

Chem 452 - Fall 2012 - Quiz 2

(Take Home, due Monday, 22. Oct)

You may discuss with others strategies for answering these questions, but what you hand in should represent your own work. You must show all calculations to receive full credit. Units are very important.

- According to the Michaelis-Menten equation, what is the v_o/V_{\max} ratio when $[S] = 3 K_M$?
- If $K_M = 3 \text{ mM}$, and $v_o = 35 \text{ } \mu\text{mol}/(\text{mL}\cdot\text{s})$ when $[S] = 3 \text{ mM}$, what is the velocity, v_o , for the reaction when $[S] = 18 \text{ mM}$?
- The following kinetic data were obtained for an enzyme in the absence of an inhibitor, and in the presence of two different inhibitors, (A) and (B), each at a concentration of 10.0 mM . Assume the total enzyme concentration, $[E]_T$, is the same for each experiment.

[S] {mM}	without inhibitor v_o { $\mu\text{mol}/(\text{mL}\cdot\text{s})$ }	with inhibitor A v_o { $\mu\text{mol}/(\text{mL}\cdot\text{s})$ }	with inhibitor B v_o { $\mu\text{mol}/(\text{mL}\cdot\text{s})$ }
0.0	0.0	0.0	0.0
1.0	3.6	3.2	2.6
2.0	6.3	5.3	4.5
4.0	10.0	7.8	7.1
8.0	14.3	10.1	10.2
12.0	16.7	11.3	11.9

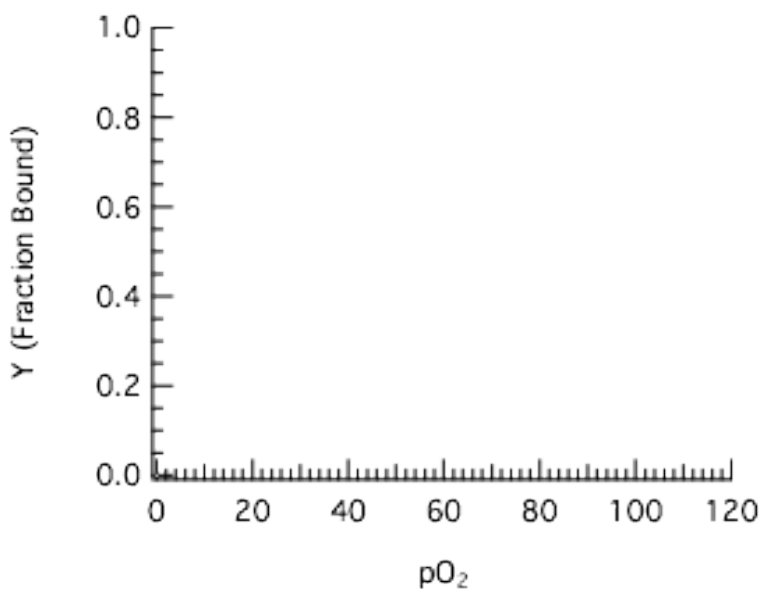
- Determine V_{\max} and K_M for the uninhibited enzyme.
 - Determine the type of inhibition and the dissociation constant, K_I , for inhibitor binding to the enzyme, for the two experiments that contain an inhibitor.
4. *Hexokinase* catalyzes the first reaction in glycolysis and phosphorylates D-glucose to D-glucose 6-phosphate using ATP as the source of the phosphate:



Under conditions of $pH 7$, 25°C and a *Hexokinase* concentration of $3.0 \text{ nmol}/\text{mL}$, the K_M for *Hexokinase* for the substrate glucose was determined to be $3.0 \times 10^{-4} \text{ M}$. When the glucose concentration was set to $160 \text{ } \mu\text{M}$, the initial rate of the reaction was found to be $65.0 \text{ } \mu\text{mol}/(\text{mL}\cdot\text{s})$.

- What is V_{\max} for *Hexokinase* under these conditions?
- What is the *turnover number* for *Hexokinase* under these conditions?
- What is the *catalytic efficiency* for *Hexokinase* under these conditions?

- d. Does *Hexokinase* display “catalytic perfection” under these conditions?
- e. What determines the ultimate speed limit of an enzyme-catalyzed reaction? That is, what is it that imposes a physical limit on catalytic perfection?
- f. In a sentence, describe *Hexokinase* based on its Enzyme Commission (EC) number. For example, the EC number for the enzyme *Chymotrypsin* is 3.4.21.1, which tells us that *Chymotrypsin* (3.4.21.1) is a hydrolase (3.4.21.1) and serine type endopeptidase (3.4.21.1) that cleaves peptide bonds (3.4.21.1).
5. Both myoglobin and hemoglobin function as oxygen binding proteins,
- Each contains an Fe^{2+} ion, which desires to interact with six ligands. Describe the six ligand interactions that an Fe^{2+} ion in oxymyoglobin.
 - The *distal histidine*, while not one of the ligands for the Fe^{2+} ion, nonetheless plays some important roles with respect to oxygen binding by hemoglobin. Describe two of these.
 - Using the axes provided below, illustrate how the binding of oxygen to myoglobin differs from that for hemoglobin. Draw your curves showing myoglobin with a P_{50} of 5 torr and showing hemoglobin with a P_{50} of 25 torr (Be sure to label your curves.)



- Explain how the behaviors illustrated above optimize myoglobin and hemoglobin for their different physiological roles.
- If the $p\text{O}_2$ in the lungs is 100 torr, and the $p\text{O}_2$ in active muscles is 25 torr, assuming a *Hill coefficient* of $n = 2.8$ for hemoglobin, what percentage of the O_2 picked up by the hemoglobin in the lungs will be released to the myoglobin in the muscles?
- When muscles are actively oxidizing food stuffs to extract the chemical energy they need for muscle contractions, they produce acidic byproducts, which decreases the $p\text{H}$ in the muscle tissues.
 - Describe the effect that this has on the structure of hemoglobin.
 - Describe the effect that this has for the P_{50} for hemoglobin.