Due Monday February 29, 2016

1. (10 pts, 15 min) Cocaine, a potent drug, is inactivated by base catalyzed ester hydrolysis, generating the inactive species benzoic acid and ecgonine methyl ester, as shown in the reaction scheme to the right. Note that this reaction proceeds through a tetrahedral transition state and that this transition state is in equilibrium with cocaine (As would be the case for (ES) and (EX^+)). Antibodies that bind cocaine can be generated by injecting cocaine that is attached to a protein into a mouse. The protein allows the immune system to generate antibodies against cocaine since the drug injected by itself will not generate antibodies. Antibodies of this type are useful in drug detoxification because they will bind cocaine in the blood and prevent it from interacting with receptors in the brain.

If the phosphate analog of cocaine (shown above) is attached to the protein and injected into the mouse, then antibodies that recognize only the transition state are obtained. The phosphate compound is a transition state analog – it is similar in structure to the transition state. These antibodies increase the rate of the degradation of cocaine are obtained and are therefore catalytic antibodies (often referred to as Abzymes). Why are these antibodies catalytic? [Hint: The reaction rate depends on the concentration of the transition state, how will adding the Abzyme affect the concentration of the transition state?]

2. (8 pts, 10 min) You work for a pharmaceutical company that produces a key synthetic intermediate by enzymatic means. This process uses an enzyme called alcohol dehydrogenase. You have two sources of this enzyme, from yeast or from mushrooms. The steady-state kinetic parameters for these two enzymes are listed in the table shown above.

<table>
<thead>
<tr>
<th></th>
<th>Km = 0.1 mM</th>
<th>kcat = 1000 sec^{-1}</th>
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</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>Km = 0.01 mM</td>
<td>kcat = 100 sec^{-1}</td>
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i) Which enzyme is more adept at performing the chemical step? Why? (1 pt)
ii) Which enzyme binds its substrate more tightly? Briefly justify your answer. (1 pt)
iii) Calculate the rate of product produced for each enzyme assuming a substrate concentration of 0.001 mM. Which enzyme has a higher velocity, i.e. produces more product/unit time. **Assume the enzyme concentration is 1 nM and the volume is 1L.** (2 pts)

iv) Which of the following three parameters: K_m, k_cat, or the ratio of k_cat/K_m, would be more useful to compare the ability of one enzyme over another with respect to product production, assuming a low substrate concentration (|S|<|K_m|)? Briefly justify your answer. (4 pts)

3. (15 pts, 15 min) The plot to the right shows the amount of product produced (µmoles) as a function of time (sec) in an enzyme reaction. The substrate concentrations were 1, 5 and 10 mM.

i) Obtain the initial rate, in units of µmoles product/second, of product formation for all three substrate concentrations. (3 pts)
ii) Plot the initial velocity versus substrate concentration for these data (e.g. plot a velocity curve). Be sure to label the axis (3 pts)
iii) Without constructing a double reciprocal plot, estimate K_m and V_max from the plot in part iii. Briefly describe your approach and note any approximations you may have made. (2 pts)
iv) Now create a double reciprocal plot (1/v versus 1/[S]) to obtain V_max and K_m. How close was your estimate in part iv to the 'true' value. Why do they differ? (3 pts)
vi) Assuming that the enzyme concentration was 5 nM, and that the volume of the reaction was 1 ml, calculate the turn-over number, or k_cat, for this enzyme (2 pts).
4. (8 pts, 15 min) Steady-state enzyme kinetic data was collected using elastase, which is a serine protease. Three different substrates were used, Ala-NP, Leu-NP, and Phe-NP (reaction with Ala-NP is shown to the right). In all three substrates, the enzyme is recognizing the amino acid to the left of the ester, and cleaving the ester. The released nitrophenol ion is bright yellow, allowing measurement of the rate of product formation. The $K_M$ and $k_{CAT}$ parameters for these three substrates are shown in the table on the right.

Explain the trend in both $K_M$ and $k_{CAT}$ for the different substrates. Illustrate your answer with a simple cartoon like drawing (similar to that shown in question 5 below, but with corrections), that illustrates the interaction of elastase with peptide substrates. Your diagram should include some information regarding the specificity pocket (e.g. large or small, non-polar or polar) and the three residues in the catalytic triad, in an appropriate location with respect to the bound substrate. You may find it helpful to visit the Jmol page on Elastase to gain an understanding of the interaction with the sidechain of each substrate with the elastase’s specificity pocket (see problem set links).

5. (14 points, 20 min) The following question will require you to visualize some trypsin structures using Jmol. The URL to the Jmol page can be found on the problem set page. These enzymes have various mutations (amino acid changes) and we are going to assume that they affect either substrate specificity or the catalytic mechanism, but not both (clearly an oversimplification!).

Depending on your last name, select the appropriate mutant enzyme (i.e. Trp2-Trp6) and answer the following questions (if working in a group, select the enzyme that corresponds to the member of your group whose last name is closest to the beginning of the alphabet).


i) State which residue was altered in your assigned protein and how it was altered (Sample answer: Asp189 to Ala) (2 pts).

ii) State whether your mutation will affect either catalysis (i.e. $V_{MAX}$) or the specificity of the enzyme (i.e. $K_M$). Briefly justify your answer (5 pts).

iii) Draw two lines on a double reciprocal plot, one that would represent the wild-type (unmodified) enzyme and one that would represent your altered enzyme (2 pts).

iv) Sketch your enzyme, indicating the location of the amino acid change in your particular enzyme. The level of detail that you should provide is indicated in the sketch on the right. Note that this sketch has several errors and missing functional groups (e.g. atoms are missing from the sidechain of the substrate). Your sketch should portray the correct structure of the enzyme as you would find in the starting (ES) complex (5 pts).

6. (6 pts, 10 min) You measure the $K_M$ (dotted red line) and $V_{MAX}$ (solid black line) for an enzyme (a hypothetical enzyme, not a serine protease) as a function of pH and obtain the data shown in the graph on the right (upper plot). What can you deduce about the most likely residues in the active site? Which type(s) of residues are involved in the mechanism? Which type(s) of residues are involved in interacting with the substrate.

7. (5 pts, 10 min) An aspartyl protease (i.e. NOT a serine protease) has two aspartic acid residues in its active site. The pKa of each of these groups was measured and found to be 4.0 and 6.0. The effect of pH on activity was measured and the data is shown on the right (lower plot). What is the protonation state of each Asp residue in the active form of the enzyme. Justify your answer with reference to the activity versus pH plot.