Biochemistry I , CHEM 4400 Exam 2 10:00 Fall 2013 Dr. Stone Name_____

rate forward = $k_{forward}$	Carbonic acid Ka = 1.6 x 10	Hydrogen carbonate Ka = 4.68 * 10^{-11}
rate reverse = $k_{reverse}$	Acetic acid Ka = 1.78 *10	K_{eq} for the reaction of carbon dioxide with water = 1.69 x 10
K_{eq}	Phosphoric acid Ka = 7.25 *10^{-3}	
$\Delta G = \Delta H - T\Delta S$	Dihydrogen phosphate Ka = 1.38 * 10	kcat = $\frac{\quad}{h}$ k = 1.381 x 10 h = 6.626 x 10
$\Delta G = \Delta G°' + RT\ln Q$ Q = [products]/[reactants]	Monohydrogen phosphate Ka = 3.98 * 10	R = 8.315 J/mol*K

Multiple choice, 3 points each: Circle the correct answers on this test.

1. Why is SDS (sodium dodecyl sulfate) added to the acrylamide gel for the electrophoresis of proteins?
    a. To provide ampholytes to enhance the separation of charged proteins
    b. To denature the proteins
    c. To add a uniform amount of negative charges
    d. To add a uniform amount of positive charges
    e. To denature the polyacrylamide
    f. None of these
    g. More than one of these, **circle all** correct answers

2. In an aqueous solution, protein folding is driven to the most stable conformation by by two **major** factors. One is the formation of the maximum number of hydrogen bonds. The other is the:

    a. formation of the maximum number of hydrophilic interactions.
    b. maximization of ionic interactions
    c. maximization of entropy of water molecules
    d. placement of polar amino acid residues around the exterior of the protein.
    e. None of these

3. Which of the following is true about the Edman degradation system of sequencing polypeptides?

I) The Edman degradation system is carried out on a machine called an Edmanator.
II) The Edman degradation system will work on any size polypeptide.
III) In the Edman degradation system the amino-terminal residue is labeled with 1-fluro,2,4-dinitrobenzene and the polypeptide is hydrolyzed with 6M HCl to its constituent amino acids.
IV) In the Edman degradation system the amino-terminal residue is labeled with phenylisothiocyanate, cleaved with trifluroacetic acid, purified and identified in each successive cycle.

A) I and II B) II and IV C)I, II and III D) IV only E) none of these

4. Why is a peptide treated with dithiothreitol and iodoacetate prior to SDS PAGE?
    a. This breaks the crosslinks in collagen
    b. This oxidizes the disulfide bonds to form iodosulfoxides
    c. This oxidizes tryptophan so it can be measured by UV absorbance
    d. This oxidizes disulfide bonds and acetylates the resultant thiol groups
    e. None of these

5. What is isoelectric focusing used for?
    a. Determine the pI of a protein
    b. Determine the pKa of a protein
    c. Separate proteins based on their molar mass
    d. Separate proteins based on their hydrophobicity
    e. None of these
    f. More than one of these, circle **all correct** answers

6. What effect does carbonic anhydrase have on the equilibrium concentrations of carbon dioxide, water and carbonic acid?

I. Carbon dioxide increases
II. Carbon dioxide decreases
III. Carbonic acid increases
IV. Carbon dioxide does not change

A. I and III B. II and IV C. I, II and III D. IV only E. None of these

7. Which filamentous protein(s) has/have very little cysteine, and has/have crosslinks formed by hydroxylysine.

A. Keratin B. Keratin and Elastin C. Collagen and Keratin D. Collagen

8. Which of the following binding constants is for a protein that has the lowest affinity for its ligand?

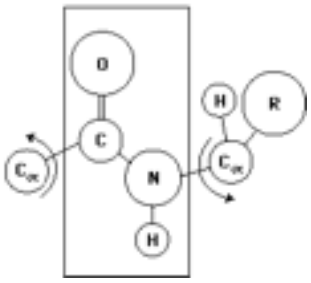
A.) $K_d = 1.0 \times 10^{8}$
B) $K_a = 1.0 \times 10^{-10}$
C) $K_a = 1.5 \times 10^{9}$
D) $K_d = 2.0 \times 10^{-8}$
E) None of these, they are all the same

Short answer section (3 points each) : Give specific details in brief answers.

9. What chemical is used to only determine the N-terminus amino acid?

10. What chemical is used to sequence all of the amino acids in a peptide?

11. In the diagram below, what does the plane drawn behind the peptide bond indicate?



12. Where are the amino acid R (side chains) groups in an alpha helix?

13. The three dimensional structure of a biochemical macromolecule is formed and maintained by noncovalent interactions. What are three types of intermolecular forces?

14. What two amino acids are commonly found in the middle of β turn?

Matching questions (5 points each): Put the number of the best match in each blank.

15. (5 points) Match the following protein terms:

A) Hsp-70_____
B) Disulfide interchange _____
C) Chaperonins _____
D) Peptide bond hydrolysis_____
E) Peptide bond isomerization_____

1. catalyzed by peptidyl prolyl isomerase
2. catalyzed by protein disulfide isomerase
3. prevents denaturation of proteins due to increased temperature
4. elaborate protein complexes that hydrolyze ATP in the process of folding proteins
5. catalyzed by chymotrypsin

16. Match the following techniques with how they are used to study proteins.

A) nuclear magnetic resonance ____
B) mass spectroscopy_____
C) X-ray crystallography_____
D) circular dichroism spectroscopy _____
E) SDS PAGE_____
F) Edmund degradation_____

1. determine exact mass of proteins and the sequence of peptides
2. determine protein structure using diffraction patterns and a computer program
3. measure amounts of protein secondary structures
4. determine structure of a protein in solution
5 determine molar mass
6. determine the sequence of peptides

This section requires longer answers, be specific and give details.

1. (10 points) The equilibrium constant K for the binding of oxygen to myoglobin is $3 \times 10^{-6}\ M^{-1}$. The rate constant for the combination of O_2 with myoglobin in $9 \times 10^{6}\ M^{-1}s^{-1}$.

a. What is the rate constant for the dissociation of O_2 from oxymyoglobin?

b. What is the mean duration of the oxymyoglobin complex? (What is the average length of time that the oxygen stays bound to myoglobin?)

2. (10 points) The following peptide was determined to have a cysteine as the N-terminus amino acid. It was then cleaved with an enzyme and the resulting peptides were separated by anion exchange chromatography. For all of the peptides, the N terminus amino group pKa is 8 and the C terminus carboxyl pKa is 3.4.

The fragments are:
ANWINET
DYNALSK
CAR

a. What is the order of elution from the anion exchange column at pH 5.5:
1st.

2nd

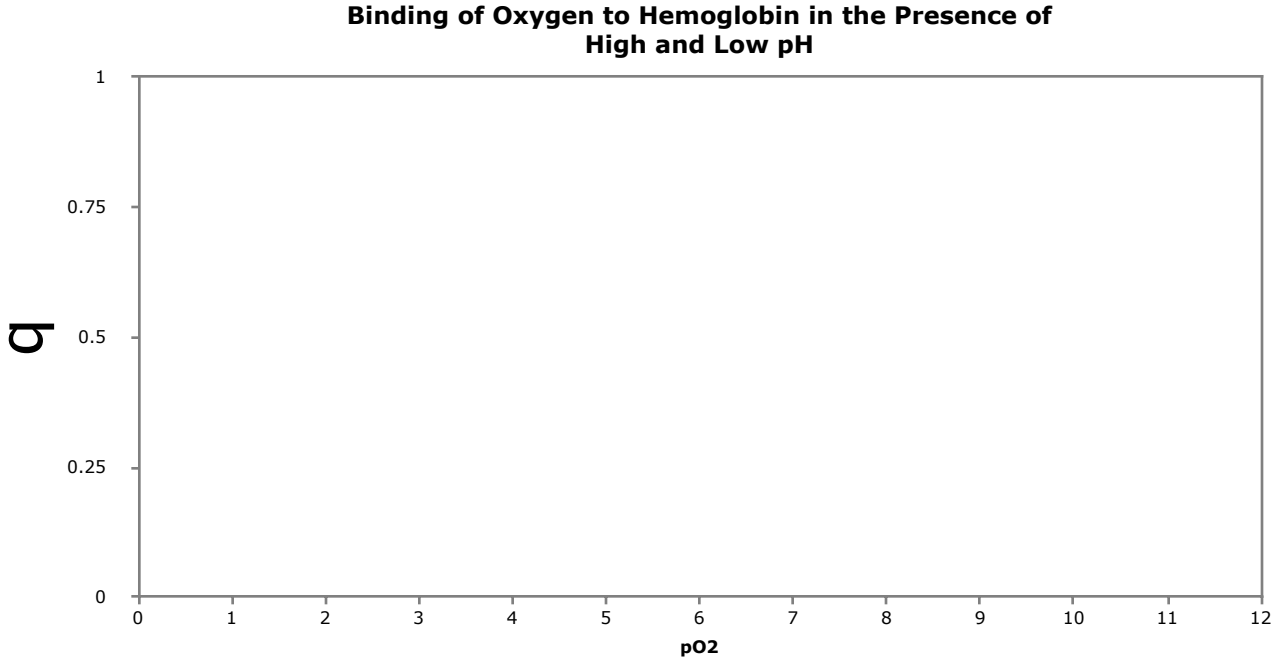
3rd.

b. What enzyme was used to cleave the peptide?

c. Can you determine the sequence of the original peptide? What is it? (Or, what are the possibilities?)

3. (5 points) a. Draw the graph of θ vs pO_2, showing the binding of oxygen at pH 7.6 and at pH 7.2 (one line for each condition).

b. How does pH change hemoglobin and thus alter its affinity for oxygen? (Be specific, more details = more points.)



4. (8 points) In hemoglobin, the pKa value for histidine (His HC-3) changes depending on the conformation of hemoglobin. When hemoglobin is in the T form, the pKa for this histidine is 8.1, in the R form, the pKa is 6.0. In the T form, this histidine forms a salt bridge. In the R form, it does not.

a. Draw the complete structure of histidine showing the equilibrium between the protonated and deprotonated forms.

b. Explain why this histidine has two different pKa values.

5. (15 points) Carbon dioxide is produced in the tissues during metabolism.

I. Fully describe the intermolecular forces that exist

a. between carbon dioxide and water.

b between bicarbonate ions and water.

II. Which is more soluble in water: Carbon dioxide or bicarbonate ions? Why?

III. Fully describe how carbon dioxide is transported from the tissues to the lungs.

IV. "Houston, we have a problem": Lack of fresh air (like being stuck on Apollo 13, or not breathing due to an overdose of narcotics) will cause carbon dioxide levels to rise. Why is this dangerous?