7.05 Biochemistry

Examination #3

April 29, 2019

NAME:______________________________

You have 90 minutes to complete the exam

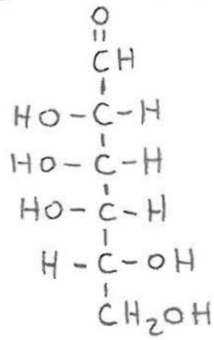
Please have your MIT ID visible on your desk

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

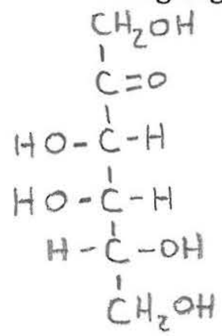
TA USE ONLY	
Question 1	____
Question 2	____
Question 3	____
Question 4	____
Total	____

QUESTION 1 (30 points)

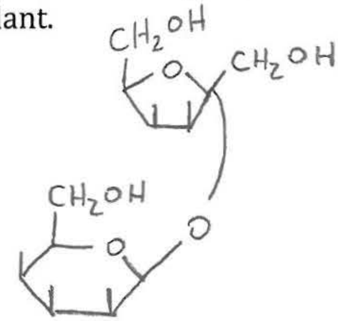
While spending the summer working at Woods Hole, you extract sugars from a marine organism and find that the following sugars are abundant.



Talsose
(i)



Tagatose
(ii)



Disaccharide (talsose + tagatose)
(iii)

A. (7 points) Circle the roman numeral that corresponds to the sugar(s) above which best answer each question (there may be 0, 1 or >1 answer for each question).

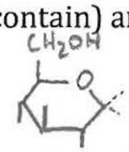
Which sugar(s) is/are(or contain) an aldose? (i) (ii) (iii)

Which sugar(s) is/are(or contain) a ketose? (i) (ii) (iii)

Which sugar(s) is/are(or contain) a pentose? (i) (ii) (iii)

Which sugar(s) is/are(or contain) a D sugar? (i) (ii) (iii)

Which sugar(s) is/are a reducing sugar? (i) (ii) (iii)

Which sugar(s) is/are(or contain) an epimer of glucose (shown here )? (i) (ii) (iii)

Which sugar(s) is/are drawn in the form most likely to be found in the organism? (i) (ii) (iii)

B. (3 points) What is the formal name of the dissacharride (iii)?

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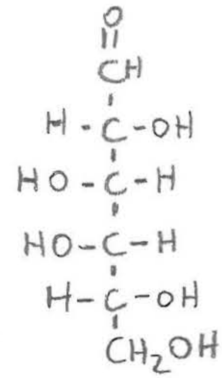
(Question 1 continued)

You also identify α-D-galactose-containing polymers in the organism. (The open chain structure of galactose is shown for reference)



galactose

C. (3 points) Draw α-D-galactopyranosyl-(1-4)-α-D-galactopyranose.

To better understand carbohydrate metabolism in this organism, you sequence the genome, and identify both an isomerase and an epimerase with homology to enzymes that are used to convert glucose to other sugars in humans.

D. (4 points) You purify each enzyme and determine that talsose is a substrate. Assuming the enzyme produces one of the other abundant sugars in the organism as a product, state the product of each reaction.

Talsose $\xleftrightarrow[\text{epimerase}]{}$

Talsose $\xleftrightarrow[\text{isomerase}]{}$

E. (5 points) You reason that the dissacharride (iii) is a storage sugar for the organism, and that it will not be able to be metabolized by predatory mammals even if they have enzymes that act on the monosaccharides. Describe which properties of the sugar lead you to these conclusions (i.e. what makes it a good storage sugar, and what might prevent metabolism by some other organisms).

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(Question 1 continued)

F. (8 points) You further characterize carbohydrate metabolism in the marine organism, and hypothesize that it stores talsose carbon as a polymer of repeating α-D-galactopyranosyl-(1-4)-α-D-galactopyranose units (that you drew in part C). Assuming that the reactions are analogous to those used to store glucose-1-phosphate as glycogen, describe a pathway to store talsose-1-phosphate as the galactose polymer. It is not necessary to provide enzyme names, draw chemical structures, or show reaction mechanisms. However, be sure to specify each of the reactions involved in your pathway and any cofactors required.

QUESTION 2 (20 points)

An enzyme, nucleotide diphosphate kinase (NDPK) catalyzes the following reaction:

$$\text{CTP} + \text{GDP} \leftarrow\rightarrow \text{CDP} + \text{GTP} \qquad\qquad \Delta G^{o\prime} = 0 \text{ kcal/mol}$$

A. (2 points) At equilibrium, which of the following will be true for the NDPK reaction? (circle your answer)

$\text{CTP} < \text{GTP}$ $\qquad$ $\text{CTP} > \text{GTP}$ $\qquad$ $\text{CTP} = \text{GTP}$

Recall that succinic thiokinase catalyzes the following TCA cycle reaction:

$$\text{succinyl-coA} + \text{GDP} + P_i \leftarrow\rightarrow \text{succinate} + \text{GTP} + \text{coA}$$
$$\Delta G^{o\prime} = -0.8 \text{ kcal/mol}$$

B. (2 points) At equilibrium, which of the following will be true for the succininc thiokinase reaction? (circle your answer)

$\text{GDP} < \text{GTP}$ $\qquad$ $\text{GDP} > \text{GTP}$ $\qquad$ $\text{GDP} = \text{GTP}$

If you mix 1 mM each of succinyl-coA, GDP, CDP, and P_i in a reaction with succinic thiokinase and NDPK:

C. (2 points) Will you have more CTP than GTP at equilibrium? Briefly explain your answer.

D. (2 points) Will you have more CTP than CDP at equilibrium? Briefly explain your answer.

E. (2 points) If you add twice as much NDPK enzyme as succinic thiokinase, will you produce more CTP? Briefly explain why or why not.

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(Question 2 continued)

F. (3 points) If you add 10 M CTP and 1 M CDP to a reaction with 1 mM each of succinyl-coA, GDP, and P_i where both succinic thiokinase and NDPK are present, approximate the ratio of GTP/GDP you expect to find when the reaction reaches equilibrium. Briefly explain your answer.

G. (7 points) If you add 10 M CTP and 1 M CDP to the reaction with 1 mM each of succinate, GTP, and coA where both succinic thiokinase and NDPK are present, will production of succinyl-coA be favored? Describe how you arrived at your answer. (Hint: the concentration of CTP and CDP added here are the same as in part F.)

ln(1000) = 6.9
ln(100) = 4.6
ln (10) = 2.3
ln (1) = 0
ln (0.1) = -2.3
ln (0.01) = -4.6
ln (0.001)= -6.9
RT = ~0.5 kcal/mol

QUESTION 3 (30 points)

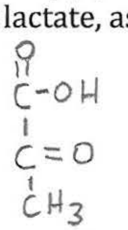
Over the summer you take a job at a brewery that specializes in "sour" beers, because they want to use your knowledge of biochemistry to help make different products.

A. (2 points) Addition of citrate is a common way to make foods taste sour, so you suggest adding citrate to the beer. Assuming that citrate can be taken up by the yeast, and that yeast regulate glycolysis in the same way as mammals, should you add the citrate at the start of fermentation (when you add the glucose), or after all the glucose has been fermented into ethanol? Briefly explain your reasoning.

B. (2 points) Will stressing the yeast to make them consume more ATP make the rate of fermentation faster or slower? Assume yeast regulate glycolysis in the same way as mammals, and briefly explain your reasoning.

To produce "sour" beers without adding citrate, you engineer yeast (that normally ferment glucose to ethanol) to also express any additional enzymes needed to produce lactate. You confirm this modified yeast ferments glucose to both lactate and ethanol.

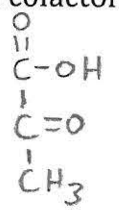
C. (2 points) Show the reaction(s) needed to convert pyruvate (structure provided below) to lactate. It is not necessary to provide enzyme name(s) or show reaction mechanism(s) (i.e. no arrow pushing). However draw the chemical structure for the product lactate, as well as any intermediates, and specify if cofactors are required.



pyruvate

(Question 3 continued)

D. (3 points) Show the reaction(s) needed to convert pyruvate to ethanol (CH_3CH_2OH). It is not necessary to provide enzyme name(s) or show reaction mechanism(s) (i.e. no arrow pushing). However draw the chemical structure for any intermediates and specify if cofactors are required.

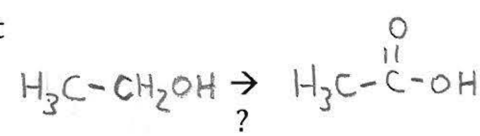


pyruvate

E. (2 points) Will the availability of oxygen affect either lactate or ethanol production? Briefly explain your answer.

Your ethanol and lactate producing yeast makes "sour" beer, but some of your customers are uncomfortable consuming a product from a genetically modified organism. Thus, you try to modify conditions so the original ethanol-only fermenting yeast will produce some acetate (as yet another way to make the beer sour). You wait until all the sugar is fermented, and bubble some oxygen into the beer so the yeast can metabolize some of the ethanol to acetate.

F. (3 points) Show the reaction(s) needed to convert ethanol to acetate (shown to the right). It is not necessary to provide enzyme names, draw chemical structures, or show reaction mechanisms (i.e. no arrow pushing). However please make clear what steps are involved, specifying any intermediates and cofactors that are required.



ethanol acetate

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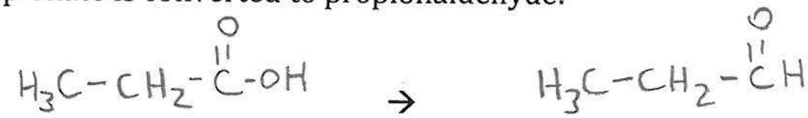
(Question 3 continued)

You are successful in increasing acetate production by adding oxygen, but find the amount of acetate produced is variable and look for an alternative to oxygen that will accomplish the same task. Someone ordered propionate as an experimental flavoring agent, and you decide to add it to your culture. You find that it does increase acetate production from ethanol even in conditions where oxygen is absent. You also notice that propionate is converted to propionaldehyde:



propionate → propionaldehyde

G. (3 points) Provide an explanation for how the above reaction allows increased acetate production.

You try adding both oxygen and propionate to the yeast culture and now propionaldehyde is no longer produced and all the ethanol (and propionate) is converted to CO_2. You also notice that if propionate is <u>not</u> added to the culture, the ethanol is no longer metabolized to CO_2. You astutely recognize that your yeast must be deficient in the enzyme needed to generate glyoxylate from isocitrate.

H. (3 points). Why is glyoxylate production necessary for yeast to convert acetyl-coA from ethanol to CO_2?

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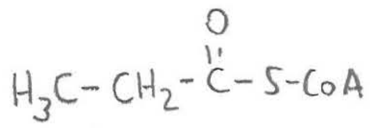
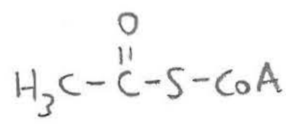
(Question 3 continued)

I. (10 points). Show the reaction(s) needed to convert both acetyl-coA (which is produced from the ethanol) and propionyl-coA (which is produced from the propionate) to CO_2. It is not necessary to provide enzyme names, draw chemical structures, or show reaction mechanisms (i.e. no arrow pushing). However please make clear what steps and cofactors are involved.



propionyl-coA



acetyl-coA

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QUESTION 4 (20 points)

As a UROP, you work on a project to develop novel formulations to better control the release of drugs taken by mouth. You determine that when some fatty acids are included in the pill formulation, a drug to treat diabetes is released in a way that is more desirable for patients. Your formulation includes the following fatty acids:

10:0	10:2 $\omega^{3,5}$	10:3 $\Delta^{2,7,9}$	18:0	18:2 $\Delta^{8,11}$
(i)	(ii)	(iii)	(iv)	(v)

A. (5 points) Circle the roman numeral that corresponds to the fatty acid(s) above which best answer each question (there may be 0, 1 or >1 answer for each question).

Which fatty acid(s) is/are unsaturated?	(i)	(ii)	(iii)	(iv)	(v)
Which fatty acid has the highest melting temperature?	(i)	(ii)	(iii)	(iv)	(v)
Which fatty acid(s) is/are most likely to be found in cells (either free or part of a lipid)?	(i)	(ii)	(iii)	(iv)	(v)

B. (3 points) Rank the fatty acids in terms of energy that can be derived from complete oxidation of the fatty acid to CO_2 (with 1 being the least energy released and 5 being the most).

____	____	____	____	____
(i)	(ii)	(iii)	(iv)	(v)

C. (2 points) Draw fatty acid (ii).

D. (2 points) Your formulation is successful, however there is a desire for a liquid rather than pill formulation and you are asked to tweak the fatty acids accordingly. What properties of the fatty acids can you change to make them more likely to be a liquid at room temperature?

(Question 4 continued)

E. (8 points) Show the steps needed to convert fatty acid (iii) to an 8:2 $\Delta^{5,7}$ fatty acid. It is not necessary to provide enzyme names or show reaction mechanisms (i.e. no arrow pushing); however, please make clear what steps and cofactors are involved.

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7.05 General Biochemistry
Spring 2020