Activity 35 Oxidative Phosphorylation

PRE-ACTIVITY ASSIGNMENT

- [6-14C]-Glc is glucose that has been labeled at the number 6 carbon with radioactive C-14. If radioactive CO2 is produced from metabolism of such a glucose, through which metabolic pathway did it proceed?

  Citric acid cycle

- What is 2,4-dinitrophenol and what is its effect on metabolic pathways?

  It is a chemical uncoupler that allows H^+ to be transported across the inner mitochondrial membrane without passing through ATP synthase. It dissipates the proton gradient without producing ATP.

IN-CLASS ACTIVITY

Critical Thinking Questions

1. The transfer of phosphate groups must be stoichiometric because of the direct transfer of the phosporyl group from a donor molecule to ADP. In oxidative phosphorylation, the transfer of the phosphoryl group is not direct, but rather the energy needed to drive this endergonic process is harnessed indirectly. The energy comes from the proton gradient established when ETC simultaneously transfers electrons and pumps protons. The number of protons translocated per reducing agent is not necessarily an integer. Furthermore, the proton gradient can be depleted by uncoupler proteins or small molecules.
2. An uncoupler allows protons to move from the intermembrane space back into the mitochondrial matrix without passing through ATP synthase. Uncouplers can be small, lipid soluble molecules that carry protons through the membrane (which is normally impermeable to protons) or proteins that create a proton channel. If protons bypass ATP synthase on their way back into the matrix, the energy associated with the transmembrane proton gradient is dissipated as heat as opposed to being harnessed to synthesize ATP. Therefore, ATP synthesis decreases or stops even as ETC continues to function.
3. Since we know that PK activity is increased 10-fold in proliferating versus resting thymocytes and PK requires ADP, there is a possibility that PK could compete with mitochondrial oxidative phosphorylation for ADP. If this were true, we would expect to see flux through TCA/ETC/Ox Phos back up in proliferating thyomocytes. DNP, which dissipates the mitochondrial proton gradient without the action of ATP synthase, would be expected to cause a great increase in flux through TCA/ETC since the process could move forward without being linked to ADP utilization by ATP synthase. If we look at the $^{14}CO_2$ production from glucose labeled at carbon 6 (indicates TCA) in proliferating thymocytes we see a value of 0.93 for untreated and 3.02 for treated. This relatively small increase suggests that ADP competition is the not cause of aerobic glycolysis.
4. PMS is an artificial electron acceptor. In the absence of PMS, oxidized FAD and NAD^+ are regenerated by electron transport for use in the TCA cycle. If FAD and NAD^+ are in short supply, TCA will slow down or stop. In the presence of PMS, $FADH_2$ and NADH can be oxidized without having to go through ETC. This means that if a limited supply of FAD and NAD^+ is the reason why proliferating thymocytes prefer glycolysis to TCA, then addition of PMS should greatly increase flux through TCA since these oxidized species will no longer be limiting. Indeed this is what we see. In proliferating thymocytes, $^{14}CO_2$ production from glucose labeled at carbon 6 (indicates TCA) increases 12-fold from 0.93 in the absence of PMS to 11.7 in the presence of PMS.
5. Observations described in 3 and 4 above.
6. For the electron transport chain to continue operating, a proton must be pumped into the intermembrane space, but as the concentration of the protons in the intermembrane space increases it becomes more difficult to pump a proton into the intermembrane space and requires a larger ΔG. The coupled reaction that is driving the proton pumping is the transfer of electrons though the electron transport chain. Eventually the electron transport chain

will halt because the transport of electrons is linked to the pumping of the proton and the overall process slowly acquires a positive ΔG.

7a. Yes, electron transport through complex IV provides enough free energy to pump electrons into the intermembrane space to create a proton gradient. Complex V takes advantage of this gradient to synthesize ATP.

7b. If ATP synthase is inhibited, protons will not be able to be transported back into the matrix and ATP will not be made. Since Complex IV is still able to transfer electrons and pump protons, the proton concentration in the intermembrane space will become artificially high. As the proton concentration becomes higher, more free energy will be required to pump protons.

Electron transport and proton pumping are coupled in the electron transport complexes. Therefore, if not enough energy is generated by transferring electrons to power proton pumping, both electron transport and proton pumping will cease. In complex IV, proton pumping appears to occur when electrons are transferred from cytochrome a3 to O_2, although the mechanism remains unknown. Therefore, all redox centers in complex IV will continue to pass electrons on to the terminal electron acceptor at the a_3 / O_2 interface until the free energy generated by this final transfer is no longer great enough to pump protons. Therefore, spectral data after inhibitor was added would indicate all centers reduced as electron transport and proton pumping ceased.

POST-ACTIVITY SKILL EXERCISES

a. If ATP synthase is inhibited, protons will not be able to be transported back into the matrix and ATP will not be made. Since the ETC complexes are still able to transfer electrons and pump protons, the proton concentration in the intermembrane space will become artificially high. As the proton concentration becomes higher, more free energy will be required to pump protons.

Electron transport and proton pumping are coupled in the electron transport complexes. Therefore, if not enough energy is generated by transferring electrons to power proton pumping, both electron transport and proton pumping will cease. Some of the complexes in ETC generate more free energy from passing electrons than others. Therefore, one would expect that complexes with relatively small differences in standard reduction potential (complexes II and III) would remain reduced and complexes with relatively large differences in standard reduction potential (complexes I and IV) would be oxidized. Ultimately, the concentration of protons in the intermembrane space may become so great that all electron transport and proten pumping ceases resulting in all complexes being reduced.

b. Both an ATP synthase inhibitor and an artificial electron acceptor will act to create an artificially high proton concentration in the intermembrane space. Therefore, downstream of complex III, the overall effect described above would be the same. Complex IV would be oxidized since no electrons would be passed to it from complex III.