Faculty Science

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M.Sc. III Sem Paper- H3002 (Phytochemistry and Metabolism),

Topic- Electron Transport Chain and Oxidative Phosphorylation

Energy-rich molecules (e.g. glucose, fatty acids and amino acids) are metabolized by a series of oxidation reactions finally yielding CO_2 and water. The metabolic intermediates of these reactions donate electrons to specific coenzymes nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) to form the energy-rich reduced coenzymes, NADH and $FADH_2$. These reduced coenzymes which in turn donate a pair of electrons to a specialized set of electron carriers, collectively called the electron transport chain. As electrons are passed down the electron transport chain, they lose much of their free energy. Part of this energy can be captured and stored by the production of ATP from ADP and inorganic phosphate (Pi). This process is called oxidative phosphorylation. The remainder of the free energy not trapped as ATP is used to drive ancillary reactions such as Ca^{++} transport into mitochondria, and to generate heat.

The electron transport chain is present in the inner mitochondrial membrane and is the final common pathway by which electrons derived from different fuels of the body flow to oxygen. Electron transport and ATP synthesis by oxidative phosphorylation proceed continuously in all tissues that contain mitochondria.

Organization of the electron transport chain

The inner mitochondrial membrane can be disrupted into five separate protein complexes, called Complexes I, II, III, IV, and V. Complexes I–IV each contain part of the electron transport chain.

1. NADH- ubiquinone oxidoreductase (Complex I)
2. Succinate- ubiquinone oxidoreductase (Complex II)
3. Ubiquinone- cytochrome c oxidoreductase (Complex III)
4. Cytochrome c oxidase (Complex IV)

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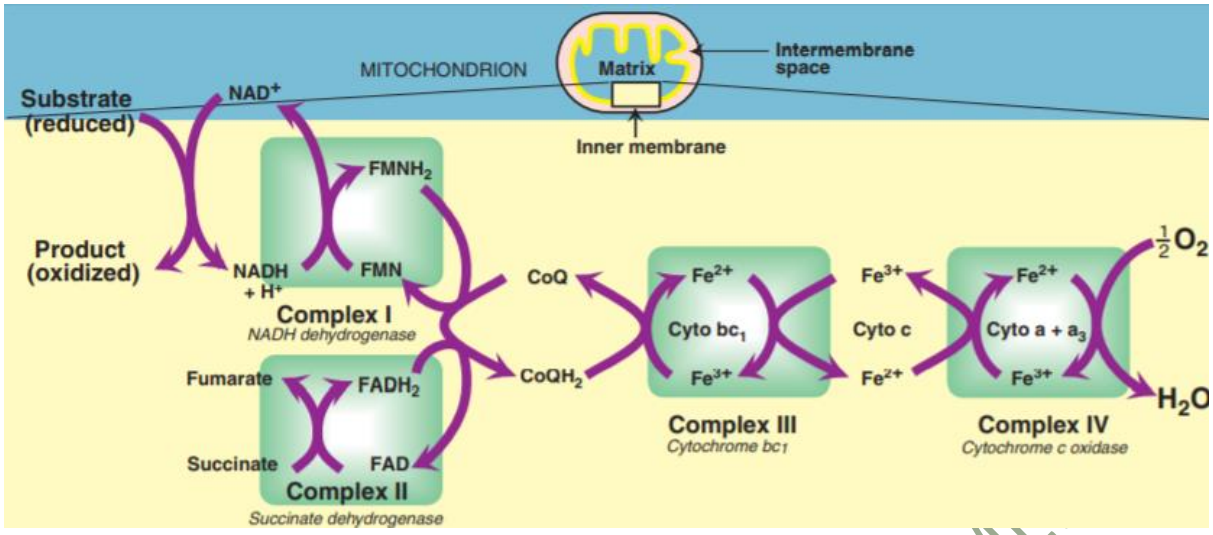

Electron Transport Chain

Each complex accepts or donates electrons to relatively mobile electron carriers, such as coenzyme Q and cytochrome c. Each carrier in the electron transport chain can receive electrons from an electron donor, and can subsequently donate electrons to the next carrier in the chain. The electrons ultimately combine with oxygen and protons to form water. This requirement for oxygen makes the electron transport process the respiratory chain, which accounts for the greatest portion of the body's use of oxygen. Complex V is a protein complex that contains a domain (F_0) that spans the inner mitochondrial membrane, and a domain (F_1) that appears as a sphere that protrudes into the mitochondrial matrix. Complex V catalyzes ATP synthesis and is known as ATP synthase.

Reactions of the electron transport chain

All members of this chain are proteins except coenzyme Q. These may function as enzymes.

1. Formation of NADH: NAD^+ is reduced to NADH by dehydrogenases that remove two hydrogen atoms from their substrate. Both electrons but only one proton (a hydride ion H^-) are transferred to the NAD^+, forming NADH plus a free proton, H^+.

2. NADH-ubiquinone oxidoreductase (complex I): The free proton plus the hydride ion carried by NADH are next transferred to NADH dehydrogenase, a protein complex embedded in the inner mitochondrial membrane. Complex I consist of a molecule of flavin mono nucleotide (FMN) that accepts the two hydrogen atoms ($2e^- + 2H^+$), becoming $FMNH_2$. NADH dehydrogenase also contains 8 iron-sulfur clusters forming prosthetic groups of iron-sulfur proteins or nonheme iron proteins. Two most common types are 2Fe-2S and 4Fe-4S.

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These are necessary for the transfer of the hydrogen atoms to the next member of the chain, coenzyme Q (ubiquinone).

3. Coenzyme Q: Coenzyme Q is a quinone derivative with a long, hydrophobic isoprenoid tail. It is also called ubiquinone because it is ubiquitous in biologic systems. It is a mobile carrier and can accept hydrogen atoms both from $FMNH_2$, produced on NADH dehydrogenase (Complex I), and from $FADH_2$, produced on succinate dehydrogenase (Complex II), glycerophosphate dehydrogenase, and acyl CoA dehydrogenase.

4. Succinate- ubiquinone oxidoreductase (complex II): It is also known as succinate dehydrogenase. This complex consists of FAD and Fe-S clusters. Electrons from succinate enter the electron transport chain via $FADH_2$ to the Fe-S clusters. They are then transferred to coenzyme Q. This transfer of electrons is not associated with a significant decrease in free energy, so protons are not pumped across the membrane at complex II. One of the important functions of this complex is to reduce the leakage of electrons to O_2 so that Reactive Oxygen Species (ROS) are not produced. Coenzyme Q transfers electrons to Complex III. Coenzyme Q, then, links the flavoproteins to the cytochromes.

5. Cytochromes: The remaining members of the electron transport chain are cytochromes. Each contains a heme group (a porphyrin ring plus iron). The cytochrome iron is reversibly converted from its ferric (Fe^{+++}) to its ferrous (Fe^{++}) form as a normal part of its function as a reversible carrier of electrons. Electrons are passed along the chain from coenzyme Q to cytochromes b and c (Complex III), c, and a + a_3 (Complex IV). Cytochrome c is associated with the outer face of the inner membrane and, like coenzyme Q, is a mobile carrier of electrons.

6. Ubiquinone-cytochrome c oxidoreductase (Complex III): The complex is also known as Cytochrome bc1 complex. This complex contains two b-type cytochromes and one C_1-type cytochrome and a Rieske-type Fe-S protein. Besides these, several types of protein subunits are also present. This complex transfers electrons from coenzyme Q to cytochrome c. Transfer of two electrons from coenzyme Q to cytochrome-c is accompanied by translocation of four protons from the matrix to the inter membrane space side of the inner mitochondrial membrane. Cytochrome-c is able to carry only one electron at a time to Complex IV

6. Cytochrome c oxidase (Complex IV): This cytochrome complex is the only electron carrier in which the heme iron has an available coordination site that can react directly with O_2, and so also is called cytochrome oxidase. It also includes two heme groups, heme α and heme α_3 and two Cu centers, Cu_A and Cu_B.

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Electrons removed from cytochrome c pass through Cu_A center, heme α and then to heme α_3-Cu_B and finally to O_2. For every four electrons transferred, four H^+ are consumed at the matrix side to produce two molecules of water. Besides, the energy of electron transport is coupled to translocation of protons from the matrix side of the membrane to the intermembrane space. For transport of each electron, one proton is translocated across the inner mitochondrial membrane. The overall the transported electrons, O_2 and free protons are brought together and O_2 is reduced to water.

Site-specific inhibitors: Site-specific inhibitors prevent the passage of electrons by binding to a component of the chain, blocking the oxidation/reduction reaction. Rotenone (Insecticide) and amytal inhibit electron transport at complex I. Antimycin A (an antibiotic) inhibits electron transport at complex III. Cyanide, azide and carbon monoxide inhibit electron transport at complex IV. Therefore, all electron carriers before the block are fully reduced, whereas those located after the block are oxidized. Inhibition of electron transport inhibits ATP synthesis because these processes are tightly coupled. Incomplete reduction of oxygen to water produces reactive oxygen species (ROS), such as superoxide (O^{--}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). ROS damage DNA and proteins, and cause lipid peroxidation. Enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase are cellular defenses against ROS.

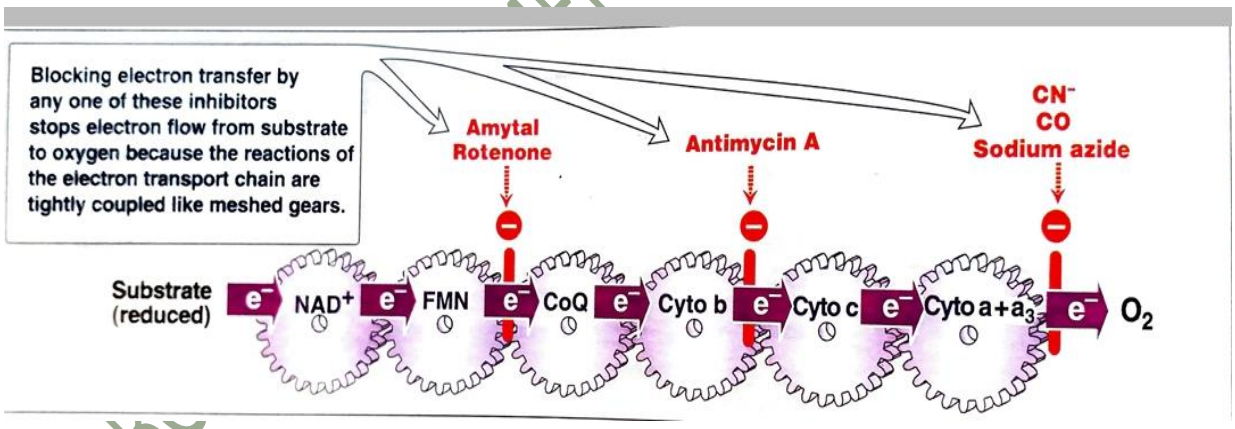

Diagram showing the site-specific inhibitors of ETC and normal direction of electron flow

Release of free energy during electron transport

Free energy is released as electrons are transferred along the electron transport chain from an electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant). The electrons can be transferred as hydride ions (H^-) to NAD^+, as hydrogen atoms (•H) to FMN, coenzyme Q, and FAD, or as electrons (e^-) to cytochromes.

Redox pairs: Oxidation (loss of electrons) of one compound is always accompanied by reduction (gain of electrons) of a second substance. For example, the oxidation of NADH to NAD^+ accompanied by the reduction of FMN to $FMNH_2$. Such oxidation-reduction reactions can be written as the sum of two separate half-reactions, one an oxidation reaction and the other a reduction reaction. NAD^+ and NADH form a redox pair, as do FMN and $FMNH_2$. Redox pairs differ in their tendency to lose electrons. This tendency is a characteristic of a particular redox pair, and can be quantitatively specified by a constant, E_0 (the standard reduction potential), with units in volts.

Standard reduction potential (E_0): The E_0 of various redox pairs can be ordered from the most negative E_0 to the most positive. The more negative the E_0 of a redox pair, the greater the tendency of the reductant member of that pair to lose electrons. The more positive the E_0, the greater the tendency of the oxidant member of that pair to accept electrons. Therefore, electrons flow from the pair with the more negative E_0 to that with the more positive E_0. The components of the electron transport chain are arranged in order of increasingly positive E_0 values.

ΔG^0 is related to ΔE_0: The change in free energy is related directly to the magnitude of the change in E_0:

$\Delta G^o = - n F \Delta E_0$

n = number of electrons transferred (1 for a cytochrome, 2 for NADH, $FADH_2$, and coenzyme Q)

F = Faraday constant (23.1 kcal/volt. mol)

ΔE_0 = E_0 of the electron-accepting pair minus the E_0 of the electron-donating pair

ΔG^o = change in the standard free energy

ΔG^o of ATP: The standard free energy for the phosphorylation of ADP to ATP is +7.3 kcal/mol. The transport of a pair of electrons from NADH to oxygen via the electron transport chain produces 52.58 kcal. Therefore, more than sufficient energy is available to produce three ATP from three ADP and three Pi (3 x 7.3 = 21.9 kcal/mol), sometimes expressed as a P:O ratio (ATP made per O atom reduced) of 3:1. The remaining calories are used for ancillary reactions or released as heat. P:O for $FADH_2$ is 2:1 because Complex I is bypassed.

Oxidative phosphorylation

The transfer of electrons down the electron transport chain is energetically favored because NADH is a strong electron donor and molecular oxygen is an avid electron acceptor. However, the flow of electrons from NADH to oxygen does not directly result in ATP synthesis.

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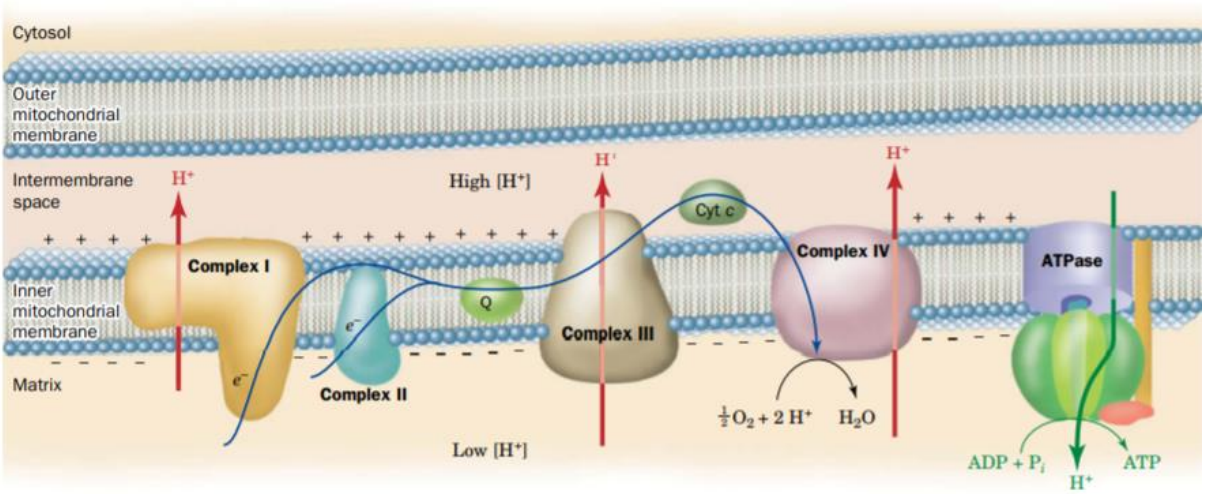
Chemiosmotic hypothesis- The chemiosmotic hypothesis (also known as the Mitchell hypothesis) explains how the free energy generated by the transport of electrons by the electron transport chain is used to produce ATP from ADP + Pi Proton pump: Electron transport is coupled to the phosphorylation of ADP by the transport ("pumping") of protons (H^+) across the inner mitochondrial membrane from the matrix to the intermembrane space at Complexes I, III, and IV. This process creates an electrical gradient (with more positive charges on the outside of the membrane than on the inside) and a pH gradient (the outside of the membrane is at a lower pH than the inside. The energy generated by this proton gradient is sufficient to drive ATP synthesis. Thus, the proton gradient serves as the common intermediate that couples oxidation to phosphorylation.



The coupling of electron transport and ATP synthesis

ATP synthase: The enzyme complex ATP synthase (Complex V) synthesizes ATP using the energy of the proton gradient generated by the electron transport chain. It is also called F_1/F_0 ATPase because the isolated enzyme can catalyze the hydrolysis of ATP to ADP and Pi. The chemiosmotic hypothesis proposes that after protons have been pumped to the cytosolic side of the inner mitochondrial membrane, they re-enter the matrix by passing through a channel in the membrane-spanning domain (F_0) of Complex V, driving rotation of F_0 and, at the same time, dissipating the pH and electrical gradients. F_0 rotation causes conformational changes in the extra-membranous F_1 domain that allow it to bind ADP + Pi, phosphorylate ADP to ATP, and release ATP.

Oligomycin: This drug binds to the F_0 domain of ATP synthase, closing the H^+ channel, preventing re-entry of protons into the mitochondrial matrix, and thus preventing phosphorylation of ADP to ATP. Because the pH and electrical gradients cannot be dissipated in the presence of this drug, electron transport stops because of the difficulty of pumping any more protons against the steep gradients.

This dependency of cellular respiration on the ability to phosphorylate ADP to ATP is known as respiratory control, and is the consequence of the tight coupling of these processes. Inhibition of one process inhibits the other. Respiratory control also results from decreased availability of ADP or Pi.
Uncoupling proteins (UCP): UCPs occur in the inner mitochondrial membrane of mammals, including humans. These carrier proteins create a "proton leak," that is, they allow protons to re-enter the mitochondrial matrix without energy being captured as ATP. The energy is released as heat, and the process is called non shivering thermogenesis. UCP1, also called **thermogenin,** is responsible for the heat production in the brown adipocytes of mammals. UCP1 is activated by fatty acids. Brown fat, unlike the more abundant white fat, uses almost 90% of its respiratory energy for thermogenesis in response to cold in the neonate, and during arousal in hibernating animals. However, humans appear to have little brown fat (except in the new born), and UCP1 does not appear to play a major role in energy balance.

Synthetic uncouplers: Electron transport and phosphorylation can also be uncoupled by compounds that increase the permeability of the inner mitochondrial membrane to protons. The classic example is **2,4-dinitrophenol**, a lipophilic proton carrier that readily diffuses through the mitochondrial membrane. This uncoupler causes electron transport to proceed at a rapid rate without establishing a proton gradient, much as do the UCPs. Again, energy is released as heat rather than being used to synthesize ATP. In high doses, **aspirin** and other salicylates uncouple oxidative phosphorylation. This explains the fever that accompanies toxic overdoses of these drug.

In plant there are three alternate mechanisms known, through which NADH/NADPH are oxidized. Electrons are transported through them without translocation of any protons. So, these sites of electron transport are not associated with any ATP synthesis**. External NADPH dehydrogenases** are also present towards the intermembrane space in addition to the both internal NADH dehydrogenase, one which is inhibited by rotenone and the other which is not inhibited by rotenone.

Alternate Oxidase: In plants an additional mitochondrial electron transport system is present in which electrons are removed from the reduced ubiquinol and are directly passed to this alternate oxidase (AOX / AOD) bypassing Complex III and Complex IV. Alternate oxidase is also known as ubiquinol oxidase. This pathway is not associated with any ATP generation. Unlike animals, respiration in plants is not inhibited by cyanide. AOX is inhibited by salicylhydroxamic acid (SHAM). The pathway is known as cyanide resistant respiration. The pathway is

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also known as SHAM pathway. In animals, respiration drops to 1% in presence of cyanide. Electrons from the Complex I are fed off to ubiquinone and from there they are directly passed to AOX, which pass the electrons to O_2 reducing it. With the transfer of four electrons, O_2 is reduced to H_2O. The energy released during electron transport is not conserved in ATP synthesis, rather is lost as heat. Role of the cyanide resistant respiration may be in the higher temperature conditions of the plant so as to help volatilize the compounds which help to attract the pollinators, or it may have a role in energy spill over or in the stress conditions.

References:

1. Taiz L, Zeiger E, (2003) in "Plant Physiology". 3rd edition, Sinauer Associates, USA.
2. Richard A. Harvey, Denise R Ferrier (2014) in "Lippincott's Illustrated Reviews: Biochemistry". 6th edition, Lippincott Williams &Wilkins, USA.
3. Bios Instant Notes Biochemistry, 3rd edition (2007). Garland Science, USA.
4. Voet D, Voet J (2018) in "Biochemistry". 5th edition. J. Wiley & Sons, USA.

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