C483 Exam 3
Spring 2017

Name Key Seat Number _____

Student ID _____

Page 11 of this exam contains equations and other helpful information. You may remove page 12 from the exam for scratch paper.

This exam contains 110 points. The highest score you may earn on this exam is 100 points.

1 _____/20pts

2. _____/10pts

3. _____/20pts

4. _____/10pts

5. _____/10pts

6. _____/10pts

7. _____/10pts

8. _____/10pts

9. _____/10pts

Total:

Regrading: All requests for regrades must be submitted in writing within 48 hours of the return of the exam. You must explicitly state what has been misgraded and why it is an error. The entire exam will be regraded, which could result in points being added or deducted overall.

Section 1: Reading guides (50 points)

1. 20 pts. Fill in the blanks (2 points each.)

A. Muscle cells lack the enzyme glucose-6-phosphatase, which means that muscle glycogen stores cannot be used to raise blood sugar.

B. Complex I catalyzes transfer of high energy electrons from NADH to Q through a series of redox centers.

C. ATP is synthesized in the ATP synthase through the binding-change mechanism.

D. The pentose phosphate pathway utilizes epimerases enzymes to transform sugars into a different stereoisomer.

E. The Q cycle allows Complex III to transport twice as many protons across the inner membrane of the mitochondria than would be possible without it.

F. Cholesterol synthesis is regulated at the enzyme HMG-CoA reductase, which catalyzes the formation of mevalonate.

G. The enzyme acetyl CoA carboxylase is allosterically regulated by malonyl CoA.

H. The glyoxylate pathway can be used by plants and some bacteria to make net glucose from acetyl CoA.

I. TPP is the cofactor necessary for the decarboxylation step of

J. αKG DH and isocitrate DH are two enzymes that are regulated in the citric acid cycle.

2. 10 pts. Write True or False (1 points each)

A. True Substrate-level phosphorylation occurs in both glycolysis and the citric acid cycle.

B. False Mammals cannot convert oxaloacetate into net glucose.

C. True The non-oxidative phase of the pentose phosphate pathway allows 5-carbon sugars to be made into 3- and 6-carbon sugars reversibly.

D. False TPP is the cofactor necessary in the citric acid cycle reaction that produces α-ketoglutarate.

E. False Citrate is a downregulator of fatty acid synthesis.

F. True The transformation of pyruvate to acetyl CoA is a redox reaction.

G. True In the electron transport chain, electrons are passed successively to compounds that have greater reduction potentials.

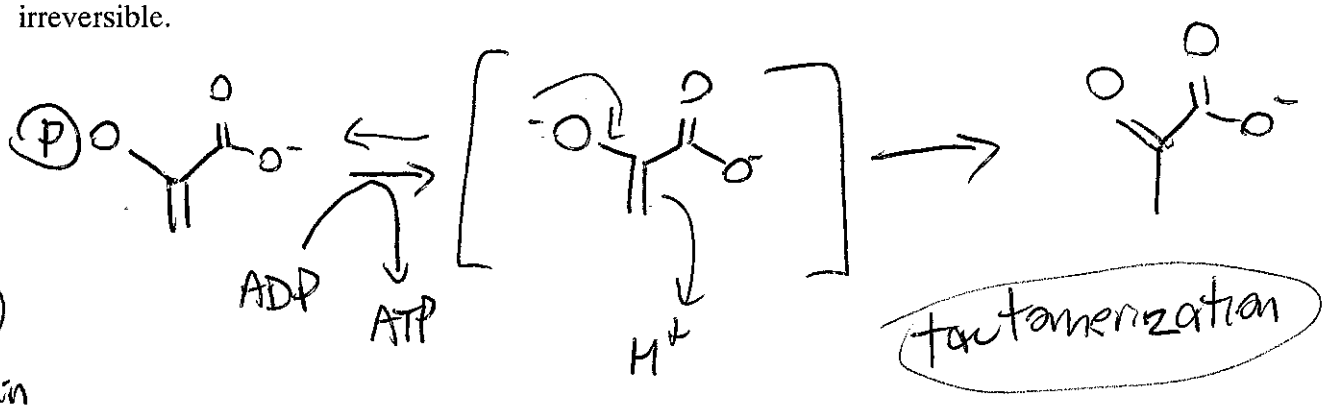
H. True The carbon source for biosynthesis of both fatty acids and cholesterol is acetylCoA.

I. True Acetyl CoA is activated for synthesis of fatty acids by a carboxylation reaction.

J. True In humans, the ATP synthase can produce 3 ATP for every 8 protons that cross the membrane through its *c* subunits.

3. 20 pts. Short answer (5 points each)

A. The final step of glycolysis, catalyzed by pyruvate kinase, involves formation of ATP and production of pyruvate. Draw the reaction catalyzed by pyruvate kinase and use it to explain the chemical driving force that allows this reaction to produce ATP and be thermodynamically irreversible.



rxn +3

explain +2

B. Use data from the end of the exam to calculate the standard free energy of the reaction catalyzed by phosphoglycerate kinase.

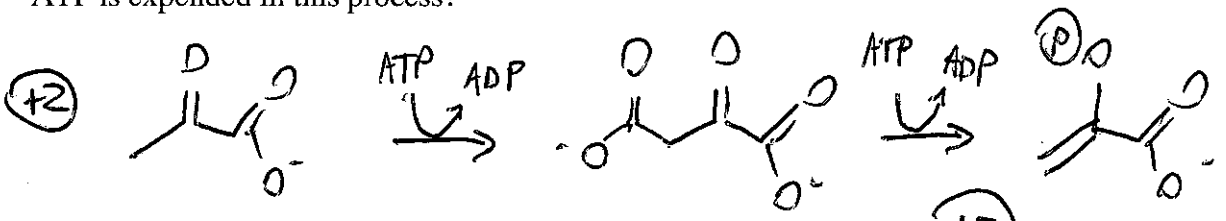
1,3-bisphosphoglycerate + ADP → 3-phosphoglycerate + ATP

$\Delta G^{o'} = -49.4$ KJ/mol

$+ 30.5$ KJ/mol

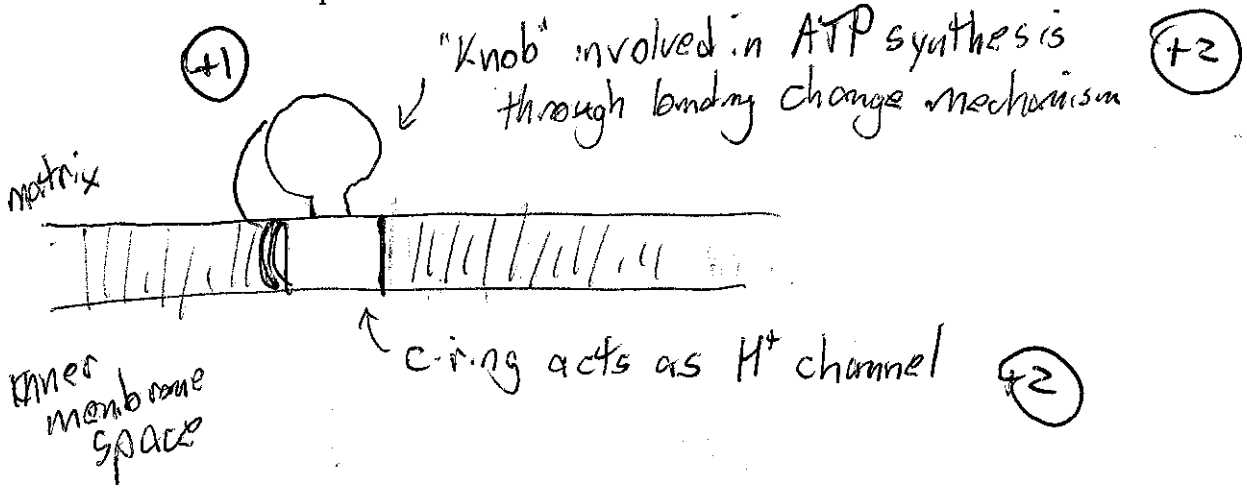
-18.9 KJ/mol

C. Draw the two reactions needed to produce phosphoenolpyruvate from pyruvate. How much ATP is expended in this process?



+1 2 ATP equivalents

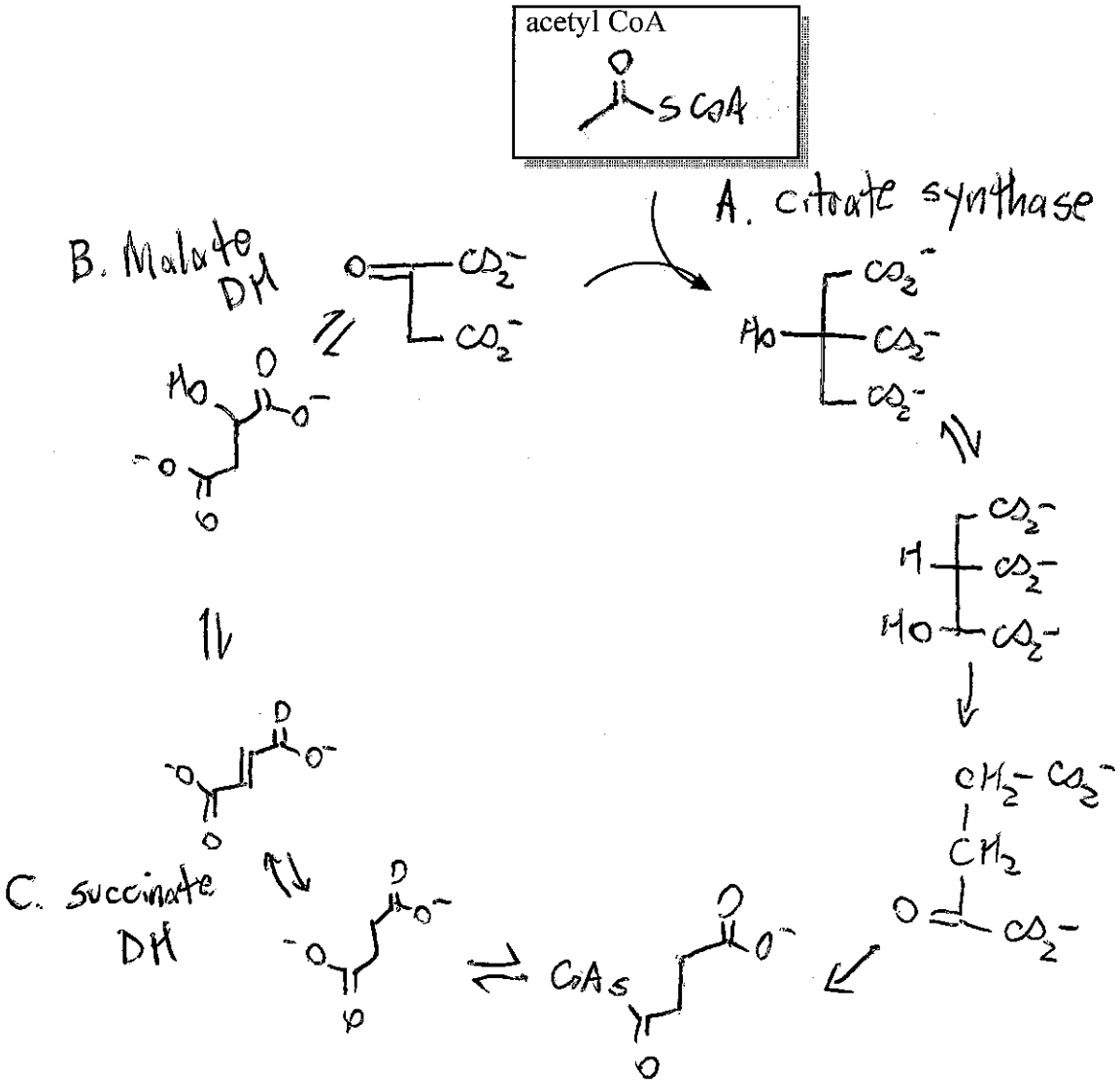
D. Draw a simple schematic of ATP synthase in terms of its F_0 and F_1 components. What is the main function of each component?



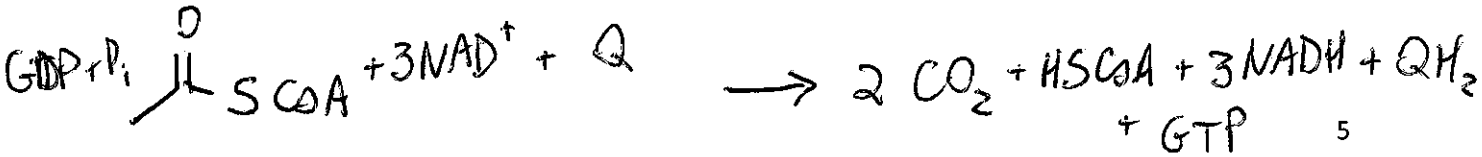
Section 2: Problems (10 points each)

4. Draw the structures of acetyl CoA and all intermediates in the citric acid cycle. Indicate which steps are reversible and irreversible. In your figure, write the names of the three enzymes described here:

A. catalyzes a hydrolysis reaction
B. catalyzes formation of NADH in a reversible reaction
C. an integral membrane enzyme



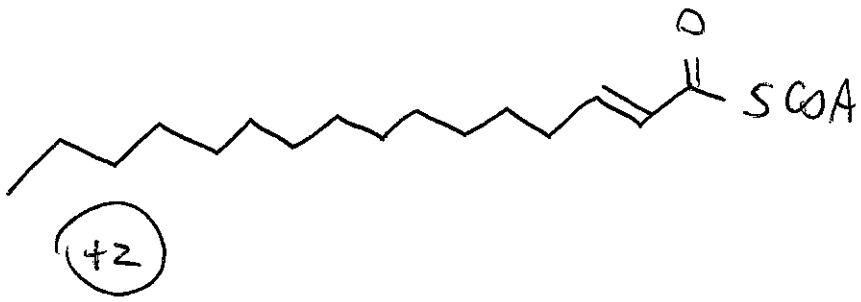
Write the next reaction for the complete combustion of acetyl CoA under aerobic conditions.



5. The enzyme acyl CoA dehydrogenase catalyzes the first step of β-oxidation of fatty acids such as palmitate (16:0). It is analogous to succinate dehydrogenase in many ways.

A. Draw the product of the reaction of palmitate catalyzed by acyl-CoA dehydrogenase and indicate the prosthetic redox cofactor utilized by this enzyme.

(+2) FAD



B. Theoretically, oxidative phosphorylation could yields about 2.3ATP per oxidation catalyzed by acyl-CoA dehydrogenase. (Experimentally, it is closer to 1.5ATP.) Explain how this theoretical P/O ratio was calculated with reference to the electron transport chain and ATP synthase.

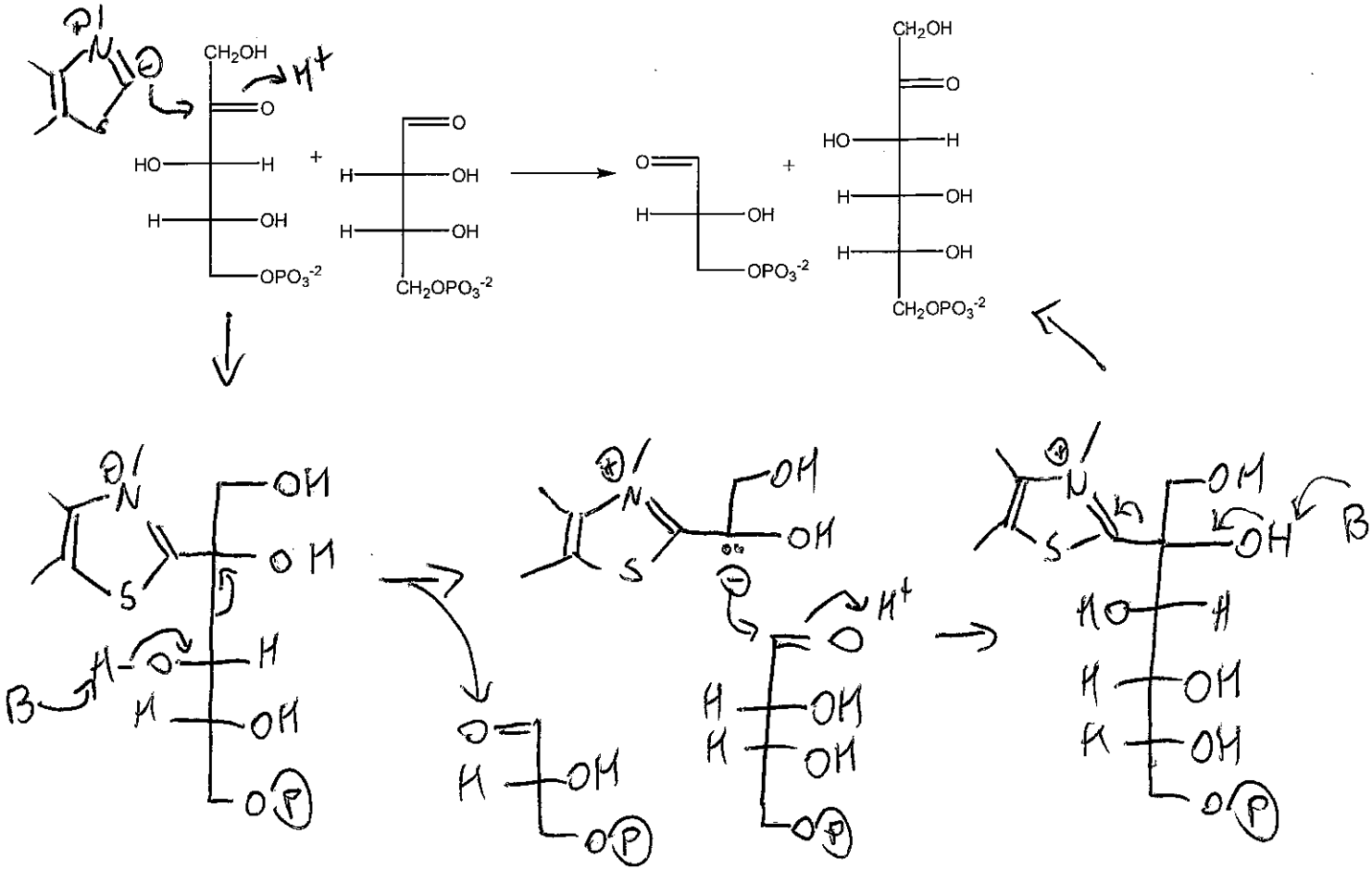
(+6)

ATP synthase can synthesize 3 ATP per rotation, which requires 8 H^+ in humans.

Electrons from acyl CoA dehydrogenase enter through the Q pool then Complexes 3+4, pumping 6 H^+s per pair of electrons.

$$\frac{P}{O} = \frac{3\ ATP}{8\ H^+}\left(\frac{6\ H^+}{2\ e^-}\right)\left(\frac{2\ e^-}{\frac{1}{2}O_2}\right) = 2.3\ \frac{ATP}{\frac{1}{2}O_2}$$

6. Provide an arrow mechanism for this reaction of the pentose phosphate pathway, catalyzed by either a transaldolase or transketalase.. Cofactor structures are given at the end of the exam if you need them.



7. What is the change in reduction potential for the oxidation of ubiquinol by cytochrome *c* when the reaction of QH_2/Q is 10 and the ratio of cyt $c(Fe^{+3})$/cyt $c(Fe^{+2})$ is 5?

Q/QH_2 $\mathcal{E}' = \mathcal{E}^{\circ\prime} - \frac{RT}{nF} \ln \frac{A_{(red)}}{A_{(ox)}}$

$= .045\,V - \frac{RT}{2F} \ln\left(\frac{QH_2}{Q}\right)^{10}$

$= .045\,V - .030\,V = \boxed{0.015\,V}$

Fe^{+3}/F^{+2}

$\mathcal{E}' = 0.235\,V - \frac{RT}{2F} \ln\left(\frac{1}{5}\right)^2$

$= \boxed{0.276\,V}$

$\Delta\mathcal{E}' = 0.276\,V - 0.015\,V = \boxed{0.261\,V}$

* Error in book answer also accepted (0.181 V)

What is the change in free energy for this process?

$\Delta G' = -nF\Delta\mathcal{E}'$

$= -2\left(96{,}485\,\frac{J}{mol\,V}\right)(0.261\,V)$

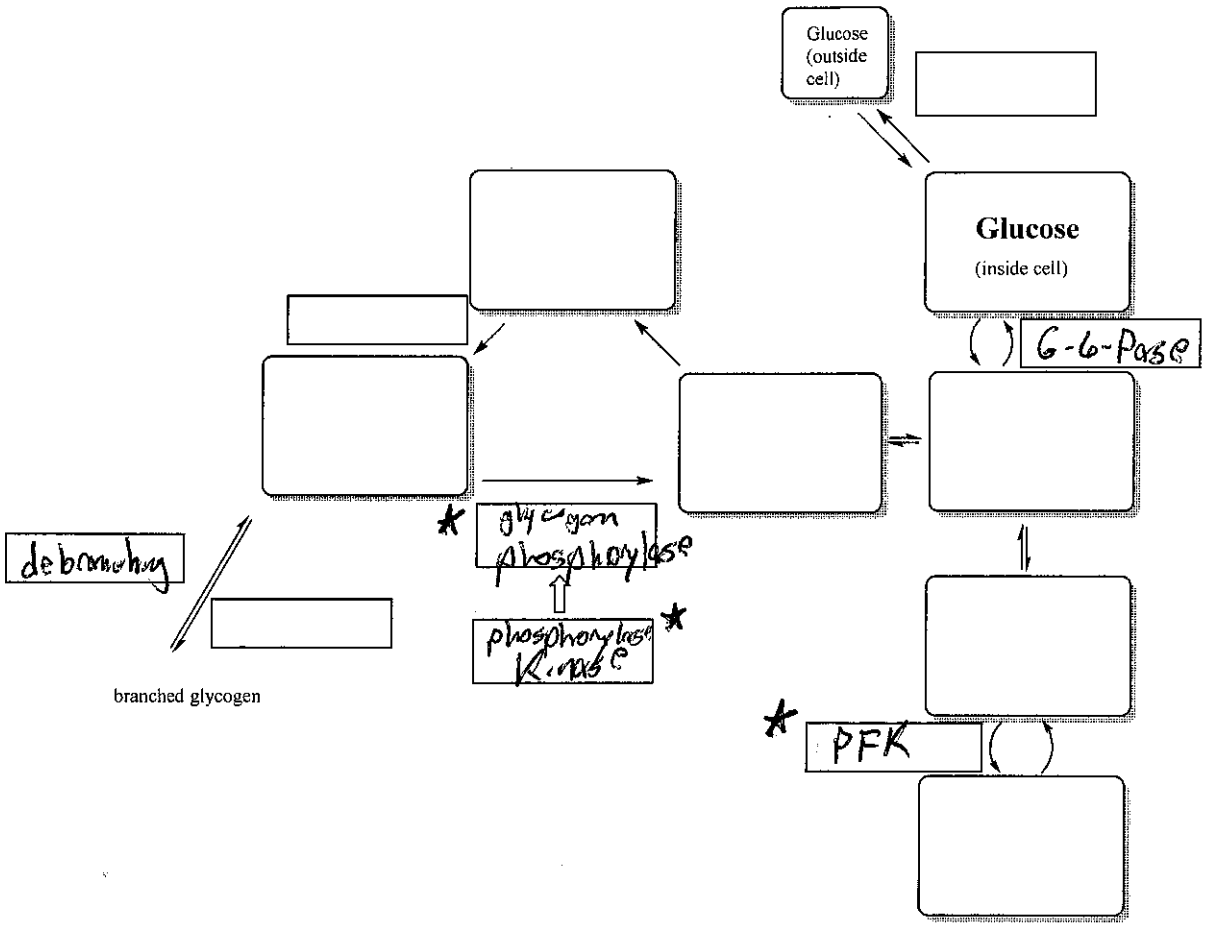
$= -50\,\frac{KJ}{mol}$

* Error in book also accepted $\left(-34.4\,\frac{KJ}{mol}\right)$

8. Two distinct glycogen storage diseases have very similar symptoms: slight excess of glycogen (with normal structure) in some tissues, limited ability to perform strenuous exercise, normal blood sugar ranges.

A. Fill in the following enzymes in the boxes of the figure below.

phosphorylase kinase, phosphofructokinase, debranching enzyme, glycogen phosphorylase, glucose-6-phosphatase



B. On the chart, mark two enzymes that could have deficiencies with these symptoms. If they are particular tissue isozymes, indicate the tissue. Use the chart to explain the symptoms from a biochemical perspective.

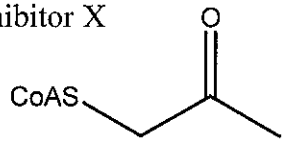
Tissue = muscle, not liver

Biochem basis: for phosphorylase, inability to mobilize glycogen storage for fast consumption by muscle

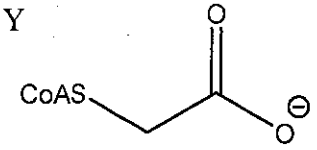
: For PFK, lowers glycolysis ability limits muscle ability to utilize carbs, so fast fuel source is unavailable

9. Section 3: Case study (10pts) A research team is investigating two inhibitors of citrate synthase, shown below.

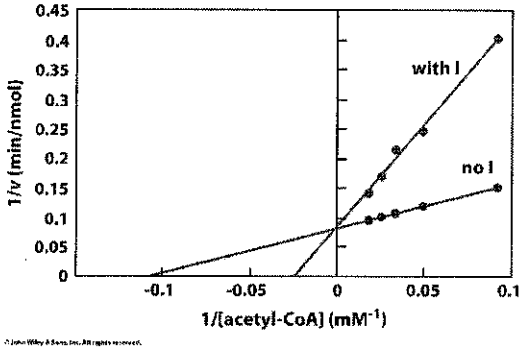
Inhibitor X



Inhibitor Y

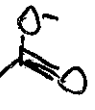


A. Kinetic data for Inhibitor X is shown below. What type of inhibitor is this? Refer to its structure to explain why this makes sense.



It is a competitive inhibitor. (+2)
It is structurally related to the substrate acetyl CoA. (+1)

B. The team has proposed that Inhibitor Y is a transition state analog for the rate determining step of the reaction catalyzed by citrate synthase. What is the rate limiting step of the reaction, and why is it reasonable to suggest that Inhibitor Y is a transition state analog?

Citrate synthase activates acetyl CoA as a nucleophile. (+2) CoAS  , so this inhibitor mimics the high energy intermediate: CoAS  . Likely to bind tighter than a substrate analog. (+2)

C. The team also tested the kinetics of the enzyme pyruvate carboxylase when treated with Inhibitor X, but they saw no effect. Why did they expect that the kinetics of pyruvate carboxylase might be affected by Inhibitor X?

Acetyl CoA is an allosteric activator of pyruvate carboxylase, so a structural analog might also be an allosteric activator. (+3)

Data Tables and equations:

Standard Free Energy Change for Phosphate Hydrolysis

Compound	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
ATP → AMP + PP_i	−45.6
Phosphocreatine	−43.1
ATP → ADP + P_i	−30.5
Glucose-1-phosphate	−20.9
PP_i → 2 P_i	−19.2
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

[TABLE 15-1] Standard Reduction Potentials of Some Biological Substances

Half-Reaction	$\mathcal{E}^{\circ\prime}$ (V)
	0.815
	0.48
	0.42
Cytochrome a_3 (Fe^{3+}) + e^- ⇌ cytochrome a_3 (Fe^{2+})	0.385
Cytochrome a (Fe^{3+}) + e^- ⇌ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + e^- ⇌ cytochrome c (Fe^{2+})	0.235
Cytochrome c_1 (Fe^{3+}) + e^- ⇌ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) + e^- ⇌ cytochrome b (Fe^{2+}) (*mitochondrial*)	0.077
Ubiquinone + 2 H^+ + 2 e^- ⇌ ubiquinol	0.045
Fumarate$^-$ + 2 H^+ + 2 e^- ⇌ succinate$^-$	0.031
FAD + 2 H^+ + 2 e^- ⇌ $FADH_2$ (*in flavoproteins*)	~0.
Oxaloacetate$^-$ + 2 H^+ + 2 e^- ⇌ malate$^-$	− 0.166
Pyruvate$^-$ + 2 H^+ + 2 e^- ⇌ lactate$^-$	− 0.185
Acetaldehyde + 2 H^+ + 2 e^- ⇌ ethanol	− 0.197
S + 2 H^+ + 2 e^- ⇌ H_2S	− 0.23
Lipoic acid + 2 H^+ + 2 e^- ⇌ dihydrolipoic acid	− 0.29
NAD^+ + H^+ + 2 e^- ⇌ NADH	− 0.315
$NADP^+$ + H^+ + 2 e^- ⇌ NADPH	− 0.320
Acetoacetate$^-$ + 2 H^+ + 2 e^- ⇌ 3-hydroxybutyrate$^-$	− 0.346
Acetate$^-$ + 3 H^+ + 2 e^- ⇌ acetaldehyde + H_2O	− 0.581

Source: Mostly from Loach, P. A., in Fasman, G. D. (ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. I, pp. 123–130, CRC Press (1976).

$$\Delta G = RT \ln\frac{[X]final}{[X]initial} + ZF\Delta\psi \qquad \Delta G^{\circ\prime} = -nF\Delta\mathcal{E}^{\circ\prime} \qquad \Delta G = -nF\Delta\mathcal{E}$$

$R = 8.314$ J/ mol · K

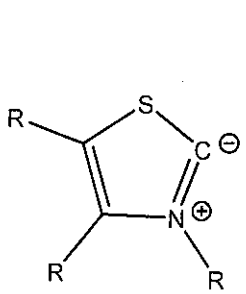
$$\mathcal{E} = \mathcal{E}^{\circ\prime} - \frac{RT}{nF} \ln\frac{A(reduced)}{A(oxidized)}$$

$F = 96{,}485$ J/V · mol

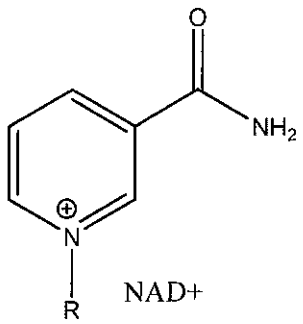
$\Delta G^{\circ\prime} = -RT \ln K_{eq}$

$$\Delta G_{reaction} = \Delta G^{\circ\prime}_{reaction} + RT \ln\frac{[products]}{[reactants]}$$

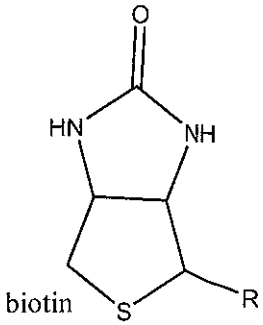
Cofactor Structures:



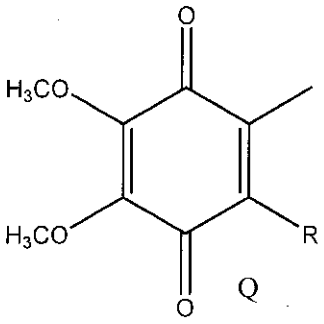
TPP



NAD+



biotin



Q

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