Biochemistry 461
February 16, 1995
Exam #1
Prof. Jason Kahn

Your Printed Name:_____

Your SS#:_____

Your Signature:_____

You have 75 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Some information which may be useful is provided on the bottom half of the next page.

Explanations should be brief (one or two sentences).

You will need a calculator for this exam. If you do not have one, full credit will be given for answers which are entirely numerical expressions.

No other study aids or materials are permitted.

Do not write anything on the top half of the next page.

Score: Question 1: _____ out of 16

Question 2: _____ out of 18

Question 3: _____ out of 15

Question 4: _____ out of 15

Question 5: _____ out of 18

Question 6: _____ out of 18

Total: _____ out of 100

Do Not Write Above This Line

Possibly Useful Information:

$\Delta G = -nF\Delta E$, where F = 96500 J/(V•mole), $n$ = number of electrons transferred

R = 8.31 J/(mole•K)

T = 298 K today

$K_a = [H^+][A^-]/[HA]$ for the dissociation of HA

$K_w = 10^{-14}$

1. (16 points) Triethylamine, $(CH_3CH_2)_3N$, or TEA, is commonly used in chromatography buffers. The $pK_a$ corresponding to the reaction below is 11.0.

$$(CH_3CH_2)_3NH^+ \rightleftharpoons (CH_3CH_2)_3N + H^+$$

(a; 7 pts) Using the information above and the equation for water self-dissociation ($K_w$), calculate the pH of a 1M solution of TEA. Specify the predominant ionic form of TEA in this solution.

Predominant ionic form of triethylamine: _____

(b; 3 pts) Derive the Henderson-Hasselbach relationship from the definition of the acid dissociation constant for an acid HA. (This part of the problem doesn't concern TEA specifically).

(c; 4 pts) Use the Henderson-Hasselbach relationship to calculate the pH of the above 1M TEA solution after the addition of 0.8 moles of the strong acid HCl. The volume of the solution remains constant at 1 liter.

(d; 2 pts) Why is triethylammonium bicarbonate ($TEAH^+/HCO_3^-$) a useful buffer for chromatography? Which component provides buffering capacity near pH 7?

2. (18 points) Bioenergetics. You have discovered a bacterium whose metabolism uses the reduction of pyruvate$^-$ by $H_2$ as an energy source. The bug couples this reaction to the phosphorylation of creatine to give creatine-phosphate. Relevant standard reduction potentials and free energies of phosphate hydrolysis are given in the list below, and see the information on page 2. Assume we are at pH 7, the biochemical standard state.

| | |
|---|---|
| pyruvate$^-$ + 2 $H^+$ + 2 $e^-$ $\rightleftharpoons$ lactate$^-$ | $E°' = -0.185$ V |
| $H^+ + e^- \rightleftharpoons {}^1/_2\ H_2$ | $E°' = -0.421$ V |
| Creatine-phosphate $\rightleftharpoons$ Creatine + $P_i$ | $\Delta G°' = -43.1$ kJ/mole |

(a; 6 pts) Write the balanced reaction for the redox reaction between pyruvate$^-$ and $H_2$ and calculate $\Delta E°'$, $\Delta G°'$, and $K_{eq}$ for the reaction.

(b; 1 pt) What is $\Delta G°'$ for the phosphorylation of creatine?

(c; 3 pts) Assume that the bug uses the reduction of one molecule of pyruvate$^-$ to drive phosphorylation of one molecule of creatine. Write the overall equation for the coupled reactions and calculate $\Delta G°'$.

(d; 4 pts) In the cell, the reactants and products are not at their standard state concentrations. Write the equation giving the observed $\Delta G$ for the reaction in (c) as a function of $\Delta G°$ and the concentrations of pyruvate$^-$, lactate$^-$, $H_2$ (in atm), creatine, creatine-phosphate, and $P_i$.

(e; 4 pts) Given the cellular concentrations listed below, calculate the maximum possible concentration of creatine-phosphate for which the coupled redox and phosphorylation reactions will still proceed spontaneously.

[creatine] = 2 mM  [$P_i$] = 3 mM  [pyruvate$^-$] = [lactate$^-$] = 1 mM  [$H_2$] = 1 atm

3. (15 points) Amino acids and peptides. Please read the whole question (a-d) first and put your answer in one diagram.

   (a; 10 pts) Write the covalent structure of the tetrapeptide Tyr-Arg-His-Ala, with all functional groups in their predominant ionic forms at pH 7. Specify the stereochemistry at $C_\alpha$ for the N-terminal amino acid using  and  bonds.

   (b; 2 pts) Write the 1-letter code and the full name of each of the four amino acids below the structure.

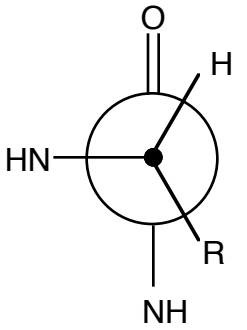
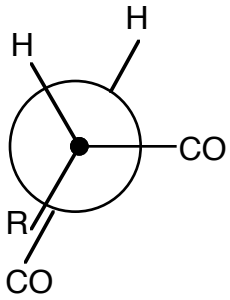
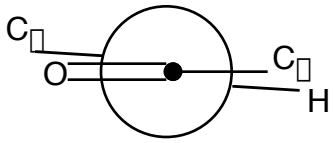
   (c; 1 pt) Indicate with a P the side chain functional group which is sometimes found to be phosphorylated in proteins.

   (d; 2 pts) Indicate the bonds which are cleaved by trypsin (T) and chymotrypsin (C).

4. (15 points) Amino acid conformation and the Ramachandran plot.

(a; 6 pts) Newman projections for the three bonds along the polypeptide backbone for an amino acid are given below. Circle the one appropriate choice corresponding to each diagram on the list below the diagram. It may help you to draw a dipeptide first.

(b; 3 pts) In the spaces given, specify which atom is the front and which is the rear in each projection, selecting from among the choices ($C_\alpha$, amide N, carbonyl C) for each answer.

| | | |
|---|---|---|
|  |  |  |
| Circle one: $\phi$ = 60°<br>$\phi$ = -90°<br>$\phi$ = -30°<br>$\phi$ = 180°<br>*trans*-peptide bond | Circle one: $\phi$ = 120°<br>$\phi$ = 30°<br>$\phi$ = -60°<br>$\phi$ = 180°<br>*cis*-peptide bond | Circle one: $\phi$ = 180°<br>$\phi$ = 30°<br>$\phi$ = 120°<br>$\phi$ = -90°<br>*trans*-peptide bond |
| Front atom: _____ | Front atom: _____ | Front atom: _____ |
| Back atom: _____ | Back atom: _____ | Back atom: _____ |

(c; 4 pts) Give a brief explanation for why the conformation shown in the left-hand diagram above is not in an allowed region of the Ramachandran diagram.

(d; 2 pts) The middle diagram above represents an allowed conformation for glycine, but not for other amino acids. Why?

5. (18 points) You need to determine the sequence of a linear, unbranched decapeptide (10 amino acids). The following experiments have been done:

   1. Amino acid analysis gives one each of the following 10 amino acids: Trp, Glx, Met, Arg, Lys, Cys, Phe, Pro, Tyr, Ala.
   2. Cyanogen bromide (CNBr) cleavage gives two pentapeptides (5 aa's).
   3. Trypsin cleavage gives two tripeptides (AMR and QFK), and a tetrapeptide.
   4. Chymotrypsin cleavage gives a dipeptide QF, a single amino acid, and a heptapeptide (7).
   5. The C-terminal residue is identified as Cys using carboxypeptidase C, which removes amino acids non-specifically, one by one, from the C-terminal end of a polypeptide.

   (a; 12 pts) Give the two possible sequences for the decapeptide, using the three-letter code, remembering the effect of proline on cleavage by the proteolytic enzymes.

   1. N-terminus–____–____–____–____–____–____–____–____–____–____–___–C-terminus

   2. N-terminus–____–____–____–____–____–____–____–____–____–____–___–C-terminus

   (b; 6 pts) You are given the products of the trypsin digestion (#3 above). Briefly describe two ways to resolve the final sequence ambiguity using this material.

6. (18 pts) Proteins are usually folded at low temperature ($T < T_m$, the so-called "melting temperature") and unfolded at high temperature ($T > T_m$). During folding, hydrophobic amino acid side chains are removed from solvent and buried inside the protein. The questions below refer to the folding reaction written as follows:

$$\text{Unfolded Protein} \rightleftharpoons \text{Folded Protein}$$

(a; 1 pt) What is the sign of $\Delta G$ for this reaction at $T < T_m$? Write "pos" or "neg":_____
(b; 1 pt) What is the sign of $\Delta G$ for this reaction at $T > T_m$? Write "pos" or "neg":_____
(c; 4 pts) Write the equation relating $\Delta G$, $\Delta H$, and $\Delta S$, define each (e.g. $\Delta E$ = change in energy), and describe briefly (< 10 words) what each of these state functions typically represents at the molecular level.

(d; 6 pts) Assuming that $\Delta H$ and $\Delta S$ are constant over the temperature range of interest, what does the fact that proteins unfold at high temperature tell us about the signs of $\Delta H$ and $\Delta S$?

Give a brief explanation of your reasoning:

Sign of $\Delta H$ (pos/neg/can't tell): _____

Sign of $\Delta S$ (pos/neg/can't tell): _____

One more page...

(e; 6 pts) The entropy change for folding of the polypeptide can be split into contributions from the entropy of folding for the polypeptide itself ($\Delta S_{protein}$) and the entropy for the accompanying change in water structure ($\Delta S_{water}$).

What is the sign of $\Delta S_{protein}$ (pos/neg): _____. Give a one-sentence explanation for your answer.

What is the sign of $\Delta S_{water}$ (pos/neg): _____. Give a one-sentence explanation for your answer.