Trapping the ATP binding state leads to a detailed understanding of the F₁-ATPase mechanism

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The rotary motor enzyme F₆F₁-ATP synthase uses the proton-motive force across a membrane to synthesize ATP from ADP and Pᵢ(H₂PO₄⁻) under cellular conditions that favor the hydrolysis reaction by a factor of 2 × 10⁸. This remarkable ability to drive a reaction away from equilibrium by harnessing an external force differentiates it from an ordinary enzyme, which increases the rate of reaction without shifting the equilibrium. Hydrolysis takes place in the neighborhood of one conformation of the catalytic moiety F₁-ATPase, whose structure is known from crystallography. By use of molecular dynamics simulations we trap a second structure, which is rotated by 40° from the catalytic dwell conformation and presents the state associated with ATP binding, in accord with single-molecule experiments. Using the two structures, we show why Pᵢ is not released immediately after ATP hydrolysis, but only after a subsequent 120° rotation, in agreement with experiment. A concerted conformational change of the αβ₃ crown is shown to induce the 40° rotation of the γ-subunit only when the β₂ subunit is empty, whereas with Pᵢ bound, β₆ serves as a latch to prevent the rotation of γ. The present results provide a rationalization of how F₁-ATPase achieves the coupling between the small changes in the active site of β₆p and the 40° rotation of γ.

F₁-ATPase | chemomechanical coupling | ATP waiting state | molecular dynamics | Pᵢ release

The molecular motor F₆F₁-ATP synthase is composed of two domains: a transmembrane portion (F₆), the rotation of which is induced by a proton gradient, and a globular catalytic moiety (F₁) that synthesizes and hydrolyzes ATP. The primary function of the proton-motive force acting on F₆F₁-ATP synthase is to provide the torque required to rotate the γ-subunit in the direction for ATP synthesis (1, 2). The catalytic moiety, F₁-ATPase, has an αβ₃ “crown” composed of three α- and three β-subunits arranged in alternation around the γ-subunit, which has a globular base and an extended coiled-coil portion (3) (Fig. 1A). F₁-ATPase by itself binds ATP and hydrolyzes it to induce rotation of the γ-subunit (in the opposite direction from that for synthesis) on the millisecond time scale under optimum conditions (4, 5). All of the α- and β-subunits bind nucleotides, but only the three β-subunits are catalytically active. The original crystal structure (3) of F₁-ATPase from bovine heart mitochondria (MF₆) led to the identification of three conformations of the β-subunit: β₆ (empty), β₆p (ATP analog bound), and β₆p (ADP bound) (Fig. 1A). In the known structures of F₁-ATPase, which apparently are near the “catalytic dwell” state, the state in which catalysis occurs (6, 7), the β₆ subunit conformation is partly to fully open and is very different from those of the β₆p and β₆p subunits, which are closed and very similar to each other (SI Appendix, SII).

Searching for the ATP Waiting State

Because no X-ray structure is available for the ATP waiting state, we searched for it by molecular dynamics (MD) simulations with an external torque applied to the γ-subunit in the hydrolysis direction while introducing different conformations of the β₆p subunit in the αβ₃ crown, in accord with suggestions from single-molecule experiments (8). The results are shown in Fig. 2 (see Methods and SI Appendix, SI2 for details of the simulations). In Fig. 2, we refer 200° for the γ-rotation angle of the catalytic dwell state and 240° for the ATP waiting dwell state, respectively, to stress that the hydrolysis of an ATP, denoted as ATPγ, bound after the ATP waiting dwell at 0°, takes place at the 200° catalytic dwell state (see Fig. 1B for the rotation angle of γ relative to the αβ₃ complex). The initial simulation used the “Walker” crown structure [Protein Data Bank (PDB) ID code 1BMF] (3), in which the β₆p subunit is closed with the angle (B° C°) formed by helices B and C equal to 21.6°, and the γ-subunit structure of Gibbons et al. (PDB ID code 1E79) (9) (Fig. 1; see Methods for system preparation). It was represented by an all-atom model based on the CHARMM program (10), combined with a coarse-grained plastic network model (PNN) (11, 12). Even for an applied torque of 2,500 pN·nm, much higher than is generated in the normal function (13), the γ-subunit, which has an initial rotation angle of 200°, stalled at an angle of about 220°. In the present work, we define the rotation angle of γ as the angle formed between an instantaneous vector and a reference vector, each defining the orientation of γ relative to the three β-subunits for the instantaneous configuration from MD or the reference Walker structure, respectively (see Methods and SI Appendix, SI2 for angle definition). Major clashes between residues γS12-I16 and β₆p,348-I338, near the DELSEED motif, prevented further rotation (SI Appendix, SIII and Fig. S1). When the external torque was removed, the γ-subunit returned to within 2.5° of the crystal orientation (see Fig. S1).

Significance

F₁-ATPase is a motor protein that converts the free energy of binding of ATP and its hydrolysis products ADP and Pᵢ into a mechanical force for γ-subunit rotation. It is the catalytic moiety of F₆F₁-ATPase, which synthesizes ATP. There are two metastable states along each 120° rotation of the γ-subunit, one associated with ATP hydrolysis (the “catalytic dwell”) and the other with ATP binding (the “ATP waiting dwell”). We use molecular simulations to determine the ATP waiting dwell structure. With this structure and the catalytic dwell X-ray structure, we develop an atomic-level model of the coupling between ATP hydrolysis and γ-subunit rotation. The molecular-level understanding of this motor will aid in its use in nanomachines and cancer therapy.

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and subunit having B^C equal to 31° and rotated. Comparisons of the observed conformations, as well as the binding-release of ligands and the hydrolysis of ATP. Starting from the binding of an ATP* to the β subunit in the ATP waiting state (0°), rotation of the γ-stalk by 200°, 40°, 80°, and 220° leads to the transition of the β subunit after the simulations with the applied torque. The values of the B^C angle in the β subunit are shown for each trajectory to indicate the stage of binding closing opening during the transition from the β subunit (B^C = 21.6°) to the ββ subunit (B^C = 48.6°) conformation. The B^C angles were maintained at their initial values by the PNM restraining potentials during the simulations.

Fig. 1. (A) Fγ-ATPase. The three β-subunits and the γ-subunit are shown (α-subunits are not shown for clarity): βH (yellow), ββ (orange), βTP (gold), and γ (purple). To define the ββ subunit conformation we use the angle between helix B (β163-176) and helix C (β1190-2204). The two helices are highlighted: helix B (blue) and helix C (gray); the B^C angle is depicted as a red angle. The βH6 helix, whose orientation was reported to undergo a 20° change during the 40° substep γ-rotation, is highlighted in red. The force acts on the Cx atom of MF, γ-M25 (shown as a red sphere). The direction of the force is determined as the cross-product of the radial vector of γ-M25-Cx and the rotational axis (green). (B) Proposed 360° rotation cycle of Fγ-ATPase showing the subunit conformations, as well as the binding-release of ligands and the hydrolysis of ATP. Starting from the binding of an ATP* to the β subunit in the ATP waiting state (0°), rotation of the γ-stalk by 200°, 40°, and 220° leads to the transition of the β subunit after the simulations with the applied torque. The values of the B^C angle in the β subunit are shown for each trajectory to indicate the stage of binding closing opening during the transition from the β subunit (B^C = 21.6°) to the ββ subunit (B^C = 48.6°) conformation. The B^C angles were maintained at their initial values by the PNM restraining potentials during the simulations.

Fig. 2. Rotational angle of the γ-subunit as a function of time during the relaxation simulations (see text) for different B^C angle values of the β subunit after the simulations with the applied torque. The values of the B^C angle in the β subunit are shown for each trajectory to indicate the stage of binding closing opening during the transition from the β subunit (B^C = 21.6°) to the ββ subunit (B^C = 48.6°) conformation. The B^C angles were maintained at their initial values by the PNM restraining potentials during the simulations.

To check the trapping simulation, we used an alternative protocol (SI Appendix, SI2) and experimental data from Merz et al. (8), who estimated that the helix-6 angle of the β subunit rotated by 20° in the ATP waiting state. The β subunit and the γ-subunit were subjected to a biased simulation (16) and it was found that for the partly open structure of the β subunit (helix-6 angle equal 20°, B^C = 23°) the γ-subunit had rotated by 40° to reach the 240° state. A number of interactions stabilize the 240° state (see Fig. 3 and SI Appendix, SI4 for details and a comparison with the interactions in the trapped structure). The structure was then subjected to all-atom explicit water MD simulations with no PNM (see SI Appendix, SI5 for details). Throughout the simulation (20 ns), the γ-stalk stayed near 240°, supporting the fact that it is a (locally) stable state. In SI Appendix, Fig. S2 A and B, we show the structure from the simulation, and compare it with the structure at the catalytic dwell. The conformations of the β subunit for the two states differ as expected. Comparisons of the β subunit with various
and ADP release takes place on the millisecond scale. The interactions between the β-subunits have been suggested as triggers for the β-subunit repulsion, have been suggested as triggers for the β-subunit repulsion. 

R133 does not change its C-terminal helix orientation during the 40° substep rotation, the large fluctuations of the orientation angle in the measurements suggest that the small change of βE observed in the MD simulation is below their resolution. We use this experimentally derived structure for the analysis that follows. Because the structure of βDP is closest in rmsd to βHO (SI Appendix, Fig. S2C), we denote the conformation of the partly open βDP as βHO-like (or simply βHO for brevity when no confusion arises).

**Timing of P<sub>i</sub> Release**

The results from free-energy simulations and multiple MD simulations (SI Appendix, S6 and Fig. S3) indicate that any of ATP hydrolysis, ADP and/or P<sub>i</sub> release, can lead to the partly open βHO-like conformation required to reach the ATP waiting state at 240° from the catalytic dwell (200°). All of these, as well as ADP and P<sub>i</sub> repulsion, have been suggested as triggers for the γ-rotation (19–21). To investigate possibilities for the actual mechanism, we calculated the probability of ligand release, particularly of P<sub>i</sub>, from different conformations of the β-subunits with different ligand occupancies. Given the results shown in Fig. 4 and additional experimental data [particularly Watanabe et al. (21)], we summarize in Fig. 1B the rotation cycle of the subunit conformations and their occupations. For an ATP, denoted as ATP*, bound after the ATP waiting dwell at 0°, the release of P<sub>i</sub>* generated from the ATP* is shown to occur after an additional 120° rotation of the γ-subunit to 320° from the catalytic dwell at 200° where the ATP* hydrolysis takes place. This contrasts with an earlier conclusion, also based on single-molecule experiments, that P<sub>i</sub> is released immediately after ATP* hydrolysis at 200° (20, 22, 23).

Because the P<sub>i</sub> and ADP release takes place on the millisecond time scale, too long to be sampled in accessible simulation times at 300 K (nanoseconds), we use the high-temperature multicycle enhanced sampling (MCES) method (see Methods) to accelerate the events. This approach has been used previously to provide meaningful results on ligand release from proteins and its dependence on their conformation [e.g., CO from myoglobin by Elber and Karplus (24); P<sub>i</sub> from myosin by Cecchini et al. (25)].

MCES was used here to explore the ligand release probabilities of P<sub>i</sub> and ADP from subunits with the conformations βE, βDP, and βHO-like as part of the αβγ-complex. For βE and βDP, we considered a structure at or near the catalytic dwell (200°), based on the X-ray structure of Braig et al. (PDB ID code 1E1R) (26); for βHO, we considered the ATP waiting dwell (240°), based on the model structure reported here. Fig. 4 shows the probabilities of

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**Fig. 3.** Interactions between the γ-subunit and the surrounding subunits for a representative structure of the simulated ATP waiting state. The color scheme is the same as Fig. 3. Residues from the γ-subunit are labeled in blue, and residues from other subunits are labeled in red. Each residue involved in the interaction is shown in stick representation.

**Fig. 4.** Probability of P<sub>i</sub> release as a function of the temperature applied to P<sub>i</sub> in the MCES simulations (see Methods): P<sub>i</sub> release from β<sub>ε</sub> in the catalytic dwell (200°; red), and the release from ADP and P<sub>i</sub> bound β<sub>E</sub> at 200° (blue). The angle definition is provided in Fig. 1B. The results test the two competing models for P<sub>i</sub> release: one from β<sub>δ</sub> immediately after its cleavage from bound ATP and the other from β<sub>ε</sub> after the rotation of γ by 120° to reach the next catalytic dwell, during which the P<sub>i</sub> release from β<sub>δ</sub> is prevented. The latter model corresponds to the reaction cycle proposed in the present work and Watanabe et al. (21). We also show the release probabilities of P<sub>i</sub> and ADP from the β<sub>ε</sub> conformation at the ATP waiting dwell with different ligand contents: P<sub>i</sub> release in the presence of ADP (green), P<sub>i</sub> release in the absence of ADP (pink), and ADP release in the presence of P<sub>i</sub> (black).
P$_i$ release from β-subunits as a function of the temperature of the multiple copies of P$_i$. In the Braig et al. structure used for the catalytic dwell state (200°), P$_E$ is occupied by P$_i$; βDP by ADP, AlF$_3$ (which was replaced with P$_i$), and Mg$^{2+}$; and βTP by ATP analog and Mg$^{2+}$. The P$_i$ present in P$_E$ was produced during the catalytic dwell at 80° from an ATP bound at ~120°. Because P$_i$ has a high release probability from P$_E$ at a temperature as low as 250 K, it is very weakly bound. Interestingly, in one of the all-atom explicit water MD simulations (see below), we observed the spontaneous release and rebinding of P$_i$ from P$_E$ (Movie S1). The release and rebinding accompany a large fluctuation of the P-loop structure. This finding is consistent with the present P$_i$ release data. By contrast, a much higher temperature (1,500 K) is required for a significant release probability of P$_i$ from the closed βDP subunit in the catalytic dwell structure (200°). This result indicates that in the 200° structure, release of P$_i$ from P$_E$ is the dominant process and that release of P$_i$* from βDP immediately after its cleavage from ATP* does not occur to a significant extent, in accord with Fig. 1B and the proposal of Watanabe et al. (21).

In the ATP waiting state (240°), where P$_E$ is empty (Fig. 1B), βDP has opened more to become βHO, but P$_i$* release is even more hindered than in the 200° structure as long as ATP* (and Mg$^{2+}$) is present (Fig. 1B); i.e., release of P$_i$* at 240° would be possible only after ATP* has been released (also see SI Appendix, SI7, SI8, and Fig. S4). To confirm this result, we performed a set of MCES simulations for βHO in the 240° structure, in which ADP and P$_i$ were both represented by multiple copies and thus competed for release. As expected, ADP* is released at a significantly lower temperature than P$_i$* (Fig. 4). Once ADP* is no longer present, P$_i$* is released easily (SI Appendix, Fig. S5). However, as shown by Adachi et al. (20) and Martin et al. (27), ADP* is released only during (or after) the rotation of the γ-subunit by another 80° to the catalytic dwell at 320° when P$_E$ has opened further to P$_E$ (see Fig. 1B legend). Very recently, Czub and Grubmüller have shown by MD simulations that P$_E$ closes spontaneously to βHO during the 80° rotation in the synthesis direction in the absence of ligand (ADP or P$_i$) in P$_E$ (28). However, because changes of the ligand occupation of each β-subunit during the rotation were not taken into account in the simulations, an understanding of the entire sequence of events that occur during the 80° rotation is not possible based on their results. Nevertheless, the results are consistent with the mechanism that the βHO → βE conformational transition and the rotation of γ to the catalytic dwell occur during or after the release of ADP*. Taken together, the P$_i$ release simulations show that P$_i$* is released after the βHO → βE transition is completed as part of the rotation from 320° to 360°.

**Coupling Between ATP Hydrolysis, P$_i$ Release, and γ-Rotation**

An essential element for understanding F$_1$-ATPase function is knowledge of the mechanism by which the torque for γ-rotation is generated. The dominant factor in the 80° rotation from the waiting dwell is known to be ATP binding to βE subunit and its closure to form βTP (12, 29), in which repulsive van der Waals interactions contribute dominantly in the generation of torque. On the other hand, our understanding of the 40° rotation is much more limited (20, 30). To explore the mechanism, we carried out targeted molecular dynamics (TMD) (31) simulations with the model generated in this paper for the ATP waiting structure as the target (see Methods). As a reference, a TMD simulation was performed, starting with the catalytic dwell structure, and a perturbation was applied to the γ- and βDP subunits of the entire αβ$_i$γ-complex to induce the 40° rotation of γ and the transition of βDP to βHO required to reach the model ATP waiting structure. The simulation produces a structure with the γ-subunit rotated by 40° and βDP partially open; the structure differs slightly from the model ATP waiting structure in the orientations of the C-terminal hth motifs of βE and all of the α-subunits (SI Appendix, Fig. S6). The change of the βE structure during or after the 40° rotation is in accord with the results of Watanabe et al. (30) that in the neighborhood of the ATP waiting state, the affinity for ATP changes with γ-rotation, implying a change in the βE structure, and the all-atom explicit water MD simulation (SI Appendix, Fig. S2A). Because the TMD perturbation was applied only to γ and βDP, the structural changes of the other parts of the αβ$_i$γ-complex reflect their spontaneous response to the rotation of γ and the partial opening of βDP. The resulting structure was used as the target structure in subsequent TMD simulations, where the perturbation was applied only to various parts of the αβ$_i$γ complex, with or without P$_i$ in the βE subunit (Fig. 5; see legend for details). With βE empty, we obtained the striking result that transformations of all α- and β-subunits are required to induce the 40° rotation (Fig. 5). As shown in the figure, when fewer elements of the αβ$_i$γ crown are transformed (e.g., all β-subunits), only intermediate rotation of the γ-subunits is produced during the simulation. Moreover, with P$_i$ present in P$_E$, the P$_i$ stays bound in the active site throughout the entire TMD simulation and only a 10° rotation of γ was achieved even with the full αβ$_i$γ transformation in the simulation (Fig. 5, orange; and see Movies S2 and S3). These results show that the presence of P$_i$ in the βE subunit blocks the γ-rotation.

**Dynamic Lock by P$_i$**

To determine the mechanism of the βE (P$_i$) lock, the structure and dynamics of the αβ$_i$γ-complex in the catalytic dwell state with different occupations of βDP and P$_E$ were studied by all-atom explicit water MD simulations (see SI Appendix, S15 for details). In the simulations, the binding pockets of βTP and all α-subunit are occupied by ATP, whereas βDP and P$_E$ have different occupations: In the prehydrolysis state simulation, ATP occupies βDP and P$_i$ occupies βE; in the posthydrolysis state simulation, ADP and P$_i$ occupy βDP and P$_i$ occupies βE; and in

![Fig. 5. γ-Rotation angles from the TMD simulations (see text). The no-P systems are systems with empty βE/γ + βDP simulation (black), βEβ simulation (blue), βEβ + P$_i$ simulation (green), all β simulation (purple), all β + α simulation (yellow). The simulation time is shown in nanoseconds, and the γ-rotation angle is defined as in Pu and Karplus (12). Except for the γ + βEβ simulation, the TMD simulations continued for 1 ns and were followed by 1-ns unperturbed simulations to relax the system; during the latter all these systems reached a plateau for the rotation angle of γ. In the γ + βEβ TMD simulation, it took 2 ns before the rmsd distance to the 240° rotated structure fell below 0.75 Å; this was followed by a 1-ns relaxation simulation as in the other cases.](diag.png)
the postrelease state simulation, $\beta_{DP}$ is occupied by ADP and P, and $\beta_L$ is empty. (See Fig. 1B and its legend for identification of the three states.) The structural comparisons reveal that during or after the hydrolysis of ATP in $\beta_{DP}$ and the release of P from $\beta_E$, small changes occur in the C-terminal hth motif and at the intersubunit interfaces of the subunits (SI Appendix, Figs. S2E and S7, and Movie S4). In addition, there are significant differences in the dynamics, as evidenced in the cross-correlation maps of the $\alpha_{i\beta_i}$-complex; they are shown in SI Appendix, Fig. S8C. Details of the structural and dynamic changes are given below. 

SI Appendix, Fig. S8 shows the cross-correlation maps of the entire $\alpha_{i\beta_i}$ complex for the prehydrolysis state (SI Appendix, Fig. S8A), posthydrolysis state (SI Appendix, Fig. S8B), and the postrelease state (SI Appendix, Fig. S8C). In comparing the simulation of the posthydrolysis state to that of the prehydrolysis state, there is a rigid-body rotation of the C-terminal hth motif of $\alpha_{DP}$ toward $\beta_{DP}$ in the former, relative to the latter. This rotation is caused by the cleavage of ATP into ADP and P in $\beta_{DP}$ (SI Appendix, Fig. S74). The rotation increases the contact between the two subunits and leads to a more closed $\alpha_{DP}$-$\beta_{DP}$ interface, as is evident from the increased buried surface area (SI Appendix, Fig. S9). It also leads to enhanced positive cross-correlation between the two subunits without a significant change of the intrasubunit cross-correlation of $\beta_{DP}$ (compare SI Appendix, Fig. S8 A and B). A similar closure of the $\alpha_{DP}$-$\beta_{DP}$ interface is observed experimentally. In SI Appendix, Fig. S7B, the X-ray structure with the transition-state analog (26) is superimposed on the structure with the ATP analog (14). The superposition shows that the C-terminal domain of $\alpha_{DP}$ is rotated toward $\beta_{DP}$ for the transition-mimic state to make the interface tighter (SI Appendix, Fig. S7B, Left), in agreement with the simulations. The origin of this structural change appears to involve the displacement of $\alpha_{DP}$, which moves to interact with the P, after it is cleaved from the ATP. SI Appendix, Fig. S7A shows the displacement of $\alpha_{DP}$ upon the cleavage of ATP in the simulations and a similar displacement in the transition-state mimic structure (SI Appendix, Fig. S7B; also see SI Appendix, Fig. S7C for the changes of interactions at the interface between the two subunits). In this interpretation, $\alpha_{DP}$ functions as a sensor that probes the progress of the hydrolysis reaction in $\beta_{DP}$ and dynamically links the two subunits ($\beta_{DP}$ and $\alpha_{DP}$). This is consistent with mutation experiments, which suggested that $\alpha_{DP}$ is involved in the rearrangement of the $\alpha_{DP}$-$\beta_{DP}$ interface upon ATP hydrolysis and the catalytic cooperativity of the enzyme (32).

We also find a noticeable difference between the cross-correlation maps of the posthydrolysis and postrelease states, i.e., there is an increase of intrasubunit correlation of both $\alpha_E$ and $\beta_L$ in the postrelease state (SI Appendix, Fig. S8C). The cross-correlation maps of $\alpha_E$ and $\beta_L$ in the postrelease state show cross-correlations that extend over the C-terminal and nucleotide binding domains (SI Appendix, Fig. S8 C and D), suggesting that the two domains behave like a rigid body. This difference in the dynamics of $\beta_L$ is of interest because the differences between the $\beta_L$ structures with or without P or a P analog are found to be negligible (SI Appendix, SI9 and Fig. S10). The anticorrelation between $\alpha_E$ and the C-terminal domain of $\beta_L$ has also increased significantly (compare SI Appendix, Fig. S8 B and D). This result indicates that the two subunits move concertedly but in opposite directions. In this case, $\alpha_{DP}$ could play an important role in controlling the dynamics of $\alpha_E$ and $\beta_L$, similar to the role of $\alpha_{DP}$ in ATP hydrolysis. In this mechanism, the interaction between $P_i$ in $\beta_E$ and $\alpha_{DP}$ keeps $\alpha_E$ close to $\beta_L$ and away from $\beta_{DP}$, preventing $\alpha_L$ from responding to the change of $\beta_{DP}$, thus blocking the rotation of $\gamma$. Once $P_i$ leaves the binding pocket, the interaction is lost, so that $\alpha_L$ and in particular its C-terminal domain are able to respond to the change occurring in $\beta_{DP}$ and the rotation of $\gamma$.

To test the proposed mechanism, we have performed an additional TMD simulation. The simulation was carried out with the TMD perturbation applied to all $\alpha$- and $\beta$-subunits but without the interaction between $P_i$ in $\beta_E$ and $\alpha_{DP}$. If these interactions were important in blocking the $\gamma$-rotation, it would be expected that $\gamma$ would rotate further in their absence than when the interactions between $P_i$ in $\beta_E$ and $\alpha_{DP}$ were present. The simulation produced a $\gamma$-rotation that is larger (close to 20°) than the simulation with the $P_i$-$\alpha_{DP}$ interaction present, but then it falls back to the lower rotation angle during the subsequent relaxation simulation (SI Appendix, Fig. S11). The result confirms the proposed role of the interaction between $P_i$ and $\alpha_{DP}$ in blocking $\gamma$-rotation. The result also suggests that interactions (within or between $\beta_E$ and $\alpha_E$), other than the interactions between $P_i$ in $\beta_E$ and $\alpha_{DP}$, are important in preventing the rotation—for example, reducing the increase of the intrasubunit cross-correlation in the $\beta_L$ and $\alpha_E$ subunits and the increase of the anticorrelation between them, which occurs upon the release of $P_i$. In this regard, we note that $\alpha_E$ is the subunit forming the most extensive surface contacts with $\gamma$ among the $\alpha$-subunits and has an extensive surface contact with $\beta_{DP}$ (SI Appendix, Fig. S9). The surface contacts of $\alpha_E$ with $\gamma$ are as extensive as the contact between $\beta_{DP}$ and $\gamma$, which is the largest surface contact among all $\beta$.

Taken together, the present analysis shows how the interactions between $\beta_E$ and $\alpha_E$, including the interaction between $P_i$ and $\alpha_{DP}$, act as a “dynamic lock” to keep the protein in the prerotated catalytic dwell state. Only after $P_i$ in $\beta_E$ is released is $\alpha_E$ freed from $\beta_E$ and able to fully engage with $\beta_{DP}$ to complete the concerted conformational transition of the $\alpha_{DP}$-$\beta_{DP}$ complex by which the $\gamma$-subunit rotates to reach the ATP waiting dwell state. Such dynamic locks have been proposed for different systems by Laity et al. for zinc finger proteins (33) and by Young et al. for c-Src (34).

Concluding Remark

The present study provides a structural model for the ATP waiting state of F$_1$-ATPase, in agreement with single-molecule experiments which have suggested that it does not coincide with any of the known crystal structures. Knowledge of this structure, combined with that of the state in which catalysis takes place, makes possible the development of a detailed atomic-level description of the coupling between the binding and hydrolysis of ATP and the $\gamma$-subunit rotation induced by the conformational changes of the $\alpha$- and $\beta$-subunits. The suggested tests of the proposal structure and a possible method for trapping it in a crystallographically accessible conformation should stimulate experimental studies (see SI Appendix, SI10 and SI11 for details).

Methods

Forced Rotation Simulation for Finding the ATP Waiting State. The structure of the minimal rotary complex $\alpha_{i\beta_i}$ was prepared based on the $\alpha_{i\beta_i}$ subcomplex of the 1BMF complex (3) and the $\gamma$-subunit of the 1E79 structure (9) by a procedure similar to that of Ma et al. (35). The CHARMM11 all-atom force field (36) and the EEF1 implicit solvation model (37) were used to describe the protein system and water solvation, respectively. In addition, the coarse-grained PNM (11, 12), in which each PNM node was assigned to the corresponding C$_\gamma$ atom position of the protein, was used to stabilize the protein conformation in the presence of the high forces used in the simulation. The system was first heated from 0 K to 300 K in 60 ps and then equilibrated at 300 K for 300 ps (see details in SI Appendix, SI2). The MD simulations were carried out with a 2-fs integration time step and SHAKE (38) applied to the bonds involving hydrogen atoms. The temperature was controlled using the Langevin thermostat.

After equilibration at 300 K for 300 ps, a large external torque was applied to drive the rotation of the $\gamma$-subunit in the hydrolysis direction (counter-clockwise as seen from the membrane). Using the PULL command of the CHARMM program (10), an external force of 2,500 pN was applied to the C$_\gamma$...
atom of residue γM25. Residue γM25 was identified by Pu and Karplus to provide a key contact point for the torque generation (12). In the forced rotation simulation, the external torque was applied only when backward rotation is detected. In that way, the simulation was biased toward the hydrolysis direction only when γ rotates backward but not when the forward rotation occurs spontaneously; the γ-rotation angle was checked at each update step (at every 1 ps). The γ-rotation angle is defined as in Pu and Karplus (12) using the αβ(18MFM)-(γE79) Walker structure as the reference structure for the catalytic dwell state, and a similar definition was used in the subsequent steps. Nat Struct Mol Biol 20(3). The direction of the force was determined as the instantaneous cross-product between the radial vector of the residue γM25 (perpendicular to the rotational axis) and the rotational axis itself (Fig. 1A). See SI Appendix, S12 for details of forced rotation simulations and definition of the γ-rotation angle.

P. Release Simulations. The 200° rotated system was prepared using the αβγδ subcomplex of the 1E18 structure (26) and the γ-subunit from the 1E79 structure (9). For the 240° state, the starting structure was the present ATP waiting state model structure. For the P, release MCES simulation (25), the P, molecule was replicated 30 times by using the BLOCK module of the CHARMM program. In all simulations, P, was treated as doubly protonated (H3P2O4), which was found to be favored in the active site of β-subunit (40). The interaction between the multiply copied P, and both the protein and the solvent was scaled by a factor that is inversely proportional to the number of P, copies, whereas each P, has no interaction with other P, molecules. The remaining interactions were not scaled. The temperature of P, was controlled by attaching each P, to a separate Langevin thermostat, while the remainder of the system was maintained at 300 K. At each P, temperature, the MCES simulation was repeated 40 times (SI Appendix, S17). Each simulation was started with different initial velocities and ran for 2 ns with a 1-fs integration time step. SHAKE was applied to constrain bonds involving hydrogen atoms.

Targeted MD Simulations of the Coordinated Conformational Transition of the αβγδ-Complex. The 40° step rotation was simulated by applying the TMD simulation method (31). The TMD simulation was first carried out with the 200° rotated catalytic dwell structure, which was prepared for the MCES P, release simulations. The TMD perturbation was applied to the nonhydrogen atoms of the γ and βP subunits of the entire αβγδ-Complex for the βP → βP0 transition and the 40° γ rotation; the ATP waiting model structure was the target structure. Subsequently, using this TMD-produced structure as the target structure for the αβγδ complex, a set of TMD simulations was carried out with the TMD perturbation applied to various parts of the αβγδ, crown with or without P, in βP and without any perturbation to γ (see Fig. 5 legend for the notation of each TMD simulation). In the simulations the rmsd distance to the target structure was decreased by 0.2 × 10^-6 Å at each MD step until the rmsd reached a value lower than 0.75 Å.

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6. Maragakis P, Karplus M (2005) Large amplitude conformational change in proteins controlled by attaching each P, to a separate Langevin thermostat, while the remainder of the system was maintained at 300 K. At each P, temperature, the MCES simulation was repeated 40 times (SI Appendix, S17). Each simulation was started with different initial velocities and ran for 2 ns with a 1-fs integration time step. SHAKE was applied to constrain bonds involving hydrogen atoms.

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