Biochemistry 2

Amino acids

For 2nd year students,
Biology Department, College of Sciences,
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الكيمياء حياتية ٢

الاحماض الامينية

المرحلة الثانية- قسم علوم الحياة
الجامعة المستنصرية

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Acid base properties of A.As

Since all amino acids have the acidic carboxyl and basic amino groups, they have acid-base properties. Their molecular species can be affected by the pH of the solution. At very low pH, the predominant molecular form will be fully protonated, H_3N^+-HCR-COOH. When the pH increases toward the physiological pH, the most acidic part of the A.A will deprotonate and the pH will equal to the pKa of the protonated group. For example, Gly will lose a proton from COOH group and be H_3N^+-HC-CH_3-COO^-. This could be better understanding through studying the titration curve of amino acids. The titration curve depends on adding equivalent amount of NaOH to a very acidic solution of A.As (low pH) and follows the deprotonation steps of that A.As, in order to know the ionic nature of A.As in aqueous solution at different pHs. The titration curve of Gly in Figure 11 illustrates this relationship where 2 distinct stages due to 2 deprotonations stages are shown. Each stage includes interconversion from one form to another. The first stage started at a pH lower than 2, where both the carboxylic and amino group are fully protonated, so the net charge will be +1. The titration with NaOH, encourages the carboxylic group to loss its proton, therefore the net charge of Gly will be 0 (zwitterion), Herein, the 1st stage of deprotonation the has completed at pH around 5.97, which is called isoelectric point (pI) and it reflects the zwitterion status of the Gly (NH_3^+-HCH-COO $^-$). The A.A is now ready to loss another proton by will

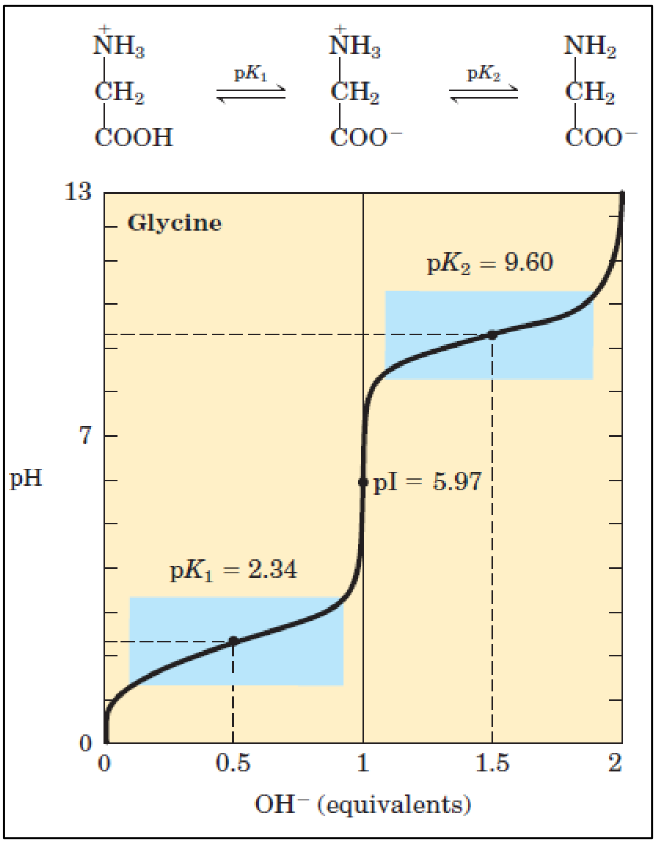


Figure 11. Titration curve of Gly.

start at a pH higher than 10, where the amino group will lose its proton and Gly will be negatively charged.

At the midpoint of each titration stage, the pH is equal to the pKa of the protonated group. In Figure 11, the pH in the midpoint of the 1st stage is equal to 2.34, hence the p*Ka* (or pK_1) of the protonated COOH is = 2.34. The pH in the midpoint of the 2nd stage is equal to 9.6, correlates to the p*Ka* (pK_2) of the amino group NH_3^+. The titration curve is fully completed at pH of around 12 where the predominant form of Gly is NH_2-HCH-COO $^-$.

Because pI is a very important point in the titration curve refer to the equilibrium form of that A.A, it should be determined accurately. For Gly, which has 2 deprotonation steps only, with no ion-able sidechain, the pI will be the mean of pK_1 and pK_2 as following:

$$\mathbf{pI = \frac{p\mathit{K}1 + p\mathit{K}2}{2} = \frac{3.34 + 9.60}{2} = 5.97}$$

The titration curve for Ala in Figure 12, demonstrates similar relationships. Starting from a fully protonated state, the pK_a of -CO_2H is 2.34, the zwitterion forms increases at isoelectric point (pI) equal to pH 6.01. This behavior can be noticed generally for simple (di-functional) A.As.

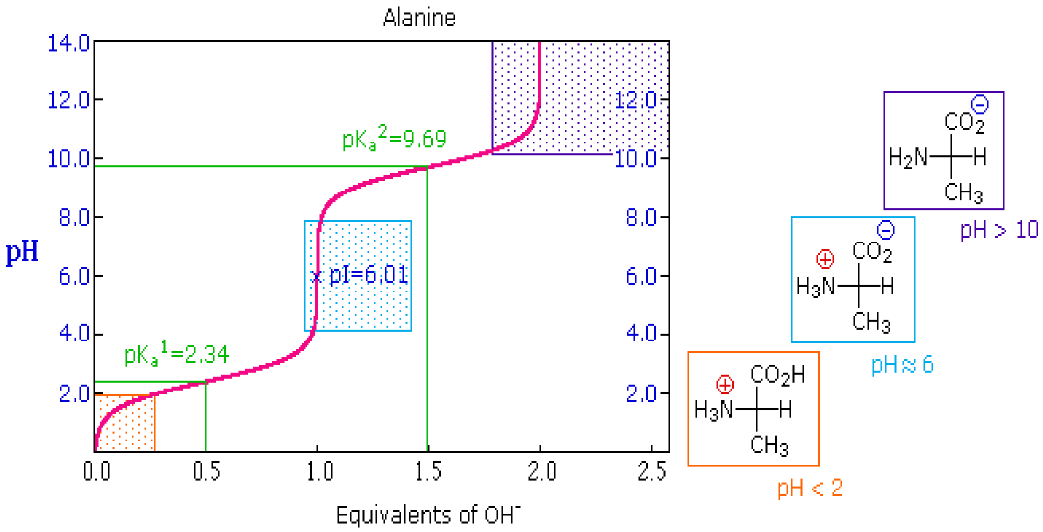


Figure 12. Titration curve of Ala.

Some A.As have ionized R group, which is either additional acidic (COO^-) or basic (NH_3^+), therefore, there is another p*Ka* related to the R group, which is pK_R. For example, Figure 13 shows the titration curve for Gly.

Answer:

For Glu, let us see the Figure below, where 3 dissociation steps.
Therefore, pI is equal to:

*pK_1 carboxylic acid **1** = 2.3*
*pK_2 carboxylic acid **2** (R group) = 4.3*
pK_R amino group = 9.6

$$pI = \frac{pK1 + pK2}{2}$$

$$pI = \frac{2.3 + 4.3}{2}$$

$$pI = 3$$

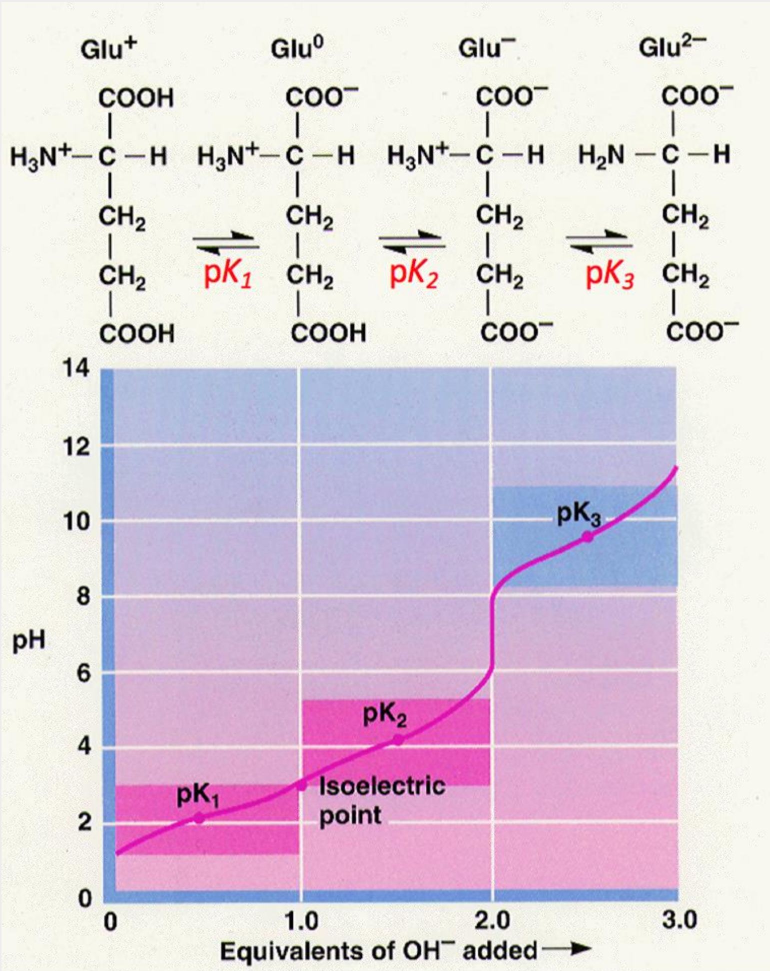


Figure 13. Titration curve of Glu.

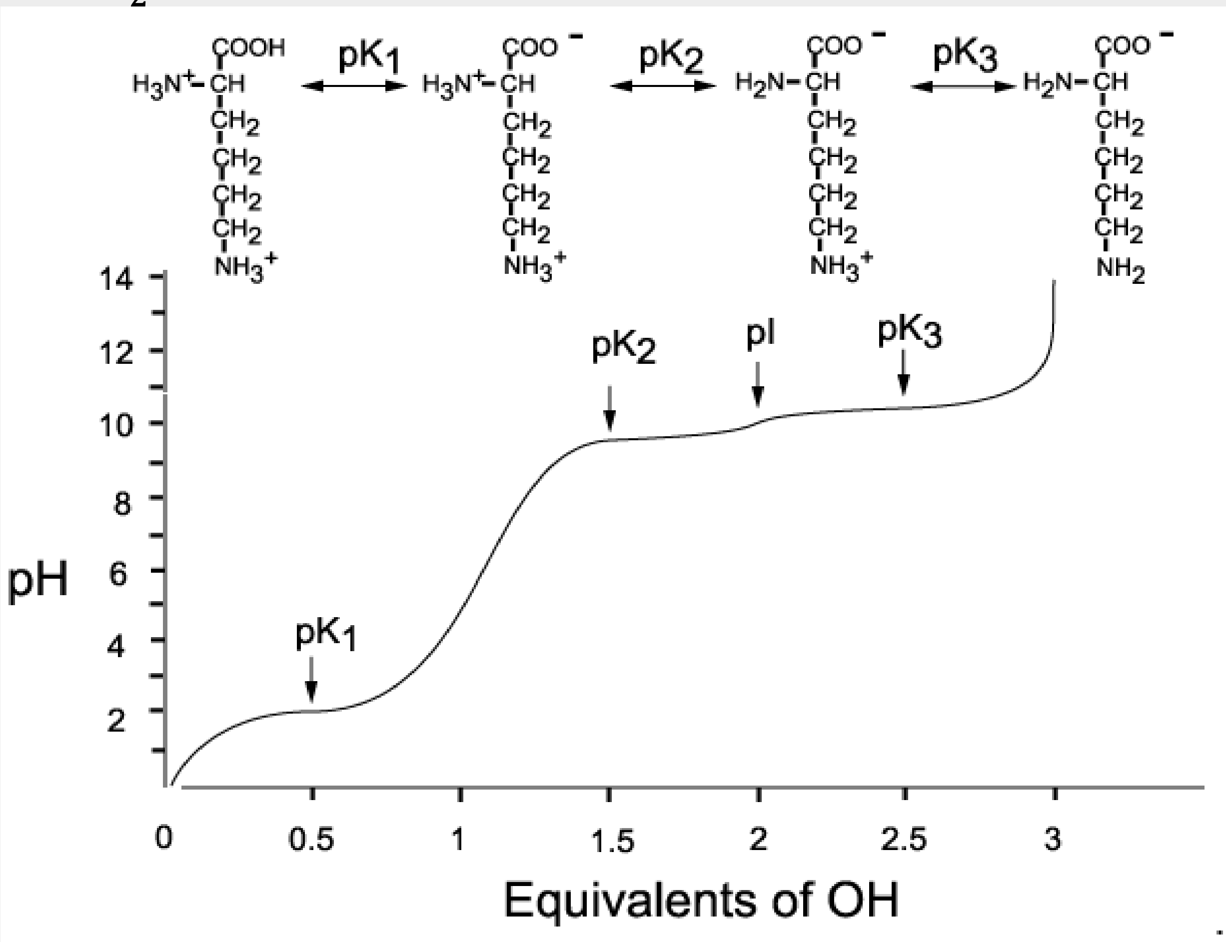
<u>Practice</u>: Predict the titration curve and calculate the pI of the A.As Lys? Tyr? Arg? His?

<u>Answer:</u>
For Lys, see the Figure below, where 3 dissociation steps.
Therefore, pI is equal to:

pK_1 carboxylic acid = 2.2
*pK_2 amino group **1** = 9.0*
pK_3 amino group 2(R group) = 10.5

$$pI = \frac{pK2 + pK3}{2}$$

$$pI = \frac{9 + 10.5}{2} = 9.75$$



Answer:

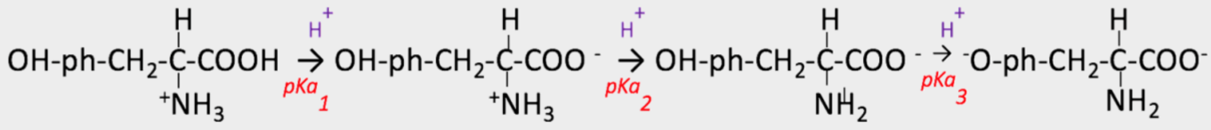
For Tyr, see the Figure below, where 3 dissociation steps.
Therefore, pI is equal to:

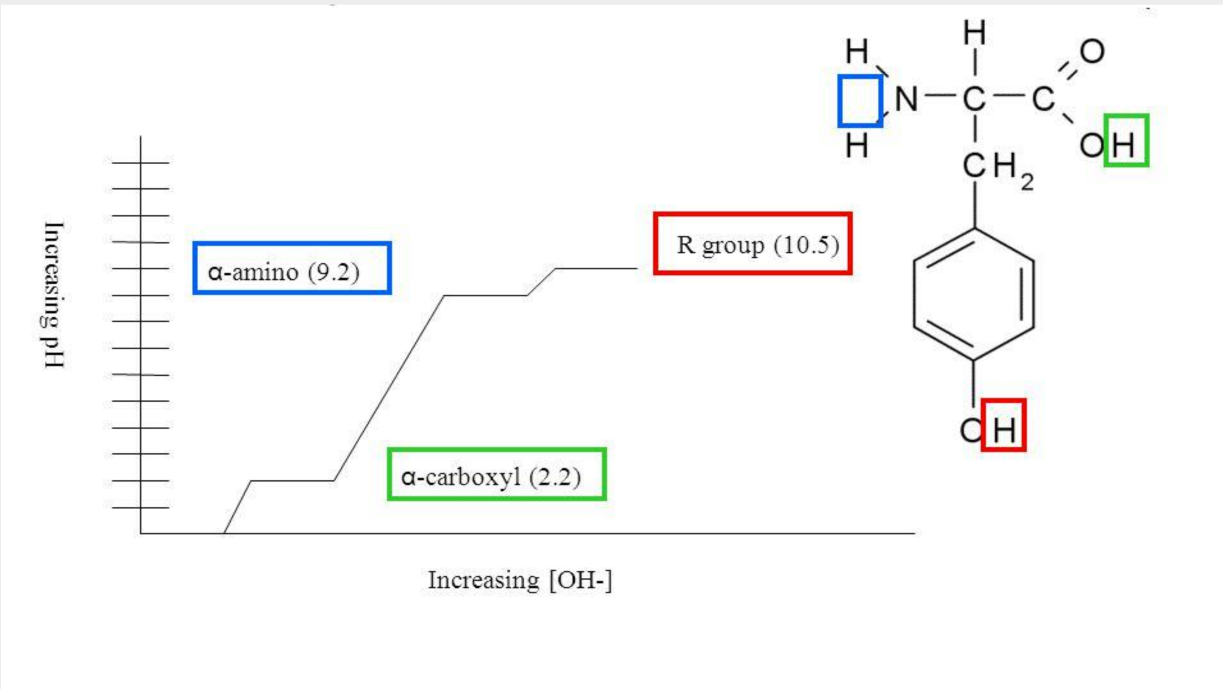
pK_1 carboxylic acid ***1*** *= 2.2*
pK_2 amino group = 9.6
pK_3 hydroxyl (R group) = 10.5

$$pI = \frac{pK1 + pK2}{2}$$

$$pI = \frac{2.2 + 9.6}{2}$$

$$pI = 5.9$$





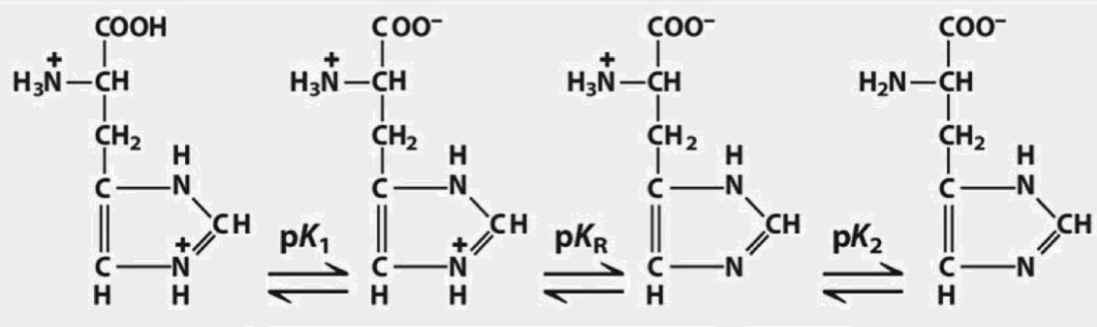
Answer:
For His, see the Figure below, where 3 dissociation steps.
Therefore, pI is equal to:

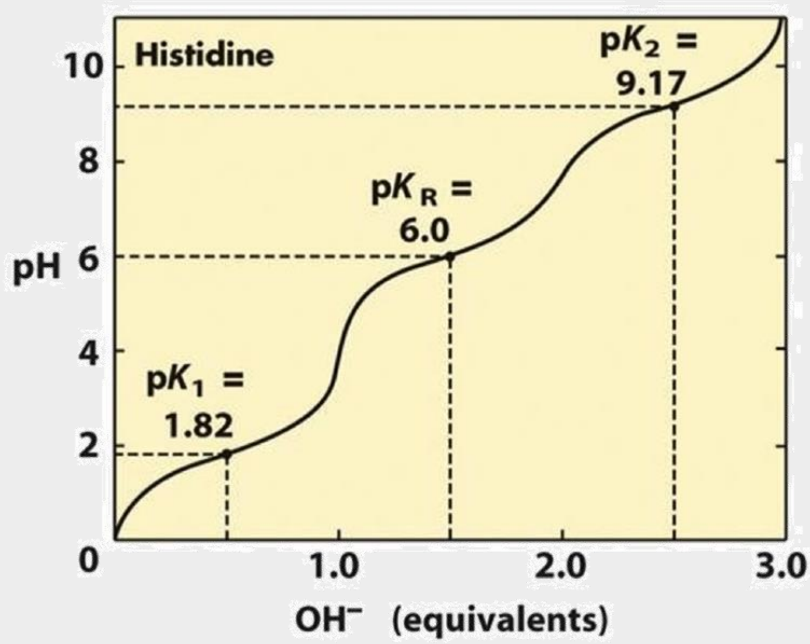
pK_1 carboxylic acid = 1.82
pK_2 amino group = 9.17
pK_R hydroxyl (R group) = 6.0

$$pI = \frac{pK1 + pK2}{2}$$

$$pI = \frac{1.82 + 9.17}{2}$$

$$pI = 5.49$$

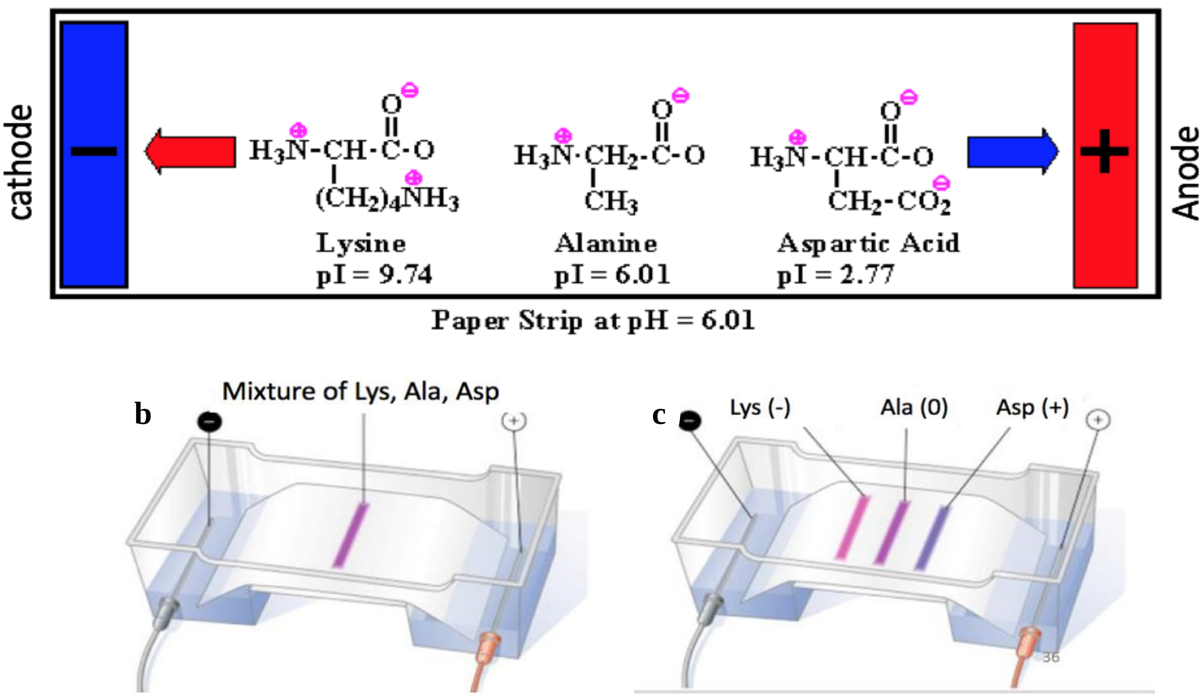




Electrophoresis

Electrophoresis technique is widely used to separate A.As and protein solutions according to their charge when they immerged in an electrical field. Principally, all A.As are ionized in aqueous solutions and have different R groups, thus, they migrate toward the complementary electrode when immerged in an electrical field. In the electrophoresis, the positively charged A.As migrates toward the negative electrode, but the negatively charged A.As moves toward the positive electrode, see Figure 13. For example, in Figure 13a, there are 3 different A.As immerged in a solution with a pH of 6.1 and connected to electrodes. Because Lys is positively charged at any pH below its pI (9.74), therefor it will migrate toward the negative electrode (cathode). Whilst, Asp is negatively charged at pH above its pI (2.77), therefore it will migrate toward the positive electrode (Anode). Ala has a pI equal to the pH of the solution (6.01), so it will stay in the middle.

a



Practice: What will be the direction of movement in the electrophoresis for each of the following Val? Asp? And His? if they immerged in a solution of pH 2 and 6?