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Biochemistry I, Spring Term 2000 - Second Exam:

This exam has a total of 100 points and is divided into two sections. You must do ALL of the questions.

There are a total of 10 pages in this exam. 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:

Part A: _____

B1 _____

B2 _____

B3 _____

B4 _____

B5 _____

B6 _____

B7 _____

TOTAL _____

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

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

α'=1 for competitive inhibition

α'>1 for non-competitive inhibition

For ([E]+[S]<-->[ES]-->[E]+[P])

$V_{max}=k_2[E_T]$

$K_M=(k_{-1}+k_2)/k_1$

R=8.1 cal/mol-deg

T=300K, RT=2.5 kJ/mol @ 300K

$\Delta G^O=-RT\ln K_{eq}$

$\Delta G=\Delta H-T\Delta S$

$\mu=\mu^O+RT\ln[X]$

$S=R\ln W$

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

$Y=K_A[L]/(1+K_A[L])$

Scatchard Plot: $\nu/[L]=n/K_D-\nu/K_D$

Hill Plot: *log*(Y/(1-Y)) versus *log*[L]

$A=\varepsilon Cl$

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Section A (28 pts): (2 pts/question). Circle the letter corresponding to the **best** answer.

1. The specificity of a ligand binding site on a protein is based on
   a) the absence of inhibitors.
   b) the presence of metal ions, such as Mg.
   c) the opposite chirality of the binding ligand.
   d) complementarity between the protein and ligand.

2. Positive cooperative binding can be identified by
   a) a non-hyperbolic binding curve.
   b) a Hill plot with a slope less than one.
   c) a Hill plot with a slope greater than one.
   d) part a) and c).

3. The dissociation constant is
   a) a measure of how easily the HIV protease dimer combines to form active protease.
   b) the inverse of the Hill coefficient.
   c) the inverse of the association constant.
   d) related to the enthalphy change due to ligand binding.

4. In hemoglobin, O_2 and bis-phosphoglycerate:
   a) are homotropic and heterotropic allosteric effectors, respectively.
   b) are heterotropic and homotropic allosteric effectors, respectively.
   c) have equal K_D values
   d) do not influence each others binding to the protein.

5. In oxy-hemoglobin (Hb), the Fe (II) is coordinated to
   a) four nitrogens of heme and to the proximal His of Hb.
   b) four nitrogens of heme and to a water molecule.
   c) four nitrogens of heme and to an O_2 molecule.
   d) choice a and c.

6. The active site of an enzyme
   a) always remains rigid and does not change shape.
   b) is found at the center of globular enzymes.
   c) is complementary to the rest of the molecule.
   d) none of the above.

7. The transition state of a catalyzed reaction ($X^{\ddagger}$) is
   a) a highly-populated intermediate on the reaction pathway.
   b) higher in energy than that of an uncatalyzed reaction.
   c) lower in energy than that of an uncatalyzed reaction.
   d) lower in energy than the reaction substrate.

8. A competitive inhibitor of an enzyme is usually:
   a) a highly reactive compound.
   b) a metal ion such as Hg^{2+} or Pb^{2+}.
   c) structurally similar to the substrate.
   d) water insoluble.

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9. Which of the following comparisons between Serine and Aspartate Proteases is true.
   a) both show an optimal pH for activity
   b) both use a His residue to activate the nucleophile.
   c) both form an acyl-enzyme intermediate
   d) both cleave the same substrates with equal rates.

10. The pKa of an ionizable side chain (e.g. His), can be determined with NMR because
   a) protonated imidazole has twice the number of protons.
   b) unprotonated imidazole is invisible in the spectrum.
   c) the chemical shift of His differs from His^{+}.
   d) None of these choices are correct.

11. The analysis of enzyme kinetics using steady-state methods
   a) is an accurate view of the reaction immediately after substrates are added.
   b) provides an accurate description of the reactions at all times.
   c) can only be used if the product does not inhibit the enzyme.
   d) can seldom be applied to most enzymes.

12. In the following kinetic scheme: $[E]+[S] \xrightarrow{K_{EQ}} [ES] \xrightarrow{k_1} [EA] \xrightarrow{k_2} [EB] \xrightarrow{k_3} [E]+[P]$
If k_2 is much, much slower than k_1 or k_3, then the observed maximal velocity, V_{MAX} is:

   a) k_2E_t
   b) k_1E_t
   c) k_3E_t
   d) $k_2K_{EQ}E_t$

13. The specific activity is
   a) a measure of the total enzyme activity.
   b) the total mass of the protein in a purification.
   c) not important in protein purification.
   d) a useful criteria for monitoring purity.

14. In cation exchange chromatography, proteins are separated on the basis of their:
   a) molecular weight.
   b) positively charged sidechains.
   c) different isoelectric points.
   d) negatively charged sidechains.

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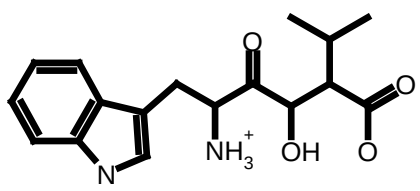
Section B:

B1 (18 pts) : A drug that inhibits the HIV protease has the following structure:



i) Circle the bond that would be cleaved by the HIV protease in the normal substrate (1 pt).

ii) Based on the structure of this inhibitor, briefly describe two of the forces, or energetic components, that might stabilize the drug-protein complex. Make *specific* reference to functional groups on this compound (4 pts).

The binding of this drug to the HIV protease has been measured by equilibrium dialysis. The amount of protein *and* ligand were quantified by UV absorption. The HIV protease dimer contains 4 Trp residues, while the drug contains one. You can assume that the extinction co-efficient (ε) of Trp is 10,000 $M^{-1}cm^{-1}$. Two different drug concentrations were used, giving the following data. The concentration of the HIV protease dimer was 10μM, you should also assume a path length of 1cm.

(**Hint**: The UV absorption inside the dialysis bag includes absorption from the protein as well as the ligand. So, calculate the UV absorption from the protein first, and subtract that from the total absorption to give the UV absorption due to the ligand)

UV absorption outside of the dialysis bag	UV absorption inside the dialysis bag
0.1	0.55
1.0	1.49

iii) What wavelength of light would you have used to measure the absorption of UV light? (1 pt)

iv) Using the 1st set of data, calculate the dissociation constant, K_D. Don't forget the units! (5 pts)

v) What is the free energy of binding of this drug to the protease (assume T=300K)? (2 pts)

B1- Continued

vi) Using the 2nd set of data, determine if the binding of the drug is cooperative or non-cooperative. Support your argument with a calculation. (3 pts)

vii) *Calculate* how large of an effect the replacement of the Trp sidechain in the above drug with a phenyl group will have on the affinity (i.e. K_{EQ}) of the drug to the enzyme. Assume that both the Trp and Phe sidechain will bind in a hydrophobic pocket on the enzyme. The free energy of transfer of Trp from a non-polar environment to water is +12 kJ/mol while that of Phe is +10kJ/mol. (2 pts)

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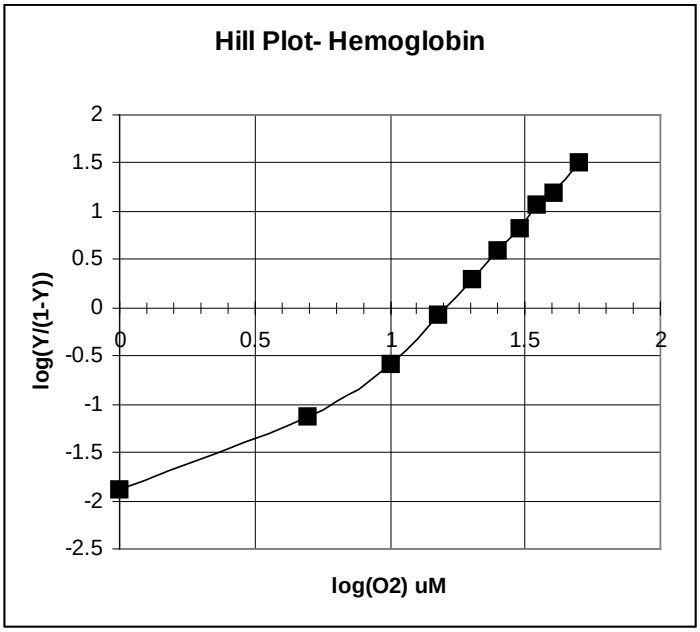
B2:(12 pts) A Hill plot for the binding of oxygen to Hemoglobin is shown below. This binding data was obtained in the presence of 10 μM bis-phosphoglycerate.

i) On the basis of this plot, what is the approximate K_D for oxygen binding. (2 pts) (**Hint:** The oxygen concentration is in given in μM).



ii) What is the Hill coefficient? Briefly explain how you obtained your answer (2 pts)

iii) The binding of protons to hemoglobin decreases its O_2 affinity 10 fold, but does not change its cooperativity. Sketch, on the same plot to the right, the Hill plot that would be obtained in the presence of protons. Label the curve with "iii" (2 pts)

iv) A reduction in the concentration of bis-phosphoglycerate to 1 μM increases the affinity by 10 fold and changes the Hill coefficient to a value of 3.5. Sketch, using a dashed line, the Hill plot under these conditions. Label the curve with "iv" (3 pts)

v) Answer **ONE** of the following:

a) Briefly explain the molecular nature of cooperative oxygen binding in hemoglobin. Why is this effect important in normal oxygen transport?(3 pts).

OR

b) Explain the molecular nature of the effect of bis-phosphoglycerate on the binding of oxygen to hemoglobin. Why is this effect important in the regulation of oxygen transport. (3 pts)

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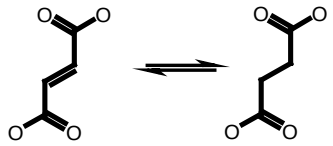
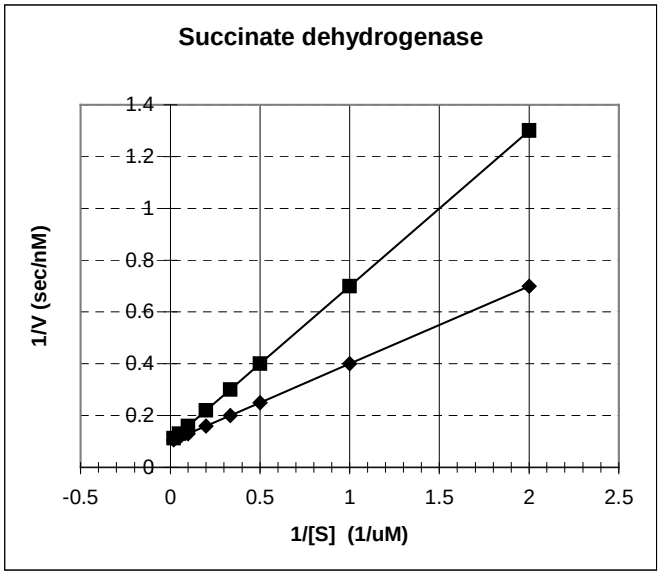
B3:(16 pts) The enzyme, succinate dehydrogenase, performs the following chemical transformation:



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:

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) Calculate V_{MAX} for the uninhibited reaction.(2 pts)

iv) Estimate, using the graph above, the K_M for the substrate.(3 pts)

v) Given that the inhibitor concentration was 10μM, what is the dissociation constant for the inhibitor?(4 pts)

vi) Draw a line on the above graph that would represent the Lineweaver-Burk plot for reactions performed in the presence of 5 μM inhibitor(2 pts).

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

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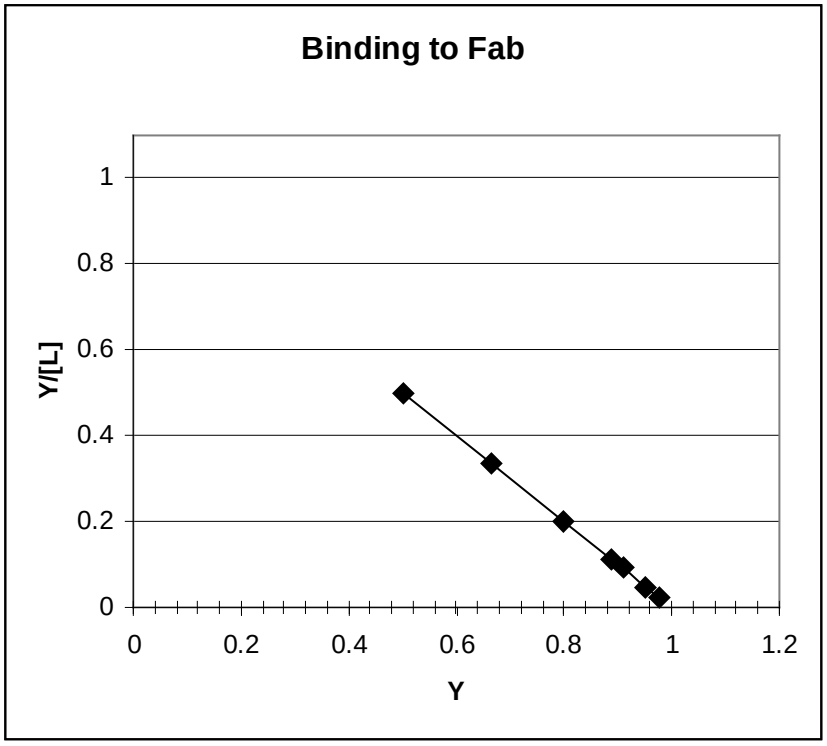
B4:(4 pts) The Scatchard plot for the binding of dinitrophenyl to the Fab fragment of an immunoglobulin is shown below (the ligand concentration is in μM);



i) Estimate the binding affinity, K_{EQ}, from this graph? Briefly describe how you arrived at your answer (2 pts).

ii) How would the above plot change if this experiment was performed on an intact antibody, instead of the Fab fragment? Re-label the x and y axis and, if necessary, re-plot the data (2 pts).

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B5:(10 pts) The color handout shows the active site of Trypsin, a serine protease. The bound substrate is drawn with thin lines while residues from the protein are drawn with thicker sticks. The residue that is responsible for the substrate specificity (Asp189) is labeled. The other three residues, labeled A, B, and C, comprise the catalytic triad.

i) How would the specificity of Trypsin change if Asp189 was replaced by Methionine (if you don't remember the structure of Methionine, draw what you think the structure is and then proceed with the question.)(2 pts)

ii) The effect of replacing Asp189 with Met would have the largest effect on the K_M or on V_{MAX}? Briefly explain your reasoning.(2 pts)

iii) Which residue (A, B, or C) is the nucleophile in this reaction?(2 pts)

iv) Briefly explain how the other two residues in the catalytic triad increase the reactivity of the nucleophile.(4 pts)

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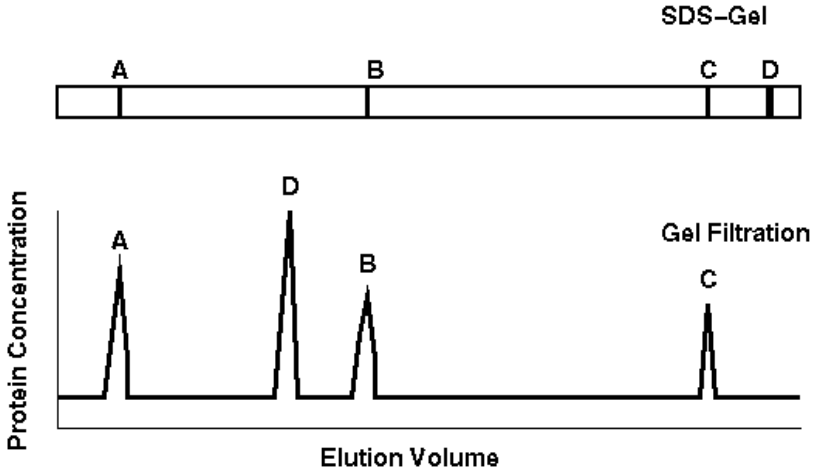
B6 (5 pts). The activity of the HIV protease is highest at pH 5. Explain this observation with specific reference to the ionization properties of the active site residues in this enzyme.

B7 (7pts) : To the right is an image of an SDS-PAGE gel (turned sideways). The top of the gel is on the left, the bottom on the right. A total of four bands were observed on this gel (labeled A, B, C, and D). The elution profile for the same mixture of proteins from a gel filtration (size exclusion) column is shown underneath the gel. The same protein has the same label in both experiments.



i) Why do most of the bands in the SDS-PAGE gel "line-up" with most of the peaks from the gel filtration column (i.e. A matches A, B matches B, etc.) (3 pts)

ii) Provide a reason why the protein labeled 'D' does not follow this pattern. That is, its migration distance on the SDS-PAGE gel is apparently unrelated to its elution volume on the gel-filtration column.(2 pts)

iii)Assume that you know the molecular weight of proteins A and C, briefly describe, for **either** gel filtration or SDS-PAGE, how you would obtain the molecular weight of the other proteins.(2 pts)