Name Key

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.

1. According the Michaelis-Menten equation, what is the v_o/V_{max} ratio when [S] = 3 K_M?

2/2

Starting with the Michaelis-Menten equation:

$$K_M\left(1+\frac{[I]}{K_I}\right)$$

Substitute [S]= 3 K_M

$$\frac{v_o}{V_{max}}=\frac{3\,K_M}{1\,K_M+3\,K_M}$$

$$=\frac{3}{1+3}$$

$$\frac{v_o}{V_{max}}=\frac{3}{4}$$

2. If K_M = 3 mM, and v_o = 35 µmol/(mL•s) when [S] = 3 mM, what is the velocity, v_o, for the reaction when [S] = 18 mM?

2/2

Starting with the Michaelis-Menten equation:

$$v_o=\frac{V_{max}[S]}{K_M+[S]}$$

We could substitute the values we have for K_M, v_o and [S] and solve for V_{max} or we could simply recognize that since both [S] and K_M equal 3 mM, v_o = 35 µmol/(mL•s) must be the half-maximum velocity, which makes V_{max} = 70 µmol/(mL•s).

Since [S] = 18 mM is equal to 6 K_M,

$$v_o=\frac{V_{max}[S]}{K_M+[S]}$$

$$\frac{v_o}{V_{max}}=\frac{6\,K_M}{1\,K_M+6\,K_M}=\frac{6}{1+6}=\frac{6}{7}$$ (see Problem 1)

$$v_o=V_{max}\left(\frac{6}{7}\right)=(70\ \mu mol/(mL\cdot s))\left(\frac{6}{7}\right)=60\ \mu mol/(mL\cdot s)$$

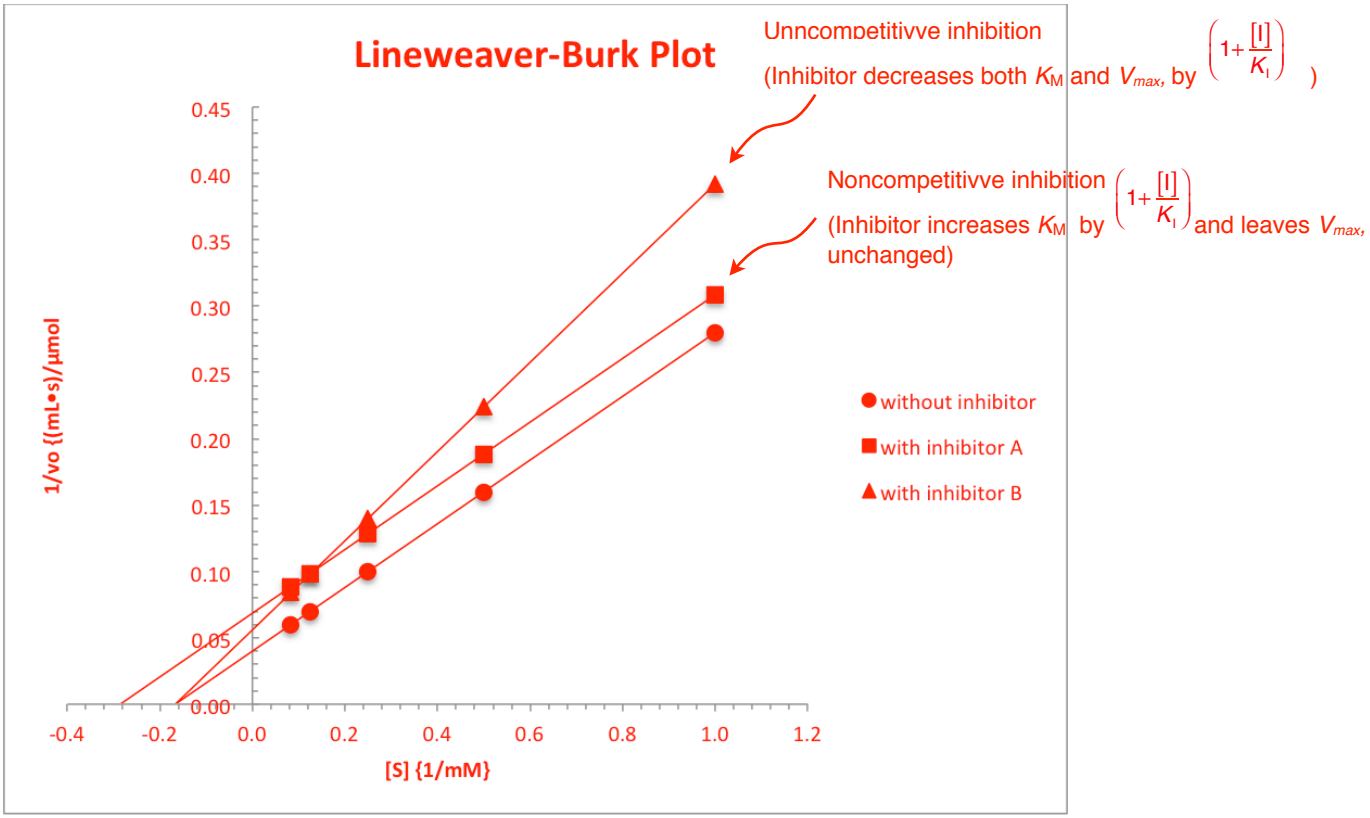
3. The following kinetic data were obtained for an enzyme in the absence of an inhibitor, and in the presence of two different inhibitors, (A) and (B), each at a concentration of 10.0 mM. Assume the total enzyme concentration, $[E]_T$, is the same for each experiment.

10/10

[S] {mM}	without inhibitor v_o {µmol/(mL•s)}	with inhibitor A v_o {µmol/(mL•s)}	with inhibitor B v_o {µmol/(mL•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

a. Determine V_{max} and K_M for the uninhibited

without inihibitor		with inibitor A		with inhibitor B	
[S]	vo	[S]	vo	[S]	vo
0.0 mM	0.0 µmol/(mL•s)	0.0 mM	0.0 µmol/(mL•s)	0.0 mM	0.0 µmol/(mL•s)
1.0 mM	3.6 µmol/(mL•s)	1.0 mM	3.2 µmol/(mL•s)	1.0 mM	2.6 µmol/(mL•s)
2.0 mM	6.3 µmol/(mL•s)	2.0 mM	5.3 µmol/(mL•s)	2.0 mM	4.5 µmol/(mL•s)
4.0 mM	10.0 µmol/(mL•s)	4.0 mM	7.8 µmol/(mL•s)	4.0 mM	7.1 µmol/(mL•s)
8.0 mM	14.3 µmol/(mL•s)	8.0 mM	10.1 µmol/(mL•s)	8.0 mM	10.2 µmol/(mL•s)
12.0 mM	16.7 µmol/(mL•s)	12.0 mM	11.3 µmol/(mL•s)	12.0 mM	11.9 µmol/(mL•s)
1/[S]	1/vo	1/[S]	1/vo	1/[S]	1/vo
1.000 1/mM	0.280 (mL•s)/µmole	1.000 1/mM	0.309 (mL•s)/µmole	1.000 1/mM	0.392 (mL•s)/µmole
0.500 1/mM	0.160 (mL•s)/µmole	0.500 1/mM	0.189 (mL•s)/µmole	0.500 1/mM	0.224 (mL•s)/µmole
0.250 1/mM	0.100 (mL•s)/µmole	0.250 1/mM	0.129 (mL•s)/µmole	0.250 1/mM	0.140 (mL•s)/µmole
0.125 1/mM	0.070 (mL•s)/µmole	0.125 1/mM	0.099 (mL•s)/µmole	0.125 1/mM	0.098 (mL•s)/µmole
0.083 1/mM	0.060 (mL•s)/µmole	0.083 1/mM	0.089 (mL•s)/µmole	0.083 1/mM	0.084 (mL•s)/µmole
Determined values					
K_M =	6.0 mM	$(K_M)_{app}$ =	3.5 mM	K_M =	6.0 mM
V_{max} =	25.0 µmol/(mL•s)	$(V_{max})_{app}$ =	14.6 µmol/(mL•s)	$(V_{max})_{app}$ =	17.9 µmol/(mL•s)
K_I =		K_I =	14.0 mM	K_I =	25.0 mM



b. 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.

Inhibitor A is an uncompetitive inhibitor. We know this because both K_M and V_{max} are decreased by the same amount, which is $\left(1+\frac{[I]}{K_I}\right)$:

Can solve for K_I using either $(V_{max})_{app} = \frac{V_{max}}{\left(1+\frac{[I]}{K_I}\right)}$ or $(K_M)_{app} = \frac{K_M}{\left(1+\frac{[I]}{K_I}\right)}$

$$\frac{V_{max}}{(V_{max})_{app}} = \left(1+\frac{[I]}{K_I}\right)$$

$$K_I = \frac{[I]}{\left(\frac{V_{max}}{(V_{max})_{app}}-1\right)} = \frac{10\text{ mM}}{\left(\frac{25.0\ \mu\text{mol/(mL•s)}}{14.6\ \mu\text{mol/(mL•s)}}-1\right)} = 14.0\text{ mM}$$

c. Inhibitor B is a non competitive inhibitor. We know this because only V_{max} is decreased by a factor of $\left(1+\frac{[I]}{K_I}\right)$:

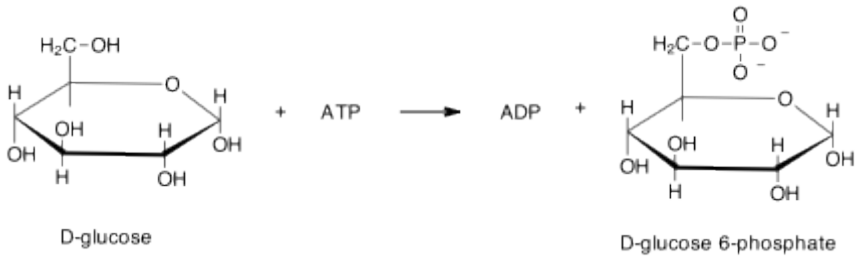
Can solve for K_I using $(V_{max})_{app} = \frac{V_{max}}{\left(1+\frac{[I]}{K_I}\right)}$

$$\frac{V_{max}}{(V_{max})_{app}} = \left(1+\frac{[I]}{K_I}\right)$$

$$K_I = \frac{[I]}{\left(\frac{V_{max}}{(V_{max})_{app}}-1\right)} = \frac{10\text{ mM}}{\left(\frac{25.0\ \mu\text{mol/(mL•s)}}{17.9\ \mu\text{mol/(mL•s)}}-1\right)} = 25.0\text{ mM}$$

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:

6/6



D-glucose D-glucose 6-phosphate

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

a. What is V_{max} for *Hexokinase* under these conditions?

$$v_o = \frac{V_{max}[S]}{K_M+[S]} = \frac{V_{max}[S]}{K_M+[S]}$$

$$V_{max} = v_o\frac{(K_M+[S])}{[S]} = \left(65.0\ \frac{\mu\text{mol}}{\text{mL•s}}\right)\left(\frac{(3.0\text{x}10^{-4}\text{ M}+160\ \mu\text{M})}{160\ \mu\text{M}}\right) = 186\ \frac{\mu\text{mol}}{\text{mL•s}}$$

b. What is the *turnover number* for *Hexokinase* under these conditions?

$$\text{turnover number} = k_{cat} = \frac{V_{max}}{[E]_{total}} = \frac{186\,\frac{\mu mol}{mL \cdot s}}{3.0\,\frac{nmol}{mL}} = \frac{186 \times 10^{-6}\,\frac{mol}{mL \cdot s}}{3.0 \times 10^{-9}\,\frac{mol}{mL}} = 62{,}000\,/\,s$$

c. What is the *catalytic efficiency* for *Hexokinase* under these conditions?

$$\text{catalytic efficiency} = \frac{k_{cat}}{K_M} = \frac{62{,}000/s}{3.0 \times 10^{-4}\ M} = 2.1 \times 10^{8}\,/\,(M \cdot s)$$

d. Does *Hexokinase* display "catalytic perfection" under these conditions?

The catalytic efficiency is greater than $10^8/(M \cdot s)$, which puts it in the range that qualifies it to be considered "catalytic perfection".

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?

When an enzyme is catalytically perfect, the reaction rate has become dependent on the rate at which the substrate is able to diffuse into the active site. This rate places an upper limit of $10^8/(M \cdot s)$ to $10^9/(M \cdot s)$ on the catalytic efficiency of an enzyme catalyzed reaction. Beyond this, there is nothing that evolution can do to further increase the rate at which the enzyme catalyzes the reaction.

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).

The E.C. number for *Hexokinase* is 2.7.1.1, which tells us that *Hexokinase* (2.7.1.**1**) is a transferase (**2**.7.1.1) that transfers a phosphate group (2.**7**.1.1) to an alcohol group as the acceptor (2.7.**1**.1) to produce a phosphate ester.

5. Both myoglobin and hemoglobin function as oxygen binding proteins,

5/5

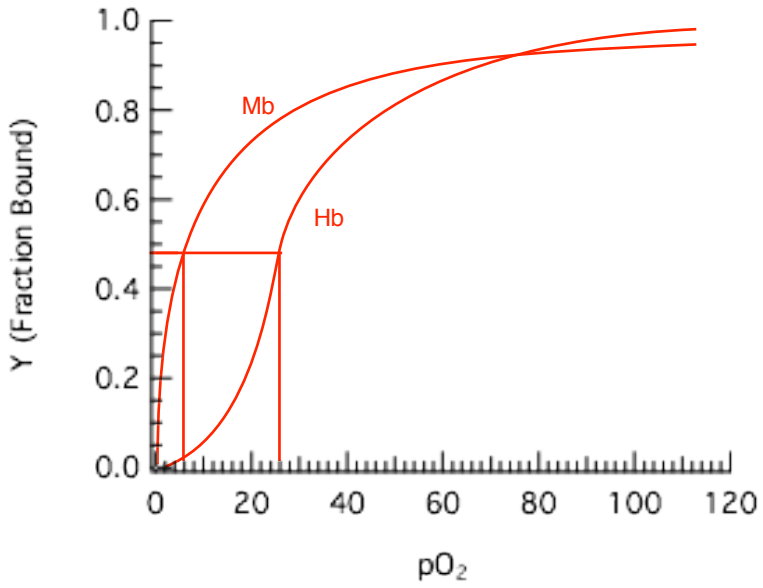
a. Each contains an Fe^{2+} ion, which desires to interacts with six ligands. Describe the six ligand interactions that an Fe^{2+} ion in oxymyoglobin.

The six ligand from an octahedral around the Fe^{2+}. Four of the ligands are the nitrogens provided by the porphyrin ring and all lie in the same ring. The fifth ligand is a nitrogen from a histidine side chain (the proximal histidine) and the six ligand is used bind the oxygen molecule.

b. 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.

The distal histidine's side chain imidazole sits near the site where the O_2 binds. (1) It hydrogen bonds to the bound O_2 and helps prevent it being release as a destructive super oxide radical (O_2^-), which would also leave the iron as Fe^{3+}, and kill the myoglobin as well. (2) The distal histidine also forces the sixth ligand that binds to the heme group to bind at an angle. This is not an issue for the intended oxygen ligand, which prefers to bind at an angle, but it, in particular, lowers the affinity for the toxic carbon monoxide (CO), which prefers to bind straight on, at right angles to the heme group.

c. 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.)



d. Explain how the behaviors illustrated above optimize myoglobin and hemoglobin for their different physiological roles.

The oxygen-binding role for Hb is to circulate in the blood and pick up O_2 in the lungs and deliver it to the tissues, such as muscles, where it passes the O_2 over to a Mb molecule, which then holds on to it until needed. The binding curve for Hb shows that it can become nearly fully saturated with O_2 when in the lung, where the pO_2 for oxygen is around 100 torr. As the O_2-bound Hb moves out the the tissues, the pO_2 levels fall. In the process, the Hb's affinity for O_2 falls off more rapidly than that for Mb. This helps to optimize Hb's ability to then transfer its O_2 cargo to an awaiting Mb molecule.

e. If the pO_2 in the lungs is 100 torr, and the pO_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?

The fraction bound by both Mb and Hb are described by the following equations:

For Mb: $Y = \dfrac{pO_2}{P_{50} + pO_2}$ and for Hb: $Y = \dfrac{(pO_2)^n}{(P_{50})^n + (pO_2)^n}$, where n is the Hill coefficient.

In the lung, Mb: $Y = \dfrac{pO_2}{P_{50} + pO_2} = \dfrac{100 \text{ torr}}{5 \text{ torr} + 100 \text{ torr}} = 0.95 = 95\%$

Hb: $Y = \dfrac{(pO_2)^n}{(P_{50})^n + (pO_2)^n} = \dfrac{(100 \text{ torr})^{2.8}}{(25 \text{ torr})^{2.8} + (100 \text{ torr})^{2.8}} = 0.98 = 98\%$

In the muscles, Mb: $Y = \dfrac{pO_2}{P_{50} + pO_2} = \dfrac{25 \text{ torr}}{5 \text{ torr} + 25 \text{ torr}} = 0.83 = 83\%$

Hb: $Y = \dfrac{(pO_2)^n}{(P_{50})^n + (pO_2)^n} = \dfrac{(25 \text{ torr})^{2.8}}{(25 \text{ torr})^{2.8} + (25 \text{ torr})^{2.8}} = 0.50 = 50\%$

The fraction of O_2 that is bound by Hb in the lung that will be released when it reaches the muscle is 0.98 - 0.50 = 0.48, or 48%

f. When muscles are actively oxidizing food stuffs to extract the chemical energy they need for muscle contractions, they produce acidic byproducts, which decreases the *pH* in the muscle tissues.

i. Describe the effect that this has on the structure of hemoglobin.

When the *pH* decreases the hydrogen ion concentration increases, causing ionizable groups on the Hb to become protonated. In particular, this alters the charge/charge and hydrogen bonding interactions that exist along the interface between the αβ dimers in the intact protein. These interactions, in turn, stabilize the tense state of Hb, which has the weaker affinity for O_2. This consequently, allows Hb to deliver more O_2 to the tissues.

ii. Describe the effect that this has for the P_{50} for hemoglobin.

sThese interactions, in turn, stabilize the tense state of Hb, which has the weaker affinity for O_2. This consequently, allows Hb to deliver more O_2 to the tissues.

25/25