

September 17, 1999

Name \_\_\_\_\_

ID number \_\_\_\_\_

## CH461/561 Exam 1

The multiple choice questions are worth 1 point each; there are 16 of them. The problems are worth 15 points each. There are 5 of them, and you must answer 4 for full credit. The amount of points for each section is indicated to the left by a circled number.

The maximum amount of points is thus 76.

**Extra credit:** If you answer all 5 problems, one of them will be used for extra credit. You will get half credit for that problem. If you wish, you may indicate which problem you want for extra credit. If you do not indicate it, I will use the problem in which you scored the least points.

Multiple choice: \_\_\_\_\_

Problems: \_\_\_\_\_

Extra credit: \_\_\_\_\_

Total: \_\_\_\_\_

Note: No notes or books of any sort may be used during the exam.

Given:  $R = 1.987 \text{ cal/K mol}$

The table of pKa's for amino acids (following page)  
The periodic table (in the room)

I have neither given nor received aid on this exam.

\_\_\_\_\_  
(signature)

1. The reaction between glucose and inorganic phosphate to form glucose-6-phosphate has a  $G^\circ$  of +3.3 kcal/mole at 298 K. Which of the following statements is TRUE?
  - A. This reaction will occur spontaneously only if the glucose-6-phosphate concentration is much greater than the glucose concentration.
  - B. This reaction will occur spontaneously only if the inorganic phosphate concentration is very low.
  - C. This reaction is at equilibrium.
  - D. This reaction will not occur spontaneously under standard conditions.
  
2. At pH 7 under physiological conditions,  $G^\circ'$  is approximately -7.5 kcal/mole for the hydrolysis of ATP. This strongly favorable free energy results from
  - A. a very favorable entropy change that overcomes an unfavorable enthalpy change.
  - B. a very favorable enthalpy change that overcomes an unfavorable entropy change.
  - C. a combination of favorable entropy and enthalpy changes.
  - D. a favorable entropy change that overcomes the overall unfavorable effect on the free energy of pH 7 compared to pH 0.
  
3. Most of the change in energy in chemical reactions is due to a change in
 

A. vibrational energy.	B. translational energy.
C. electronic energy.	D. rotational energy.
  
4. As the temperature increases,
  - A.  $T S$  becomes more positive, but  $G$  remains the same.
  - B.  $T S$  becomes more negative, but  $G$  remains the same.
  - C.  $G$  becomes more positive.
  - D.  $G$  becomes more negative.
  
5. At what pH's will the average charge on the phosphate species be: -0.5, -1.0, -1.5? Use pKa values of 2.12, 7.21, and 12.7 for phosphoric acid.
  - A. 2.12, 7.21, 12.7
  - B. 2.12, 4.67, 7.21
  - C. 4.67, 9.96, 12.7
  - D. 7.21, 9.96, 12.7
  - E. none of the above

6. Which is true for an X-H:Y hydrogen bond in biological systems?
- A. X and Y typically are any combination of nitrogen and/or oxygen atoms
  - B. It is the strongest when the axis of the orbital containing the lone pair and the axis of the X-H sigma bond meet at an angle less than  $90^\circ$
  - C. It is the strongest when the axis of the orbital containing the lone pair and the axis of the X-H sigma bond are co-linear
  - D. a and b
  - E. a and c
7. Why are histidyl residues frequently utilized when mechanisms are proposed for enzymatically catalyzed reactions?
- A. Because of the partial aromatic character of its side chain.
  - B. Because of the cyclic nature of its side chain.
  - C. Because of the heterocyclic nature of its side chain.
  - D. Because it can function as a proton donor or acceptor near physiological pH.
  - E. Because of the planar nature of its side chain.
8. Proline is unique among the amino acids because
- A. it is the only amino acid whose alpha carbon is not chiral.
  - B. it exists naturally in two diastereomeric forms.
  - C. its alpha amino group is a primary amine.
  - D. its alpha amino group is a secondary amine.
  - E. its alpha amino group is a tertiary amine.
9. Which structure is unique to collagen?
- A. the alpha helix
  - B. the double helix
  - C. the triple helix
  - D. the beta structure
  - E. the beta barrel
10. Which of the following statements is true?
- A. all fibrous proteins have quaternary structure
  - B. all globular proteins have quaternary structure
  - C. all proteins have quaternary structure
  - D. the interactions between the eight helical regions is partially responsible for the quaternary structure of myoglobin
  - E. only proteins with more than one subunit have quaternary structure
11. The alpha helix and the beta sheet have much in common. Which of the following do the alpha helix and beta sheet not have in common?
- A. both are regular structures
  - B. both are secondary structures
  - C. both rarely contain proline
  - D. both are compact structures
  - E. both have hydrogen bonding

12. In a site directed mutagenesis experiment a particular Leu residue in a protein was changed to various other residues. Which of the following changes would have the greatest impact on the structure of the protein.
- A. Val            B. Asp            C. Ala            D. Ile            E. Phe
13. The number of hydrogen bonds per amino acid is:
- A. greater in parallel beta sheet than antiparallel beta sheet  
B. greater in antiparallel beta sheet than parallel beta sheet  
C. the same number in parallel and antiparallel  
D. hydrogen bonds are not a factor in parallel and antiparallel structures
14. Which of the following is not true about the common  $\alpha$ -helix?
- A. the structure is right handed  
B. the distance along the helix axis is 5.4 Å per turn  
C. there are an integer number of amino acids per turn  
D. the amino acid R-groups are on the outside of the helix  
E. it is stabilized by hydrogen bonding
15. Enzymes regulate the reactions that occur within living cells by
- A. providing the energy for these reactions to occur.  
B. accelerating the rate of the particular cell reactions that they catalyze.  
C. making cell reactions more thermodynamically favorable.  
D. providing the raw materials for cell reactions.
16. Identify the relationship between cysteine and cystine.
- A. carboxylic acid and amide  
B. amide and carboxylic acid  
C. thiol and disulfide  
D. disulfide and thiol  
E. both are aromatic

Answer Sheet for Test "Exam 1-Mult choice", 9/16/99

No. in No. on

Q-Bank   Test   Correct Answer

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2	8	1	D
2	10	2	C
2	11	3	C
2	22	4	D
3	13	5	B
3	22	6	E
4	9	7	D
4	10	8	D
5	3	9	C
5	5	10	E
5	10	11	D
5	13	12	B
5	19	13	C
5	20	14	C
1	12	15	B
4	1	16	C

## Problems

- 1) Your assignment is to make the following buffer:  
50 mM potassium phosphate (pH 7.0)

You have at your disposal the following items:

solid  $\text{KH}_2\text{PO}_4$   
pure phosphoric acid ( $\text{H}_3\text{PO}_4$ )  
1M HCl  
1M KOH  
distilled water  
a weigh balance  
a pH meter  
spatulas, weighing paper, pipettes, beakers, graduated  
cylinders, *etc.*

Use these as the pKa's for phosphoric acid: 2.1, 7.2, 12.7

Describe exactly how you will make the buffer.

2) The following reactions occur during glycolysis to convert glycerate 3-phosphate to pyruvate, generating 1 ATP in the process. Each step is catalyzed by a specific enzyme. For each reaction, I have indicated the standard free energy changes, as well as the estimated free energy changes occurring at the physiological concentrations of the reactants and products in a human erythrocyte.

	$\Delta G^{\circ}$ (kcal/mol)	$\Delta G'$ (kcal/mol)
glycerate-3-phosphate		
↓	+1.1	+0.19
glycerate-2-phosphate		
↓	+0.4	-0.79
+ H <sub>2</sub> O		
phosphoenolpyruvate		
ADP + H <sup>+</sup>		
↓ + ↓	-7.5	-4.0
ATP		
pyruvate		

- a) Briefly, why are  $\Delta G^{\circ}$  and  $\Delta G'$  different in each case?
  
- b) Does the overall conversion of glycerate-3-phosphate to pyruvate occur spontaneously in the erythrocyte?
  
- c) If the steady-state concentration of glycerate-2-phosphate in the erythrocyte is 10 mM, then what is the concentration of glycerate-3-phosphate?

d) What is  $K_{eq}$  of the second reaction?

e) Given that the standard free energy of hydrolysis of ATP under physiological conditions is  $-7.5$  kcal/mol, calculate the standard free energy of hydrolysis of phosphoenolpyruvate.

3) Consider the following oligopeptide (written in the 3-letter code):

## Val-Lys-Pro-Cys-Tyr

a) Draw its full chemical structure, as it would exist at pH = 7. Indicate all bonds (those to hydrogen are optional; *i.e.* you could write methylene as CH<sub>2</sub> rather than H-C-H). You do not need to indicate stereochemistry.

b) The pK<sub>a</sub>'s of the amino and carboxyl termini are 9.1 and 3.4, respectively. What is the pI of the peptide? Over what pH range will this peptide be neutral? (For the purposes of this question, the range where ≥50% of the peptide is neutral.)

c) You react the peptide with dansyl chloride, hydrolyze it in 6M HCl, and recover two different fluorescent products. Draw their structures:

4) You are working for the new start-up biotechnology company BioBucks™, and the biochemists have just identified a new polypeptide hormone, adipocyte growth factor (AGF). Considering the obvious marketing potential, the company is eager to get the gene for this protein. But to do that, they need your protein chemistry team to determine the sequence of AGF. The team starts by treating the polypeptide with performic acid, after which it still behaves as a single polypeptide. This polypeptide is purified and then treated with chymotrypsin. The products are separated and purified, and each member of the team is given a chymotryptic fragment to analyze. You are given the peptide named CT3 to work with.

You take CT3, treat it with trypsin, and obtain 3 products (named CT3-T1, CT3-T2, and CT3-T3).

You take CT3, treat it with cyanogen bromide (CNBr), and obtain 2 products (named CT3-CN1 and CT3-CN2).

You treat all of these peptides with 6M HCl (100°C, 24 hours), perform amino acid analysis after *o*-phthaldehyde derivatization, and obtain the results tabulated below ("Amino acid composition").

Peptide	Amino acid composition	UV?	form at pH=7
CT3	2 x E <sup>1</sup> , G, K, M, R, T, cysteic acid, ? <sup>2</sup>	yes	(not done)
CT3-T1	M, R, T, cysteic acid	no	neutral
CT3-T2	E, G, K	no	neutral
CT3-T3	E, ? <sup>2</sup>	yes	neutral
CT3-CN1	E, R, T, cysteic acid, ? <sup>2</sup>	yes	(not done)
CT3-CN2	E, G, K, homoserine	no	(not done)

<sup>1</sup> 2 moles of glutamate for each mole of the other amino acids

<sup>2</sup>? = not one of the 20 natural amino acids (degradation product?)

After finding that the CT3 peptide has a significant UV absorbance at 260 - 290 nm, you put each of the peptides in a UV-visible spectrophotometer to find which peptides share this characteristic. (Results noted above.)

During your separation of the tryptic fragments by isoelectric focussing, you find that all three peptides are neutral at pH 7.

Treatment of the CT3-CN2 with FDNB (fluorodinitrobenzene) followed by acid hydrolysis gives DNP-Gly and DNP-Lys. Similar treatment of CT3-CN1 yields DNP-Thr.

One of your colleagues has determined that her peptide has the C-terminus of the protein, so you know that the C-terminus of CT3 is not the C-terminus of AGF.

a) Using the information given above, determine the sequence of the peptide. (You may give it in the 1-letter code or 3-letter code.)

b) In the latter stages of protein sequencing, someone cleaves AGF with chymotrypsin without prior performic acid treatment. While many of the chymotryptic fragments isolated after this procedure are identical to the ones already purified and sequenced by the various members of your group (including you, hopefully), a new peptide is also isolated (CT10). After treatment with performic acid, CT10 is converted to 2 peptides, CT3 and CT5. CT5 was determined by another member of your team to have the following sequence:



How do you explain the existence of CT10 and what does this say about the structure of the native AGF protein?

c) In the real world, you would not have done so much work to determine the sequence of CT3. Using one particular reaction, the entire sequence could be determined, in principle. Name the reaction and the reagent used.

5) You isolate a new protein and determine its sequence. When you run the sequence through your secondary structure prediction computer program, it predicts that the protein has 8  $\alpha$  helices and 8  $\beta$  strands, in an alternating pattern:

N— $\beta$ — $\alpha$ — $\beta$ — $\alpha$ — $\beta$ — $\alpha$ — $\beta$ — $\alpha$ — $\beta$ — $\alpha$ — $\beta$ — $\alpha$ — $\beta$ — $\alpha$ — $\beta$ — $\alpha$ —C

("N" and "C" refer to N- and C-termini and "--" refers to possible loop regions)

You note that the predicted  $\beta$  segments have a high density of hydrophobic amino acids, while the predicted  $\alpha$  segments have both hydrophobic and hydrophilic amino acids.

a) Given this information and what you know about protein structure, what is the most likely type of structure attained by this protein?

b) Based on the sequence, the protein should have a molecular weight of 34 kDa, but the purified protein behaves as if it has a molecular weight of 68 kDa. How do you explain this?

c) Label the following 2° structural elements with the direction of the polypeptide chain (N  $\rightarrow$  C) and the hydrogen bonds that stabilize them. Which of these 3 would contribute to the structure of your new protein?

**(sorry — no electronic files of these)**