BI/CH 422/622 on 2/22/22

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic — Exam-1 material

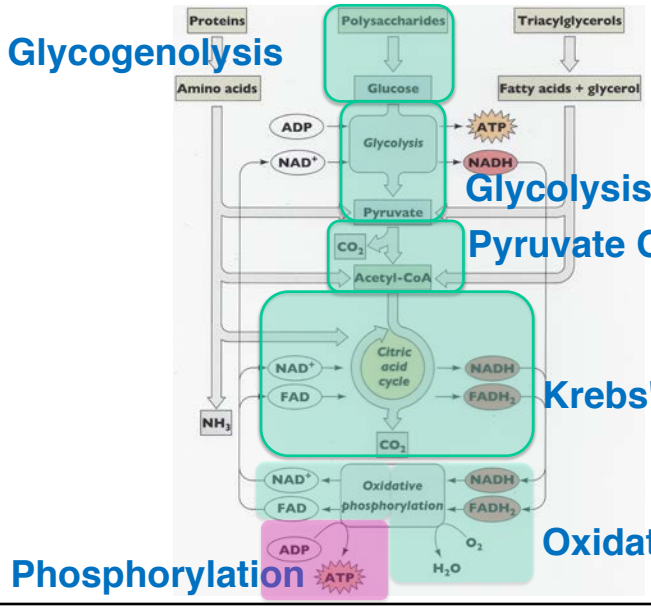
Fermentations — Exam-2 material
Pyruvate
- pyruvate dehydrogenase (ox-decarbox; S-ester)

Krebs' Cycle
- How did he figure it out?
- Overview
- 8 Steps
  - Citrate Synthase (C-C)
  - Aconitase (=, -OH)
  - Isocitrate dehydrogenase (ox-decarbox; =O)
  - Ketoglutarate dehydrogenase (ox-decarbox; S-ester)
  - Succinyl-CoA synthetase (sub-level phos)
  - Succinate dehydrogenase (=)
  - Fumarase (-OH)
  - Malate dehydrogenase (=O)
- Energetics
- Regulation
- Summary

Oxidative Phosphorylation
- Energetics
- Mitochondria
- Transport
- Electron transport
  - Discovery
  - Four Complexes

Oxidative Phosphorylation

Oxidative Phosphorylation



Glycogenolysis

Glycolysis

Pyruvate Oxidation

Krebs' Cycle

Oxidative Phosphorylation

Oxidative Phosphorylation

Learning goals:

- Function of electron-transport chain in mitochondria…... make water (finish the reaction: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$)
- Building up the proton-motive force
- Synthesis of ATP in mitochondria, chloroplasts, & bacteria

- Fuels for the cell, in the form of reduced carbon compounds (sugars), have been burned to carbon dioxide.
- Electrons from reduced fuels are transferred to cofactors, which get reduced as NADH or $FADH_2$.
- In oxidative phosphorylation, energy from NADH and $FADH_2$ is used to make ATP.

…... But how?

Oxidative Phosphorylation

Energy of the reduced cofactors

Is there enough energy in NADH & $FADH_2$ to drive the synthesis of ATP?

Each ATP synthesis is about +7.3 kcal/mol (opposite of hydrolysis)
We can do this calculation two ways:

1. Calculate the $\Delta E_o'$ needed to get 1 ATP made: compare to $\Delta E_o'$ of NADH→O_2
2. Calculate the $\Delta G^{o'}$ for the NADH→O_2 : compare to the $\Delta G^{o'}$ for ATP synthesis

#1

$\Delta E_o'$ of ATP

$$\Delta G^{o'} = -n\mathcal{F}\Delta E^{o'}$$

$$\frac{\Delta G^{o'}}{-n\mathcal{F}} = \Delta E_o'$$

$$\frac{+7.3\ \text{kcalmol}^{-1}}{-(2)(23.06\text{V}^{-1}\text{kcalmol}^{-1})} = -0.16\text{V}$$

$\Delta E_o'$ of ATP = –0.16V

$\Delta E_o'$ of NADH→ ½ O_2

$$\Delta E_o' = E_o'_{(reduction)} - E_o'_{(oxidation)}$$

$$= +0.82\ \text{V} - (-0.32\ \text{V})$$

$$= +1.14\ \text{V}$$

7.1 times more energy in $2e^-$ than needed to drive the synthesis of ATP

Oxidative Phosphorylation

Energy of the reduced cofactors

$\Delta G^{o'} = -n\mathcal{F}\Delta E_o'$
Nernst Equation

Oxidative Phosphorylation

Energy of the reduced cofactors

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#2

$\Delta G^{o'}$ of NADH→ ½ O_2

[NADH + H^+ + ½ O_2 ⇌ NAD^+ + H_2O]

$\Delta E_o' = E_o'$ (*reduction*) $- E_o'$ (*oxidation*)

$= +0.82\ V - (-0.32\ V)$

$= +1.14\ V$

$\Delta G^{o'} = -n\mathcal{F}\Delta E_o'$

$= -(2)(23.06 V^{-1} kcal mol^{-1})(+1.14 V)$

$\Delta G^{o'} = -52.6$ kcal/mol

$\Delta G^{o'}$ of ATP

7.2 times more energy in $2e^-$ going from NADH to oxygen than needed to drive the synthesis of ATP

6.5 times more energy in $2e^-$ going from $FADH_2$ to oxygen than needed to drive the synthesis of ATP

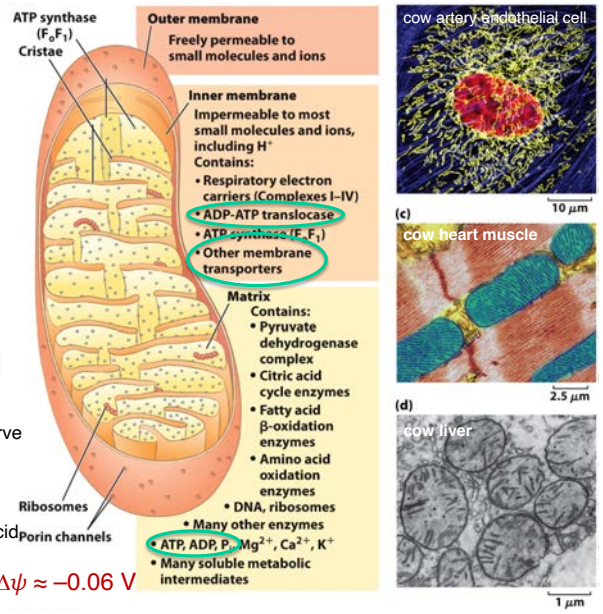
$\Delta G^{o'} = +7.3$ kcal/mol

Oxidative Phosphorylation

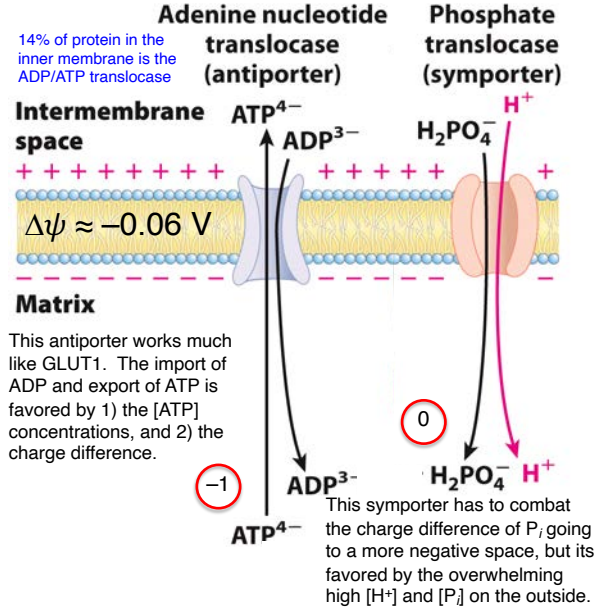
Structure of Mitochondria

Double membrane leads to four distinct compartments:

1. Outer membrane:
   - relatively porous membrane; allows passage of metabolites
2. Intermembrane space (IMS):
   - similar environment to cytosol
   - higher proton concentration (lower pH) +++++
3. Inner membrane
   - relatively impermeable, with proton gradient across it
   - location of electron transport chain complexes
   - Convolutions called cristae serve to increase the surface area.
4. Matrix
   - location of the citric acid cycle and parts of lipid and amino-acid metabolism
   - lower proton concentration (higher pH) -----



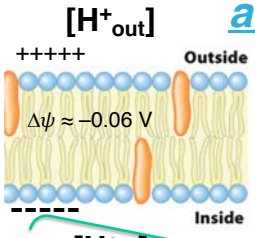
Oxidative Phosphorylation



- These translocases cost the membrane potential, which must be restored; costs 25% of Electron Transport.
- These translocases require energy from both the electrochemical gradient across inner mitochondrial membrane plus the proton gradient. Together they are called the Proton Motive Force.
- What generates this gradient?
- How much energy does it take to pump H^+ out?

Oxidative Phosphorylation

Energy required to pump a single proton *against* a pH gradient



$H^+_{in} \rightleftharpoons H^+_{out}$

$$\Delta G' = RT\ln\frac{[H^+]_{out}}{[H^+]_{in}} + z\mathcal{F}\Delta\psi$$

Switch the sign here because reaction is opposite that of transport

Top
$\ln[H^+]_{out} = 2.3\log[H^+]_{out}$
$= -2.3\ pH_{out}$

Bottom
$\ln(1/[H^+]_{in}) = 2.3\text{-}\log[H^+]_{in}$
$= +2.3\ pH_{in}$

$pH_{out} \approx 6.75$
$pH_{in} \approx 7.5$

$= RT2.3(pH_{in} - pH_{out})$
$= 5.7(7.5 - 6.75)$
$= 5.7(0.75)$
$= 4.3$ kJ/mol
$= 1.0$ kcal/mol

$= z\mathcal{F}\Delta\psi$
$= (+1)(96480)\ \Delta\psi$
$= (+1)(96480)(+0.06)$
$= 5.8$ kJ/mol
$= 1.4$ kcal/mol

$\Delta G' = 1.0 + 1.4 = 2.4$ kcal/mol
As a consequence, it will take ~3 protons per ATP.

Oxidative Phosphorylation

P/O ratios

Using tissues rich in mitochondria like pigeon muscle, and eventually using isolated mitochondria, biochemists would add different carbon compounds and measure two things:

1) Oxygen consumption
2) Amount of ATP made

The ratio of ATP synthesized to the oxygen ($\frac{1}{2}O_2$) consumed was termed the P/O ratio

The $\frac{1}{2}O_2$ represents $2e^-$ going through electron transport. The P/O ratio for various fuel molecules provided to cells/mitochondria were measures as:

NADH was ~3
Pyruvate was ~3
Succinate was ~2
Ascorbate was 1

$\Delta G^{\circ\prime}$ of NADH→ $\frac{1}{2}$ O_2

$\Delta G^{\circ\prime}$ = –52.6 kcal/mol

$\Delta G^{\circ\prime}$ = +7.3 kcal/mol 7.3 x P/O of 3 = 22 kcal/mol

22/53 = 42% efficient

This is the energy recovered from the complete oxidation

What makes this proton motive force?

Electron Transport

Electron Transport

Electron-Transport Chain Complexes Contain a Series of Electron Carriers

- When it was realized that isolated mitochondria are capable of respiration (oxygen consumption when provided fuels), biochemists began purifying them and their components.
- The first things purified were redox compounds and small stable proteins:
  - NADH
  - flavin mononucleotide (FMN)
  - flavin adenine dinucleotide (FAD)(bound to protein; flavoproteins)
  - iron-sulfur clusters
  - Coenzyme Q (Ubiquinol)
  - cytochromes *a*, *b*, or *c*

- Once purified, they were analyzed by measuring their E_o'.
- Order of transfer of electrons is dependent on E_o':

TABLE 19-2 Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

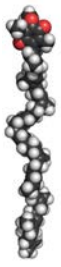
Redox reaction (half-reaction)	E_o' (V)
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
FAD-E + 2 H· + 2e- → $FADH_2$-E	−0.02
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$) FeS	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome*c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$½O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.817

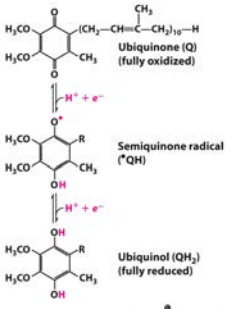
Big Drop! Big Drop! Big Drop!

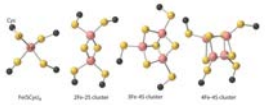
NADH ⟶ Q ⟶ Cyt *b* ⟶ Cyt c_1 ⟶ Cyt *c* ⟶ Cyt ($a + a_3$)

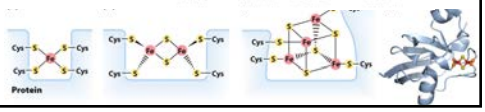
Electron Transport

- NAD^+, FMN, and FAD accept electrons. The flavin nucleotides can accept one or two electrons, and can also donate one electron at a time to acceptors that can only accept single electrons
- Ubiquinone, also called Coenzyme Q, is an isoprene lipid that readily accepts electrons. Upon accepting two electrons, it picks up two protons to give an alcohol, ubiquinol ($CoQH_2$). Its found IN the inner membrane.
- Iron-sulfur complexes (Fe-S) that can only carry one electron at a time (role is different than in aconitase).





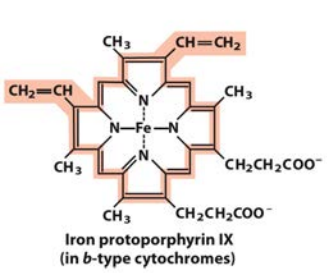


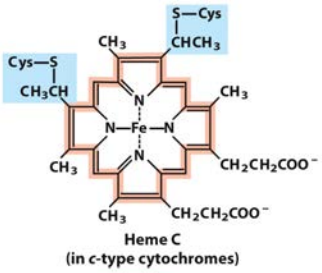


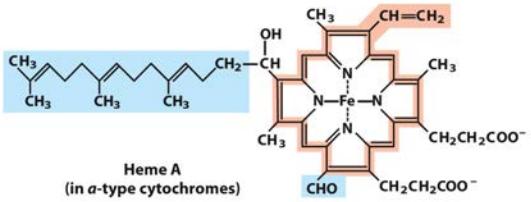
Electron Transport

Cytochromes

- Small one-electron carrier proteins
- Iron-coordinating porphyrin-ring derivatives
- *b*/b_1, *c, or a*/a_3 differ by ring additions

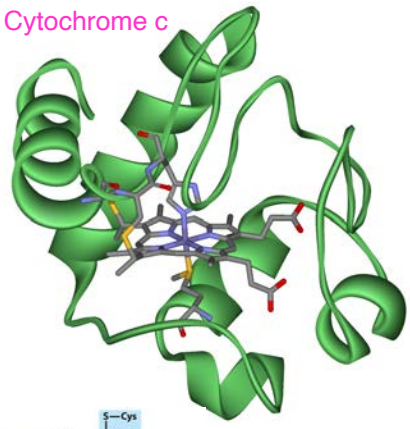


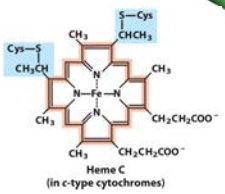




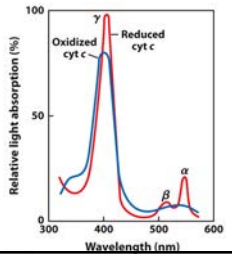
Electron Transport

Cytochrome c





- Mobile electron carrier; a peripheral membrane protein
  - Cytochrome *c* moves through the intermembrane space (IMS).
- A soluble heme-containing protein
- Heme iron can be either ferrous (Fe^{2+}, reduced) or ferric (Fe^{3+}, oxidized).
- Cytochrome *c* carries a single electron.
- The two redox forms have different spectra:

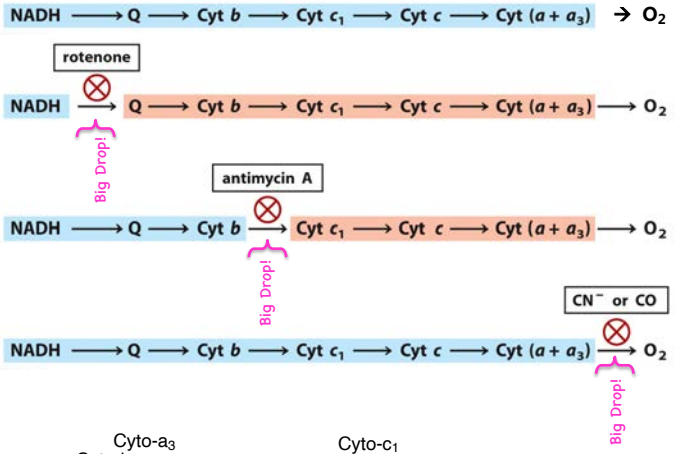


- Intense Soret band near 400 nm absorbs blue light and gives cytochrome *c* an intense red color.

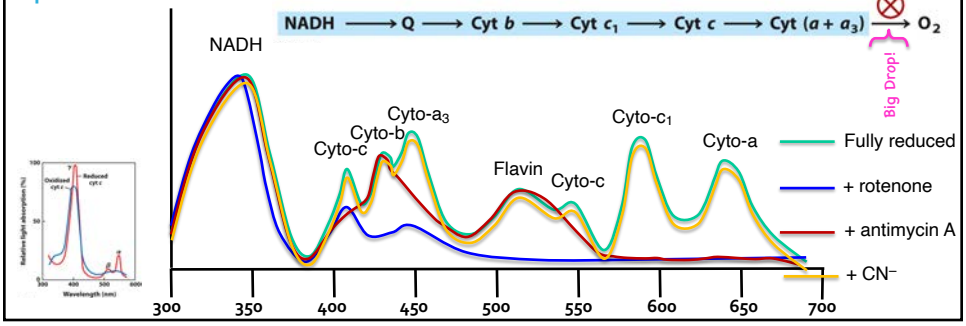
Electron Transport

Inhibitors of Electron Transport

- Inhibitors all stop ET and ATP synthesis: very toxic!

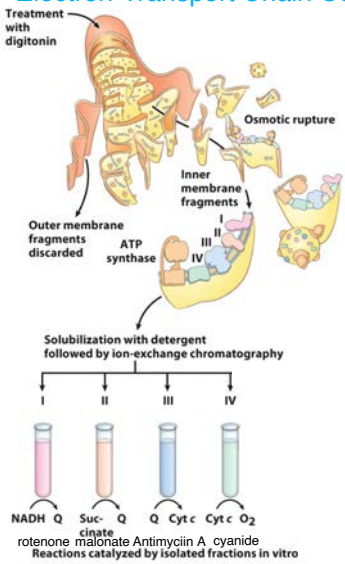


Spectral work



Electron Transport

Electron-Transport Chain Complexes Contain a Series of Electron Carriers



- Better techniques for isolating and handling mitochondria, and isolated various fractions of the inner mitochondrial membrane
- Measure E_o'
- They corresponded to these large drops, and they contained the redox compounds isolated previously.
- When assayed for what reactions they could perform, they could perform certain redox reactions and not others.
- When isolated, including isolating the individual redox compounds, and measuring the E_o' for each, it was clear that an electron chain was occurring; like a wire!
- Lastly, when certain inhibitors were added, some of the redox reactions could be inhibited and others not. Site of the inhibition could be mapped.