

BIOSC 1820
Metabolic Pathways and Regulation
Spring, 2012
Prof. Jeffrey L. Brodsky
Mid-Term Exam
March 1, 2012

NAME: _____

Unless indicated otherwise, each question is worth 2 points – Good luck!

1. THE REAL WORLD

A. The components that make-up the Citric Acid Cycle in a cyanobacteria were recently reported in a paper that appeared in the journal *Nature*. Interestingly, the organism lacks α -ketoglutarate dehydrogenase and succinyl-CoA synthetase. However, it still makes succinate. The authors of this paper then found that two enzymes existed in this organism in place of α -ketoglutarate dehydrogenase and succinyl-CoA synthetase. The first enzyme performs a non-oxidative decarboxylation and the second is a dehydrogenase.

Based on this information, propose a metabolic pathway by which the reactant for α -ketoglutarate dehydrogenase can be converted into succinate. Draw the structures of the reactants/products of these two enzymes, and if appropriate, denote the name of any cofactors (double credit).

B. Based on this information, approximately how many ATP equivalents are “lost” compared to mammals for each turn of this cycle? (In brief, please provide your reasoning)

C. What is the full name of the cofactor that most likely performs the non-oxidative decarboxylation, and what is the name of the enzyme that we have already discussed that performs the same type of reaction?

2. It is becoming increasingly clear that cancer cells “prefer” to make ATP in a non-oxidative manner, thus generating lactic acid. One way this happens is by reduced activity of the enzyme that catalyzes the final favorable step in glycolysis.

A. Draw the structures of the reactant and the product in the reaction catalyzed by this enzyme:

B. Based on the mechanism by which this enzyme is regulated in the liver, propose a mechanism that cancer cells use to reduce the enzyme's activity:

C. As a result of this altered metabolic "decision" in cancer cells, it is thought that more glucose-6-phosphate is available to the cells. This, in turn, reduces both oxidative damage and the generation of reactive oxygen radicals that might otherwise kill the cancer cells. Draw the structures of the reactants and products for the reaction that uses glucose-6-phosphate and generates a product that can then be used, indirectly, to relieve the cell of oxygen radicals (the structures of any required co-factors can be abbreviated):

3. Cholera leads to a horrible loss of body fluids and electrolytes through uncontrolled diarrhea. This is the result of the phosphorylation of an ion channel in the cells that line the small intestine, which when activated releases chloride into the lumen of the small intestine. When chloride leaves these cells, water also leaves the cells, and diarrhea results. Interestingly, the way that cholera triggers this event is by constitutively activating an α subunit in a G protein.

A. Propose a candidate for the kinase that activates the intestinal chloride channel:

B. Recently, a protein known as “GoLoCo” was found to bind to and stabilize Gα-GDP. Would an increase in the amount of this protein in intestinal cells make the effects of cholera better or worse? Why?

4. The activity of lipoprotein lipase is highly regulated, and in fact some diseases result from an inability to properly synthesize and secrete this enzyme to the extracellular surface of the plasma membrane.

A. Draw the structures of the reactants and the products of the reaction that is catalyzed by lipoprotein lipase (a generic substrate and the reactants can be depicted):

B. Suppose you wanted to do gene therapy and use a virus to introduce the enzyme into the endothelial cells of people who expressed only ~50% of the normal amount of lipoprotein lipase. Which metabolite might the transcriptional promoter respond to that you would want to use to drive the transcription of the message encoding the lipase?

5. As we discussed, during starvation, ketone bodies serve as a means to provide energy to cells in the central nervous system.

A. What is the name of the enzyme that performs the first step in ketone body synthesis?

B. One ketone body is acetoacetate. Draw the structures of the reactants and the products by which acetoacetate is converted into a useful metabolite in the brain. Be sure to include the structures of both reactants and products:

C. If this reaction were running at a high rate in the brain, do you think the activity of the Citric Acid Cycle would increase or decrease? Why?

2. MULTIPLE CHOICE

i. Which of the following molecules leads to the inhibition of the activity of phosphofructokinase-1 in the liver?

- A. citrate**
- B. AMP**
- C. fructose-2,6-bisphosphate**
- D. insulin**
- E. More than one of the above is correct**

ii. Which of the following vitamins/cofactors is not a component of the pyruvate dehydrogenase complex?

- A. thiamine pyrophosphate
- B. glutathione
- C. lipoic acid
- D. niacin
- E. pantothenic acid

iii. An increase in the activity or the amount of each of the following would increase the mobilization of fatty acids in adipocytes, except:

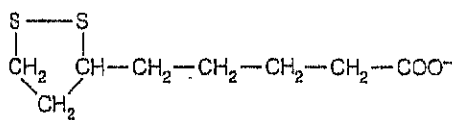
- A. monoacylglycerol lipase
- B. phosphorylated perilipin
- C. protein kinase A regulatory subunit ("R")
- D. epinephrine
- E. adenylyl cyclase

iv. Which of the following sugars would not react with sodium borohydride (NaBH_4)?

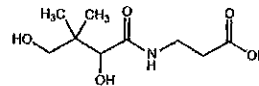
- A. D-glucose
- B. D-fructose
- C. D-mannose
- D. inositol
- E. L-lactate

v. Which of the following molecules is conjugated onto fatty acids and is required for fatty acid transport into the mitochondria?

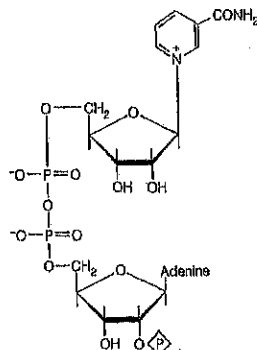
A.



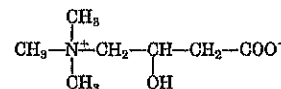
B.



C.

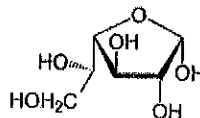


D.



vi. Which of the following terms best describes the classification of the depicted sugar?

- A. D-glucopyranose
- B. D-galactofuranose
- C. L-aldopentose
- D. D-fructose
- E. Bob the Sugar



vii. Which of the following properties does not apply to ATP?

- A. The free energy change for hydrolysis to ADP is -31.5 kJ/mol
- B. It has a low activation energy barrier for hydrolysis
- C. The ionization of a phosphate in ADP contributes to the favorable free energy of hydrolysis
- D. In almost all cases Mg^{2+} is associated with ATP in cells
- E. None of the above, they all apply to this wacky molecule

3. THINK ABOUT IT....

A. Why does pyruvate carboxylase play a more important role than the malic enzyme as an anapleurotic reaction for the Citric Acid Cycle?

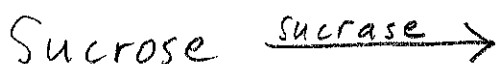
B. Which do you think would cause a more severe disease: People who lack glycogen phosphorylase or the debranching enzyme? Why?

C. Based on the following Table, what is the ΔG for the transfer of 2 electrons from dihydrolipoamide acetyltransferase into the next enzyme within the pyruvate dehydrogenase complex? (You can assume that Faraday's constant, \mathcal{F} , is 100 kJ/V•mol) (double credit)

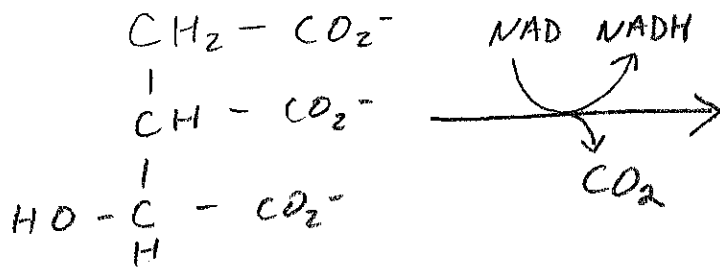
Half-reaction	E° (V)
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}$	0.816
$\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+}$	0.771
$\text{NO}_3^- + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	0.421
Cytochrome <i>f</i> (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome <i>f</i> (Fe^{2+})	0.365
$\text{Fe}(\text{CN})_6^{3-}$ (ferricyanide) + $\text{e}^- \rightarrow \text{Fe}(\text{CN})_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}_2$	0.295
Cytochrome <i>a</i> (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome <i>a</i> (Fe^{2+})	0.29
Cytochrome <i>c</i> (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome <i>c</i> (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome <i>b</i> (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome <i>b</i> (Fe^{2+})	0.077
Ubiquinone + $2\text{H}^+ + 2\text{e}^- \rightarrow$ ubiquinol + H_2	0.045
Fumarate ²⁻ + $2\text{H}^+ + 2\text{e}^- \rightarrow$ succinate ²⁻	0.031
$2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2\text{H}^+ + 2\text{e}^- \rightarrow$ butyryl-CoA	-0.015
Oxaloacetate ²⁻ + $2\text{H}^+ + 2\text{e}^- \rightarrow$ malate ²⁻	-0.166
Pyruvate ⁻ + $2\text{H}^+ + 2\text{e}^- \rightarrow$ lactate ⁻	-0.185
Acetaldehyde + $2\text{H}^+ + 2\text{e}^- \rightarrow$ ethanol	-0.197
FAD + $2\text{H}^+ + 2\text{e}^- \rightarrow$ FADH ₂	-0.219
Glutathione + $2\text{H}^+ + 2\text{e}^- \rightarrow$ 2 reduced glutathione	-0.23
S + $2\text{H}^+ + 2\text{e}^- \rightarrow$ H ₂ S	-0.243
Lipoic acid + $2\text{H}^+ + 2\text{e}^- \rightarrow$ dihydrolipoic acid	-0.29
NAD ⁺ + $\text{H}^+ + 2\text{e}^- \rightarrow$ NADH	-0.320
NADP ⁺ + $\text{H}^+ + 2\text{e}^- \rightarrow$ NADPH	-0.324
Acetoacetate + $2\text{H}^+ + 2\text{e}^- \rightarrow$ β -hydroxybutyrate	-0.346
α -Ketoglutarate + $\text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow$ isocitrate	-0.38
$2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$ (at pH 7)	-0.414
Ferredoxin (Fe^{3+}) + $\text{e}^- \rightarrow$ ferredoxin (Fe^{2+})	-0.432

4. THOSE CRAZY REACTIONS (or, WHY I BOUGHT A BUNCH OF ^\$#@! INDEX CARDS)—as always, draw the structures AND give the names of the products of the following reactions:

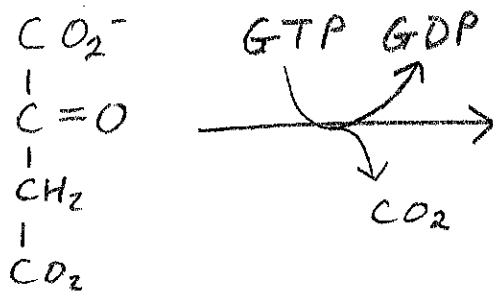
A.



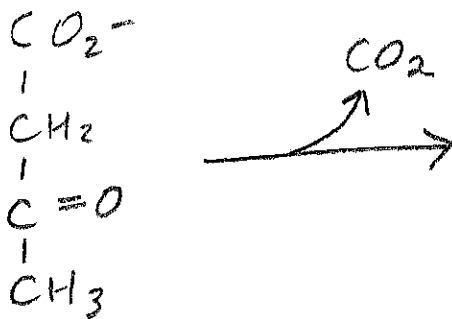
B.



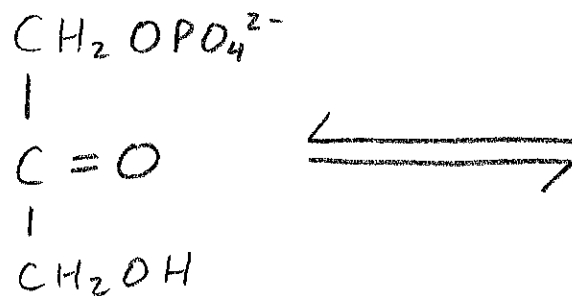
C.



D.

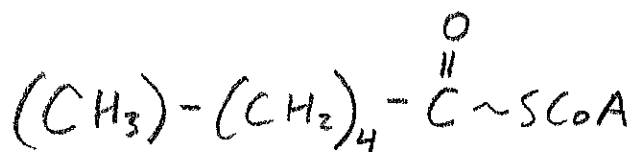


E.



5. SHORT ANSWER

A. How many ATPs could, in theory, be produced from the complete oxidation of the following fatty acid (assume that $\text{NADH} = 3 \text{ ATPs}$ and $\text{FADH}_2 = 2 \text{ ATPs}$)?



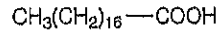
B. If a liver cell expressed a mutant form of Protein Kinase A that was constitutively active, would phosphofructokinase-2 be active or inactive? And, what would the effect be on glycolysis in that cell?

What are the names of the two enzymes that we have discussed that have a phosphorylated histidine as a key component of their reaction mechanisms?

C. _____

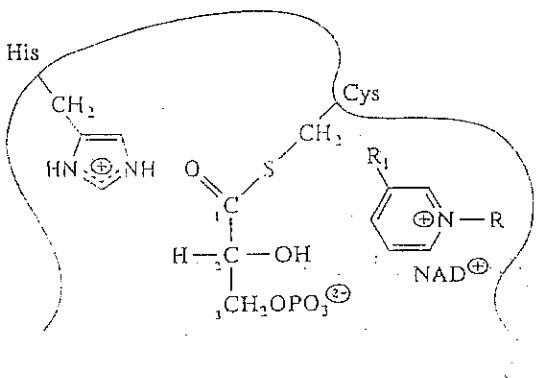
D. _____

E. What is the name of the following fatty acid?



F. This enzyme is activated by an insulin-dependent phosphatase and catalyzes the committed step in the synthesis of fatty acids:

G. The following depicts an intermediate in the reaction catalyzed by which enzyme?



Number incorrect:

$$75 - (2 \times \underline{\quad}) = \boxed{\quad}$$