Rotation Mechanism of V_1-ATPase

Vacuolar ATPases (V-ATPases) function as ATP-driven ion pumps, which are located on membranes of various living organisms. Human V-ATPase is a promising drug target for osteoporosis and cancer metastasis. V_1-ATPase (the hydrophilic part of V-ATPase) is a rotary motor, in which a central stalk DF complex rotates inside a catalytic A_3B_3 complex with the ATP hydrolysis. We have solved the A_3B_3 complex and the V_1-ATPase from the bacterium *Enterococcus hirae*, and have proposed a model of the rotation mechanism based on their asymmetric structures.

The ion-translocating rotary ATPases (V-ATPases and F-ATPases) have evolved from a common ancestral enzyme. F-ATPases in mitochondria, chloroplasts and oxidative bacteria function as ATP synthase (ATP is the major energy currency of life) and V-ATPases in acidic organelles and plasma membranes of eukaryotic cells function as H^+ pumps. These ATPases have similar overall structures that consist of a hydrophilic portion (F_1 and V_1) and a membrane-embedded ion-transporting portion (F_o and V_o), and they have a similar reaction mechanism that occurs through rotation. *E. hirae* V-ATPase transports Na^+ or Li^+, instead of H^+. This is composed of nine subunits that are homologous to the corresponding subunits of eukaryotic enzymes. The catalytic part of the V_1 consists of a hexameric arrangement of alternating A- and B-subunits (A_3B_3), and V_1-ATPase is composed of A_3B_3 and central stalk DF complex.

We determined the crystal structures of the nucleotide-free A_3B_3 (eA_3B_3) and AMPPNP-bound A_3B_3 (bA_3B_3) at first by using BL41XU, SPring-8 [1]. Both of the A_3B_3 hexamers assembled asymmetrically, but different combinations of conformation were contained. In eA_3B_3, one of the three A adopts a closed conformation (A_C), which shifts the structure into the center of the A_3B_3, whereas the other two A adopt similar open conformations (A_O and $A_{O'}$), Similarly, one of the three B shows a closed conformation (B_C) compared to the others (B_O and $B_{O'}$) (Fig. 1**a**-**d**). The conserved nucleotide-binding sites were located between the three different combinations: A_OB_C, $A_{O'}B_O$ and $A_CB_{O'}$ pairs. In bA_3B_3, AMPPNP molecules were bound at two $A_CB_{O'}$ each, and not at the other A_OB_C (Fig. 1 **e**-**h**).

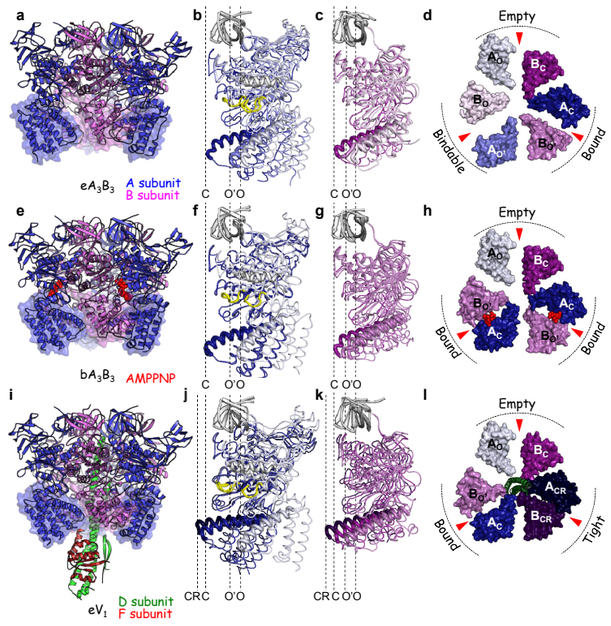

Figure 1: Structure of the A_3B_3 and A_3B_3DF complexes **a**, Side view of the nucleotide-free A_3B_3 structure (eA_3B_3). **b**, **c**, Superimposed structures at the N-terminal β-barrel (white) of the three structures of A (b) and B (c) in eA_3B_3. Open (O and O') and closed (C) conformations of A and B are shown in light and darker colors, respectively. **d**, Top view of the C-terminal domain (shown in **a** as transparent surface) of eA_3B_3 from the N-terminal β-barrel side. Red triangles indicate the nucleotide-binding sites. **e**-**h**, Structures of the AMPPNP-bound A_3B_3 complex (bA_3B_3), and **i**-**l**, Structures of the nucleotide-free A_3B_3DF complex (eV_1) viewed and colored as in **a**-**d**.

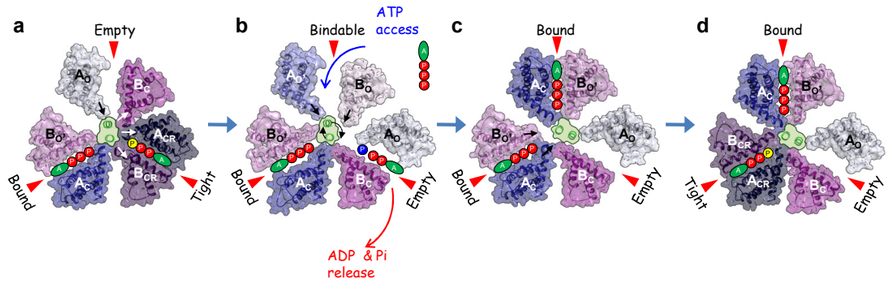

Figure 2 : Rotation model of V_1-ATPase. Top view of the C-terminal domain viewed as in Fig. 1 **d**, **h** and **l**. ATP with yellow "P" in **a** and **d** represents an ATP molecule that is committed to hydrolysis. The blue "P" in **b** represents a phosphate molecule after ATP hydrolysis. **a**, The AMPPNP-bound V_1: Two ATPs are bound in the "Bound" and "Tight" forms at first. 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_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 AMPPNP-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.

Compared with conformations of these pairs, we designated A_OB_C and $A_CB_{O'}$ as the "Empty" and "Bound" form, respectively. The $A_{O'}B_O$ in eA_3B_3 seemed to change to $A_CB_{O'}$ by binding with the AMPPNP molecule. We designated this unique $A_{O'}B_O$ of eA_3B_3 as the "Bindable" form. These asymmetries suggest that the formation of A_3B_3 imposes a restriction on the AB to induce 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 determines the order of nucleotide binding.

Next, we determined the crystal structures of the V_1-ATPase (A_3B_3DF) by using BL-1A, AR-NE3A and AR-NW12A. The asymmetry of A_3B_3DF was increased. By the interaction with the DF complex, the "Bindable" form ($A_{O'}B_O$) of eA_3B_3 changes to the "Bound" form ($A_CB_{O'}$) of V_1 and the "Bound" form (A_CB_O) of eA_3B_3 changes to the new "Tight" form ($A_{CR}B_{CR}$) of V_1. The new "Tight" form is composed of closer conformations of A and B (A_{CR} and B_{CR}, respectively). In the "Tight" form, the position of conserved Arg350 on B_{CR}, which helps ATP hydrolysis, seemed to approach the nucleotide binding site. The ATP hydrolysis is stimulated by this approach triggered by the movement of Arg350, which is induced by binding between the DF complex and A_3B_3.

Based on these asymmetric structures, a rotation model of V_1-ATPase was proposed. In AMPPNP-bound V_1 (Fig. 2 **a**), two ATP molecules are bound in the "Bound" and "Tight" form at first. The "Tight" form with the ATP molecule is presumed to be the hydrolysis awaiting state, therefore the ATP hydrolysis in the "Tight" form initiates the rotary reaction. 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_3) 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. However, the interaction between the DF and "Tight" form might prevent these structural changes, and an intermediate state may exist instead of the state of Fig. 2 **b**. After that, the new ATP molecule approaches and binds to the "Bindable" form, and the conformation changes to the "Bound" form; as a result, the A_3B_3 portion has two "Bound" forms with two ATP molecules and one "Empty" form, the structure of which corresponds to bA_3B_3, and then the DF rotates (Fig. 2 **c**). At last, the "Bound" form from the beginning changes to the next "Tight" form, induced by DF binding (Fig. 2 **d**) and the V_1-ATPase returns to the initial state (Fig. 2 **a**).

REFERENCE

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BEAMLINES

BL-1A, AR-NE3A and AR-NW12A

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