

Biochemistry I, Spring Term 2001 - Second Exam:

There are a total of 7 pages in this exam. The total number of points is 70. You should spend about 1 min/1.5 points in answering any question.

Please check that you have all the pages and write your name on every page before you begin. Use the space provided to answer the questions.

Grade:

For $([E]+[S] \rightleftharpoons [ES] \rightarrow [E]+[P])$

Part A: _____

 $V_{\max}=k_2[E_T]$ $K_M=(k_{-1}+k_2)/k_1$

B1 _____

B2 _____

B3 _____

B4 _____

B5 _____

 $R=8.3 \text{ J/mol-deg}$ $T=300\text{K}, RT=2.5 \text{ kJ/mol @ } 300\text{K}$ $\Delta G^0 = -RT \ln K_{eq}$ $\Delta G = \Delta H - T\Delta S$ $u = u^0 + RT \ln [X]$ $S = R \ln W$ $pH = pK_a + \log([A^-]/[HA])$

TOTAL _____

 $Y = K_{EQ}[L]/(1 + K_{EQ}[L])$ $Y = [L]/(K_D + [L])$ $Y = [PL]/([P] + [PL])$

Scatchard Plot:

 $v/[L] = n/K_D - v/K_D$ $Y/[L] = 1/K_D - Y/K_D$ Hill Plot: $\log(Y/(1-Y))$ versus $\log[L]$ $A = \epsilon Cl$

$$v = \frac{\frac{V_{MAX}}{\alpha'} [S]}{\frac{\alpha'}{\alpha'} K_M + [S]}$$

$$\alpha = 1 + ([I]/K_I)$$

 $\alpha'=1$ for competitive inhibition $\alpha'>1$ for non-competitive inhibition

Section A - 8 Multiple Choice. 24 points.

1. K_m and K_D are similar in that:
 - a) they refer to the concentration of ligand or substrate in a biochemical process.
 - b) they both relate to ligand binding measurements, $K_m = 1/K_D$
 - c) they both reflect half-way points in a biochemical process.
 - d) answers a and c.

2. In both hemoglobin and myoglobin the oxygen is bound to.
 - a) the iron atom in the heme group.
 - b) the magnesium atom in the heme group.
 - c) a hydrophobic pocket in the protein.
 - d) the BPG binding site

3. A protein that binds two ligands in a highly *cooperative* manner will:
 - a) show a sigmoidal binding curve
 - b) show a hyperbolic binding curve
 - c) show a linear Scatchard Plot
 - d) answers b and c are correct.

4. A protein that shows infinite cooperative for binding of 4 ligands will
 - a) show a Hill coefficient (n_h) of 0
 - b) only be found in either the unliganded form or the fully liganded form.
 - c) show a Hill coefficient (n_h) of 1
 - d) show a Hill coefficient (n_h) of 4/2

5. The active site of an enzyme
 - a) provides amino acid side chains that become modified during the reaction.
 - b) provides amino acid side chains that are complementary to the substrate
 - c) provides amino acid side chains that are complementary to a non-competitive inhibitor
 - d) provides amino acid side chains that are always charged

6. Which of the following features are **not** shared between Serine and Aspartate Proteases
 - a) both use an amino acid side chain (Ser or Asp) as a nucleophile
 - b) both use a base to activate the nucleophile.
 - c) both require water to complete the catalytic cycle.
 - d) both show specificity for certain amino acid sequences.

7. In an protein purification scheme, which of the following usually increases during the procedure?
 - a) the total protein concentration.
 - b) the enzyme activity of the desired enzyme.
 - c) the specific activity
 - d) the amount of the desired enzyme.

8. The final result of an X-ray diffraction study of protein crystals is
 - a) the size of the protein crystal.
 - b) the wavelength of the X-rays used in the experiment.
 - c) the x, y, and z coordinates of most of the atoms in the protein.
 - d) the phi and psi angles of the peptide bond.

B1: Please answer **one** of the following **three** questions:(8 pts)

i) Briefly discuss how the different oxygen binding properties of hemoglobin and myoglobin are important for the normal transport of oxygen. Be sure to discuss the most important structural features of both proteins.

ALTERNATIVELY Briefly discuss the role of bis-phosphoglycerate (BPG) in the adaptation of oxygen transport to high altitude. Be sure to discuss the most important structural features of this process.

OR

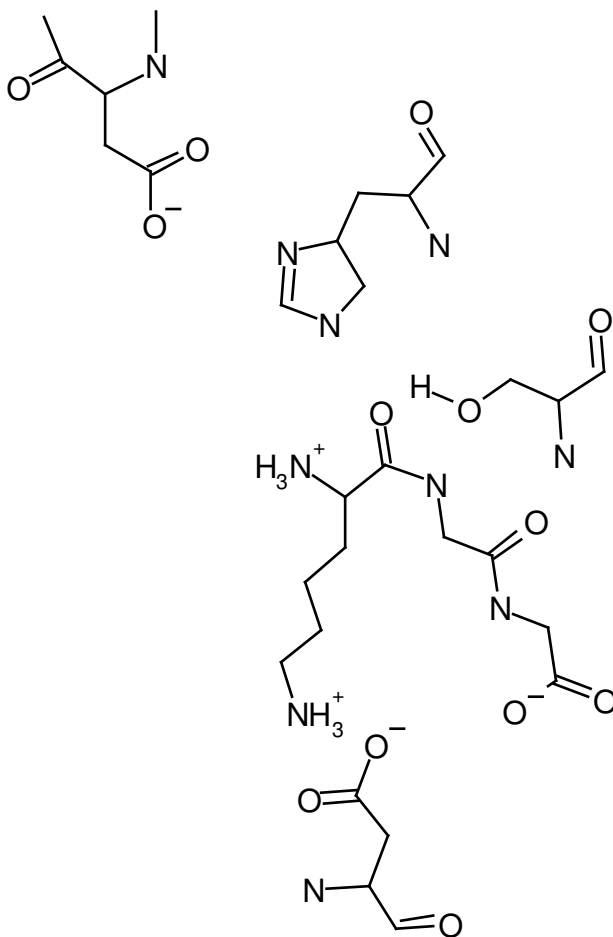
ii) Briefly discuss transition state theory as it applies to the rate enhancement of enzymatic reactions. Provide one concrete example of how enzymes might affect the energy of the transition state.

OR

iii) Briefly discuss the key differences between a competitive and a non-competitive enzyme inhibitor. Which form of inhibition might be used to regulate biochemical processes? Why?

B2: Trypsin Mechanism.(12 points)

The chemical drawing on the left shows the active site of the enzyme trypsin. This is the ES complex with a substrate Lysine-Glycine-Glycine bound. Please answer all of the following questions:



i) Label the three residues that form the catalytic triad. You don't need to indicate the sequence number, just the amino acid name.(3 pts)

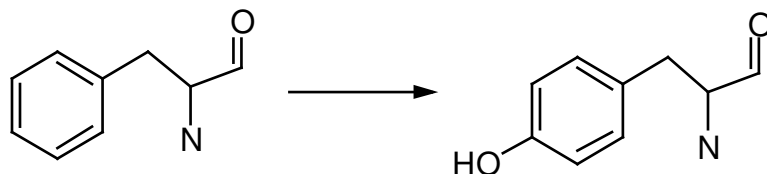
ii) Briefly describe the role of **one** of the residues from part **i** in the catalytic cycle.(2 pts)

iii) Label the residue that is responsible for the substrate specificity of trypsin. Again, you don't need to give the residue number, just the amino acid type. Circle your label to distinguish it from part i.(2 pts)

iv) How would modify the enzyme to change its specificity such that it would efficiently cleave peptides containing Methionine (side chain: $-\text{C}-\text{C}-\text{S}-\text{CH}_3$). Sketch your modification below and briefly support your answer with explicit reference to the molecular forces that would be involved in the recognition of the Methionine. **ALTERNATIVELY** you can discuss how you might modify HIV proteases inhibitors to be effective against a virus that has altered Val81 to Phenylalanine in the protease (5 pts).

NAME: _____

B3:(13 pts) The enzyme, phenylalanine hydroxylase, performs the following chemical transformation:

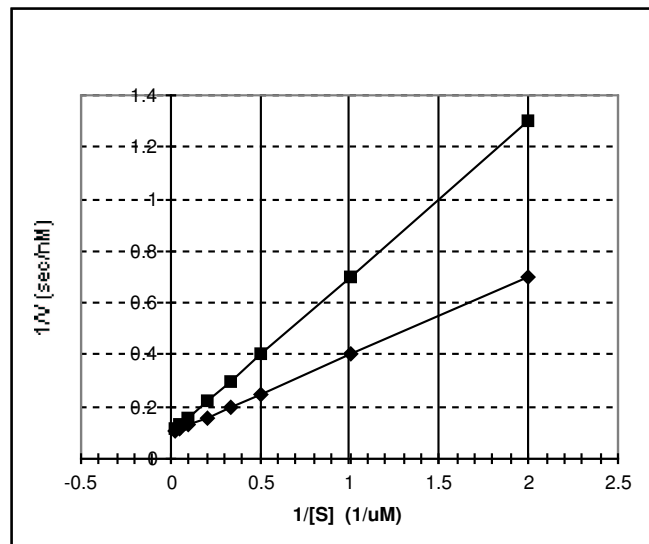


A series of kinetic measurement was performed for the inhibited and un-inhibited reaction. These data were plotted on the following Lineweaver-Burk (double reciprocal) plot. ($1/v$ is plotted versus $1/[S]$)

The substrate concentrations are in μM and the enzyme activity is in units of nM product produced/second.

i) Clearly label the curve that represents the data obtained in the absence of the inhibitor (-I) and the data obtained in the presence of the inhibitor (+I) (2 pts).

ii) What kind of inhibitor is this compound (ie. competitive, non-competitive)? Briefly Justify your answer? (2 pts)



iii) Obtain V_{MAX} for the uninhibited reaction. Briefly explain how you determined V_{MAX} (2 pts)

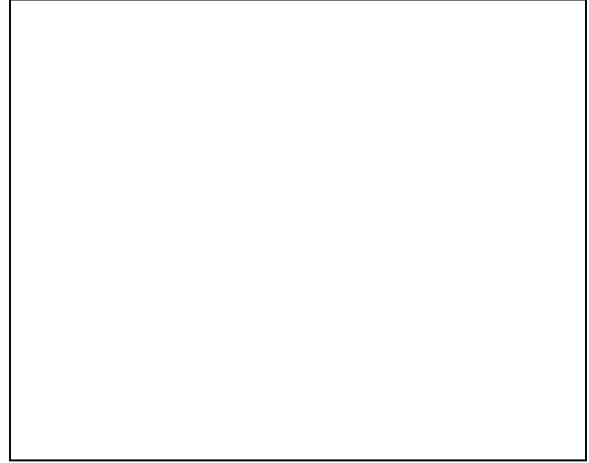
iv) Estimate, using the graph above, the K_M for the substrate. Briefly explain your approach (4 pts).

v) Given that the inhibitor concentration was $5\mu\text{M}$, what is the dissociation constant (K_I) for the inhibitor? (2 pts)

vii) On the basis of the type of inhibition and the substrate of the reaction, draw the chemical structure of a plausible inhibitor (1 pt).

B4. Answer **one** of the following three questions (6 pts).

i) Briefly discuss the effect of pH on the activity of either Trypsin or the HIV protease. You can assume that the pK_a for His in Trypsin is 7.0 and that for the two Asp residues in the HIV protease to be 3.0 and 6.0 respectively. In your discussion, provide a brief sketch in the box provided of how you might expect the enzyme activity (V_{max}) to change as a function of pH. Be sure to indicate the enzyme you have elected to discuss.



OR

ii) Select **one of the following** chromatography methods and briefly discuss how the technique works and the property of the protein that is exploited by the method to separate different proteins. Also indicate a method to elute to protein from the particular resin if necessary.

- a) Cation exchange chromatography
- b) Affinity chromatography
- c) Gel Filtration chromatography

OR

iii) Briefly discuss why, in the case of a dimeric protein, the native molecular weight is obtained after gel filtration but the subunit molecular weight is obtained with SDS gel electrophoresis.

B5. (7 Pts). The following data was acquired in an equilibrium dialysis experiment. In all cases the protein concentration inside the dialysis bag was 1 μM ($1 \times 10^{-6} \text{ M}$):

$[\text{L}]_{\text{Free}}$	$[\text{L}]_{\text{inside}}$	Y	$Y/[\text{L}]_{\text{Free}}$	$\text{Log}[\text{L}]$	$\text{Log}(Y/(1-Y))$
0.1 μM	0.20 μM	0.09	1.00	-1.0	-1.0
1.0 μM	1.50 μM	0.50	0.50	0.0	0.0
3.0 μM	2.75 μM				
10 μM	10.9 μM	0.90	0.09	1.0	1.0

i) Determine the fractional saturation, Y, for a ligand concentration of 3.0 μM . Please show your work.(2 pts)

ii) Is this binding cooperative or non-cooperative? If necessary you may want to plot the data on the grid below, but a plot may not be necessary. Regardless of your approach, please justify your answer.(3 pts)

iii) Determine the K_D for binding from these data using any method you choose. But show your work.(2 pts)

