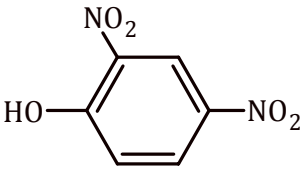
Chemistry 5.07
Problem Set 8 2013

Problem 1. All oxidation steps in the pathway from glucose to CO_2 result in the production of NADH, except the succinate dehydrogenase (SDH) step in the TCA cycle, which yields $FADH_2$. How can you explain this exception, considering that generating NADH at this step would allow production of more ATP per molecule of glucose degraded? Using the table of redox potentials on the last page, calculate $\Delta G^{o'}$ and K_{eq} if the SDH reaction used NAD^+ instead of FAD. What property unique to FAD allows the SDH reaction to occur?

Problem 2. Organisms that live deep in the ocean or deep in the earth are unable to use oxygen as a terminal electron acceptor. As with early life, before plants and cyanobacteria generated molecular oxygen, these organisms use inorganic salts to accept electrons at the end of their electron transport chain. You discover one of these organisms; it thrives on sulfate (SO_4^{2-}). It also makes soluble flavins (e.g., FAD and FMN) but lacks NAD+. Keep in mind that flavins can do the same chemistry as NAD+ but they tend to be attached to proteins, which protect them from destructive oxidation; it is thought that this organism evolved soluble flavins and, because it never encounters oxygen, those soluble flavins are able to do all of the redox chemistry that in our world is done by bound flavins and NAD+. The organism apparently benefits in its specific niche by not having to make NAD+.

The organism has an inner and outer plasma membrane, so it can do electron and proton transport as discussed in class. Recent studies found the following redox active organic molecules in the inner membrane of this bacterium: a protein containing FMN bound, cytochrome a, cytchrome b, cytochrome c, cytochrome c_1 and ubiquinone.

a. Using the information in the Table of Redox Potentials given at the end, write the anticipated order of the electron carriers in the inner membrane. Show the electron flow from $FADH_2$ to the ultimate electron acceptor.
b. Calculate the maximum number of moles of ATP ($\Delta G^{o'}_{\text{hydrolysis}}$ = -30.5 kJ/mol) that could be synthesized by oxidation of a mole of $FADH_2$ in this organism via its ETC; assume the $E^{o'}$ of $FADH_2$ is -0.22 V.
c. You are working with a culture of your bacterium in the laboratory. You add 2,4-dinitrophenol (DNP) (shown below) to a concentrated culture of the organism. Assume that the pKa of DNP is 5.2 and that this compound has easy access to all parts of the cell, including its membranes. Would the temperature of the cell culture medium go up or down following the addition of DNP? Please explain your answer in detail. It may be helpful to draw a picture.



2,4-dinitrophenol (DNP)

Problem 3. The following question is unusual. Sometimes I ask questions like this in order to "round out" an exam. It has a lot of parts and they each can test a concept I hope you carry away from the course. I want you to defend, contradict or otherwise respond to the following statements. I have double spaced the statements so that you can edit them if a word here or there would make them accurate.

a. The compound 2,4-dinitrophenol is in the problem above. I an idea -- this agent might help our football team gain weight, they would become Division I, and MIT would be so rich that it would no longer charge tuition. Would this be a good idea (the part about gaining weight)? Explain.

b. Molecular oxygen, NAD+, cytochrome C (Fe2+) and coenzyme Q are mobile electron carriers. NAD+ is reduced to NADH or NADPH in the TCA cycle and delivers its single electron directly to coenzyme Q, in the mitochondrial matrix, and then coenzyme Q (as its one or two electron-reduced forms) then delivers the electron to cytochrome C, which gives it to a flavin, which freely floats in the hydrophilic middle of the mitochondrial inner membrane.

c. An electron is transferred from reduced cytochrome C (Fe2+) to complex IV, also known as cytochrome C reductase, and it travels via iron-sulfur centers and hemes until it reaches the site where it participates in the two electron reduction of O_2 to water. The heat produced in this exothermic reaction boils water and creates a steam engine that turns the shaft inside Complex V, allowing synthesis of ATP and GTP.

d. Oxidation of reduced flavins generates only two ATPs, rather than the three you get from NADH. The reason is because NADH adds its electron to the electron transport chain too far upstream of O_2. FADH2 is closer to oxygen physically and in its reduction potential, so it is able

to generate a more robust proton gradient and hence more ATP. The electron can jump about 50 Angstroms from center to center during its path to oxygen.

e. The concentration of ATP in the mitochondrial matrix is the primary factor that determines the activity of the FoF1 ATP synthase. If you work hard, your ATP level drops and the lower level of ATP draws pre-made ATP off of the alpha subunit of an alpha-beta dimer on the ATP synthase. Once the ATP site is vacant, protons flow, which makes the T site able to bind ADP and Pi, the precursors to ATP. The actual synthesis of ATP occurs because the Pi forms a "high energy" intermediate, which transfers the phosphorous to ADP in a coupled reaction.

f. The back side of the TCA cycle (form succinate to oxaloacetate) looks a lot like some other pathway. Which one?

g. If I were Mother Nature and wanted to make a phospholipid, I would start with dihydroxyacetone phosphate from glycolysis, convert it to glycerol-3-phosphate, and then decorate the hydroxyl groups with fatty acids. To get the fatty acids to form ester linkages with the glycerol, I would adenylate the hydroxyls on the glycerol moiety and let them be attacked by the carboxylate oxygen of the fatty acid. Indeed this is the best way to make a phospholipid. (We have not covered lipid biosynthesis yet, so you have to do a bit of research in the book; what I am looking for here is the chemical logic you would use to make a phospholipid.)

Table of Redox Potentials			
Oxidant	Reductant	n	E_0', V
Acetate + CO_2 + $2H^+$	Pyruvate + H_2O	2	-0.70
Succinate + CO_2 + $2H^+$	α-Ketoglutarate + H_2O	2	-0.67
Acetate + $3H^+$	Acetaldehyde + H_2O	2	-0.60
O_2	O_2^-	1	-0.45
Ferredoxin (oxidized)	Ferredoxin (reduced)	1	-0.43
$2H^+$	H_2	2	-0.42
Acetoacetate + $2H^+$	β-Hydroxybutyrate	2	-0.35
Pyruvate + CO_2 + H^+	Malate	2	-0.33
NAD^+ + H^+	NADH	2	-0.32
$NADP^+$ + H^+	NADPH	2	-0.32
FMN (enzyme-bound) + $2H^+$	$FMNH_2$ (enzyme-bound)	2	-0.30
Lipoate (oxidized) + $2H^+$	Lipoate (reduced)	2	-0.29
1,3-Bisphosphoglycerate + $2H^+$	Glyceraldehyde -3- phosphate + P_i	2	-0.29
Glutathione (oxidized) + $2H^+$	2 Glutathione (reduced)	2	-0.23
FAD + $2H^+$	$FADH_2$	2	-0.22
Acetaldehyde + $2H^+$	Ethanol	2	-0.20
Pyruvate + $2H^+$	Lactate	2	-0.19
Oxaloacetate + $2H^+$	Malate	2	-0.17
α-Ketoglutarate + $\dot{N}H_4$ + $2H^+$	Glutamate + H_2O	2	-0.14
Methylene blue (oxidized) + $2H^+$	Methylene blue (reduced)	2	0.01
Fumarate + $2H^+$	Succinate	2	0.03
CoQ + $2H^+$	$CoQH_2$	2	0.04
Cytochrome *b* (+3)	Cytochrome *b* (+2)	1	0.07
Dehydroascrobate + $2H^+$	Ascorbate	2	0.08
Cytochrome c_1 (+3)	Cytochrome c_1 (+2)	1	0.23
Cytochrome *c* (+3)	Cytochrome *c* (+2)	1	0.25
Cytochrome a (+3)	Cytochrome a (+2)	1	0.29
$^{1/2}O_2$ + H_2O	H_2O_2	2	0.30
Ferricyanide	Ferrocyanide	2	0.36
Nitrate + $2H^+$	Nitrite + H_2O	2	0.42
SO_4^{2-} + $2H^+$	SO_3^{2-} + H_2O	2	0.48
Cytochrome a_3 (+3)	Cytochrome a_3 (+2)	1	0.55
Fe (+3)	Fe (+2)	1	0.77
$^{1/2}O_2$ + $2H^+$	H_2O	2	0.82

Figure by MIT OpenCourseWare.

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