Biochemistry I, Fall 2005 Name:_____

Exam II - Face Page

The following equations and constants may be useful:

Ligand Binding: $Y = \frac{[ML]}{[M]+[ML]}$ $Y = \frac{[L]}{K_D + [L]}$ $Y = \frac{K_A[L]}{1 + K_A[L]}$

Scatchard Plot: Y/[L] vs Y
Y/[L] = $-Y/K_D + 1/K_D$
$\nu/[L] = -\nu/K_D + n/K_D$

Hill Plot: log(Y/(1-Y)) vs log[L]
Hill Equation: $\log(Y/(1-Y)) = \log K\pi + n_h \log[L]$

Enzyme Kinetics:

For (E + S <--> ES → E + P)

$V_{MAX} = k_{cat}[E_T]$
$K_M = (k_{-1}+k_{cat})/K_1$

Michaelis-Menton equation: $v = \frac{V_{MAX}[S]}{K_M + [S]}$

Steady State Equation for Enzyme Inhibition: $v = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_M + [S]}$

Double Reciprocal Plot: $\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$

Competitive Inhibition: $\frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$

Noncompetitive Inhibition: $\frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{\alpha'}{V_{MAX}}$

$\alpha = 1+[I]/K_I$ $\alpha' = 1+[I]/K_I'$
$\alpha' = 1$ for competitive inhibition
$\alpha' > 1$ for noncompetitive inhibition

$$\alpha = \frac{slope([I]>0)}{slope([I]=0)} \qquad \alpha' = \frac{y-\text{int}([I]>0)}{y-\text{int}([I]=0)}$$

General Thermodynamics

T = 300K and pH = 7.0 unless otherwise stated.
R = 8.3 J/mol-K S = R ln W
$\Delta G = \Delta H - T\Delta S$ $\Delta G = -RT \ln K_{eq}$

Section A (24 pts, 3 pts/question). Circle the letter corresponding to the best answer.

1. A ligand that binds a macromolecule more tightly than another ligand probably
   a) has a faster on rate.
   b) has a faster off rate.
   c) has a slower on rate.
   d) has a slower off rate.
2. In the binding of O_2 to hemoglobin, 2,3-bisphosphoglycerate
   a) increases the K_D.
   b) stabilizes the tense state of hemoglobin.
   c) is an allosteric inhibitor.
   d) all of the above.
3. When the Hill coefficient, n_h, is greater than 1,
   a) $K_{D1} < K_{D2}$.
   b) $K_{D1} > K_{D2}$.
   c) the binding of the first ligand competes with the binding of the second.
   d) the binding of the second ligand competes with the binding of the first.
4. The affinity with which a macromolecule binds to its ligand can be determined
   a) only at high ligand concentrations.
   b) using a Hill plot.
   c) using a Scatchard plot.
   d) both b and c.
5. An enzyme increases the rate of a reaction by
   a) lowering the free energy of the product(s).
   b) increasing the free energy of the reactant(s).
   c) lowering the free energy of the transition state.
   d) both a and b.
6. The Michaelis-Menton equation
   a) applies to enzyme catalyzed reactions only when substrate is not saturating.
   b) applies to enzyme catalyzed reactions only when [S] >> Km.
   c) only holds for enzyme catalyzed reactions at steady state.
   d) does not apply in the presence of a noncompetitive inhibitor.
7. During the purification of an enzyme,
   a) the V_{MAX} of the enzyme should increase.
   b) the specific activity should increase.
   c) the fraction with the lowest specific activity is carried through to the next step.
   d) both a and b.
8. The rate of migration of a protein in an SDS-PAGE gel
   a) depends on its charge.
   b) depends on its molecular mass.
   c) depends on its charge to mass ratio.
   d) is unaffected by the presence of disulfide bonds between subunits.

B1. (18 pts) Please answer **three** of the following **five** questions. (6 pts each)

1. Explain how an enzyme stabilizes the transition state. Please use the terms enthalpic and entropic in your explanation.

   <u>Enthalpic</u>: An enzyme stabilizes the transition state by directly binding to the distorted transition state intermediate. (3 pts) In the case of the serine proteases, two backbone amide protons in the enzyme's oxy-anion hole interact electrostatically with the negatively charged oxygen in the tetrahedral intermediate.

   <u>Entropic</u>: It would be energetically unfavorable to bring together the reactive groups and the substrate together in close enough proximity to allow the formation of the transition state if these were free in solution. The required decrease in entropy would be too large. Because the active site residues (the catalytic triad) are already positioned in the enzyme, there is no entropic cost associated with positioning these groups. (3 pts)

2. An enzyme lowers the free energy difference between the reactants and the transition state by 30 kJ/mol. What is the rate enhancement provided by the enzyme?

   Transition state theory states that reaction rate is proportional to the transition state complex ($[X^{\pm}]$ or $[EX^{\pm}]$). Because it is assumed that $[X^{\pm}]$ and $[EX^{\pm}]$ are in equilibrium with [S] and [ES], respectively, an equilibrium constant K_{eq} for formation of $[X^{\pm}]$ and $[EX^{\pm}]$ can be written as $K_{eq}=[X^{\pm}]/[S]$ and $K_{eq}[EX^{\pm}]/[ES]$, respectively. Thus v_e (the reaction rate for the enzyme catalyzed reaction) α $K_{eq}[S]$, and v (the reaction rate for the uncatalyzed reaction) α $K_{eq}[ES]$

   Therefore the rate enhancement $\frac{v_e}{v}$ **equals** $\frac{e^{-\Delta G_E/RT}[ES]}{e^{-\Delta G/RT}[S]} = e^{(\Delta G - \Delta G_E)/RT}$ **(3 pts)**

   The reaction is accelerated by a factor of $e^{(30{,}000 J/mol\text{-}K)/8.3 J/mol*300K}=e^{12}$ or **171,000! (3 pts)**

3. Why is it important that an enzyme does not bind its substrate too tightly (ie, that the equilibrium constant, K_A, of ES complex formation is not too large)?

   An ES complex that is of sufficiently low energy would result in the accumulation of both enzyme and substrate in a thermodynamic pit. **The energy required to reach the transition state in this situation may become too large and even approach that of the uncatalyzed reaction. (6 pts) (4 pts were given if it was simply stated that the reaction would not proceed forward from ES to E+P; full credit was reserved for those who mentioned energy in their explanation).**

4. What is the purpose of a Scatchard plot? What is its relationship to a saturation binding curve? Under what assay condition is it imperative to use a Scatchard plot?

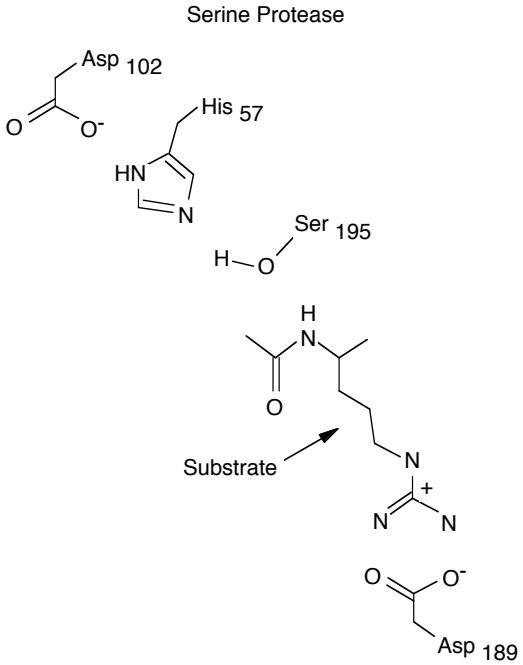
   The Scatchard plot is a linearization of the saturation binding curve (2 pts)

that allows a more accurate determination of K_D (or K_A) from the slope of the line. (2 pts) Importantly, it allows extrapolation of the experimental data to infinite ligand concentrations. It is necessary to use a Scatchard plot when the fractional saturation at high ligand concentrations cannot be experimentally determined (ie, ligand is limiting). (2 pts)

5. What property of proteins are utilized in their separation by either anion or cation exchange chromatography? How are the bound proteins eluted from the column?

Anion exchange and cation exchange resins bind proteins or regions of proteins with a net negative or positive charge, repectively. (3 pts) Bound proteins are eluted from the resin with increasing salt, which effectively shields the electrostatic charges on both the resin and the protein, thereby abolishing their interaction. (3 pts)

B2. (16 pts) The figure to the right shows the active site of the serine protease trypsin.



Part A: Select **three** of the **four** residues and *briefly* discuss its role in the catalytic mechanism. (12 pts)

Asp 102:

Stabilizes the positive charge that develops on His 57 when His 57 deprotonates Ser 195.

His 57:

Activates both Ser 195 and H_2O to nucleophiles by abstracting a proton from both. (Later it acts as an acid by donating a proton to the amino terminus of the cleaved P2 product).

Ser 195:

The first nucleophile in the catalytic mechanism. After activation by His 57, it performs a nucleophilic attack on the electro-positive carbonyl carbon, causing cleavage of the peptide bond. After the first stage, Ser 195 is covalently attached to the substrate as an acyl-enzyme intermediate.

Asp 189:

Lines the substrate binding pocket and defines the specificity of Trypsin by interacting electrostatically with the positive charge on the Lys or Arg side chain of the substrate.

Part B: Select **one** of the following **two** participants in the mechanism and briefly discuss its role. (4 pts)

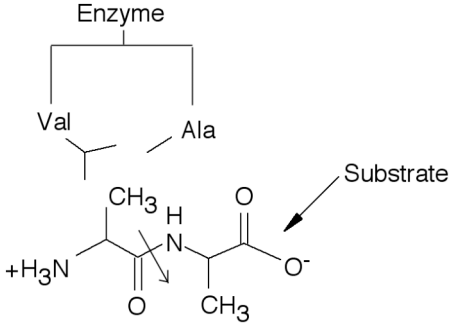
Oxy-anion hole:

Backbone amide protons (partially positively charged) that interact electrostatically with the negatively charged, oxy-anion transition state intermediate. This interaction stabilizes the transition state intermediate, lowering its free energy.

H_2O:

The second nucleophile in the catalytic mechanism. After activation by His 57, OH^- attacks the carbonyl carbon, cleaving the acyl-enzyme intermediate and leading to transfer of the P1 product from Ser195 to itself.

B3. (26 points) Elastase is a serine protease that cleaves peptide bonds following Ala and Gly residues. Shown below is a peptide substrate interacting with the residues (Val and Ala) that line the enzyme's substrate binding pocket.



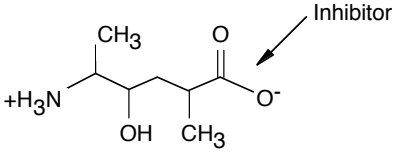
i) Indicate the scissile bond (the bond to be cleaved by the enzyme).
(See diagram) (2 pts)

ii) Elastase exhibits a K_m of 10 μM and a k_{cat} of 1000 s^{-1} for the peptide. What is V_{MAX} when the enzyme concentration $[E_T]$ = 10 nM?
$V_{MAX}=k_{cat}[E_T]$ = $(1000s^{-1})(10\ nM)$ = **10 μM/sec (2 pts)**

iii) A mutant form of the enzyme with a Val to Asp alteration in the substrate binding pocket has been generated. Is this mutation more likely to affect K_m or k_{cat}? *Briefly* justify your answer.

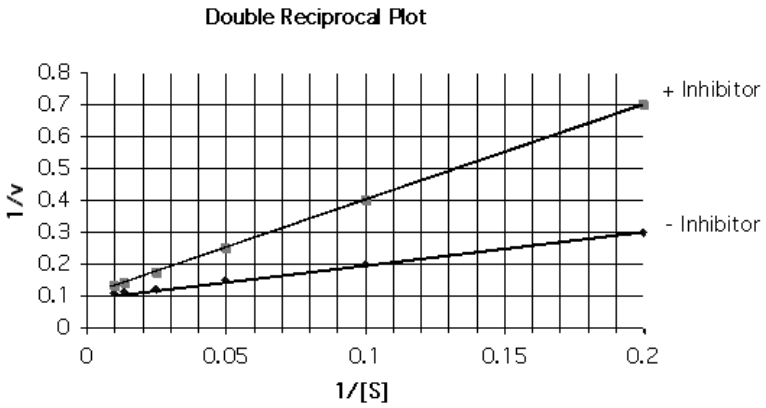
The mutation is predicted to affect substrate binding but not catalysis. Thus only the K_m, largely a measure of substrate binding affinity and similar to K_D for ligand binding, should be affected. K_{cat}, a measure of the ability of the enzyme to perform catalysis, should not be affected. (6 pts)

iii) Elastase is inhibited by the compound shown below (labeled inhibitor). Is this inhibitor likely to be a competitive or a noncompetitive inhibitor? Explain your reasoning. (6 pts)



The compound is likely to be a competitive inhibitor because it is structurally very similar to the actual substrate (4 pts) but lacks the scissile bond (2 pts).

iv) Shown to the right is a double reciprocal plot for elastase in the absence or presence of 10 μM of the above inhibitor. What is the affinity of the inhibitor for the enzyme?



Even if (iii) was answered incorrectly, the fact that the two plots converge at the same y-int indicates that V_{MAX} is unaffected by the inhibitor. Therefore the inhibitor is competitive and its K_I can be determined from α, the degree of inhibition.

α = slope (+ inh) / slope (-inh) = 3. From α, KI is calculate as follows: $\alpha = 1+[I]/KI$.

Thus K_I, the K_D of inhibitor binding to the enzyme, is $[I]/\alpha-1 = 10\ \mu M/(3-1) = 5\ \mu M$.

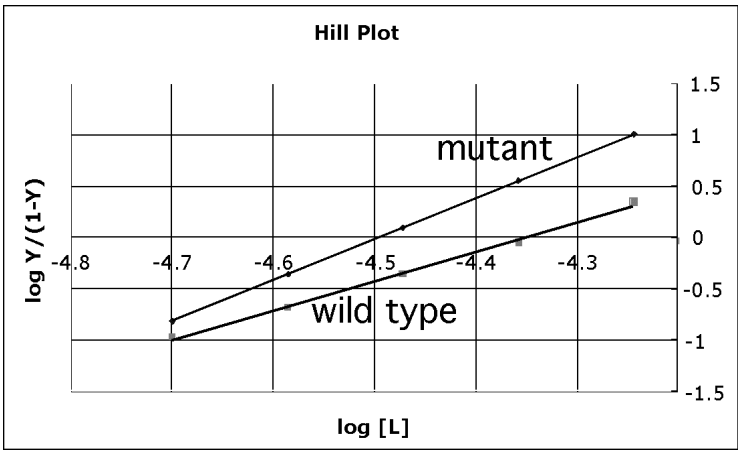
v) Does the inhibitor bind to the ES complex? *Briefly* justify your answer.

No, the inhibitor does not bind ES because it binds to the same site that the substrate binds. (4 pts)

B4. (16 pts) Below is a graph depicting the Hill plot of normal hemoglobin (wild type) and a mutant form of hemoglobin. Both wild type and mutant forms consist of four subunits; each subunit binds one O_2.

i) Which hemoglobin binds O_2 with a higher degree of cooperatively? *Briefly* justify your answer.

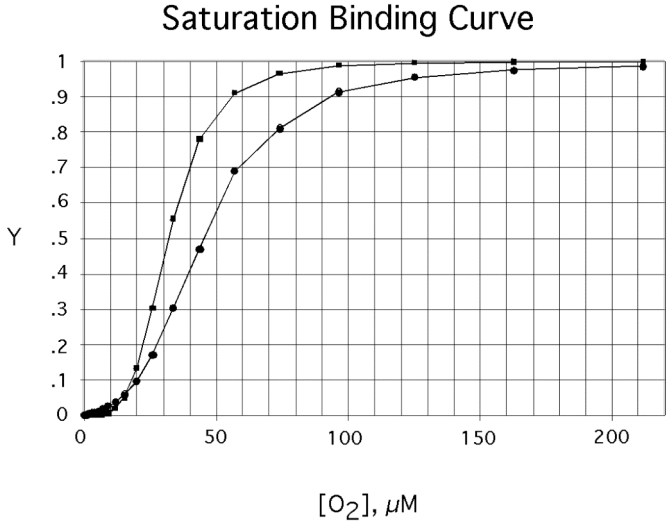
The mutant binds more cooperatively because the slope of the mutant Hill plot, n_h, is greater than that of the wild type. (4 pts)



ii) Which hemoglobin has a higher overall affinity for O_2? *Briefly* justify your answer.

The 'average' K_D of the mutant, $10^{-4.5}$ M, is lower than that of the wild type, $10^{-4.35}$ M. Therefore its affinity for O_2 is higher. (4 pts)

iii) To the right is a graph depicting two saturation binding curves. Based on the information provided above, indicate which curve represents the wild type protein and which curve represents the mutant protein.



The curve on the left corresponds to the mutant because the mutant has a lower average K_D as well as a steeper rise in fractional saturation with increasing ligand concentration, indicative of a greater degree of cooperativity in ligand binding. (4 pts)

iv) Below are five possible liganded forms of hemoglobin. Circle the forms likely to be present when a mutant form of hemoglobin with a Hill coefficient, n_h, of 4 is half saturated with O_2. (4 pts)

For a macromolecule that binds ligand with infinite cooperativity, the binding is all or none and only the unliganded and fully liganded forms are expected. At half saturation, half the molecules would be expected to have no ligand bound while half the molecules would be expected to have four ligands bound.