BCHM 463 **Your Name:** Key

Biochemistry and Physiology **ID #:**

Exam II, November 4, 2002 **Prof. Jason Kahn**

You have 50 minutes for this exam.

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

You may use 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.

Explanations should be concise and clear.

Honor Pledge: 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."

Possibly useful information:

Michaelis-Menten equation: $v_0 = V_{max}[S]/(K_M + [S])$

Type of inhibition	Apparent K_M	Apparent V_{max}	Apparent V_{max}/K_M
Competitive	αK_M	V_{max}	$(1/\alpha)\ V_{max}/K_M$
Uncompetitive	$(1/\alpha')K_M$	$(1/\alpha')\ V_{max}$	V_{max}/K_M
Mixed	$(\alpha/\alpha')K_M$	$(1/\alpha')\ V_{max}$	$(1/\alpha)\ V_{max}/K_M$
Noncompetitive ($\alpha=\alpha'$)	K_M	$(1/\alpha')\ V_{max}$	$(1/\alpha)\ V_{max}/K_M$

$\alpha = 1 + ([I]/K_I)$ $\quad \alpha' = 1 + ([I]/K_{I'})$

$V_{max} = k_{cat}\,[E]_{total}$

RT = 2476 J/mole today

$\Delta G = \Delta G^{\circ\prime} + RT\ln Q$, where Q has the form of an equilibrium constant

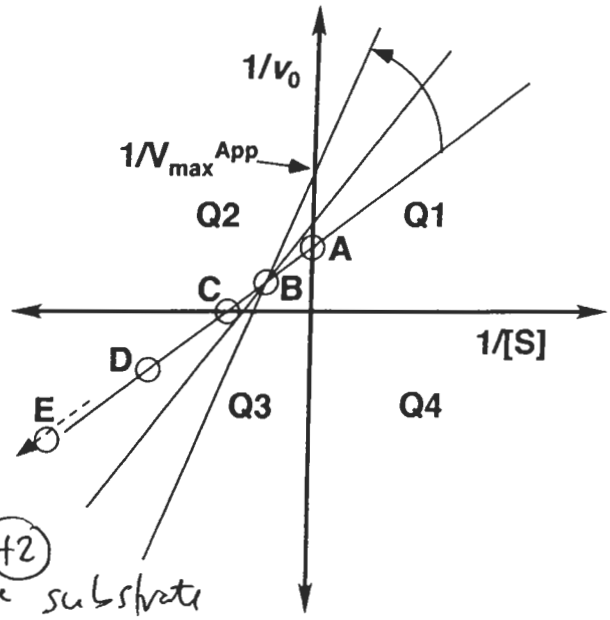
$\Delta G = -n F \Delta E$, where F = 96500 J/(V•mole), n = number of electrons transferred

$\Delta G^{\circ\prime}$ = -30.5 kJ/mole for $ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+$

1. Michaelis-Menten Kinetics (25 pts):

A schematic graph of Michaelis-Menten kinetics in the presence of an inhibitor is shown below. The lines are drawn through experimental data (not shown) in the absence of inhibitor and at increasing inhibitor concentration, as indicated by the curved arrow.

The lines here intersect at point B, representing mixed inhibition, but in general they could intersect at any point from A to E. Point E, at infinity in the indicated direction, represents pure uncompetitive inhibition.



(a; 7 pts) If the lines had intersected at point A instead, discuss what this would have meant in terms of what you observe as you add more and more substrate to the reaction at different inhibitor concentrations. What kind of inhibition would intersection at A represent?

The y-axis refers to infinite substrate (+2) concentration. Intersection at A would mean that at infinite substrate concentration all reactions reach the same V_{max} (+3), that inhibitor is swamped out. This represents competitive inhibition. (+2)

(b; 6 pts) What two types of inhibition are being mixed to give mixed inhibition? Why do we consider non-competitive inhibition to be a special case of mixed?

Mixed is a mixture of competitive and uncompetitive.
(+1 for either, (+3) for both)

B-C-D are all mixed- noncompetitive is the special case where Km is (+2) unaffected, whereas in general for a mixed inhibitor (+1) Km can go up or down. In other words, mixed represents intersection envelope between A and B, and C is just the one special case of unaffected Km.

(SP)

(c; 7 pts) In which quadrant do all of the experimental data points lie? Why can't the lines ever intersect in Quadrant 1 (Q1) for a simple M-M enzyme? Why can't they intersect in Q4?

(+2) All in Q1 - ⊕ v_o, ⊕ [S]

(+3) Can't intersect in Q1 because that would mean that for some ~~substrates~~ [ ] the velocity would be greater for the inhibited reaction

(+2) Can't intersect in Q4 because the lines can never enter Q4! That would correspond to a ⊖ v_o for a real [S].

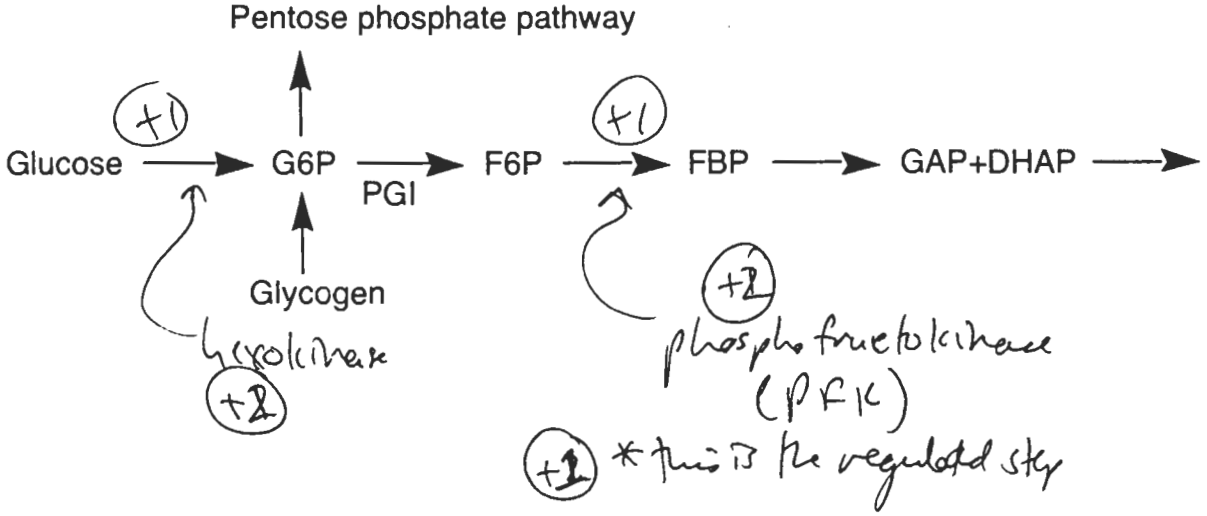
(d; 5 pts) What is the appropriate measure of quality for an enzyme, and what is the physical process that limits the performance of enzymes (answer is about 16 characters)?

(+3) k_{cat}/K_m (or any variant)

(+2) Diffusion

2. Glycolysis (23 pts):

(a; 10 pts) The story so far of glycolysis is sketched below. Indicate the two steps that are strongly favorable energetically, and name the enzymes that catalyze these steps. We identified one of these steps as the crucial control point for glycolysis (at least among the reactions we have studied so far). Which one is it, and what is the chemical equation (write out chemical names for the glycolytic intermediates) for the reaction it catalyzes?



[fructose-6-phosphate + ATP ⇌ fructose-1,6-bisphosphate + ADP

(+3) [+2 If there are any errors, +1 if no ATP]

(b; 8 pts) Explain why a step which is roughly at equilibrium is not a good candidate for regulation, couching your answer in terms of flux control. Also, give one reason why the other thermodynamically favorable step in (a) is not as highly regulated as the one you identified.

$A \rightarrow B \underset{v_r}{\overset{v_f}{\rightleftharpoons}} C \rightarrow D$

(+3) for this idea

A step that is at equilibrium has v_f and $v_r \gg$ flux. Therefore reducing the possible flux requires a very large decrease in the enzymatic activity. Don't put a dam in the middle of a lake!

(+2)

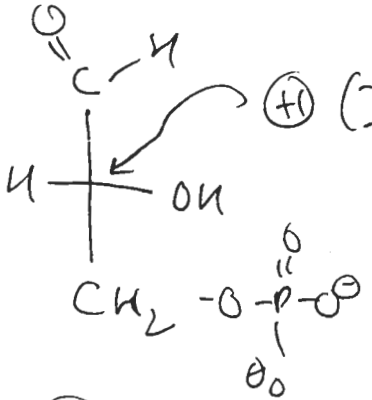
- Flux is constant through a pathway!

The hexokinase reaction is not as highly regulated because

(1) it's not a committed step for glycolysis – there are other sources of G6P, and other fates for it

(+3) for either (2) Presumably it is advantageous to phosphorylate + retain glucose even if there is no current need for it.

(c; 5 pts) Draw D-glyceraldehyde-3-phosphate (GAP) and indicate the stereocenter that would be inverted to give L-glyceraldehyde-3-phosphate.



(+1) (It's the only stereocenter!)

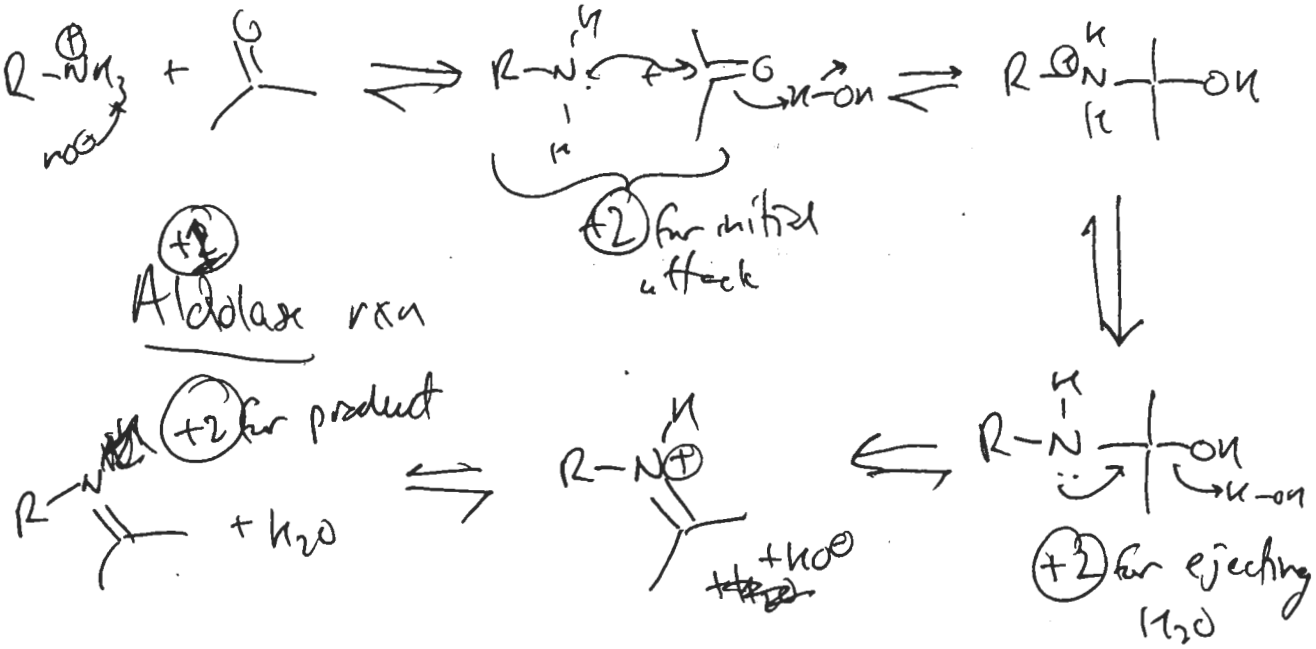
+1 for an aldehyde
+1 for a phosphate
+2 for correct

(+4)

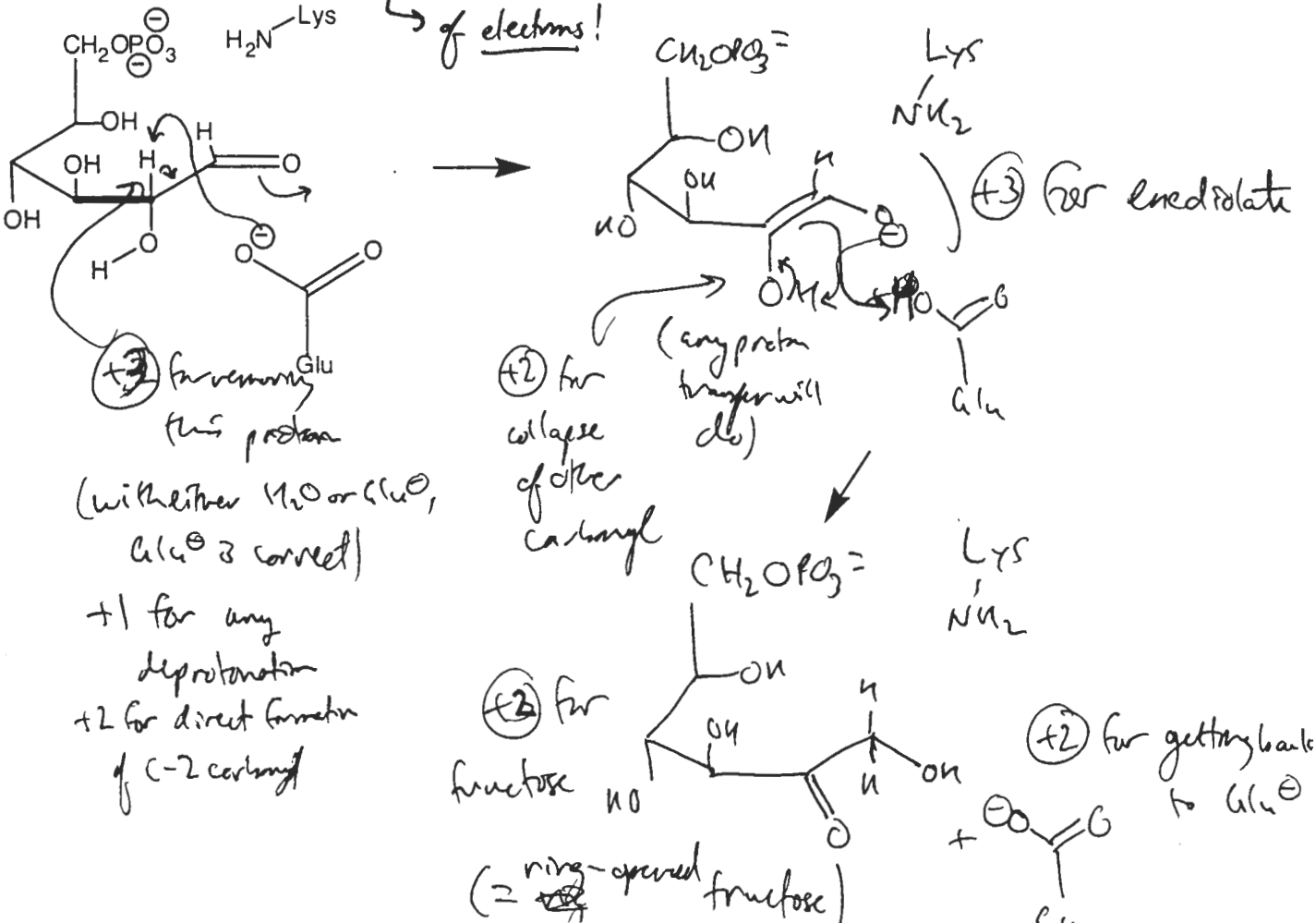
TV

3. Enzymatic Catalysis (22 pts):

(a; 7 pts) Draw the mechanism for the base-catalyzed formation of a Schiff's base between lysine and acetone. You may represent the protonated form of lysine as RNH_3^+. Where have we seen something like this in glycolysis?



(b; 12 pts) One intermediate in the phosphoglucose isomerase (PGI) reaction mechanism is shown below. Draw the arrow-pushing and intermediates for the next two steps toward fructose. Lysine is not involved. Protonation of an enediolate does not count as a step.].



(c; 3 pts) What kind of catalysis and what type of reaction does the mechanism in (b) represent?

(+1) general acid / general base

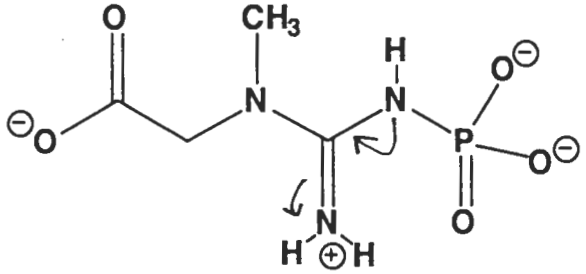
(+2) Isomerization

- ⊖ charge "stable" on enolates, ⊕ on Schiff's bases - or resonance-stabilized carbanion, carbocation

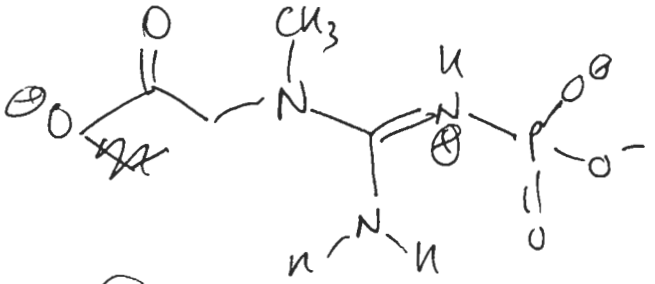
Rules for mechanisms
- Arrows are electrons. Draw from lone pairs or bonds.
- 4 bonds to carbon
- filled octets w/ consistent charges
- Don't make unstabilized carbanions or carbocations
- Return enzyme to original state
- Typical n-philes are O, N

4. Bioenergetics and Baseball (30 pts):

Creatine supplementation is used by athletes to enhance their muscles' ability to sustain maximal exertion, as in weight lifting and home run hitting. Supplementation can increase creatine concentration in the muscle by 30% or so, to a total body burden of about 150 g (MW is 121). [Creatine has the added advantages of being legal and also relatively safe.] Creatine phosphate is a "high-energy" molecule, i.e. it has a large negative standard free energy for phosphate hydrolysis ($\Delta G^{\circ\prime}$ = -43.1 kJ/mol). Its structure is shown below.



(a; 9 pts) Draw a resonance form for creatine phosphate that illustrates one reason why it is "high-energy," and state what reason it illustrates. Name one other driving force that we discussed that contributes to highly exergonic bond hydrolysis in general.



(+3) for anything with a ⊕ charge on the central N

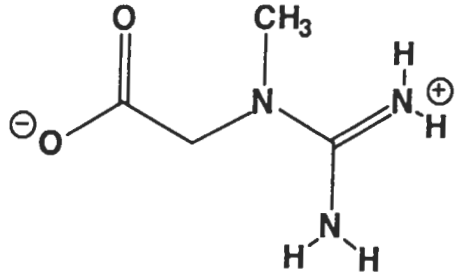
[+1 for idea but charge balance wrong]

- Competing resonance or inductive destabilization of the electronegative nitrogen

(+3) for either

(+3) for solvation or charge separation of products

The creatine phosphate hydrolysis products at pH 7 are creatine (below) and P_i:



(b; 6 pts) Creatine phosphate hydrolysis can be used to drive the synthesis of ATP from ADP and P_i (though P_i would not actually be a free intermediate in the reaction). Write down the chemical equations for creatine phosphate hydrolysis, ATP hydrolysis in the appropriate direction, and the net chemical equation for the coupled reactions. Calculate $\Delta G^{\circ\prime}$ given that the $\Delta G^{\circ\prime}$ for ATP hydrolysis to give ADP + P_i is -30.5 kJ/mol.

(+1) creatine-Ⓟ + H_2O ⇌ creatine + P_i $\Delta G^{\circ\prime} = -43.1$ kJ/mol

(+1) ADP + P_i ⇌ ATP + H_2O $\Delta G^{\circ\prime} = +30.5$ kJ/mol

(+2) creatine-Ⓟ + ADP ⇌ creatine + ATP $\Delta G^{\circ\prime} = -12.6$ kJ/mole (+2)

(c; 6 pts) Given the following concentrations, calculate the actual ΔG (at pH 7) for the ATP synthesis reaction above. Creatine phosphate = 60 mM, creatine = 30 mM, ATP = 1 mM, ADP = 0.1 mM.

(+2) $\Delta G = \Delta G^{\circ\prime} + RT \ln \dfrac{[\text{creatine}][\text{ATP}]}{[\text{creatine-Ⓟ}][\text{ADP}]}$

$= -12.6$ kJ/mole $+ 2.476$ kJ/mole $\ln \dfrac{(30)(1)}{(60)(.1)}$ (+2) for substitution

$(60)(.1) = 5$

$= -8.6$ (+2) for answer

(20)

(d; 9 pts) Why don't athletes use the "high-energy" creatine phosphate instead? Answer in terms of thermodynamics, assuming that a 30 g Snickers bar can considered to be all glucose (MW = 180), and that aerobic metabolism of glucose provides approximately 38 moles of ATP (from ADP) per mole of glucose. Your calculations need not be exact.

(+3) for any calculation of ATP synthesis

$$\frac{30\text{ g glucose}}{180\text{ g/mole}} = \frac{1}{6}\text{ mole glucose} \times 38 = 6\text{ moles ATP synthesis}$$

(+3) for comparison to creatine-P amounts

There's only ~ 1.25 moles of creatine in the body and the supplement is ~ 0.25 moles.

(-3) for idea

Thus the additional energy in creatine phosphate is [+1] trivial relative to normal intake. It's the added anaerobic capacity [+2] that's desirable.

4/9 for idea with no calculations

Score:	1. Michaelis-Menten Kinetics (25 pts):	_____
	2. Glycolysis (23 pts):	_____
	3. Enzymatic Catalysis (22 pts):	_____
	4. Bioenergetics and Baseball (30 pts):	_____
	Total: out of 100	_____