

## Biochemistry I, Spring Term 2001 - Second Exam answer key

### Section A - 8 Multiple Choice. 24 points.

1.  $K_m$  and  $K_D$  are similar in that:

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

2. In both hemoglobin and myoglobin the oxygen is bound to.

- 3 pts** 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:

- 3 pts** a) show a sigmoidal binding curve
- b) show a hyperbolic binding curve [true for non-cooperative binding]
- c) show a linear Scatchard Plot [true for non-cooperative binding]
- 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
- 3 pts** b) only be found in either the unliganded form or the fully liganded form.
- d) show a Hill coefficient ( $n_H$ ) of 1
- 1 pt** d) show a Hill coefficient ( $n_H$ ) of 4/2

5. The active site of an enzyme

- 1 pt** a) provides amino acid side chains that become modified during the reaction.[only true for serine proteases]
- 3 pts** 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

- 3 pts** 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 in 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.
- 1 pts** b) the enzyme activity of the desired enzyme. [although it doesn't increase, it should stay at the same level.
- 3 pts** c) the specific activity
- ) 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.
- 3 pts** c) the x, y, and z coordinates of most of the atoms in the protein.
- 2 pts** 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.

**Hemoglobin and myoglobin:**

- both have Fe/Heme for oxygen binding
- myoglobin has one oxygen binding site and binds non-cooperatively
- hemoglobin has four sites, binding of oxygen to the first site increases the affinity to subsequent sites - positive cooperativity.
- Positive cooperativity allows full saturation of hemoglobin at the lungs and almost complete release of oxygen at the tissue.

**Bis-phosphoglycerate:**

- Hemoglobin has a single BPG site in deoxy-form
- Site is positively charged, interacting with neg charges on BPG
- BPG stabilizes the deoxy form of hemoglobin, reducing overall affinity
- The reduction in oxygen affinity results in more efficient delivery of oxygen to the tissues at high altitude

**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.

- Rate of reaction is proportional to the concentration of the transition state:
- Enzymes lower the free energy of the transition state, increasing the population of the transition state, therefore increasing the rate.
- Examples:
  1. Serine proteases - Asp/His aid in charge delocalization. Oxyanion hole stabilizes tetrahedral intermediate
  2. HIV protease - proximity of two Asps in the active site decreases the entropy of the transition state compared to the uncatalyzed reaction. Another way of thinking about this is the changes of finding two Asp residues next to a peptide bond in solution is quite rare.

**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?

- Competitive inhibitor binds to the active site and is structurally similar to the normal substrate. Because high levels of substrate can displace the inhibitor, there is no change in  $V_{max}$ , but an increase in  $K_m$
- Non-competitive inhibitor does not bind at the active site. Its binding changes the conformation of the active site. This change in conformation will change  $V_{max}$  and may change  $K_m$
- Either of the above could be used for regulation in biochemical pathways. A competitive inhibitor might be used in feed back inhibition, where a product of a metabolic pathway may resemble the 1st substrate. Non-competitive inhibitors can modify the activity of an enzyme via allosteric control, allowing control of pathways by compounds that don't resemble any of the chemicals produced by the pathway.

**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)

**Starting from the top:**

- **Asp102**
- **His57**
- **Ser195**

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

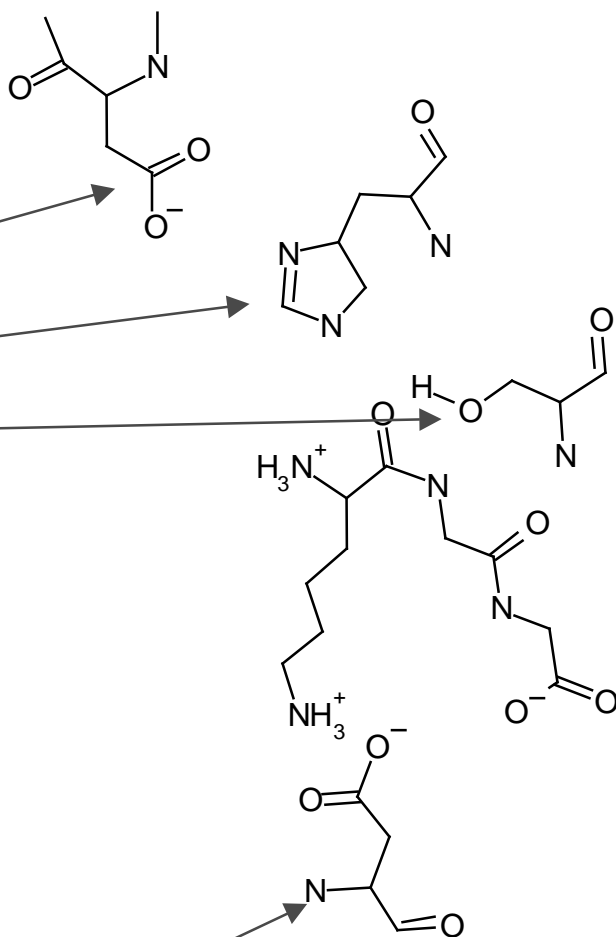
- **Ser:** nucleophile that attacks peptide bond
- **His:** Activates Ser by deprotonation of side chain
- **Asp:** Facilitates protonation of His residue by charge delocalization

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)

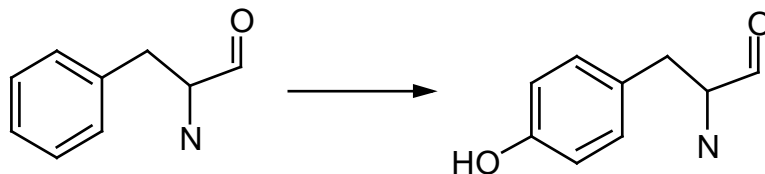
- This is the bottom-most residue, Asp189

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 Val82 to Phenylalanine in the protease (5 pts).

- Replacing the Lysine group in the substrate with Methionine would change the positively charged sidechain to non-polar one of slightly shorter length. To insure that this substrate will bind, you should replace Asp189 with a non-polar group that will have good contacts with the Met sidechain. These would be, in order of suitability: Met, Leu, Ile, Val, Ala. The contribution to binding would include hydrophobic forces and van der Waals interactions.
- In the HIV protease, Val82 contacts a phenyl ring on the drug via a non-polar interaction. If the Val is changed to a Phe, then the interaction will be weakened because the increase in the size of the sidechain (Val to Phe) will push the drug out of the binding pocket. To restore binding affinity, the phenyl group on the drug should be replaced with an isopropyl group (i.e. sidechain of Valine)



**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  $1/[S]$ )

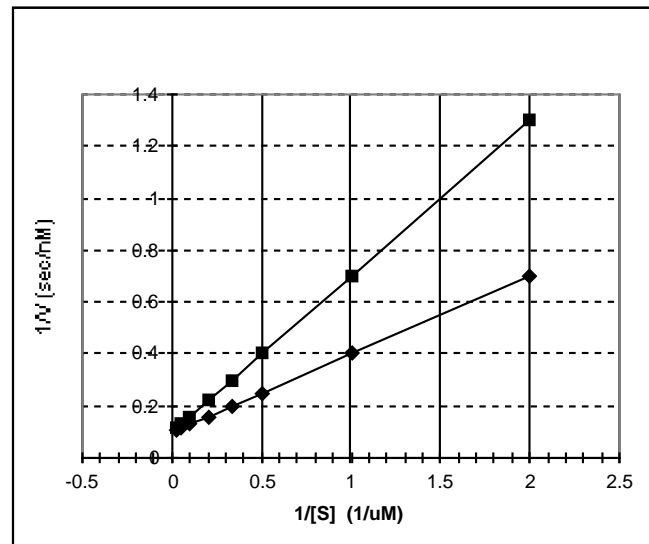
The substrate concentrations are in  $\mu\text{M}$  and the enzyme activity is in units of  $\text{nM}$  product produced/second.

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).

- The upper curve is the one with inhibitor. Since this is a  $1/v$  plot, higher values represent smaller reaction velocities.

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

- This is competitive inhibition since  $V_{\text{MAX}}$  is not affected (both curves have the same y-intercept).



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

- $V_{\text{max}}$  is given by  $1/y$  intercept. Or  $1/0.1 = 10 \text{ nmol/sec}$ .

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

- There are two ways of doing this:
  - The x-axis gives  $1/K_m$ : Intercept is about -0.3, giving a  $K_m$  of  $3.3 \text{ uM}$
  - The slope of the line is  $K_m/V_{\text{max}}$ : slope =  $0.3/1$ . Therefore  $K_m = 3 \text{ uM}$

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

- Since the  $K_m$  observed in the presence of the inhibitor is  $\alpha K_m$ , you need to find the  $K_m$  in the presence of the inhibitor. Again, there are two ways of doing this, from the x-intercept or the slope. Using the slopes is easiest, since the ratio of the two slopes (with and without inhibitor) is  $\alpha$ . The ratio of the slopes is 2. Since  $\alpha = 1 + ([I]/K_I)$ , the inhibitor concentration is equal to the  $K_I$ , or  $5 \text{ uM}$  in this case.

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).

- Anything that was similar to the substrate (or product) would work. Here are some samplings:
  - replacement of OH on phenyl ring with NH, or F, or C or ...
  - replacement of N on backbone with oxygen or hydrogen or carbon ...
  - replacement of phenyl ring with cyclohexane

**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.

- Trypsin: The enzyme is inactive if the His is protonated. Therefore the activity will be low for pH values  $< pK_a$  (7.0), equal to 50% of its value at pH 7.0, 90% of its value at pH 8.0 and close to 100% of its value at higher pH values.
- HIV protease: This enzyme has two Asp residues, one must be deprotonated and the other must be protonated for activity. Therefore, reduced activity would be observed for pH values less than 3 or greater than 6. The highest value of activity would be observed at pH 4.5, the pH value that would give the highest fraction of the active species. The curve would be 'bell shaped'

**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

- Negatively charged beads bind positively charged proteins
- Elute with salt or pH changes to reduce electrostatic interaction

## b) Affinity chromatography

- Beads have a ligand (or antibody) on their surface to which a specific protein binds to.
- Elution with excess ligand or change in salt/pH/solvent polarity to weaken interaction

## c) Gel Filtration chromatography

- Beads have small pores in them that allow smaller proteins to enter the pores while larger ones flow by. Therefore separation by native size occurs. There is no need to change the solvent conditions to elute the protein since the proteins do not stick to the beads.

**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.

- Gel filtration is usually performed under conditions such that the protein is not denatured. In the case of SDS gel electrophoresis the proteins are denatured by the SDS, destroying quaternary (and tertiary) interactions.

**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  $\mu\text{M}$  ( $1 \times 10^{-6} \text{ M}$ ):

$[\text{L}]_{\text{Free}}$	$[\text{L}]_{\text{inside}}$	Y	$Y/[\text{L}]_{\text{Free}}$	$\text{Log}[\text{L}]$	$\text{Log}(Y/(1-Y))$
0.1 $\mu\text{M}$	0.20 $\mu\text{M}$	0.09	1.00	-1.0	-1.0
1.0 $\mu\text{M}$	1.50 $\mu\text{M}$	0.50	0.50	0.0	0.0
3.0 $\mu\text{M}$	3.75 $\mu\text{M}$				
10 $\mu\text{M}$	10.9 $\mu\text{M}$	0.90	0.09	1.0	1.0

i) Determine the fractional saturation, Y, for a ligand concentration of 3.0  $\mu\text{M}$ . Please show your work. (2 pts)

- $Y = [\text{PL}] / ([\text{P}] + [\text{PL}]) = [\text{PL}] / (\text{total protein})$
- The total amount of bound ligand is given by the difference between  $[\text{L}]_{\text{inside}}$  and  $[\text{L}]_{\text{free}}$ , or 0.75  $\mu\text{M}$  in this case. Since the total protein is 1  $\mu\text{M}$ ,  $Y = 0.75$ .

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)

- There are two easy ways to check for cooperative versus non-cooperative binding:
  1. How much does Y change from 0.5 with  $[\text{L}]$  is reduced 10 fold from  $K_D$ ? If a 10 fold drop in  $[\text{L}]$  changes Y from 0.5 to 0.1, then the binding is non-cooperative (this is equivalent to saying that a 100 fold change in ligand concentration about  $K_D$  changes Y from 0.1 to 0.9).
    - If the change in Y was greater (i.e. Y is less than 0.1 for the 10 fold change in  $[\text{L}]$ ), then the binding is positively cooperative
    - If the change in Y was smaller (i.e. Y is greater than 0.1 for the 10 fold change in  $[\text{L}]$ ), then the binding is negatively cooperative
  2. Is the slope of the Hill plot when the plot crosses the x-axis = 1? If so the binding is non-cooperative. In this case the slope is:

$$d\log(Y/1-Y)/d\log[\text{L}] = 1.$$

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

- The  $K_D$  is the ligand concentration that gives  $Y = 0.5$ . *This is true regardless of the nature of the binding.* For non-cooperative binding, this is the actual  $K_D$ . In the case of cooperative binding, this ligand concentration is the 'average'  $K_D$ . The three methods you could have used to get  $K_D$  are:
  1. Ligand concentration when  $Y = 0.5$ ,  $[\text{L}] = 1 \mu\text{M}$ ,  $K_D = 1 \mu\text{M}$
  2. Ligand concentration when Hill plot crosses the x-axis ( $\log(Y/(1-Y)) = 0$ ), this occurs at  $[\text{L}] = 1 \mu\text{M}$ .
  3. Slope of Scatchard plot. The Scatchard plot is  $Y/[\text{L}]$  versus Y. The slope obtained from this data is  $-0.81/0.81 = -1 \mu\text{M}$  (There was a typo on the 1st item of the  $Y/[\text{L}]$  column, it should have read 0.90, if this caused you to lose points, please see me.)