Bio 30

Problem set:
Krebs cycle/Oxidative phosphorylation/Photosynthesis

1. Make sure that you understand the answer to question 17-2.

2. If radioactive Acetyl CoA, with the two carbon atoms of the acetyl group labelled with 14C, condense with oxalacetate, do you expect to see two moles of radioactive carbon dioxide released in the subsequent first turn of the Krebs cycle?

Nope. It takes a second cycle before you see any 14C released.

3. Check out problem 17-7 in the textbook. For a wonderful eye-opener, calculate the number of molecules of oxaloacetate in a mitochondrion if the latter is a sphere 2 microns in diameter.

4. It's not complicated, but make sure that you are clear on the answer to problem 17-11.

5. In dihydrolipoyl dehydrogenase, the isoalloxazine ring of the FAD is in almost direct contact with the nicotinamide ring of NAD, in marked contrast to the situation in succinate oxidase. Speculate on why these two cofactors are in such close proximity.

The close proximity suggests that protons may be transferred along with electrons (a hydride anon) from FADH2 to NAD.

6. Speaking of succinate oxidase, its structure, once determined, was not a surprise. On the contrary, it was essentially the same as the structure of bacterial fumarate reductase, which catalyzes the reverse reaction in bacteria. One big difference, however, was the heme in succinate oxidase near the ubiquinone binding site. That heme is absent in fumarate reductase, and the authors suggested that the heme might act as a safeguard against superoxide generation. Why would this safeguard be unnecessary in fumarate reductase?

Fumarate reductase would normally operate when the bacteria were experiencing anaerobic conditions, with no oxygen around. In the presence of oxygen, the cycle runs in the other direction.

7. Mitochondria maintain a ΔpH of about 1 pH unit between the matrix and the cytoplasmic sides of the inner mitochondrial membrane, and also maintain a potential difference $\Delta\psi$ of about 0.1-0.2 V, inside negative.

- Calculate the ΔG for transfer of one proton down this electrochemical gradient.

About 5 kcal/mole

- How can ATP be made if this is the available free energy?

More than one H+ ions are transported for each ATP synthesized.

- What do you expect to happen to these values in the presence of oligomycin, and why?

You expect both the pH gradient and the potential gradient to increase. The synthesis of ATP consumes those gradients, reducing their value; when the ATP synthase is blocked by oligomycin, continuing electron transport (which is not blocked directly by oligomycin) will continue to pump protons.

- Quantitatively estimate the values you might expect to see in the presence of oligomycin.

To a first approximation, those gradients will increase until they reach a level where the cost of pumping protons out of the matrix against this gradient equals or exceeds the energy released by the transfer of electrons from NADH to O2. Taking the reduction potentials from the text (p. 532) suggests that the energy available from that source is about 1.14 V, or 52.6 kcal/mole. If you estimate that about 10 protons are pumped per NADH oxidized, implying that there is a driving force of about 10.5 kcal/electron. The values above suggest that the standing gradients represent a cost of

$\Delta G' = 2.3RT\,\Delta pH + F\Delta\Psi$ or 1.37 kcal/mole + 2.3 kcal/mol

= 3.7 kcal/mole (about 1/3 of that available from NADH oxidation)

with about 37% contributed by ΔpH , and 63% by $\Delta\Psi$.

If those proportions are maintained, the proton pumping will stop when ΔpH reaches about 3, and $\Delta\Psi$ is about 0.3-0.6 volts!

- Quantitatively estimate the values you might expect to see in the presence of valinomycin (assuming ample supplies of K+).

In the presence of valinomycin, the voltage gradient would not build up, and the system would go merrily along, pumping protons until the pH gradient alone blocked further movement, which would not occur until the ΔpH reached a value of about 9! By this time, it is reasonable to assume that the mitochondrion would be ill.

8. Antimycin blocks electron transfer between QH2 and cytochrome c. It does so by blocking the exchange of electrons between QH2, or Q- (the semiquinone) and cytochrome b_H. Transfer between QH2 and cytochrome c can also be blocked by removal (by extraction) of the Fe-S protein. When these matters were examined in detail, it was found that the latter treatment blocked reduction of cytochrome c1, but not reduction of cytochrome b_H or b_L. However, addition of antimycin to Fe-S deficient complex III blocked this reduction of the two cytochrome b hemes. Reconstitution of the Fe-S back to the deficient complex resulted in the restoration of cytochrome b reduction. Explain these results in terms of the Q cycle.

9. Consider the following subject for contemplation. Most of the dehydrogenation steps in glycolysis and the Krebs cycle use NAD+ (Eo' for NAD+/NADH=-0.32V) as the electron acceptor, but succinate dehydrogenase doesn't - it uses a bound FAD (Eo' = 0.05V) instead during the catalysis of the conversion of fumarate to succinate (Eo'=0.03V). Why is FAD a more appropriate acceptor inthis case? Another question: given the E'_os for the FAD/FADH2 and succinate/fumarate pairs, why does succinate dehydrogenase continuously transfer electrons in the direction of succinate -> QH2?

Although there is not enough energy in the succinate->fumarate step to drive NAD+ reduction, there is (barely) enough to drive FAD -> FADH2 reduction. However, these are about equally balanced reactions, which should leave them at a standstill. However, electrons flow continuously in the direction of QH2 because reoxidation of QH2 at the expense of cytochrome c is energetically quite favorable (ΔG = -9.2 kcal/mole), draining away QH2. In a larger sense, the overall favorable free energy associated with the turning of the citric acid cycle also drains away fumarate. Both processes conspire to insure that electrons flow in the direction of succinate -> FAD

10. A traditional problem: One of the critical analytical approaches used to establish the Calvin cycle was the isolation and positional analysis of radioactive carbon atoms in newly synthesized glucose. To get an idea of how this method works, try it in reverse. Imagine that you could isolate and analyze glucose at various stages after the introduction of 14C-labelled CO2. Predict the order in which you expect the atoms of glucose to become radioactive, assuming that incorporation occurs by the Calvin cycle as outlined in the lecture and text.

Working from the handout, showing the reductive pentose phosphate cycle: Radioactivity will appear first in the C-1 carbon of PGA, and thus in the C-1 of Gal3P, where it will appear in the C-4 carbon of F6P and thus into C-4 of glucose. Radioactivity will flow almost immediately into the DHAP pool in the C-1 (unphosphorylated) position of DHAP, and thence into the C-3 position of glucose. Label in GAl3P will flow by the transketolase into the C-3 position of RuDP, and thus back into the C-1 position of PGA, which already carries label. However, once label reaches the DHAP pool, label will flow immediately into C-3 position of SDP, and from there into the C-1 of Xu5P and thence into the C-3

position of PGA. From there, label will flow into, first, the C-6, and then through DHAP into the C-1 position of glucose. However, labelled F6P, once it acquires label through DHAP in the C-4 position, will pass a labelled carbon into the C-1 position of E4P. (Label will flow earlier into the C-2 position, but from there flows back into the C-1 of PGA). From the C-1 position of E4P, label passes through the C-4 position of SBP into the C-2 of RuBP, where it labels the C-5, and then the C-2 position of glucose. Labelling order in glucose is thus predicted to be C-4, C-3, C-6, C-1, C-5, and C-2.

11. a) Show that the reduction of CO2 to glucose is essentially a 4 electron reduction reaction. Show, in addition, that the carbon atoms in glyceraldehyde-3-phosphate are already at the reduction level of glucose.

In CO2, the oxidation state of the carbon atom is +4, while in glucose (C6H12O6), the average oxidation state is 0 (+ 1 at C1, 0 at C2-C5, -1 at C6). The reduction per carbon is thus a four electron reduction (+4 -> 0). In glyceraldehyde (C3H6O3), the oxidation state is again 0 (on average).

b) In the operation of the Calvin cycle, there seems to be only one reduction reaction intervening between the rubisco reaction and glyceraldehyde-3-phosphate, involving only the 2 electrons of NADPH, in apparent contradiction of your demonstration in part a). Resolve this conflict.

In PGA (C3H6O4), the oxidation state of the carbons is +3, 0, and -1. Essentially, the 4 electron excess oxidation status of CO2 has been distributed between *two* molecules of PGA. Each of these must be reduced in a two electron transfer from NADH to yield the GAl3P products which go on to become glucose.

12. Propose an experiment, using the Hill reaction, to show that PSII is the photosystem upstream of the cytochrome bf complex (assuming that you already expect one photosystem at each end of the electron transport chain).

The cytochrome bf complex requires electrons, which originate in water. The Hill reaction uses an artificial electron acceptor as a sink for electrons liberated from water by light. PSII is the photosystem which absorbs light at 680 nm. If PSII is the photosystem upstream of cytochrome bf, then the Hill reaction should be catalyzed by the photosystem which absorbs light at 680 nm. This prediction can be tested by measuring the wavelength dependence for O2 evolution in the Hill reaction: it should not proceed with light of wavelengths longer than 680 nm.