

Biochemistry 461, Section 0101

Your Name: _____

May 17, 1995

Final Exam

Your SS#: _____

Prof. Jason Kahn

Your Signature: _____

You have 120 minutes for this exam.

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

Explanations should be concise.

You will need a calculator for this exam.

No other study aids or materials are permitted.

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

Look over the entire exam before starting. You can often answer later parts of a question even if you have been unable to do the beginning.

The exam is worth 200 points, divided into 7 questions. You must do question 1 and question 2, but then do only FOUR of the remaining questions. Indicate which 4 we are to grade.

Exams will be available for viewing on May 19, from 3:30-5:00 p.m.

Final grades will be made available by e-mail or the MARS system, not over the phone. If you want to have your grade posted outside my office, listed according to the last four digits of your SS#, place a check mark in this box:

Scratch Space:

Score: Question 1: _____ out of 35

Question 2: _____ out of 25

Question 3: _____ out of 35

DO FOUR QUESTIONS CHOSEN FROM 3-7

Question 4: _____ out of 35

Question 5: _____ out of 35

Question 6: _____ out of 35

Question 7: _____ out of 35

Total: _____ out of 200

Do Not Write Above This Line

Free energy for transfer of an ion A from outside a membrane to inside:



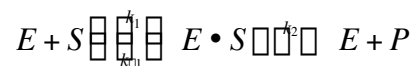
$$\Delta \overline{G}_A = RT \ln \frac{[A]_{in}}{[A]_{out}} + Z_A F \Delta \psi$$

where $RT = 2.58$ kJ/mole at 37°C ,

Z_A = charge on ion A, $F = 96.5$ kJ/(mole·V), and

$\Delta \psi$ = membrane potential in volts

Michaelis-Menten kinetic scheme:



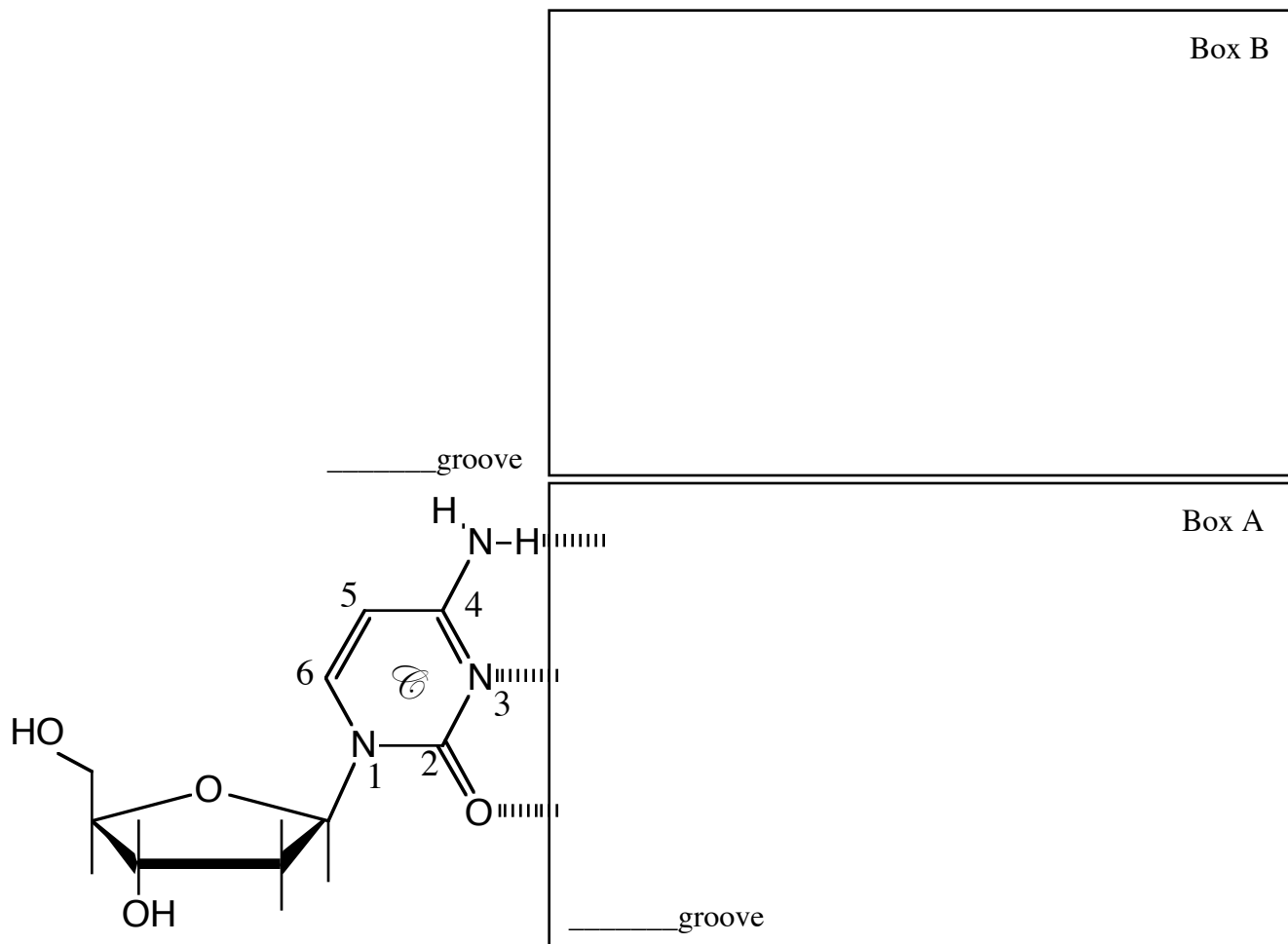
Definition of an acid dissociation constant: $\text{p}K_a = -\log_{10}K_a$, where $K_a = [\text{H}^+][\text{A}^-]/[\text{HA}]$

$\Delta G = \Delta G^\circ + RT \ln Q$, where Q has the form of an equilibrium constant. At equilibrium $\Delta G = 0$ and $Q = K_{eq}$, so $\Delta G^\circ = -RT \ln K_{eq}$.

1. Nucleic acid structure and hydrogen bonding (35 pts)

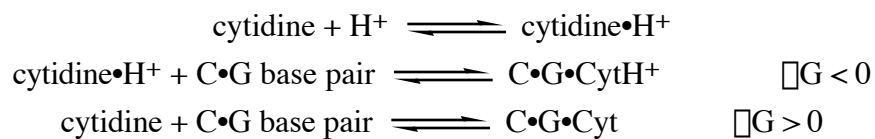
Triple-helical DNA has been proposed as a possible highly-selective therapeutic agent for the control of gene expression. The formation of triple-helical DNA relies on specific hydrogen bonds formed between the third strand and the major groove edge of a Watson-Crick base pair.

- (a) (8 pts) The structure of cytidine is given below. Draw its Watson-Crick partner guanosine in Box A below, denoting the deoxyribose ring with “dR”. Label the major and minor groove edges of the W-C base pair.
- (b) (12 pts) Cytidine can be protonated at N-3, with a pKa of about 4.2 for the protonated nucleoside, i.e. for the reaction $\text{Cytidine} + \text{H}^+ \rightleftharpoons \text{Cytidine} + \text{H}^+$. In box B below, draw N-3-protonated cytidine hydrogen-bonded to the major groove edge of the guanosine in Box A, with the cytidine N-4 amino group interacting with the guanosine 6-oxo group and the proton on N-3 interacting with guanosine N-7. This time, draw the Haworth projection for the deoxyribose ring, assuming that the relative orientation of the cytosine base and the sugar is the same as that of the cytidine shown below. Indicate the 3' and 5' hydroxyl groups.



(c) (4 pts) In this triple helix, in which base triples like the one shown are stacked and linked by phosphodiester backbones, is the third strand (box B) parallel or anti-parallel to the first strand (the C shown)? How do you know?

(d) (4 pts) Given the reaction scheme below, why is the pKa for third-strand cytidine found to be 5.5 instead of 4.2 (qualitative answer expected)?



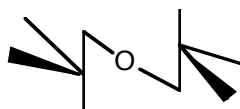
(e) (4 pts) Would you expect the pKa for protonation of the first-strand cytidine N-3 to be less than or greater than 4.2? Why?

(f) (3 pts) Finally, given the acid-base properties of the triple helix, can you identify a potential problem (one of many) with using third-strand DNA as a drug?

2. Carbohydrates (25 points)

(a) (9 pts) Name the three main functions for which carbohydrates are used in the cell. For each of these functions, give an example of a carbohydrate-containing molecule involved.

(c) (10 pts) Draw the structure of lactose N-linked to asparagine, using the chair form for the sugar rings. Lactose is a disaccharide, [*O*- β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucopyranose], where galactose is the C-4 epimer of glucose. Lactose is attached to Asn via a β linkage from the glucose. The β -(1 \rightarrow 4) linkage between the monosaccharide units is given below to get you started.



(d) (6 pts) Penicillin interferes with an essential bacterial growth process. What is this process (short answer, no structures)? How do resistant bacteria survive penicillin treatment?

3. Bioenergetics and Membrane transport (35 pts).

- (a) (8 pts) You are studying a transport system which pumps calcium (Ca^{++}) out of cells. The outside concentration of calcium is always 1 mM, the inside concentration is 0.1 μM , and the membrane potential is -50 mV (inside negative). Calculate ΔG for transporting one calcium from inside to outside.
- (b) (4 pts) If the free energy for ATP hydrolysis is -35 kJ/mole under these conditions, how many Ca^{++} ions can be transported per ATP hydrolyzed?
- (c) (8 pts) Assuming a constant membrane potential, what is the smallest attainable calcium concentration inside the cell, assuming one ATP hydrolyzed per calcium ion pumped?
- (d) (4 pts) You add valinomycin ion carriers to your experiment. This has the effect of eliminating the membrane potential by allowing free flow of K^+ across the membrane. What is the new value for ΔG for calcium transport?
- (e) (8 pts) Instead of valinomycin, you add a calcium channel and inhibit the calcium pump. Assuming that the cell is able to maintain the original -50 mV membrane potential, what is the equilibrium concentration of calcium inside the cell?
- (f) (3 pts) Now you add both the valinomycin and the calcium channel and inhibit the pump. What is the new equilibrium concentration of calcium inside the cell?

4. Protein Methods (35 pts):

A peptide was isolated from a cockroach, in a lab with a broken Edman sequencer. The amino acid composition is as follows: Glx, Asx, 2 Cys, Val, Phe, Met, 2 His, Tyr.

Trypsin digestion on this peptide gave no products. The entomologist decided to do some protein modification in order to obtain tryptic fragments. Since the Edman degradation was not available, she also decided to attempt to determine the peptide sequence using anion-exchange and cation-exchange chromatography.

- (a) (7 pts) When cysteine is treated with bromoethylamine ($\text{BrCH}_2\text{CH}_2\text{NH}_3^+$), the resulting adduct is cleaved by trypsin. Realizing that the cysteine $-\text{SH}$ group is a good nucleophile, draw the structure of the side chain of the adduct and explain why trypsin recognizes it:

With this technique in hand, we can proceed to the analysis of the peptide. The diagram provided below for your answer contains some hints. You can get some credit for (c) through (e) even if (b) is wrong.

The following experiments were performed:

- Reduction of disulfide bonds with DTT gave a hexapeptide and a tetrapeptide.
 - From ion-exchange chromatography, the hexapeptide was found to be uncharged at pH 8 and the tetrapeptide was found to have a charge of -2 .
 - Treatment of the mixture of the two reduced peptides with trypsin gave two tripeptides and two dipeptides.
 - These four modified peptides were analyzed by ion-exchange chromatography. At pH 8, their net charges were determined to be 0, $+1$, $+1$, and -2 , in no particular order. At pH 6, the peptide which had been neutral at pH 8 had a charge of $+1$, and at pH 4.5 it had a charge of $+2$.
 - CNBr cleavage of the hexapeptide gave a tetrapeptide and a dipeptide.
 - Carboxypeptidase digestion of the C-terminal residues of the original compound gave two products, Asp and His.
 - Chymotrypsin cleavage of the original compound gave a dipeptide, a free tyrosine, and a disulfide-linked fragment.
- (b) (15 pts) Write the amino acids in the proper locations on the picture below.
- (c) (2 pts) Indicate the position of the disulfide linkage.
- (d) (5 pts) Indicate the positions of chymotrypsin cleavage, CNBr cleavage, and cleavage of the reduced and modified peptides with trypsin.
- (e) (6 pts) Indicate the charges of the four tryptic fragments at pH 8.



Scratch space for this or other questions:

5. Protein structure, folding, and evolution (35 pts):

Most globular proteins have a close-packed hydrophobic core and a hydrophilic surface. This problem concerns the structure, folding, and function of an essential, tetrameric, allosteric enzyme which binds inorganic phosphate, P_i .

The table below shows some of the amino acids from the human, rat, and fruit fly enzymes. In addition, mutants (indicated by the asterisks) have been engineered (via site-directed mutagenesis) for each enzyme. The unfolding T_m , the K_S (binding constant, k_1/k_{-1}) for phosphate, and the Hill coefficient for allosteric regulation of catalysis (k_2) are given; the R and T states bind substrate equivalently but the T state catalyzes the subsequent reaction much more slowly than the R state. The concentration of P_i in each organism is 1 mM. Mutant sequences and their effects are in **bold** in the table.

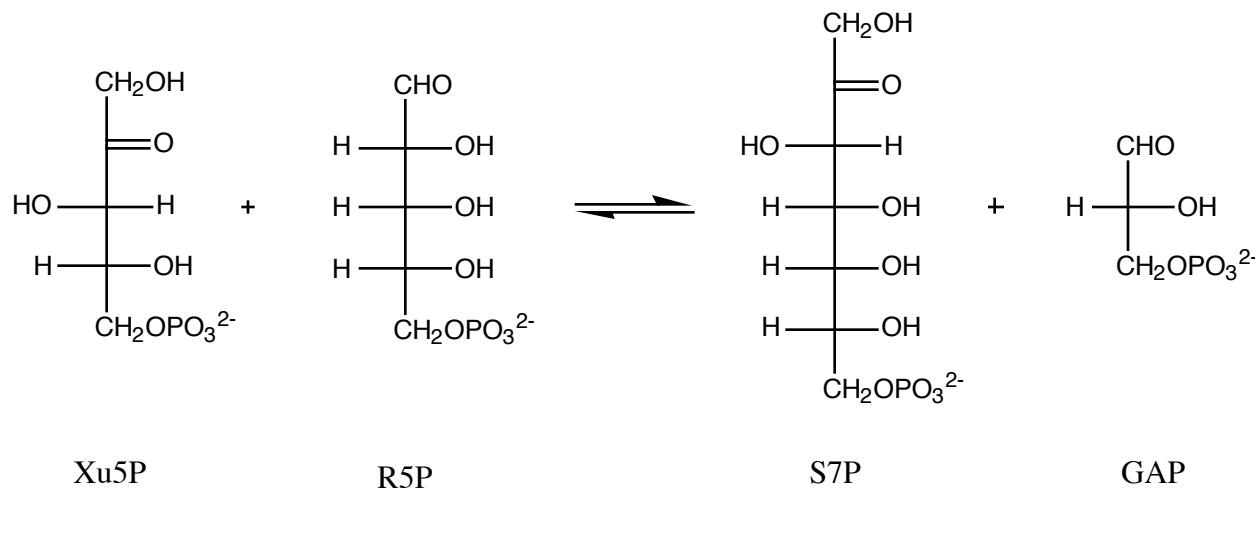
Enzyme	Amino Acid Sequences: Residue # (where it is)				Enzyme Properties		
	27 (core)	30 (core)	57 (surface)	72 (surface)	T_m (°C)	$K_S(P_i)$, mM	Hill n
Human	Gly	Val	Lys	Phe	45	0.5	2.7
Human*	Gly	Ala	Lys	Phe	32	0.5	2.7
Rat	Ala	Ile	Lys	Phe	45	0.5	2.7
Rat*	Ile	Ala	Glu	Phe	45	10	2.7
Fly	Ala	Val	Arg	Tyr	45	0.5	2.7
Fly*	Ala	Lys	Arg	Asp	20	0.5	1.1

- (a) (7 pts) The crystal structure of the Human* mutant enzyme shows that a cavity has been created in the hydrophobic core by changing Val \square Ala. Why might this destabilize the folding of the protein?
- (b) (4 pts) In the Rat* enzyme, switching Ile and Ala has no effect on stability. Why not?
- (c) (4 pts) If switching the amino acids at residues 27 and 30 has no effect on stability, and if it were shown furthermore to have no effect on function, why hasn't this mutation occurred naturally?

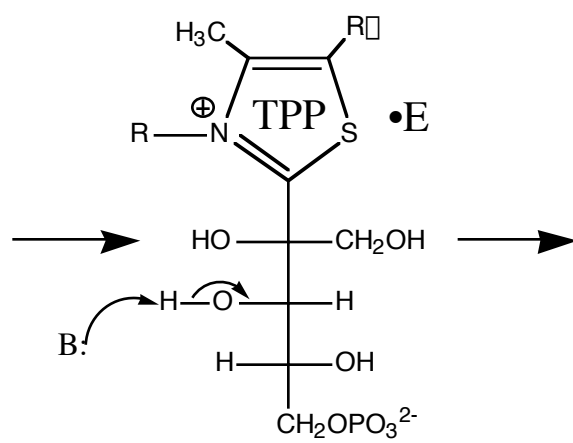
- (d) (6 pts) Clearly the side chains of residues 27 and 30 interact. If they are part of a repeating secondary structure (not a turn) in the protein core, is it more likely to be an α -helix or a β -sheet? Why?
- (e) (4 pts) Explain why the Val \rightarrow Lys mutation in Fly* is strongly destabilizing.
- (f) (6 pts) If you had to guess, which surface residue interacts with phosphate and which one is part of the interface between subunits? Explain your reasoning.
- (g) (4 pts) Draw the structure of arginine.

6. Carbohydrates and the Thiamine Pyrophosphate Mechanism (35 points)

The enzyme transketolase catalyzes the reaction below, the transfer of a two-carbon unit from the pentose phosphate xylulose-5-phosphate (Xu5P) to ribose-5-phosphate (R5P), yielding glyceraldehyde-3-phosphate (GAP) and sedoheptulose-7-phosphate (S7P). The enzyme uses a TPP cofactor.

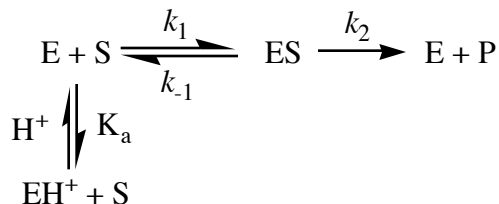


- (a) (2 pts) On the line below each sugar, indicate whether it is an aldose or a ketose.
- (b) (5 pts) Why are sugars often phosphorylated in the cell?
- (c) (5 pts) Why doesn't Xu5P exist in a stable ring-closed form, whereas R5P does?
- (d) (3 pts) Name a reaction other than the transketolase above which uses TPP.
- (d) (20 pts) The structure of an early key intermediate in the transketolase reaction mechanism is given below, with some of the electron-pushing arrows needed to take it on to the next step. Draw the entire mechanism, starting with a nucleophilic attack of TPP on the carbonyl of Xu5P, for the conversion Xu5P + R5P \rightarrow S7P + GAP as above. You need not indicate general acid/general base catalysis, but push arrows carefully.



7. Michaelis-Menten Kinetics and the Effect of pH (35 points):

The effect of pH on enzyme-catalyzed reactions can be treated mathematically in a manner analogous to competitive and uncompetitive inhibition. We will derive the Michaelis-Menten equation for the simple case diagrammed below, in which we assume that free enzyme can be protonated, that the resulting $E \cdot H^+$ is incapable of binding substrate, but that the ES complex cannot be protonated:



K_a refers to the acid dissociation reaction $EH^+ \rightleftharpoons E + H^+$.

- (a) (4 pts) Does this scheme resemble competitive, uncompetitive, or mixed inhibition? Explain your reasoning briefly.

The Michaelis-Menten equation is derived using the steady-state approximation and the conservation equation for enzyme concentration.

- (b) (4 pts) What is the physical meaning of the following conservation equation (not a trick question)?

$$[E]_{\text{total}} = [E] + [ES] + [EH^+]$$

- (c) (6 pts) Write down the expression for the rate of change of $[ES]$ ($d[ES]/dt$) as a function of $[E]$, $[S]$, $[ES]$, k_1 , k_{-1} , and k_2 . Write down the expression for the rate of product formation (v_0 or $d[P]/dt$). Which expression is set to zero under the steady state approximation (circle one): $d[ES]/dt$ $d[P]/dt$

- (d) (4 pts) Give an expression for $[EH^+]$ as a function of $[E]$, $[H^+]$, and K_a .

Substitution of this expression into the conservation equation and rearrangement yields the following equation for $[E]$ in terms of $[ES]$, $[E]_{\text{total}}$, $[H^+]$, and K_a (you don't have to derive it):

$$[E] = \frac{([E]_{total} - [ES])}{1 + [H^+]/K_a}$$

- (f) (7 pts) [I recommend skipping the derivation and going on to part (g) if you're short on time] Using the steady state approximation, the expression for $d[ES]/dt$, and the expression for $[E]$ above, solve for $[ES]$ and derive an equation of the form below for the rate of reaction as a function of the elementary rate constants, $[E]_{total}$, $[S]$, $[H^+]$, and K_a :

$$v_o = \frac{k_2[E]_T[S]}{[S] + K_M}, \text{ where } K_M = \frac{k_{-1} + k_2}{k_1}$$

- (g) (4 pts) In the expression from part (f) for the Michaelis-Menten rate, referring to your answer for part (f) or part (a), what is the value of α as a function of $[H^+]$ and K_a ?

- (h) (6 pts) Sketch the pH dependence of v_0 for this enzyme assuming $pK_a = 5$, for $[S] = K_M$.

