Crystal Structure of the Rotor from V-ATPase Molecular Motor

Vesicular ATPase (V-ATPase) is an ion-translocating rotary motor found in the membranes of acidic organelles and plasma membranes of eukaryotic cells. ATP hydrolysis causes the rotation of the central rotor complex, which is composed of the central stalk subunits (D-, F-, and d-subunits) and a membrane-embedded c-ring. We have determined the crystal structure of DF complex from Enterococcus hirae and assessed subunit–subunit interactions between V₁-ATPase subunits. The long coiled-coil motif of the D-subunit was similar to the motifs of other known rotary complexes. The short β-hairpin region of the D-subunit seems to be preserved in V₁-ATPase and further involved in the regulation of V-ATPases.


Figure 1
Schematic model of Enterococcus hirae V₁-ATPase. The DF complex is shown by the dotted line. Na⁺ is transported by the pathway which consists of the α-subunit and the c-ring, from cytoplasm to periplasm.

Figure 2
Crystal structure of the DF complex of Enterococcus hirae V₁-ATPase. (A) Cartoon representation of the DF complex. D- and F-subunits are shown in green and dark red, respectively. (B) D-subunit is presented in blue to red from the N to C terminus. The β-hairpin region is shown by the dotted box.

We have determined the crystal structure of the DF complex from Enterococcus hirae at 2.0 Å resolution [1]. The structure of D comprises a long pair of N- and C-terminal helices (approximately 100 Å), which are twisted into a left-handed coiled-coil, with a short β-hairpin region (residues 89–108) [Fig. 2 (A, B)]. The coiled-coil structure seems to be conserved in A₁-ATPase and eukaryotic V₁-ATPase from sequence alignment, and is similar to both the γ-subunit of bovine F₁-ATPase and FIJ (a component of the flagellar type III protein export apparatus) with low sequence similarity. Thus, the left-handed coiled-coil structure seems to be conserved in these rotary complexes, but the short β-hairpin region of D is unique and seems to be important for specific functions of V₁-ATPases. To evaluate this assumption, we prepared a deletion mutant that lacked the β-hairpin region with F, and further tested the binding affinity of the mutant DF to A₁β₃ and its effect on the ATPase activity. As a result, it was found that the β-hairpin region is not essential for ATPase activity, but it stimulates the activity by approximately two-fold. Additionally, we assessed the subunit–subunit interactions between V₁-ATPase and the d-subunit by using the Biacore system (surface plasmon resonance). The binding affinity (K₈ = 82 nM) of d and DF was lower compared with other subunit–subunit interactions (A₁β₃-D, K₈ = 0.8 nM; A₁β₃-D-F, K₈ = 3.2 nM). Previous studies on eukaryotic V₁-ATPases showed that the enzyme was regulated by the reversible binding of V₁-ATPase. The weak interaction between the DF complex and the d-subunit might be responsible for the regulation of V-ATPases.

In the present study, the β-hairpin region involved in the regulation of V-ATPase was identified. This region seems to be conserved also in eukaryotic V-ATPase. Therefore, our results might help to elucidate the regulatory mechanism of V-ATPase.

REFERENCE

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