Medical Committee
The University of Jordan

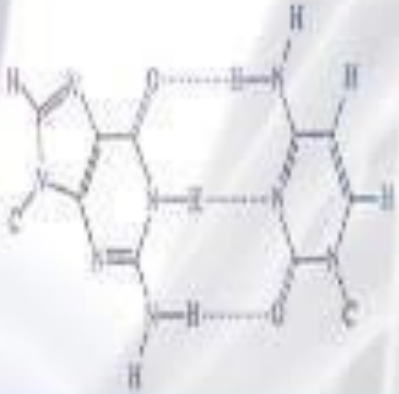
SLIDE SHEET

LECTURE#: 12

DR.NAME: Nafeth Abu-Tarboush

DONE BY: Moath Elbalawe

Biochemistry



Majida Al-Fogaraa'

Oxidative Phosphorylation 2

This lecture is a continuation for the oxidative phosphorylation.

-This process occurs at the inner mitochondrial membrane (IMM) through protein complexes which are fixed within this membrane.

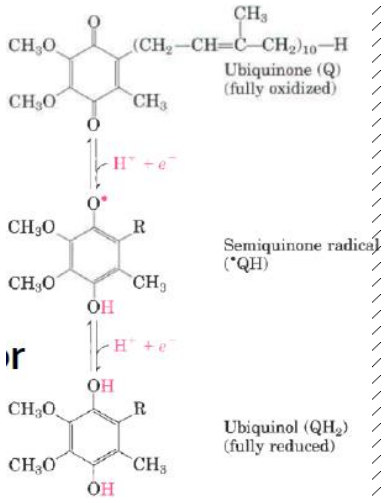
- This process is based on extracting the energy from high energy molecules (NADH and FADH2) *by moving the electrons* gradually from compounds with lower reduction potential (more negative) to compounds with higher reduction potential (more positive), eventually to the oxygen (which has the highest positive reduction potential) forming water.

-The energy produced from the movement of these electrons to lower energy states is used to pump hydrogen ions (H+/ protons) across the IMM to the intermembranous space making a potential gradient which would be used to produce the ATP molecules.

-Electron carriers move the electrons from one complex to another. These carriers should normally be lipid soluble to be able to move freely within the membrane (IMM). One of the carriers is the ubiquinone and the other one is cytochrome C (cytochrome C is water soluble, not lipid soluble, so it transfers the electrons while it is on the outer surface of the IMM).

*Ubiquinone:

Also called coenzyme Q (Co-Q) or simply, quinone (Q). It is a cyclic structure with 2 ketone groups, which hold the electrons, and repeated isoprene units (6-10 isoprenes) which make the carrier lipid soluble. Its structure interchanges between the oxidized, free radical and reduced states. Co-Q is similar to flavins (e.g.: FAD) in producing a free radical state.



-Co-Q is commercially available and it is given to patients with myocardial infractions , **why ?**

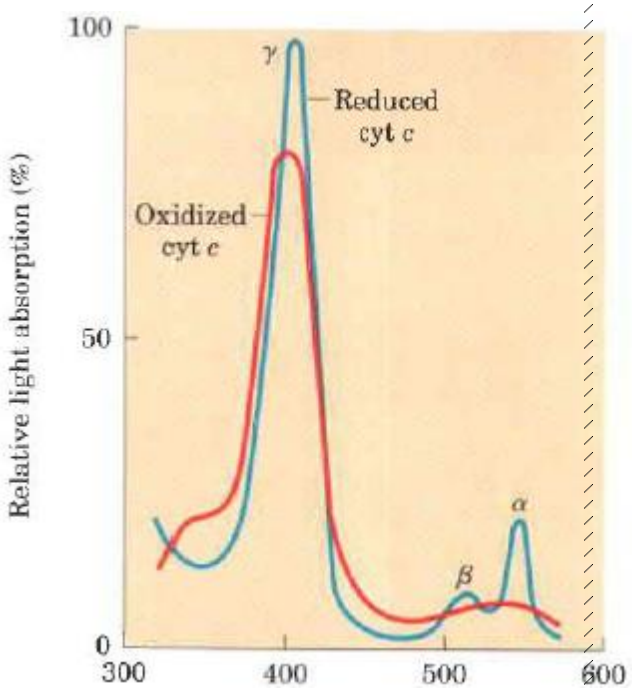
- to help the injured cells in producing more ATP by moving electrons faster to the protein complexes (from 1 & 2 to 3) and the ETC will be faster . (Co-Q is given orally; cytochrome C, which is the other electron carrier between the protein complexes (3 & 4), cannot be given orally as it is a protein and it would be degraded in the GI tract , so it can't be used as Co Q).

1. The (fully) oxidized, ubiquinone (Q), state is when the molecule is carrying no electrons on it.
2. The free radical state, semiquinone radical (.$QH^{\cdot}$), when the carrier is carrying one electron by reducing one ketone group to a hydroxyl group (also an H+ is added), while the other ketone group is converted to a free radical state.
3. The (fully) reduced state, ubiquinol (QH2), where the two electrons (with the two H+) had been added to the Q in a sequential manner (in two steps rather than one), converting the two ketone groups to hydroxyl groups.

Cytochromes:

- Any protein that transfers electrons using a heme group is a cytochrome.
- Different heme groups make different cytochromes (which are the proteins that contain the heme), e.g.: cytochrome A has heme a. ---- - The heme group is made of a porphyrin ring with the iron ion in the center.
- The heme groups differ from each other by the groups which are attached to the porphyrin ring (side chains.
  -For example :
  *Heme a has an isoprene unit attached to the porphyrin ring
  * heme b has vinyl groups (with double bonds)
  * heme c has thiol groups, which are used for attachment to cysteine residues in proteins.

Light absorption in the cytochromes:



-Cytochromes exhibit absorption for the visible light because of the heme groups; different cytochromes absorb different wavelengths of visible light (because of their different heme groups).

-And within the cytochrome itself, its oxidized state (Fe+3/ ferric) absorbs different wavelengths of light than its reduced state (Fe+2/ ferrous).

-The oxidized state of the heme appears red and its reduced state appears colorless. To know the absorbed wavelengths of light, a Spectrophotometer is used.

-When the Spectrophotometer is used (as in the picture), the ***oxidized state*** of the cytochrome shows one band of absorbed light and generally has an absorption band at 400 nm with small differences between different types of cytochromes.

-While the reduced cytochrome show three bands (alpha, beta and gamma). The type of the cytochrome is determined from the alpha band in the reduced cytochrome.

***The alpha band in cytochrome A is at 600 nm, B is at 560nm, and C is at 550 nm**.

-Also, the Spectrophotometer shows different subtypes of cytochromes which share the same type of heme, having minor differences in the wavelength of the alpha band (around one or two nanometers) ,e.g.: cytochrome C 550 and cytochrome C551.

Notes:

- Most common heme group is heme b which is found in myoglobin and hemoglobin.

- A heme group can carry only one electron, because iron has only two states, either Fe+2 (ferrous) or Fe+3 (ferric).
- Cytochromes are classified according to type of their heme group and their light absorption on spectrophotometer.

Requirements of oxidative phosphorylation:

1. ATP synthase
2. Electron donors (NADH and FADH2) and electron acceptor (oxygen)
3. Electron carriers (protein complexes and the carriers)
4. Intact IMM, so we could have an electrochemical gradient across the membrane, i.e. it must not be leaky to H+ in order to maintain this gradient and use it in making ATP.

Note :

*Difference in energy which is produced by the movement of two electrons from one complex to another is around 16 kcal, and this difference is used to pump protons across the membrane .

-We are saying 2 electrons as NADH and FADH2 carry two electrons.

*There is no connection between complex I and complex II , that's why CoQ is needed to carry electrons to complex 3.

Now, we will talk about each complex in electron transport chain (ETC) individually:

-Because of the difference in potential that results from the transferring of the two electrons from complex I to Co-Q ,pumping of 4 protons (H+) from the matrix to the intermembranous space will result, and this will only happen for NADH (not FADH2 which does not pass through complex I). When the electrons pass from Co-Q to complex III, the generated difference in potential will result in pumping of another 4 protons from matrix to intermembranous space, and when electrons reach complex IV and oxygen is reduced to water, and the generated potential from transfer of electrons from cytochrome C to complex IV

will result in pumping the last 2 protons from the matrix to the intermembranous space.

In conclusion:

1. Electrons that come from NADH and move until reaching oxygen result in pumping of ten H+, and the protons come back from intermembranous space to the matrix through ATP synthase, where each four H+ moving through the ATP synthase result in the production of one molecule of ATP
2. So the two electrons that move from the NADH and reach oxygen results in generating **2.5** ATP.
3. Because FADH2 does not reach complex I like NADH, the first four protons that are pumped by complex I are not involved in the oxidation process of FADH2, and we only include the protons that are pumped through the other complexes (four protons through complex III and two protons through complex IV), so as a result, six protons will be pumped. And as you know, each four protons will result in producing one ATP, so 1.5 ATP will be produced from the transfer of two electrons from FADH2 to oxygen.

Complex I	4 H^+
Complex II	Zero
Complex III	4 H^+
Complex IV	2 H^+
4 H^+ → 1 ATP 1 NADH → 10 H^+ → 2.5 ATP 1 $FADH_2$ → 6 H^+ → 1.5 ATP	

Complex I:

It is called NADH dehydrogenase; because it oxidizes NADH (which comes from kreb's cycle to NAD^+, it is a large complex, quaternary structure (made of 25 polypeptides, some of these polypeptides are made from nuclear genes and others are from mitochondrial genes).

It's a transmembrane protein (within the IMM), it has an attachment domain within the matrix; where NADH initially bind to FMN then through several Fe-S clusters till it reaches Co-Q to donate its electrons.

FMN (Flavin Mononucleotide) is tightly bound to this complex; because it is a flavin molecule and all flavins must be tightly bound to a protein, and their reduction potential varies according to the protein bound to it.

The two electrons pass from NADH to FMN, then through seven Fe-S clusters, after that they go to the Co-Q (the electron carrier) which has a higher reduction potential, thus energy would be produced (16 kcal), and this energy will move **four H+** to the intermembrane space.

Complex II:

Also called succinate dehydrogenase, and it is a member of the oxido-reductase enzymes family (it oxidizes $FADH_2$ and reduces Co-Q). It is the only peripheral complex (on the inner side of IMM); so it does not have a transmembrane channel for proton pumping, because the difference in energy that is generated from transferring electrons from $FADH_2$ to Co-Q equals approximately **zero**.

It is a flavo-protein, and it is the linkage between TCA (Kreb's) cycle and the ETC (electron transport chain).

After FAD is reduced to $FADH_2$ (during the converting of succinate to fumarate), two electrons from $FADH_2$ are donated to Q, converting it to QH_2 (two H+ are taken from the matrix).

On the outer side of the IMM, there is a certain protein called electron transfer flavoprotein (ETF). This protein brings electrons (which might come from fatty acid oxidation) from the cytoplasm to the Co-Q, converting Q to QH_2 which would continue its way to complex III.

Complex III:

Called cytochrome BC1, because it contains two cytochromes (hemes): cytochrome B (contains BH heme and BL heme groups), and cytochrome C1 (contains heme c1). It is a dimer structure and each part of the dimer

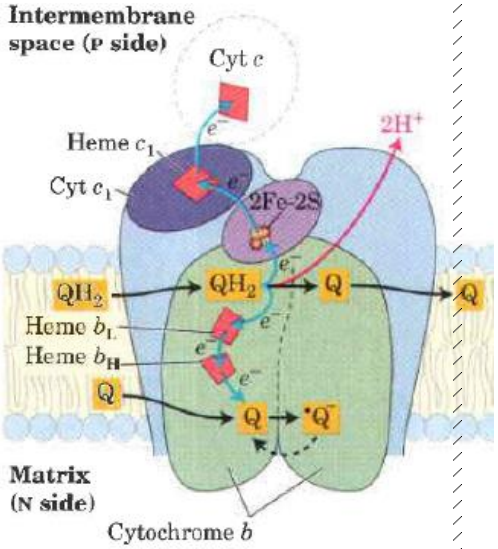
consists of eleven subunits (ie: the complex has 2 cytochrome C1 and 2 cytochrome B because of the dimer structure).

It has two binding sites for Co-Q, one at the outer side of the IMM (reduced Q) and the other is at the inner side of IMM (oxidized Q).

This complex gets the electrons from QH_2 and donates them to cytochrome C, which results in pumping out $4H^+$.

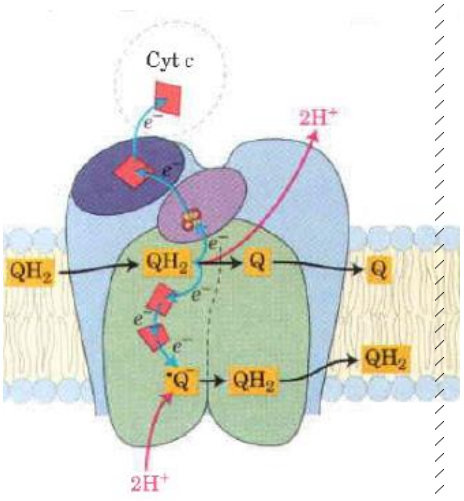
The Q-cycle:

The Co-Q comes to complex III (from complex I and complex II) in its reduced state as ubiquinol (QH_2). **It gets oxidized to Q and loses two H+ and two electrons.**



- One electron is transferred to the iron-sulfur center then this electron goes to the heme c1 (at cytochrome C1) then it goes to cytochrome C (which has the capability to carry one electron only, this cytochrome will go through the intermembrane space to complex IV).
- The two protons from the QH_2 will be released into the intermembrane space directly.
- The other electron passes through BL heme then BH heme and then it reaches an oxidized ubiqinone (Q) at the cytoplasmic side of the complex, converting it to the free radical state (·QH). This occurs in the first part of the cycle.

The second part of the cycle is similar to the first part of it; another QH_2 molecule comes to the outer side of the complex, gets oxidized to Q, one electron goes to the iron-sulfur center then this electron goes to the cytochrome C1 then it goes to cytochrome C ending at complex IV; two protons get released to the intermembrane space; the second electron goes to cytochrome B (passing through heme BL and heme BH) and it is transferred to the semiubiquinone (·QH)



- Converting of Q to QH_2 at cytochrome B requires addition of electrons as well as protons; the two protons are acquired from the mitochondrial matrix.

The net of this cycle is as follows:

- Two QH_2 were converted to Q and one Q was converted to QH_2, so the total is that one QH_2 was converted to Q.
- Four protons were pumped to the intermembrane space; two protons per each QH_2.
- Two electrons were transferred to complex IV in a sequential manner (each electron is carried by one cytochrome C).

Complex IV:

Called cytochrome C oxidase, it is the place were oxygen molecule (O_2) is reduced to two water molecules (most of the oxygen that we inhale is converted to water).

Within this complex, the electron acceptors are two heme groups (heme a and heme a3) and two copper atoms (CuA and CuB, copper is a transition metal just like the iron, so it can lose or gain electrons by moving from reduced Cu^{+1} to oxidized Cu^{+2} and vice versa). Complex IV also has a site for binding the oxygen.

Electron movement through complex IV:

CuA→ heme a → heme a3+ CuB

Heme a and CuA are not so near to each other so the electrons move through them in a sequential manner, while heme a3 and CuB are very close to each other so they form one unit and can share the electrons with each other.

That was it for this lecture…

Done by Moath Elbalawe

Co-written by Nedal AlSabatin

Dedications to Eyad Al-Ajlony.