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Biochemistry

● Sheet ○ Slides

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Title: Oxidative Phosphorylation complexes

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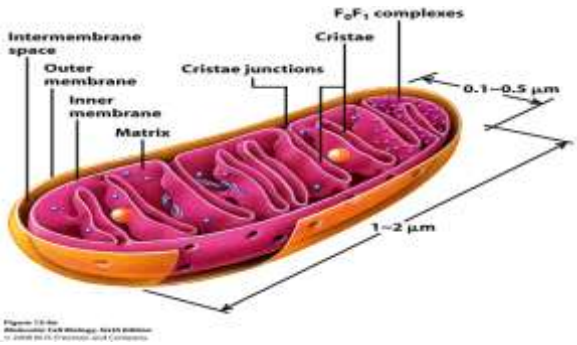
The doctor started the lecture by introducing the mitochondria structure.
The mitochondria have an **outer membrane**, **inner membrane**, and **matrix**.



Starting with the **OMM** it is permeable to small molecules, which their MW is 5000 Delton or less through special channels called porins, which are nonspecific to certain molecules.
However, the **IMM** is impermeable to even the smallest thing in life, Protons H+, so special carriers are needed.

All the energy metabolism reactions occur inside the **matrix** of the mitochondria except for one process that occurs inside the cytosol, which is the <u>glycolysis.</u>

<u>Where does oxidative phosphorylation occur?</u>

-It is the last stage of energy metabolism.

REMINDER OF THE STAGES OF THE ENERGY METABOLISM:

1) Ingestion digestion, absorption, reaching the cells.
2) Acetyl-CoA formation through different metabolic pathways.
3) The use of Acetyl-CoA through citric acid cycle.
4) Oxidative phosphorylation process.

As the name implies the oxidative phosphorylation it consists of two processes:

a) Series of redox reactions (we are using the redox reaction for the sake of ATP generation)
b) Phosphorylation process.

Oxidative phosphorylation has 3 major steps:

- Electron transport chain. Flow of electrons coming from the citric acid cycle represented by NADH and $FADH_2$ coming to the electron transport chain.
- Series of electron carriers, enzymes in the inner mitochondria membrane, which accept these electrons and then transfer them in a series of reactions.
- Movement of electrons according to their reduction potential will generate a difference in energy, which will be used to pump protons from the matrix to the inter membranous space across the inner mitochondria membrane.

  Electrons move because of the energy difference between them, this energy difference are used whenever electron moves from one place to another in order to pump protons outside the IMM.
  Protons are pumped outside, creating pressure on the outer surface of IMM where there is more protons now. They create a difference in concentration, more protons outside than inside, so there is a larger difference in the charge. There is a difference of protons inside and out as well as a difference in charge, a potential called the electrochemical potential is created.
  This pressure must be released.

As we mentioned before, IMM is impermeable to anything even to protons so we have a special carrier, ATP synthase, where a flow of protons go through in the OP, which provides the free energy the synthesis of ATP.

The OP happens when electrons from NADH and $FADH_2$
The NADH came from the citric acid cycle, it is soluble and can move freely in solution, which it flows until it finds a receptor and binds to it. It binds to an enzyme that can receive the electrons from NADH.

$$NADH \xrightarrow{\text{Oxidation}} NAD+$$

The enzyme that receives the NADH is called NADH oxidoreductase (**Complex1**). Complex 1 is a big complex and has 25 polypeptides chain. The complex has many electrons carrier molecules. In order for electrons to move through proteins, it requires certain structures, for instance, Hemes, have iron do Fe^{+2} can be converted to Fe^{+3}. Other structures are Flavins, $FADH_2$ and FMN. And Iron Sulphur bluster is another structure that can help electrons move through proteins. Iron can go from $iron^{+2}$ to $iron^{+3}$ and vice versa.

Complex 2 (Succinate dehydrogenase)
Converts succinate to fumarate, and it is the only direct connection between electron transport chain and citric acid cycle (6^{th} reaction in TCA)
Electrons came from succinate the enzyme that converts it to fumarate is succinate dehydrogenase. The electrons then go to $FADH_2$ inside within the complex 2 and they move inside through electron carrier molecules amongst them iron sulphuric blasters. We have electrons here and here. There is no relation or connection between complex1 and complex2

Coenzyme quinone
Electrons from **complex 1** and **complex 2** do not move in between and have no relation or connection between the two. They both go to **complex 3** directly.
When talking about electron transport chain we are talking about series of oxidation-reduction reactions through enzymes, proteins that move through the membrane.

- Note that integral proteins cannot move through membrane it's too rigid.

There has to be a structure to help the electrons move through the membrane. Co enzyme Q "quinones", these structures are able to go through oxidation-reduction reactions. Electrons in complex 1 and complex 2 can float through the membrane until it reaches complex 3.

Complex 3 (Cytochrome BC1 complex) Have Heme B and Heme C.
Cytochrome BC1 complex, quinone oxidoreductase. In order to move electrons from complex 3 to complex 4, we need certain structures in the peripheral side of the outer surface of the inner mitochondria membrane there is an electron carrier molecule, which is a protein called cytochrome C contains Heme C, that can move one electron at a time.

Cytochrome C takes electron from complex 3 and sends it to complex 4. Complex 4 contains Heme it can connect to oxygen. Heme cannot connect to oxygen unless it is reduced. The affinity of oxidized heme to oxygen is very low. So it cannot connect to oxygen and must be reduce first. Special enzymes in your blood that always keep heme reduced in Haemoglobin. If it wasn't reduced or there is genetic deficiency for that enzyme, "methemoglobinemia occurs".

> Electrons reach complex 4 (cytochrome C oxidase) where there are hemes that are reduced and connect to oxygen, creating H+ and converting oxygen to H_2O. Electrons move from complex1 to co-enzyme Q because there is a difference in energy, which is used to pump protons outside matrix across the inner mitochondrial membrane.

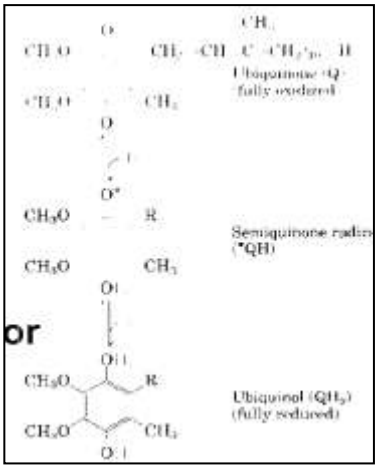
NADH gives 2 electrons to complex 1 to coenzyme Q, pumps 4 protons outside the matrix. When electrons move from complex 2 to co-enzyme Q, the energy between them is "0 kcal". No difference in energy, so no protons are pumped. It is <u>designed not to be integral</u>. Electrons move, but no energy to pump protons out.

When electrons move from complex 3 to cytochrome C they create difference in energy. Two electrons from NADH or $FADH_2$ move and <u>4 protons</u> are pumped out. The 2 electrons from complex 1 or complex 2 reach complex 3. The 2 electrons pump <u>4 protons</u> by the energy difference between them. When they reach complex 4, the difference of energy can move out <u>2 protons</u>. In conclusion the NADH pumps <u>10 protons</u> in total 4+4+2.

Every 4 protons when they move to the matrix are able to generate 1 ATP. That is why the ratio NADH gives 2.5 ATP (10/4). However, $FADH_2$ didn't go to complex 1 so it has only 6 protons so ratio would be 1.5ATP (6/4).

There are 3 types of electron transfer reactions in the oxidative phosphorylation process:

a) Direct electron transfer, where the electron moves to the other structures directly. It occurs in any structure containing heme, which will convert the iron^{+2} to iron^{+3} and it gains electrons or loses them.
b) Move of electrons through H, in (NADH) as H- (hydride ion).
c) Electrons can be transfer directly through structures such as NADH, NADPH, and flavins.
   Flavins: Structure like FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide)
   All flavins can accept 1 electron or 2 electrons on the other hand NADH can accept 2 electrons at a time.
   Other carrying molecules are the quinone (ubiquinone), which carry the electrons from complex 1 to complex 3 and from complex 2 to complex 3.
   The structure of the quinone is cyclic diene structure chemistry wise. Cycle bounded with two O through double bound
   The structure in the Co-enzyme Q has a long hydrophobic chain or long isoprenoid chain … why?



- So it can be soluble within the membrane, it needs to be able to move. Same with flavins , co-enzyme Q transfer through the intermediate state, which have free radical. The first electron moves to semi-quinone state then is converted to ubiquinol.
ubiquinone which is oxidized have two oxygen in the oxidized state, semi-quinone with one hydrogen and free radical. When fully reduced, is the ubiquinol state when it becomes an alcohol it can accept 2 electrons in a sequential manner from two different sources as FADH2 and can move in free radical state.

Sometimes ubiquinone can be prescribed to people with myocardial infarction and recovered. WHY?
Heart attack is when blood stops flowing (block) properly to part of the heart and the heart muscle is injured due to not enough oxygen supply.
The cells that nourish the heart muscle and arteries become blocked due to many reasons. People who have recovered from heart attacks have weaker hearts, by giving them co-enzyme Q, you are facilitating and enhancing the movement of electrons in the mitochondria in the heart muscle. You moved them faster, more molecules that are able to move electrons, you will generate more ATP.

Cytochromes, other molecules, which can set electrons and lose them and can go through oxidation-reduction reactions. Any enzyme that can gain or lose electrons and contain heme is called cytochrome.
Cytochromes are of many types according to the heme it contains.

Cytochromes A, B and C, are available in oxidative phosphorylation process.
Heme A has two types in **complex 4**, which are *Heme A* and *Heme A3*.
Complex 3 has *Heme B&C*.
What moves the electrons from complex 3 to complex 4 is cytochrome C. When Heme moves from the oxidation state to the reduction state it changes in its light reflection length, spectrophotometer. Any materiel you put in a solution and expose it to light it will absorb some light and reflect some so it gives special bands. Difference between oxidized heme and reduced heme. First is shown in red and the second is in blue. **Oxidized heme** gives one band, wavelength is about 400nm. **Reduced heme** gives 3 bands, starting from highest wavelength: alpha, beta and gamma. According to where the alpha band's wavelength occurs, we name the cytochrome in different name.
Example1, Cytochrome C550: is an enzyme that can gain and lose electrons and have Heme C and when the heme in the reduced state it gives Alpha band centred over 550 nm.
Example2: Cytochrome B 562, when it is reduced, gives alpha bands centred at 562 nm. (Just to know)

Heme can transfer one electron at a time. Ubiquinone transfers 2 electrons at a time.

Reduction potential of heme is variable depending on the protein it contains.

Other electron carrier molecules are iron sulphur clusters, special structures in where iron bounds with sulphur in a way, for instance, one iron with 4 sulphurs, or 2 irons with 4 sulphurs …etc.

They transfer electrons one at a time. Electrons move through iron, which can only move one electron at a time.

There are at least 8 types of iron sulphur proteins in the electron transfer chain. 7 of them exist in complex 1 at least. The structure varies and depends on the organisms and cells. Their main goal is to set electrons and lose them. They can destruct the protein, that's why there has to be a special construction that can go through oxidation-reduction reactions. Reduction potential is variable depending on the protein. If you have any electron carrier molecule you can't find it free in the solution, it'll be with more than one protein, thus reduction potentials is variable. In iron sulphur clusters the reduction potential is also variable and can reach -650 millivolt to +450 millivolt.

Oxidative phosphorylation requirements are:
1. Electron donors, NADH $FADH_2$.
2. Electron acceptor, final electron acceptor oxygen (without it there is no process)
3. Proteins that can transfer the electrons through a series of reactions to reach the final electron acceptor.
4. Intact mitochondrial membrane. The difference in energy exists to move the protons. If the membrane is leaky, the protons will move back. Thus, no difference in gradient is created. NO ATP!
5. Go through certain structures to generate ATP. (ATP synthase)

Energy difference from the movement of electrons equals to "16kcal per 2 electrons moving", for example from complex1 to CoQ , used to pump out protons out, but not available in complex 2 to CoQ

- Complex 1: NADH dehydrogenase

-NADH-Q oxidoreductas.
-Have more than 25 polypeptide chains.
-Electrons from NADH enter carrier molecules in order to transfer it to quinone, these molecules are: *iron sulphur clusters* as we mentioned earlier 7 of them are present in complex 1, in addition *FMN* is the <u>first structure</u> to receives the electrons from NADH. In case of FMN it is slightly bound as in the case of most flavins so it won't destroy the protein it resides in (free radical state)

- Complex 2:

It is present in the inner mitochondria membrane, where the Krebs cycle starts and in step 6 it is catalysed by succinate dehydrogenase "complex 2".
Difference in energy in succinate to fumarate is nearly 0 calories, thus no protons are pumped out.

Other flavin proteins have $FADH_2$ structure can move electrons to electron transport chain, called electro transfer flavoproteins, exist in the cell.
So keep in your mind that NADH and $FADH_2$ are not the only source of electrons that comes from the Krebs cycle (from cytosol + mitochondria ---> ubiquinone)

- Complex 3: cytochrome BC1 complex: Q-cytochrome C oxidoreductase.

A very big complex contains two identical monomers (dimer) bounded together; the complex has 11 sub units. We call it BC1 complex because it has heme B and heme C1. Iron sulphur clusters are present as well for electron transport.

- Q cycle:

When electrons reach complex 3:
Complex 3 have two binding sites one is close to outer surface of the inner mitochondria membrane and the other is close to the inner surface of the inner mitochondria membrane. One is close to inter membranous space and the other is close to the matrix.
The one close to the inter membranous space has high affinity for the reduced quinone , the quinol "the alcohol with 2 electrons". The binding site close to matrix has a high affinity to the oxidized quinone, but with less affinity to semi-quenon and more less affinity to the reduced form.
Each bind to its binding site and have 2 electrons and are set in the heme close to it, it gives each heme (Heme C1) one electron at a time --> cytochrome C. The other electron binds with another heme close to it (heme B) away from the process, it is set on B then goes to the binding site of the oxidized state of quinone. Now it is in the free radical state since it has one electron. Another quinol repeats the cycle. Gives an electron to iron sulphur clusters--> heme C1--> cytochrome C. "Have 2 electrons moving". An extra electron sets on heme B, now the one in free radical state has 2 extra electrons (fully reduced state) it is now a quinol.
Two molecules of REDUCED quinols are needed to complete the cycle but we are regenerating one in the end. By the end the net use is one quinol.

- Complex 4:

Complex 4 structures: Has 2 copper sites and 2 "hemes, Heme A, Heme A3, CopperA, Copper B".
Cytochrome C gives electrons to copper A (close to surface). In copper A electrons move to Heme A, a little far, then they bind to "heme A3 and copper B". Copper B is a site which is kinda close to heme A3 and they work as one centre so they can share electrons , while copper A and heme A work independently .