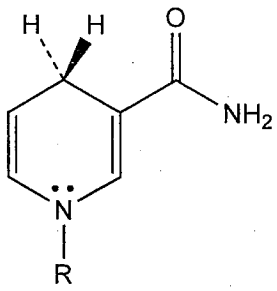
 Name Key

Biochemistry 5700
Fall, 2011
Exam 4

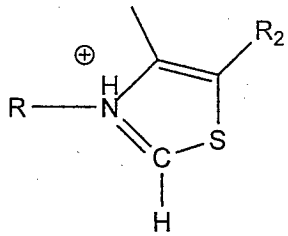
INSTRUCTIONS:
Do not begin until 10:30 AM. The exam must be turned in by 11:20 AM.

PLEASE USE THE FOLLOWING ABBREVIATED STRUCTURES AS THE BASIS FOR DRAWING MECHANISMS INVOLVING THESE COFACTORS. Note cofactors will undergo structural changes (e.g. oxidation, reduction, deprotonation, acylation, etc. during catalysis, so modify the structures as needed as you draw mechanisms.
Note: when asked to write balanced chemical equations, you need not balance protons in the reactions.

Abbreviated cofactor structures



(1)



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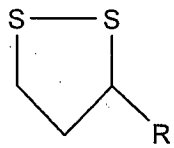

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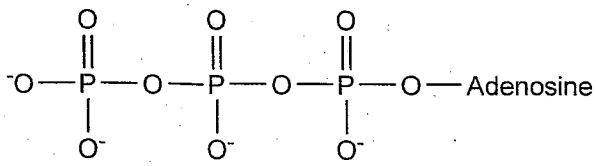

(4)

FAD

(5)

HSCoA

(6)

 Name _____

1. (3 points). The reactions of glycolysis beginning with glucose and ending with pyruvate consume 2 ATP molecules, form 4 ATP molecules via substrate level phosphorylation, and form 2 NADH molecules.

2. (3 points) The conversion of **2 mol** of pyruvate to **6 mol** of CO_2 via pyruvate dehydrogenase and the citric acid cycle also yields 8 mol of NADH, 2 mol of $FADH_2$ (bound to succinate dehydrogenase) , and 2 mol of GTP. 1. (16 points)

3. (10 points) For each of the enzymes listed below, name an intermediate (e.g., cis-enediolate, schiff base adduct, thiohemiacetal, etc) that the enzyme forms during the course of catalysis. If the enzyme does not form an intermediate, write "none".

Term	Intermediate formed during catalysis
phosphoglucokinase	pentavalent intermediate (or none?)
Triose phosphate isomerase	cis-ene-diolate
Aldolase	schiff base adduct
Enolase	none- concerted (or carbanion)
phosphoglycerate mutase	dephospho enzyme (diphosphorubstrate)
glyceraldehyde-3-phosphate dehydrogenase	thiohemiacetal or acyl thioester
Pyruvate decarboxylase	hydroxyethyl TPP
malate dehydrogenase	none- concerted, or alkoxide
aconitase	cis-aconitate
Citrate synthase	resonance stabilized carbanion of acetyl-CoA

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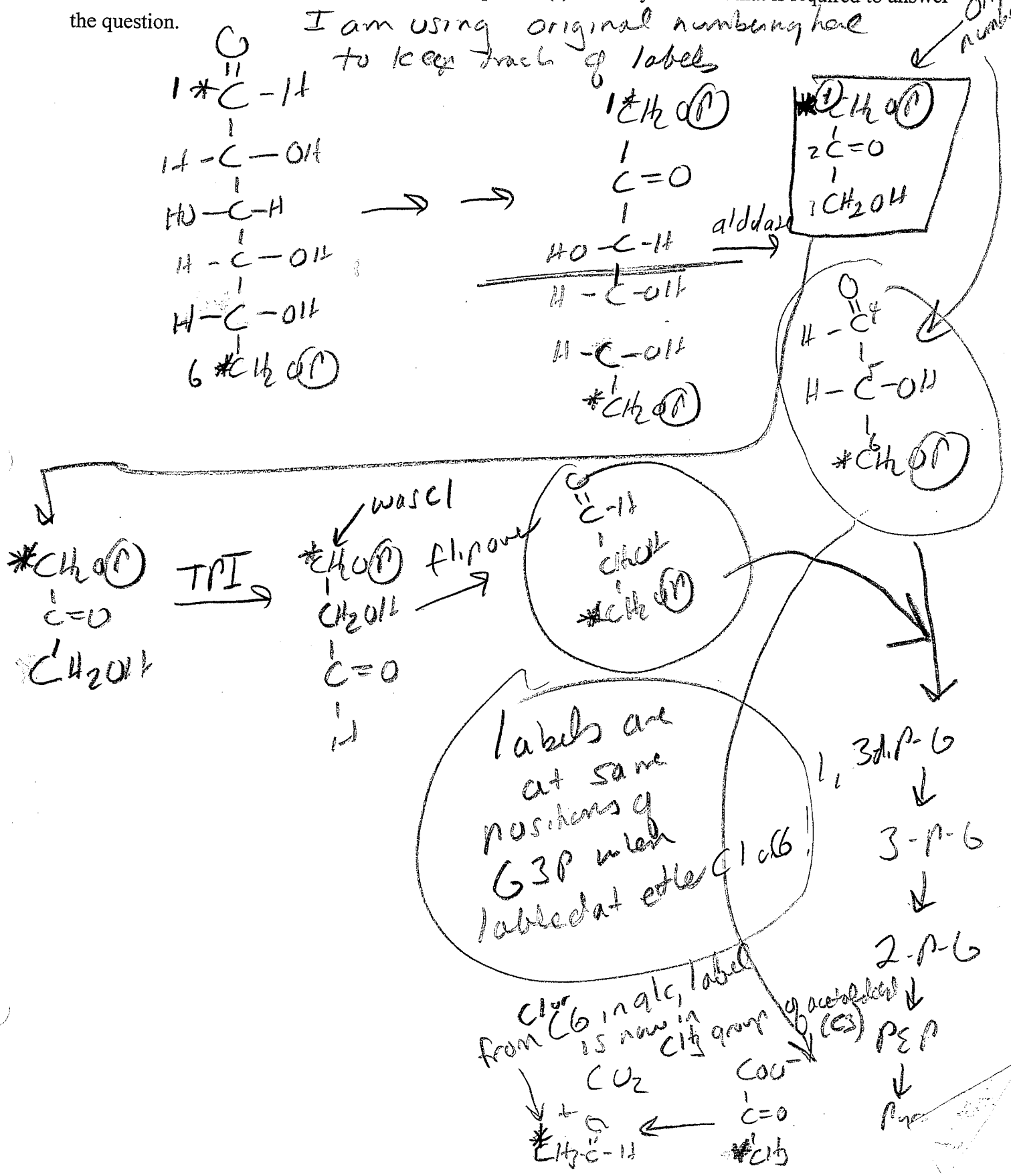
(4) 10 points. You add glucose that is radiolabelled with ^{14}C in the C1 position to yeast cells growing anaerobically. After complete conversion of glucose to produce acetaldehyde and CO_2 as fermentation products, where will the $^{14}CO_2$ label appear? If you had labeled the glucose with ^{14}C at the C6 position instead where would the label have appeared in product(s)? Show your work that is required to answer the question.

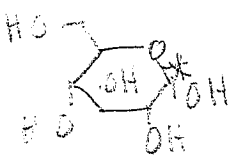


Student answer

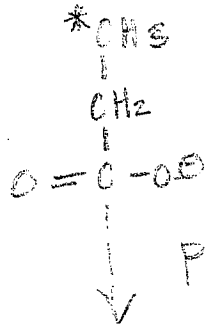
 Name [redacted]

(4) 10 points. You add glucose that is radiolabelled with ^{14}C in the C1 position to yeast cells growing anaerobically. After complete conversion of glucose to produce acetaldehyde and CO_2 as fermentation products, where will the ^{14}C label appear? If you had labeled the glucose with ^{14}C at the C6 position instead where would the label have appeared in product(s)? Show your work that is required to answer the question!

glucose C1 will be at pyruvate C3 (methyl)



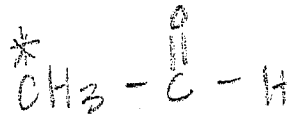
glycolysis →



* = radioactive label

pyruvate

pyruvate decarboxylase



+ CO_2 will come off.

-If non labeled glucose at C1 then the label would show on the methyl carbon of acetaldehyde

-If you labeled glucose at C6 then the label would show at the same position (methyl carbon) of acetaldehyde. The C6 position on glucose will become equivalent with the C1 position after step 5 (triosphosphate isomerase) of glycolysis. This is the step where dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate.

good

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5. (3 points) What are the general names (e.g. alkyl phosphate, guanidinium phosphate) for the high energy organic compounds used for substrate level phosphorylation in glycolyis and the citric acid cycle? acyl phosphate, enol phosphate, CoA thioester

6. (5 points) Explain what insights into mechanistic strategies employed by metabolic enzymes were obtained from comparisons of the crystal structures of hexokinase with and without glucose bound.

induced fit - binding of glucose closes the enzyme (clamps down on substrates), excluding water so the (γ) of ATP is specifically transferred to C6 OH of Glc. induced fit reorients active site so necessary AA residues are in correct positions for catalysis

7. (8 points) Write the balanced chemical equation (names, not structures) for the overall reaction catalyzed by the pyruvate dehydrogenase multienzyme complex. Then write the balanced chemical equations for the reactions catalyzed by the individual components (E1, E2, E3) of the pyruvate dehydrogenase multienzyme complex. Note that the reactions catalyzed by the three individual enzymes should add together such that the overall reaction is obtained.

(a) overall reaction: pyruvate + CoASH + NAD+ → acetyl-CoA + CO_2 + NADH

(b) E1: pyruvate + TPP → hydroxyethyl TPP

(c) E2: hydroxyethyl TPP + ox. lipoamide + HSCoA → reduced lipoamide + acetyl-CoA

(d) E3: reduced lipoamide + NAD+ → NADH + ox. lipoamide

8. (2 points) Write a balanced chemical equations (no structures) for any reaction that involve phosphorolysis. $(glycogen)_n + P_i \rightarrow (glycogen)_{n-1} + Glc\text{-}1\text{-}P$

3 to choose from: Compound 1: glyceraldehyde-3-P + P_i + NAD+ → 1,3-BP-glycerate + NADH

Compound 2: succinyl-CoA + GDP + P_i → succinate + GTP + HSCoA

9. (2 points) Name two glycogen precursors (can be a monosaccharide, disaccharide and/or polysaccharide) for which entry into glycolysis will require a "mutase" enzyme.

1) glycogen (Glc 1-P → Glc 6-P)
2) galactose " " "
3) lactose → Glc

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Student L

6. (5 points) Explain what insights into mechanistic strategies employed by [illegible] obtained from comparisons of the crystal structures of hexokinase with and without glucose bound.

When the crystal structures of hexokinase with and without glucose bound were compared, it was clear that there was a conformational change between the two. With glucose not bound to the enzyme, the active site is completely exposed. With glucose bound, there is a conformational change that blocks entry into the active site. This keeps water from coming into the active site. This phenomenon is known as "induced fit" 5

7. (8 points) Write the balanced chemical equation (names, not structures) for the overall reaction catalyzed by the pyruvate dehydrogenase multienzyme complex. Then write the balanced chemical equations for the reactions catalyzed by the individual components (E1, E2, E3) of the pyruvate dehydrogenase multienzyme complex. Note that the reactions catalyzed by the three individual enzymes should add together such that the overall reaction is obtained.

(a) overall reaction: Pyruvate + HSCoA + NAD^+ → (TPP, lipoamide, Mg^{2+}) → CO_2 + Acetyl-CoA + $NADH$

(b) E1: Pyruvate + NAD^+ → (TPP, Mg^{2+}; lipoamide) → Acetyl-lipoamide + CO_2 + $NADH$

(c) E2: Acetyl lipoamide + HS-CoA + FAD^+ → Acetyl-CoA + $FADH_2$ 8

(d) E3: $FADH_2$ + NAD^+ → FAD^+ + $NADH$

8. (2 points) Write a balanced chemical equations (no structures) for any reaction that involve phosphorolysis. thiohemiacetal 2

Glyceraldehyde 3-P + P_i → (Cys-S-H) → 1,3 diphosphoglycerate

9. (2 points) Name two ~~glycogen~~ glucose precursors (can be a monosaccharide, disaccharide and/or polysaccharide) for which entry into glycolysis will require a "mutase" enzyme. 2

glycogen galactose

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 Name _____

10. (8 points) Name the enzyme from the citric acid cycle that catalyzes each of the following reactions:

(a) dehydrogenation of a C-C bond — Succinate DH

(b) carbon-carbon bond formation — citrate synthase

(c) distinguishes prochiral groups on a substrate — aconitase

(d) oxidative decarboxylation not requiring TPP — Isocitrate DH

(e) substrate level phosphorylation — Succinyl-CoA synthetase

(f) alcohol dehydrogenation — malate DH

(g) oxidative decarboxylation not involving TPP — Isocitrate DH

(h) oxidative decarboxylation involving TPP — α-ketoglutarate DH

(11) (4 points) Circle the correct term (e.g. either liver or muscle, activation or inactivation) from the underlined choices to complete the sentences.
Glucose binds an allosteric site on glycogen phosphorylase in the **liver/muscle** which results in **activation/inactivation** of the enzyme, while AMP binds an allosteric site on glycogen phosphorylase in the **liver/muscle** which results in **activation/inactivation** of the enzyme.

(12) (6 points) What is the correct sequence of events involved in hormonal activation of glycogen breakdown in the muscle? Number the following steps in the order in which they occur (1 to 7).

Step	step
Production of cAMP	4
Conformational change in G protein	2
Activation of glycogen phosphorylase	7
Binding of epinephrine to cell receptor	1
Activation of phosphorylase kinase	6
activation of protein kinase	5
Migration of G_α to adenylyl cyclase	3

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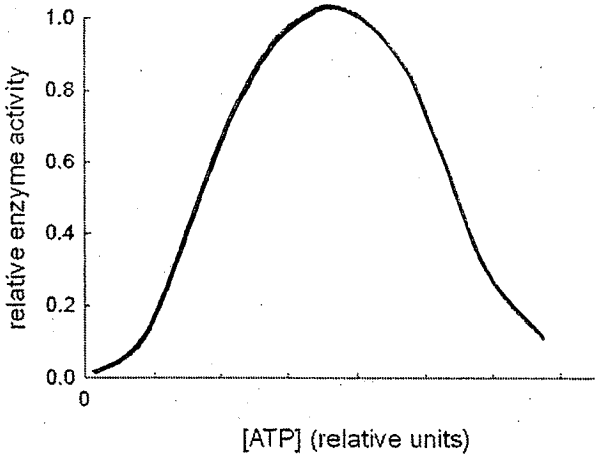
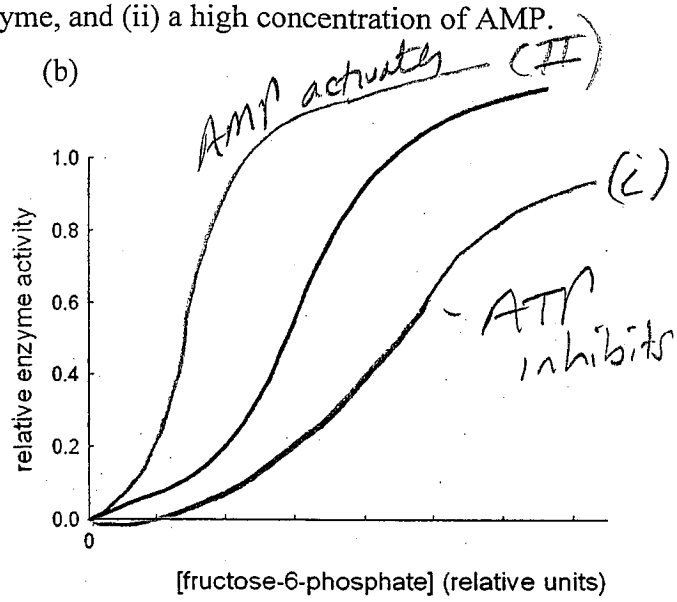
13. (8 points) The following are plot frames for the enzyme phosphofructokinase. Note the units of the x-axis (variable substrate) for each plot. To each plot, add the curve that will be seen as the variable substrate concentration is increased to a concentration much higher than K_m while the concentration of the fixed substrate is held constant. Then, to plot (b), add additional lines that reflect the effects of adding (i) a high concentration of ATP to the enzyme, and (ii) a high concentration of AMP.

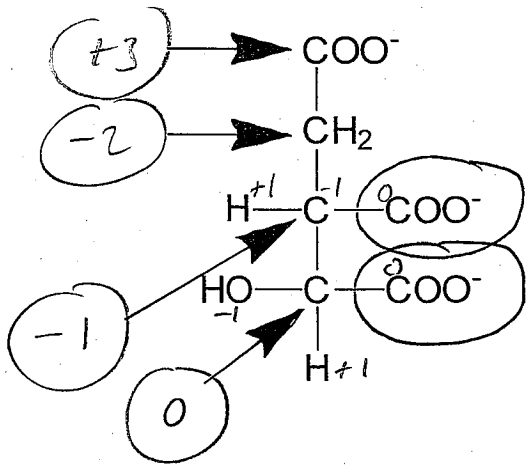
(a)



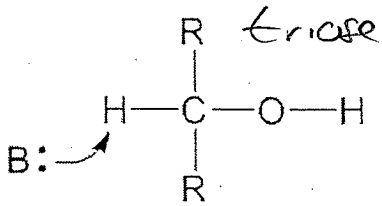
(b)



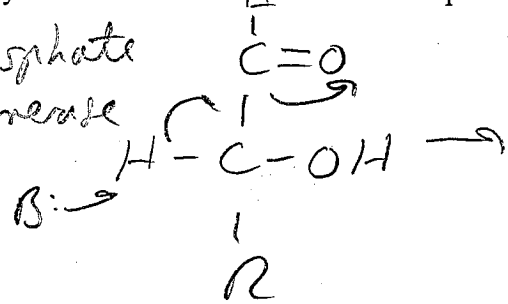
14. (8 points) Assign correct oxidation numbers to the indicated carbon atoms in isocitrate. Then, circle the C atoms that are released as CO_2 during one turn of the citric acid cycle



15. (2 points) The following general base abstraction would be used as the first electron pushing step for which enzyme(s) of glycolysis? Pay attention to the structural details provided for the substrate.



triose phosphate isomerase



→ cisenediolate

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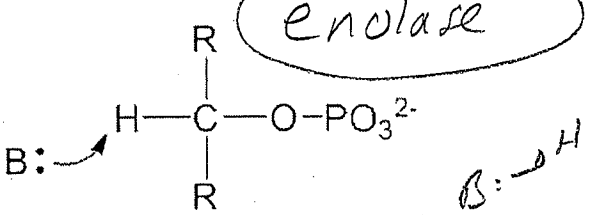
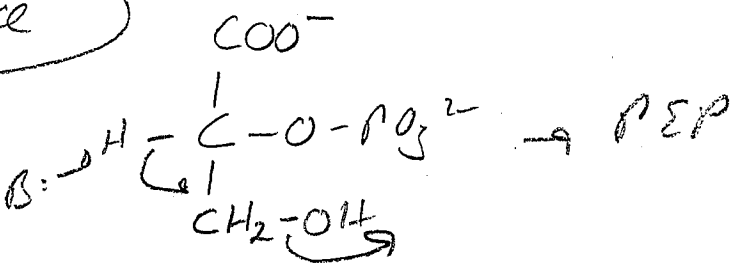
 Name _____

16. (2 points) The following general base abstraction would be used as the first electron pushing step for which enzyme(s) of glycolysis? Pay attention to the structural details provided for the substrate.

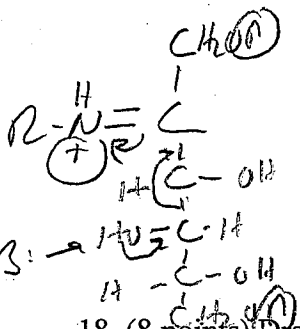
enolase

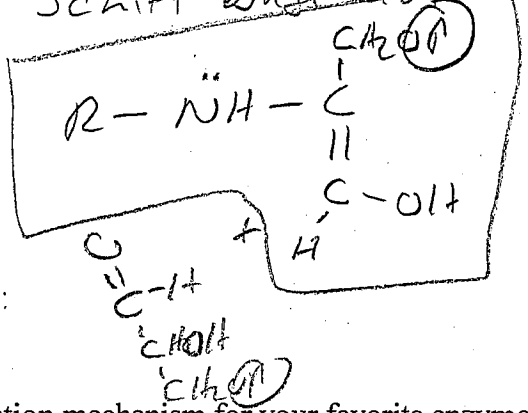


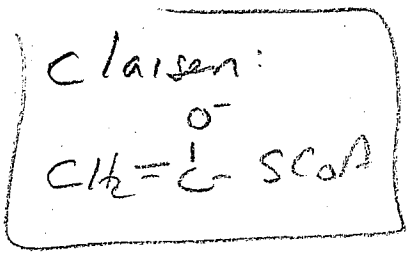


17. (6 points) Draw the structures of the <u>resonance stabilized carbanions</u> that are formed as intermediates by the enzymes aldolase and citrate synthase.

aldulase: Schiff base adduct of DHAP:







18. (8 points) Draw a reaction mechanism for your favorite enzyme of glucose metabolism that catalyzes the dehydrogenation of an alcohol. Draw the mechanism in the physiologically important direction (oxidation or reduction, depending on which enzyme you choose).

lactate dehydrogenase, malate dehydrogenase, G-3-P DH,

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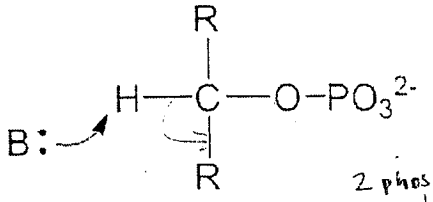
 Name Student

16. (2 points) The following general base abstraction would be used as the first electron pushing step for which enzyme(s) of glycolysis? Pay attention to the structural details provided for the substrate.

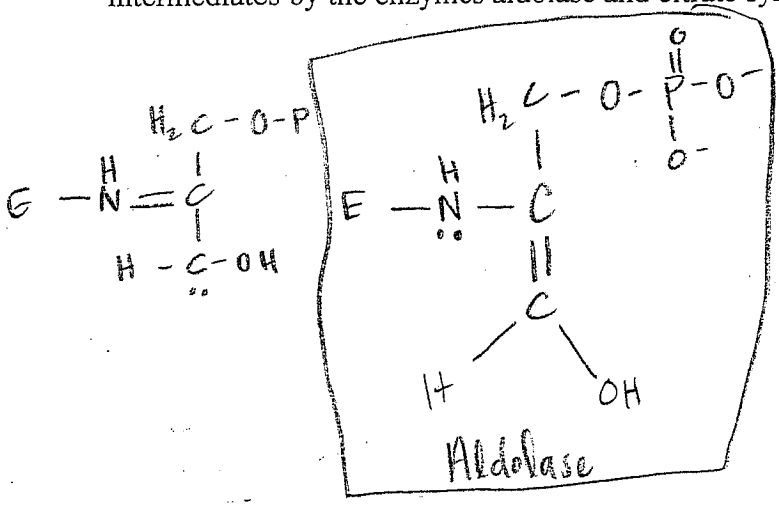


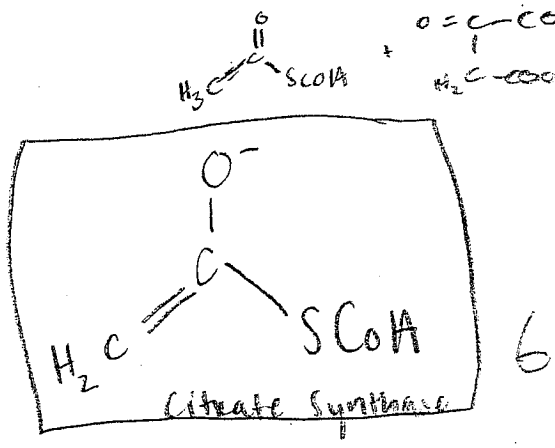
Enolase

2

2 phospho glycerate
↓
phosphoenol pyruvate

17. (6 points) Draw the structures of the resonance stabilized carbanions that are formed as intermediates by the enzymes aldolase and citrate synthase.





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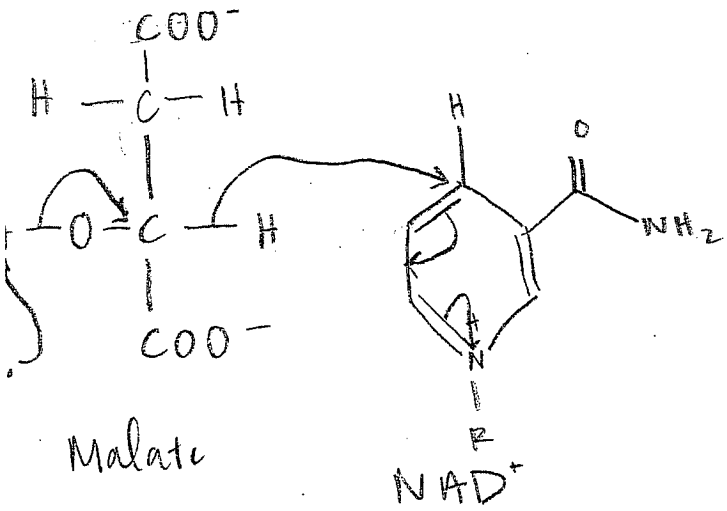
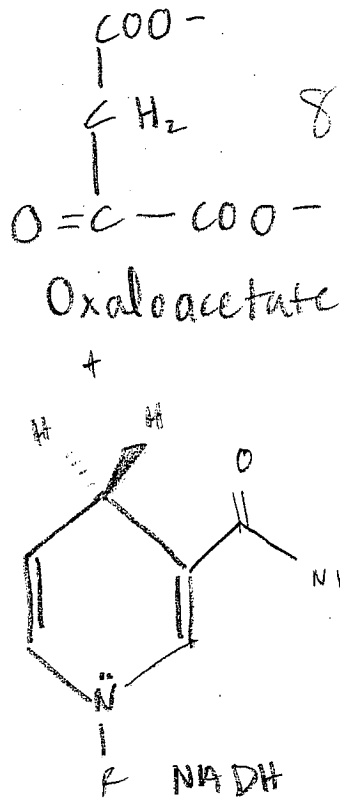
18. (8 points) Draw a reaction mechanism for your favorite enzyme of glucose metabolism that catalyzes the dehydrogenation of an alcohol. Draw the mechanism in the physiologically important direction (oxidation or reduction, depending on which enzyme you choose).



Malate Dehydrogenase



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