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?

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.

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?

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.

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

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

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

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

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?

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.

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.

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.

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).