

Name:

## Chem 464 Biochemistry

*Multiple choice (4 points apiece):*

1. The reaction of two amino acids to form a dipeptide is a(n):
  - A) cleavage.
  - B) condensation.
  - C) group transfer.
  - D) oxidation-reduction.
  - E) rearrangement.
2. Osmosis is:
  - A) movement of a nonpolar solute molecule across a membrane.
  - B) movement of a polar solute molecule across a membrane
  - C) movement of a charged solute molecule (ion) across a membrane.
  - D) movement of a gas molecule across a membrane.
  - E) movement of a water molecule across a membrane.
3. Which of the following is true about the properties of aqueous solutions?
  - A) Hydrogen bonds form readily in aqueous solutions.
  - B) Charged molecules are generally insoluble in water.
  - C) An increase in pH from 5.0 to 6.0 reflects an increase in the hydroxide ion concentration ( $[\text{OH}^-]$ ) of 20%.
  - D) A decrease in pH from 8.0 to 6.0 reflects a decrease in the proton concentration ( $[\text{H}^+]$ ) by a factor of 100.
4. Which of the following statements about aromatic amino acids is correct?
  - A) On a molar basis, tryptophan absorbs more ultraviolet light than tyrosine.
  - B) The major contribution to the characteristic absorption of light at 280 nm by proteins is the phenylalanine R group.
  - C) Histidine's ring structure results in its being categorized as aromatic or basic, depending on pH.
  - D) The presence of a ring structure in its R group determines whether an amino acid is aromatic or not.
  - E) All are strongly hydrophilic.
5. In a conjugated protein, a prosthetic group is:
  - A) a part of the protein that is not composed of amino acids.
  - B) a fibrous region of a globular protein.
  - C) a subunit of an oligomeric protein.
  - D) synonymous with "protomer."
  - E) a nonidentical subunit of a protein with many identical subunits.
6. In the  $\alpha$  helix the hydrogen bonds:
  - A) are perpendicular to the axis of the helix.
  - B) occur mainly between electronegative atoms of the R groups.
  - C) occur mainly between electronegative atoms of the backbone.
  - D) occur only between some of the amino acids of the helix.
  - E) occur only near the amino and carboxyl termini of the helix.
7. The three-dimensional conformation of a protein may be strongly influenced by amino acid residues that are very far apart in sequence. This relationship is in contrast to secondary structure, where the amino acid residues are:
  - A) always side by side.
  - B) generally near each other in sequence.
  - C) generally on different polypeptide strands.
  - D) generally near the polypeptide chain's amino terminus or carboxyl terminus.
  - E) restricted to only about seven of the twenty standard amino acids found in proteins.

8. *Calculation* (10 points): Calculate the pH of a buffer that contain 5 grams of  $\text{NaH}_2\text{PO}_4$  and 5 grams of  $\text{Na}_2\text{HPO}_4$ . The  $\text{pK}_a$  for the deprotonation of  $\text{H}_2\text{PO}_4^-$  is 6.86.

$$\text{Molar mass NaH}_2\text{PO}_4 = 23 + 2(1) + 31 + 4(16) = 120$$

$$\text{Moles NaH}_2\text{PO}_4 = 5/120 = .0417$$

$$\text{Molar mass Na}_2\text{HPO}_4 = 2(23) + 1 + 31 + 4(16) = 142$$

$$\text{Mole Na}_2\text{HPO}_4 = 5/142 = .0352$$

$$\text{pH} = \text{pK}_a + \log (\text{A}^-/\text{HA})$$



Volume of buffer doesn't matter because we have the same

volume

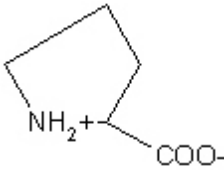
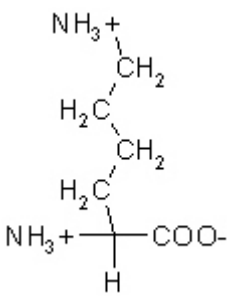
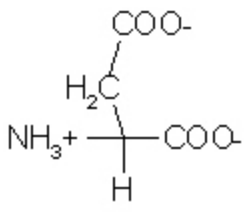
In both the numerator and denominator of the A-/HA term

$$\text{pH} = 6.86 + \log (.0352/.0417)$$

$$6.86 + (-.0736)$$

$$= \mathbf{6.79}$$

9. (20 points) *Fill in the following table:*

Name	Proline	Lysine	Aspartate (or Aspartic acid)
3 letter abbreviation	Pro	Lys	Asp
1 letter abbreviation	P	K	D
Structure			
side chain $\text{pK}_a$ (if ionizable)	NA	10.53	3.65
General classification (nonpolar, polar, etc)	Nonpolar (Polar in older texts)	Basic or Positively charged	Acidic or Negatively charged

Longer questions:

10. (10 points) Peptide SADTEST

Make a rough sketch of the titration curve of the peptide

What is the charge of this peptide at pH 1

What is the charge of this peptide at pH 12

What is the pI of this peptide?

Ser-Ala-Asp-Thr-Glu-Ser-Thr

N terminal pKa 9-10, I'll use 9.5

COOH terminal pKa 2-4, I'll use 3

AA's with acid/base functions

Asp pKa 3.65

Glu pKa 4.25

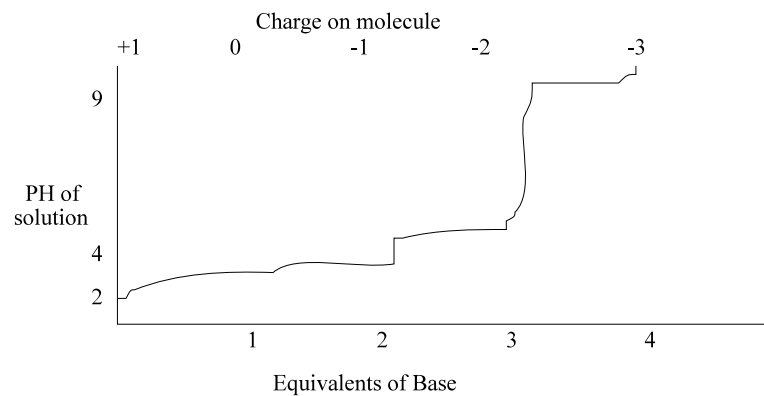
At pH 1 this will be fully protonated

NH<sub>3</sub><sup>+</sup> Ser Ala Asp(COOH) Thr Glu(COOH) Thr - COOH(terminal) Net +1

At pH 12 this will be fully deprotonated

NH<sub>2</sub> Ser Ala Asp(COO<sup>-</sup>) Thr Glu(COO<sup>-</sup>) Thr - COO<sup>-</sup>(terminal) Net -3

Titration curve



$$pI = (3 + 3.65) / 2 =$$

3.325

11. (10 points) William Astbury discovered that the x-ray pattern of wool shows a repeating structural unit spaced about 5.2Å along the length of the wool fiber. When he steamed and stretched the wool the x-ray pattern showed a new repeating structural unit of 7.0Å. Steaming and stretching the wool, then letting it shrink gave an x-ray pattern consistent with the original spacing of about 5.2Å.

1. Interpret Astbury's observations

2. When wool sweaters or socks are washed in hot water or heated in a dryer they shrink. Under the same conditions, silk does not shrink. Explain.

Wool with a 5.2Å repeat would be typical of an α-keratin structure which is primarily α-helical. When this is steamed and stretched, the peptides are extended into the β conformation with the 7.0Å repeat.

Similarly, when wool is processed to make yarn it is stretched into the β conformation. Heating the β-conformation wool yarn in sweaters and socks helps it to go back into its native α helical conformation and when this happens the wool shrinks.

Silk on the other hand, is made with amino acids that prefer the β conformation, and these amino acids cannot adopt the shorter α helical structure. Thus when silk is heated it remains in the extended β conformation and no shrinking can occur.

12. (12 points) We discussed several chromatographic methods for purifying proteins, and several electrophoresis methods to analyze proteins. Discuss in as much detail as possible EITHER three or more different chromatographic methods OR three or more different electrophoresis methods.

#### Chromatographic methods

*Ion Exchange Chromatography* - The column contains a matrix of 'plastic' beads. Included in the molecular structure of the beads are chemical groups with either a + charge (anion exchanger) or groups with a negative charge (cation exchanger). When a protein solution is passed through the column molecules with the opposite charge are attracted to the matrix and bound to the column while compounds of the same charge pass directly through the column. To remove the proteins that are retained by the column, you can either increase the ionic strength of the medium, and screen out the charge-charge interactions that bind materials to the column, or you can change the pH of the solution to change the charge state of either the protein or the exchange resin so the protein is no longer bound to the matrix and washed through the column.

*Gel Chromatography* - In this case the matrix is composed of 'beads' that contain holes of a set size. Protein molecules that are larger than these holes pass around the beads and elute quickly. Protein molecules that are smaller than the beads can enter into the beads and travel through the interior of the bead, so it takes them longer to pass through the column. The protein's elution time correlates with the log of the protein's molecular weight. Thus if a column is calibrated with a set of known molecular weight standards, the molecular weight of the target protein may be determined as well as a separation from proteins of different sizes.

*Affinity Chromatography* - In affinity chromatography a small molecule that is normally bound by the protein you are trying to purify is covalently linked to the molecular structure of the matrix. As the protein passes through the matrix it binds this small molecule and, in turn, becomes bound to the matrix. All the other proteins that don't bind this small molecule pass through the matrix and are not retained by the column. Your target protein can then be eluted from the column by washing the column with a large excess of the binding molecule. This technique gives the best 1-step purification for a protein, but is also the most difficult because it requires foreknowledge of the binding molecule, and some extra chemistry to link the binding molecule to the matrix.

#### Electrophoresis Methods

*Native protein electrophoresis* - In this technique your protein is placed on an agarose or polyacrylamide gel in its native conformation, and it is subjected to an electrical potential that moves it through the gel matrix. Because the shapes and charges of native proteins can vary widely, the speed at which the protein moves through the gel cannot be directly correlated with any one physical property of the protein. Thus this technique can give information on how pure a protein is, but it doesn't give any direct information of the physical state of the protein.

*SDS Gel Electrophoresis* - In this technique your protein is denatured in a solution of SDS (sodium dodecyl sulfate- a detergent) before it is placed on an agarose or polyacrylamide gel and subjected to an electrical potential that moves it through the gel matrix. The SDS denaturation unravels the protein into a random coil and coats it with a negative charge of uniform density. Because of this, the protein's mobility through the gel is inversely related to the log of the protein's molecular weight. Thus the position of the protein in gel at the end of the electrophoresis period can tell you the molecular weight of the protein. The shapes and charges of native proteins can vary widely, the speed at which the protein moves through the gel cannot be directly correlated with any one physical property of the protein. Thus this technique can give information on how pure a protein is, but it doesn't give any direct information of the physical state of the protein.

*Isoelectric focusing* - In this technique a pH gradient is built into the agarose or polyacrylamide gel matrix through which the protein will be electrophoresed. Since the protein's net charge depends on the pH of the solution around it, the protein travels through the gel until it finds the spot where the pH of the gel matches the protein's pI. At this point the protein has no net charge and is no longer mobile. By using a small pH electrode you can determine the pH of the gel where the protein is immobilized and thereby determine the pI of the protein.

*2D gel electrophoresis* - Here a protein mixture is first subjected to isoelectric focusing so all the

proteins are separated by their  $pI$ 's. This entire gel then is placed onto the side of a second gel that contains SDS, and the proteins are electrophoresed into the new gel based on their molecular weight. In the end you get protein separation in two dimensions, one of which tells the protein's isoelectric point, and the other dimension that tells the protein's molecular weight.

12. (Take home - 10 points) A protein fragment yields the following data

A. When reacted with FDNB and hydrolyzed the only 2,4-dinitrophenyl derivatized amino acid found is Serine.

B. When cleaved with Cyanogen bromide the following peptides are obtained:

SEPIRVLTGAAGIAYSLLYSIGNGSVFGKDQPIILVLLDITPM  
 ELQDCALPLLKDVIATDKEEIAFKDLDVAILVGSM  
 ERKDLLKA  
 GVLDGVL  
 PRRDGM  
 M

C. When cleaved with Trypsin the following peptides are obtained:

DQPIILVLLDITPMMGVLDGVLMEQDCALPLLK  
 VLVLTGAAGIAYSLLYSIGNGSVFGK  
 DLDVAILVGSMPR  
 DVIATDK  
 EEIAFK  
 DGMER  
 SEPIR  
 DLLK  
 R  
 K  
 A

What is the sequence of the protein fragment?

Part A says that the N terminus is a Serine. In B the cleavage point is on the COOH side of methionines. In C the cleavage point is on the COOH side of all R and K

Lining up the peptides we have the following sequence

SEPIRVLTGAAGIAYSLLYSIGNGSVFGKDQPIILVLLDITPMMGVLDGVLMEQDCALPLLKDVIATDKEE  
 IAFKLDVAILVGSMPRRDGMERKDLLKA