

MCB102 / Metabolism
Problem Set #3
Spring 2008

These are example problems, which are similar to those you may see on the final exam.

QUESTION 1: TRUE/FALSE. Circle the correct answer, but if the answer is FALSE provide a statement that corrects the one given.

- (i) The mitochondrial PMF can be used to drive transport reactions across the membrane, e.g., ADP/ATP exchange.

TRUE

FALSE

- (ii) Ornithine, Arginine, and Aspartate are all amino acids.

TRUE

FALSE

- (iii) Pyruvate makes alanine following transamination in the ~~liver~~ in a parallel reaction to that used in the Cori Cycle to recover glucose from lactate.

TRUE

FALSE

MUSCLE

- (iv) The QH₂ produced by NADH oxidation in Complex I is equal in energy to the QH₂ produced by FADH₂ oxidation via Complex II. (Explain why this so and what it means for the final ATP yield for the oxidation of each type of reduced co-factor.)

TRUE

FALSE

Not intended for T/F Question. See Below.

- (v) The heme cofactor in the protein cytochrome c can accept ~~two~~ electrons.

TRUE

FALSE

one

- (vi) ~~The F₁ subunit of the F₁F₀ ATP Synthase~~ is an example of an uncoupler.

Dinitrophenol

TRUE

FALSE

[Sorry, but T/F question iv is a short answer really. My mistake. Anyway, if QH₂ is made either from succinate or NADH oxidation it will, of course, have the same redox potential. The production of QH₂ via Complex I pumps 4 net protons to the intermembrane space, but the same is not true for oxidation of succinate via Complex II (where no protons are pumped). Thus the 4 proton differential means that 1 less ATP can be produced for succinate oxidation as compared to NADH oxidation.]

the cytosol and

The Urea Cycle occurs in the matrix of mitochondrial so that it may couple by product fumarate to conserve energy during urea production. T/F

re-use fumarate by Citric Acid Cycle **TRUE** converting to Aspartate via **FALSE**

(vii) The electron transport chain minimizes producing radical intermediate species, like semiquinones, because they cause damage to cellular machinery.

hydroxy radicals OR superoxide **TRUE** **FALSE**

(viii) Lipid and sterol biosynthesis are likely regulated since they are energetically costly anabolic pathways.

TRUE **FALSE**

QUESTION 2: FILL IN THE BLANKS.

(i) What is the name of the oxidized form of Coenzyme Q in the electron transport chain?
Ubiquinone (Q)

(ii) What is the name of the reduced form of Coenzyme Q in the electron transport chain?
Ubiquinol (QH₂)

(iii) What is the name of the Complex that creates water from oxygen?
Complex IV--the Cytochrome c oxidase

(iv) The cell can mop up hydroxyl radicals made as by-products of respiration using an enzyme called:
Glutathione peroxidase

QUESTION 3: SHORT ANSWER.

- (i) What essential nutrient is found in both the Acyl Carrier Protein (ACP) used in fatty acid biosynthesis and the CoA cofactor used in fatty acid catabolism?

Pantethine OR panthothenic acid OR 4'-phosphopantethine

- (ii) How might a rotary mechanism favor ATP release from the F1Fo ATP Synthase of the inner membrane of mitochondria?

An irregularly shaped "shaft" linked to the Fo proton pore rotates relative to the F1 proteins, which are arranged in a ring, when H⁺ ions flow through Fo. The conformation of each b subunit changes sequentially, as it interacts with the rotating shaft. Each of the 3 b subunits is in a different stage of the catalytic cycle at any time, allowing ATP to be released by stage 3.

- 1. a loose conformation in which the active site can loosely bind ADP + Pi**
- 2. a tight conformation in which substrates are tightly bound and ATP is formed**
- 3. an open conformation that favors ATP release.**

- (iii) Describe why NADH can release 10 net protons to the intermembrane space. Convert these protons into ATP explaining how you arrived at your final ATP production figure for those protons.

As the high energy electron from NADH is brought down in energy via Complex I, III, and IV, protons are pumped from the matrix to the intermembrane space of the mitochondria. Consensus says:

3 H⁺ → 1 ATP (in matrix)

1 H⁺ required to get Pi in for ADP + Pi → ATP

=====

4H⁺ / ATP

Thus 10 H⁺ / 4 H⁺ /ATP = 2.5 ATP/NADH

- (iv) Describe the regulation of cholesterol biosynthesis.

The HMG-CoA Reductase enzyme catalyzes the rate-limiting step committing hydroxymethylglutarate-CoA (HMG-CoA) to sterol and cholesterol biosynthesis. Hormonal control leads to changes in phosphorylation of HMG-CoA Reductase, where insulin upregulates by dephosphorylation and glucagon down regulates by phosphorylation. Excess cellular cholesterol can cause the HMG-CoA Reductase protein to be proteolytically degraded. Also the protein is under strict transcription control when cholesterol levels dip transcription goes up and when cholesterol levels are high transcription goes down.

- (v) Why is PLP a great cofactor for the transamination reactions?

Pyridoxal phosphate (PLP), produced from vitamin B6, is a cofactor in transaminases that can catabolize amino acids. PLP-dependent enzymes use PLP to covalently bind the substrate amino acid via its amine group, forming a Schiff base--a strong electrophile. The amine is removed from the donor amino acid due to the strong electron withdrawing power and can be passed to the acceptor α-keto acid via a reversal of Schiff base formation process.

(vi) Explain how the uncoupler DNP operates when administered to respiring mitochondria.

Uncoupling reagents (like DNP) dissipate the mitochondrial proton gradient (ΔpH), thereby shutting down ATP synthesis, while electron transport continues unregulated, allowing oxygen consumption to continue, and liberating NADH/FADH₂ oxidation free energy as heat.

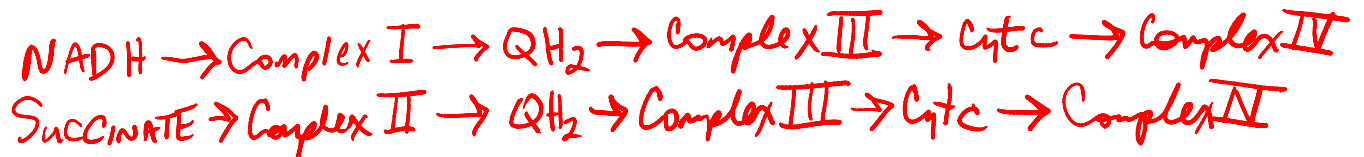
(vii) How is the Urea Cycle regulated?

Arginine stimulates (by allosteric control) an enzyme that makes N-acetyl-glutamate from Acetyl-CoA and glutamate (NAG). NAG then allosterically upregulates carbamoyl phosphate synthase I enzyme, leading to stimulation of the urea cycle.

(viii) Draw out the products and reactants of the urea cycle. Circle the steps that require the hydrolysis of ATP.

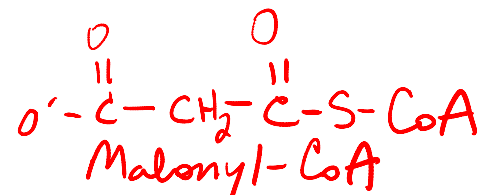
(see Figure on Pg 666 of Lehninger) The carbamoyl phosphate synthase I step and the argininosuccinate synthetase step, which are the ATP-dependent steps, and they should be circled.

(ix) Explain the two possible paths electrons may take when flowing from either NADH or succinate to oxygen.



(x) What is malonyl-CoA and where does it belong in metabolism?

Malonyl-CoA is required for each cycle in fatty acid biosynthesis; it is made using the enzyme biotin carboxylase using biotin cofactor, acetyl-CoA, ATP, and bicarbonate.



QUESTION 4: ENERGETICS CALCULATIONS. Show your work.

- (i) How many ATP are consumed in making a C₁₆ saturated fatty acid from acetyl-CoAs? In your answer explain what cofactors are used to synthesize fatty acids and why?

(ATP REQ. STEP #1) To make each malonyl-CoA requires 1 ATP and an Acetyl-Co and enzyme that uses the co-factor, biotin; (ATP REQ. STEP #2) A keto bond must be reduced by NADPH (2.5 ATP); (ATP REQ. STEP #3) A C=C double bond must be reduced by NADPH (2.5 ATP). NADPH is used over NADH, since NADH levels are low in the cytosol, where synthesis occurs. 4'-Phosphopantethenic acid from Acyl Carrier Protein (ACP) is a thiol-ended tether that is used in the transfer and catalysis reactions, and from the thiol end, malonyl groups can be transferred to the growing acyl chain in each step. Seven condensations are required, which is 6 x 7 = 42 ATP.

- (ii) With a basic calculation of PMF, show that for a pH gradient of 1 unit and a membrane potential of 180 mV how much energy (in % yield) would be stored from the original oxidation of NADH if only 8 protons were transferred to the intermembrane space?

$$\begin{aligned}\Delta G_{\text{PMF_perH}^+} &= 2.3 RT \Delta\text{pH} + nF\Delta\Psi \quad (n_{\text{H}^+} = +1 \text{ and } RT = 2.5 \text{ kJ/mol at } 300 \text{ K}) \\ &= 2.3 \times 2.5 \times 1 + (+1) \times (96.5 \text{ kJ/V/mol}) \times (0.180 \text{ V}) = 23.2 \text{ kJ/mol}\end{aligned}$$

For 8 protons, total $\Delta G_{\text{PMF}} = 8 \times 23.2 \text{ kJ/mol} = 185.6 \text{ kJ/mol}$

NADH redox potential = -0.32 V; O₂ redox potential = 0.816 V

Net redox reaction for oxidation of NADH has $\Delta E^{\circ} = +1.14 \text{ V}$

$$\Delta G^{\circ} = -nF\Delta E^{\circ} = -2 \times (96.5 \text{ kJ/V/mol}) \times (+1.14 \text{ V}) = -220 \text{ kJ/mol}$$

$$185.6 / 220 \times 100 = 84\% \text{ yield}$$