How ATP is Synthesized at “Energy-Coupling” Membranes: Chemiosmotic Coupling

Dale Sanders

23 February 2009
Objectives

By the end of the lecture you should understand…

1. the notion that NADH oxidation can be coupled to ATP synthesis via a transmembrane electrochemical potential for protons (or protonmotive force, PMF);

2. how the energy stored in transmembrane solute potential differences and ion potential differences can be quantified in terms of kJ/mol or mV;

3. how uncouplers increase the rate of electron transport;

4. that the PMF is used in a wide array of biological systems to transduce free energy from one form to another.
Reading

As in previous lectures, all the topics today are well covered by the standard big biochemistry textbooks. An example is


Also useful for a more in-depth treatment is

Nicholls, DG & Ferguson, SJ (2002) *Bioenergetics* 3 pp. 46 & 47 and Chapter 4
How is ATP Synthesis Coupled to the Redox Reactions?

NADH \rightarrow \text{Redox} \rightarrow \frac{1}{2}O_2 + 2H^+ \rightarrow H_2O

NAD^+ \rightarrow ADP + P_i \rightarrow \text{Phosphorylation} \rightarrow ATP

Phosphorylation is energized indirectly, via generation of a H\(^+\) potential across the inner mitochondrial membrane:
**The chemiosmotic hypothesis** (Peter Mitchell, 1960s):

1. The coupling complexes in the redox chain are $H^+$ pumps.
2. Low membrane permeability to $H^+$ allows build up of transmembrane $H^+$ potential.

Note: Redox enzymes + ATP synthase are discrete elements in membrane.
A hydraulic analogy of chemiosmotic coupling

Energy input
Mains electricity
e⁻ transport

Pump
H₂O pump
H⁺ pump

Coupling energy:
Potential energy difference for H₂O H⁺

High potential H₂O H⁺

Energy transducer
Water wheel/dynamo
ATP synthase

Energy output
Light
ATP

Low potential
H₂O H⁺
Economic Exploitation of Hydraulic Energy Coupling

http://www.antonine-education.co.uk/physics_gcse/Unit_1/Topic_4/hydroelectric_dam.jpg
The F-Type ATP Synthase

Two sectors

ADP binding site

Rotation

biologicinstitute.org/2008/04/03/perspectives/
Quantifying the Free Energy in Transmembrane Solute Potentials

(i) Uncharged solute:

Chemical potential of \( S \) = \( \mu_s = \mu^0_s + RT \ln [S] \)  

Chemical potential difference, inside with respect to outside is

\[
\Delta \mu_S = (\mu_S)_i - (\mu_S)_o = RT \ln \left( \frac{[S]_i}{[S]_o} \right)
\]
\[ \Delta \mu_S = (\mu_S)_i - (\mu_S)_o = RT \ln \left( \frac{[S]_i}{[S]_o} \right) \]

\[ = RT \times 2.303 \log_{10} \left( \frac{[S]_i}{[S]_o} \right) \]

e.g. if \([S]_i = 10 \text{ mM}, [S]_o = 1 \text{ mM}\]

\[ \Delta \mu_S = 8.314 \times 298 \times 2.303 \times 1 = +5.7 \text{ kJ/mol} \]

Note:
1. Units are those of Gibbs free energy.
2. Polarity: + ve value says that reaction \( S_o \rightarrow S_i \) is “uphill”
   Conversely, \( S_i \rightarrow S_o \) is “downhill”
   Will release 5.7 kJ/mol
Quantifying the Free Energy in Transmembrane Solute Potentials

(ii) Charged solute (ion):

\[ [S^z]_o \leftrightarrow [S^z]_i \]

\[ \psi_o - \psi_i = \Delta \psi \]

Electrochemical potential of \( S^z \) = \( \bar{\mu}_S = \bar{\mu}_o^S + RT \ln\left[\frac{[S^z]_i}{[S^z]_o}\right] + zF\psi \)  

(3)

Electrochemical potential difference, inside with respect to outside is

\[ \Delta \bar{\mu}_S = (\bar{\mu}_S)_i - (\bar{\mu}_S)_o = RT \ln\left[\frac{[S^z]_i}{[S^z]_o}\right] + zF\Delta \psi \]  

(4)
\[ \Delta \mu_S = (\mu_S)_i - (\mu_S)_o = RT \ln \left( \frac{[S^2]_i}{[S^2]_o} \right) + zF\Delta \psi \]  

(4)

Units of Eq. 4: J/mol.

To convert to volts (recall \( \Delta G \rightarrow E_m \)): \( \div \) F. Thus

\[ \frac{\Delta \mu_S}{F} = \frac{RT}{F} (2.303 \log_{10} \left( \frac{[S^2]_i}{[S^2]_o} \right) + z\Delta \psi \]  

(5)

For the special case of the proton (\( H^+ \)), \( z = +1 \);

\[ \log_{10} [H^+]_i = -pH_i \; ; \; \log_{10} \left\{ \frac{1}{[H^+]_o} \right\} = pH_o \; ; \]

\[ \frac{RT (2.303)}{F} = \frac{(8.314)(298)(2.303)}{96500} = 0.059 \text{ V} = 59 \text{ mV} \]

Thus can rewrite Eq 5 as

\[ \frac{\Delta \mu_{H^+}}{F} = 59 \left( pH_0 - pH_i \right) + \Delta \psi \]  

(6)

**The University of York**
\( \Delta \mu_H/F \) is Known as The Protonmotive Force (PMF)

From Eq 6 we can write

\[
\text{PMF} = 59 \,(pH_o - pH_i) + \Delta \psi
\]  

Units: mV

A measure of the free energy in the electrochemical potential difference for protons across a membrane;

An important biological parameter because so much of energy transduction in biology is through the PMF
The PMF in coupled mitochondria

\[ \text{pH}_{\text{cytosol}} = \text{pH}_0 = 7.5 \; ; \; \text{pH}_{\text{matrix}} = \text{pH}_i = 8.0 \]

\[ \Delta \psi = -170 \; \text{mV} \; \text{(inside negative)} \]

From Eq 7,

\[ \text{PMF} = 59 \; (7.5 - 8.0) + (-170) \]

\[ = -30 - 170 = -200 \; \text{mV} \]

Proton flows, stoichiometries and energetics

**Note:**
1. Total of 10 \( \text{H}^+ \) pumped out per 2e\(^-\) flowing through redox chain
2. 4 mol \( \text{H}^+ \) used directly for each mol ATP synthesised
Requirement for > 1 mol H⁺/mol ATP

Energy available for ATP synthesis is

\[ \Delta \tilde{\mu}_H = (\text{PMF})(F) = (-0.2)(96500) = -19.3 \text{ kJ/mol} \]

But \( \Delta G'_{\text{ATP}} = -48.8 \text{ kJ/mol} \) (Lecture 6)

i.e. for ATP synthesis, +48.8 kJ/mol is required

Reaction is

\[ nH^+_o + ADP + P_i \leftrightarrow nH^+_i + ATP \]

Energetically, this reduces to

\[ nF(\text{PMF}) + \Delta G'_{\text{ATP}} < 0 \text{ for ATP generation.} \]

By coupling ATP synthesis to translocation of 4 H+/ATP,

\[ (-19.3)(4) = -77.2 \text{ kJ} \] is available for each mol of ATP

This is more than enough energy!!
H⁺ Flows and Impact on Redox-Phosphorylation Coupling

1. Why do redox reactions speed up when ADP is added? [See slide 20, Lecture 12]

Answer:
ADP facilitates flow of H⁺ through ATP synthase. This decreases the PMF slightly. Decrease in opposing driving force enables faster operation of redox coupled H⁺ pumps.
2. The action of uncouplers

[A brilliant prediction of the chemiosmotic hypothesis]

Some compounds (e.g., 2, 4 dinitrophenol)
(i) abolish ATP synthesis “uncouple”
(ii) speed up respiration

Chemiosmotic explanation:
Uncouplers catalyse passive $H^+$ flow:
They are “protonophores”: abolish PMF
Thus (i) no driving force for ATP synthesis
(ii) no force opposing redox reactions
How Uncouplers Work

ATP synthase

Respiratory complexes

Uncoupler

\[ H^+ \]

cytosol

matrix
Membrane-bound ATP Synthase is Ubiquitous at Energy-Coupling Membranes

A. Mitochondria + aerobic bacteria:

B. Obligate anaerobic bacteria e.g. Streptococcus lactis:

C. Thylakoids: Pumps + ATP synthase inverted compared with mitos
ATP Synthase and the PMF: Recurring Themes in Bioenergetics

From previous slide…

Although energy sources and mechanisms of $\text{H}^+$ pumps are variable,

presence and mechanism of ATP synthase is conserved through evolution.

Furthermore (next slide)…

Bacteria have evolved alternative physiological uses for the PMF…
A. Solute uptake

Free energy stored in PMF is transduced into solute gradient energy by the carriers

B. Swimming: Some bacteria swim towards attractants

<table>
<thead>
<tr>
<th>Organism</th>
<th>Speed (µm/s)</th>
<th>Body length/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli Champion</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>E. coli Sprinter</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Flagellum ROTATES
Rotation is powered by PMF

E. coli
Summary

1. NADH oxidation is coupled to ATP synthesis via generation of a PMF.

2. The energy stored in ion potential differences can be expressed in terms of kJ/mol or mV and comprises electrical and chemical components.

3. For H\(^+\), PMF = 59(pH\(_0\) - pH\(_i\)) + Δψ (in mV).

4. Uncouplers (and ADP to some extent) tend to increase the rate of e\(^-\) transport by dissipating the PMF.

5. The PMF is also used to generate ATP at bacterial cell membranes and thylakoids.... and.....

6. ...powers bacterial solute uptake and swimming.