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MCDB 108B - Biochemistry
Winter 2010

Midterm

February 10, 2010

Answer Key.

Make sure your exam contains pages numbered 1 through 5. Answer all questions. The point allocation for each question is approximate.

$\Delta G^\circ = -2.3RT \log K_{eq}$
$2.3RT = 1.4$ kcal/mol at 37°C

Question	
1	_____/10
2	_____/12
3	_____/5
4	_____/10
5	_____/10
6	_____/18
7	_____/20
total	_____/85

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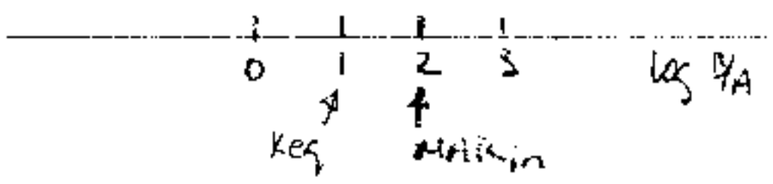
1. (10) Answer the following questions. In some cases the answer may be "unknown".

a. What is the value of ΔG at equilibrium? Explain.
$\Delta G = 0$ At equil^m, no work can be done. Therefore ΔG must be 0.

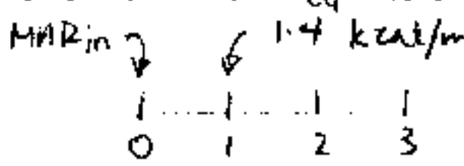
b. What is the value of ΔG if $K_{eq} = 10$? Show your work or Explain.
unknown. To get ΔG both K_{eq} & the MAR$_{in}$ must be known.

c. What is the value of ΔG if the MAR = 100, $K_{eq} = 10$? Show your work or Explain.



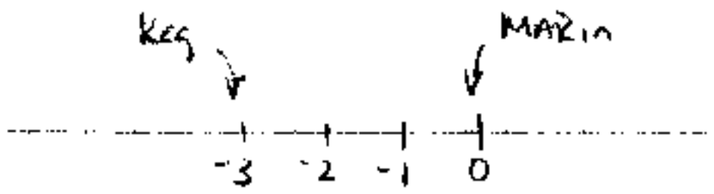
So: $\Delta G = +1.4$ kcal/mol

d. What is the value of K_{eq} if $\Delta G^\circ = -1.4$ kcal/mol? Show your work or Explain.



$\therefore K_{eq} = 10$ because ΔG° is neg

e. What is the value of ΔG° if $K_{eq} = 0.001$? Show your work or Explain.



$\Delta G^\circ = +4.2$ kcal/mol

2. (12) Consider the following sequence of metabolic reactions. The value for ΔG and ΔG° for each reaction (in kcal/mol) is given as shown:

ΔG:		1.4		1.1		−8.4		-1.4	0	
	A	⇆	**B**	⇆	**C**	⇆	**D**	⇆	**E**	⇆ **F**
ΔG°:		-7.0		1.1		0.2		0	-0.6	

Answer the following questions. Do not use an equation, rather you must explain all answers intuitively. You can show a line graph of the MARs to explain your answers.

a) What reaction step controls the overall flux through the entire pathway during steady-state flow? Explain. C ⇌ D because ΔG for this step is the most large & neg.

b) What is the K_{eq} for the reactions A ⇆ B and D ⇆ E?
A ⇌ B $\Delta G^\circ = -7.0$ (10^5- fold difference in MAR between init & equil^m cond^s)
init = 1/1 equil^m ∴ = $\underline{\underline{10^5/1}}$

D ⇌ E $K_{eq} = 1$ (MAR$_{in}$ & Keq position are the same)

c) What is the MAR_{ss} for A ⇆ B and B ⇆ C?
A ⇌ B $K_{eq} = 10^5$ ∴ MAR$_{in}$ = 10^6 (1.4 kcal/mol in the positive direction)

B ⇌ C $\Delta G = \Delta G^\circ$ ∴ MAR$_{in}$ = 1/1

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d) The MAR_{ss} is at equilibrium for which reaction?

$E \rightleftharpoons F$ $\Delta G = 0$ ∴ the rxn must be at equil^{m}.

3. a) For the reactions:

PEP + H_2O → PYR + PO_4	ΔG = X
ADP + PO_4 → ATP + H_2O	ΔG = + 12 kcal/mol
~~ATP + H_2O → ADP + PO_4~~	~~ΔG = -12.0 kcal/mol~~
PEP + ADP → pyruvate + ATP	ΔG = -4.0 kcal/mol.

X = − 16 kcal/mol.

What would be the value for ΔG for the *hydrolysis* of PEP (transfer of PO_4 to H_2O instead of to ADP) if it were to occur in cells? Show all work (2).

b) In cells, glucose-6-P can be formed from glucose-1-P or, alternatively, from fructose-6-P:

glucose-1-P ⇆ glucose-6-P	ΔG° = -1.7 kcal/mol
fructose-6-P ⇆ glucose-6-P	ΔG° = 0.4 kcal/mol

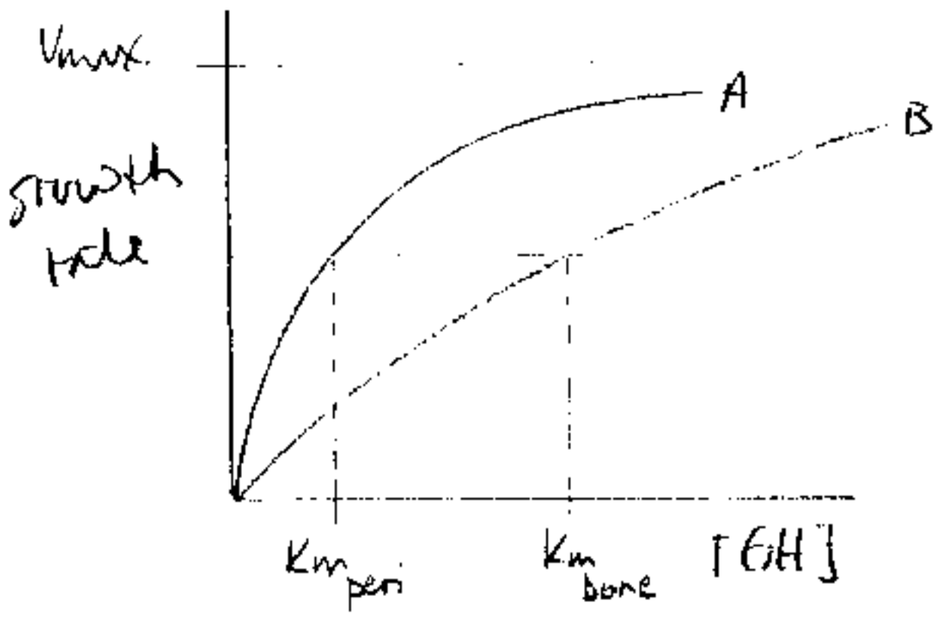
Show how you would set up the equation to calculate the ratio of fructose-6-P to glucose-1-P at equilibrium? Show all your work except the answer (3).

G1P ⇌ G6P ⇌ F6P ΔG° total = −2.1 kcal/mole

ΔG° −1.7 −0.4

$\log K_{eq} = \frac{-2.1}{-2.3RT} = \frac{-2.1}{-1.4}$ $K_{eq} = 10^{(2.1/1.4)}$

4. (10) a) Growth hormone (GH) binds to a cell surface receptor and, at high concentration, stimulates both bone and peripheral organs to grow at the same maximum rate. At low concentrations, however, growth hormone causes primarily peripheral organs to grow, while bone is significantly less responsive. Describe how nature has likely achieved this differential response to GH biochemically. Show the graphs that describe the kinetics of growth rate vs. GH concentration for both bone and peripheral organs. Explain how to interpret your graphs.



A - peripheral organs
B - bone

Two different receptors for GH. On bone, Km is high, binds GH poorly. On peripheral organs, Km is low, binds GH tightly. Vmax is the same for both.

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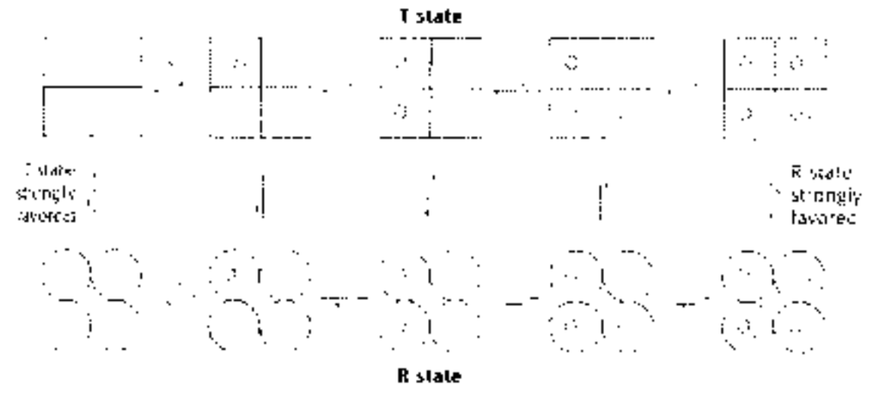
b) What is the equation that describes growth rate as a function of GH concentration? Explain intuitively the meaning of this equation. From your graphs what is the fold difference in GH affinity and maximum growth rate, between one receptor type versus the other. Show and Explain.

$$\text{growth rate} = V_m \times \frac{[GH]}{K_m + [GH]}$$

Growth rate is always some proportion of the max (V_m). That proportion is a function of the [GH] and affinity (K_m)

Fold difference looks to be about 2.5 fold. (from working at a).

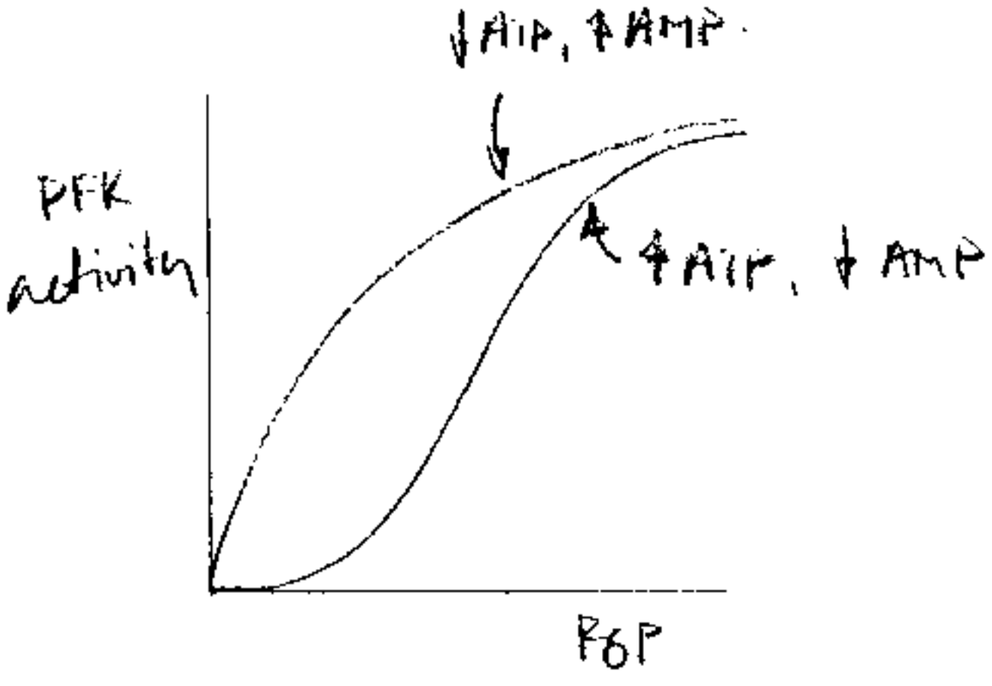
5. a) (4) Consistent with the following figure, what are the 2 crucial properties of this system that produce cooperative binding of O_2?



1. In absence of O_2 T must greatly predominate.

2. O_2 greatly favors binding to R than to T.

b) (6) PFK is a cooperative enzyme that classically obeys the concerted model. Draw the graphs of PFK activity vs [F-6-P] expected at high and low energy charge in the cell. Describe the biochemical mechanism that is responsible for the shapes of these graphs.



At ↑E·C, ATP favors T form, but only R is active. ∴ Activity must be in cooperation with conversion of T → R. ∴ Sigmoidal curve.

At ↓E·C all is in the R form. ∴ Michaelis-Menten kinetics

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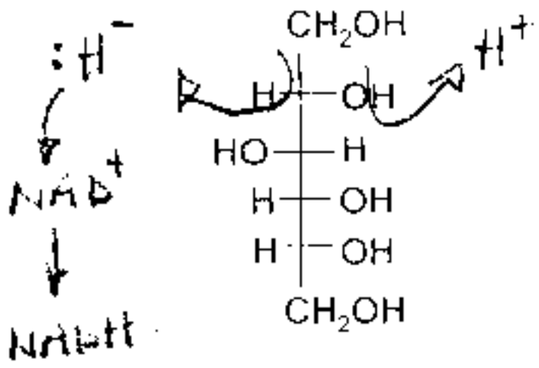
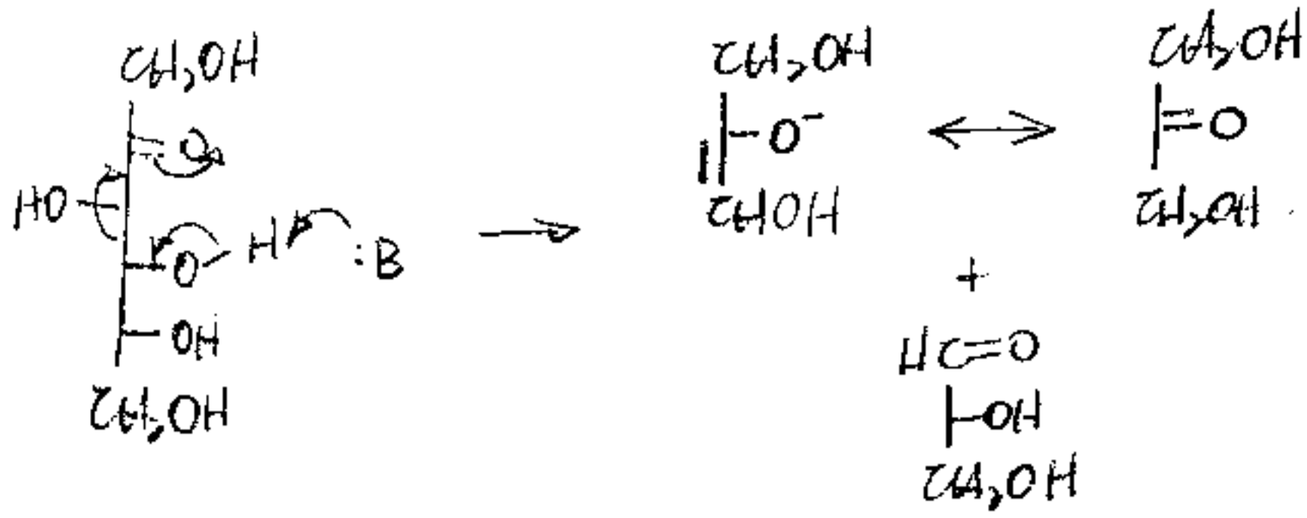
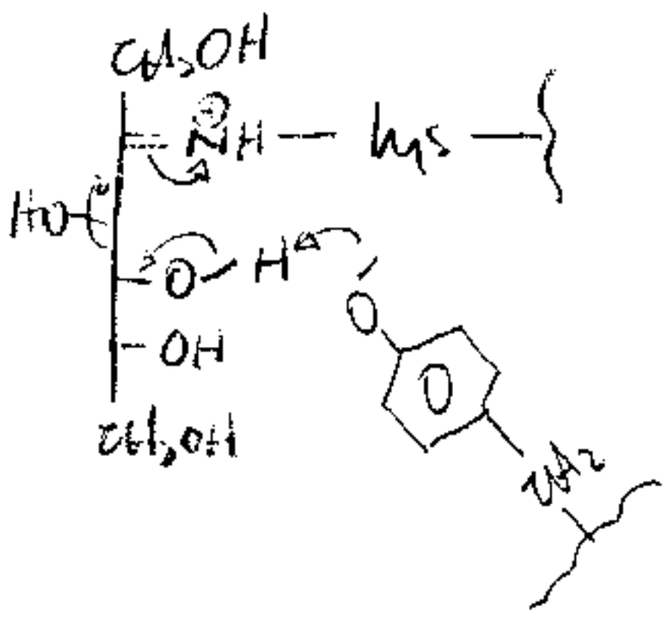
6. a) (6) Shown below is a hypothetical 6-carbon compound. You wish to modify it such that cleavage into two 3-carbon compounds is favorable. Show the exact organic chemistry involved in this modification, and indicate all cellular co-factors that may be required. Secondly, show the organic chemical mechanism of the cleavage reaction itself, and explain why the prior modification makes cleavage favorable. Draw all products.

① Must oxidize α-OH to -C(=O)- grp





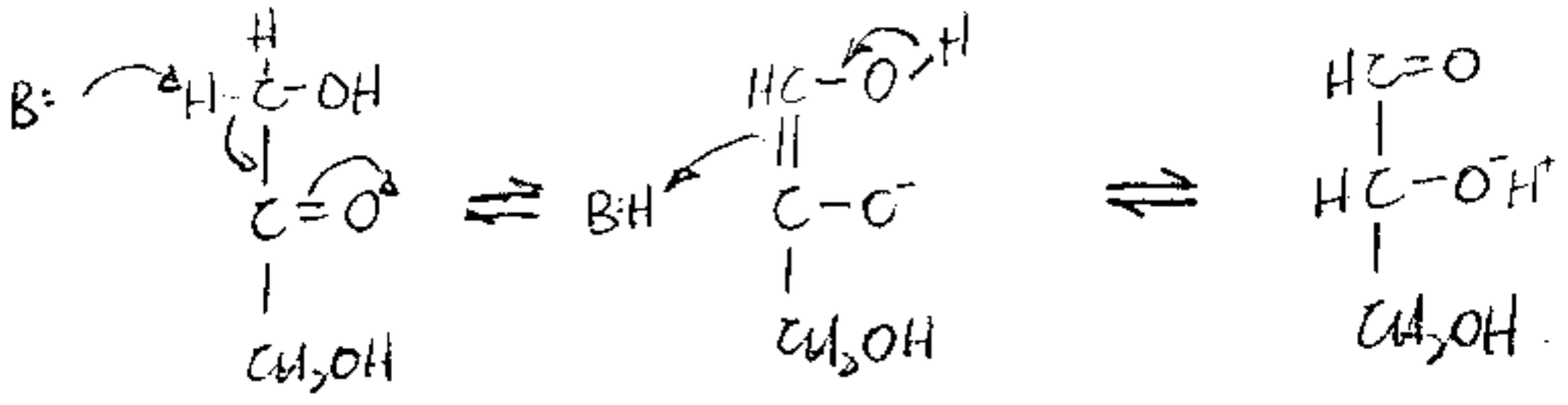
b) (6) Aldolase performs the same chemistry (as in (a)) ~10^{12}-fold faster than occurs in solution. What are 2 main reasons for this enormous rate enhancement? Show the corresponding organic chemistry, and explain.



① Better electron acceptor by =N⁺H-

② Intrinsic Tyr residue provides strong base

c) (6) The products of the aldolase reaction are DHAP and G3P. Show the organic chemistry by which DHAP is converted to G3P, the latter which continues through glycolysis. As part of your answer, draw the intermediate that mediates this process.



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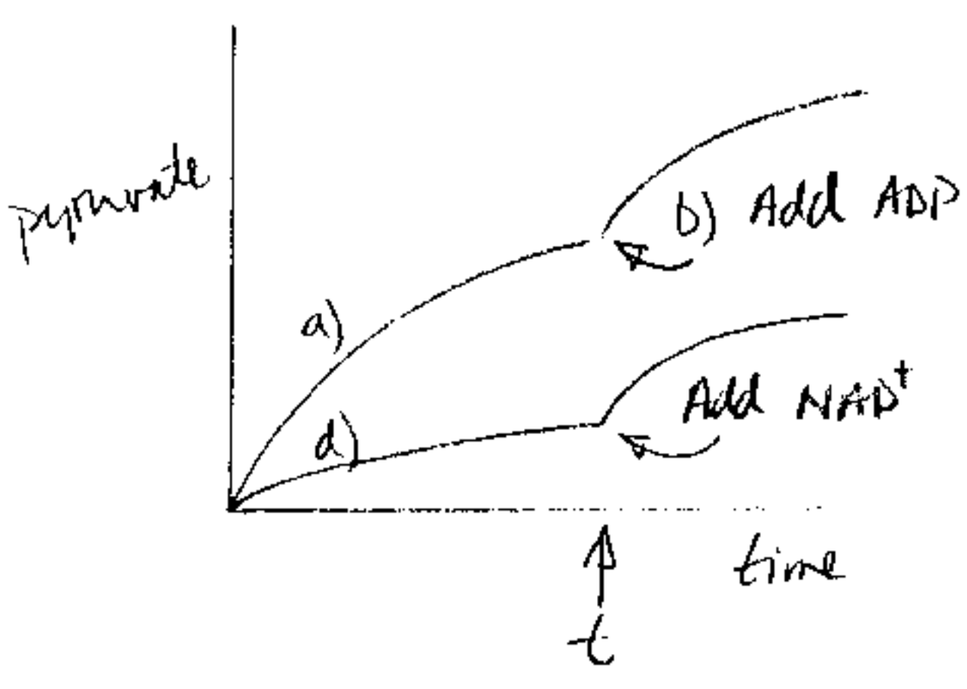
7. (a) A cytosolic extract from rabbit muscle contains all of the enzymes and co-factors required to carry out glycolysis in a test tube. Draw the expected graph of pyruvate production (y-axis) against time (x-axis) after adding an excess amount of glucose to the extract (3).

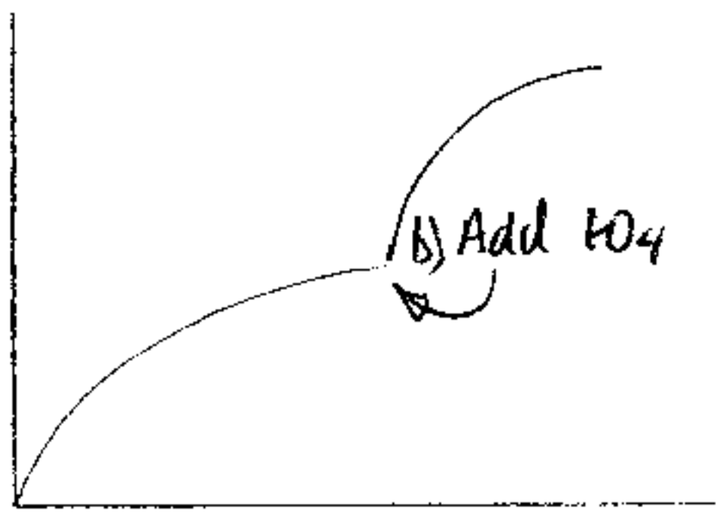
b) As you expect, pyruvate production slows down over time due in part to changes in the levels of PO_4 and ADP. If the original concentrations of PO_4 and ADP were unknown, how would you test which one is more severely limiting? Draw the expected results of your experiment on your graph. Explain fully (5).

c) In separate experiment, you start with fresh extract and you supplement it with excess PO_4 and ADP before you add glucose. You find that the rate of pyruvate production still slows down even though PO_4 and ADP are plentiful! What is the likely reason for this? (It is not due to a change in pH). Explain the proposed mechanism (3).

d) In yet another experiment, a specific inhibitor of the following reaction is added before addition of glucose. How is this inhibitor expected to affect the rate of pyruvate production? Draw the graph. What is the mechanism? How would you convincingly demonstrate your hypothesis? (5)

e) The same inhibitor has no effect on extracts prepared from yeast. Explain why. Draw the reactions and the chemical structures that allow yeast to carry out glycolysis in the presence of this inhibitor. (4)

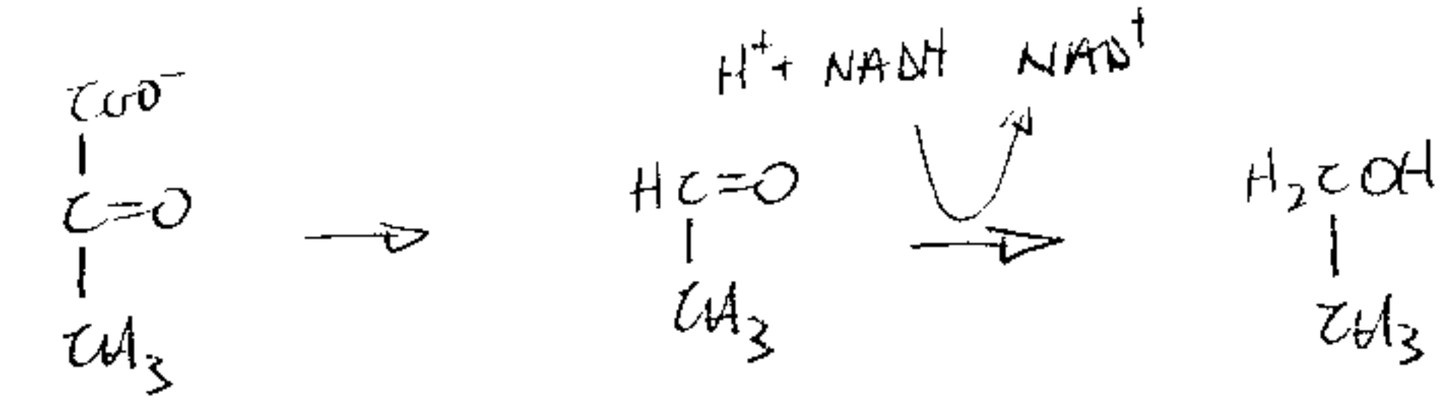




b) Add ADP to rxn observe increase in pyruvate formation.
Add PO_4 to a second rxn,
which ever produces the greatest burst in pyruvate formation is the most severely limiting

c) ATP is building up. ATP ⊖ PFK, PK by promoting the T form.

d) Reduction of pyr to lactate requires NADH to regenerate NAD^+ to keep glycolysis running. Inhibition of LDH prevents regeneration of NAD^+ thus pyruvate production slows down. Confirm by adding back NAD^+ and observing a burst in pyr production.



pyruvate acetaldehyde ethanol

This reaction in yeast regenerates NAD^+ by does not require LDH, and therefore proceeds in the presence of an LDH inhibitor