~7.4) the $-NH_2$ group remains basic and protonated while the -COOH group is acidic and remains deprotonated.

Amino acids can act both as acid or a base, i.e., they can exist both in their cationic form and anionic form. Additionally, amino acids can also exist in a dipolar state, which is known as the zwitterionic form. In this dipolar state, amino group is protonated and carboxyl group is deprotonated and these dipolar ions can act as either an acid (proton donor) or a base (proton acceptor). Such substances have dual character and are called as ampholytes.



Cationic form   Zwitterionic form   Anionic form

3. 3 Ionizable groups in enzymes

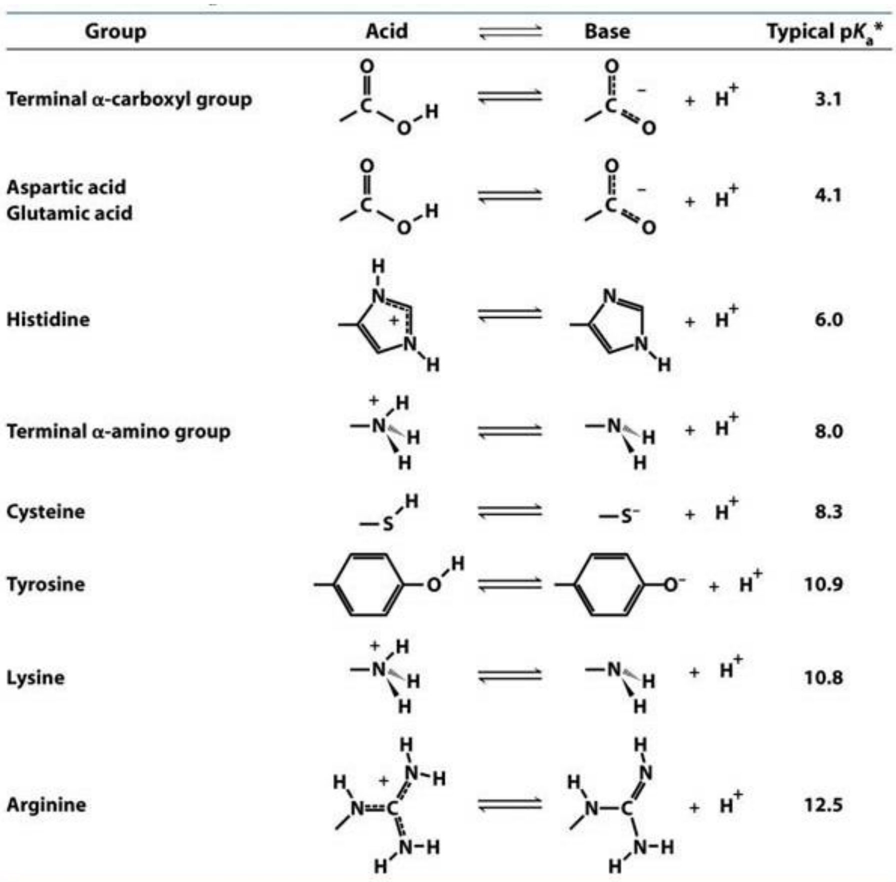
Having understood the existence of various forms of amino acids in solution, we now take a look at the various ionizable groups which can be present in enzymes. These are as follows:

- A *terminal* α-amino in each peptide or protein which can accept a proton
- A *terminal* α-carboxyl in each peptide or protein whcih can donate a proton

- Seven of the amino acids- arginine, aspartic acid, cysteine, glutamic acid, histidine, lysine and tyrosine; have ionizable side chains. These are able to donate or accept protons.

The typical pK_a values for the R group of these amino acids are given in Table 1.

Table 1. Typical pKa values of ionizable groups in enzymes



Group	Typical pK_a*
Terminal α-carboxyl group	3.1
Aspartic acid Glutamic acid	4.1
Histidine	6.0
Terminal α-amino group	8.0
Cysteine	8.3
Tyrosine	10.9
Lysine	10.8
Arginine	12.5

*pK_a values depend on temperature, ionic strength, and the microenvironment of the ionizable group.

3.4 Henderson - Hasselbalch Equation

The predominant molecular species of amino acids present in an aqueous solution will depend on the pH of the solution. In order to determine the nature. The **Henderson - Hasselbalch Equation** helps in the determination of the molecular and ionic species that are present in aqueous solutions at different pH's. The equation is represented as:

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

Where pKa is the acidity of a specific conjugate acid (HA).

Titration curves for ionizable groups in three different types of amino acids are shown below. These are amino acids with no ionizable side chain (Glycine), amino acids with carboxylic acid side chain (Glutamic acid) and amino acids with amine side chain (Lysine).

3.5 Titration curve of Glycine

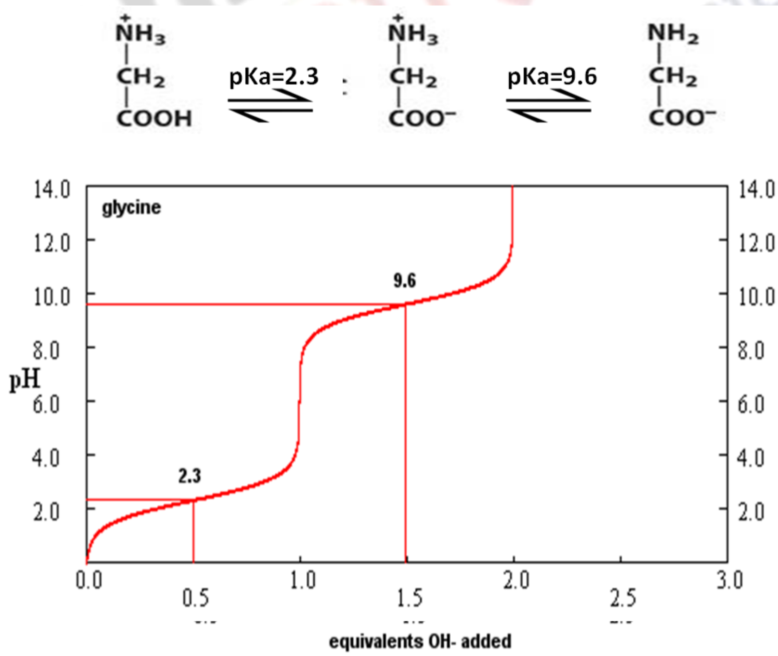


Fig. 2 Ionization and Titration curve of glycine

The amino acid glycine has no ionisable side groups. It has only two ionisable groups, a carboxyl group and amino group. When titrated with a strong base (NaOH), the titration curve so obtained is as shown in Fig. 2. Two steps are involved corresponding to the deprotonation of the two groups with NaOH.

1) At low pH, the amino acid is completely protonated and exists as $^{+}NH_3$-CH_2-COOH

2) As the titration continues, at first midpoint (pH = 2.34), there is equimolar concentration of $^{+}NH_3$-CH_2-COOH and $^{+}NH_3$-CH_2-COO^-

3) The mid-point of any titration is the point of inflection where pH = pKa of the protonated group. For glycine, the first point of inflection occurs at pH = 2.34 so pKa at this point equals 2.34. Thus, the carboxyl group of glycine has a pKa of 2.34.

4) The second point of inflection is reached at pH = 5.97. At this point, deprotonation of carboxyl group is complete and that of amino group has just started. At this pH, glycine is present as a dipolar ion $^{+}NH_3$-CH_2-COO^-

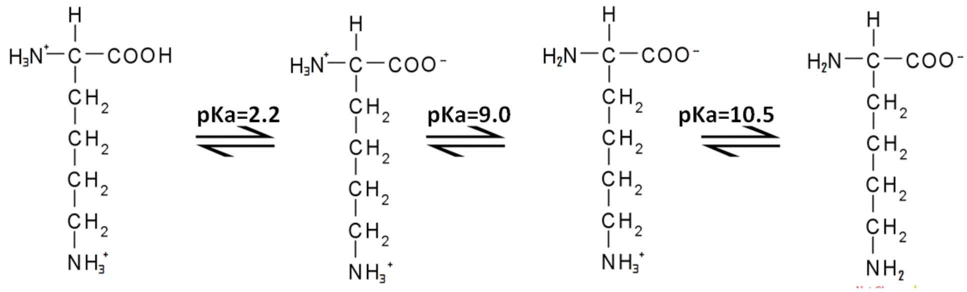
5) As the titration continues, deprotonation of amino group takes place. At mid point, the pH = 9.60 so pKa for amino group is 9.60. Also, at this point, there exists an equimolar concentration of proton donor and proton acceptor forms.

6) As the titration proceeds further, around pH = 12, the deprotonated form NH_2-CH_2-COO^- is found in abundance.

Similarly titration curves for lysine and glutamic acid are shown below.

3.6 Titration curve for lysine

A Gate



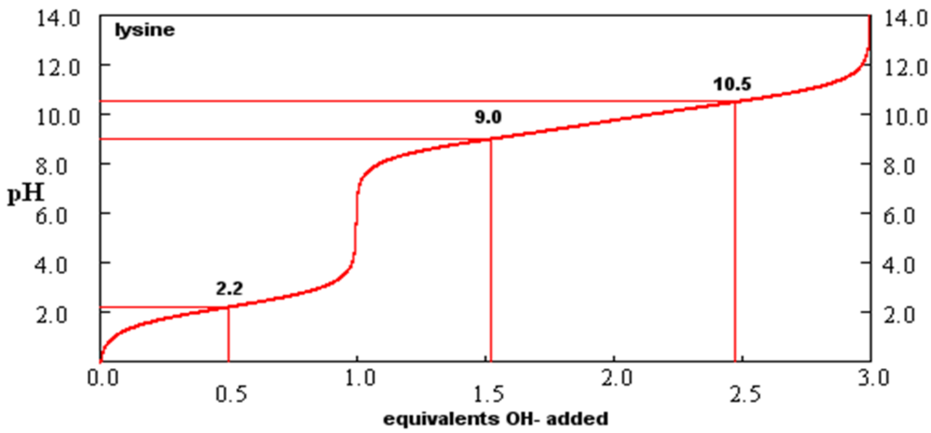


Fig. 3 Ionization and Titration curve of lycine

Lysine is a basic amino acid, with a 3rd ionizable functional group, the R group. The titration curve of lysine is shown in Fig. 3 above. As the concentration of the hydroxide ion increases, the hydrogen ion concentration decreases. At the first pKa, the dissociation of the alpha-carboxyl takes place. At the second pKa, the dissociation of the alpha-amino occurs while at the third pKa, the dissociation of the R-group amino takes place. At each pKa, the solution resists change in pH with addition of more and more hydroxide. The pI occurs where lysine has no net charge. Thus, pI= $(pK_2+pK_3)/2$= (9+10.5)/2. Thus pI is reached at pH 9.75

3.7 Titration curve for glutamic acid

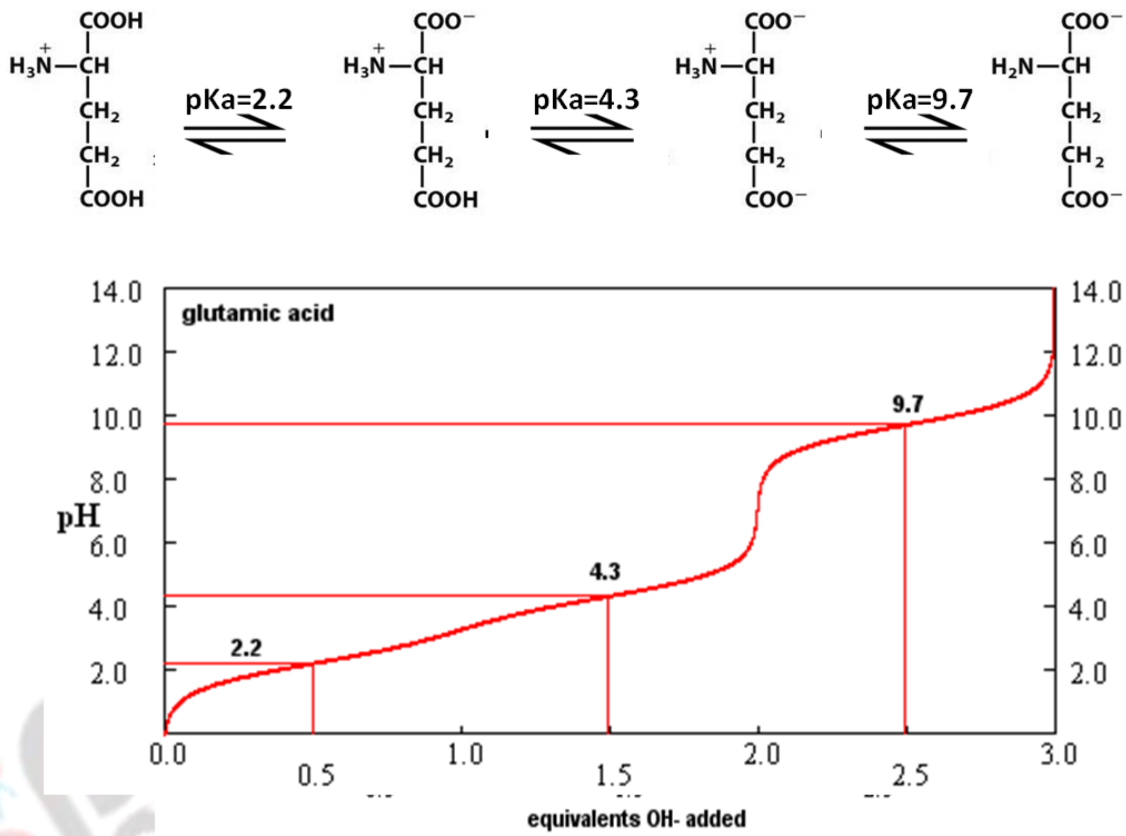


Fig. 4 Ionization and Titration curve of glutamic acid

Glutamic acid is an acidic amino acid, with a 3rd ionizable functional group, the R group. The titration curve of glutamic acid is shown in Fig. 4 above. At lower pH (<pH = 2.0), the glutamic acid carries an overall positive charge. As base is added (one equivalent), the group which has the lowest pKa is deprotonated. This therefore results in no net charge on a glutamic acid. The pI (isoelectric point) is therefore obtained at pH = (2.2 + 4.3)/2 = 3.25. With further addition of base, the second acidic proton starts getting removed from the -COOH of the R-group. This amino acid bears only a formal negative charge at physiological pH (7.0).

3.8 Significance of titration curves

1) Calculation of pKa values of ionizing groups.

2) Identification of regions of buffering for the amino acid. For example, it can be said that glycine has two regions of buffering centered around pH 2.34 and 9.60, respectively.

3) Determination of electric charge on an amino acid. This point at which the net electric charge on an amino acid is zero is called as the ***isoelectric point*** and is designated as pI.

4) With increasing number of ionizable groups in amino acids, the titration curves become more and more complex due to increase in number of ionization steps.

4. Summary

In this lecture we learnt about:

- Amino acids can act both as acid or a base. Additionally, amino acids can also exist in a dipolar state, which is known as the zwitterionic form.
- Functional groups of amino acids in enzymes present have the ability to readily ionize.
- Enzymes have a terminal amino group and a carboxyl group which are ionizable and seven specific other amino acids which have ionizable side chains. These include arginine, aspartic acid, cysteine, glutamic acid, histidine, lysine and tyrosine.
- The nature of the molecular and ionic species present in aqueous solutions at different pH's is determined by Henderson - Hasselbalch Equation.
- Titration curves help in calculation of pKa values of ionizing groups, identification of regions of buffering for the amino acid and determination of electric charge on an amino acid.