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Review

Infrared spectroscopic studies on the V-ATPase ☆

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ABSTRACT

V-ATPase is an ATP-driven rotary motor that vectorially transports ions. Together with F-ATPase, a homologous protein, several models on the ion transport have been proposed, but their molecular mechanisms are yet unknown. V-ATPase from *Enterococcus hirae* forms a large supramolecular protein complex (total molecular weight: ~700,000) and physiologically transports Na^+ and Li^+ across a hydrophobic lipid bilayer. Stabilization of these cations in the binding site has been discussed on the basis of X-ray crystal structures of a membrane-embedded domain, the K-ring (Na^+ and Li^+ bound forms). Sodium or lithium ion binding-induced difference FTIR spectra of the intact *E. hirae* V-ATPase have been measured in aqueous solution at physiological temperature. The results suggest that sodium or lithium ion binding induces the deprotonation of Glu139, a hydrogen-bonding change in the tyrosine residue and rigid α-helical structures. Identical difference FTIR spectra between the entire V-ATPase complex and K-ring strongly suggest that protein interaction with the I subunit does not cause large structural changes in the K-ring. This result supports the previously proposed Na^+ transport mechanism by V-ATPase stating that a flip-flop movement of a carboxylate group of Glu139 without large conformational changes in the K-ring accelerates the replacement of a Na^+ ion in the binding site. This article is part of a Special Issue entitled: Vibrational spectroscopies and bioenergetic systems.

1. Introduction

Biological systems contain various active transport machineries, where uni-directional transports of ions and substrates are achieved across the membrane [1–6]. Mechanism of such molecular machineries is one of the central questions in bioenergetics. Transporters and pumps are membrane proteins, which include specific pathway of ions and substrates. However, the pathways cannot be fully connected between the two sides of the membrane, because the gradient formed by active transport will be collapsed. This is an important aspect in distinguishing transporters and pumps from channels. The former needs energy input, which ensures the uni-directionality of transport across the membrane. Light and chemical energies by specific chemical reactions are used to drive these molecular machines. In particular, the energy gained by ATP hydrolysis is ubiquitously used in biological systems.

V-ATPases couple ion movement with ATP hydrolysis, whose structure and mechanism resemble those of F-ATPases [7]. V-ATPases have a globular catalytic domain, V_1 (equivalent to F_1), where ATP is hydrolyzed. This domain is attached by central and peripheral stalks to the intrinsic membrane domain, V_o (equivalent to F_o), which pumps ions across the membrane. It is generally accepted that electrostatic interaction at the glutamate (aspartate) residues within the H^+ (or Na^+)-binding sites of the c-ring and the conserved arginine residue of the a-subunit of V_o (or F_o) is crucial for ion-transport mechanism. Several ion transporting mechanism models of F-ATPases have been reported before obtaining the precise structure of the F_o part. First, Junge et al. have proposed 'two-half-channel' model [8]. In the ion pumping mode, rotation of the c-ring by ATP hydrolysis energy via F_1-ATPase brings an occupied ion-binding site into the interface between c-ring and a-subunit. The a-subunit provides for 'exit' and 'entry' of the ions via aqueous half-channels to periplasm and from cytoplasm, respectively (Fig. 1a). Second, NMR studies of the monomeric c-subunit in organic solvent mixtures at various pH values have suggested that the process of pumping ions involves large conformational changes which involve swiveling of the outer helix of subunit c of *Escherichia coli* F-ATPase (Fig. 1b) [9]. Third, 'single channel' model has also been proposed for the F-ATPase from *Propionigenium modestum*, where the Na^+-binding site is placed close to the membrane center, but Na^+ can exchange through an intrinsic c-ring channel connecting the ion-binding sites to the cytoplasm (Fig. 1c) [10]. In the pumping mode, the bound ion of

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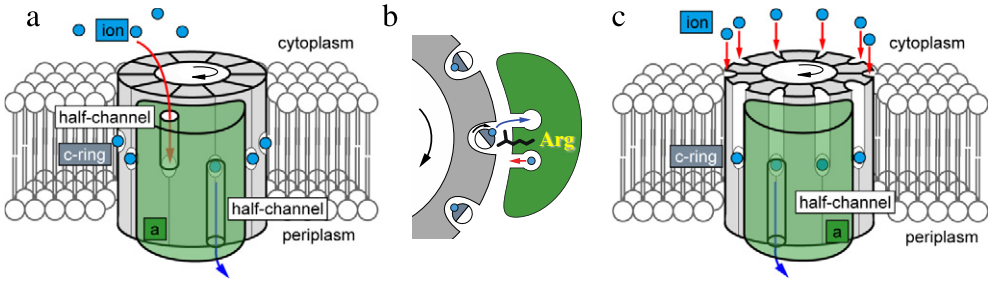

Fig. 1. Schematic models of ion-transporting mechanism of F-ATPases. The direction of the rotation in these models as shown in black arrows is in the pumping mode but not in ATP synthesis mode. Ions, c-ring and a-subunit are shown in blue spheres, gray and green cartoons, respectively. (a) 'Two-half-channel' model; side view (b) 'helix swiveling' model; top view from the cytoplasm at the level of the ion-binding sites (c) 'single-channel' model; side view. See text for details.

the c-ring releases at a half-channel to the periplasm of a-subunit between the interfaces. Recent progresses suggest that the 'two-half-channel' model without swiveling of the helix of c-ring is most favorable [11,12], but the precise mechanism of ion translocation by F (or V)-ATPase remains unclear because of the lack of the atomic structure of the membrane protein complex.

V-ATPase from *Enterococcus hirae* physiologically transports Na^+ and Li^+ [13]. This enzyme is encoded by nine *ntp* genes (*ntp*FIKECGABD) organized in the *ntp* operon [14]. Amino acid sequences of Ntp-F, -I, -K, -E, -C, -G, -A, -B, and -D were homologous with those of the subunits G, a, c, E, d, F, A, B, and D of eukaryotic V-ATPases, respectively [15]. The V_1 domain responsible for ATP-driven rotation consists of the Ntp-A, -B, -C, -D, -E, and -G subunits (Fig. 2). In V_1, the three A subunits and the three B subunits are arranged alternately around a central D subunit. V_o domain, which utilizes the rotation of V_1 for the transport of Na^+ (or Li^+), is composed of oligomers of 16-kDa NtpK, which form a membrane rotor ring (K-ring), and a single copy of the NtpI subunit. The V_1 and V_o domains are connected by a central stalk, which is composed of NtpD, NtpG, and NtpC subunits, and two peripheral stalks, which are composed of NtpE and NtpF subunits of V_1. ATP hydrolysis induces the rotation of the central stalk and the attached K-ring, which causes

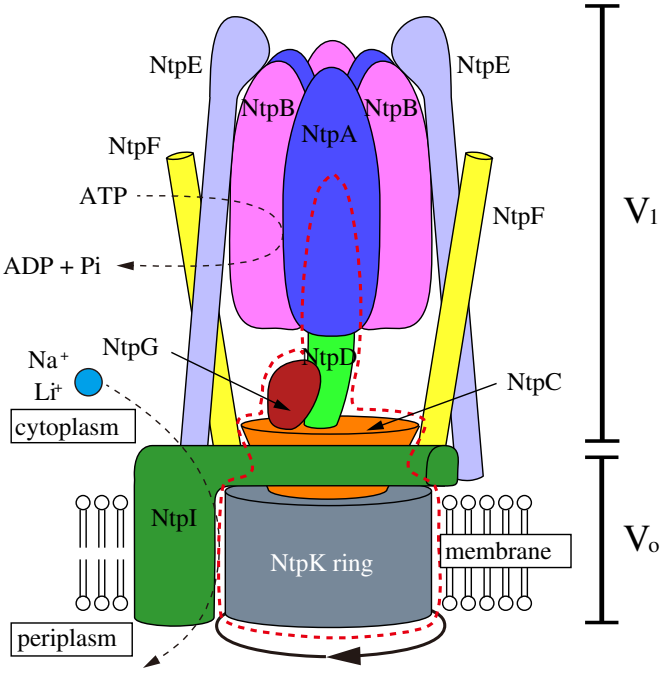

Fig. 2. Schematic model of *E. hirae* V-ATPase. A side view of the structure model of the V-ATPase complex. ATP hydrolysis induces the rotation of the central stalk with K-ring (indicated with the dotted red line), which causes ion (Na^+ or Li^+) pumping at the interface between the K-ring and NtpI.

Na^+ (or Li^+) pumping at the interface between the K-ring and NtpI subunit.

2. X-ray crystal structures of membrane rotor K-ring of *E. hirae* V-ATPase

In 2005, the crystal structure of the Na^+-bound K-ring was reported as the first high-resolution ring structure (Fig. 3a and b) [16]. Ten sodium ions are bound to the specific binding pockets each of which is composed of five oxygen atoms 2.2–2.3 Å distant, four of them in the side chains of T^{64}, Q^{65}, Q^{110} and E^{139}, and the fifth in the main chain carbonyl of L^{61}. They are located on the external surface of the ring, and the hydrophobic surface arrangement places the Na^+-binding site close to the center of the bilayer. Recently, the crystal structure of the Li^+-bound K-ring was also reported (Fig. 3c) [17]. The overall structure of the Li^+-bound K-ring is almost identical (RMSD for all atoms; 0.09 Å) to that of Na^+ bound K-ring except for the ion binding pocket of each K-subunit (Fig. 3b and c). Each Li^+ is surrounded by five oxygen atoms in Helix 2, 3 and 4 which all contribute to the Li^+-binding pocket as seen in the Na^+ bound K-ring structure. The distances between Li^+ and the oxygen atoms are slightly shorter than those between Na^+ and the oxygen atoms. Thus, the ion binding pocket of the K-ring specifically binds Na^+ or Li^+ using identical binding pocket with slightly different metal — oxygen distances.

The ion transport mechanism has been discussed on the basis of these structures; however, X-ray crystal structures lack information on hydrogen atoms. Of the five residues involved in Na^+ binding to the K-ring only one, an essential glutamate (E^{139}) is conserved in all H^+-transporting V-ATPases. The other four residues (L^{61}, T^{64}, Q^{65} and Q^{110}) are not conserved. It is widely accepted that the H^+ binding/release in H^+ transport of V-ATPase occurs via protonation/deprotonation of the carboxyl group of the glutamate residue. The K-ring whose carboxyl group should be protonated at acidic pH does not bind $^{22}Na^+$ with the same high affinity at alkali pH. Analysis of inhibition by H^+ ions suggested that the K-ring binds Na^+ and H^+ competitively [17]. The K_i value of H^+ corresponds to the acid dissociation constant (K_a) of the E^{139}, the pK_a of which is 5.5. However, we have not observed ATP-driven H^+ uptake activity into proteoliposomes reconstituted with the whole V-ATPase complex at pH 5.5 in the effective absence of Na^+. It proved impossible to obtain crystals of H^+ bound K-ring under acidic conditions, as the K-ring was unstable at less than pH 4.5. Therefore, precise structural differences such as protonation states of carboxylate residues and hydrogen-bonding structures of amino-acid side chains remained unclear.

3. ATR FTIR spectroscopy of membrane proteins

Stimulus-induced difference Fourier-transform infrared (FTIR) spectroscopy is a powerful tool to investigate protein structural changes accompanying biologically important functional processes. This method has been extensively applied to photo-active proteins [18–21], and