The torsional mechanism of energy transfer in ATP synthase

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ATP synthase (or F_1F_0 ATPase) is the central enzyme in biological energy conversion, synthesizing ATP from ADP and inorganic phosphate using the free energy derived from protonmotive force. This large enzyme complex (with an overall molecular weight of 520,000 in *Escherichia coli*) consists of two major parts: a water-soluble F_1 containing three α, three β and one copy each of the γ, δ and ε subunits and a membrane-embedded F_0 composed of one a, two b and twelve c subunits. The mechanism of coupling proton translocation through F_0 to γ-ε subunit rotation in F_1 is unknown. Here we propose and formulate the novel concept of a torsional mechanism for energy transfer and ATP synthesis in ATP synthase. We show how the protonmotive torque is transmitted from F_0 to F_1 and elucidate how energy is stored and transferred in this remarkable enzyme. The recent observation that an enzyme form where all three catalytic sites are occupied by bound nucleotide occurs during ATP synthesis provides strong experimental support for our proposed mechanism. A motion picture of the cycle of events at the catalytic sites of ATP synthase is also presented for the first time.

ADENOSINE triphosphate synthase (ATP synthase or F_1F_0 ATPase) is the central enzyme in biological energy conversion that is almost three billion years old and is present in the membranes of mitochondria, chloroplasts and bacteria with amazingly similar structure and function in different species. It couples proton translocation through its membrane-embedded, hydrophobic domain, F_0 to the synthesis of the energy carrier molecule, ATP from ADP and inorganic phosphate in its soluble, hydrophilic headpiece, F_1. The *Escherichia coli* multisubunit assembly consists of a globular domain, F_1 (with composition $\alpha_3\beta_3\gamma\delta\varepsilon$) and an intrinsic membrane domain, F_0 (with composition ab_2c_{12}) linked by two slender stalks[1–6], as illustrated in Figure 1. The central stalk is formed by the ε subunit and part of the γ subunit, while the peripheral stalk is constituted by the hydrophilic portions of the two b subunits of F_0 and the δ subunit of F_1. The proton channel is formed by the a and c subunits in F_0 and the catalytic binding sites are predominantly in the β subunits of F_1 at the α–β interface[1–5]. Recent structural[1,2], biochemical[3,5,7], spectroscopic[8,9] and microscopic[10,11] studies indicate that conformational changes arise from rotation of the γ-ε subunit in a static barrel of the $\alpha_3\beta_3$ subunits in ATP synthase (Figure 1), making it the smallest molecular machine (rotor radius ≈ 1 nm) known to mankind. Recently, we have shown how the protonmotive force can be converted to a protonmotive torque in F_0, how the torque causes conformational changes in F_1, and how the conformational changes lead to ATP synthesis[12]. Here we elucidate, for the first time, the mechanism of energy storage and transmission from F_0 to F_1 in ATP synthase.

The torque generated in the F_0 subunit by the mechanism proposed in our recent work[12] is transmitted to the central connecting stalk, which is attached to the centre of the c rotor. Since the c subunit has 9–12 copies, the torque generation is quantized in steps of 9–12; however the three-fold symmetry of F_1 indicates that this number should be either 9 or 12. A nonequilibrium thermodynamic analysis of ATP synthesis[13] shows this number to be 12, which implies that (a) synthesis of one molecule of ATP requires the translocation of four protons, (b) the c rotor rotates in 12 discrete steps, and (c) 12 protons have to be pumped out for 3-hydroxybutyrate (P/O = 3) and 8 protons for succinate (P/O = 2) by the redox pump per oxygen atom consumed to ensure maintenance of a steady state.

Thus, according to our mechanism proposed here, the bottom of the central stalk rotates in twelve discrete 30° steps. The F_1 crystal structure[1], the decay of the polarization anisotropy parameter[8,9], and the epifluorescence microscopy experiments[10,11] indicate that the γ subunit rotates in three discrete steps of 120°. Figure 2 illustrates the angular displacement predicted from our mechanism as a function of the number of protons. The apparent disparity at the top and bottom of the stalk can be understood by considering it as a shaft that is free to

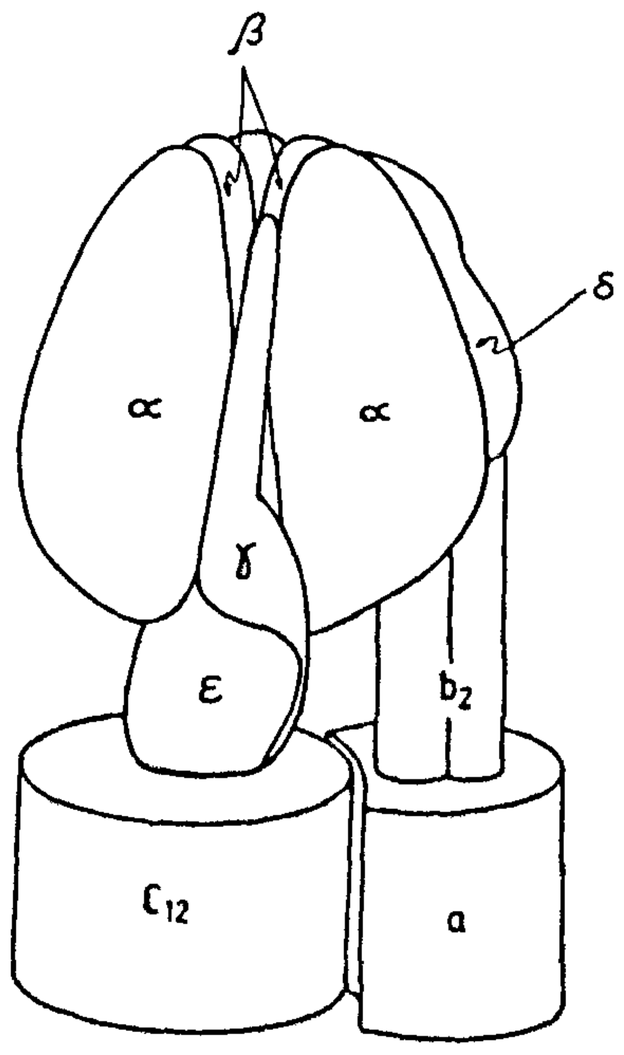

Figure 1. Diagram of the *Escherichia coli* ATP synthase enzyme complex.

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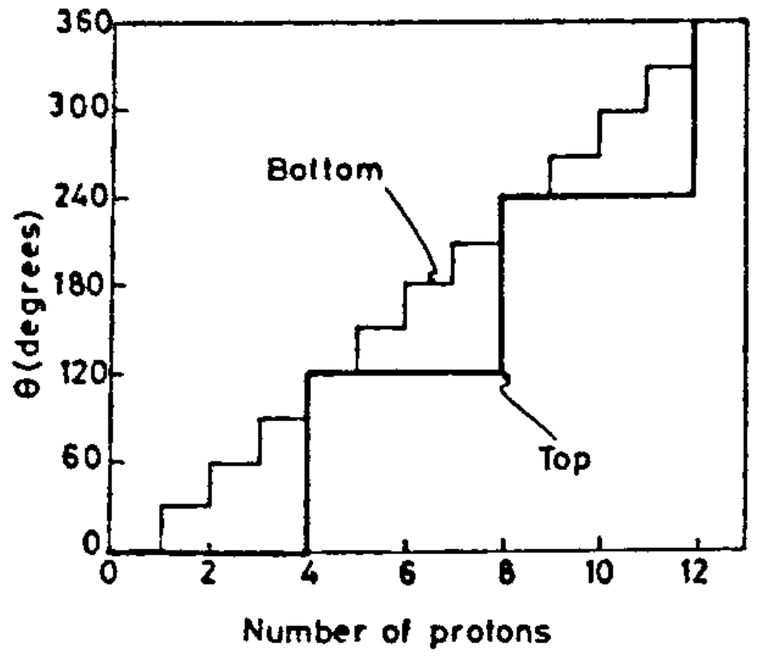

Figure 2. Rotation of the top and bottom of the stalk as a function of the number of protons as predicted by the proposed torsional mechanism of energy transfer in ATP synthase.

rotate at the bottom but is constrained at the top. These constraints are the interactions of the stalk with the catalytic sites. The bottom of the shaft rotates relative to the top, causing *torsion* in the shaft, leading to generation of a stress that is proportional to the angle of twist and storage of torsional energy in the shaft. Thus, the energy of the proton flux is stored as torsional energy in the γ subunit. This torsional energy is further used to cause conformational changes at the catalytic sites, which leads to ATP synthesis. The passage of protons increases this stress and torsional energy. When they are large enough, the constraints are broken and the top of the shaft rotates to a new angular position where there is zero torsional strain. This breakthrough stress is attained upon passage of 4 protons, in accord with the observed H^+/ATP ratio. Thus, our proposed torsional mechanism resolves the apparent disparity and also explains the mechanism of torque transmission and energy storage while the bottom of the stalk is rotating but the top is stationary. This torsional mechanism of energy transfer is a novel concept in bioenergetics.

The recently published tryptophan fluorescence experiments of Senior and colleagues[14] show that during ATP synthesis there occurs an enzyme form in which all three β-subunits contain bound nucleotide (the 'three-nucleotide state'). In contrast, the crystal structure of the Walker group[1] contains bound nucleotide in only two β-subunits (the 'two-nucleotide state'). In the crystal structure, the empty β-subunit possesses a substantially different conformation from the other two, which are distinct but similar to each other. One of the sites in this crystal structure binds the ATP analogue AMP–PNP and is designated as β_{TP}; another site binds ADP and is denoted by β_{DP}, while the empty, distorted site is called β_E. In our view, the truest test of any mechanism of ATP synthesis is to interpret, explain and reconcile these apparently contrasting observations.

We now show how our proposed torsional mechanism of energy transfer and ATP synthesis is consistent with both observations and how the ATP synthase switches from the two-nucleotide state to the three-nucleotide state and back again. Consider an instant in time when the F_1 subunit is in the two-nucleotide state. At this instant, the conformations adopted by the three β-subunits are: loose (β_{TP}), tight (β_{DP}), and open (β_E)[12]. MgADP enters in β_E, but in the absence of the binding site, it cannot bind to it. As proton translocation takes place in the F_0 subunit, the bottom of the shaft, consisting of the ε subunit and the bottom of the γ subunit rotates. This causes the interaction of the ε-subunit and the Glu-381 of β_E to break, allowing the MgADP to bind. The top of the shaft remains stationary in this interval of time; consequently, the conformations of the β_{TP} and β_{DP} sites remain unchanged. Thus, at this particular instant, all the three β-subunits contain bound nucleotide. This is the three-nucleotide state reported recently[14]. With further rotation of the bottom, the constraints at the top are broken, and the entire shaft rotates to a new position, where ε interacts with the Glu-381 of β_{DP} and converts it to the open conformation, leading to the release of MgATP. The change in γ–β interactions converts the β_E site to the loose conformation and the β_{TP} site to the tight conformation. Thus, the enzyme regains the two-nucleotide state and the cycle starts all over again. Therefore, the observation of two different bound nucleotide states provides strong experimental evidence for the torsional mechanism of energy transfer in ATP synthase.

In summary, we have proposed the *first, complete, unified* mechanism for the synthesis of ATP by protonmotive (or sodiummotive) force in ATP synthase, the smallest known molecular machine. Recently[12], we have shown how the protonmotive force can be converted to a protonmotive torque in F_0, how the torque causes conformational changes, and how these conformational changes lead to ATP synthesis. Here we have shown how the protonmotive torque is transmitted from F_0 to F_1. We have also elucidated the mechanism of energy storage and transfer in ATP synthase. The observation of two different bound nucleotide states in ATP synthase provides strong experimental support to our proposed mechanism. Several mechanisms[15–19] dealing with *torque generation* have been proposed, but none of them addresses the question of *torque transmission and energy storage* in ATP synthase. Only one other researcher has presented a plausible mechanism of coupling proton transport to ATP synthesis[20] (the question of energy storage, however, was not really answered and was left as a puzzle). In his mechanism, a relative movement between the γ–ε complex and the c subunits is envisaged. However, the mechanism is not in accordance with recent crosslinking studies[21], which demonstrate that the γ subunit can be crosslinked to c subunits without loss of ATPase activity. Recent experimental results on the ATPase activity as a function of *N*-ethylmaleimide modification of c subunits[22] in ATP synthase are also inconsistent with the relative movement

proposal[20]. In fact, crosslinking can be used in a novel way to experimentally verify our proposed mechanism: crosslinks can be created within the γ subunit in such a manner that although they permit assembly of the enzyme, the relative movement between parts of the γ subunit is hampered. According to our proposed mechanism, such crosslinks will impair the ability of the enzyme to synthesize ATP. In conclusion, the torsional mechanism of energy transfer and ATP synthesis is the *first* mechanism that addresses the issue of *energy storage and transfer* in ATP synthase.

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Ground penetrating radar ice thickness measurements of Dokriani bamak (glacier), Garhwal Himalaya

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Ice thickness measurements of Dokriani bamak (glacier) were carried out with a ground penetrating radar (GPR) pulse EKKO IV Sensor Software, Canada. This is the first attempt to estimate the ice thickness of an Indian Himalayan glacier by GPR. Profiling by GPR was carried out at a central frequency of 12.5 MHz with 2 m step size. Three distinct reflection patterns were observed; (i) glacier ice (GI), (ii) subglacial lodgment till (SLT) produces complex series of reflections; and (iii) bedrock (BR). Crevasses are distinguished by the laterally compressed synclinal hyperbolas stacked one over the other. The glacier ice thickness measured by GPR ranged from 15 m to 120 m. Ice thickness calculated theoretically and by GPR were very close. The computed total volume of glacier ice was 283.6×10^6 m^3.

IN November 1995, ground penetrating radar (GPR) profiling was carried out to measure ice thickness of Dokriani bamak (bamak meaning glacier in local dialect of the area). Pulse EKKO IV radar of Sensor Software, Canada, was used to carry out the profiling. GPR sounding was carried out from the snout, the accumulation zone of the glacier. Nearly 30% to 40% of the glacier could not be approached by the GPR profiling team as the glacier is intensely dissected by crevasses. Therefore, special efforts were made to measure the glacier ice thickness at the base of the icefall and above it, to have a realistic estimate of ice thickness of the glacier as far as possible. Profiling along the central line and across the glacier has provided us with a fair amount of data to understand the morphological feature of the bedrock. The GPR profiling of subglacial lodgment till and bedrock profile has facilitated in the interpretation and understanding of the subglacial hydrology and sediment transfer. With intensive GPR profiling of the glacier, it would be possible to estimate the volume of glacier ice with a higher degree of confidence.

Dokriani bamak is one of the valley glaciers in Bhagirathi river basin, that has nearly 282 glaciers. The Dokriani bamak glacier lies to the west of famous Gangotri glacier, the source of Ganga River (Figure 1). It extends from 30°50′ to 30°52′ N and 78°47′ to 78°50′ E. The total length of glacier is 5 km. It has a catchment area of 15.1 sq km, out of which 5.76 sq km is covered with ice, 3.94 sq km is covered by permanent snowfield,

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