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Mechanical Control of ATP Synthase Function: Activation Energy Difference between Tight and Loose Binding Sites[†]

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ABSTRACT: Despite exhaustive chemical and crystal structure studies, the mechanistic details of how F_oF_1-ATP synthase can convert mechanical energy to chemical, producing ATP, are still not fully understood. On the basis of quantum mechanical calculations using a recent high-resolution X-ray structure, we conclude that formation of the P−O bond may be achieved through a transition state (TS) with a planar PO_3^- ion. Surprisingly, there is a more than 40 kJ/mol difference between barrier heights of the loose and tight binding sites of the enzyme. This indicates that even a relatively small change in active site conformation, induced by the γ-subunit rotation, may effectively block the back reaction in β_{TP} and, thus, promote ATP.

F_oF_1-ATP synthase is a large multisubunit complex enzyme responsible for most of the ATP synthesis (*1*, *2*) in membranes of bacteria, chloroplasts, and mitochondria. The F_1 domain consists of $\alpha_3\beta_3\gamma\delta\varepsilon$ subunits, with the three $\alpha\beta$ pairs surrounding the central α-helical coiled coil γ-subunit. The ATP synthesis can be summarized as $ADP + P_i \leftrightarrow ATP + H_2O$ (with $P_i = H_2PO_4^-$) which occurs in catalytic sites formed mainly in the three β-subunits at the $\alpha-\beta$ interfaces (*3*). This ATP-producing reaction occurs due to a rotation of the γ-shaft, inducing conformational changes in the surrounding α/β-subunit pairs according to the unbinding and rotational coupling mechanism proposed by Boyer and others (*4*−*8*). It has been demonstrated that in the presence of excess ATP the isolated F_1 part can hydrolyze ATP which results in a reverse rotation of the γ-subunit (*6*). Despite extensive experimental (*1*−*13*) and theoretical studies (*11*, *14*−*16*), however, the mechanistic details of the reaction are not fully understood.

In the first crystal structure of F_1 (*8*), or F_1-ATPase, the three catalytic sites contained an ATP analogue in one site, an ADP in the other, and an empty third site. Because of this and ATP binding affinity measurements (*9*, *10*), the sites are termed the "tight binding site" (β_{TP}), the "loose binding site" (β_{DP}), and the "empty site" (β_E), respectively. In short, during ATP hydrolysis, each subunit pair changes conformation along the repeating $\beta_E \rightarrow \beta_{TP} \rightarrow \beta_{DP} \rightarrow \beta_E$ cycle, where each of these steps is associated with a 120° rotation of the γ-subunit (*3*, *11*−*13*). In a recent high-resolution (1.9 Å) X-ray structure of bovine heart mitochondrial F_1-ATPase, the β_{DP} and β_{TP} binding sites display only minor structural differences, with the largest variation corresponding to an ∼1−1.5 Å relative displacement of αArg-373 and a ∼0.7 Å shift of the nucleophilic water molecule (*17*). Previous structural studies also showed that the transition state analogue of ATP hydrolysis (Al- or probably $MgF_3\cdot$ADP) (*18*, *19*) is formed in the β_{DP} site, whereas ATP (AMP-PNP) was bound in β_{TP}. In addition, single-molecule imaging studies indicate that ATP hydrolysis likely takes place at an ∼80° rotational substep from β_{TP} toward β_{DP}, during an ∼1 ms event (*12*). These observations suggest that ATP bound to the loose binding site is the nucleotide that is likely to hydrolyze through a transition state, as also proposed previously (*17*). Nevertheless, it is not known how the small changes between the two sites mentioned above would influence the reaction coordinate of ATP synthesis and/or hydrolysis.

To investigate whether there are important underlying energetic and structural variations taking place in these two sites (*17*), we applied a quantum mechanical (QM)[1] active site approach (*20*−*23*) using the Gaussian 03 software package (*24*). The employed B3LYP (*25*) hybrid density functional showed accurate results in previous studies involving Mg-coordinated phosphate hydrolysis (*20*, *23*); nevertheless, its performance was compared on a similar smaller model to MP2 calculations, which resulted a very good correlation (Supporting Information). The active site models were extracted from the X-ray structure of the enzyme (*17*) and were treated using QM [at the B3LYP/6-311++G(d,p)//B3LYP/6-31G(d) level of theory], while polarization effects from the rest of the protein were considered using the integral equation formalism polarizable continuum solvent model (*26*). Zero-point energy corrections were acquired from frequency calculations, which were also used to confirm the nature of the obtained critical points. As the two active sites are very similar in structure, the QM models of β_{TP} and β_{DP} sites were chosen to be identical in composition, containing 152 atoms. This, beside geometric properties, allows also the direct energetic comparison of the reaction steps in the two different sites. The reaction coordinate is expressed for an ATP hydrolysis path based on crystal data providing a starting geometry for the initial state of the bound ATP analogue (Figure 1); however, results are also used to aid in interpretation of ATP synthesis. Considering the initial step where the reacting nucleophilic water molecule and ATP are coordinated prior to hydrolysis, the results show an almost equal relative stability between the two sites, with an only 3.5 kJ/mol difference favoring the β_{DP} conformation. This is in line with previous results (*17*, *18*), namely, that the local level of

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[1]Abbreviations: TS, transition state; QM, quantum mechanics.

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stabilization of ATP is high in both β_{DP} and β_{TP}. It would also suggest that slight variations of residual positions have no significant effects on the stability of ATP bound in the catalytic site; however, it illustrates the relative enthalpic stability of ATP only between the two sites and cannot be directly related to binding affinity studies (*3*, *9*, *10*). Considering the final, ADP + P_i, state along the reaction coordinate, there is a larger (15.7 kJ/mol) difference between the two sites favoring the conformation in the β_{DP} site. This is most likely due to the different coordination of the P_i by βArg-260 (Figure 2). While in the β_{TP} site the latter forms an N-H−O-P type H-bond, in the β_{DP} site this guanidine rotates; therefore, the same hydrogen forms a H-bond with βGlu-188, and its position is shifted by ~0.9 Å (ArgN-H−O-Glu, 2.87 Å for β_{TP} and 1.91 Å for β_{DP}). Overall, the hydrolysis in the β_{TP} site is very slightly endothermic (3.3 kJ/mol), with ATP synthesis being favored, while in the β_{DP} site, it is somewhat exothermic (−9.1 kJ/mol), with the ADP + P_i state preferred. In general, the small energetic differences between the initial and end states of hydrolysis are in reasonable agreement with the experimental $K_{eq} \sim 1-3$ values observed during unisite catalysis (*3*, *9*, *10*).

Surprisingly, there is a significant difference in the γP−O bond-forming or -breaking step, as the relative stability difference is > 40 kJ/mol for the two TSs (Figure 1). The structure of the TS is composed of a planar metaphosphate (PO_3^-) ion in both active sites as suggested by obtained bond orders. The barrier heights obtained for β_{DP} and β_{TP} are 56.9 and 97.8 kJ/mol, respectively.

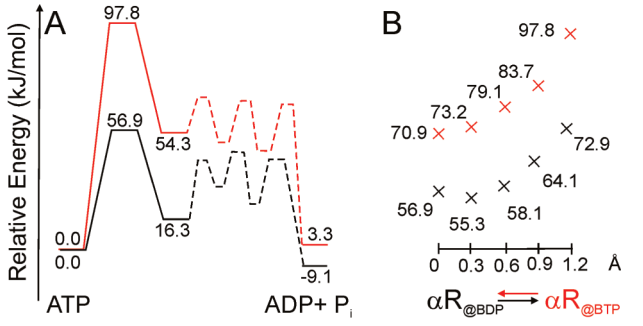

FIGURE 1: (A) Relative energies along the schematic reaction coordinate for β_{DP} (black) and β_{TP} (red). Putative intermediate states are shown as dotted lines. (B) Effect of the αArg-373 position on the barrier height as tested for both β_{DP} (black) and β_{TP} (red) by using gradual shifts toward its position in the other site. Values are relative to that of the ATP-bound state in β_{DP} and β_{TP}, respectively.

The former can be compared to the free energy barrier of ~52.3 kJ/mol which is derived from the experimental hydrolysis rate (4.1×10^3 s^{-1}) (*12*) using classical transition state theory.

After the TS, the reaction arrives to an intermediate in which βGlu-188 is protonated and HPO_4^{2-} is coordinated to the carbonyl group of αSer-344, where the relative difference between the two sites is still 38 kJ/mol. Such a behavior of the "catalytic carboxylate" βGlu-188 is not surprising, as already early experimental studies showed that it acts as a general base to polarize a water molecule to make the nucleophilic attack on γ-P of ATP (*8*) and the mutation of the equivalent βGlu-190 in thermophilic F_1 to Gln eliminated ATPase activity completely (*27*).

To further investigate the difference in barrier heights, in the β_{DP} TS geometry αArg-373 was gradually shifted in four steps from its position into a relative position close to that in β_{TP}, while the rest of the structure remained the same. The newly obtained TS structures were fully optimized (Figures 1 and 2). The same procedure was performed for the β_{TP} TS, where αArg-373 was shifted "backward" toward its β_{DP} position. In both cases, a considerable change was observed, as the barrier height increased an overall 16.0 kJ/mol for the $\beta_{DP} \rightarrow \beta_{TP}$ shift and decreased 26.9 kJ/mol for the $\beta_{TP} \rightarrow \beta_{DP}$ shift (Figure 1). During both scans, only a slight change in barrier height is observed when the position of αArg-373 is close to that in β_{DP}, which suggests that an active site conformation near the β_{DP} geometry could be already appropriate for hydrolysis. This is in line with single-molecule studies, which show that hydrolysis most likely takes place after an ~80−90° rotation of the γ-subunit, when the occupied active site is likely to be in a conformation close to β_{DP}. The TSs show somewhat dissimilar structural properties in the two sites (Figure 2 and SFigure 2 of the Supporting Information). The $H_2O-\gamma P$ distances are 2.13 and 2.17 Å and the γP−O-ADP distances 2.36 and 2.50 Å for β_{DP} and β_{TP}, respectively. The $H_2O-\gamma P-O$-ADP line is slightly closer to linear in β_{DP}, the O−P−O angle being 173.9°, while in β_{TP}, it is 171.7°. The distances between the two oxygens involved in bond formation and/or breakage are ~4.49 and ~4.67 Å for β_{DP} and β_{TP}, respectively. The obtained values for β_{DP} are in close agreement with X-ray studies using Mg-ADP and Al-fluoride to mimic transition state formation (~4.4 Å) (*13*). Note that due to the difference in composition, a quantitative comparison cannot be made.

One of the most significant differences between the TSs is the coordination of the αArg-373 side chain on the nucleophilic

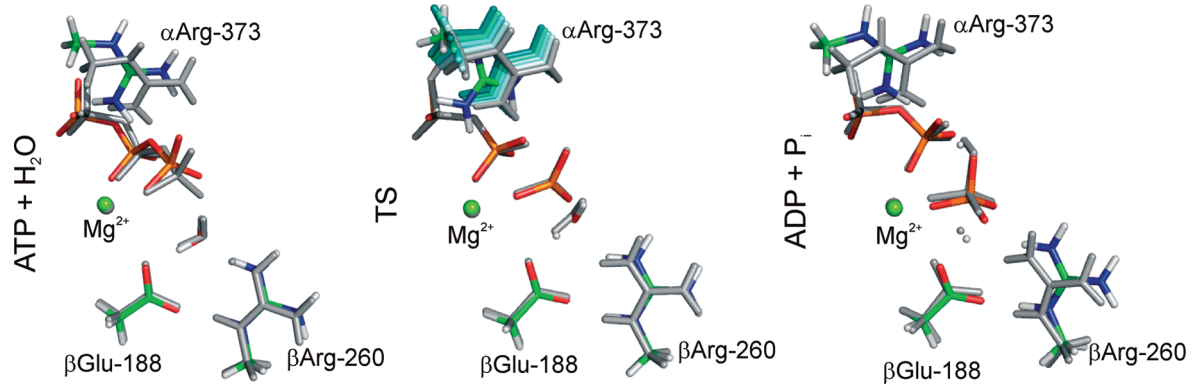

FIGURE 2: Superimposed structures of selected parts from the active site models of β_{DP} (gray) and β_{TP} subunits (colored), describing the obtained calculated geometries for the initial state with ATP bound (left), the TS (middle), and the final state with ADP + P_i (right). In the TS structure, the scanned positions of αArg-373 are also shown as darkening shades of cyan. Some of the H−X (X = O or N) bonds are elongated due to strong H-bonds; these hydrogens are illustrated as nonbonded spheres. Illustrations were made using PyMol (*29*).

oxygen of ADP. With respect to β_{DP}, in the case of β_{TP} this residue is shifted away from the active site by ~1.1 Å. As this residue tightly holds the γ-phosphate of the ground state-bound ATP with two H-bonds, its side chain needs to swing to let PO_3^- leave ATP (Figure 2 and SFigure 3 of the Supporting Information). On the basis of the sensitivity of the barrier height to its relative position, we conclude that αArg-373 may have a fundamental role in destabilizing the bond forming and/or breaking TS and consequently control hydrolysis. However, an energetic difference is still present between the TS in the β_{TP} site with αArg-373 shifted closer to PO_3^- and the TS in the original β_{DP} site, which suggests that other structural differences are also present. Note that the side chain of αArg-373 in β_{TP} is fully extended in the crystal structure (average side chain dihedrals of ~173°) (*17*); thus, a larger shift toward the PO_3^- would require considerable changes in an expanded region making it unlikely to occur. βArg-260 and αSer-344 coordinate the nucleophilic water relative to the γP ($H_2O-\gamma P$ distances of 3.40 Å in β_{DP} and 3.87 Å in β_{TP}), which likely assists the destabilization of the TS in β_{TP}, as also proposed previously (*17*).

Overall, the discrete energetic difference between the two catalytic sites indicates that in F_oF_1-ATP synthase the rotation of the γ-subunit controls the reaction mechanically by driving the geometry and consequently electrostatic effects in the active site into a state where the backward reaction, i.e., ATP hydrolysis, is unlikely to happen due to an increased barrier. Considering hydrolysis in F_1-ATPase under unisite catalysis, it takes place rather slowly because most likely the slow conformational change of the α/β-subunits coupled to reverse rotation of the γ-subunit preserves ATP until a favorably small activation energy is reached. The catalytic cooperativity appearing during bisite or trisite catalysis is required to enhance the conformational change and hasten the reaction by several orders of magnitude (*3*).

In this paper, we demonstrate with theoretical methods that there is a large difference in barrier heights to ATP hydrolysis between the tight and loose binding sites of F_1-ATPase. On the basis of our results, we conclude that the reaction most likely proceeds through a TS with a planar metaphosphate ion in an active site geometry close to that of the β_{DP} subunit. The barrier height in the latter can be compared to the free energy barrier derived from the experimental reaction rate (*12*). The large difference in barrier height is significant considering the relatively small structural differences between the geometries of the tight and loose binding sites. It is most likely due to the shifted relative position of the αArg-373 residue. The results are in line with previous structural studies, suggesting that ATP bound in the β_{DP} site is likely to form a transition state and then be hydrolyzed (*17*, *18*). Current results indicate that the rotation of the γ-subunit acts as a mechanical control which practically inhibits ATP hydrolysis in the β_{TP} subunit. This is also supported by single-molecule studies that show that hydrolysis in F_1-ATPase takes place after an $\sim 80-90^o$ rotation of the γ-subunit (*12*). In the near future, we aim to study the remaining reaction steps to understand the full mechanism of this mechano-enzyme system. These likely involve rotation or transprotonation of the inorganic phosphate aided by the highly conserved αSer-344 OH side chain, shown to make a contribution to transition state formation (*28*).

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SUPPORTING INFORMATION AVAILABLE

Full ref *24* and computational and model details. This material is available free of charge via the Internet at http://pubs.acs.org.

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