Rotation of Escherichia coli F₁-ATPase

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By applying the same method used for F₁-ATPase (TF₁) from thermophilic Bacillus PS3 (Noji, H., Yasuda, R., Yoshida, M., and Kinoshita, K., J. r. (1997) Nature 386, 299–302), we observed ATP-driven rotation of a fluorescent actin filament attached to the γ subunit in Escherichia coli F₁-ATPase. The torque value and the direction of the rotation were the same as those observed for TF₁. F₁-ATPases seem to share common properties of rotation irrespective of the sources.

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Driven by a downhill flow of protons, F₀F₁-ATPase synthesizes ATP (1). It can also catalyze the reverse reaction, hydrolysis of ATP pumping protons in the opposite direction. ATP synthesis and hydrolysis occur at the membrane-protruding portion, F₁, with a subunit composition of αβγδε. Isolated F₁-ATPase exhibits strong ATP hydrolysis activity, hence called F₁-ATPase, and its αβγδ subcomplex is the minimum stable ATPase-active unit (2). F₀, the membrane-embedded portion, mediates the proton-translocation through the membrane. The γ subunit of F₁-ATPase is a major component of the “shaft” connecting F₁ to F₀ and is responsible for the exchange of energy between the two portions (3). Boyer proposed a rotational movement of γ subunit within F₁-ATPase during the coupling reaction (4). This rotational model gained strong support from a crystal structure of F₁-ATPase in which the central γ subunit is surrounded by an αβδγ cylinder (5). Biochemical and spectroscopic studies (6–8) suggested a rotation which was then proven by its direct observation in a single αβγδγ subcomplex of F₁-ATPase from thermophilic Bacillus PS3 (TF₁) (9). Attachment of a fluorescent actin filament to the γ subunit enabled us to observe its counterclockwise rotation (as viewed from F₀ side) under an inverted fluorescence microscope. The analysis of the rotation at low ATP concentrations revealed that this enzyme is a highly efficient stepping-motor with discrete 120-degree rotation driven by each ATP hydrolysis (10). To confirm that these properties of the rotation are common features irrespective of the source of the enzyme, we applied the same experimental approach to F₁-ATPase (EF₁) from Escherichia coli.

MATERIALS AND METHODS

Strain and plasmids. EF₁ containing 6 additional His residues (His-tag) at the N-termini of the three β subunits and a γK107C mutation (corresponding to γS107C of TF₁) for the rotation assay was obtained by expression of the plasmid pKH7 in E. coli strain DK8. pKH7 was constructed from plasmids pACWU1.2 (pHis-tag/Cys-less) (11) and pSK7 (pHis-tag) (12). γK109 in pACWU1.2 was substituted with Cys by PCR using oligonucleotide primers, 5'-CCG ACT GCC TTC AAG CCG ACC TTC A-3' and 5'-CCG ACT GCC TTC AAG CCG ACC TTC A-5'. 

Isolated F₁-ATPase (EF₁) at the N-termini of the three His-tag/Cys-less) was subcloned into pACWU1.2 to yield pKH3 (pHis-tag/K109C). pKH3 was constructed from plasmids pACWU1.2 and pSK7. A 660-bp DNA fragment coding for the N-terminal region of β (His-tag/C137S) was subcloned into pACWU1.2 to yield pKH4 (pHis-tag/Cys-less). The KpnI-PmeI fragment coding γK109C of pKH3 was subcloned into pKH4 to yield pKH7 (pHis-tag/K109C). In all cases, correct mutations were confirmed by DNA sequencing.

Protein preparations. E. coli strain DK8 was transformed with plasmid pKH7. Cells were collected at O.D₅₉₅ = 0.8. Membranes were purified as described by Wise (13). EF₁, was extracted by EDTA treatment and applied to an anion-exchange column (Tosoh Fractogel TSK-DEAE 650(S), Toyo Soda, J apan) equilibrated with buffer A (50 mM Tris·HCl, 2 mM EDTA, 10% (v/v) methanol, pH 7.8). EF₁ was eluted stepwise with 75 mM, 150 mM, and 500 mM Na₂SO₄, buffer B. The 75–150 mM Na₂SO₄ fraction containing EF₁ was diluted 4-fold with buffer A and applied to Ni-NTA agarose (Qiagen). EF₁ was eluted with 100 mM imidazole in buffer A. The yield of purified EF₁ was around 10 mg starting from a 8-liter culture. The purified EF₁ was stored at –80°C.

The torque was measured using a fluorescence microscope (14). The Δγ value was calculated by dividing the number of actin filaments rotated by the number of EF₁ molecules extracted from the membranes.

The Δγ value from EF₁ was 5% in comparison with 10% from TF₁.
was biotinylated at the sole Cys, γK109C and conjugated with streptavidin. Rabbit skeletal actin filaments were biotinylated and stained with phalloidin-tetramethylrhodamine B (9).

Rotation assay. Proteins were infused into a flow chamber constructed with two coverslips (10). A solution (0.5% 2-mercaptoethanol, 0.2 mg/ml glucose oxidase, 30 U/ml catalase, 33 mM glucose, 2 mM MgCl₂, and 2 mM MgATP) was infused into the flow chamber and observed on an inverted fluorescent microscope (IX70, Olympus, Japan) at 22°C. Images were recorded with an intensified CCD camera (ICCD-350F, Video Scope, USA) on an 8 mm video tape. Rotating filaments with the axis at one end were selected, and the rotation angle was calculated from the centroid of the filament image with an analyzing software, NIH image.

RESULTS AND DISCUSSION

Using a mutant EF₁ in which all Cys residues had been substituted by Ala (11), we introduced further mutations similar to the mutant TF₁ used previously (9), as follows. At the N-termini of β subunit, His-tags were added to immobilize EF₁ on Ni-NTA-coated coverslips. The γK109C mutation was introduced and the single Cys residue was biotinylated for the attachment of the actin filament through streptavidin. SDS–PAGE analysis indicated that our preparation of EF₁ had lost most of the δ subunit and the amount of the ε subunit was also substoichiometric (data not shown). Upon applying EF₁ to the rotation assay, the enzyme concentration would be less than nM, thus causing further loss of ε due to the nM range Kᵋ (14). The observed rotational properties would be, therefore, mainly derived from the αβγ subcomplex of EF₁.

EF₁ was infused into the flow chamber after conjugation with streptavidin. Then, we infused the actin filament into the flow chamber to attach it to the streptavidin-conjugated EF₁ immobilized on the surface of the coverslip and observed with the fluorescent microscope. In the presence of ATP, some of the actin filaments rotated continuously with the axis at one end of the filament (Fig. 1a). Rotating filaments with the axis near the middle were also observed (Fig. 1b). The direction of rotation was counterclockwise without exception, the same direction as observed for TF₁. The

FIG. 1. Sequential images of a rotating actin filament attached to the γ subunit in EF₁ at 2 mM ATP. (a) A rotating filament with the axis at one end. The filament length, 2.2 μm; rotational rate, 0.77 rps; time interval between images, 66 ms. (b) A rotating filament with the axis close to the middle of the filament. One length from the axis to tip, 1.2 μm; another length, 0.7 μm; rotational rate, 3.1 rps; time interval between images, 33 ms. Scale bar, 5 μm in a and b.

FIG. 2. Time-course of the rotation of the γ subunit. Images of the rotating actin filaments were recorded with the intensified CCD camera, and the centroids were analyzed. Each line represents the anti-clockwise rotation of one filament.
The time-courses of the rotation of individual actin filaments are shown in Fig. 2. The filaments rotated continuously for several minutes and the rotational rate was up to 6.7 revolutions/sec. As hydrolysis of one ATP is assumed to drive a 120-degree rotation (10), 83 revolutions/sec were expected from the ATPase activity (42 μmole/min/mg; 250 turn-over/sec) of the free (not immobilized) EF₁ in solution determined by Pi measurement. The reason for the slow rate of observed rotation might be the hydrodynamic friction against rotating actin filament that would limit the rotational velocity of the γ subunit (10). Longer actin filaments rotated slower (Fig. 3). Nevertheless, the rotational torque values calculated from the rotational rate and length of individual actin filaments were constantly at around 40 pNnm. This value well agrees with the value obtained for the rotation of TF₁ (10).

Now F₁-ATPases from two sources, E. coli and thermophilic Bacillus PS3, have shown rotation. They are powerful motors, which exert torques of 40 pNnm irrespective of the load. Rotation assay of EF₁ will be useful due to the wealth of genetic and physiological studies on EF₁. The rotation assay will be applied to mitochondrial and chloroplast F₁-ATPases in the near future. It will reveal common properties of rotational coupling of F₁,F0-ATPases as well as specific functions for each enzyme from different sources.

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REFERENCES