STEPPING ROTATION OF F_1-ATPASE: HOW AN ATP-DRIVEN MOLECULAR MACHINE MAY WORK

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Splitting ATP into ADP and phosphate liberates energy, but how this energy can drive molecular machines in the cell is not fully understood yet. In general, ATP-driven molecular machines operate in three stages: (1) binding of ATP to a catalytic site, (2) splitting of ATP in the catalytic site, and (3) release of products (ADP and phosphate) from the catalytic site. Here we show experimentally that, at least for the rotary motor F_1-ATPase, stage (1) is the major force-producing step: most of mechanical work of this motor is done in stage (1). Stage (3) also confers some force (work). But the splitting stage (2), unlike the splitting reaction in solution, is more or less reversible and thus does not contribute much energy.

F_1-ATPase is a portion of the enzyme ATP synthase that synthesizes ATP from ADP and phosphate using proton flow across a membrane as the energy source [1]. Isolated F_1-ATPase, which hydrolyzes ATP instead of synthesizing it, consists of $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ subunits. In a crystal structure [2], the γ subunit is at the center and is surrounded by a cylinder made of $\alpha_3\beta_3$ subunits. In 1997, we showed [3] that the isolated F_1-ATPase is a rotary motor, as proposed by Boyer [1], in which the central γ subunit rotates against the surrounding $\alpha_3\beta_3$ subunits when ATP is hydrolyzed in the three, catalytic β subunits. Reverse rotation of the γ subunit in ATP synthase, powered by the proton flow, is supposed to result in the ATP synthesis in the β subunits [1]. The F_1 rotation was visualized on a microscope by attaching an actin filament, a long rod that served as a marker, to the γ subunit while fixing the hexamer cylinder to a glass surface [3]. The sense of rotation was in accord with the crystal structure of F_1 [2] that indicated sequential ATP hydrolysis on the three β subunits. Imaging F_1 rotation has so far revealed the following mechanical properties of this molecular motor: (a) the rotation consists of discrete 120° steps each driven by hydrolysis of one ATP molecule [4,5]; (b) the mechanical work done in each step is constant and is 80-90 pN·nm which is comparable to the free energy obtained by hydrolysis of one ATP molecule; (c) the torque (rotary force) of this motor is approximately constant against the stepping angle [6]. Points (a) and (b) imply that the energy-conversion efficiency of this ATP-driven molecular motor can reach ~100% [4]. Precise correspondence between the hydrolysis reaction and mechanical rotation, however, could not be established in these earlier studies.

Recently, we have attached a 40-nm colloidal gold bead to the γ subunit and imaged its rotation at 8,000 frames per s [7] (see Fig. 1). At a saturating ATP concentration of 2 mM, the motor rotated at 130 revolutions per second (~8,000 revolutions per minute at 23°C for the motor obtained from a thermophilic bacterium). The 120° steps were clearly resolved even at this full speed, and the speed during stepping exceeded 100,000 revolutions per minute. At lower speeds, the motor showed distinct ~90° and ~30° substeps, each taking only a fraction of a millisecond (Fig. 2). Analysis of the substep kinetics has suggested the scheme in Fig. 3a: the 90° substep is driven by ATP binding, and the 30° substep by product release. Between 90° and 30° substeps are two ~1 ms reactions that are mechanically silent, which may correspond to hydrolysis and release of a product. The substeps are not resolved at 2 mM ATP, because a 90° substep immediately follows a 30° substep.

From the scheme in Fig. 3a and the previous observation that the torque of this motor is angle-independent, we can deduce the potential energy for the γ rotation as shown in Fig. 3b. In the one-

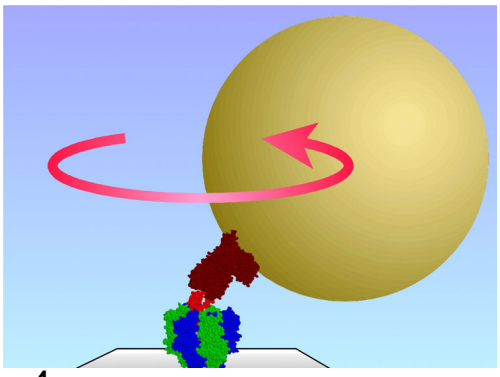

Figure 1. Imaging F_1 rotation through a gold bead [7]. The cylinder made of three α (blue) and three β (green) subunits was fixed on a glass surface, and a 40-nm gold bead was attached to the central γ subunit (red) through streptavidin and BSA (brown) that served as glue. When the bead was attached obliquely as shown in the figure, rotation of the γ subunit resulted in a circular movement of the bead image. The rotation angle was estimated from the circular trajectory of the bead movement.

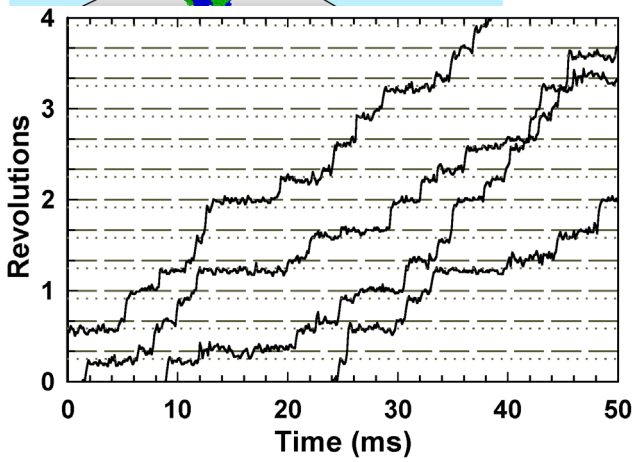

Figure 2. Substeps in the rotation of F1-ATPase at 20 μM ATP [7]. All curves are continuous, the latter curves being shifted to save space. Long dashed lines are drawn at intervals of 120°, and dotted lines are drawn 30° below the dashed lines.

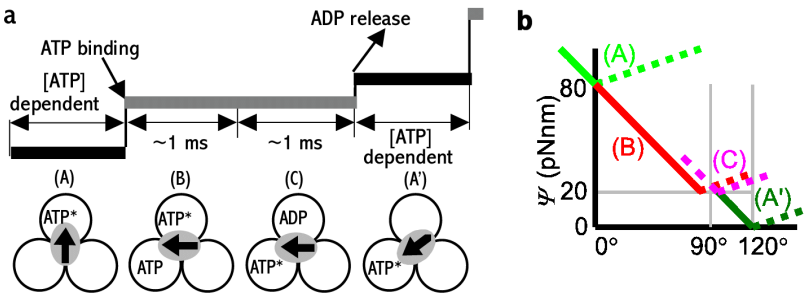

Figure 3. Kinetics of substeps (**a**) and deduced rotational potential (**b**). ATP* stands for ATP or ADP + phosphate; ADP may by phosphate or ADP + phosphate. Colored lines in **b** represent the angle-dependent potential energy for γ rotation. (A)-(A') are potential energies for the corresponding chemical states in **a**. Adapted from ref. 7.

nucleotide state (A) in Fig. 3a, the potential energy is minimal at the equilibrium angle, which is taken as 0°. Binding of a second ATP (B) produces a potential that is downhill towards the angle 90° ahead, with a constant slope indicating a constant torque. Upon the release of the last hydrolysis product, the potential energy returns to that in (A) except for the 120° difference; the potential in (A') is again linearly downhill at least over the interval between 90° and 120°.

That ATP binding drives the 90° substep implies, by the law of action and reaction, that the reversal of a 90° substep (B→A in Fig. 3a) would reduce the affinity for ATP of the β subunit on the left of the arrow in Fig. 3a. The magnitude of the affinity decrease can be estimated from the potential diagram in Fig. 3b, and is more than 2,000,000 fold [7]. Likewise, reversal of a 30° substep (A'→C) accompanies an increase of >100 fold in the affinity for ADP of the β subunit on the right of the arrow. These affinity changes can account for ATP synthesis when the γ subunit is forced to rotate clockwise in Fig. 3a (by the action of proton flow through ATP synthase). Starting from A' in Fig. 3a, the β subunit on the right of the arrow will pick up ADP from the medium when the γ subunit turns 30° clockwise. Further clockwise rotation by 90° will reduce the affinity for the previously synthesized ATP on the left of the arrow, and this ATP is released into the medium. This scheme indicated by our

experiment is an embodiment of the binding change mechanism for ATP synthesis proposed by Boyer many years ago [1].

ATP and (ADP + phosphate) are in equilibrium in the catalytic site, and thus synthesis or hydrolysis of ATP on the enzyme do not accompany significant change in free energy (synthesis and hydrolysis are freely reversible on the enzyme). Myosin also hosts ATP and (ADP + phosphate) in equilibrium. Many ATP-driven molecular machines may adopt this strategy: by binding ATP tightly, they stabilize the ATP form such that its free energy is comparable with that of ADP + phosphate. Then, much of the free-energy drop must occur in the ATP binding step. An efficient molecular machine should convert this maximal energy into work. The role of hydrolysis per se is mainly to reset the machine for the next round of cycle, by separating ATP into two entities and thereby allowing them to dissociate easily. For the completely reversible F_1 motor, hydrolysis may play an additional role of determining the sense of rotation [6,7].

Rotation of F_1-ATPase is also powered by GTP or ITP, but not by UTP or CTP [8]. Mechanical characteristics of GTP- or ITP-driven rotation appear to be quite similar to those of ATP-driven rotation. It thus seems that the protein contains in it a precisely preset rotary mechanism, which a nucleotide either enables to proceed or fails to activate.

References

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