Biochemistry 463, Summer II **Your Name:** _____

University of Maryland, College Park **Your SID #:** _____

Biochemistry and Physiology **Profs. Doug 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 concise and clear. 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:

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

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

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

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

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

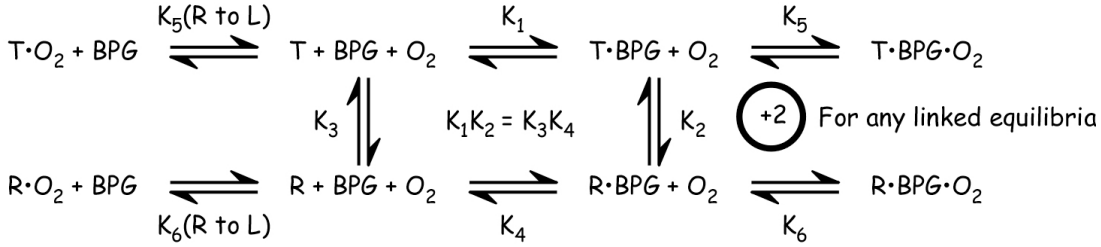
"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

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.

We assume BPG binding does not change O_2 binding to T or R (K_5 and K_6) and $K_5 << K_6$

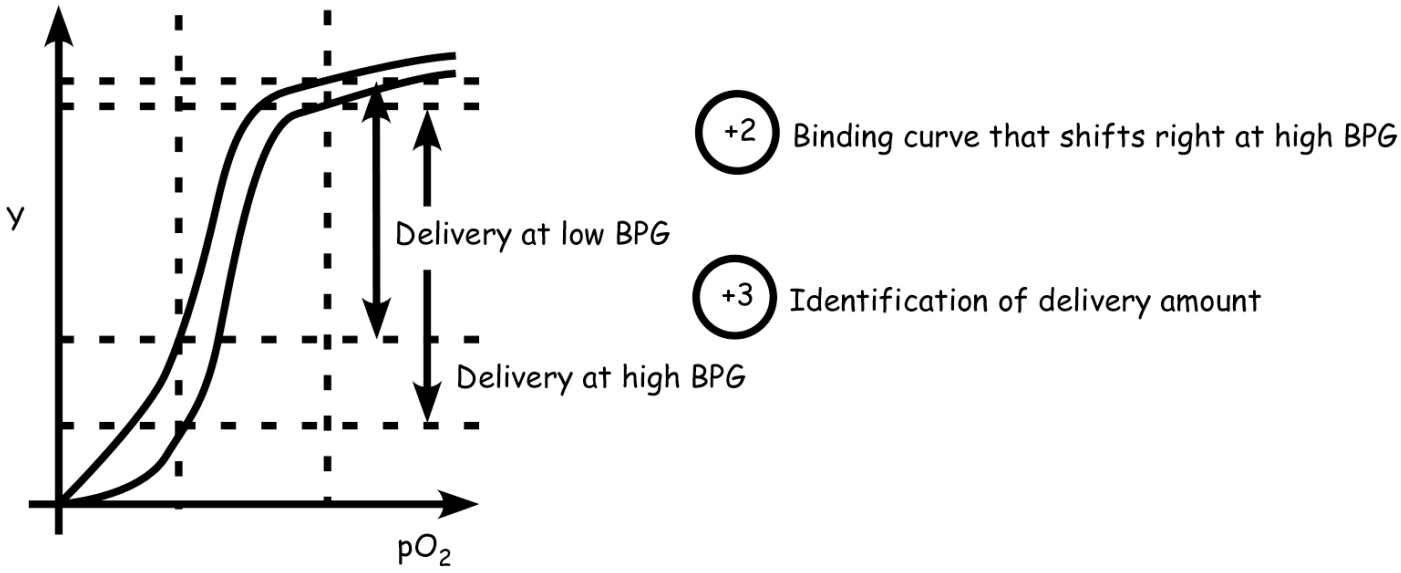


+2 Since T binds BPG much better than R does, $K_1 >> K_4$, so $K_2 << K_3$, meaning BPG binding stabilizes the T state.

+2 BPG binding decreases the overall free energy and increases the population of the T state, so the overall K_{bind} is closer to $K_5 << K_6$, poorer binding of O_2.

Score for the page_____

(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.

+2 Extreme aerobic performance is not achieved at high altitude because athletes can't train work hard enough at low pO2.

+2 Live High, Train Low allows athletes to get the benefit of added BPG and EPO from living at high altitude, while training at the intensity permitted by high pO2.

+2 Devices: example: A house or tent that decreases air pressure during sleep. Or – exercising at high altitude with supplemental O2. [+1 for a car or plane.]

(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:

+1 for Yes No or Maybe

+2 for any reasonable statement like:

It should be banned because it is expensive and that means an unfair advantage.

It should be allowed because there is no doping involved, just simulating what you could otherwise do by driving up and down.

It should be allowed because athletes should be allowed to take/do anything they want – they know the risks

It should be banned because to much BPG/EPO is associated with strokes.

It should be allowed but regulated as to apparent altitudes

Score for the page_____

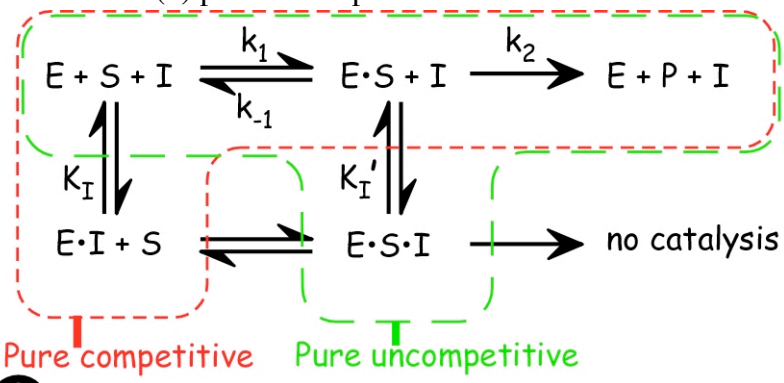
2. (30 pts) Enzymatic catalysis, Michaelis-Menten kinetics, and Inhibition

(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?

+3 The SSA is the idea that the concentration of the intermediate remains constant throughout most fo the reaction (our example was d[ES]/dt = 0)

+3 The intermediate must have a rapid decay path available. [This means that very soon after it starts building up it will reach a steady state concentraiton.]

(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.



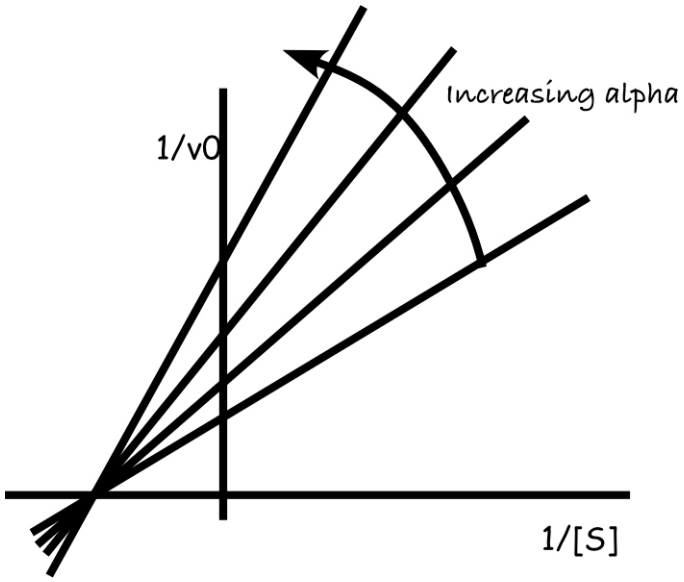
+2 for each. Okay to include only the KI or KI' equilibrium

(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?

+2 for a graph with four lines on it.

+2 for correct intersection: on x axis

+4 for: Dialyze away or run a size exclusion column to remove the inhibitor. If the enzyme recovers it was a reversible inhibitor. If the enzyme is permanently inactivated by the inhibitor, it is an irreversible reagent.



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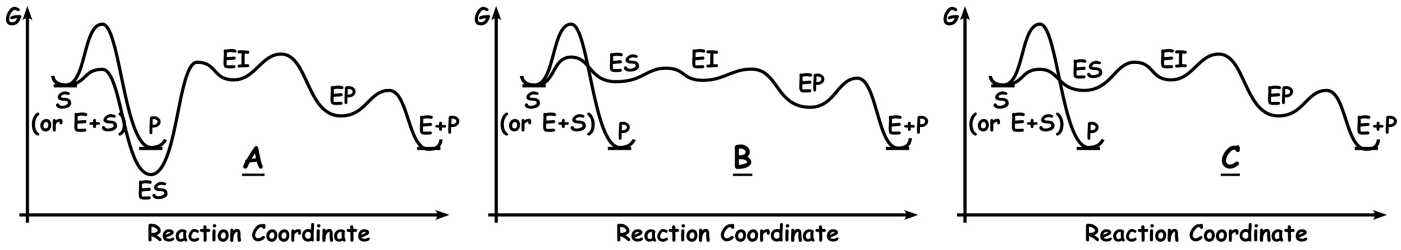
(d; 5 pts) From among the graphs below, identify which two free energy profiles describe enzymes and which one describes a binding protein, and give the reason behind your answer (one reason total).

Enzymes: _B__ and _____C___ Binding protein: ___A____ (+2 for all 3, +1 for one)



Reasoning:

+3 The enzymes must bind the transition state more tightly than they do the ground state S. I looked for the smallest overall barriers.

(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?___B (+1)___

Catalytic perfection is the idea that the enzyme catalyzes its reaction every time (+2) it encounters (diffuses into) a substrate.

We look for kcat/Km (+2) approaching the diffusion controlled limit.

The formation of ES complex E + S -> ES (+2) is limited by diffusion, so if the enzyme is going to catalyze reactions at the diffusion controlled rate, the initial formation of ES must be fast but also rate limiting – if it were not, the subsequent slower step would slow the overall reaction down to slower than diffusion controlled.

Score for the page_____

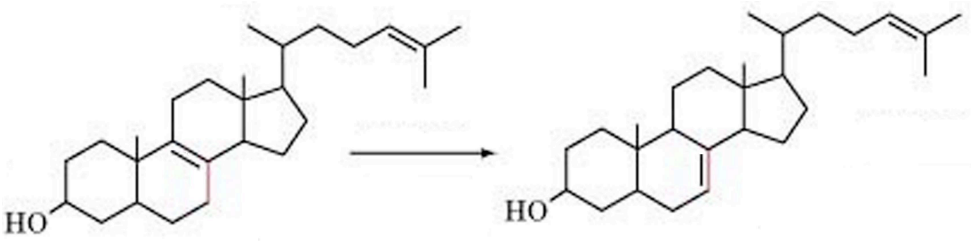
3. (8 points) Reaction Types

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

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

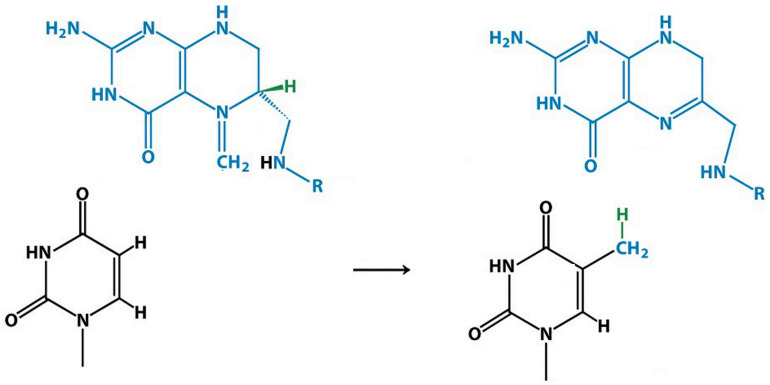
Examine each of the reactions given below and identify the reaction type that best describes the reaction shown. (Note – the complete, balanced reaction is not necessarily shown!)

(a) Reaction type: ___Isomerization_____

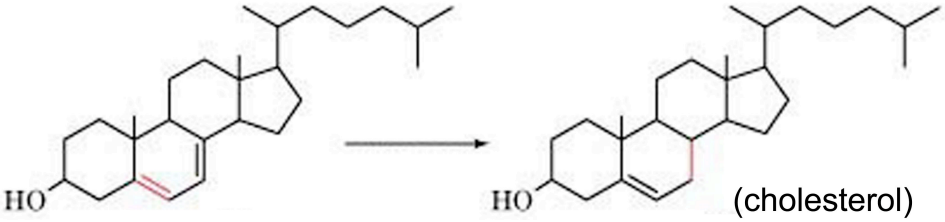


(b) Reaction type: __Addition/removal of functional groups_____

(c) Reaction type: __Group transfer_____



(d) Reaction type: __Oxidation/reduction_____



(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 $\Delta G'°$ for ATP hydrolysis at 25 ºC (298 K):

$$ATP + H_2O \Leftrightarrow ADP + P_i$$

Show your work. Remember that R = 0.0083 kJ/mol K.

1) calculate $\Delta G'°$ for the two reactions: $\Delta G'° = -RT \ln(270) = -13.84$ kJ/mol

$\Delta G'° = -RT \ln(890) = -16.79$ kJ/mol

$\Delta G'°$ for ATP hydrolysis is their sum = $-13.84 + -16.79$ = **-30.6 kJ/mol**

K'_{eq} for ATP hydrolysis = $\exp(-\Delta G'°/RT)$ = **2.4×10^5**

(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.

Calculate the [Pi] that makes $\Delta G = 0$, using $\Delta G = \Delta G'° + RT \ln Q$. Use $\Delta G'° = +30.6$, since the reaction is now ATP synthesis.

$\Delta G = 0 = \Delta G'° + RT \ln Q = \Delta G'° + RT \ln \{[ATP] / [ADP][Pi]\}$

$-\Delta G'° /RT = \ln \{[ATP] / [ADP][Pi]\}$

$\exp(-\Delta G'° /RT) = [ATP] / [ADP][Pi]$

[Pi] $= [ATP] / \{[ADP] (\exp(-\Delta G'° /RT))\} = 10^{-6} / (10^{-3} \times 4.229 \times 10^{-6}) =$ **236 M (a lot)**

Score for the page_____

(#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^+ ⇔ 1,3-bisphosphoglycerate + NADH + H^+ $\Delta G'^o$ = +6.3 kJ/mol

1,3-bisphosphoglycerate + ADP ⇔ 3-phosphoglycerate + ATP $\Delta G'^o$ = −18.8 kJ/mol

Suppose the cellular concentrations are:

[GAP] = 1mM [1,3-BPG] = 1 mM

[NAD^+] = 1 mM [NADH] = 1 mM

[ADP] = 1 mM [3-phosphoglycerate] = 1 mM

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

1) Find $\Delta G'^o$ for the coupled reactions:

GAP + P_i + NAD^+ + ADP ⇔ 3-PG + NADH + ATP + H^+ $\Delta G'^o$ = −12.5 kJ/mol

2) Again, find [Pi] for which $\Delta G = 0$:. Proceed as in previous part:

$$\exp(-\Delta G'^o/RT) = \{[3\text{-PG}][\text{NADH}][\text{ATP}] / [\text{GAP}][NAD^+][\text{ADP}][\text{Pi}]\}$$

$$157 = \{(10^{-3}\ \text{M})\ (10^{-3})\ (3 \times 10^{-3}) / (10^{-3})\ (10^{-3})\ (10^{-3})\ [\text{Pi}]\}$$

[Pi] = $(3 \times 10^{-3}) / [(10^{-3})\ (157)$ = **0.0191 M** (not nearly as much)

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.

i) phosphoglucose isomerase **ii) aldolase** **iii) pyruvate kinase**

Answer 1:

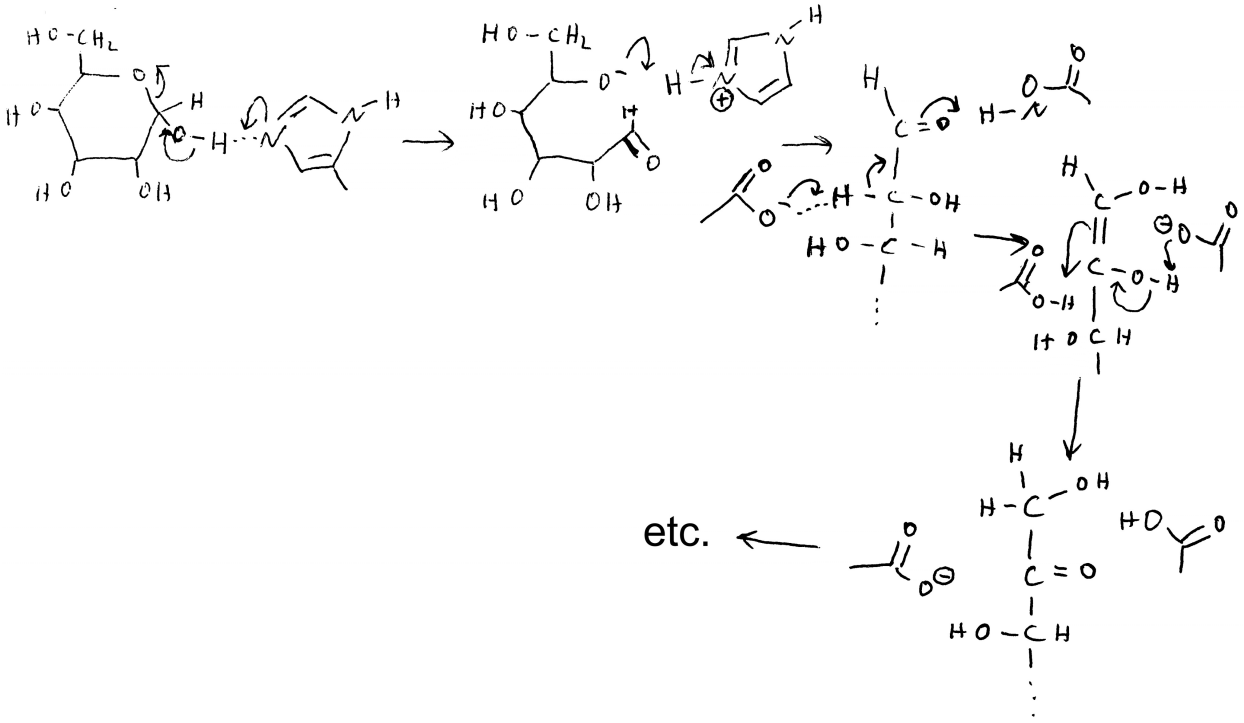
See textbook, p. 436, Fig. 16.2

Score for the page_____

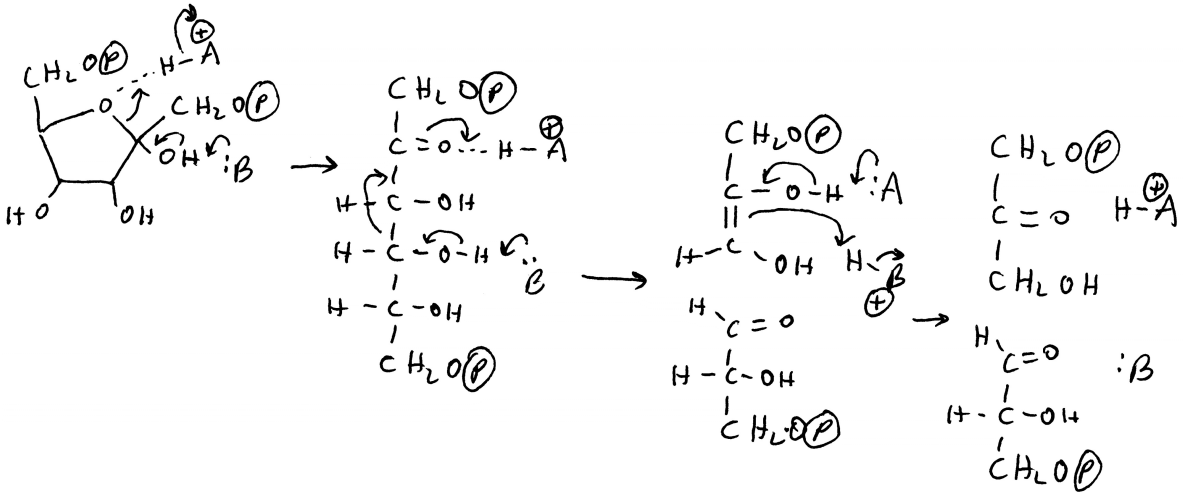
#5a, Answer 2:

(#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.

Possible mechanism for phosphoglucose isomerase:



Possible mechanism for aldolase:



(:B is a base, such as His or Glu; H-A is an acid such as a protonated His or Glu)

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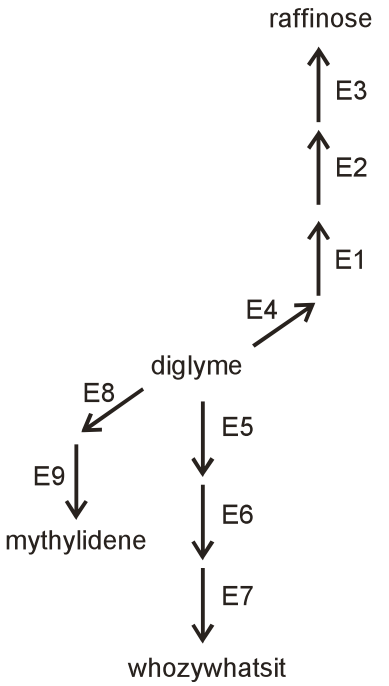
6. (8 points) Regulation of Metabolism

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.



E5. Whozywhatsit would feed back to inhibit the enzyme that catalyzes the first committed step in its biosynthetic pathway .

(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.

E4. Raffinose inhibits E4 for the same reason as in part (a), above – E4 catalyzes the first step in the pathway that leads to synthesis of raffinose.

Mythylidene might activate E4, since, if the mythylidene concentration increases due to its synthesis from diglyme, it might activate a pathway that converts diglyme to something else, such as raffinose.

Score for the page_____