SECTION 2

Amino Acids and the Primary Structure of Proteins

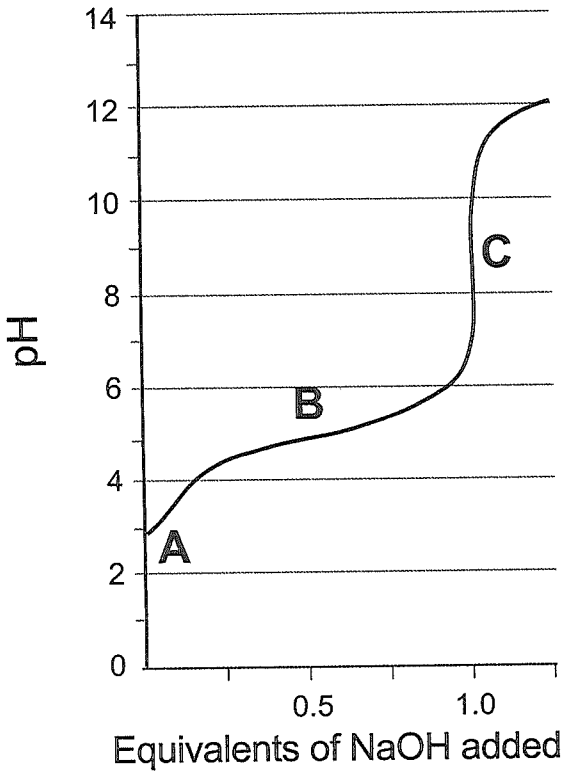
PRE-ACTIVITY ASSIGNMENT

1. Produce a reading outline for the chapter on amino acids and the section that introduces the peptide bond. Commit to memory the structures of the amino acids.
2. Draw a titration curve for the amino acid lysine using the pK_as of 2.2, 9.0 and 10.0 for the ionizable groups of lysine. Use the titration curve in Model 1 and its description as a model for your drawing. Label the buffering regions and equivalence points. Draw the structures for the primary species of lysine at all the buffering regions and equivalence points you include in your graph. You might find it helpful to answer questions 1 and 2 to complete this.
3. Define the term ***buffering region*** and describe how you could locate the buffering region on a titration curve.
4. Define the term ***equivalence point*** and describe how you could locate the equivalence point on a titration curve.

Model Titration Curve

The graph below is a titration curve in which a solution of NaOH is added to a solution of propanoic acid (HPr). (This type of titration is often covered in general chemistry.) At point A the primary form present is the conjugate acid (HPr). As OH^- is added, it forms water by combining with protons in the solution. This reduces the concentration of HPr and produces the conjugate base propionate (Pr–). The Ka for propanoic acid is 1.3×10^{-5} and the pK_a for propanoic acid is 4.89. When the pH of a solution of HPr is 4.89 (the pH equals the pK_a which is point B on the graph), the concentration of the conjugate acid, HPr, and the concentration of the conjugate base Pr– are equal. For acids and bases, it is always true that the concentrations of the respective conjugate acid/base pair are equal when the pH is equal to the pK_a of the conjugate acid. This is usually called the buffering region. Point C on the graph is the equivalence point. At this point the molar amount of monoprotic acid in the original solution is equal to the molar amount of OH^- added, i.e. the equivalents of protons equals the equivalents of base added.

Model 1 Titration of propanoic acid with NaOH



IN-CLASS ACTIVITY

Why

Amino acids are monomers from which proteins are constructed. Understanding the structure and characteris-tics of amino acids and the peptide bond that covalently links them to form peptides will aid in understanding larger, more complex protein structures. Proteins carry out a multitude of different and important functions. The great variety in function is accomplished through a complex and variable polymeric structure. Comprehending protein structure will give you a better understanding of how proteins carry out their roles.

Outcomes

1. Use the acid/base characteristics (pK_a data) of the 20 amino acids found in proteins to determine the charge of an amino acid at a given pH.
2. Determine the pI of a small peptide.
3. Identify the peptide bond and describe the structural features that characterize a peptide bond.
4. Use information processing skills to draw conclusions about chemical characteristics of complex molecules.

Plan

1. Form teams as instructed.
2. The person whose hometown is the most distant from here assumes the role of team manager. The team manager should assign remaining roles.
3. Answer the Critical Thinking Questions.
4. Prepare the spokesperson to articulate two discoveries the group has made that would help others better process information in this chapter.

Critical Thinking Questions

1. Quickly review questions a through c below.
   a. What is the letter on the model titration curve of HPr that corresponds to the point where pH equals the numerical value of the pK_a for HPr?

   b. Is the titration curve horizonal or vertical at this point that corresponds to pH = pK_a?

   c. What species of molecules are present at the point noted in question 1b?

2. The textbook values for pK_as for each ionizable group of lysine are variable. For the purpose of this exercise, use the values of: 2.2 (α-COOH), 9.0 (α-NH_3^+), and 10.0 (R group).

   a. Will the titration curve for lysine be horizontal or vertical at the points where pH = pK_a?

   b. What species of molecule are present at each pH = pK_a value? Draw them.

   c. Using the pK_a data for lysine, determine the charge on a sample of the amino acid lysine at pH 1, at pH 9.0, at pH 12.

   pH 1

   pH 9.0

   pH 12

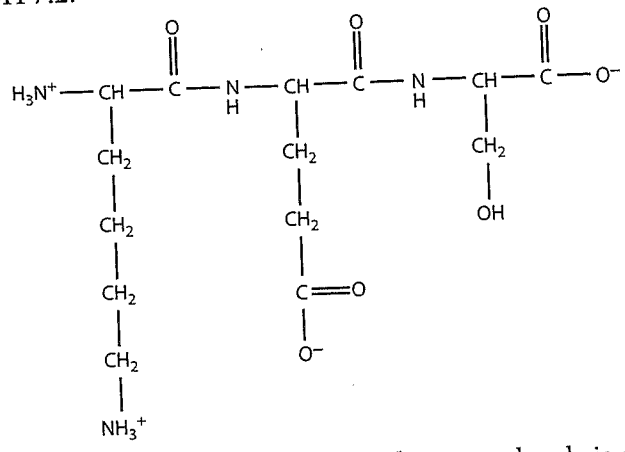
3. From your reading and discussion, come to and record a common definition of ***pI***.

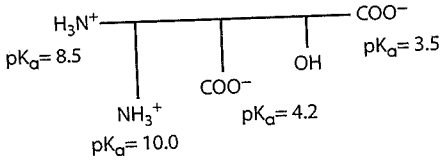
4. While the definition of pI is straightforward, applying the definition is more difficult. Work with your group to establish the pI of lysine and finalize a drawing for the titration curve of lysine. Do not look in the book to verify your answer until you have made your own determination. You might find it helpful to examine Model 2 to establish the pI.

5. Refer to Model 2 for information relevant to this question. A sample of the peptide Lys-Glu-Ser has a net charge of zero between what two pH values? What is the pI of Lys-Glu-Ser?

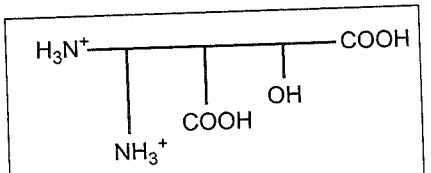
Model 2:

The pl of a peptide is determined by examining the ionizable groups. The protonated and unprotonated forms of each ionizable group are in equilibrium. Consider the peptide Lys-Glu-Ser shown below at pH 7.2. The complete structure is on on the left and a stylized structure with just the ionizable groups is on the right. While the N-terminal is depicted as protonated, a sample of Lys-Glu-Ser is composed of a population of molecules and within that population some molecules may contain a non-protonated N-terminal group at pH 7.2.



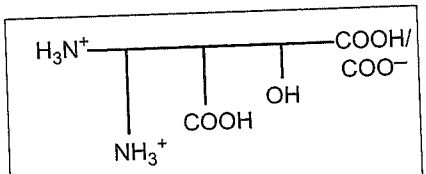


In the stylized structures below, only one molecule is drawn. However each diagram represents a collection of many molecules. Therefore "half protonated" implies that half the molecules present are protonated and half are not.



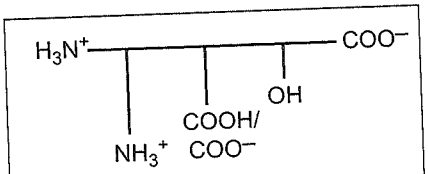
At pH=1 all groups are protonated.

NET charge = **+2**



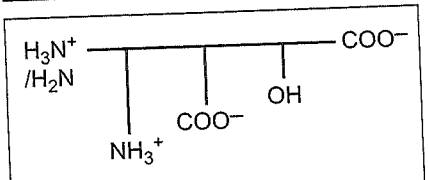
At **pH=3.5** the C terminal is half protonated and half deprotonated.

NET charge = **+1.5**



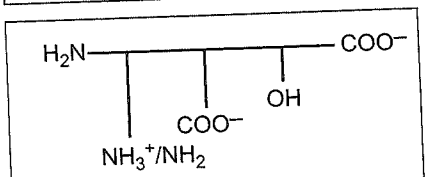
At **pH=4.2** the R group carboxyl is half protonated, half deprotonated.

NET charge = **0.5**



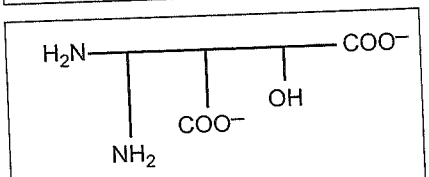
At **pH=8.5** the N terminal is half protonated and half deprotonated.

NET charge = **–0.5**



At **pH=10.0** the R group amino of lysine is half protonated, half deprotonated.

NET charge = **–1.5**



At **pH=12** all groups are deprotonated.

NET charge = **–2.0**

NOTE: In peptides and proteins, the N-terminal and C-terminal groups have different pK_as from the parent amino acid. The pK_a of the N-terminal is about 8.5 whereas C-terminal pK_a is about 3.5

6. Would Lys-Glu-Ser have the same pI as Ser-Glu-Lys? Explain.

7. For a protein, how do you think you might estimate the pI?

8. Draw a dipeptide (use R_1 and R_2 for the side chain R groups) and the resonance structures of the peptide bond.

9. Recall the geometry about atoms that participate in double bonds or partial double bonds. What atoms form the rigid plane of the peptide bond (which atoms are coplanar)?

10. How do you expect the rigid plane of the peptide bond to impact folding?

POST-ACTIVITY SKILL EXERCISES

1. Draw the appropriate titration curve for the tripeptide Met-Lys-Val on graph paper starting at pH 1 and ending at pH 12. On the curve label the pK_as and the pI. Below the titration curve, using structures, show the equilibria that occur at the buffering region(s) and the equivalence point(s).

2. Draw the structure of the peptide Arg-Met-His-Val-Glu and label the coplanar atoms in one peptide bond.

3. Estimate a pI for the peptide given in question 2, above.