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Invited Minireview

Rotation of the γ Subunit in F_1-ATPase; Evidence That ATP Synthase Is a Rotary Motor Enzyme*

Ryohei Yasuda,[1] Hiroyuki Noji,[2] Kazuhiko Kinosita, Jr.,[1] Fumihiro Motojima,[2] and Masasuke Yoshida[2,3]

ATP-dependent, azide-sensitive rotation of the γ subunit relative to the $\alpha_3\beta_3$ hexagonal ring of ATP synthase was observed with a single molecule imaging system. Thus, ATP synthase is a rotary motor enzyme, the first ever found.

KEY WORDS: ATP synthase; F_1-ATPase; rotary motor; single molecule imaging; loose coupling.

INTRODUCTION

ATP synthase couples ATP synthesis and hydrolysis to proton electrochemical gradients in energy-transducing membranes. It is composed of two separable parts, a membraneous portion, F_o, which mediates proton translocation, and a peripheral portion, F_1-ATPase, which catalyzes ATP hydrolysis (Boyer, 1997). F_1-ATPase and F_o are connected by a relatively narrow stalk-like structure which can mediate energy exchange between ATP synthesis/hydrolysis at F_1-ATPase and the proton flow at F_o. A hypothetical rotational catalytic mechanism of ATP synthase, proposed by Boyer and his colleagues (Gresser *et al.*, 1982), gained support from the crystal structure of mitochondrial F_1-ATPase in which the coiled-coil α helices of the γ subunit extend from the $\alpha_3\beta_3$ subassembly into the stalk region (Abrahams *et al.*, 1994). Then, large motion of the γ subunit relative to the surrounding $\alpha_3\beta_3$ subassembly, interpreted as rotary motion, was suggested from cryoelectron microscopic (Gogol *et al.*, 1990), biochemical (Duncan *et al.*, 1996) and polarized spectroscopy techniques (Sabbert *et al.*, 1996). However, these results cannot discriminate ATP-induced rotational freedom of the γ subunit from unidirectional rotation and exclusive evidence has been awaited.

SETUP

It is very difficult to design an experiment to prove unidirectional rotation by observing average behaviors of a large number of F_1-ATPase molecules, therefore, we developed a method for imaging rotary motion of a single F_1-ATPase molecule as shown in Fig. 1. We used an engineered $\alpha_3\beta_3\gamma$ subcomplex of thermophilic F_1-ATPase from *Bacillus* strain PS3. In the crystal structure of mitochondrial F_1-ATPase, the N-termini of β subunits are opposite to the stalk region of the γ subunit that links F_1-ATPase to F_o. To fix the $\alpha_3\beta_3\gamma$ subcomplex on a glass plate, ten histidines (his-tag) linked to the N-termini of the β subunits were used, γ-Ser106, which is presumably in the exposed stalk region of the γ subunit, was replaced with Cys. α-Cys193 in the α subunit, the only Cys in the wild-type $\alpha_3\beta_3\gamma$ subcomplex, was replaced with Ser. The mutant $\alpha_3\beta_3\gamma$ subcomplex was expressed in *Escherichia coli*, and purified (Matsui and Yoshida, 1995). Its ATPase activity was the same as that of the wild-type subcomplex, 52 ATPs hydrolyzed per sec. The introduced Cys was reacted with biotin-maleimide. After a glass plate was coated with horseradish peroxidase conjugated with Ni-NTA, the $\alpha_3\beta_3\gamma$ subcomplex was fixed on it through his-tags of three β subunits. Fluorescently labeled, biotinylated actin filament was fixed to the γ subunit through streptavidin and

[1] Department of Physics, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kouhoku-ku, Yokohama 223, Japan.
[2] Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama 226, Japan.
[3] To whom correspondence should be addressed; e-mail: myoshida@res.titech.ac.jp.

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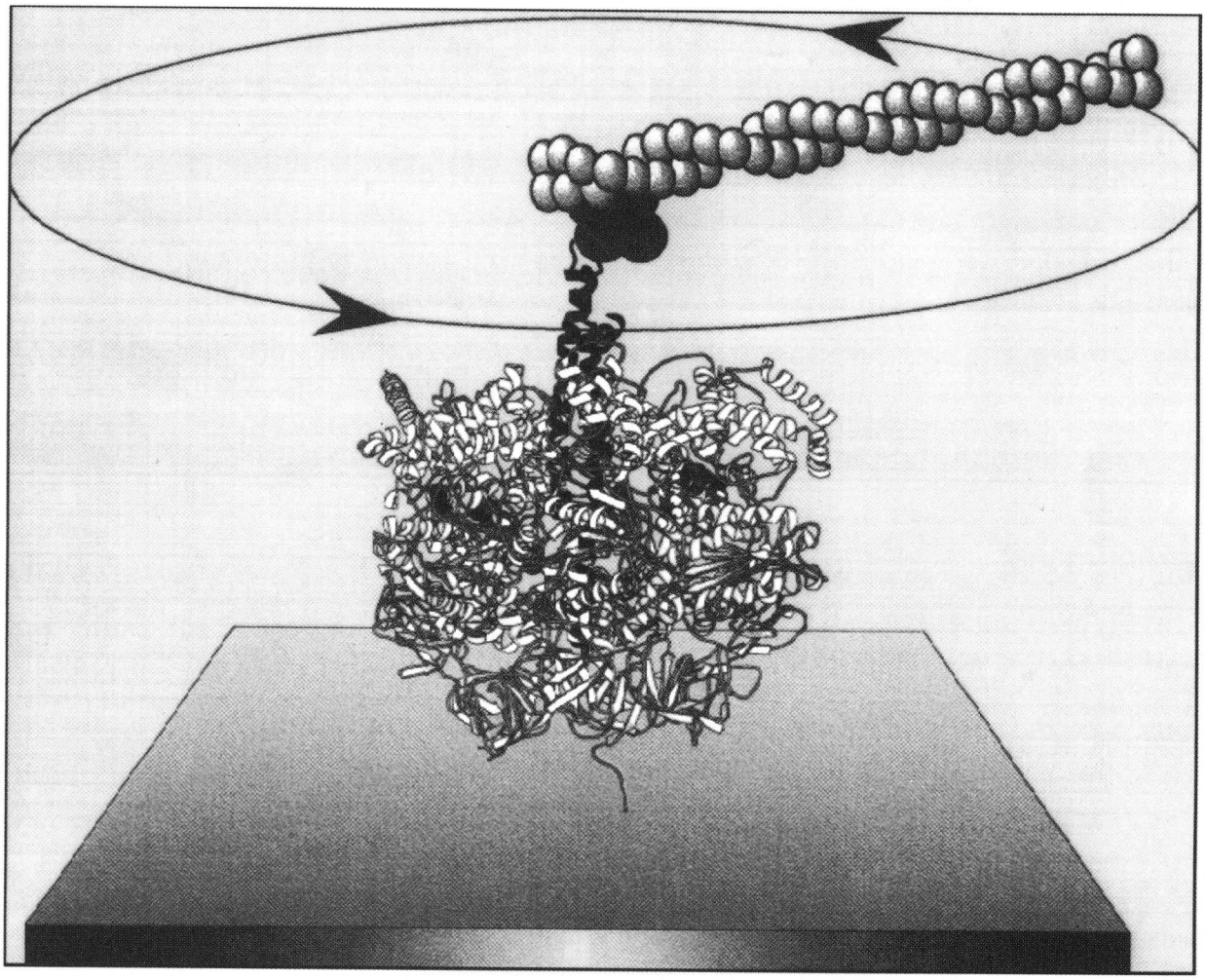

Fig. 1. Schematic illustration of fixed $\alpha_3\beta_3\gamma$ subcomplex of F_1-ATPase from thermophilic bacteria used for observation of the rotation of the γ subunit. The crystal structure of mitochondrial F_1-ATPase (Abrahams *et al.*, 1994) is adopted to represent the thermophilic $\alpha_3\beta_3\gamma$ subcomplex. His-tag's which are supposed to fix the subcomplex to the glass plate are not shown. An actin filament is attached to the γ subunit through biotin–streptavidin–biotin.

observed with an epifluorescent microscope (Noji *et al.* 1997).

ROTATION

Rotating actin filaments were found in the field of the microscope in the presence of 2 mM ATP. On average, one out of 70 filaments was rotating. Most of the rotating filaments had their rotation axis at one edge of the filament while others had the axis at the center region and rotated like a propeller. Some filaments rotated for more than 100 turns. This rotation means that the γ subunit is rotating in the center of the $\alpha_3\beta_3$ subassembly. Filaments rotated counterclockwise without exception. The $\alpha_3\beta_3\gamma$ subcomplex was fixed to the glass plate in an "upside-down" direction and the F_o side of the intact ATP synthase was above the glass plate. Therefore, counterclockwise rotation of the filaments corresponds to the counterclockwise rota-

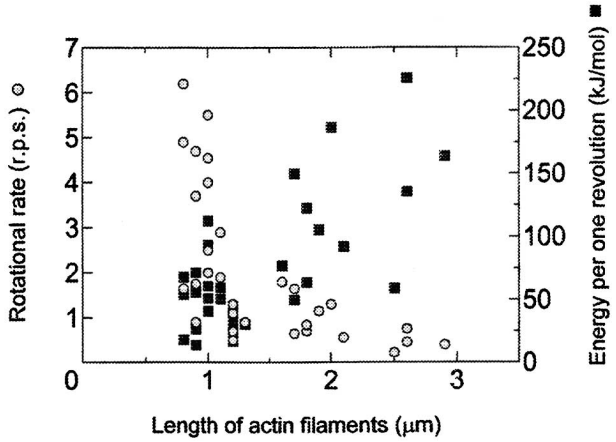

Fig. 2. Rotational rate and calculated energy required for one revolution for each filament plotted as a function of actin filament length. Note that $\Delta G^{0\prime}$ of ATP hydrolysis is ~30 KJ/mol.

tion of the γ subunit in the center of ATP synthase when viewed from the F_o side. Thus, ATP hydrolysis drives the rotation in such a direction that, in the crystal structure, the γ subunit interacts sequentially with the three forms of β subunits in this order: empty → ADP → AMP–PNP. This sequence is equivalent to the catalytic transition in one β subunit in the order, AMP–PNP → ADP → empty forms, the order expected in the ATP hydrolysis reaction. Rotary motion was often paused at the same angle probably obstructed by nearby proteins. The rate of rotation was different from one filament to another but, for each filament, it was usually almost constant. In the absence of ATP, no rotary motion was observed except one or two rare revolutions of some actin filaments by Brownian motion. Azide is an inhibitor of ATPase activities of F_1-ATPase and the $\alpha_3\beta_3\gamma$ subcomplex, and no rotating actin filament was found when sodium azide was added to the ATP solution. The result described here provides evidence that ATP synthase is a rotary motor enzyme, the first ever found. Only the bacterial flagella motor has been shown to be a rotary protein machine in the biological world, but it is not an enzyme.

ENERGETICS

The fastest rate of rotation observed was about 6 revolutions per sec (r.p.s.) (Fig. 2). If three ATP's are hydrolyzed per one revolution and specific activity of the fixed subcomplex is the same as that of the unfixed subcomplex, the $\alpha_3\beta_3\gamma$ subcomplex used in this experiment should turn at 17 r.p.s. Hydrodynamic friction against the rotating filament could be responsible for the observed slow rotation. Indeed, the rotation rates of longer filaments are slower in general when compared with those of shorter filaments (Fig. 2). The slower movement under higher load suggests that mechanical performance of rotary motion of the F_1-ATPase is continuously variable. A calculation predicts that energy required for one revolution of some filaments exceeds 150 kJ/mol. Note that $3 \times \Delta G^{0\prime}$ of ATP hydrolysis is ~90 kJ/mol. These features of the rotation raise a possibility that ATP hydrolysis and the rotation are "loosely" coupled in F_1-ATPase and hence in ATP synthase. It is obvious that we need more definite experiments to evaluate several factors such as probable slippage between γ subunit and the filament, actual ΔG value of ATP hydrolysis, and viscosity of water near the glass surface.

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