Name: **Key**

Chem 4512/6502 Exam 3 April 14, 2016

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Please put your name at the top of every page.

There are **6 multi-part questions** to this exam.
Please look over the whole exam before you start answering questions. The maximum points for each question are indicated. There are 100 points possible. Your whole answer **MUST** be written on the space(s) provided. All programmable calculators must be **cleared** of any memory of previous work related to this class.

Potentially helpful information is provided

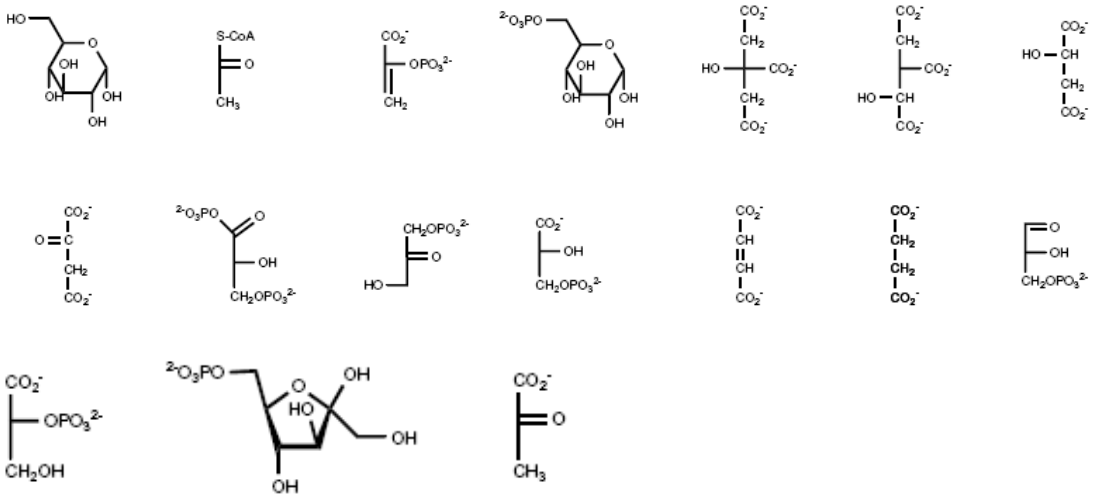
You are expected to strictly adhere to Georgia Institute of Technology Honor Code.

Georgia Institute of Technology Honor Code.

I have neither given nor received help on this work.

Signature **date**

Potentially helpful information:



Energy Charge (EC):	Change in Free Energy:
$\frac{[ATP]+0.5[ADP]}{[ATP]+[ADP]+[AMP]}$	$\Delta G' = \Delta G^{o'} + RT \ln \frac{[product]_{actual}}{[substrate]_{actual}}$ *At equilibrium (when $\Delta G' = 0$):* $\Delta G^{o'} = -RT \ln \frac{[product]_{equilibrium}}{[substrate]_{equilibrium}}$

USEFUL CONSTANTS:
R (gas constant) **= 8.315 J•mol^{-1}•Kelvin^{-1} = 8.315 x 10^{-3} kJ•mol^{-1}•Kelvin^{-1}**
F (Faraday Constant) **= 96.48 kJ•V^{-1}•mol^{-1}**

If temperature = 25 °C, absolute temperature T = 298 K (Assume this temperature unless problem states otherwise.)

ATP $\rightleftharpoons$ ADP + Pi $\quad \Delta G^{o'} = -30.5$ kJ/mol

Section 1: Answer <u>all</u> questions in this section. Each question is worth 2 points.

I. Complete the following statement correctly: FAD is a prosthetic group, …..

a) …it is readily exchanged with the solvent.
b) …it is loosely associated with the protein.
c) …it is an artificial substitute for NADH.
d) ... it is tightly bound to the enzyme.
e) …it needs to be cleaved to activate the enzyme.

II. Which combination of cofactors is involved in the conversion of pyruvate to acetyl-CoA?

a) Biotin, FAD, and TPP
b) Biotin, NAD^+, and FAD
c) NAD^+, biotin, and TPP
d) Pyridoxal phosphate, FAD, and lipoic acid
e) TPP, lipoic acid, and NAD^+

III. Cyclic 3', 5'-AMP increases the rate of glycogen breakdown (glycogenolysis) by

a) promoting the formation of a phosphorylated form of glycogen phosphorylase.
b) serving as a substrate for glycogen phosphorylase.
c) serving as a precursor of 5' AMP which is a cofactor for glycogen phosphorylase.
d) furnishing phosphate for the phosphorolysis of glycogen.

IV. In eukaryotes, the enzymes that catalyze the reactions of citric acid cycle are located in.

a) the cell nucleus.
b) the endoplasmic reticulum.
c) the mitochondria.
d) the lysosomes.
e) the cytosol.

V. Malonate is a competitive inhibitor of succinate dehydrogenase. If malonate is added to a mitochondrial preparation that is oxidizing pyruvate as a substrate, which of the following compounds would you expect to decrease in concentration?

a) Citrate
b) Fumarate
c) Isocitrate
d) Pyruvate
e) Succinate

VI. The citric acid cycle enzyme _____ contains an iron-sulfur cluster.

a) aconitase
b) isocitrate dehydrgenase
c) succinyl-CoA synthetase
d) malate dehydrogenase
e) citrate synthase

VII. Below are the standard reduction potentials (E'°) for two conjugate redox pairs:
Pyruvate-/lactate- E'° = –0.185v NAD+/NADH E'° = –0.320v
Which of the following is true?

a) The pyruvate/lactate conjugate redox pair has a greater tendency to lose electrons than the NAD+/NADH redox pair.
b) Pyruvate has a greater affinity for electrons than NAD+.
c) NAD+ is a reducing agent.
d) Under standard conditions, NAD+ is more likely to be converted to NADH, than pyruvate is to be converted to lactate.
e) none of the above.

VIII. The chemical energy generated by mitochondrial electron transport results from which of the following?

a) Excess H^+ in the matrix
b) A H^+ gradient across the inner membrane
c) The formation of thioesters in the matrix
d) A conformational change in the inner membrane

IX. In the reoxidation of QH_2 by purified ubiquinone-cytochrome *c* reductase (Complex III) from heart muscle, the overall stoichiometry of the reaction requires 2 mol of cytochrome *c* per mole of QH_2 because:

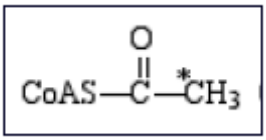
a) cytochrome *c* is a one-electron acceptor, whereas QH_2 is a two-electron donor.
b) cytochrome *c* is a two-electron acceptor, whereas QH_2 is a one-electron donor.
c) cytochrome c is water soluble and operates between the inner and outer mitochondrial membranes
d) heart muscle has a high rate of oxidative metabolism, and therefore requires twice as much cytochrome *c* as QH_2 for electron transfer to proceed normally.
e) two molecules of cytochrome *c* must first combine physically before they are catalytically active.

X. Transketolases transfer carbon units:

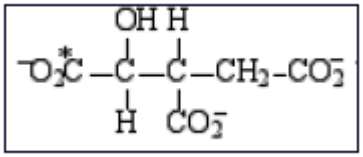
a) 1.
b) 2.
c) 3.

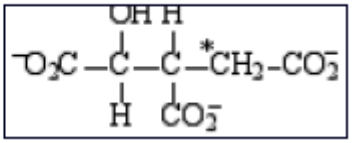
d) 4.
e) 5.

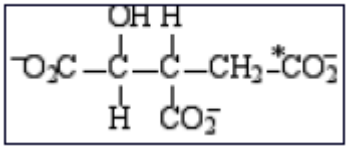
XI. If acetyl-CoA labeled with 14C, as shown in the figure to the right, were used as the substrate for the citric acid cycle, which of the following intermediates would be produced during the first round of the cycle?

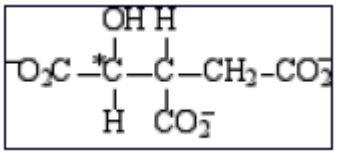


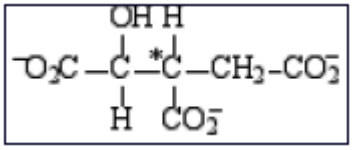
b)











XII. Given that the standard reduction potential of oxaloacetate is –0.166 V and the standard reduction potential of NAD+ is –0.315 V. What is the DE°' for the oxidation of malate by NAD^+:

$$\text{Malate} + NAD^+ \rightarrow \text{Oxaloacetate} + NADH + H^+$$

a) –4.81 V
b) + 4.81 V
c) –0.149 V
d) +0.149 V
e) +0.0523 V

XIII. Because of photosynthesis

a) **the atmosphere is rich in oxygen gas**
b) plants produce carbon dioxide
c) animals can get energy directly from the sun
d) all of the above

XIV. Cyclic electron flow in chloroplasts produces:

a) ATP and O_2, but not NADPH.
b) ATP, but not NADPH or O_2 .
c) NADPH, and ATP, but not O_2 .

d) NADPH, but not ATP or O_2 .
e) O_2, but not ATP or NADPH.

XV. When O_2 instead of CO_2 enters in Calvin cycle, _____ is directly produced in addition to 3-phosphoglycerate.

a) CO_2
b) Sedoheptulose-1,7-bisphosphate (S-1,7-P)
c) 2-Phosphoglycolate (2PG)
d) Ribulose-5-phosphate (R5P)
e) None of them

XVI. In plants and cyanobacteria, Photosystem II _____, and Photosystem I _____.

a) oxidizes O_2…reduces FAD
b) oxidizes H_2O…reduces FAD
c) oxidizes H_2O…reduces NAD^+
d) oxidizes H_2O...reduces $NADP^+$
e) none of the above

XVII. This enzyme is responsible for generating the minor product of glycogenolysis

a) UDP-glucose pyrophosphorylase.
b) Glycogen synthase
c) Glycogen phosphorylase
d) Phosphoglucomutase
e) Glycogen debranching enzyme

XVIII. This enzyme is responsible for eliminating limit branch during glycogenolysis

a) UDP-glucose pyrophosphorylase.
b) Glycogen synthase
c) Glycogen phosphorylase
d) Phosphoglucomutase
e) **Glycogen debranching enzyme**

XIX. Which of the following is MOST often associated with free fatty acid transport in human blood?

a) Albumin
b) Globulin
c) Cholesterol
d) Sphingolipid
e) Mucopolysaccharide

XX. With respect to the β-oxidation of palmitate which is true?

A) β-oxidation results in production of 8 acetyl-CoA, 7 NADH and 7 FADH2
B) β-oxidation results in production of 9 acetyl-CoA, 9 NADH and 9 FADH2
C) β-oxidation results in production of 8 acetyl-CoA, 8 NADH and 8 FADH2
D) β -oxidation results in production of 9 acetyl-CoA, 8 NADH and 8 FADH2

Section 2 (20 points): Answer <u>all</u> questions in this section.

2a (6 pts). Match the phrase on the left with the letter of the answer on the right that bests matches the description of fatty acid (FA) metabolism (only *one* answer best matches each description):

_i__ photosynthetic water splitting enzyme
_d__acts as template for initiating glycogen
_e__ catalyzes the redox reaction
_j__ transfer photons to the photosynthetic reaction centers
_b__ catalyzes the commitment step in FA synthesis
_g__ subcellular location of FA degradation

a. palmitoyl CoA
b. acetyl CoA carboxylase
c. cytosol
d. glycogenin
e. dehydrogenase
f. PEP carboxykinase
g. mitochondrial matrix
h. coenzyme Q (QH2)
i. OEC
j. Light harvesting complexes

2b (2 pts). Which respiratory chain complex does not produce enough energy to pump protons?

Ans: complex II or Succinate-coenzyme Q Oxidoreductase

2c (3 pts). What are the three different conformational stages of the αβ units of the F1 subunit of the F_1F_o-ATPase?

Ans - O: Open state, L: Lose state and T: Tight state

3a (2 pts). What is the effect of uncoupler molecules such as dinitrophenol on the production of (i) NADPH) and (ii) ATP in a chloroplast?
Ans:

(i) No Effect
(ii) Reduction in ATP production

3b (2 pts). How many ATP molecules are needed to fix a molecule of CO_2 in (i) C3 plant (normal plant) and (ii) in a C4 plant?
Ans:

(i) 3 ATPs
(ii) 5 ATPs

3c (2 pts). What are the ATP and NADPH produced by chloroplasts used for when light is absent

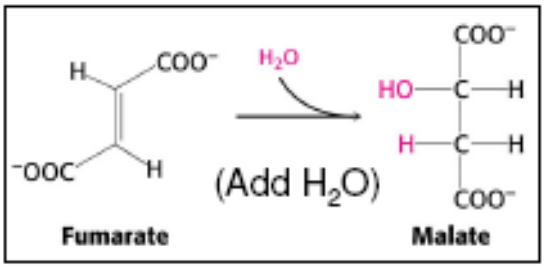
Ans: make carbohydrate via the Calvin cycle

3f (3 pts). Give the primary products of proton capture in the light reaction of photosynthesis.

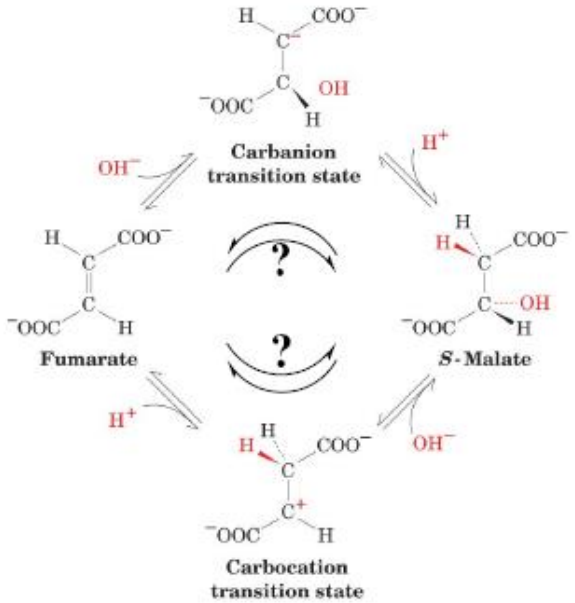
Ans: O_2, NADPH and ATP.

Section 3: Questions 4 through 6. Answer <u>any two</u> questions in this section. Each question is worth 20 points

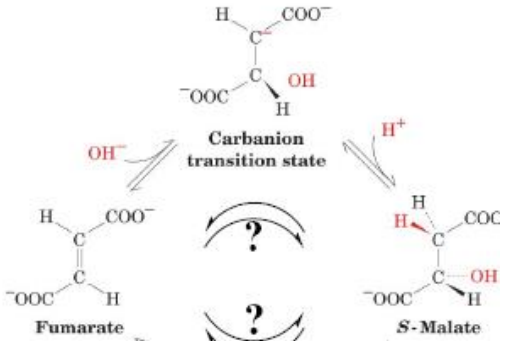
4 (20 points)
4a. The citric acid pathway enzyme fumarase facilitates the highly stereospecific hydration of fumarate to give malate as shown below:



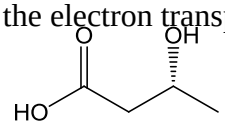
i (2 pts). Provide the two possible mechanistic pathways for the hydration of fumarate as catalyzed by fumarase. Be sure to clearly identify the transition state of either pathway.



ii (2 pts). Based solely on the principles of organic chemistry, which of these pathways is preferable?



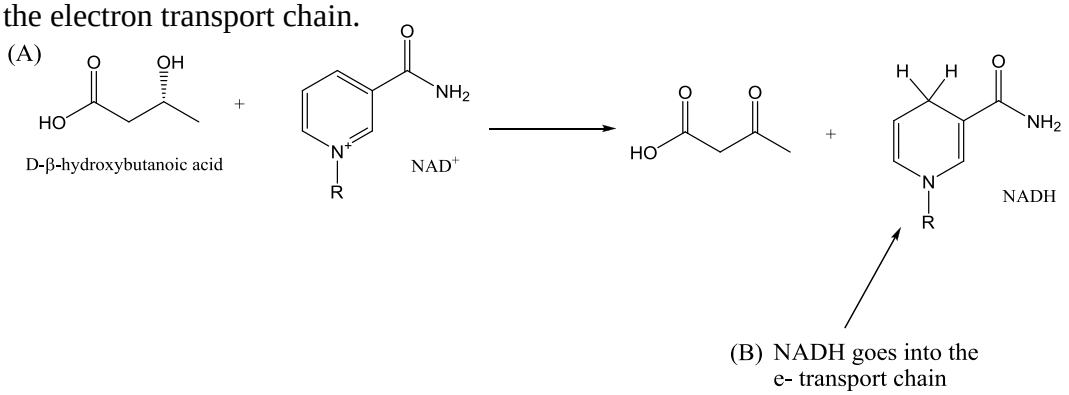
4b. It has been observed that the infusion of the ketone body D-β-Hydroxybutyrate (DβHB) in mice confers partial protection against dopaminergic neurodegeneration through a mechanism that involves the electron transport chain.



D-β-hydroxybutanoic acid

(i) (2 pts). Propose a plausible transformation of DβHB that may jump-start electron transport.

(ii) (1 pt). Identify the product resulting from the transformation of DβHB that goes into the electron transport chain.



4c (3 pts). Peroxisomes (the cytoplasmic organelle containing enzymes, especially catalase) shorten very long chain fatty acids via β-oxidation and thus prepare them for complete degradation by the mitochondrial β-oxidation system. The first enzyme in the pathway is peroxisomal fatty acyl-CoA oxidase. Give the overall reaction for this enzyme.

Ans:

Overall reaction: **Fatty acyl-CoA + O_2 → *trans*-Δ^2-enoyl-CoA + H_2O_2**

4d (6 pts). Koop and Lehninger are pioneering scientists whose work led to the elucidation of fatty acid catabolism. Succinctly describe Knoop's and Lehninger's experiments and the conclusions from these studies (limit to four sentences for each experiment).

Koop's Experiment: Fed dog with even- and odd-chain fatty acids (FAs) with ω-phenyl group and analyzed urine sample collected from these dogs for metabolites. Concluded that FAs are metabolically oxidized at their β-carbon atom (hence β-oxidation), resulting in the release of a C-2 specie per β-oxidation cycle.

Lehninger's Experiments: Added FAs to homogenized rat liver in the presence and absence of ATP. He observed that the FAs are metabolized only in the presence of ATP. Concluded that FA has to be activated to commence β-oxidation

4f.

(i) (2pts) What compounds control the flux through the Pentose Phosphate Pathway?

Ans: NADPH

(ii) (2 pts) A deficiency in G6P dehydrogenase leads to which condition? How might this be a good thing to some people?

Ans: Hemolytic anemia (Favism is acceptable); the decrease in glutathione production means less nutrition for the malaria parasite.

5 (20 points).

5ai (1 pt). Write the name of the **enzyme** responsible for the following TCA cycle reaction.

Malate Dehydrogenase

5aii (1 pt). What type of reaction does this represent?

Oxidation (A redox reaction is acceptable)

5aiii (2 pts). Name the enzymes that catalyze the step(s) in the TCA cycle where ATP is made.
Succinyl-CoA Synthetase

5b (3 pts). Malonate is a competitive inhibitor of succinate in the succinate dehydrogenase reaction. Explain why increasing the oxaloacetate concentration can overcome malonate inhibition.

Competitive inhibition can be overcome by adding more substrate, in this case succinate. Oxaloacetate overcomes malonate inhibition because it is converted to succinate by the reactions of the TCA cycle.

5c. The enzyme isocitrate dehydrogenase catalyzes the formation of the first of the three NADH molecules produced by the citrate cycle. Two relevant standard half reactions and their reduction potentials are written below.

α-ketoglutarate + CO_2 + $2H^+$ + 2e → isocitrate	E°' = –0.380 V
NAD^+ + H^+ + $2e^-$ → NADH	E°' = –0.320 V

i (2 pts). Write out the net reaction for this important redox conversion *in the citrate cycle*.

isocitrate + NAD^+ → α-ketoglutarate + CO_2 + NADH + H^+

ii (3 pts). Calculate the change in standard reduction potential ($\Delta E^{o'}$) for the reaction. Show your work and state units.

$\Delta E^{o'} = (\Delta E^{o'}_{\text{e- acceptor}}) - (\Delta E^{o'}_{\text{e- donor}})$

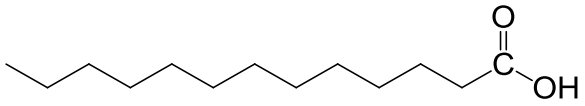
$\Delta E^{o'} = (\Delta E^{o'}_{NAD+}) - (\Delta E^{o'}_{\text{isocitrate}})$

$\Delta E^{o'} = (-0.320\ V) - (-0.380\ V) = 0.06\ V$ (Must be positive! Must have correct units.)

iii (1 pt). Indicate whether this reaction is favorable or unfavorable *under standard conditions*.

Favorable

5d (7 pts). Tridecanoic acid (shown below) is an unusual, odd-chain fatty acid (13-C) used in the creation and/or manufacturing of fragrance. In its activated form, tridecanoic acid is a substrate for β-oxidation:



i. How many times is the β-oxidation pathway repeated during oxidation of a 13-C fatty acid? ___**5**_____

How many each of NADH, (ii) __**5**____, (iii) $FADH_2$___**5**___, and Acetyl CoA (iv)__**5**____ are produced, per 13-C fatty acid, in the β-oxidation pathway?

v. What is the last product of degradation of tridecanoic acid? – **Propionyl CoA**---------

What are the, (vi) Catalytic and (vii) Noncatalytic consequences of the build-up of the last product (identified in question iii) in the TCA cycle?

Ans:
Catalytic: Supplies succinyl-CoA and this increases the catalytic activity of the cycle
Non-catalytic: Converted malate which is transported to the cytosol where it undergoes oxidative decarboxylation by malic enzyme to yield pyruvate.

6 (20 points).

6ai (2 pts). What drives the production of ATP by the chloroplast ATP Synthase?

Ans: Proton gradient

6aii (2 pts). What molecule is the final electron acceptor in ***cyclic*** phosphorylation?

Ans: Chlorophyll

6aiii (2 pts). In photosystem II, P680 transfers an electron to phyophytin upon photoexcitation. How is this electron replaced?

Ans: Oxidation of water to O_2 (or the splitting of water) at the OEC (or Mn center) will supply electron to photosystem II

6b. Light activation of PSI leads to the reduction of ferredoxin which then reduces not only $NADP^+$ to form NADPH, but also thioredoxin, which uses the electrons to reduce disulfide bridges in several Calvin cycle enzymes, leading to their activation.

(i). (2 pts) What turns off these Calvin cycle enzymes when the sun goes down?

Ans:The disulfide bridges are spontaneously oxidized in the absence of reduced thioredoxin. (Acceptable - Transition state analog CA1P, synthesized in the absence of sunlight by plants, inhibits RuBP Carboxylase)

(ii). (2 pts) What explains the dependence of the Calvin cycle enzyme Rubisco on light activation of PSII?

Ans: Light activated electron transport through PSII is required for proton pumping into the thylakoid space (lumen) from the stroma. This results in an increase in stromal pH and efflux of Mg^{2+} to the stroma to balance the charge. Rubisco activity is stimulated by this increase in pH and elevated [Mg^{2+}] in the stroma.

(iii). (2 pts) Why is it possible for chloroplasts to absorb much more than 8 photons per O_2 molecule evolved?

Ans: When cyclic electron flow occurs, photoactivation of PSI drives electron transport independently of the flow of electrons derived from water. Thus, the oxidation of H_2O by PSII is not linked to the number of photons consumed by PSI.

6c (8 pts). Very briefly define the following terms or concepts (Your answer can contain **no more** than 20 words):

i. Complex III Q-Cycle

An unbalanced proton pump (of the 4 protons transferred, two are from the matrix and two are from UQH2)

ii. Chemiosmotic theory

Endergonic Synthesis of ATP from ADP and Pi, catalyzed by ATP Synthase through a H+ gradient coupled to e- transport.

iii. β-carotene in photosynthesis

Accessory Pigment that covers spectral regions where chl do not absorb

vi. Special pair of PS II

Recipient of e^- from OEC

Or

Strong oxidant in the photosynthetic Two-Center Electron Transport