Crystal structure of membrane-bound pyrophosphatase, a primary proton pump

Membranes consist of a lipid bilayer and proteins essential for biological functions such as solute transport, signal transduction and numerous metabolic reactions. Vacuoles play many important roles in metabolism and growth of plants. In plants, vacuoles occupy most of the cell volume and accumulate various molecules. There are several transport systems on the plant vacuolar membrane. One important function is to store protons transported from cytoplasm, which maintains the pH balance in cytoplasm and acidifies the vacuole lumen [1].

Since the interior environment of a vacuole is acidic, pumping protons into it is against the chemical gradient. A characteristic feature of plants is that two proton pumping proteins, vacuolar H^+-ATPases (V-ATPases) and H^+-translocating pyrophosphatases (H^+-PPases), coexist on plant vacuolar membranes [2]. To generate a pH gradient, V-ATPase uses ATP as an energy source while H^+-PPase utilizes PP_i, a by-product of many biosynthesis reactions. Both enzymes acidify the vacuolar lumen and establish an electrochemical proton gradient across the vacuolar membrane. However, the structure and function of V-ATPase have been widely studied, whereas those for H^+-PPase are still unclear.

H^+-PPase is primarily found in higher plants, several protozoa, some bacteria, and archaebacteria and shares a high degree of homology of the amino acid sequence with land plants [3]. To understand the three-dimensional structure and detailed mechanisms underlying the enzymatic mechanism and proton translocation reactions of H^+-PPases, we reported the first crystal structure of a *Vigna radiata* H^+-PPase (*Vr*H^+-PPase) in a complex with a non-hydrolyzable substrate analogue, imidodiphosphate (IDP), at 2.35 Å resolution [4]. Structure analysis was carried out using beamlines BL13B1 and BL13C1 in NSRRC, and **BL12B2** and **BL44XU** in SPring-8.

As shown in Fig. 1(a), the overall structure of *Vr*H^+-PPase is a homodimer with a non-crystallographic 2-fold symmetry, and both the N- and C-termini face the vacuolar lumen. The cytosolic region of *Vr*H^+-PPase has many hydrophilic residues, whereas the vacuolar region of the protein protruding out of the membrane is relatively smaller. The root mean square deviation is 0.32 Å among the Cα atoms between the two monomers. Each monomer contains 16 transmembrane helices (M1-M16) and folds into a rosette manner with two concentric walls, the six inner TMs and ten outer TMs. A single IDP molecule in each monomer was located at the core of the inner wall near the cytosolic region, constructing a substrate binding pocket (Fig. 1(b)).

The substrate/IDP binding site is a funnel-shaped pocket formed by six center TMs with an unusually acidic environment resulting from the presence of several acidic residues. In addition, the IDP molecule is surrounded by one K^+ and five Mg^{2+} ions at the binding site. All these Mg^{2+} ions possess octahedral coordination that mediates the interactions between those acidic residues and IDP. These binding interactions confine the substrate completely and precisely at the active site for the hydrolysis of PP_i. Furthermore, a detailed comparison of the pyrophosphate-binding pocket between *Vigna radiata* H^+-pyrophosphatase and water-soluble *Escherichia coli* pyrophosphatase (*Ec*PPase) suggests that membrane-bound H^+-PPases and soluble PPases utilize different strategies to trap the nucleophile for PP_i hydrolysis [5].

Following PP_i hydrolysis, proton translocation will be carried on in H^+-PPase. However, the proton pathway is not found in the dimerization interface as previously thought. A unique proton translocation pathway was made by six center transmembrane helices in each monomer by demonstrating the charged residues in the *Vr*H^+-PPase (Fig. 2 (a)). In the *Vr*H^+-PPase complex, four charged residues, R242-D294-K742-E301, are sequentially lined up and buried in the transmembrane domain exactly beneath the PP_i binding pocket (Fig. 2(b)). The pathway can be vertically dissected into three parts: the

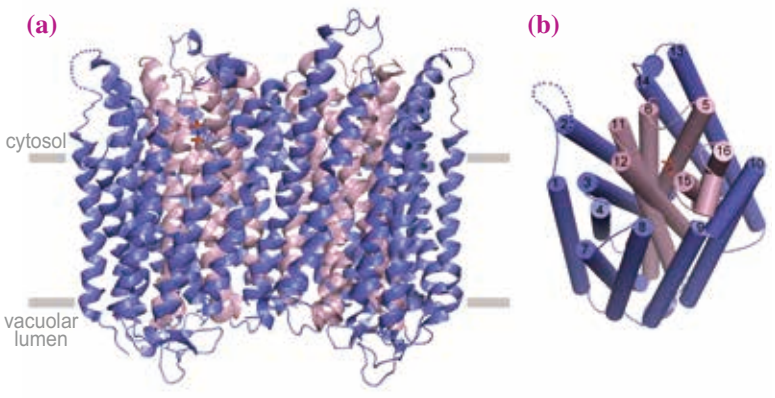

Fig. 1. **(a)** Crystal structure of *Vr*H^+-PPase-IDP complex. **(b)** 16 TMs of *Vr*H^+-PPase.

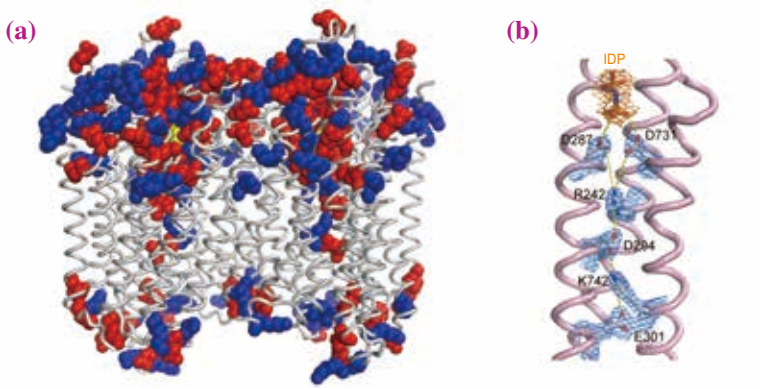

Fig. 2. **(a)** Charged residues of *Vr*H$^+$-PPase-IDP complex. **(b)** Electron density map around the proton transport pathway.

cytosolic region of the channel is solvent-accessible for proton uptake; the middle of the channel is a narrow, compact, and water-restricted route for proton translocation; the vacuolar region is slightly open to enable proton release. Glu301 was completely embedded inside the transmembrane region acting as a constricted neck. Beyond this glutamate are several hydrophobic residues that keep protons of high concentration inside the vacuole from entering the channel. Similar to other proton pumping proteins, two water molecules are confined in the channel of H$^+$-pyrophosphatase to assist the passing of protons. Through a series of protonation and deprotonation among acidic and basic amino acids and bound water molecules in the channel, protons can be translocated from donors and acceptors in a sequential manner.

On the basis of the structure described above and other biochemical studies, a working model for proton translocation in *Vr*H$^+$-PPase was suggested (Fig. 3). Three possible states are indicated: resting state (R-state/ligand free), initiated state (I-state/PP_i bound) and transient state (T-state/ P_i hydrolyzed). In the absence of a substrate (R-state), the structure of the binding pocket in H$^+$-PPase (Fig. 3(a)) is more flexible and rather open to the solvent. In contrast, the lumenal portion of center TMs might orientate into a closed conformation to avoid H$^+$ back reflux to cytosol. Upon substrate (or IDP in our structure) access to the binding site, an initiated state for PP_i hydrolysis (I-state) was formed (Fig. 3(b)). The center transmembrane helices (such as M6 and M16) on the cytosolic side transform into a closed conformation to lock PP_i in the substrate binding pocket. Meanwhile, the lumenal portion of these TMs would be changed to a semi-open conformation for the following H$^+$-translocation. Our structure presents the H$^+$-PPase-IDP complex in an I-state. When PP_i is hydrolyzed, the free phosphate is generated, subsequently followed by proton production (Fig. 3(c)).

In summary, we present that the *Vr*H$^+$-PPase complex is a new transmembrane proton pumping protein of a distinctive folding with an inhibitor IDP bound inside each subunit. A detailed PP_i binding site and the proton pumping pathway are located in close proximity and along a continuous route in the core of *Vr*H$^+$-PPase. A working model is accordingly proposed to reveal the association of PP_i hydrolysis and proton translocation between H$^+$-PPase.

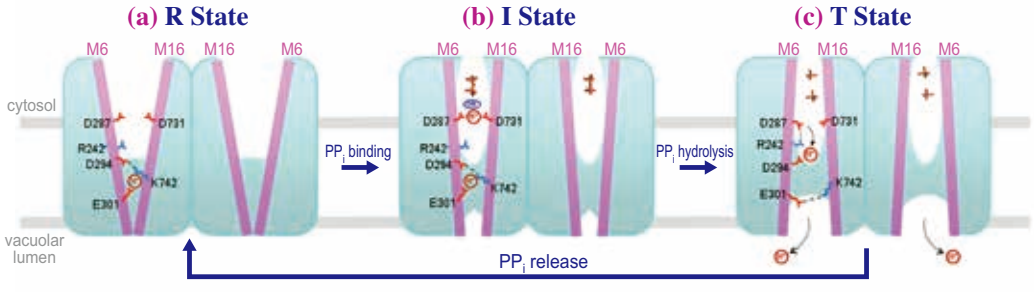

Fig. 3. Working model of proton pumping in *Vr*H$^+$-PPase. **(a)** R state: resting state. **(b)** I state: initiated state. **(c)** T state: transient state.

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References

[1] F. Marty: Plant Cell **11** (1999) 587.
[2] P. A. Rea and R. J. Poole: Annu. Rev. Plant Physiol. Plant Mol. Biol. **44** (1993) 157.
[3] M. Maeshima: Biochim. Biophys. Acta **1465** (2000) 37.
[4] *S.M. Lin, J.Y. Tsai, C.D. Hsiao, Y.T. Huang, C.L. Chiu, M.H. Liu, J.Y. Tung, T.H. Liu, R.L. Pan, and Y.J. Sun: Nature **484** (2012) 399.*
[5] V. R. Samygina *et al.*: J. Mol. Biol. **366** (2007)1305.