P. G. Sem II, CC7, Unit 1, submit 1-3

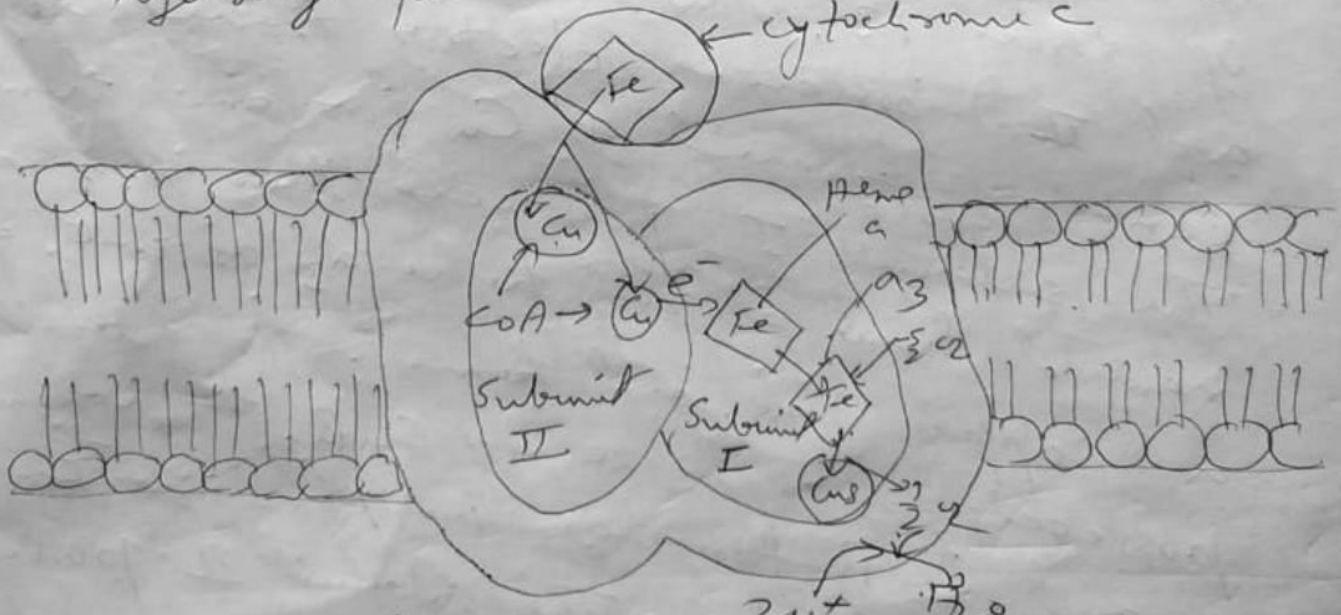
Mitochondrial Electron Transport chain & Oxidative Phosphorylation (Part III)

By
Dr. Sunita Kumari Sharma
Associate Prof & Head
Maharaja College Ara

Contd. from Part II —

METC Complex IV → This Complex is also called Cytochrome Oxidase. It induces oxidation of Cytochrome C (reduced form) and using mitosolic protons brings about reduction of molecular O_2 to H_2O. Cytochrome Oxidase is a complex metalloprotein, protruding in both mitosol and intermembrane space. It Comprises 13 submits, 2 hemes and 3 copper ions. Of these 13, two submits (submit I and II) possesses electron transferring centers formed of Cu ions and hemes. The submit I possesses two hemes, named heme a and heme a_3 as well as a copper ions. ~~and hemes~~. The submit I possesses two hemes and CuB. The heme a and CuB forms an independent Fe-Cu binuclear Center. The submit II possesses four Copper ions present in the interacts with R groups of Cysteinyl

and aspartyl residues of protein. The pair of Cu ions forms another binuclear Center named Cu A. The domain of subunit II protruding into intermembrane space possesses a binding site for cytochrome c and also the binuclear Center Cu A. The electrons flow in single from two reduced cytochrome c to the two Cu ions of binuclear Center, Cu A. Both Copper ions share each electron equally. From Cu A, electron moves to heme a and then to Fe-Cu binuclear Center. This induces binding of O_2 to heme a_3, which is followed by reduction of O_2 by an electron to O_2^-, a superoxide, and by another electron and mitosolic proton pair to H_2O_2, forming hydroxyl-free radical and H_2O.



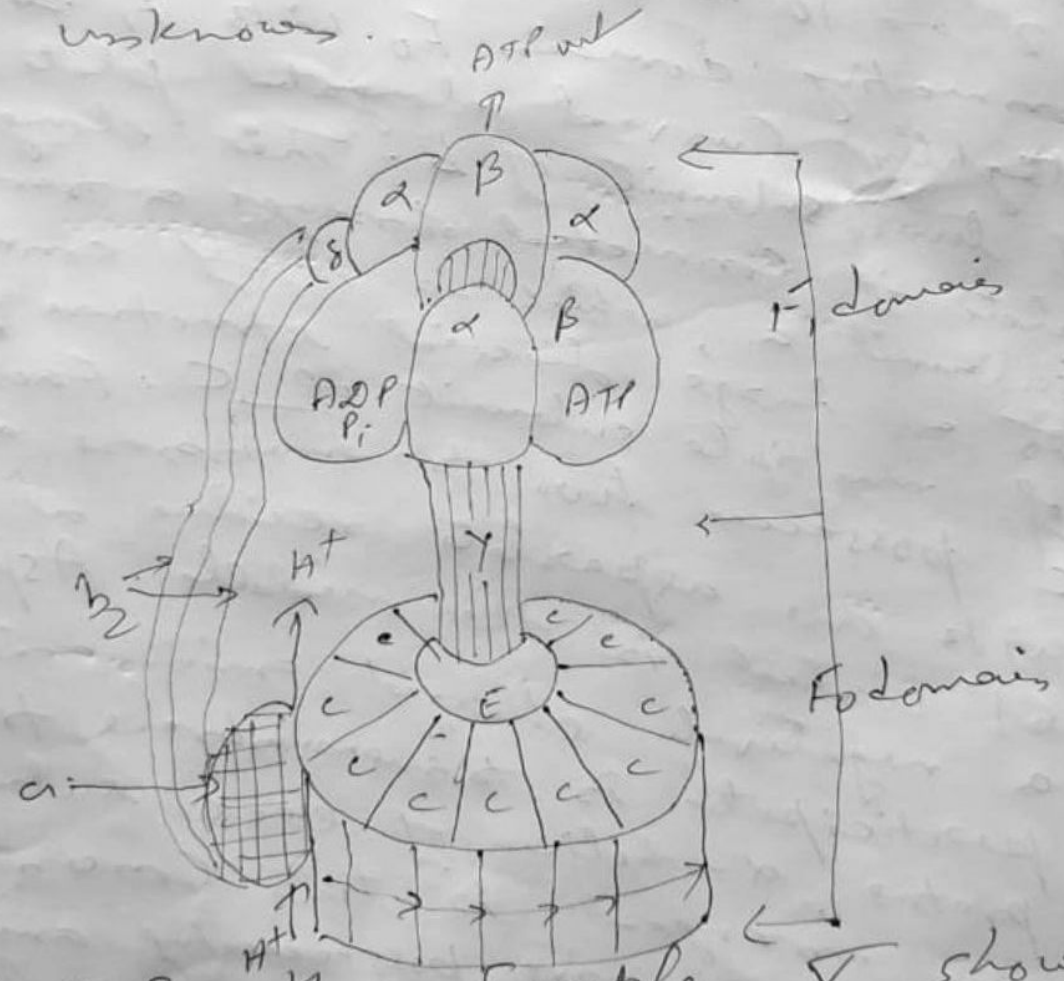
Simplified Representation of Complex IV (Cytochrome Oxidase) showing subunit I & II.

Structure of Complex V (ATP Synthase/ATPase)

Complex V (MW 480-500 kDa) is functionally both ATP Synthase and ATPase. It is constituted of two domains, F_o & F_1. F_o domain is so named as this factor is sensitive to oligomycin (O) and F_1 is named for it was the first (1) factor (F); identified from mitochondria, which participate in oxidative phosphorylation.

F_o domain is integral part of IM and is responsible for transmembrane transfer of protons. It comprises three different types of subunit a, b and c. numbering 1, 2 and 10-12 subunits, respectively. Connecting domain F_o with F_1 is a slightly curved elongated plate formed of two subunit of b. This elongated plate is connected through a transmembrane subunit a, which is itself connected to a large discoid structure, formed of multiple subunit of c. Each subunit c possesses two transmembrane α helices with one aspartate residue at position 61 located in the middle. Aspartate, a negatively charged amino acid, participate in transmembrane transfer of protons, α-helices of each subunit c are arranged in two concentric rings, inner ring possessing amino terminal and outer ring carboxy terminal. Located centrally in F_o disk is a small disk like subunit E connected to a long axis of γ subunit that

possesses through F_1 domain centrally. The F_1 domain possesses five types of subunit α, β, γ, ε and δ. The α and β types possess three subunits, each arranged alternately around centrally located axial γ subunit. The two subunits of b (b_2) form an elongated curved structure that connects subunit a of F_0 with subunit δ, which is connected to an α subunit of F_1 domain. Through both α and β subunits pass binding sites for ADP and ATP; the functional significance of binding of the two nucleotides with α subunit is unknown.



ATP Synthase Complex V showing F_0 and F_1 domains

Mechanism of ATP Synthesis –

The flow of electrons through Electron Transport chain creates high Concentration of protons in the intermembrane space due to pumping of protons by Complexes I, III and IV. This creates a proton gradient across IM with negative electrochemical potential on mitosolic surface of IM. The proton gradient creates a proton motive force that pushes protons through subunit a and c of Fo channel. while passing through Fo channel, protons bind the negatively charged R group of aspartate located at position 61 of subunit a. This caused C-subunits to rotate along with the attached ε and γ subunits. Each of the β-subunit, while undergoing conformational change, is β-subunit inducing binding of ATP and Pi in one β-subunit phosphorylation of ADP forming ATP in the second and release of ATP from the third β-subunit, one after the other in three step sequence. Each of the β-subunit, while undergoing conformational change picks up ADP and Pi and brings about phosphorylation of ADP to ATP and releases ATP formed. Thus, total of 3 ATPs are

synthesized in one complete rotation of γ-subunit. It is thus seen that protons pumped into intermembrane space by respiratory chain complexes I, III and IV are used by ATP synthase for ATP synthesis.

Thus, for transfer of every three protons, one ATP is synthesized. As we know, oxidation of a reduced NAD and reduced FAD by respiratory chain complexes generates 10 protons and 6 protons, respectively, which generate more than 3 (10/3) and 2 (6/3) ATP, respectively. However, there is loss of protons across IM due to leakage, utilization in mitochondrial buffering and utilization in substrate transfer. Thus, the no. of protons available for ATP synthesis is subject to correction. On the basis of different studies it has been established that four protons are utilized in the synthesis of one ATP. Thus oxidation through respiratory chain complexes of one reduced NAD generates 2.5 (10/4) ATP and one reduced FAD generates 1.5 (6/4) ATP.

$$NADH + H^+ \xrightarrow{\text{ETC complexes}} 10H^+ \rightarrow 10/4 = 2.5 \text{ ATP.}$$

$$FADH_2 \xrightarrow{\text{ETC complexes}} 6H^+ \rightarrow 6/4 = 1.5 \text{ ATP.}$$

Total = 4.0 ATP

Inhibition of ATP Synthesis

ATP Synthesis is inhibited by some chemicals that block transfer of some electrons at specific step of respiratory chain or protons flow through F_o domain or phosphorylation of ADP to ATP.

Flow of electrons through respiratory chain. Complexes inducing pumping of protons into intermembrane space from mitosol is blocked by different chemicals. such as Rotenone, Carboxin, antimycin A, Carbon monoxide, oligomycin and 2,4 - dinitrophenol.

Barbiturates (amytal, piercidin, halothane etc.) block transfer of electrons from Fe-S center of complex I to Coenzyme Q. Carboxin blocks transfer of electrons from complex II to coenzyme Q, Antimycin A blocks electron transfer through complex III. Carbon monoxide, cyanides, H_2S all act as poisons and block electron transfer at heme a_3 - Cu center of Complex IV. Cyanide binds oxidized form of heme a_3 (Fe^{3+}) and prevent transfer of electrons from heme a to heme a_3 (Fe^{+2}). whereas CO binds reduced form of heme a_3 (Fe^{+2}) and prevent transfer of electrons from heme a_3 to O_2. Oligomycin prevent flow of electrons through F_o domain, thus preventing

rotation of C-disk and attached γ-axil. Thus phosphorylation of ADP into ATP is inhibited at β_1 submit. Atractyloside inhibit transfer of ADP into mitosol and ATP out of mitosol, thereby preventing phosphorylation of ADP into ATP.

Uncouplers such as 2,4-dinitrophenol, prevent phosphorylation of ADP into ATP without interfering with electron transfer through respiratory chain. Dinitrophenol being weak acid gets easily protonated utilizing high proton present in intermembrane space. These protonated dinitrophenol being hydrophobic (lipophilic) get diffused into mitosol having low protein Concentration, loses protons. This abolishes proton gradient across IM, inhibiting proton flow through F_o domain, ATP Synthesis by β-submit of F_1 domain is impaired.