Rotation mechanism of V_1-ATPase

Vacuolar ATPases (V-ATPases) function as proton pumps, which are involved in many processes such as bone resorption and cancer metastasis, and these membrane proteins represent attractive drug targets for osteoporosis and cancer. Their structures resemble those of F-ATPases, which function as ATP synthase in mitochondria, chloroplasts and bacteria. These ATPases consist of a hydrophilic portion (F_1 and V_1) and a membrane-embedded ion-transporting portion (F_o and V_o), and have a similar reaction mechanism that occurs through rotation. F_1-ATPase has been investigated in detail, and the molecular mechanism has been proposed on the basis of crystal structures of the complex [1], and extensive single-molecule observation of the rotation [2]. Similar V_1-ATPase experiments have been conducted using the *Thermus thermophiles* enzyme, which functions physiologically as an ATP synthase. The crystal structures of the A_3B_3DF (V_1) complex at low resolution suggest differences in its structure and interactions compared with F_1-ATPases [3]. Single-molecule analyses of V_1-ATPase also suggest differences in torque generation and the coupling scheme of the rotation mechanism compared with F_1 [4]. *Enterococcus hirae* V-ATPase acts as a primary ion pump similar to eukaryotic V-ATPase, which transports Na^+ or Li^+, instead of H^+ ions. Recently, we have succeeded in obtaining the crystal structures of A_3B_3 and V_1-ATPase complexes at high resolution, which enabled the generation of a model of the rotational mechanism.

We determined the crystal structures of the nucleotide-free (2.8 Å) A_3B_3 (denoted as eA_3B_3) and the AMP-PNP-bound (3.4 Å) A_3B_3 (denoted as bA_3B_3) using beamline **BL41XU** (Fig. 1(a,c)) [5]. In eA_3B_3, one of the three A subunits adopts a closed conformation (denoted as A_C), which shifts the structure into the center of the A_3B_3 ring, whereas the other two A subunits adopt similar open conformations (denoted as A_O and $A_{O'}$) (Fig. 1(b)). Similarly, one of the three B subunits shows a closed conformation (denoted as B_C) compared with the others (denoted as B_O and $B_{O'}$). The conserved nucleotide-binding sites are located between the three different combinations: A_OB_C, $A_{O'}B_O$, and $A_CB_{O'}$ pairs. On the other hand, in bA_3B_3, AMP-PNP:Mg molecules bind at two $A_CB_{O'}$ each, and not at the other A_OB_C (Fig. 1(d)).

We designated these A_OB_C pairs as the 'empty' form on the basis of their apparent very low affinity for AMP-PNP:Mg, and these $A_CB_{O'}$ pairs as the 'bound' form take the ATP-bound form. The $A_{O'}B_O$ in eA_3B_3 seems to change to $A_CB_{O'}$ upon binding with AMPPNP:Mg. We designated this unique $A_{O'}B_O$ pair of eA_3B_3 as the 'bindable' form. These asymmetries suggest that the formation of the A_3B_3 hexamer ring imposes a restriction on the AB pair and induces conformational changes that cooperatively generate one empty (ATP-unbound form), one bindable (ATP-accessible form), and one bound (ATP-bound form) conformation, which in turn determine the order of nucleotide binding in the ring in the right-handed rotational orientation viewed from the top of the V_1 complex.

Next, we crystallized and solved the crystal structure of the nucleotide-free (2.2 Å) V_1-ATPase (denoted as eV_1) (Fig. 2(a)) and the AMP-PNP-bound (2.7 Å) V_1-ATPase (denoted as bV_1). A and B subunits assembled asymmetrically and a central axis composed of D and F subunits penetrated into the cavity of the A_3B_3 hexamer.

The structures of eA_3B_3 and eV_1, which should have been induced by interaction with the DF complex, are compared. The eV_1 has an empty form (A_OB_C) and a bound form ($A_CB_{O'}$), but the bound form of eV_1 is positioned as the site of the bindable form of eA_3B_3 when both empty forms are superimposed. Therefore, the DF binding seems to induce a change from the bindable eA_3B_3 to the bound form, similar to the conformational changes of the eA_3B_3 induced by AMP-PNP binding. The remaining AB pair of eV_1 represents a more tightly packed structure composed of closer A and B subunit conformations approaching the center of the A_3B_3 ring. This is not observed in the structure of the A_3B_3 complex (Fig. 2(b)). We designated the

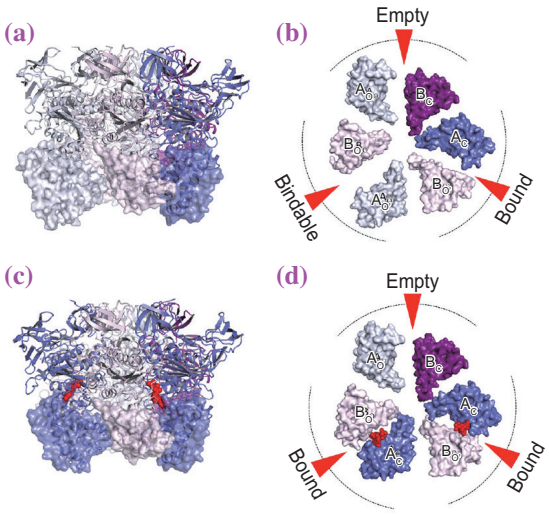

Fig. 1. Structure of the A_3B_3 complex. **(a)** Side view of the nucleotide-free A_3B_3 structure (eA_3B_3). **(b)** Top view of the C-terminal domain (shown in (a) as a transparent surface) of eA_3B_3 from the N-terminal β-barrel side. Red triangles indicate the nucleotide-binding sites. **(c)**-**(d)** Structures of the AMP-PNP-bound A_3B_3 complex (bA_3B_3).

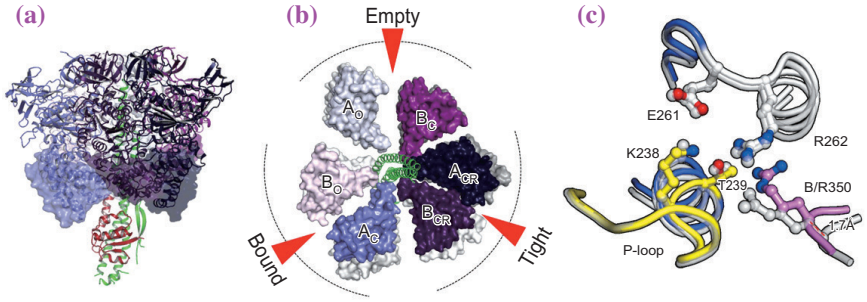

Fig. 2. Structure of the A_3B_3DF complex. **(a)** Side view of the nucleotide-free A_3B_3DF structure (eV_1). **(b)** Top view of the C-terminal domain of eV_1, as in Fig. 1(b), superimposed at the empty form onto that of transparent eA_3B_3 (gray). **(c)** Nucleotide-binding sites. Tight form in eV_1 (color) and bound form in eA_3B_3 (gray).

new conformations as the 'tight' form ($A_{CR}B_{CR}$), and DF complex binding seems to change the bound form of eA_3B_3 to the tight form. In bV_1, the overall structure of bV_1 was similar to that of eV_1, although the binding sites of bound and tight forms were occupied with AMP-PNP:Mg.

In the tight form, the position of the conserved Arg-finger (Arg350 of B_{CR}), which helps ATP hydrolysis, was closer to the nucleotide-binding site than that in the bound form (Fig. 2(c)). Thus, the ATP hydrolysis is stimulated by this approach triggered by the movement of the Arg-finger, which is induced by binding between the DF complex and A_3B_3.

On the basis of these asymmetric structures, a rotation model of V_1 was proposed (Fig. 3). The V_1-ATPase is bound to two ATP:Mg molecules in the bound and tight forms (Fig. 3(a)). Bound ATP in the tight form is awaiting ATP hydrolysis. When ATP is hydroid in the tight form, V_1-ATPase starts the rotary reaction. The conformation of the A_3B_3 part in V_1-ATPase may return to eA_3B_3 in a cooperative manner (Fig. 3(b)). Thus, while the tight form changes to the empty form with the release of ADP and phosphate, the empty form changes to the bindable form. However, the interaction between the DF and the tight form might prevent these structural changes, and an intermediate state may exist instead of the state of eA_3B_3. After that, the new ATP molecule binds to the bindable form, and the conformation changes to the bound form. Thus, the state may be similar to that of bA_3B_3 (Fig. 3(c)). Then, the DF rotates, and the bound form from the beginning changes to the next tight form vie interaction with the DF complex. In brief, the V_1-ATPase returns to the initial state (Fig. 3(d)).

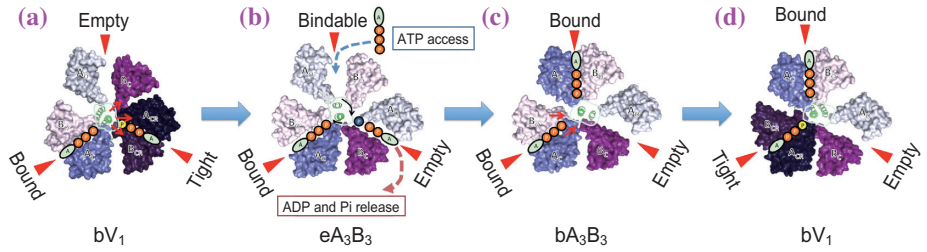

Fig. 3. Rotation model of V_1-ATPase. Top view of the C-terminal domain viewed as in Figs. 1(b, d) and Fig. 2(b). ATP with yellow "P" in (a) and (d) represents an ATP molecule that is committed to hydrolysis. The blue "P" in (b) represents a phosphate molecule after ATP hydrolysis. **(a)** The AMP-PNP-bound V_1: first two ATPs are bound in the bound and tight forms. The reaction is triggered by the ATP hydrolysis in the tight form. **(b)** The nucleotide-free A_3B_3: by the conversion to ADP and phosphate, the conformation of the A_3B_3 part in V_1-ATPase may return to eA_3B_3 (ground structure of A_3B_3complex) in a cooperative manner. The tight form changes to the empty form with the release of ADP and phosphate and the empty form changes to the bindable form. **(c)** The AMP-PNP-bound A_3B_3: by new ATP binding to the bindable form, the conformation changes to bA_3B_3, which has two bound forms with two ATP, and then the DF rotates. **(d)** The bound form from the beginning changes to the next tight form, induced by DF binding, and the V_1-ATPase returns to the initial state with 120° rotation.

Kano Suzuki[a,*] and Takeshi Murata[a,b]

[a] Department of Chemistry, Chiba University
[b] JST/PRESTO

*E-mail: r2sd3103@chiba-u.jp

References

[1] J. P. Abrahams *et al.*: Nature **370** (1994) 621.
[2] H. Noji *et al.*: Nature **386** (1997) 299.
[3] N. Numoto *et al.*: EMBO Rep. **10** (2009) 1228.
[4] H. Imamura *et al.*: Proc. Natl. Acad. Sci. USA **102** (2005) 17929.
[5] *S. Arai, S. Saijo, K. Suzuki, K. Mizutani, Y. Kakinuma, Y. Ishizuka-Katsura, N. Ohsawa, T. Terada, M. Shirouzu, S. Yokoyama, S. Iwata, I. Yamato and T. Murata: Nature* ***493*** *(2013) 703.*