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Chapter 21

Electron Transport and Oxidative Phosphorylation

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Overview

- Oxidation of NADH and $CoQH_2$ produced in TCA cycle by O_2 is very exergonic.
- Some of the energy of oxidation is captured by synthesis of ATP from ADP and P_i
- The capture of energy requires a "coupling" of oxidation to phosphorylation
- Coupling occurs through an intermediate proton electrochemical gradient across the inner mitochondrial membrane.

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Energetics of Redox Reactions

- Oxidation-Reduction (Redox) reactions can be written as two "half-cell" reactions.

$$A_{ox} + B_{red} \rightarrow A_{red} + B_{ox}$$

can be written as the sum of:

$$A_{ox} + e \rightarrow A_{red}$$

and $$B_{red} \rightarrow B_{ox} + e$$

- These "half-cell" reactions can be physically separated and the electron transferred by an electrical circuit. (See Fig 21.2)

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Energetics of the Voltaic Cell

- When the voltage difference between two half cell reactions is measured, the cells are at equilibrium.
  - $\Delta G_{overall} = \Delta G_{chemical} + \Delta G_{electrical} = 0$
  - $\Delta G_{chemical} = -\Delta G_{electrical} = -nF\,\Delta E$

(under standard state conditions, ΔG^{o}' and ΔE_{o}')

n = # of electrons, F = the Faraday, 96,485 coulombs/mol

(Recall that 1 coulomb-volt = 1 Joule)

The measurement of the voltage difference between two half cells is therefore a way to measure the free energy change of the chemical reaction. It tells you the amount of "useful work" (in this case electrical work) you can get out of the process. By conventions established, a spontaneous process (where ΔG is negative) has a positive value for ΔE.

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Standard Reduction Potentials

- Voltage differences are additive

  If voltage between A and B is 0.15, and between B and C is 0.23, then the voltage between A and C would be 0.38
- Therefore we can create a scale of "relative" voltages by picking a "standard" half cell, and setting it to 0.0, measuring everything else relative to it.

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Standard Reduction Potentials, con't.

- The standard electrode is chosen as the "hydrogen" electrode, for the half cell reaction:

  $H^{+} + e^{-} \rightleftharpoons \frac{1}{2} H_{2\,(1\ atm)}$ ($E_{o} = 0.0$ volt)
- Voltages of half cells measured against this cell are "standard reduction potentials" the tendency for reduction to occur.
  - *The higher the number, the more easily reduced, or the stronger the substance is as an oxidizing agent.*

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Standard Reduction Potentials, con't.

- Reduction potentials vary with concentration, just as free energy change does.

for $A_{ox} + e \rightarrow A_{red}$

$$E = E_o - \frac{RT}{nF} \ln \frac{[A_{red}]}{[A_{ox}]} \quad \text{or} \quad E_o + \frac{RT}{nF} \ln \frac{[A_{ox}]}{[A_{red}]}$$

so correcting the hydrogen electrode to the "biological standard state"

$$E_o' = E_o + \frac{RT}{nF} \ln \frac{[H^+]}{[H_2]} = 0.0 + \frac{(8.3 \times 10^{-3} \text{ kJ/mol} - \text{K})(298\text{K})}{(1)(96.5 \text{ kJ/mol})} \ln \frac{[10^{-7}]}{[1]}$$

$$E_o' = E_o + (.0256)(\ln 10^{-7}) = E_o + (.0256)(-16.1) = -0.41 \text{ volt}$$

Note, the table in the book gives –0.421 volt—difference due to rounding errors.

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Standard Reduction Potentials, con't.

- Summarized in Table 21.1
- Some examples: (ignoring protons)
  - ½ O_2 + 2 e → H_2O E_o' = 0.82
  - CoQ + 2 e → $CoQH_2$ E_o' = 0.06
  - NAD + 2 e → NADH E_o' = -0.32
- Electrons flow spontaneously from **low** to **high** reduction potential.

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Energetics of NADH Oxidation

- NADH + ½ O_2 → NAD + H_2O
- NADH + → NAD + 2 e E_o' = -0.32 volt
- ½ O_2 + 2 e → H_2O E_o' = 0.82 volt
- $\Delta E_o' = E_o'_{acceptor} - E_o'_{donor}$ = 0.82-(-0.32) = 1.14 V
- $\Delta G^{o\prime} = -nF \Delta E_o'$
  = -(2)(96.5 kJ/mol-V)(1.14 V)
  = - 220 kJ/mol

Two ways to think of this. Either subtract the potential of the electron donor (the substance being oxidized) from that of the acceptor (the substance being reduced), **or** change the sign of the potential of the equation written as an oxidation and add the potential values.

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Energetics of $CoQH_2$ Oxidation

- $CoQH_2 + ½\ O_2 \rightarrow CoQ + H_2O$
- $CoQH_2 + \rightarrow CoQ + 2\ e \quad E_o' = 0.06$ volt
- $½\ O_2 + 2\ e \rightarrow H_2O \quad E_o' = 0.82$ volt
- $\Delta E_o' = E_o'_{acceptor} - E_o'_{donor} = 0.82\text{-}0.06 = 0.76$ V
- $\Delta G^{o\prime} = -nF\ \Delta E_o'$
  $= -(2)(96.5\ kJ/mol\text{-}V)(0.76\ V)$
  $= -147\ kJ/mol$

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Electron Transport Chain

- Electrons are not passed to oxygen directly, but along a series of "carriers" with intermediate reduction potentials.
- The carriers are located in protein complexes in the inner mitochondrial membrane.
- Classes of intermediate carriers include a **flavoprotein**, **Fe/S proteins,** and **cytochromes.**
- Cytochromes named because of their absorption in the visible spectrum. (See Fig. 21.9)
  - Prosthetic groups are heme groups, like hemoglobin. See Fig. 21.10

By breaking the overall large drop in energy into a series of smaller steps, the ability to "capture" the energy more efficiently is gained.

The participation of cytochromes in the process was discovered early because of the changes in the visible spectrum of tissues in various states of oxidation. The latter part of the electron transport chain was sometimes referred to as the "cytochrome chain".

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Electron Transport Chain, con't.

There are Four Complexes in the chain:

I. NADH-CoQ Reductase
   $NADH + CoQ \rightarrow NAD + CoQH_2$

II. Succinate-CoQ Reductase
   (This is the same as **succinate dehydrogenase**)
   $Succinate + CoQ \rightarrow fumarate + CoQH_2$)

III. $CoQH_2$-cytochrome c Reductase
   $CoQH_2 + 2\ cyt\ c\ (ox) \rightarrow CoQ + 2\ cyt\ c\ (red)$

IV. Cytochrome c Oxidase
   $2\ cyt\ c\ (red) + ½\ O_2 \rightarrow 2\ cyt\ c\ (ox) + H_2O$

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Topology of Electron Transport Chain

- Two mobile electron carriers transport electrons between the complexes.
  - Coenzyme Q (ubiquinone) carries electrons from complexes I and II (and other flavoprotein complexes) to complex III. It is dissolved in the membrane.
    - See Figure 21.5 for redox structures of CoQ
  - Cytochrome c carries electrons from complex III to complex IV. It is a peripheral protein located on the external face of the membrane.
    - See Figure 21.3 for a ribbon diagram.
- See Figure 21.4 for a schematic topology

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Complex I NADH-CoQ Reductase

- More than 30 protein subunits, MW 850 kD.
- Contains FMN and Fe/S centers as prosthetic groups.
- NADH binding site on matrix side.
- Detailed mechanism unknown.
  - See Figure 21.6 for postulated mechanism.
- $\Delta E_o' = 0.06-(-0.32)=0.38$ V, $\Delta G^{o'} = -73.3$ kJ/mol
- Pumps 4 H^+ across membrane for each 2 e^-

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Complex II Succinate-CoQ Reductase

- 4 protein subunits, MW 140 kD.
- Contains FAD and Fe/S centers as prosthetic groups.
  - FAD covalently bound.
- Succinate binding site on matrix side.
- $\Delta E_o' = 0.06-0.03=0.03$ V, $\Delta G^{o'} = -5.8$ kJ/mol
- Not enough energy to pump protons.

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Complex III
CoQ-Cytochrome c Reductase

- 9-10 protein subunits, MW 250 kD.
- Contains three hemes (b_L, b_H, c_1) and Fe/S centers as prosthetic groups.
- Spans membrane. (See structure, Fig 21.11)
- Cytochrome c binding site on outer face of membrane.
- $\Delta E_o' = 0.254\text{-}0.06 = 0.194$ V, $\Delta G^{o\prime} = -37.4$ kJ/mol
- Pumps two protons per 2 e⁻.

Note that the proposed mechanism in Figure 21.12 suggests two protons being removed from the matrix per pair of electrons, but **four** protons being released to the outside of the membrane (two in the first half of the cycle, two in the second half).

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Complex III, con't.

- Complex III bridges two electron carriers (CoQ) to one electron carriers (Fe of heme).
- Proposed mechanism involves a "Q cycle", that occurs in two stages.
  - It explains both the 2e to 1e transition, as well as the proton translocation.
  - See Figure 21.12 for details.

Note in the proposed Q cycle, the two cytochrome b's are not on the direct line of electron transfer, but participate in the Q recycling steps. This explains some early ambiguities in attempts to "order" the carriers in the chain. Some evidence suggested that CoQ was reduced before cytochrome b, other evidence suggested cytochrome b was reduced before CoQ.

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Complex IV
Cytochrome c Oxidase

- X-ray structure now known. (Fig 21.16)
- 13 protein subunits, MW 204 kD
- Contains two hemes (a and a_3) and two copper atoms (Cu_A and Cu_B) as prosthetic groups.
- Cytochrome oxidation at external face of membrane, oxygen reduction at matrix side.
- $\Delta E_o' = 0.816\text{-}0.254 = 0..562$ V, $\Delta G^{o\prime} = -108.5$ kJ/mol
- Pumps protons, stoichiometry uncertain, probably about 4 H^+ per 2 e^-.

Again, the proposed model suggests an unbalanced proton pump, this time with four being removed from the matrix and two being released to the outside for every two electrons passed to oxygen.

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Complex IV, con't.

- Cu_A associated with heme a, electrons accepted by Cu and passed to heme
- Cu_B associated with heme a_3 forming a **binuclear center**
- The binuclear center cycles through several oxidation states as it is first reduced, then binds oxygen, then is further reduced.
  - See the proposed model in Fig. 21.20

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Overall Model of Electron Transport

- The four complexes (and other flavoprotein complexes) are oriented in the inner mitochondrial membrane, and free to move laterally.
- Coenzyme Q is an electron carrier buried in the hydrocarbon region of the membrane.
- Cytochrome C is a peripheral membrane protein on the external face of the membrane (in the space between the inner and outer membranes).
  - See Figure 21.21 for an overall summary of this model.

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The Proton Gradient

- Mechanism of proton pumping still not understood, but some features apparent.
  - Reduction of flavoprotein and oxygen on matrix side removes protons from matrix.
  - Oxidation of $CoQH_2$ on surface leads to release of protons to outside (i.e., into inner membrane space.)
- Stoichiometry of 4 H^+ for complex I, 2 H^+ for complex III and 4 H^+ for complex IV is consistent with the energy change of these steps.
- But note the text suggests complex III and IV are "unbalanced", III takes up 2 H^+, exports 4, while IV takes up 4 H^+ and exports 2.

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The Proton Gradient, con't.

- Energy is stored in the proton gradient as both a **chemical gradient** (ΔpH) and an **electrical gradient (**$\Delta\psi$, the difference in electrical potential across the membrane).
- The work it takes to move **n** protons against this gradient is given by the relation:

for $H^+_{in} \rightarrow H^+_{out}$

$$\Delta G = \Delta G_{chemical} + \Delta G_{electrical} = nRT\ln\frac{[H^+_{out}]}{[H^+_{in}]} + nF\Delta y$$

$$= n(-2.3RT\Delta pH + F\Delta y)$$

The proton motive force, is defined as

$$\Delta p = \frac{\Delta G}{nF} = -\frac{2.3RT}{nF}\Delta pH + \Delta y$$, and the units are volts.

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What Does "Coupling" Mean

- ATP synthesis (phosphorylation) occurs when electron transport takes place.
  - Stoichiometry expressed by a P/O_2 ratio.
- Electron transport will not occur unless phosphorylation can also occur.
- "Respiratory control" is an inhibition of electron transport by the **absence** of ADP.
  - Degree of control expressed by a "respiratory control ratio".

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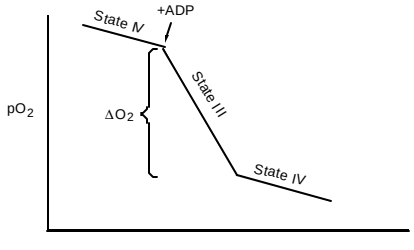

- P/O_2 Ratio = $ADP/\Delta O_2$
- Respiratory Control Ratio= Rate $_{State\ III}$/Rate $_{State\ IV}$

State III mitochondria have plentiful supply of substrate and oxygen. Rate of respiration is limited by quantity of mitochondria. State IV mitochondria have everything but the "acceptor" (ADP), and so rate of respiration is limited by the acceptor. When P/O_2 ratio is low, respiratory control ratio is also low.

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Inhibitors

- Some block particular electron transfer steps.
  - Rotenone and Amytal inhibit Complex I
  - Antimycin inhibits Complex III
  - Cyanide and azide inhibit Complex IV
- Some affect the "coupling"
  - Oligomycin and DCCD inhibit respiration only if it is coupled.
  - Dinitrophenol uncouples respiration from phosphorylation.

DCCD is dicyclohexylcarbodiimide.

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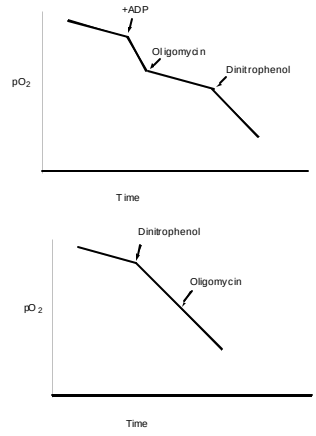

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Sites of Coupling

- Many experiments over the years suggested that coupling occurred:
  - Between NADH and CoQ
  - Between Co Q and cytochrome c
  - Between cytochrome c and oxygen
- Using glyceraldehyde-3-phosphate as a model, there was presumed to be a 1:1 stochiometry for 1 site = 1 ATP.

Oxidation of substrates that produced NADH gave a P/O_2 ratio near 3.0. Oxidation of succinate gave a P/O_2 ratio near 2.0. Oxidation of cytochrome c, or something which reduced cytochrome c, such as ascorbic acid, gave a P/O_2 ratio near 1.0.

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Models of Coupling

- The first model proposed a "high energy" chemical intermediate at each coupling site.
  - For example:
    $A_{ox} + B_{red} + Y \leftrightarrow A_{red} + B_{ox}\sim Y$
    $B_{ox}\sim Y + X \leftrightarrow B_{ox} + X\sim Y$
    $X\sim Y + P_i \leftrightarrow X\sim P_i + Y$
    $X\sim P_i + ADP \leftrightarrow ATP + X$
- This was called the "chemical coupling" hypothesis.

Years of research by many research groups were invested in trying to isolate X or Y. One problem with this hypothesis was that the chemical nature of X and Y had to be different at each coupling site.

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Models of Coupling, con't.

- Electron micrographs showed that the inner membrane of state IV mitochondria was more "condensed" than in other states.
- Circular dichroism studies indicated differences in protein conformation for the various states of mitochondria.
- This led to the "conformational coupling" model:
  - $A_{ox} + B_{red} + P_{conf.A} \leftrightarrow A_{red} + B_{ox} + P_{conf.B}$
  - $ADP + P_i + P_{conf\ B} \leftrightarrow ATP + P_{conf\ A}$
    - Where $\Delta G_{conf\ B\ \ conf\ A} < 0$

Paul Boyer was a chief proponent of the conformational coupling hypothesis. Remnants of this hypothesis remain in the description of the conformational changes which the ATP synthase undergoes during ATP synthesis.

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Models of Coupling, con't.

- Peter Mitchell's Chemiosmotic Coupling Hypothesis was the breakthrough.
- It earned him the Nobel Prize.
- He proposed that the proton gradient was the coupling intermediate.
- Few accepted the idea at first, but evidence eventually accumulated to support it, including actual measurements of ΔpH and $\Delta\Psi$.

See page 693 for further discussion of the debates over these models.

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ATP Synthase

- First discovered as an ATPase activity.
- Associated with particles on inner surface of inner membrane.
- The F_i unit was associated with the "knobs" seen in electron micrographs.
  - It catalyzes hydrolysis of ATP when isolated.
  - It also restored coupling to submitochondrial particles that lost it.
  - It is bound to an integral membrane protein complex called F_o.

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ATP Synthase, con't.

- The complex between F_1 (a peripheral membrane complex) and F_o (an integral membrane complex) is now known to be a proton pump.
- It can pump protons out of the mitochondria at the expense of ATP hydrolysis.
- But in reverse, it can tap the energy of the proton gradient to drive ATP synthesis.

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Energetics of Proton Coupling

•ΔG of ATP synthesis under cellular conditions is near 45-50 kJ/mol.

$\Delta G = nF\Delta p$, where Δp is the proton motive force

$$n\Delta p \text{ must be } > \frac{\Delta G}{F} \text{ or } \frac{50\ \frac{kJ}{mol}}{96.5\ \frac{kJ}{mol\text{-volt}}} = 0.52 \text{ volts}$$

a ΔpH of 1 and Δy of .15 corresponds to a Δp of about 0.2 volt

$$\text{Therefore n must be } > \frac{0.52}{0.2} \text{ or } > 2.6$$

Measurements suggest that n = 3, I.e. the ATP pumps three protons per ATP hydrolyzed.

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Structure of ATP Synthase

- F_1 contains 5 protein subunits
  - $\alpha, \beta, \gamma, \delta, \varepsilon$
  - stoichiometry $\alpha_3 \beta_3 \gamma \delta \varepsilon$
- F_o contains 3 hydrophobic subunits
  - a, b, c
  - stoichiometry $a_1 b_2 c_{9-12}$
- See Table 21.3, and Figures 21.24 and 21.25.

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Boyer Model for ATP Synthesis

- Three ATP binding sites, each in a different conformation.
- Each sites rotates through each conformation.
- Proton flow somehow drives this "molecular motor".
  - See Figure 21.27
- This postulate won Boyer a Nobel Prize.

According to Boyer, the energy requiring step is the conformational change that leads to release of ATP. Isotope exchange data with $H_2{}^{18}O$ showed ATP could be formed in the absence of the proton gradient. (See Figure 21.26.)

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Artificial System for ATP Synthesis

- Confirmation that a proton gradient could drive ATP Synthesis came from experiments combining the ATP synthase and **bacteriorhodopsin** into phospholipid vesicles.
- Bacteriorhodopsin pumps protons when exposed to light.
- The vesicles catalyzed ATP synthesis when exposed to light.
  - See Figure 21.28.

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Inhibitors

- Figure 21.30 summarizes effects of inhibitors.
- Oligomycin and DCCD inhibit the ATP synthase.
- Uncouplers are all lipid-soluble weak acids. They can serve as proton carriers across the membrane which discharge the proton gradient.

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Communication Across Mitochondrial Membrane

- ATP-ADP Translocase
  - Antiport exchange of ADP and ATP across membrane.
  - Exchange lowers electrical gradient. (Fig. 21.32)
- Phosphate entry by symport with H^+.
  - Transport lowers proton gradient.
- Net result is a cost of the electrochemical gradient of one proton to export the ATP.
- Therefore 4 protons needed for each ATP made.

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P/O_2 Ratios Revisited

- Stoichiometry depends on stoichiometry of proton pumps.
- Current estimates:
  - Complex I: 4 protons
  - Complex III: 2 protons
  - Complex IV: 4 protons
  - ATP synthesis (Complex V)
    - 3 protons + 1 proton for export

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P/O_2 Ratios, con't.

- For NADH reoxidation:
  - 10 protons from electron transport.
  - 4 protons needed per ATP.
  - Therefore P/O_2 ratio of 2.5.
- For succinate (or $CoQH_2$) oxidation:
  - 6 protons from electron transport.
  - 4 protons needed per ATP.
  - Therefore P/O_2 ratio of 1.5.

The text suggests that in bacteria, since no ATP needs to be transported across a membrane, that the respective P/O_2 ratios are 3.0 and 2.0, respectively.

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Oxidation of Cytoplasmic NADH

- NADH produced in the cytoplasm cannot cross the inner mitochondrial mechanism.
- There are at least two mechanisms by which it can be reoxidized by the electron transport chain.
  1. Glycerophosphate shuttle (Fig. 21.33)
     Reduces a flavoprotein, producing $CoQH_2$, and hence only 1.5 ATP.
  2. Malate-Aspartate shuttle (Fig. 21.34)
     More complex. Involves transaminases.