Rotation mechanism of V₁-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 (F₁ and V₁) and a membrane-embedded ion-transporting portion (F₀ and V₀), and have a similar reaction mechanism that occurs through rotation. F₁-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 V₁-ATPase experiments have been conducted using the Thermus thermophiles enzyme, which functions physiologically as an ATP synthase. The crystal structures of the A₃B₃DF (V₁) complex at low resolution suggest differences in its structure and interactions compared with F₁-ATPases [3]. Single-molecule analyses of V₁-ATPase also suggest differences in torque generation and the coupling scheme of the rotation mechanism compared with F₁ [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 A₃B₃ and V₁-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) A₃B₃ (denoted as eA₃B₃) and the AMP-PNP-bound (3.4 Å) A₃B₃ (denoted as bA₃B₃) using beamline BL41XU (Fig. 1(a,c)) [5]. In eA₃B₃, one of the three A subunits adopts a closed conformation (denoted as Aₒ), which shifts the structure into the center of the A₃B₃ ring, whereas the other two A subunits adopt similar open conformations (denoted as A₀ and Aₒ) (Fig. 1(b)). Similarly, one of the three B subunits shows a closed conformation (denoted as Bₒ) compared with the others (denoted as B₀ and Bₒ). The conserved nucleotide-binding sites are located between the three different combinations: A₀Bₒ, A₀Bₒ, and AₒBₒ pairs. On the other hand, in bA₃B₃, AMP-PNP:Mg molecules bind at two AₒBₒ each, and not at the other AₒBₒ (Fig. 1(d)).

We designated these AₒBₒ pairs as the ‘empty’ form on the basis of their apparent very low affinity for AMP-PNP:Mg, and these AₒBₒ pairs as the ‘bound’ form take the ATP-bound form. The AₒBₒ in eA₃B₃ seems to change to AₒBₒ upon binding with AMPPNP:Mg. We designated this unique AₒBₒ pair of eA₃B₃ as the ‘bindable’ form. These asymmetries suggest that the formation of the AₒBₒ 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 V₁ complex.

Next, we crystallized and solved the crystal structure of the nucleotide-free (2.2 Å) V₁-ATPase (denoted as eV₁) (Fig. 2(a)) and the AMP-PNP-bound (2.7 Å) V₁-ATPase (denoted as bV₁). A and B subunits assembled asymmetrically and a central axis composed of D and F subunits penetrated into the cavity of the A₃B₃ hexamer.

The structures of eA₃B₃ and eV₁, which should have been induced by interaction with the DF complex, are compared. The eV₁ has an empty form (AₒBₒ) and a bound form (AₒBₒ), but the bound form of eV₁ is positioned as the site of the bindable form of eA₃B₃ when both empty forms are superimposed. Therefore, the DF binding seems to induce a change from the bindable eA₃B₃ to the bound form, similar to the conformational changes of the eA₃B₃ induced by AMP-PNP binding. The remaining AB pair of eV₁ represents a more tightly packed structure composed of closer A and B subunit conformations approaching the center of the A₃B₃ ring. This is not observed in the structure of the A₃B₃ complex (Fig. 2(b)). We designated the

![Fig. 1. Structure of the A₃B₃ complex. (a) Side view of the nucleotide-free A₃B₃ structure (eA₃B₃). (b) Top view of the C-terminal domain (shown in (a) as a transparent surface) of eA₃B₃ from the N-terminal β-barrel side. Red triangles indicate the nucleotide-binding sites. (c)-(d) Structures of the AMP-PNP-bound A₃B₃ complex (bA₃B₃).](image-url)
new conformations as the ‘tight’ form \((A_{CR}B_{CR})\), and DF complex binding seems to change the bound form of \(eA_{3}B_{3}\) 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 \(A_{3}B_{3}\).

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 hydrolyzed in the tight form, \(V_{1}\)-ATPase starts the rotary reaction. The conformation of the \(A_{3}B_{3}\) part in \(V_{1}\)-ATPase may return to \(eA_{3}B_{3}\) in a cooperative manner (Fig. 3(b)). Thus, the tight form exchanges 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 \(eA_{3}B_{3}\). 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 \(bA_{3}B_{3}\) (Fig. 3(c)). Then, the DF rotates, and the bound form from the beginning changes to the next tight form via interaction with the DF complex. In brief, the \(V_{1}\)-ATPase returns to the initial state (Fig. 3(d)).

![Fig. 2. Structure of the \(A_{3}B_{3}DF\) complex. (a) Side view of the nucleotide-free \(A_{3}B_{3}DF\) 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 \(eA_{3}B_{3}\) (gray). (c) Nucleotide-binding sites. Tight form in \(eV_{1}\) (color) and bound form in \(eA_{3}B_{3}\) (gray).](image)

![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 \(A_{3}B_{3}\): by the conversion to ADP and phosphate, the conformation of the \(A_{3}B_{3}\) part in \(V_{1}\)-ATPase may return to \(eA_{3}B_{3}\) (ground structure of \(A_{3}B_{3}\)-complex) 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 \(A_{3}B_{3}\): by new ATP binding to the bindable form, the conformation changes to \(bA_{3}B_{3}\), 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.](image)

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