Problem Set 1

BMB 178
Fall 2015

Due 10/21/2015 (Wed)

1. Consider a general unimolecular reaction, where a substrate (S) is converted to a product (P):

\[ K_{eq} \]
\[ S \xleftrightarrow{K_{eq}} P \]

At 25°C, the equilibrium constant \( K_{eq} \) is 250 and the rate constant of the spontaneous reaction, \( k_{uncat} \), is \( 1.2 \times 10^{-4} \) s\(^{-1}\). The reaction can be catalyzed by an enzyme, E, which provides a \( 10^6 \)-fold rate enhancement without forming detectable intermediates. (20 points)

(a) Calculate the \( \Delta G^\circ \) for the spontaneous and enzyme-catalyzed reaction. (3 points)

(b) Calculate \( \Delta G^\ddagger \) for both the uncatalyzed and enzyme-catalyzed reactions. (5 points)

(c) Draw a free energy diagram to scale showing both the uncatalyzed and catalyzed reactions. Label the substrate, transition states, and product appropriately. Show and label \( \Delta G^\circ \) and \( \Delta G^\ddagger \) for both reactions. (5 points)

(d) Consider the following free energy diagram for a different enzyme catalyzed reaction.

[Diagram of free energy profile with labels for S, P, and intermediates]

Label S, P and the three intermediates on this diagram. (2 points)

The reaction progresses through three intermediates. Which intermediate would be the best structural mimic for designing an inhibitor of the enzyme? Explain your answer. (5 points)
2. Refer to the study by Andrews et al. (PLoS Biol 11(7)). The authors studied interactions between Alkaline Phosphatase (AP) and substrates to elucidate the possible mechanism of enormous rate enhancement achieved by this enzyme. (25 points)

(a) What are some strategies that AP uses to provide such an enormous rate enhancement based on previous studies? (4 points)

(b) Compare scenarios in Figure 2C and 2D. By destabilizing the ground state, the barrier for reaction from E\(\cdot\)S to E\(\cdot\)P is decreased. This of course allows the reaction to proceed faster. What additional effect does this ground state destabilization have in enzyme activity? Why is this important? Think in terms of enzyme recycling. (6 points)

(c) Why did the authors make mutations on R166? What is the function of R166 based on their structural analysis? (5 points)

(d) The authors test pH dependence of P\(_i\) binding to AP. Why does R166S AP show a bell shaped pH dependence? How do the authors verify their prediction for acidic pH region? (5 points)

(e) In the end, the authors estimate the ground state destabilization to be only 10\(^3\)-fold. How do authors justify this result? What are some other possible contributions that help AP to achieve 10\(^{27}\)-fold rate enhancement? (5 points)
3. The catalytic center of serine proteases is universally conserved. There are two major families of structures: trypsin-like serine proteases and subtilisin-like serine proteases. A typical catalytic center for subtilisin-like serine protease contains a ‘catalytic triad’ that includes a serine, a histidine, and an aspartic acid residue. (30 points)

(a) Download the crystal structure for subtilisin BPN' serine protease (PDB: 1lw6, with inhibitor). This crystal structure contains a substrate analogue, CI2, at the active site. Make a figure showing catalytic center of this enzyme with substrate as well as residues in the ‘catalytic triad’ labeled accordingly. Assuming that CI2 is a “normal” substrate, i.e. it can be cleaved by serine protease, which peptide bond would be digested based on the crystal structure? Why? (6 points)

(b) Actually, CI2 acts as an inhibitor to serine proteases. It is proposed that the extensive hydrogen bonds from residue Thr58 to Arg67 provide a stable framework that prevents hydrolysis. Identify this hydrogen bonding network and make a figure. (5 points)

(c) According to the step-wise mechanism of serine proteases, which step or steps would be inhibited by CI2? Explain how each step may potentially be affected by this inhibitor. (5 points)

(d) A series of mutations are introduced into wildtype serine protease, and their catalytic activity is determined as follows:

<table>
<thead>
<tr>
<th>Entry</th>
<th>Mutants</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$K_m$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>wildtype</td>
<td>$(6.3\pm 0.1)\times 10^1$</td>
<td>440±30</td>
</tr>
<tr>
<td>2</td>
<td>S221A</td>
<td>$(5.4\pm 0.3)\times 10^{-5}$</td>
<td>650±90</td>
</tr>
<tr>
<td>3</td>
<td>H64A</td>
<td>$(1.9\pm 0.1)\times 10^{-4}$</td>
<td>1300±150</td>
</tr>
<tr>
<td>4</td>
<td>D32A</td>
<td>$(1.8\pm 0.1)\times 10^{-2}$</td>
<td>1400±120</td>
</tr>
<tr>
<td>5</td>
<td>D32A;H64A</td>
<td>$(1.8\pm 0.1)\times 10^{-3}$</td>
<td>460±40</td>
</tr>
<tr>
<td>6</td>
<td>H64A;S221A</td>
<td>$(5.2\pm 0.2)\times 10^{-5}$</td>
<td>480±60</td>
</tr>
<tr>
<td>7</td>
<td>D32A;S221A</td>
<td>$(5.9\pm 0.3)\times 10^{-5}$</td>
<td>460±80</td>
</tr>
<tr>
<td>8</td>
<td>D32A;H64A;S221A</td>
<td>$(7.8\pm 0.3)\times 10^{-5}$</td>
<td>730±70</td>
</tr>
</tbody>
</table>

(i) Consider the $K_m$ values. What can you conclude about the role of each residue in the catalytic triad in substrate binding? (5 points)

(ii) Consider the $k_{cat}$ data. How much deleterious effect does the S221A mutation cause when this mutation is made in the wildtype protein? How much does the same mutation affect the reaction rate when D32 or H64 is already removed? Similarly, how much does the H64A mutation affect the reaction rate in the context of the wildtype enzyme vs. the D32A mutant? How do you explain this difference? Propose a model for the roles of each residue in this catalytic triad. (9 points)
4. Ketosteroid isomerase catalyzes the following reaction using three key residues: Tyr16, Asp103, and Asp40. (25 points)

(a) Draw a possible electron-pushing mechanism of the isomerization reaction. What are the functions of the three key residues? (7 points)

(b) The ionized form of equilenin shown below serves as a competitive inhibitor of the enzyme. Based on the mechanism that you have proposed above, is equilenin a substrate-analog, a transition-state analog, or a product analog inhibitor of ketosteroid isomerase? Briefly explain. (5 points)

Surprisingly, a simple phenolate molecule can bind tightly to the active site of ketosteroid isomerase. Thus, researchers attempt to separate two different stabilization effects in the transition state: the electrostatic effect caused by the oxy-anion and the positioning effect caused by the rest of the steroid molecule.

(c) Consider the electrostatic effect. Mutation of Asp103 to Ala reduces the rate constant of the reaction ($k_{cat}$) 10^4-fold. What does this result suggest about the catalytic contribution of electrostatic effects to catalysis? How much is the contribution? (5 points)

(d) If electrostatic interactions with the substrate and the enolate-like transition state can be recapitulated by binding of the phenolate molecule, what will fluorine substitutions on the phenolate ring do to the binding between the transition state and the enzyme? (8 points)