7.05 Biochemistry

Examination #4

May 23, 2019

NAME:_____

You have 90 minutes to complete the exam

Please have your MIT ID visible on your desk

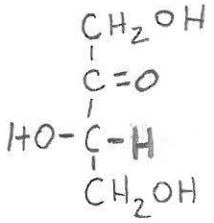
If you get stuck on a question, move on and come back to it at the end.

TA USE ONLY	
Question 1	_____
Question 2	_____
Question 3	_____
Question 4	_____
Question 5	_____

Total	_____

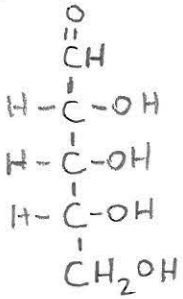
QUESTION 1 (15 points)

Consider the following sugar (L-erythrulose):



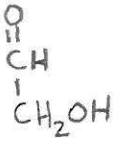
L-erythrulose

A. (4 points) Draw the products of the reaction catalyzed by Transketolase (TK) or Transaldolase (TA) if L-erythrulose is mixed with each of the following sugars (it is not necessary to specify the stereochemistry of the products):

L-erythrulose +  ------- > TK +

L-erythrulose +  ------- > TA +

B. (5 points) Draw <u>six</u> products you expect to find if L-erythrulose is mixed with glycolaldehyde and BOTH Transketolase (TK) and Transaldolase (TA)(it is not necessary to specify the stereochemistry of the products):

L-erythrulose +  ------- > TK + TA

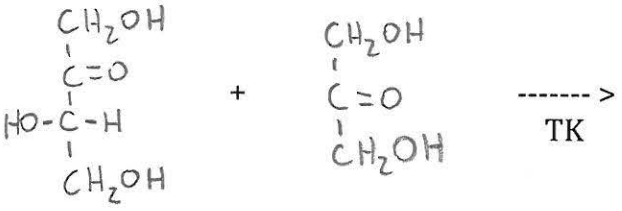
glycolaldehyde

C. (2 points) If you include 3-deazathiamin diphosphate (deazaTPP), an inhibitor of all TPP+ dependent enzymes, in the reaction described in part B, <u>circle</u> the sugar(s) in you answer to part B that will NOT be produced.

2

(Question 1 continued)

D. (4 points) If L-erythrulose is mixed with dihydroxyacetone and Transketolase (TK) what products do you expect to be produced? To answer the question, it will be important to consider the enzymatic mechanism of TK (it is not necessary to show the enzymatic mechanism or specify the stereochemistry of the products):



L-erythrulose dihydroxyacetone

QUESTION 2 (25 points)

Baker's yeast, *S. cerevisiae*, differs from many other eukaryotes in that it lacks complex I of the mitochondrial electron transport chain. Instead, these organisms express a different mitochondrial protein called Ndi1 that localizes to the inner mitochondrial membrane and can transfer electrons between NAD+/NADH and ubiquinone (UQ)/ubiquinol (UQH_2). Unlike the mitochondrial complex I found in most organisms, Ndi1 DOES NOT pump protons across the inner mitochondrial membrane when electrons are transferred from NADH to UQ.

A. (3 points) Ndi1 is a flavoprotein, using the cofactor FMN/$FMNH_2$ as an electron carrier to carry out favorable electron transfer from NADH to UQ. Rank the following electron carriers involved in this electron transfer from 1 to 3 where "1" is the carrier with the lowest standard reduction potential ($E^{\circ'}$) and "3" is carrier with the highest $E^{\circ'}$.

____ UQ/UQH_2 ____ NAD+/NADH ____ FMN/$FMNH_2$

3

(Question 2 continued)

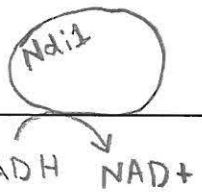
Apart from using Ndi1 instead of Complex I, the mitochondrial electron transport chain in *S. cerevisiae* is the same as the one found in most other organisms.

B. (5 points) Diagram how electrons flow from NADH to reduce oxygen (O_2) to water (H_2O). It is only necessary to include the major proteins/protein complexes involved (i.e. you do not need to specify how electrons are transferred between electron carriers within each complex). However, in your diagram make clear how electrons flow across the electron transport chain starting from Ndi1, and indicate which protein(s)/protein complex(es) pump H+ and place them in the proper location on the diagram below.

mitochondrial inter-membrane space (cytosol)

---

inner mitochondrial membrane



---

mitochondrial matrix

B. (3 points) Is the amount of ATP that can be generated from complete oxidation of pyruvate in the mitochondria by *S. cerevisiae* the same, less, or more than what can be generated by human mitochondria? Assume that coupling between mitochondrial $\Delta\Psi/\Delta pH$ and the F_oF_1-ATPase is the same in *S. cerevisiae* and humans. Circle your answer and briefly explain your reasoning.

Same Less More

C. (2 points) Is the amount of energy released by electron transfer from NADH to O_2 via the mitochondrial electron transport chain in *S. cerevisiae* the same, less, or more than that released by electron transfer from NADH to O_2 in humans?
Circle your answer and briefly explain your reasoning.

Same Less More

4

(Question 2 continued)

A lab at MIT devises a method to implant photosensitive semiconducting nanoparticles into the mitochondrial membranes of *S. cerevisiae*. When exposed to a specific wavelength of light, an electron is excited in the semiconducting material and transferred directly to ubiquinone (UQ) to reduce it to ubiquinol (UQH_2). The resulting positive charge on the semi-conducting material then extracts an electron from H_2O to generate O_2 (in a manner that is analogous to photosynthesis).

D. (4 points) Can electron transfer from UQH_2 back to O_2 to regenerate H_2O by the mitochondrial electron chain be used to net produce ATP (i.e. can *S. cerevisiae* with the photosensitive semiconducting material in their mitochondria use light to drive ATP synthesis)? Briefly explain why or why not?

E. (4 points) You measure the NAD+/NADH ratio in the mitochondria of *S. cerevisiae* with the photosensitive semiconducting material and find that it is more reduced (i.e. NAD+/NADH ratio is lower) than that found in wild-type yeast. However, if you delete Ndi1 then the mitochondrial NAD+/NADH ratio is the same with or without the photosensitive semiconducting material. Briefly explain why.

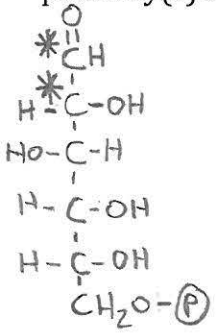
F. (4 points) Will implanting the photosensitive semiconducting material into the mitochondria of *S. cerevisiae* allow these organisms to net convert CO_2 to glucose? Explain your answer.

5

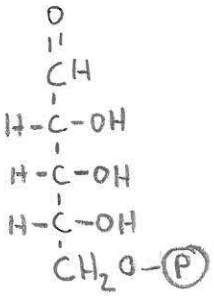
QUESTION 3 (30 points)

Your UROP project in a cancer lab is to understand how cancer cells metabolize glucose to support growth, and you take advantage of 1,2-^{13}C-labeled glucose where carbons #1 and #2 are labeled (indicated by a "*" below) with a heavy isotope.

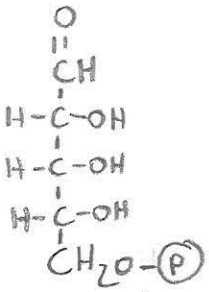
A. (8 points) You culture cancer cells in medium with 1,2-labeled glucose and analyze the ribose-5-phosphate produced in cells. You find ribose-5-phosphate where only <u>one</u> carbon is labeled, and ribose-5-phosphate where <u>two</u> carbons are labeled. Provide a pathway(s) starting with 1,2-labeled glucose-6-phosphate (shown below) to produce ribose-5-phosphate with <u>one</u> carbon labeled and ribose-5-phosphate with <u>two</u> (or more) carbons labeled. It is not necessary to draw structures, provide enzyme names, or show reaction mechanisms (i.e. no arrow pushing). It is also not necessary to specify how glucose-6-phosphate is converted into any glycolytic intermediates (just write "glycolysis"). However, specify substrates, products, and cofactors involved in any other reactions to produce the different labeled forms of ribose-5-phosphate.



1,2-labeled glucose-6-P



One carbon-labeled ribose-5-P



Ribose-5-P with two or more labeled carbons

B. (2 points) On the "one carbon-labeled ribose-5-P" drawn above, <u>circle</u> the specific carbon that <u>will</u> be labeled from 1,2-labeled glucose.

BONUS (2 points) <u>Circle</u> the carbon on "Ribose-5-P with two or more labeled carbons" drawn above that is <u>least likely</u> to be labeled from 1,2-labeled glucose.

6

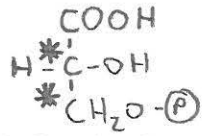
(Question 3 continued)

C. (3 points) You find that most ribose labeled in cancer cells from 1,2-labeled glucose contains <u>two or more</u> labeled carbons. What can you conclude about the relative demand of these cells for NADPH versus ribose? Briefly explain your answer.

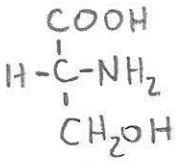
D. (2 points) Hydrogen peroxide will react with the tripeptide glutathione and promote the oxidized G-S-S-G form of the molecule. If you add hydrogen peroxide to the culture media, do you expect the amount of ribose-5-phosphate that is labeled on <u>only one carbon</u> to increase, decrease, or stay the same? Briefly explain your answer.

You evaluate other fates of 1,2-labeled glucose and notice that ~50% of the glycolytic intermediate 3-phosphoglycerate is labeled on carbons #2 and #3 (where "*" indicates the labeled carbons below). You also find some of the serine and glycine is labeled.

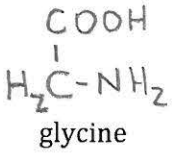
E. (5 points) Provide a pathway(s) starting with 3-phosphoglycerate to produce serine and glycine. It is not necessary to draw structures, provide enzyme names, or show reactions mechanisms (i.e. no arrow pushing). However, specify all substrates, products, and cofactors involved.



3-phosphoglycerate



serine



glycine

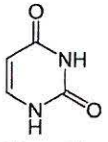
F. (2 points) On the glycine molecule structure above, <u>circle</u> the carbon(s) you expect to be labeled from 1,2-labeled glucose (via labeled 3-phosphoglycerate).

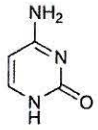
7

(Question 3 continued)

You also note that some nucleotide tri-phosphates become labeled when cells are cultured in 1,2-labeled glucose. You find that label can be incorporated into the ribose part of nucleotides, as well as some of the bases.

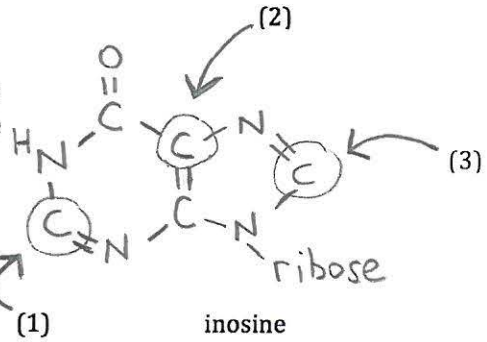
G. (4 points) Considering ONLY labeled carbon that could be incorporated FROM SERINE AND GLYCINE from 1,2-labeled glucose via the pathway you provided in E, circle each pyrimidine base below that you expect to contain labeled carbon. Very briefly explain your answer.


Uracil


Cytosine


Thymine

You find that purine bases can be labeled on multiple carbons from 1,2-labeled glucose, including the three carbons circled on the purine inosine (shown here):



inosine

H. (4 points) Briefly describe the direct source of each of the circled carbons in purine synthesis. Providing either the name or the structure of the intermediate that contributes each of the circled carbons to inosine is sufficient.

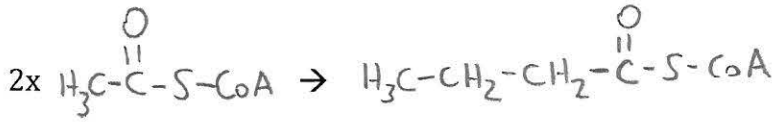
(1) (2) (3)

8

QUESTION 4 (15 points)

During your summer working at Woods Hole studying the metabolism of marine organisms, you sequence the genome of a newly discovered photosynthetic algae that thrives in waters where the surf churns against the rocky coast, and find that it lacks the enzyme acetyl-coA carboxylase (ACC), which is required for fatty acid synthesis in most organisms.

A. (7 points) Describe how 2 acetyl-coA molecules are used to generate the fully saturated four-carbon (4:0) fatty acyl-coA (shown below). It is not necessary to draw structures, specify enzyme names other than ACC, or show reactions mechanisms (i.e. no arrow pushing). However, make clear which reaction is catalyzed by ACC, and which substrates, products, and cofactors are involved in each step.



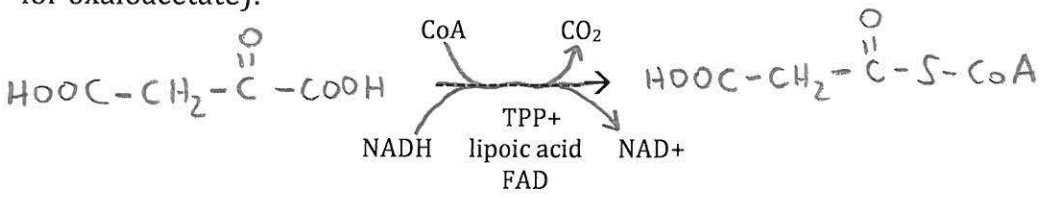
acetyl-coA 4:0 fatty acyl-coA

You provide the organism with isotope labeled acetate and find that it is incorporated into fatty acids, demonstrating that it is capable of fatty acid synthesis despite lacking the enzyme ACC, and seek to understand how this can be the case.

9

(Question 4 continued)

You test several hypotheses and discover that the organism has an enzyme complex that uses oxaloacetate as a substrate in a reaction that is analogous to that catalyzed by pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (αKGDH) and branched chain α-ketoacid dehydrogenase (BCKDH)(i.e. it has an E1 that is specific for oxaloacetate):



B. (3 points) Propose a hypothesis for why this photosynthetic organism that lives in particularly well-oxygenated water might benefit from using the above enzyme complex instead of ACC.

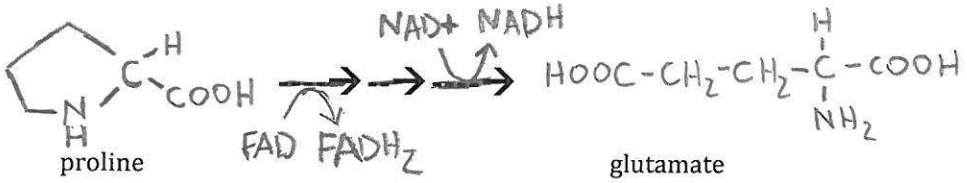
C. (2 points) Do you expect fatty acid synthesis to be regulated in the same way in this marine organism as it is in humans? Briefly explain your answer.

D. (3 points) Can the break down of amino acids be used to supply carbon for fatty acid synthesis in this organism? Does it matter if they are glucogenic or ketogenic amino acids? Briefly explain your answer, and specify any assumptions about other reactions that the organism is capable of doing if it impacts your answer.

10

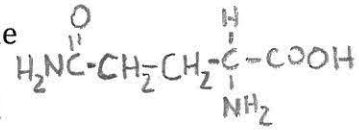
QUESTION 5 (15 points)

Recently it was discovered that some cancer cells can catabolize proline (derived from breakdown of collagen) as a source of energy to survive. Proline is converted to glutamate via a series of oxidation reactions:



proline glutamate

You add proline that contains isotopically labeled nitrogen to the culture media under conditions where the cells catabolize proline, that find that the cells excrete both ammonia (NH_3) and glutamine (shown at right) that contain labeled nitrogen.



glutamine

A. (2 points) Show the reaction that will allow glutamate with nitrogen derived from proline to produce ammonia. It is not necessary to specify enzyme names or show reactions mechanisms (i.e. no arrow pushing). However, make clear which substrates, products, and cofactors are involved.

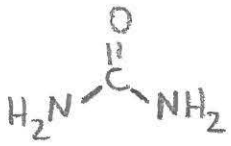
B. (3 points) Show the reaction(s) that will allow labeled nitrogen from proline to produce glutamine with <u>two</u> labeled nitrogens. You do not need to show how proline is turned into glutamate with one labeled nitrogen (it occurs via the reactions shown above). It is also not necessary to provide structures, specify enzyme names, or show reactions mechanisms (i.e. no arrow pushing). However, make clear which substrates, products, and cofactors are involved.

C. (2 points) Will the oxidation of proline in the mitochondria to derive energy require access to oxygen? Briefly explain your reasoning.

11

(Question 5 continued)

You analyze what is excreted into the blood from a proline-consuming tumor, and find that here is a large amount of glutamine. You reason that this glutamine must be metabolized in the kidney to excrete the nitrogen from proline as urea.



urea

D. (8 points) Show that reaction(s) that will allow the two nitrogens from glutamine to produce urea. It is not necessary to provide structures, specify enzyme names, or show reactions mechanisms (i.e. no arrow pushing). However, make clear which substrates, products, and cofactors are involved to incorporate the two nitrogens from glutamine into the same molecule of urea. Assume any necessary metabolites and cofactors are freely available.

12

7.05 General Biochemistry
Spring 2020