Problem Set 4

MCDB 108B

1. In the early 1900's, the B vitamins were discovered and shown to be cofactors of enzymes of intermediary metabolism. Match the vitamins with the corresponding class of chemical reaction for which they (or their derivatives) are cofactors (there may be more than one answer in each case).

i) biotin	_____	A) carboxylation
ii) pantothenic acid	_____	B) CoA synthesis
iii) thiamine pyrophosphate	_____	C) β-decarboxylation
iv) riboflavin	_____	D) α-decarboxylation
v) nicotinamide	_____	E) oxidation/reduction
vi) lipoamide	_____	F) group exchange between adjacent carbon atoms
		G) carbon-carbon desaturation
		H) none of the above

2. The following half-reactions display E_0 values as follows.

Fumarate + $2H^+ + 2e^-$ $\rightarrow$ Succinate	E_0=0.03 V
FAD + $2H^+ + 2e^-$ $\rightarrow$ $FADH_2$	E_0=0 V

What is the value for K_{eq} for the overall reaction: Succinate + FAD $\rightarrow$ Fumarate + $FADH_2$? Show all work.

3. Members of the genus Nitrobacter oxidize nitrite (NO_2^-) to nitrate (NO_3^-) using the energy released by the transfer of electrons to oxygen to drive ATP synthesis. Given the E_o values below, calculate the maximum ATP yield per mole of nitrite oxidized, given that ΔG^o = +7.3 kcal/mol for ADP + Pi → ATP.

$NO_2^- + H_20 \rightarrow NO_3^- + 2H^+ + 2e^-$	E_0 = -0.42 v
$1/2O_2^- + 2H^+ + 2e^- \rightarrow H_20$	E_0 = +0.82 v

4. Indicate true or false for the following. For those that are false, explain why, and provide the corresponding statement that is true.

__ a) O_2 is most hazardous after accepting 2 electrons, because 2 atoms of oxygen free radicals are generated.
__ b) valinomycin is a carrier of charge but not protons
__ c) nigericin is a carrier of both charge and protons
__ d) The phosphoanhydride bond of ATP is synthesized in the T state conformer of the ATP synthase.
__ e) The flow of protons through the ATP synthase drives a conformational change from the T state to the L state, the latter which binds ADP + P_i.

5. A patient suffers from a muscle disorder, dementia, and several other neurological problems. A skeletal muscle biopsy is obtained, the intact mitochondria isolated, and respiratory activities measured under the following conditions:

Number of O atoms consumed/min/mg

	Substrate	No addition	ADP + P_i	DNP
Normal	Pyruvate	20	100	100
Patient	Pyruvate	20	40	100

a) For a normal subject, explain the basis for the increase in oxygen consumption following the addition of ADP + P_i. Provide an explanation for increases in O_2 consumption when DNP (2,4-dinitrophenol) is added.

b) What possible defect in oxidation or phosphorylation could explain these results? Briefly explain.

c) Suppose that <u>fragments</u> of sonicated mitochondria from normal subjects and from the patient are used with pyruvate as substrate. How would you expect the results of the experiments to differ from those shown above? (fill in the blanks the number of O atoms consumed)

	Substrate	No addition	ADP+P_i
Normal	Pyruvate	_____	_____
Patient	Pyruvate	_____	_____

Provide a brief explanation for your answer.

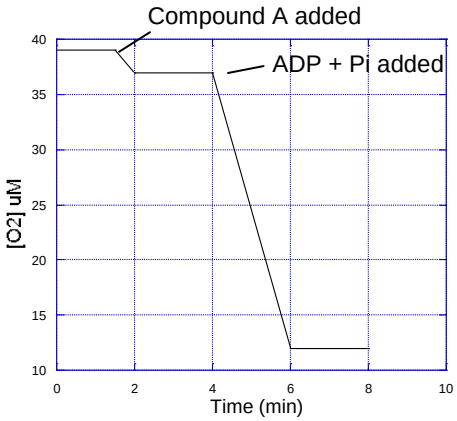
6. Compound A can donate electrons to the electron transport chain. When 100 uM compound A is added to isolated mitochondria, there is initially a small but minimal amount of O_2 consumtion, which then stops. When 50 uM ADP and 50 uM Pi are then added, robust O_2 consumption resumes, and then stops (7).

a) Explain the initial minimal O_2 consumption upon adding compound A, and why it stops.

b) Explain the robust O_2 consumption upon adding ADP and Pi and why it stops.

c) What is the P/0 ratio of compound A? Show all work.

d) How would you predict the graph to be altered if rotenone were added at time 0?



7. You are given 2 unknown samples which correspond to either oligomycin or cyanide. In an experiment using isolated intact mitochondria, you find that both samples inhibit respiration. How could you unequivocally distinguish the two using dinitrophenol (4)?

8. (6) Complex III of the electron transport chain catalyzes the one e^- reduction of cytochrome c by ubiquinol. Answer the following:

a) Explain the purpose of evolving a Q-cycle carried out by complex III.

b) In one cycle of the Q-cycle, how many molecules of QH_2 must participate? How many e-s are transferred to cytochrome C? How many protons are pumped into the intermembrane space? Where exactly do these protons come from (…this is tricky!) ?

9. You have a test tube containing purified mitochondria in a medium which is saturated with oxygen, but in which ADP and PO_4 are absent:

a) (3) Upon adding β-hydroxybutyrate, would you expect the pH of the medium to change? Would there be O_2 consumption? Briefly explain.

b) (3) If the same experiment (as in 9a) were carried out in the presence of large amounts of dinitrophenol, would you expect a pH change in the medium, and would there be O_2 consumption? Explain.

c) (3) If ATP were added instead of β-hydroxybutyrate, would you expect the pH of the medium to change? Would there be O_2 consumption? Briefly explain.

10. (5) An experimental system similar to that in question 9 is set up to measure the P:O ratios of an NAD^+ versus FAD – linked system. The conditions are as follows: purified mitochondria are incubated with PO_4 in an O_2-saturated medium. β-hydroxybutyrate and ADP (50 uM) are then added, and O_2 consumption is measured. The final concentrations of components relative to each other are as follows: [PO_4] > [β-hydroxybutyrate] > [ADP]. Next, succinate and ADP (50 uM) were similarly added together with *rotenone*, and O_2 consumption measured. Draw the corresponding graph of O_2 consumption vs. time (similar to question 6). Show how the graph would differ *if rotenone were omitted* from the experiment. Explain.