Name Key

Chem 452 - Fall 2012 - Exam II

Some potentially useful information:

pK_a values for ionizable groups in proteins: (*α-carboxyl*, 3.1; *α-amino*, 8.0; *Asp* & *Glu* side chains, 4.1; *His* side chain, 6.0; *Cys* side chain, 8.3; *Tyr* side chain, 10.9; *Lys* side chain, 10.8; *Arg* side chain, 12.5)

R (*ideal gas constant*) = 8.314 J/mol•K = 0.08206 L•atm/mol•K

Charge on one electron = 1.602×10^{-19} C

The answer to one of the questions on this exam is -3.3 kJ/mol

1. In class, we discussed a number of strategies used by enzymes to speed up the rates of the reactions they catalyze. In one or two sentences, describe a specific example for each of the following strategies, drawing from the four systems that we discussed in class. Include each of the four systems as an example for at least one of these strategies. (The systems discussed in class include, *chymotrypsin*, *carbonic anhydrase*, the *EcoRV* restriction endonuclease, and the *myosin II ATPase*.)

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a. Covalent catalysis:

The ser 195 in *chymotrypsin* serves as a nucleophile in the proteolysis reactions carried out by serine proteases. It catalyzed the hydrolysis of peptide bonds by attacking the the carbonyl carbon of the peptide bond that is being hydrolyzed. This leads to the formation of an intermediate that is covalently bonded as an ester to the ser 195. The ester is later hydrolyzed to return the enzyme to its initial state.

b. Catalysis by approximation:

Enzymes speed up reactions by placing the various players in a reaction next to one another. The catalytic triad in *chymotrypsin* provides an example. Aspartate 102 forms a hydrogen bond to histidine 57, which plays the role of a general acid base catalyst. The asp 102 orients this histidine so that it can accept a proton from the hydroxyl group of serine 195, turning it into a powerful nucleophile, which can then attack the carbonyl of the peptide bond that is to be hydrolyzed in the reaction. All of the other systems discussed could also be cited as examples.

c. Substrate specificity:

The *EcoRV* endonuclease provides a good example of specificity. It binds non-specifically to a DNA molecule, and then slides along it until finds it recognition. A number of hydrogen bonds then form with the DNA which provides sufficient free energy to kink the DNA and force the phosphate-ribose backbone into the the active site of the enzyme.

d. General acid/base catalysis:

The histidine 57 described in a. above, is one example. *Carbonic anhydrase* provides a second example with a histidine that helps to remove a proton from the active site after it has been removed from the substrate water molecule. Another examples is provided by ser 236 in the *myosin II ATPase*, which also accepts a proton (as a base) from the substrate water after if first donates a proton (as an acid) to the γ-phosphate of the substrate ATP.

e. Metal ion catalysis:

The Zn^{2+} ion in *carbonic anhydrase* is a good example. It binds the substrate water, lowers its *pK* so that it more readily gives up a proton to produce the reactive hydroxide ion. A Mg^{2+} ion plays a similar role in the mechanism for the *EcoRV* restriction endonuclease.

f. A contribution of a buffer component to the rate-limiting step in a reaction:

This was observed with *carbonic anhydrase* for which the presence of the conjugate base component of the buffer has been shown to produce a concentration dependent increase in the rate of the reaction and therefore is playing a role in the mechanism for the reaction.

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2. In 1949, Linus Pauling was the first to propose that genetically linked diseases are cause by structural defects in a protein that a mutated gene codes for. One of the first pieces of evidence to support his claim was provided by the genetically linked sickle-cell hemoglobin disease. Describe the physiological effects of this disease along with the molecular defect that has been determined as its cause.

For individuals that have this disease, their hemoglobin molecules, when in the deoxy state, have a tendency to aggregate or polymerize into long rod shaped structures resembling actin filaments. These rod structures cause the red blood cells to become distended and sickle-shaped and no longer able to deliver oxygen to the tissues. The molecular defect is a single amino acid substitution in the β-subunit, where a glutamate residue is replaced with a valine. This substitution of a charged residue with a non-polar residue creates a hydrophobic patch on the surface of the hemoglobin molecules, which promotes its aggregation with other hemoglobin molecules.

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3. Which of the following effects can be brought about when an enzyme catalyzes the following reaction:

$$S \underset{k_b}{\overset{k_f}{\rightleftharpoons}} P$$

where $K'_{eq} = \dfrac{[P]}{[S]}$, and k_f and k_b are the forward and backward rate constants.

a. The enzyme increases k_f (Yes/No). Yes

b. The enzyme decreases K'_{eq} (Yes/No). No

c. The enzyme increases k_b (Yes/No) Yes

d. The enzyme decreases the activation free energy change, $\Delta G^{\ddagger}$ (Yes/No) Yes

e. The enzyme makes the overall free energy change, $\Delta G'$, more negative (Yes/No) No

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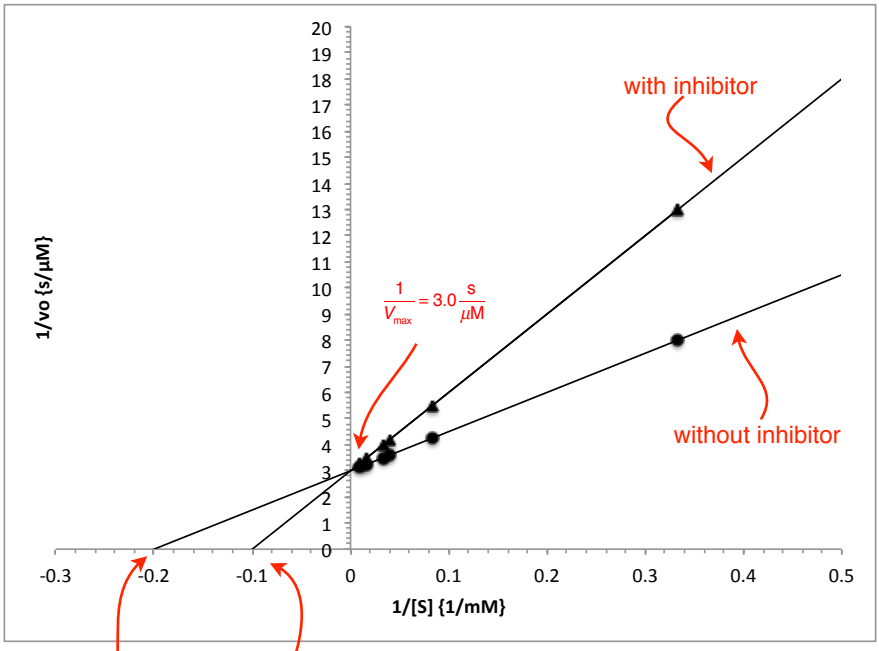
4. Shown below is a Lineweaver-Burke plot displaying the kinetics for an enzyme catalyzed reaction that was conducted with a 100 pM concentration of enzyme in both the absence and presence of a 40 μM concentration of an inhibitor.



a. What type of inhibition is displayed by this inhibitor? Competitive (K_M is affected, but not V_{max})

b. What are the values of V_{max} and K_M in the absence of the inhibitor? (Show your calculations and be sure to include units.)

$$\frac{1}{V_{max}} = 3.0\,\frac{s}{\mu M} \qquad \frac{-1}{K_M} = -0.20\,\frac{1}{mM}$$

$$V_{max} = \frac{1}{3.0}\,\frac{\mu M}{s} = 0.33\,\frac{\mu M}{s} \qquad K_M = \frac{1}{0.20}\,mM = 5.0\ mM$$

c. How many reactions does each enzyme active site catalyze per second when saturated with substrate in the absence of the inhibitor? (Show your calculations and be sure to include units.)

This number is given by the catalytic rate constant, k_{cat}, which can be determined from the V_{max} and the total enzyme concentration:

$$k_{cat} = \frac{V_{max}}{[E]_{total}} = \frac{0.33\,\frac{\mu M}{s}}{100\ pM} = \frac{0.33 \times 10^{-6}\,\frac{M}{s}}{100 \times 10^{-12}\ M} = 3{,}300\,\frac{1}{s}$$

d. Is the maximum rate for the uninhibited reaction limited by the rate at which the substrate diffuses into the active site? No Explain:

This can be assessed by looking at the catalytic efficiency, which is a second-order rate constant, and comparing it to the theoretical maximum value of ~10^8-10^9 1/M•s, which is limited by the rate at which a substrate molecule can enter the active site by simple diffusion. The catalytic efficiency for an enzyme is determined by dividing its k_{cat} value by its K_M value:

$$\text{catalytic efficiency} = \frac{k_{cat}}{K_M} = \frac{3{,}300\,\frac{1}{s}}{5.0\ mM} = \frac{3{,}300\,\frac{1}{s}}{5.0 \times 10^{-3}\ M} = 6.6 \times 10^5\,\frac{1}{M \cdot s}$$

This value for the catalytic efficiency is three orders of magnitude less than the theoretical maximum.

e. Where on the enzyme molecule, relative to the active site, does the inhibitor most likely bind? It is binding at the active site. Explain:

Because the inhibitor lowers the affinity of substrate binding to the active site (it increases the K_M) but not the rate at which the substrate, once bond, is converted to product (V_{max} stays the same), it suggests that the inhibitor is competing for the same binding site on the enzyme molecule as the substrate, *viz.*, it binds to the active site.

5. With the use of site-directed mutagenesis, hemoglobin has been prepared in which the proximal histidine residues in both the α- and the β-subunits have been replaced by glycine. The imidazole ring from the histidine residue can be replaced by adding free imidazole in solution. Would you expect this modified hemoglobin to show cooperativity in oxygen binding? Explain: d

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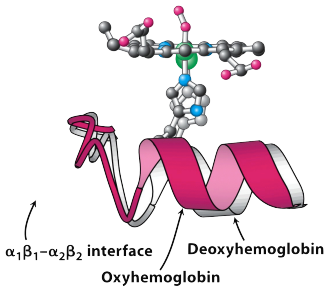
No. When O_2 binds to the heme iron of one of the four subunits, the Fe ion moves up into the plane of the heme. The imidazole ring of the proximal histidine is one of the axial ligands for the the Fe ion (the O_2 is the other). When the Fe ion moves into the ring of the heme it therefore pulls the proximal histidine along with it. This histidine is found in the middle of one of the α-helices. As a consequence, this α-helix becomes displaced when the O_2 binds and disrupts intersubunit interactions at the interface between the hemoglobin subunits. This promotes a conversion from the *tense* to the *relaxed* state in the remaining subunits which increases the binding affinity for O_2 to the heme in those subunits. Removing the imidazole side chain of the proximal histidine eliminates the cooperative binding. Replacing the histidine side chain with free imidazole does not result in a return of the cooperative binding behavior because it is not physically connected to the α-helix.



6. The graph below plots the oxygen saturation curves for hemoglobin under different conditions. The **solid** curve represents the curve observed under physiological conditions of *pH*, concentrations of CO_2, and the metabolite 2,3-bisphosphoglycerate.

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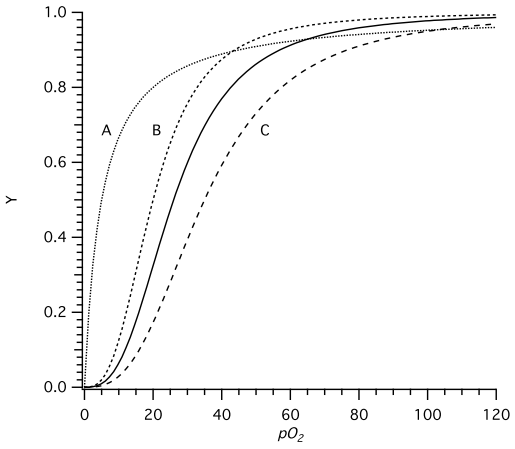
a. Which curve (A, B or C) represents that predicted when metabolic activity leads to an increase in the production of acidic byproducts in the tissues?
(Proton binding a lower *pH* stabilized the tense state. See Figure 7.19.) C

b. Which curve (A, B or C) represents that predicted when the β-subunits of hemoglobin are substituted with the γ-subunits found in fetal hemoglobin?
(The γ-subunits have a preference for the relaxed state compared to the β-subunits. See Figure 7.18.) B

c. Which curve (A, B or C) represents that predicted for the site-directed mutation that was described above in question 5.
(With the loss of cooperatively, the binding curve should revert from sigmoidal to hyperbolic.) A



7. In the second to last reaction of the citric acid cycle fumarate is converted to L-malate:

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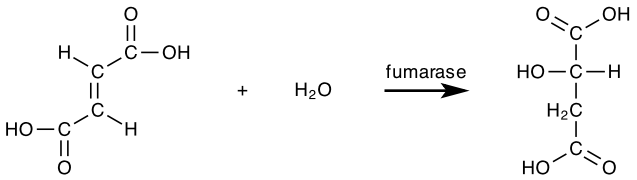

fumarate L-malate

The standard free energy change for this reaction is $\Delta G^{\circ\prime} = +15.6$ kJ/mol, which makes it an unfavorable reaction under standard state conditions.

a. This reaction is catalyzed by the enzyme *fumarase*. What class of enzyme does *fumarase* belong to? *Lyase* (The water is added across a double bond)

b. In a sentence, explain what *fumarase* alone can do to make this reaction favorable under standard state conditions?
As an enzyme catalyst, *fumarase* affects only the activation free energy and not the reaction free energy, consequently, there is nothing that the *fumarase* can do about this situation.

c. The physiological conditions found in most cells are not the standard state conditions. If the concentration of fumarate in a healthy cell at 37°C is 1.5 x 10^{-3} M, while the concentration for L-malate is 1.0 x 10^{-6} M, what is the free energy change, $\Delta G'$, for this reaction under physiological conditions? (Show your work.)

$$\Delta G' = \Delta G^{\circ\prime} + RT\ln\left(\frac{[\text{L-malate}]}{[\text{fumarate}]}\right) = 15.6\ \frac{\text{kJ}}{\text{mol}} + \left(8.314 \times 10^{-3}\ \frac{\text{kJ}}{\text{mol•K}}\right)((273+27)\text{K})\ln\left(\frac{1.0 \times 10^{-6}\text{M}}{1.5 \times 10^{-3}\text{M}}\right)$$

$$\Delta G' = -3.25\ \frac{\text{kJ}}{\text{mol}}$$

d. Is this reaction favorable under physiological conditions? Yes ($\Delta G' < 0$). Explain:
Under physiological conditions the free energy change for the reaction ($\Delta G'$) is less than zero.

8. Associate each of the terms in the left-hand column with a description from the right-hand column:

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a.	7 Kinase	1.	Is a restriction endonuclease
b.	11 Trypsin	2	The more-active state of an allosteric enzyme
c.	3 cAMP	3.	Allosterically activates protein kinase A (PKA)
d.	6 T state	4.	Is a proenzyme
e.	2 R state	5.	Halloween
f.	1 EcoRV	6.	The less-active state of an allosteric enzyme
g.	4 Zymogen	7.	Catalyzes the transfer of a phosphate from ATP to an alcohol
h.	12 Phosphorylation	8.	The (Grateful) Day of the Dead
i.	5 October 31	9.	Hydrolyzes of phosphate ester
j.	9 Phosphatase	10.	Allosterically inhibited by CTP
k.	8 November 2	11.	Activates trypsin
l.	10 ATCase	12.	Is a common covalent modification of proteins

9. Extra credit:
   a. Ask the one question that you were dying to answer on this exam, but which I failed to ask. You will receive up to 1 points for asking a clever and insightful question about enzymes or oxygen transport proteins.

+ 3

   b. You will receive up to 2 points for answering your question correctly.

100 + 3