University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Biochemistry

● Sheet ○ Slides

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Title: Cont. Oxidative Phosphorylation

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Try to enjoy it… :P

- ☒ **Complex IV (cytochrome C oxidase)**
  - ➔ It does **Oxidation** of cytochrome C
    (electrons are transported from cytochrome c (oxidized) to this complex (reduced))
  - ➔ This complex contains **4** redox components (electrons carriers) :
    2 copper sites (**A** and **B**) and 2 hemes (**A** and **A3**)
    - ❖ **What is the sequence of electron transportation between these four components?**

Copper A →heme A→copper B→heme A3

- **Copper.b** and **heme.a3** are close to each other…so they work as one unit (one centre)….we call it "**Dinuclear Centre**"
- BUT… **Copper.a** and **heme.a** are not close to each other so the oxidation reduction reactions occur in a sequential manner.

Story of oxygen:

Oxygen binds with heme.a3 in **complex IV** which is in its reduced state (the heme). Oxygen binds in order for it become reduced to H_2O. Oxygen needs 4 electrons with 4 H^+ to be reduced to H_2O (we will explain this later in this sheet). Each oxygen molecule accepts two electrons so O2 accepts 4 electrons to become 2 molecules of water.

So…. Oxygen binds with reduced heme.a3 in order to play its role as oxidizing agent, each Oxygen atom in O_2 molecule accepts 2 electrons with 2 H+ to form water…after cleavage of the bond in O_2 molecule.(we will explain this later)

→For each oxygen atom…half reaction for reduction of oxygen:

$1/2O_2 + 2H^+ + 2e \rightarrow H_2O$

Overall reaction:

$2Cyt\ aa3\ [reduced,Fe\ (II)] + 1/2O_2 + 2H^+ \rightarrow 2Cyt\ aa3\ [oxidized,\ Fe\ (III)] + H_2O$

→for each O_2 molecule…in relation to cytochrome c :

$Cyt\ c\ (reduced) + 4H^+ + O_2 \rightarrow Cyt\ c\ (oxidized) + 2H_2O$

- Km of Cytochrome c oxidase (**complex IV**) for oxygen is very low…lower than that of mayoglobin which is lower than that of **hemoglobin**. Km is a measure of affinity. Affitnity of cyt c to oxygen is much higher than others (this is reflected by its low Km)
  Km of oxygen: hemoglobin>mayoglobin>complex IV
  - ❖ **Why??**
    Hemoglobin takes oxygen from lungs and goes to donate the oxygen to mayoglobin in tissues , which must donate that oxygen to complex IV, because oxygen is the final electron acceptor in electron transport

chain.(most of times, low Km means hight affinity). **Most of the oxygen we breathe turns into water! (Exam question)**

- **Partial O_2 reduction is hazardous..why?**

Because this produces what we call Reactive Oxygen Species ROS…free radicals that are highly reactive, it can react with many components in the cell (lipids, proteins, membranes…etc.). It will ruin the cell's structure. It will convert a normal thing to an abnormal thing,, initiating the process of cancer.

☒ **Reduction of Oxygen (mechanism)**

Electrons to copper A to heme A sequentially then oxygen moves to copper b and heme A3

Remember: **Copper.b** and **heme.a3** are close to each other so they work as one centre (**Dinuclear centre**) and they can share electrons and hydrogen.

→After reaching the **dinuclear centre**:

1) First electron: goes to reduce **Copper.b** (Cu^{2+} to Cu^{+})
2) Second electron: goes to reduce **heme.a3** (Fe from Fe^{3+} to Fe^{2+})

After heme.a3 is reduced… O_2 binds with the reduced Fe. (oxygen affinity towards reduced Fe is more than oxidized Fe, so after reduction of Fe; oxygen binds to it).

Then **Copper.b** and **heme.a3** share the oxygen with each other (they work as a centre)

→This leads to a decrease in electronegativity of Fe.

3) Third electron goes to reduce **Copper.b** (here…the copper is **fully** reduced)

So this leads to cleavage of bond in O_2 … and we will have oxygen atom bound to **copper.b** and another one bound to **heme.a3 (Fe)** … after that, oxygen that is bound to **copper.b** accepts $2H^{+}$ from the matrix. Iron becomes very deficient of electrons Fe (IV)

- Most of electrons now are with **copper.b** so Fe in **heme.a3** is very deficient with electrons now (deficient with 2 electrons),,,Fe^{4+} …and this state is very rare, we can't find it except in this case and when Fe is bound to the amino acid (tyrosine)

4) Forth electron goes to reduce iron 4 (Fe^{4+}) to (Fe^{3+}) that is bound to oxygen..after that, Fe is in its fully reduced state.
   - ➔ After that, hydrogen atoms are shared between **heme.a3** and **Copper.b** … (one hydrogen goes from oxygen that is bound with **Copper.b** to oxygen that is bound with **heme.a3**)….at the end…$2H^{+}$ come from the matrix to complete production of $2H_2O$.
   - ➔ What the doctor needs from all of this: 1) 4 electrons are needed 2) copper A and heme A are not close to each other (sequential manner) 3) copper b and

heme a3 work as one center, reduction starts at copper, they eventually share electrons and hydrogens 4) they end up producing two molecules of water

- ❖ **How can we detect the right arrangement in electron transport chain, and how electrons are moving between complexes?**

✓ By measuring standard reduction potentials for molecules in electron transport chain, like: NADH, O_2, Fe-S clusters, hemes, ubiquenone etc. and estimating oxidation reduction status of them at standard conditions….refer to slide 22.
BUT…it is difficult to get actual reduction potentials in the mitochondria due to its environment. (it is difficult to find this in cells) **This is the ideal situation, but this cannot be done due to the difficulty of the process!**
With the standard electron potential we get a preliminary arrangement, in order to make sure of this arrangement we use the following method:

✓ We can do experiment of electron transport chain under anaerobic conditions (absence of O_2)…here, no ATP synthesized; because no enough electrochemical gradient for phosphorelation process.

In absence of oxygen, there is no electron acceptor, so all complexes will be reduced 1 time during movement of electrons between complexes according to their difference in reduction potential. The process cannot be repeated. In order for the process to be repeated you need oxygen to become reduced in order for complex 4 to become oxidized, so the process can start over again.

Any complex, when it is reduced it will show certain band in spectrophotometer report (every complex has certain hemes, Fe-S clusters…etc.); so in this case we can detect complex type from bands that are produced by reduction of that complex. And due to absence of oxygen, there won't be any overlapping (every complex is reduced and oxidized for 1 time only) so we can detect surely what is the arrangement of electron transport chain.

✓ If we know certain inhibitors that work on certain complexes.

- **Example**: if we inhibited complex I every component before complex I is reduced, and everything after complex I is oxidized. And when we try different inhibitors on different complexes we will know the arrangement.

☒ **<u>Pumping of protons:</u>**

Remember:

- When 2 electrons are transported from complex I to unbiquinone, $4H^+$ are pumped from matrix to the intermembranous space.
- When 2 electrons are transported from complex II to ubiquinone; there are no protons pumped.
- When 2 electrons are transported from complex III to cytochrome c , $4H^+$ are pumped from the matrix to intermembranous space.

- When 2 electrons are transported from cytochrome c to complex IV, $4H^+$ are pumped from matrix to intermembranous space.
  - ☒ **ATP synthase**
    - Composed of 2 subunits:
      F0 portion…within the membrane
      F1 portion…towards the matrix
      (please refer to slides)

- ✓ There is Gama subunit (like a stoke) which is attached to F0 portion of ATP synthase …when **F0** portion rotates…..gama subunit rotates in asymmetrical (not symmetrical) way due to its shape…so it hits beta subunits (there are 3 beta subunits) in asymmetrical way. Every time it hits a beta subunit, it causes conformational change in that subunit (from state to another)
- ☒ F1 subunit:
  1) Gama subunit (it is like a stalk,, asymmetrical) and it is attached to F0 subunit so it can rotate.
  2) Beta subunits (hitted by gama subunit) (we have 3 beta subunits) binds ADP and turns it into ATP.
  3) Alpha subunit (present for structural reasons)
     - Beta subunit:
       When gama subunit hits beta subunit it causes conformational change.
       Conformations of beta subunit: loose (**L**)…beta subunit is bound to ADP and Pi ….Tight (**T**)…beta subunit is tightly bound to ADP and Pi that are very close to each others to produce ATP (after hitting by gama subunit)…..Open (**O**)…to release the ATP (after hitting by gama subunit again)
- ☒ F0 subunit:
  1)C subunit: 12 subunits within membrane (rotating) → while rotating they are attached to A subunit.

  2)A subunit: enclosing C subunits from 1 side.
     - H^+ enters from intermembranous space through A subunit and goes to bound in C subunit→this leads to change in amino acid structure (binds with glutamate and neutralize it (it was negative))→rotation. This process continues until a full rotation is achieved.
     - After full rotation, a proton is released to matrix … and remember: during rotation, gama subunit rotates and hits beta which leads to production of ATP.
     - Remember: every $4H^+$ pumped…1 ATP molecule is produced.
  - ➔ *Energy yielded from electron transport chain:*
    NADH→-53 kcal (2.5 ATP's) → 7.3 * 2.5 → divided by -53 we get the efficiency
    $FADH_2$ →-41 Kcal (1.5 ATPs) → 7.3 * 1.5 → divided by -41 we get the efficiency

But Efficiency of these molecules is 25-30% only….so oxidative phosphorelation has low effeciency.
TCA cycle → 90% efficiency

- **Where is the lost energy ?**
  It is lost as Heat, transport ions from outside to inside, Ca transporting, phosphate transporting, other subunits, transporting of NADH from outside sometimes, transport of ATP outside, ADP…etc.

☒ **Regulation of electron transport chain:**

✓ **ADP**: allosteric regulator it is the main regulator of electron transport chain (please refer to slides)..without excess ADP →low rate of oxygen consumption (low rate of electron transport chain)….with excess ADP→high rate…then when ADP ends…Plateau (low rate)

✓ **Calcium**: When we use skeletal muscles Ca goes out to consume ATP →calcium drops (about 20% drop in calcium)→ATP formation decreases.

- **Why?? Or What is Happening??**

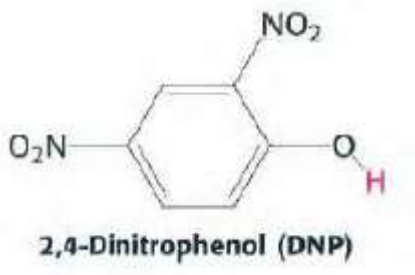
➔ You can search in wikipedia :P

- Heart is always pumping…so calcium is always have to be maintained in high concentration.

✓ Regulation through inhibitors:
Rotinon specific inhibitor for complex 1
Antimyocin ! antibioctic inhibitor for complex 3
Cyanide azide and carbon monoxide which bind to complex 4
Cyanglycosides (cyanides like amygdalin) in the seeds of fruit like apricot… if you crush them and eat them you might have cyanide poisoning.

- Redox reactions are always coupled with phosphorelation of ADP in electron transport chain, but how can we destroy this coupling??
  ➔ If H^+ that are pumped to intermembranous space are back to matrix without ATP synthase→ no phosphorelation ….we call this: "**Uncoupling**"
- This uncoupling occurs naturally or by certain inhibitors.

☒ **Uncoupling through certain inhibitors:**
Example: **Dinitrophenol**



2,4-Dinitrophenol (DNP)

The hydroxyl group can lose or gain an H. this ring can pass through the membrane lipophilic, and has an OH. It can lose or gain the H. This chemical uncoupler binds with H^+ when it is found in intermembranous space (high protons concentration) and loses H^+ after it reaches matrix (low protons concentration), so it transports H^+ from the space to the matrix without ATP synthase→no phosphorelation. (because there is no electrochemical gradient, or what we call "proton motive force").

- This drug has been banned from USA in 1938.
- It was used for patients who suffers from obesity … it decreases ATP synthesis … (most of energy that is produced from H^+ transport from space to matrix is transformed to heat not ATP molecules)…so this leads to:
  1) Body respond to ATP deficiency by degrading its components.
  2) Anabolic reactions will happen in less amounts than catabolic reactions.
- This drug was used in wars by the soviet army. The soldiers were in Siberia and it was very cold (produces heat)

❖ **Why this drug has been banned??**

Because it produces amounts of heat that we can't standardize it for people (there are certain personal variations between people, in tissue type…etc.)

In addition: it causes hemorrhage, heat in eyes →blindness.

☒ **<u>Uncoupling naturally</u>**: there are certain proteins that bind with H^+ and transport it into the matrix without ATP synthase through membrane.(uncoupling proteins)

- These proteins are used to produce heat in a regulated way…not like chemical inhibitors they are not regulated.
- We have different types of these proteins (I, II, III, IV) and they have certain distributions in certain tissues….like uncoupling protein I (thermogenin) in brown tissues in new born babies. The difference between this and DNP is that the body regulates this mechanism.
- In some populations…some people has uncoupling protein I deficiency (in their genetics of coupling proteins)…→most of energy is used in anabolic processes..not much heat is produced … no degradation of the composition of the body→obesity.

☒ **<u>oxidative- phosphorylation genetic diseases</u>**

A <u>mitochondrial DNA and OXOPHOS diseases</u>

The mitochondrial diseases are related to mitochondrial & nuclear DNA

the mitochondrial DNA is small, consists of (16569) base pair , circular double stranded DNA, It encodes for 13 subunits in the electron transport chain complexes (7 subunits of complex I , 1 subunit of complex III, 3 subunits of complex IV), it encodes two subunits of the F0 portion of the ATP-synthase)

- note: not all subunits of the ETC encoded in the Mitochondrial DNA ; most subunits are actually encoded in the nuclear DNA .
- **Maternal inheritance**: the mitochondria of any individual are from his mother, because when fertilization occur, the mitochondria of the sperm don't union with the ovum to produce the zygote so we have our mothers mitochondria :p
- **heteroplasmy** : having different numbers and kinds of mitochondria in different cells of different tissues due to replicative segregation (segregation depends on replication of the mitochondria which is independent on the nuclear DNA).(not all cells or tissues may reflect problems and mutations in mitochondria)
- The tissue which uses more mitochondria shows more diseases related to mitochondria.
- The mitochondrial DNA and OXOPHOS diseases shows more with age due to increasing accumulation of somatic mutations with time.
- Highest ATP demands: CNS, heart, skeletal muscle, kidney & liver

B Nuclear genetic disorders of oxidative phosphorylation

- Nuclear DNA encodes for 1000 proteins in the mitochondria
- Usually these disorders are transferred through autosomal recessive process
- It is expressed in all tissue due to the fact that these disorders associated with nuclear DNA so by every replication the disorder will be transferred to the 2 daughter cells in the same way.
- Highest ATP demand → more show the disorders.

Slide 33: shows diseases caused by mitochondrial DNA and others caused by nuclear DNA…these diseases cause more problems with age…the patient starts to lose his muscle tone around age of 10…..at 25-30 he will be paralyzed …after that he will die through respiratory arrest.

WE DO NOT HAVE TO KNOW ANYTING ABOUT THE DISEASES! We just need to know that there are nuclear diseases and mitochondrial based diseases! :D

Please refer to slides,,, this sheet is not enogh (enno mesh koll eshi bel slides 7aka el Dr)

GOOD LUCK!