Biochemistry 461, Section I Your Printed Name:_____

May 21, 1997

Final Exam Your SS#:_____

Prof. Jason D. Kahn

Your Signature:_____

You have 120 minutes for this exam.

The exam has 6 questions, worth 200 points. Do all 6 questions.

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

Explanations should be concise and answer the specific question asked.

You will need a calculator for this exam. No other study aids or materials are permitted.

There will be a viewing at a time and place to be announced on the class web page. Final grades will be available only through MARS.

Possibly Useful Information:

Michaelis-Menten equation: $v_0 = V_{max}[S]/(K_m + [S])$, where $V_{max} = k_2[E]_t$

Type of inhibition	Apparent K_m	Apparent V_{max}	Apparent V_{max}/K_m
Competitive	αK_m	V_{max}	$(1/\alpha)\ V_{max}/K_m$
Uncompetitive	$(1/\alpha')K_m$	$(1/\alpha')V_{max}$	V_{max}/K_m
Mixed	$(\alpha/\alpha')K_m$	$(1/\alpha')V_{max}$	$(1/\alpha)\ V_{max}/K_m$
Noncompetitive ($\alpha=\alpha'$)	K_m	$(1/\alpha)\ V_{max}$	$(1/\alpha)\ V_{max}/K_m$

$\alpha = 1 + [I]/K_I$ $\alpha' = 1 + [I]/K_{I'}$

Henderson-Hasselbach equation: $pH = pK_a + \log([A^-]/[HA])$

$\Delta G = \Delta H - T\Delta S = \Delta G^{\circ\prime} + RT\ln Q$, where Q has the form of an equilibrium constant

Nernst equation: $\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$, F = 96500 Coulomb/mole electrons

For transport of A from out to in, $\Delta G = RT\ln([A]_{in}/[A]_{out}) + Z_A F\Delta\Psi$

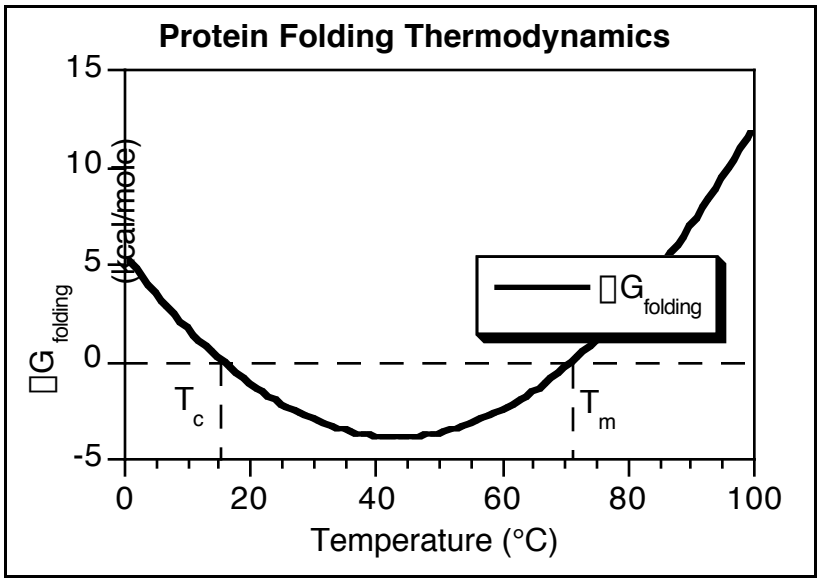
RT = 2500 J/mole today

1. (40 pts) Protein Structure and Folding.

(a; 8 pts) What is the hydrogen bond pattern of an α helix (specify functional groups on the *n* and *n* + something residues)? Describe two differences between α helices and β sheets which rationalize why it easier to make small peptides (~20 aa) which fold into α helices than it is to make peptides which fold into small β sheets and why α helices are more common folding nuclei than β sheets.

(b; 7 pts) Draw the Ala-Pro dipeptide with a *cis* peptide bond. Why is proline the only amino acid for which the *cis* form is energetically accessible? Why is spontaneous *cis* $\rightleftharpoons$ *trans* interconversion of the peptide bond slow?

(c; 25 pts) Proteins can always be denatured by heating (though for some proteins this may require temperatures > 100 °C). Some proteins also denature at low temperatures ("cold denaturation"). We want to understand the thermodynamics of these processes. One simple model uses the temperature-dependence of the hydrophobic effect: As the temperature increases, the hydrophobic effect becomes weaker, as clathrates become less enthalpically stable and less ordered. (We will assume that London forces, salt bridges, and hydrogen bonds are temperature-independent, contributing a favorable $\Delta H < 0$ and $\Delta S = 0$, while configurational ordering of the peptide chain has a temperature-independent $\Delta H = 0$ and unfavorable $\Delta S < 0$.) The graph sketches the temperature dependence of $\Delta G_{folding}$, for the reaction below:



1. (2) At the transition temperatures T_c (cold) and T_m (melting), where [U] = [N], what is ΔG for folding?

2. (3) At low temperature, $\Delta G_{folding}$ increases (goes from negative to positive) as temperature decreases through T_c. Deduce the signs of $\Delta H_{folding}$ and $\Delta S_{folding}$ at low temperature. Is folding enthalpy-driven or entropy-driven?

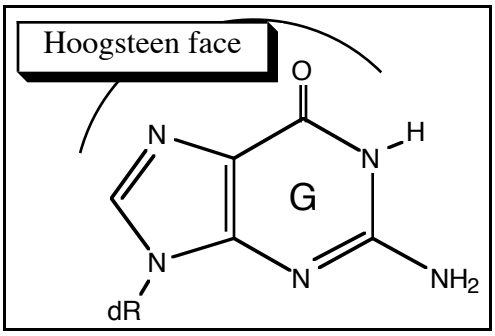
3. (6) Explain the physical origin of the signs of ΔH and ΔS from part 2.

4. (3) Around T_m, $\Delta G_{folding}$ increases as temperature increases. Deduce the signs of $\Delta H_{folding}$ and $\Delta S_{folding}$ at high temperature. Is folding enthalpy-driven or entropy-driven?

5. (6) Explain the apparently contradictory results of parts 2 and 4 using the temperature-dependent thermodynamics of the hydrophobic effect.

6. (5) Explain why proteins cold-denature in terms of the hydrophobic effect.

2. (35 points) Nucleic Acids



(a; 10 pts) Draw a possible base pair between guanosine and adenosine, with at least two hygrogen bonds. The structure of guanosine is given at the right. What makes the four Watson-Crick base pairs special?

(b; 8 pts) Seeman and Rich proposed that arginine should specifically recognize guanine and that asparagine should recognize adenine in protein-DNA complexes. Draw a reasonable recognition interaction between arginine and the Hoogsteen face of guanine (the major groove edge).

(c; 8 pts) Why is the the major groove more "informative" than the minor groove? Why is it difficult for a protein to specifically recognize the major groove of A-form helical double-stranded RNA?

(e; 9 pts) Give a chemical rationale (with a structure) for the evolutionary advantage of the DNA sugar-phosphate backbone as opposed to the RNA backbone for the genetic material.

3. (30 points) Bioenergetics, Transport

(a; 20 pts) The Na^+/K^+ ATPase pumps 3 Na^+ (sodium ions) out of the cell and 2 K^+ (potassium) in for each ATP hydrolyzed according to the equilibrium below:

$$3\ Na^+_{in} + 2\ K^+_{out} + ATP + H_2O \rightleftharpoons 3\ Na^+_{out} + 2\ K^+_{in} + ADP + P_i$$

Typical conditions are: $[Na^+]_{in}$ = 10 mM, $[Na^+]_{out}$ = 150 mM, $[K^+]_{in}$ = 120 mM, $[K^+]_{out}$ = 5 mM, and $\Delta\Psi$ = -82 mV (inside negative, drives cations in).

1. (5) What is ΔG for transporting one Na^+ ion from inside to outside?

2. (5) What is ΔG for transporting a K^+ ion from out to in?

3. (5) What is the total ΔG for the transport performed by the ATPase? This value is somewhat larger than the $-\Delta G^\circ$ = 30.5 kJ/mole available from ATP hydrolysis. How is this possible? (Hint: I haven't told you the concentrations of ATP, ADP, and P_i.)

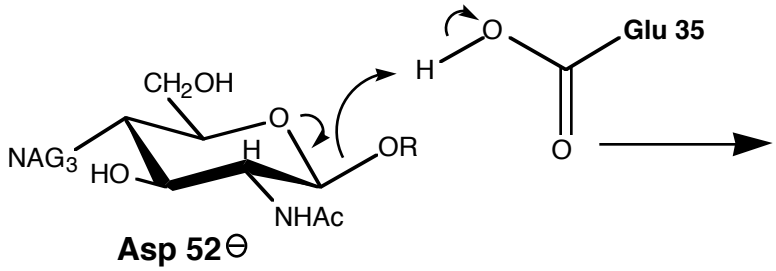
4. (5) What effect would opening a potassium channel have under these conditions? How about a sodium channel?

(b; 10 pts) The ΔG° for ATP hydrolysis is -30.5 kJ/mole and for glucose-6-phosphate hydrolyis is -13.8 kJ/mole. Calculate ΔG° and the equilibrium constant for the reaction below. What is one likely physiological function for this phosphorylation?

$$\text{Glucose} + \text{ATP} \rightleftharpoons \text{Glucose-6-phosphate} + \text{ADP}$$

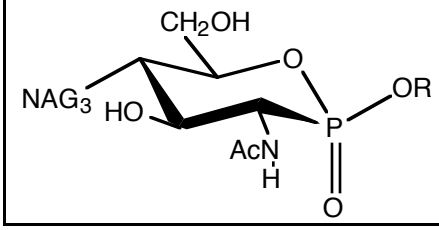
4. (35 pts) Enzymology

(a; 8 pts) The first step in the lysozyme mechanism is shown below. Draw the oxonium ion which results. What is the role of Asp 52 in the reaction?

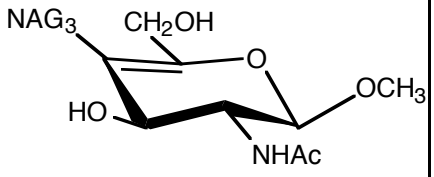


(b; 6 pts) Based on your answer to (a), which one of the following compounds could be a transition state analogue (and therefore a good inhibitor) of the enzyme? Explain your reasoning.

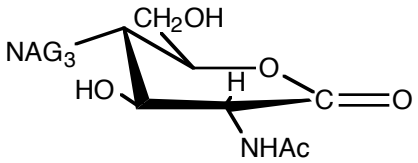
A.



B.

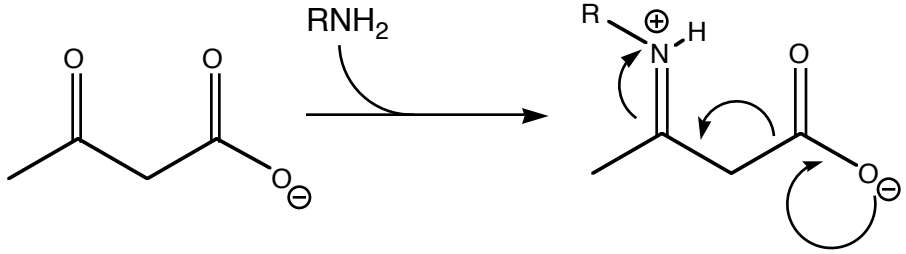


C.

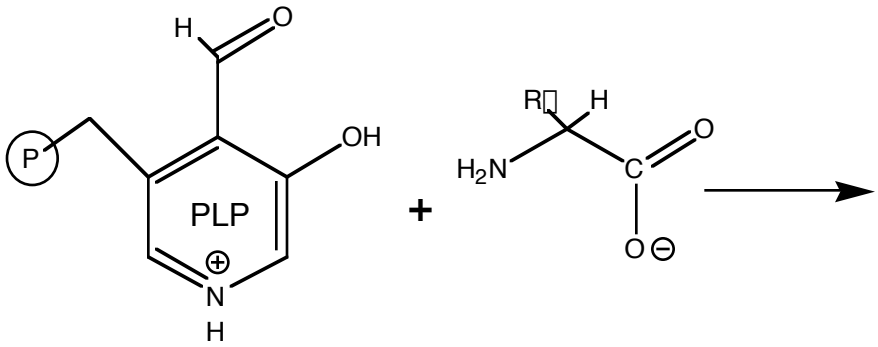


(c; 5 pts) The enzyme ATCase is allosterically activated by ATP and deactivated by CTP. In the presence of saturating amounts of the substrates aspartate and carbamoyl phosphate, how will the binding constants for each of the two allosteric effectors change?

The beginning of the mechanism for Schiff's-base catalyzed decarboxylation of a β-keto carboxylic acid is drawn below, as an aid in part (d).



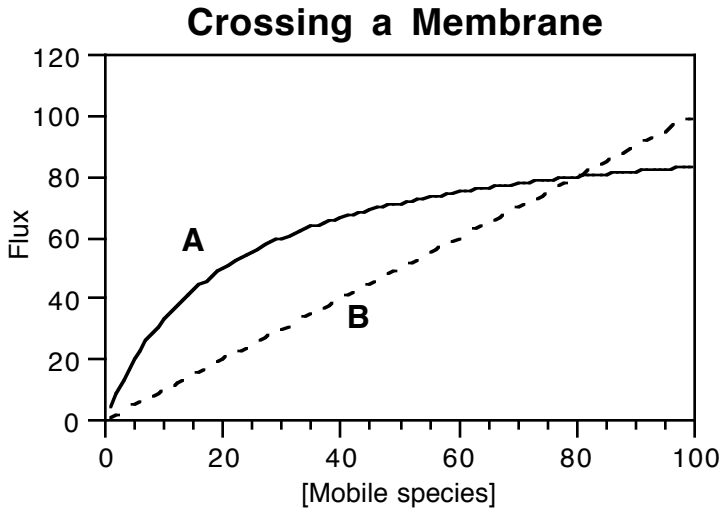
(d; 11 pts) The cofactor pyridoxal phosphate (PLP) is shown at the left below. It is a good electron sink with a reactive aldehyde [RC(=O)H]. Starting with Schiff's base formation between the amino acid below and the aldehyde moiety of PLP, propose a mechanism for decarboxylation of the amino acid to give the amine $RCH_2NH_3^+$. You need not draw out the steps in forming or hydrolyzing Schiff's bases.



(e; 5 pts) Why does a competitive inhibitor affect K_m but not V_{max} for a Michaelis-Menten enzyme?

5. (30 points) Biomembranes

(a; 6 pts) Which of the curves below (A or B) would represent the flux across a membrane for a molecule crossing by diffusion through the membrane itself, and which one would be for passive transport through a pore? Why?



(b; 6 pts) Draw the structure of phosphatidyl choline, indicating the alkyl tails with R_1 and R_2.

(c; 5 pts) Briefly describe the fluid mosaic model for biomembrane structure.

(d; 8 pts) How can we identify putative membrane-spanning □ helices from examination of protein sequences (other than by simple homology to known proteins)?

(e; 5 pts) What is the reasoning behind speculation that membranes evolved very early in the history of life?

6. (30 points) Methods for chromatography, analysis, and pedagogy

(a; 8 pts) What is an epitope tag and how is it used for adapting any protein to affinity purification?

(b; 16 pts) Given the information below for analysis of a decapeptide, write down its 1° sequence.

1. Amino acid composition: Trp, Leu, Lys, Met, Ser, Ala, Glx, Val, His, Arg
2. Trypsin cleavage gives a tetrapeptide and a hexapeptide. The tetrapeptide is VSAR.
3. Attempted Edman degradation on the intact decapeptide gives no products.
4. Chymotrypsin cleavage gives a single decapeptide, which does give products in the Edman reaction, giving an N-terminal sequence LKVS.
5. Ion-exchange chromatography shows that the peptide has a net charge of +2 at pH 7.
6. Cyanogen bromide cleavage at M gives a decapeptide whose N-terminus is H(E or Q)W.

(c; 6 pts) List either (a) a significant, uncorrected error in the lectures or the book or (b) your favorite and least favorite lectures, and very briefly why you feel that way.

Thank you very much for your attention and interest this semester.

Do Not Write Below This Line

Score:

Question 1: _____ out of 40

Question 2: _____ out of 35

Question 3: _____ out of 30

Question 4: _____ out of 35

Question 5: _____ out of 30

Question 6: _____ out of 30

Total: _____ **out of 200**