ANSWERS Problem Set 8

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 FADH2. 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?

Answer:
From the table of potentials, we see that $\varepsilon^{o'}_{NAD/NADH}$ = -0.32 V and $\varepsilon^{o'}_{FAD/FADH2}$ = -0.22 V. The reduction potential of fumarate to succinate is +0.03 V.

$\Delta G^{o'} = -nF\Delta E^{o'}$. For the NAD reaction, $\Delta G^{o'} = -2\times96485\times(-0.35) = +67.5 kJ/mol$

$\Delta G^{o'} = -RT \ln K_{eq}$; $K_{eq} = e^{-(\Delta G^{o'}/RT)}$; $K_{eq} = 1.4\times10^{-12}$ This is a VERY small constant, indicating that we get essentially no product at all.

FAD however, can accomplish the SDH reaction because, unlike NAD^+, it can be tuned to different redox potentials, depending on its protein environment and binding. Therefore, the FAD bound to SDH probably has a redox potential of at least $\varepsilon^{o'}_{FAD/FADH_2} = +0.03\ V$, which is the value required to reach $\Delta G^{o'} = 0$.

Problem 2. Organisms that live deep in the ocean or deep in the earth are unable to use oxygen as a terminal electron acceptor.….

Answer:
a. The order of electron carriers can be determined by comparison of reduction potentials. The species that is the easiest to reduce (i.e., the half reaction with the most positive reduction potential) will be the terminal electron acceptor. The electrons will flow from the species hardest to reduce to that which is the easiest to reduce. Coupled to electron transport is proton transfer through the membrane to generate a proton gradient; this stored energy can be used for work such as ATP synthesis.

A suitable terminal electron acceptor is sulfate, because it has a reduction potential higher than cytochrome a. Sulfate salts are also quite abundant in deep sea water.

The order of electron carriers will be:
FMN → CoQ → Cytochrome b → Cytochrome c_1 → Cytochrome c → Cytochrome a → Sulfate

Sulfate is the terminal electron acceptor and is reduced to sulfite.

b. In this organism, sulfate reduction to sulfate serves as the terminal electron acceptor, rather than O_2 reduction to H_2O. Hence, the 2 electron equivalents of $FADH_2$ are transferred to sulfate in the overall equation:

$$FADH_2 + sulfate \longrightarrow FAD + sulfite + H_2O$$

The half reactions are:

$FAD + 2\,H^+ + 2e^- \longrightarrow FADH_2$ $\qquad \varepsilon^{o\prime} = -0.22\,V$

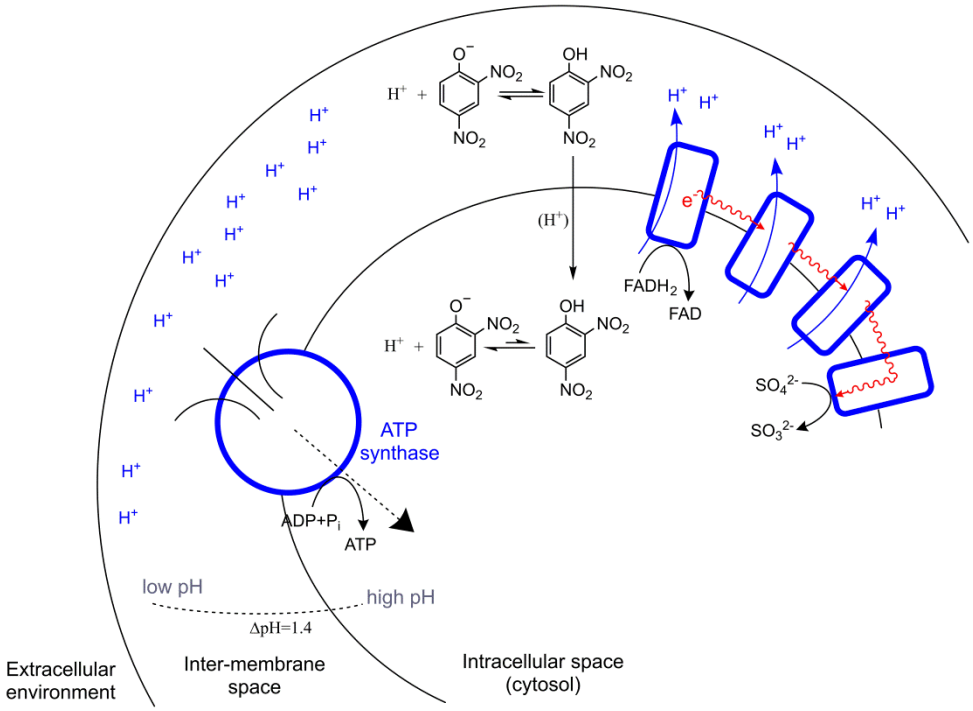
$SO_4^{2-}\,(sulfate) + 2\,H^+ + 2e^- \longrightarrow SO_3^{2-}(sulfite) + H_2O$ $\qquad \varepsilon^{o\prime} = +0.48\,V$

$\Delta E^{o\prime} = -(-0.22\,V) + (+0.48\,V) = +0.70\,V$

$\Delta G^{o\prime} = -nF\Delta E^{o\prime}$; $\Delta G^{o\prime} = -2\times96485\times0.70 = -135\,kJ/mol.$ **This is the energy obtained by the electron transport chain, available for ATP synthesis. Since it takes ≈ 30.5 kJ/mol to make 1 ATP molecule, this energy would be enough for about 4 ATPs.**

Therefore, assuming 100% efficiency, for every FADH2 oxidized, we get 4 ATPs.

c. DNP has a pK_a of 5.2 and when it is protonated, it can easily diffuse through the mitochondrial membrane. In the intermembrane space where the pH is low (close to the pKa of DNP), the concentration of the deprotonated species will be higher, but there will still be a good amount of the protonated species. The protonated form can diffuse across the membrane to the mitochondrial matrix. The pH in the matrix is higher, and hence the amount of deprotonated DNP will be higher relative to the case of the intermembrane space. Most importantly, DNP carries the protons from the intermembrane space back to the matrix, and thus has the effect of uncoupling electron transport and oxidative phosphorylation. The dissipation of the proton electro-chemical gradient results in heat gene-ration. Hence the temperature of the cell culture medium would go up.



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 test a concept I hope you carry away from the course. I want you to defend, contradict, or otherwise respond to the following statements.

Answers:

a. **They would probably lose weight, not gain weight. They would also probably die because they would be very diminished in the capacity to make ATP.**

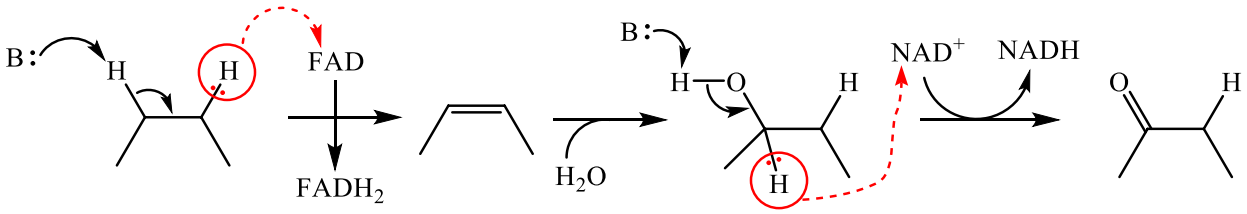
b. **Molecular oxygen, NAD^+, cytochrome C (Fe^{2+}) and coenzyme Q are mobile electron carriers. NAD^+ is reduced to NADH in the TCA cycle, and NADH delivers its two electrons to a flavin, which passes them along to [FeS] centers, and then to coenzyme Q, in the mitochondrial IM. Coenzyme Q (as its one or two electron-reduced forms) then delivers its electron to cytochrome C, which gives it to an iron-sulfur complex in cytochrome C oxidase, which is part of complex IV.**

c. **An electron is transferred from reduced cytochrome C (Fe^{2+}) to complex IV, also known as cytochrome C oxidase, and it travels via iron-sulfur centers and hemes until it reaches the site where it participates in the four electron reduction of O_2 to water. The energy produced in this exothermic reaction creates a proton gradient that turns the shaft inside Complex V, allowing synthesis of ATP.**

d. **Oxidation of reduced flavins generates only two ATPs, rather than the three you get from NADH. The reason is because NADH adds its 2 electrons to the electron transport chain far upstream of O_2. $FADH_2$ is closer to oxygen in its reduction potential, so it is able to generate a less robust proton gradient and hence less ATP. The electron can jump about 10-15 Angstroms from center to center during its path to oxygen.**

e. **The concentration of ADP 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 is converted to ADP. ADP binds to the β subunit of an alpha-beta dimer on the ATP synthase. Once the ADP site is occupied, protons flow, which makes the O site able to bind ADP and P_i, the precursor of ATP. The actual synthesis of ATP occurs because the proton flow creates a favorable environment for $\boldsymbol{ADP + P_i \rightarrow ATP}$.**

f. **The back side of the TCA cycle looks a lot like Fatty acid oxidation. Also, strangely, Fatty acid biosynthes.**



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 fatty acid moiety and let the adenylates be attacked by the hydroxyls of glycerol-3-phosphate. Indeed this is the best way to make a phospholipid.**

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