ATP synthesis is a most fundamental and versatile biological reaction that is governed by the energy-transducing membranes of mitochondria, bacteria and chloroplast via the central enzyme F0F1 ATP synthase. A lot of information has been accumulated on this fundamental process during more than 50 years of research; yet true molecular mechanistic insights are still lacking. The first comprehensive presentation of the central mechanism of oxidative and photophosphorylation was offered in Mitchell’s chemiosmotic theory [1]. It explicitly noted that the concentration difference of pH (ΔpH) and an unequal distribution of charge density i.e. a potential difference (ΔΨ), created and solely by protons, between the two aqueous phases across a biological membrane,‘ drives the process of phosphorylation. But over the period of time this theory was thoroughly scrutinized and many flaws were encountered in it [2-4]. Among one such problem, the requirement of anion / counter cation (apart from proton) has also been noticed by many researchers but their biological function could not be explained anywhere. The first proposal of such an anion requirement has been made in Nath’s torsional theory of energy transduction and ATP synthesis, wherein, a role for anion has been critically evaluated for the generation of ΔΨ. Thus, the concentration difference of proton (ΔpH) and transmembrane electric charge density of both proton and anion (ΔΨ) highlight the true driving force for ATP synthesis. Recently the experimental data in support of the torsional theory that explained the effect of anions in the process of phosphorylation has been presented [5].

The mechanism of ADP phosphorylation is catalytically controlled by the enzyme called ATP synthase, which utilizes the energy of ions to synthesize ATP. The free energy released (~35 kJ/mol) from ATP hydrolysis [6] can be coupled to drive myriad energy requiring processes like active transport, nerve conduction, muscle contraction [7] and assembly of various macromolecules. It is also a valuable target for therapeutic drugs, and a variety of thermodynamic and kinetic approaches have been applied to these problems [8-11]. This is therefore a most fundamental process in biology that also has numerous applications in health and disease [12,13].

A vast majority of research has been carried out on ATP hydrolysis but the advancements at the detailed molecular mechanistic level on ATP synthesis is still lacking. Gross models for ATP synthase functioning such as the chemiosmotic theory [14] and the binding change mechanism [15] have been proposed previously. It is only recently that a molecular mechanistic details of ion movement and torque generation in F0 [16,17], interaction of F0 and F1 for torque transmission [18,19], torsional energy storage in F1 [20], and a stepwise catalytic details of ATP synthesis in the proposed Nath’s torsional mechanism of energy transduction and ATP synthesis has been formulated in consummate detail.

According to Mitchell’s chemiosmotic theory for ATP synthesis, the driving force resides in the electrochemical potential difference (ΔμH+) that constitute the electrical potential difference (ΔΨ) and pH difference (ΔpH) across a biological membrane and is correlated by the equation ΔμH+ = F ΔΨ - (2.303RT) ΔpH. A central feature of this dogma is the assertion that membrane potential ΔΨ and transmembrane ΔpH both are equally capable of changing the rate of ATP synthesis by the same factor and are solely generated by the transport of protons across the membrane. It also emphasizes the coupling to be delocalized between two bulk equilibrium phases.

However, a breakthrough has been achieved by the systematic assessment in Nath’s torsional theory for ATP production [4,10] wherein translocation of both proton and membrane-permeable anion have been postulated for ATP synthesis that provide detailed insights into the intricacies of mechanistic events involved in ATP synthesis by the enzyme. As one of the major breakthroughs of the torsional theory, the presence of anion and proton entry half–access channels in the vicinity of F0 complex have been postulated to account for the coupled transport of anion and proton as a critical requirement for ATP synthesis. Binding of anion and proton in the vicinity of F0 complex creates a localized ΔΨ to further rotate the c-rotor that helps anion and proton to leave from F0 complex through their exit half–access channels.

The simultaneous binding and unbinding of anion and proton to their respective half –access channels causes the change in localized electrostatic potential Δ(ΔΨ) that rotates the c-rotor. The rotation of the c-rotor convert the ion–gradient energies into the torsional energy that get stored in γ-subunit, which later causes the conformational changes in F1 domain of the enzyme F0F1, ATP synthase. Thus, the torsional theory utilizes both - delocalized ΔpH (in the bulk) and localized ΔΨ (in the vicinity of F0) -to generate ATP. The localized ΔΨ created by the movement of anion generates the anionic electrical field that facilitates the binding of proton to cause the coupled transport of anion and proton in the localized region of F0 for ATP synthesis.

The torsional theory provides the intricate details of the dynamic process of ATP synthesis at the molecular level which addresses the mechanism in F0, the mechanism in F1 as well the issue of coupled transport of anion and proton for ATP synthesis in great detail [4, 10]. The F0–subunits constitute the rotor in F0 and are present in different numbers (in different models) with Asp as the predominant amino acid at the position 61. Whereas, the a-subunit acts as a stator and has Arg- and His- at 210 and 245 respectively, as the predominant amino acids. The different number of ‘c’ subunits combines to form a cylinder while the a’ subunit superimposes the ‘c’ subunits. It is generally accepted that the protons while translocating down the electrochemical gradient
interact with a specific Asp/Glu residue of subunit ‘c’ in F0 and evoke a conformational change which drives the production of ATP [21-23] in F1. The proton binding sites in ‘c’ subunit at the position 61 of Asp gets unprotonated at the interface of a-c subunits. The translocating proton interacts with the leading Asp-61. The specific, regulated anion binding sites are present in the ‘a’ subunit at the predominant His at 245, which attracts the unprotonated Asp-61 and leads to rotation of the ‘c’ subunits, moving the protonated Asp-61 into the a-c interface. Arg-210 ensures that only the leading Asp-61 is protonated and induces the proton on the new Asp-61 to jump into the matrix. The torque generated in the F1 is transmitted to the central connecting stalk. The stalk rotates in twelve discrete 30° steps (each of which has two sub-steps of 15°) while the γ-subunit rotates in three discrete steps of 120°. The energy of the proton flux is stored in the γ-subunit leading to conformational changes in F1 for ATP synthesis.

Based on unique molecular systems biology and combined engineering approach, a strong requirement of anions for the process of phosphorylation from the perspective of the torsional theory has been postulated. But this requirement of anions in driving phosphorylation cannot be addressed in Mitchell’s chemiosmotic theory, where anion does not have to play any role and protons are the sole source for driving ATP synthesis by creating a pH gradient and/or electric potential (if any). However, the role of anions as a prerequisite for localized electrical potential generation in F0 has been uniquely addressed in torsional theory, which is mandatory for ATP production. According to the torsional theory, both ΔpH and ΔΨ contribute towards the energy requirement for synthesis of ATP.

ΔpH contributes half of the energy required, while the other half comes from the ΔΨ. The mechanism postulates a dynamically interdependent and electrogenic transport of anion and proton to maintain the overall phenomenon of ADP phosphorylation as electroneutral. The flow of anion in the ‘a’ subunit of the enzyme complex generates ΔΨ. This is followed by the transport of protons through the proton half channel along its concentration gradient that consequently binds to the Asp (or Glu) amino acids of c-subunit resulting in a change in the overall ΔΨ. Hence the torsional mechanism considers the role of delocalized ΔpH and localized ΔΨ in the F0 domain of the enzyme ATP synthase. The experimental evidence to correlate the presence of anions and phosphorylation has been recently published in detail [5].

Hence, a role for anions has been emphasized for the generation of electrochemical potential and for overall electroneutral transport of anion and proton by ‘anion-coupled protonsym sequence co-transport’ for the synthesis of ATP. It has been postulated to take place by the presence of anion and proton half–access channels that describes the mechanism of the torsional theory in F0 portion at the molecular level in great detail. Moreover, the torsional theory consolidates the role of anion (coupled to proton) in clarifying the conceptual lacunae in the previously existing chemiosmotic theory (Figures 1 and 2).

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