Biochemistry 461, Section 0101 Your Name:_____

May 2, 1995

Exam #3 Your SS#:_____

Prof. Jason Kahn

Your Signature:_____

Please have photo ID available.

You have 80 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 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.

The exam is worth 100 points, 20 points per question.

Exams will be returned in class on May 9. At that time, you will also have an opportunity to fill out course evaluation forms.

The make-up exam will be held on May 11 at 10:30 a.m. in Chemistry 2507, there will be a review session May 12 at 3:30 p.m. in Chemistry 1402, and the final will be May 17 at 10:30 a.m. in Chemistry 1402

<u>You must contact me by May 10 if you intend to take the make-up exam.</u>

You may submit exam 3 for regrading any time before the beginning of the final exam.

Score: Question 1: _____ out of 20

Question 2: _____ out of 20

Question 3: _____ out of 20

Question 4: _____ out of 20

Question 5: _____ out of 20

Total: _____ out of 100

Do Not Write Above This Line

Possibly Useful Information:

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

$$A_{out} \rightleftharpoons A_{in}$$

$$\Delta\overline{G_A} = RT \ln \frac{[A]_{in}}{[A]_{out}} + Z_A F \Delta\Phi$$

where RT = 2.58 kJ/mole, Z_A = charge on A, F = 96 kJ/(mole·V), $\Delta\Phi$ = membrane potential

Michaelis-Menten kinetic scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \bullet S \xrightarrow{k_2} E + P$$

1. Michaelis-Menten Kinetics (20 points):

The table below contains data collected for the rate of an enzymatic reaction in the presence of a 4 mM concentration of inhibitor I. The graph on the next page is a double-reciprocal plot, with the data for the same enzyme in the absence of inhibitor already plotted, for the same substrate concentrations.

[S] (mM)	v_o (mM/min), [I] = 0 (already plotted)	v_o (mM/min), [I] = 4 mM
1.25	0.02727	0.01111
2	0.03750	0.01667
5	0.06000	0.03333
20	0.08571	0.06667

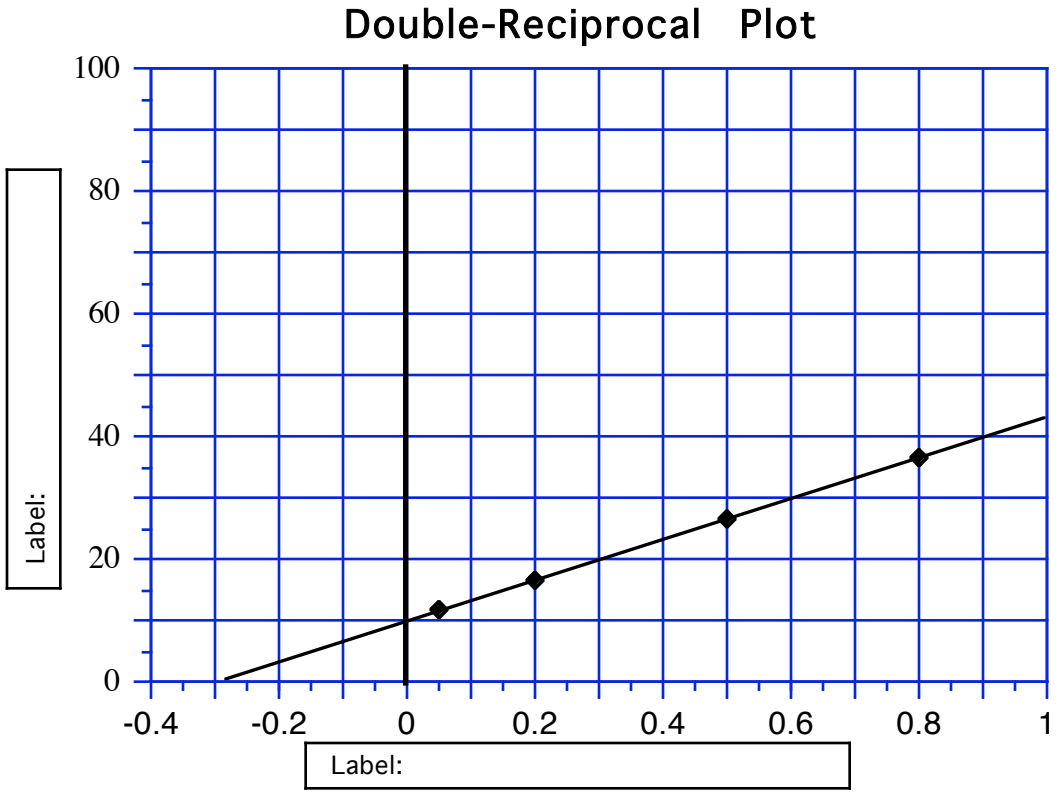
(a) (3 pts) Label the axes of the graph, including units, and give the symbolic values for the *x*-intercept, *y*-intercept, and slope of the plot (for example, the *y*-intercept is $1/V_{max}$):
x-intercept _____, *y*-intercept 1/Vmax , slope _____.

(b) (8 pts) Plot the data for the inhibited reaction on the same graph, and determine K_m and V_{max} in the presence and absence of the inhibitor. Enter the numerical values here, with units:
No inhibitor: K_m _____, V_{max} _____.
4 mM inhibitor: K_m _____, V_{max} _____.
Scratch space:

(c) (3 pts) What kind of inhibition is exhibited here? Explain how you know.

(d) (3 pts) What is/are the numerical value(s), with units if appropriate, for:
□ or/and □□ (circle which one(s) is/are appropriate) at [I] = 4 mM? _____.
K_I or/and K_I□ (circle which one(s) is/are appropriate)? _____.

(e) (3 pts) Determine k_2/K_m (uninhibited) for this enzyme or specify what further information you would need in order to do so (use the definition of V_{max}):



2. Miscellaneous short questions (20 pts):

(a) (5 pts) List two ways in which the regulation of enzymatic activity is achieved, and give an example of an enzyme which is regulated by each mechanism. Discuss briefly the advantages and disadvantages of one of these mechanisms for the cell.

(b) (5 pts) During neuronal firing, sodium channels are induced to open. Given that $[Na^+]$ outside the cell is 150 mM, that $[Na^+]$ inside the cell is 10 mM, and that the membrane potential □□ is -50 mV, determine qualitatively the direction for sodium ion flow (you do not need your calculator). Does ion flow through the channels make □□ more positive or more negative?

(c) (5 pts) List three reactions or processes performed by cofactors/prosthetic groups in biological systems and give an example of a cofactor/prosthetic group used for each. For two of your examples, name a protein, enzyme, or multi-protein complex which uses that cofactor.

(d) (5 pts) (i) Why do membranes containing unsaturated fatty acids remain fluid at lower temperatures than those with only saturated fatty acids? (ii) Why is transverse (flip-flop) diffusion of membrane phospholipids so slow relative to lateral diffusion?

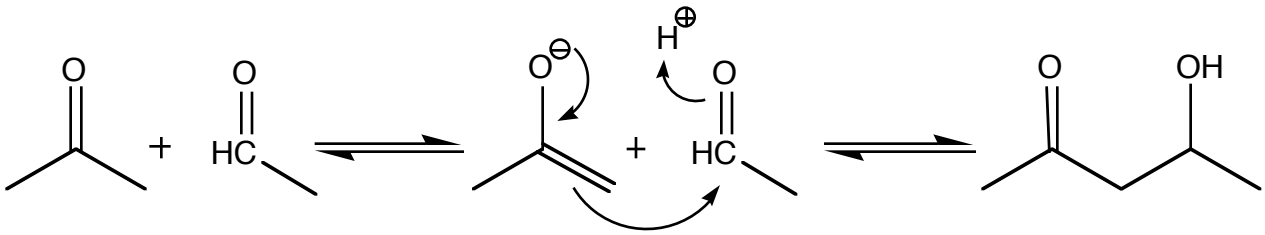
3. Structures (20 pts — part (e) is on the next page):

(a) (4 pts) Draw the thiazolium ring of thiamine pyrophosphate (TPP) and indicate the proton which is easily removed to give a carbanion.	(b) (5 pts) Draw phophatidylserine, indicating the fatty acid hydrocarbon tails with R_1 and R_2.
(c) (3 pts) Draw the structure of aspartyl phosphate.	(d) (3 pts) Draw the structure of the redox-active end of lipoic acid, in its oxidized form.

(e) (5 pts) Draw the two resonance forms of the oxonium ion intermediate in the hydrolysis of NAG_6 by lysozyme. You need only show the atoms of the six-membered ring and the substituent on C1, and you do not need to specify the conformation of the ring.

4. Catalytic Mechanisms (20 points)

The mechanism of the aldol condensation reaction between acetone and acetaldehyde to give 4-hydroxy-2-pentanone is shown below:



(a) (8 pts) Draw the mechanism for the retro-aldol reaction of 4-hydroxy-2-pentanone to give acetone and acetaldehyde, as catalyzed by ethylamine ($CH_3CH_2NH_3^+$). You do not need to write out the formation or hydrolysis of a protonated Schiff's base explicitly, but otherwise show all intermediates, push arrows, and keep track of protons.

(b) (4 pts) Now, assume that in addition to covalent catalysis a general base is involved in the reaction. Draw the participation of the general base on the scheme you have written above.

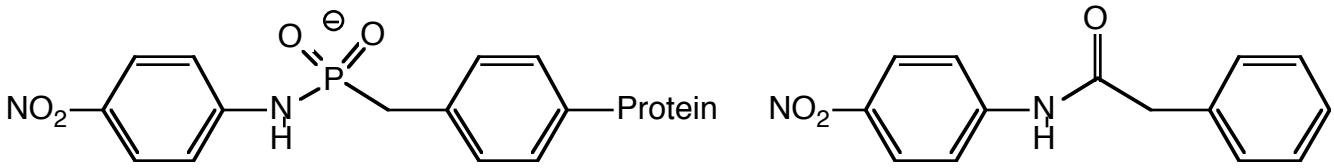
(c) (4 pts) What are the roles of the ethylamine and the general base in catalyzing the retro-aldol reaction (*i.e.* do they act as nucleophiles, via electrostatic stabilization...)?

(part (d) on next page)

(d) (4 pts) You have discovered an enzyme which catalyzes the above retro-aldol reaction. Upon incubating the enzyme with a sample of 4-hydroxy-2-pentanone prepared via ethylamine catalysis, under conditions which thermodynamically favor the acetone and acetaldehyde products, you find that half the material is rapidly converted to products while the remainder reacts very slowly. Explain this observation.

5. Catalytic Antibodies (20 pts):

The phosphonamidate on the left below, conjugated to a protein to confer immunogenicity, was used to raise monoclonal antibodies. The antibodies obtained were selected for binding to the unconjugated compound and then screened for catalytic activity using the peptide mimic on the right as a substrate.



(a) (7 pts) Draw a plausible mechanism of the reaction catalyzed by the antibody for which a reaction was observed. Indicate the intermediate which is presumably stabilized by the antibody.

(b) (2 pts) Some antibodies which bound the phosphonamidate were not observed to catalyze any change in the substrate -- why not?

(c) (3 pts) What principle of enzymatic catalysis is exemplified by the catalytic antibodies we have discussed? Would you expect the phosphonamidate in this case to inhibit the antibody-catalyzed reaction? If so, would the inhibition be competitive or uncompetitive? [This question oversimplifies the results actually obtained — for the more complete version, see *Science*, vol. 241, p. 1188.]

(d) (8 pts) Draw qualitative but careful free-energy reaction coordinate pathways for the catalyzed and uncatalyzed reactions on a single diagram, denoting substrate, intermediate(s), product(s), and antibody with S, I, P, and E respectively. Indicate $\Delta G^{\ddagger}$ for the catalyzed and uncatalyzed reactions on your diagram.