Name:

Chem 464
Biochemistry

Multiple choice (4 points apiece):

1. Which of the following statements about buffers is true?

A) A buffer composed of a weak acid of pKa = 5 is stronger at pH 4 than at pH 6.
B) At pH values lower than the pKa, the conjugate base concentration is higher than that of the acid.
C) The pH of a buffered solution remains constant no matter how much acid or base is added to the solution.
D) The strongest buffers are those composed of strong acids and strong bases.
E) When pH = pKa, the weak acid and conjugate base concentrations in a buffer are equal.

2. Three buffers are made by combining a 1 M solution of acetic acid with a 1 M solution of sodium acetate in the ratios shown below.

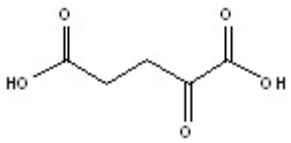
	1 M acetic acid	1 M sodium acetate
Buffer 1:	10 mL	90 mL
Buffer 2:	50 mL	50 mL
Buffer 3:	90 mL	10 mL

Which of these statements is true of the resulting buffers?

A) pH of buffer 1 < pH of buffer 2 < pH of buffer 3
B) pH of buffer 1 = pH of buffer 2 = pH of buffer 3
C) pH of buffer 1 > pH of buffer 2 > pH of buffer 3
D) The problem cannot be solved without knowing the value of pKa.
E) None of the above.

3. What is the approximate charge difference between glutamic acid and α-ketoglutarate at pH 9.5?

The structure of α-ketoglutarate is :



A) 0
B) ½
C) 1
D) 1½
E) 2

4. The formation of a peptide bond between two amino acids is an example of a(n) _____ reaction.

A) cleavage
B) condensation
C) group transfer
D) isomerization
E) oxidation reduction

5. By adding SDS (sodium dodecyl sulfate) during the electrophoresis of proteins, it is possible to:

A) determine a protein's isoelectric point.
B) determine an enzyme's specific activity.
C) determine the amino acid composition of the protein.
D) preserve a protein's native structure and biological activity.
E) separate proteins exclusively on the basis of molecular weight.

6. 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) invariably restricted to about 7 of the 20 standard amino acids.
D) often on different polypeptide strands.
E) usually near the polypeptide chain's amino terminus or carboxyl terminus.

7. Which of the following statements concerning the process of spontaneous folding of proteins is false?

A) It may be an essentially random process.
B) It may be defective in some human diseases.
C) It may involve a gradually decreasing range of conformational species.
D) It may involve initial formation of a highly compact state.
E) It may involve initial formation of local secondary structure.

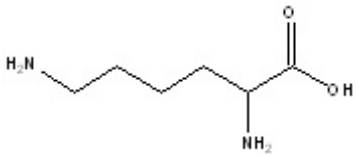
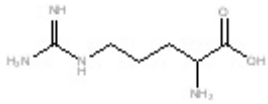
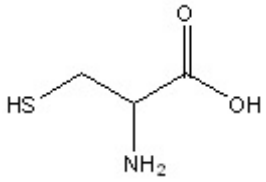
Longer questions (72 points total)

8. (10 points) Cola has a pH of about 3.0. If a Cola has a concentration of H_2CO_3 of .005M, what is the concentration of HCO_3^-, and what is the total concentration of all Carbonates if the pKa of H_2CO_3 is 3.77?

$pH=pKa + \log (A^-/HA)$
$3=3.77 + \log(X/.005)$
$-.77=\log(A^-/.005)$
$10^{-.77}=A^-/.005$
$.17=A^-/.005$
$A^- = HCO_3^- = .17(.005)=.00085$

Total Carbonate = .005 + .00085 = .00585M

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

Name	Lysine	Arginine	Cysteine
3 letter abbreviation	Lys	Arg	Cys
1 letter abbreviation	K	R	C
Structure			
side chain pK_a (if ionizable)	10.53	12.48	8.18
General classification (nonpolar, polar, etc)	Base, Positively Charged	Base, Positively Charged	Polar

10. A (6 points) There are two secondary structures that are common in both fibrous and globular proteins, describe these two structures.

α-helix - found in α-keratin, a right handed helix with 3.6 residues/per turn of the helix and a rise of 5.4 Å per turn. Hydrogen bonds are formed between the CO of a residue and the NH of the residue 4 amino acids further down the helix.

β-sheet - found in The silk fiberoin, strands of amino acids in their extended conforations but each strand is hydrogen bonded to the next strand to make sheets that are called pleated sheets because they point up and down like pleats. The side chains are located on the surface of these sheets where they can interact with other sheets.
B (4 points) Describe the one secondary structure that is found only in fibrous proteins. Why would it be difficult for this structure to occur in a globular protein?
The collagen triple helix is not found un globular proteins. In this structure the amino acid and in a fully extended conformation and then three strands of these extended amino acids are wound together in left handed triple helix. The collagen is composed of 35% glycine,11% alanine and 21% proline or hydroxypolinedue to the structural constraints of the structure. It is not found in globular proteins because is can only extend in one direction.

11. I have found a tripeptide with the sequence D-R-Z, where Z is a novel amino acid.

A. (2 points) If the charge is on the peptide is +2 at pH 1

Is Z positively charged, negatively charged or neutral AA (Circle one)

(I accepted either)

B. (2 points) The peptide has no absorbance at 280. What does this tell you about Z

Not aromatic

C. (3 points) If the peptide has a pI of 3.325, what is pKa of Z sidechain?

(3.65 + X)/2 = 3.325;X=3.00

D. (1 point) At pH 6 will peptide bind to a anion exchange column?

pH6>pI; the peptide will have negative charge and be an anion, so WILL bind

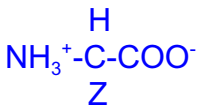
E. (2 points) Can the peptide be cleaved with any of the three cleavage methods I told you to memorize?

Yes, Submaxillary protease will cleave after the Arg.

F. (2 points) On a gel permeation column will peptide come off column before or after a 50 residue peptide?

On this column Large things come out first and smaller last, so this peptide comes out AFTER the 50 residue peptide.

G. (2 points) Z is a D- amino acid. Draw a sterochemically correct structure for this amino acid using 'Z' for the side chain



H. (1 bonus point) Propose a possible structure for Z.

With a pKa of 3 any acid functionality will do.

12. A (3 points) Describe the experiment that 'proved' that proteins do not need anything other then their own sequence to fold.

In the Anfensin experiment ribonuclease was denatured by treating with urea and β-mercaptoethanol to completely remove all structure. These two small molecules were then removed by dialysis, and the protein recovered all its activity and structure without and cellular 'machinery' present.

B.(6 points) We now know that the cell does provide some mechanisms to help proteins fold. Describe two of these mechanisms.

In the Hsp70 family of chaperons, Hsp70 protein locates regions of exposed protein and binds to them before the protein can aggregate with other misfolded proteins. The Hsp70 is then removed byHsp40 and NEF when the protein can refold under better conditions.

In the Chaperonin system a misfolded protein is placed in a massive chaperonin 'cage' where it is segregated from other proteins and given time to fold by itself.

13. (9 points) Describe three different ways to determine the sequence of a protein.

Edman Degredation - phenylisothiocynate is reacted with the protein and he N-terminal amino acid is removed. As each amino acid is removed, it is identified, and then the next amino acid is removed. This reaction does not have a 100% yield, so only peptide of up to 50 residues can be sequenced by this method.

MS/MS The protein is placed in an ESI spectrometer and a single molecular ion is passed on to a second mass spectrometer in the presence of a reaction gas. This reaction gas tend to make the molecular ion break at peptide bonds, and then the second mass specrometer determines the masses of all the fragments, and this information is used to recreate the proteins sequence. This method cannot differentiate between Leucine and Isoleucine.

DNA sequencing. Will be described in greater detail in later chapters.