Spring 2016

NAME: _____ Final Exam

The following exam questions are based on information in van Hellemond, J.J.; Opperdoes, F.R.; and Tielens, A.G.M. (2005). The Extraordinary Mitochondrion and Unusual Citric Acid Cycle in *Trypanosoma brucei*. *Biochemical Society Transactions* **33:** 967-971.

1. The authors suggest that malate metabolism is important to this parasitic organism. Malate dehydrogenase may be used to determine the concentration of malate within samples.

    a. Write out the reaction catalyzed by malate dehydrogenase.

    NADH absorbs at both 260 nm and 340 nm, while NAD^+ only absorbs at 260 nm. Imagine that you create a series of malate solutions between 0 μM and 200 μM, which you subsequently react with NAD^+ using malate dehydrogenase as a catalyst. You allow the solutions to equilibrate and record the absorbance of each at 340 nm. A plot of absorbance at 340 nm versus concentration of NADH (μM) is linear with a slope of 0.004 μM^{-1}.

    b. If a solution with an unknown amount of malate treated in identical assay has an absorbance at 340 nm of 0.34, what is the concentration of the malate in the unknown?

    c. If the unknown tested in part "b" was a 1:5 dilution, what is the concentration of malate in the original sample. [If you were unable to answer part "b", assume the answer was 6 μM and continue with the calculation for part "c"].

    d. What property of malate from a metabolic standpoint makes it an interesting molecule in the types of studies reported in the exam paper?

2. "The long-slender bloodstream form *T. brucei* has a very simple type of energy metabolism, as it is entirely dependent on degradation of glucose into pyruvate by glycolysis. Glucose is degraded to 3-phosphoglycerate inside the glycosomes [a special membrane-bound organelle for parts of glucose oxidation] and this intermediate is further degraded in the cytosol to pyruvate."...

    a. Diagram the process described by the authors for the conversion of glucose to pyruvate in the long-slender bloodstream form *T. brucei* naming molecules and enzymes (no chemical structures are needed).

    b. Fructose-2,6-bisphosphate has been found to activate <u>cytosolic</u> *T. brucei* pyruvate kinase rather than effect phosphofructokinase-1 or fructose-1,6-bisphosphatase activity within the <u>glycosome</u>. In the long-slender bloodstream form, is:

        i. Phosphofructokinase-2 ON OFF

        ii. Fructose-2,6-bisphosphatase ON OFF

3. ... “Upon transformation into the procyclic insect stage, the glycosomal metabolism is extended and part of the produced phosphoenolpyruvate is imported from the cytosol and subsequently converted into succinate via PEPCK (phosphoenolpyruvate carboxykinase), malate dehydrogenase, fumarase, and a soluble glycosomal NADH:fumarate reductase.”

    a. Diagram the process described by the authors for the conversion of phosphoenolpyruvate to succinate naming molecules and enzymes and including chemical structures.

    b. Fructose-2,6-bisphosphate has been found to activate cytosolic *T. brucei* pyruvate kinase rather than effect phosphofructokinase-1 or fructose-1,6-bisphosphatase activity within the glycosome. In the procyclic insect stage, is:

        i. Phosphofructokinase-2 ON OFF

        ii. Fructose-2,6-bisphosphatase ON OFF

4. "In addition to carbohydrate degradation, amino acids, mainly proline and threonine, are important substrates for the production of ATP in procyclic insect-stage *T. brucei*." [succinate is found to accumulate]

    a. Draw proline.

    b. The secondary amine of proline is converted into a secondary imine within the side chain (e.g., not with the α-carbon) to produce 1-pyrroline-5-carboxylate.

        i. Draw 1-pyrroline-5-carboxylate.

        ii. What class of enzyme catalyzes this type of reaction?

        iii. Suggest any additional cofactors or reactants that may be required for this reaction.

    c. Water acts as a nucleophile to break the imine. The imine nitrogen remains on the α-carbon as a primary amine, while the imine carbon is converted to an aldehyde to produce glutamate-5-semialdehyde. Draw glutamate-5-semialdehyde.

    d. The aldehyde of glutamate-5-semialdehyde is converted to a carboxylate to produce glutamate.
        i. Draw glutamate.

ii. What class of enzyme catalyzes this type of reaction?

iii. Suggest any additional cofactors or reactants that may be required for this reaction.

e. Glutamate is converted to α-ketoglutarate.
   i. Draw α-ketoglutarate.

   ii. What class of enzyme catalyzes this type of reaction?

   iii. Suggest any additional cofactors or reactants that may be required for this reaction.

f. α-Ketoglutarate is converted to succinate. Draw the steps including chemical structures, enzyme names and additional cofactors or reactants.

g. How does the parasite generate ATP from the metabolism of proline?