Biochemistry 463, Summer II   Your Name: _____

University of Maryland, College Park   Your SID #: _____

Biochemistry and Physiology   **Profs. Douglas Julin and Jason Kahn**

Exam II (100 points total)   **August 11, 2008**

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be <u>concise</u> and <u>clear</u>. I have given you more space than you should need. There is extra space on the last page if you need it.

You will need a calculator for this exam. No other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Useful Equations:

$$\Delta S_{system} - \Delta H_{system}/T \geq 0 \qquad pH = -\log([H^+]) \qquad E = mc^2 \qquad e^{i\pi} + 1 = 0$$

$$S = k\ln W \qquad \Delta G = \Delta H - T\Delta S \qquad pH = pK_a + \log([A^-]/[HA])$$

$$K_a = [H^+][A^-]/[HA] \qquad \Delta G° = -RT\ln K_{eq} \qquad \Delta G = \Delta G° + RT\ln Q$$

$$v_0 = \frac{(1/\alpha')V_{max}[S]}{(\alpha/\alpha')K_M + [S]}, \text{where } \alpha = 1 + \frac{[I]}{K_I} \text{ and } \alpha' = 1 + \frac{[I]}{K_I'}$$

<u>Honor Pledge: At the end of the examination time , please write out the following sentence and sign it, or talk to me about it:</u>

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1.   (20 pts) BPG and oxygen transport

High-altitude training can enhance the performance of endurance athletes. Part of the adaptation to high altitude is an increase in BPG levels.

(a; 6 pts) Sketch the thermodynamic linkage relationship that explains how BPG binding to the T state decreases O_2 binding to Hb.

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(b; 5 pts) Sketch oxygen binding curves showing how decreasing O_2 binding to Hb actually leads to increased O_2 delivery.
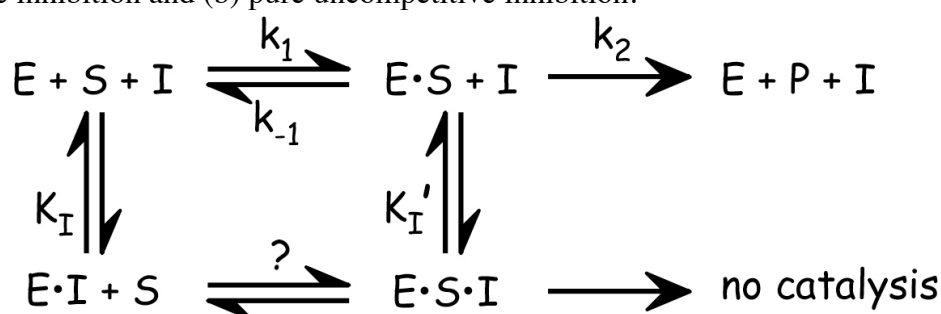
(c; 6 pts) What is the idea behind "Live High, Train Low" programs and devices? (Hint: performance records in aerobic sports are not achieved at the "High" altitude.) Briefly describe one such device.

(d; 3 pts) Do you think "Live High, Train Low" should be permitted in sports? We don't need no stinkin' litmus tests here: Yes, No, or Maybe is fine, but defend your answer briefly:

Score for the page_____

2. (30 pts) Enzymatic catalysis, Michaelis-Menten kinetics, and Inhibition

   (a; 6 pts) What is the SSA, and what must be true of an intermediate in a reaction mechanism for the SSA to be applicable to it?

   (b; 4 pts) On the scheme below, identify which interconversions (i.e. steps) are relevant to (a) pure competitive inhibition and (b) pure uncompetitive inhibition.

$$E + S + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S + I \overset{k_2}{\longrightarrow} E + P + I$$

$$K_I \updownarrow \qquad\qquad K_I' \updownarrow$$

$$E{\cdot}I + S \overset{?}{\rightleftharpoons} E{\cdot}S{\cdot}I \longrightarrow \text{no catalysis}$$

   (c; 8 pts) On one graph, sketch the Lineweaver-Burke plots for three concentrations of a non-competitive inhibitor ($\alpha = \alpha'$) as well as the uninhibited enzyme. An irreversible inhibitor has the same characteristic L-B plot as a non-competitive inhibitor. Experimentally, how could you distinguish between an irreversible inhibitor and a reversible noncompetitive inhibitor?

Score for the page_____

(d; 5 pts) From among the graphs below, identify which two free energy profiles describe enzymes and which one describes a binding protein, and give the reason behind your answer (one reason total).

Enzymes: _____ and _____   Binding protein: _____

Reasoning:
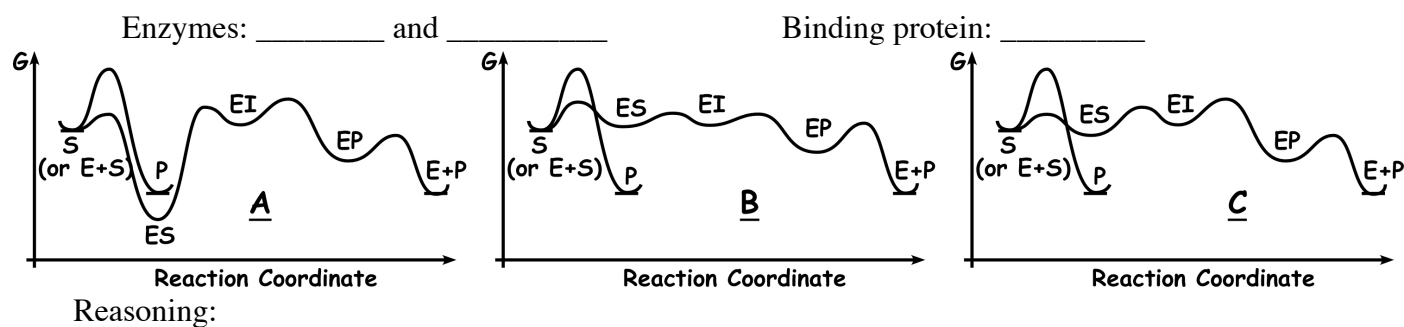
(e; 7 pts) What is the definition of catalytic perfection for an enzyme, and which kinetic parameter or combination of kinetic parameters do we evaluate to decide whether an enzyme is perfect? Which step in the basic M-M mechanism has a rate that is limited by diffusion? Which of the schemes above shows a catalytically perfect enzyme?_____

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3.   (8 points) Reaction Types

Six basic types of reaction that occur throughout metabolism are given in the textbook:

1. Oxidation-reduction   4. Group transfer
2. Ligation requiring ATP cleavage   5. Hydrolytic
3. Isomerization   6. Addition/removal of functional groups

Examine each of the reactions given below and identify the reaction type that <u>best describes</u> the reaction shown. (Note – the complete, balanced reaction is not necessarily shown!)
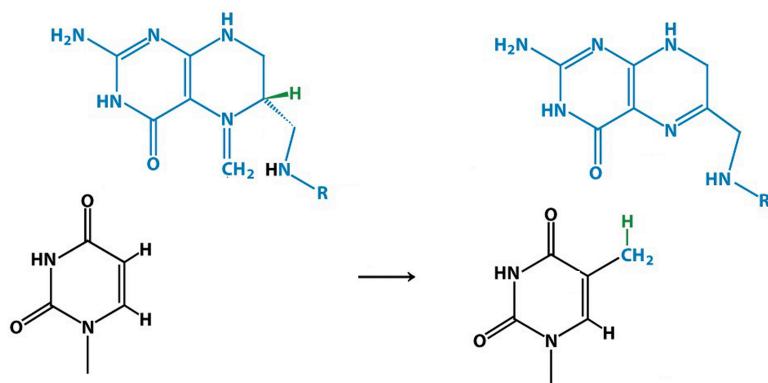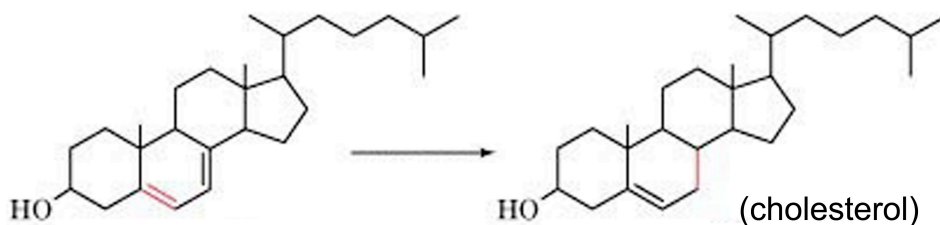
(a)   Reaction type: _____
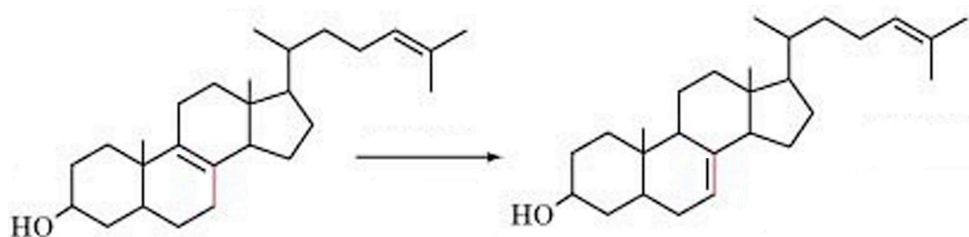
(b)   Reaction type: _____

(c)   Reaction type: _____

(d)   Reaction type: _____

(cholesterol)

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4. (14 points) Bioenergetics

(**a; 3 points**) It is very difficult to determine the equilibrium constant (K'_{eq}) for the hydrolysis of ATP by measuring the concentrations of reactants and products, since there is very little ATP present at equilibrium. Instead, K'_{eq} for this reaction can be determined by measuring K'_{eq} for the following two separate reactions:

Glucose-6-phosphate + H_2O	$\Leftrightarrow$	glucose + P_i	$K'_{eq} = 270$
ATP + glucose	$\Leftrightarrow$	ADP + glucose-6-phosphate	$K'_{eq} = 890$

Using this information, calculate the value of K'_{eq} and of $\Delta G'^{\circ}$ for ATP hydrolysis at 25 °C (298 K):
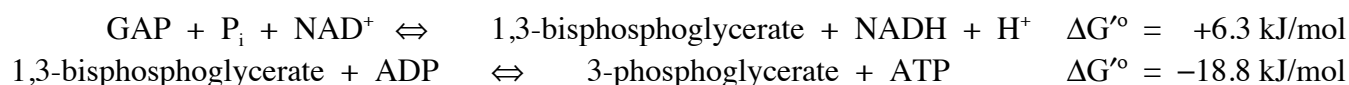
$$ATP + H_2O \Leftrightarrow ADP + P_i$$
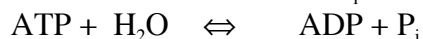
Show your work. Remember that R = 0.0083 kJ/mol K.

(**b; 4 points**) Cells contain a number of enzymes called ATPases. These are enzymes that catalyze the hydrolysis of ATP, usually coupling the free energy to some other process. Since all enzymes catalyze both the forward and reveres reactions, these ATPases are also able to catalyze the formation of ATP from ADP and P_i:

$$ADP + P_i \Leftrightarrow ATP + H_2O$$
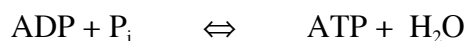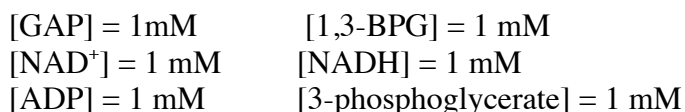
Suppose the [ADP] in the cell were 1 mM, and the cell relied on this ATPase enzyme to synthesize its ATP, by the reaction shown above. What concentration of P_i would be required for the cell to maintain $[ATP] = 10^{-6}$ M (1 µM)? Show your work.

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(#4c; 7 points) The cell actually uses the reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase to make ATP:

$$GAP + P_i + NAD^+ \Leftrightarrow \quad 1,3\text{-bisphosphoglycerate} + NADH + H^+ \quad \Delta G'^o = +6.3 \text{ kJ/mol}$$
$$1,3\text{-bisphosphoglycerate} + ADP \quad \Leftrightarrow \quad 3\text{-phosphoglycerate} + ATP \quad \Delta G'^o = -18.8 \text{ kJ/mol}$$

Suppose the cellular concentrations are:

$\quad\quad$ [GAP] = 1mM $\quad\quad$ [1,3-BPG] = 1 mM
$\quad\quad$ [NAD$^+$] = 1 mM $\quad\quad$ [NADH] = 1 mM
$\quad\quad$ [ADP] = 1 mM $\quad\quad$ [3-phosphoglycerate] = 1 mM

What [P$_i$] would be required to maintain the [ATP] = **3 mM** in the cell?

5. (20 points) Glycolysis

(a; 12 points) Listed below are three enzymes from the glycolysis pathway. For **any two (your choice)** of these enzymes, draw the complete structure (including all H atoms) of the reactants and products for the reaction catalyzed by that enzyme, and write the names of the reactants and products. You must draw the complete structure of phosphate once; after that you may just write P inside a circle (as I do in class). You may draw sugars in either the ring or open chain form. You must include molecules such as ATP, NAD$^+$, etc., if they are involved in a reaction, but you need not draw their structures.

$\quad\quad$ **i) phosphoglucose isomerase** $\quad\quad$ **ii) aldolase** $\quad\quad$ **iii) pyruvate kinase**

$\quad\quad$ **Answer 1:**

Score for the page_____

#5a, Answer 2:

(#5b; 8 points) Propose and draw a mechanism by which **either (your choice)** phosphoglucose isomerase or aldolase (**not** pyruvate kinase) might catalyze its reaction. Include in your mechanism any intermediates that might form during the reaction, "push arrows" to show how the reaction occurs, and include specific amino acid side chains that might participate in the reaction. Describe clearly in words, and/or in your drawing, what these side chains are doing in the reaction.

6.   (8 points) Regulation of Metabolism

The figure drawn below shows a hypothetical metabolic pathway. A few of the intermediate compounds are named, but most are not. The arrows indicate enzyme-catalyzed reactions, and the enzymes are indicated as E1, E2, etc. The directions of the arrows indicate the direction of the reaction under normal cellular conditions.

(a; 4 points) The well-known compound **whozywhatsit** would be a feedback, allosteric inhibitor of one enzyme in the pathway. Which enzyme? Explain, briefly.
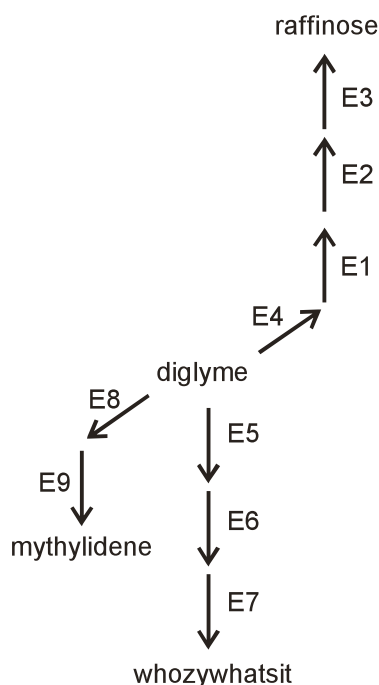
(b; 4 points) There is a second enzyme that might be inhibited (allosterically) by raffinose, but activated (allosterically) by mythylidene. Which enzyme? Explain briefly why this enzyme would be regulated in this way by these two compounds.

Score for the page_____

Page	Score
1	/5
2	/18
3	/13
4	/15
5	/10
6	/19
7	/16
8	/4
Total	**/100**

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