Crystal structure of voltage-gated proton channel

Voltage-gated proton channel, VSOP (or Hv1), plays dual roles as a voltage sensing and proton permeation. VSOP was first suggested to be involved in bioluminescence in dinoflagellates, and was identified in *Mus musculus* and *Homo sapiens* in 2006 [1,2]. VSOP is required for high-level superoxide production by phagocytes through its tight functional coupling with NADPH oxidase to eliminate pathogens. Hv1 is expressed in human sperm, where its suggested role is to regulate motility through activating alkalization-activated calcium channels. The activities of Hv1 also have pathological implications such as exacerbation of ischemic brain damage and progression of cancer.

VSOP only has a voltage-sensor domain (VSD) that consists of four-transmembrane helices, which are similar to the VSD of typical voltage-gated ion channels such as voltage-gated sodium (Nav), potassium (Kv), or calcium (Cav) channels and voltage sensor-containing phosphatases [3]. In biological membranes, VSOP is expressed as a dimer, which depends on the dimerization of the cytoplasmic coiled-coil. The forth-transmembrane helix (S4), which has periodically aligned positive charge residues such as arginines, is known to change its orientation relative to other helices in VSD upon a membrane potential change (Fig. 1(a)). Thus, VSD is an individual module-like domain; not only can it convert the membrane potential changes into several biological signals by exchanging a connected domain such as ion pore and phosphatase, but it can also permeate protons as a voltage sensor in the case of VSOP (Fig. 1(b)).

However, the mechanism of voltage sensing is unknown because all crystal structures of VSDs have been determined as activated structures since almost all typical voltage gated ion channels show the activated state in cancelled membrane potential (0 mV), which is equal to the solubilization-state using detergent for protein preparation. Although the mechanism seems to depend on membrane potential, the discussion of the resting structure is just conjecture. The predicted resting structure via computational techniques has not been completely explained. On the other hand, VSOP would be an agreeable model protein to understand the mechanism of voltage sensor, because the solubilized VSOP is considered to be closed-form and the promoter also shows proton channel activity. Moreover, VSOP activation is inhibited by Zn^{2+}, which is consistent with the property where almost all voltage sensors are inhibited by bivalent metal ions [4]. Thus, VSOP is not only the most compact voltage sensor protein but also

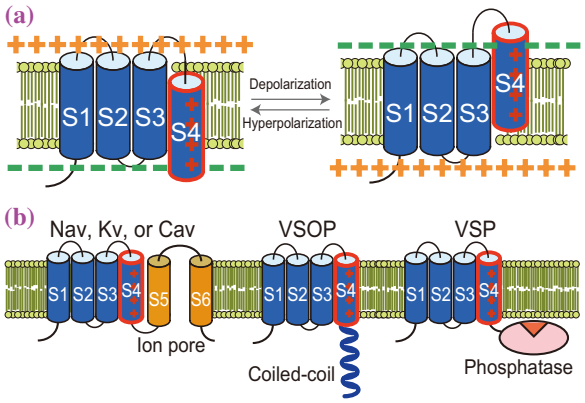

Fig. 1. S4 motion of VSD and VSD protein family. **(a)** Schematic illustration of the S4 motion model. Electrophysiological studies have shown that S4 moves upward and downward relative to other helices (S1 – S3) upon depolarization and hyperpolarization, respectively. **(b)** Voltage sensor protein superfamily contains a canonical voltage-gated ion channel, VSOP, and VSP. In particular, the property of S4's periodical aligned positively charged residues is highly conserved in this superfamily.

has a voltage sensing characteristic. In this report, we explain the crystal structure of the resting state VSOP in the presence of Zn^{2+} [5].

The crystallization construct (mHv1cc) is a chimeric channel, in which the cytoplasmic coiled-coil (Val216 – Asn269) and S2–S3 half intracellular-side (Glu149 – Phe171) are replaced with the GCN4 leucine zipper from *S. cereviece* (Arg249 – Arg281) and the intracellular portion of Ci–VSP (Asp164–Leu188), respectively. We obtained mHv1cc crystals by vapor diffusion method. All reflection data sets were collected at 90 K on **BL44XU** beamline. The initial electron density map was obtained by the multiple anomalous diffraction method. The assignments of S2 and the S4 were performed using the anomalous signals of three selenomethionines (SeMet) in the S2 and the S4 transmembrane helices. Moreover, the S1 and S3 registers were assigned using L107M/L118M and L182M mutants substituted by SeMet. As a result, we determined the crystal structure of mHv1cc at a 3.45-Å resolution in which the R and R_{free} values were refined 34.1% and 35.7%, respectively. The crystal structure of mHv1cc shows that the entire shape looks like “closed Wagasa (traditional Japanese umbrella)” (Fig. 2(a)). We also identified Zn^{2+} in mHv1cc using two wavelength data collected below and above the absorption edge of zinc (1.290 Å and 1.275 Å). Two histidine residues, His136 and His189 of mHv1cc, which are known to be critical for Zn^{2+} binding in hHv1, exist at positions that

should coordinate with Zn^{2+}. His189 was disordered and could not be modeled in the electron density map, but it also would be positioned near Zn^{2+}. In addition, we found two negatively charged residues, Glu115 and Asp119, near Zn^{2+} (Fig. 2(b)). Thus, four residues likely contribute to Zn^{2+} binding in the non-activated state or closed form because Zn^{2+} specifically inhibits the activities of VSOP.

Until now, all of these VSD crystal structures were considered to be in the activated state as evidenced by their biophysical and electrophysiological properties. The positive charged residues of S4 helices in Kv1.2–2.1 chimeric channel and other VSDs are located above a charge transfer center. In the case of mHv1cc, it seems that two of the sensor residues, Arg204 and Arg207, slide toward the inner membrane side relative to the conserved phenylalanine, Phe146, on S2 in a charge transfer center (Fig. 3(a)). This along with the Zn^{2+}-binding and the location of S4 indicates that this structure is a resting-state of the mHv1cc VSD structure.

The mHv1cc structure has two hydrophobic layers (Fig. 3(b)). The lower layer at the cytoplasmic side (HL_{in}) includes Phe146 and Phe178, while the upper layer is on the extracellular-side (HL_{ex}), and consists of four highly conserved hydrophobic residues from four helices (Val112(S1), Leu143(S2), Leu185(S3) and Leu197(S4)). HL_{ex} and HL_{in} may also prevent the penetration of water molecules, which can act as proton carriers. In our mHv1cc structure, Asp108, which is critical for proton selective permeation, seems to be in the hydrophobic layer. However, recent PEGylation experiments to detect the accessibility of maleimide-reagents suggest that Asp108 faces an aqueous vestibule [5]. The two hydrophobic layers probably play distinct roles in regulating the proton conduction pathway.

In conclusion, we not only determined the first VSOP crystal structure, but we also observed the likely resting state structure of VSD. Future elucidation of the crystal structures of VSOP at higher resolutions and those of other gating states should unravel the detailed operating mechanisms of VSOP and the general principles of VSD, which are common among voltage-gated ion channels and voltage–sensing phosphatases upon membrane potential changes.

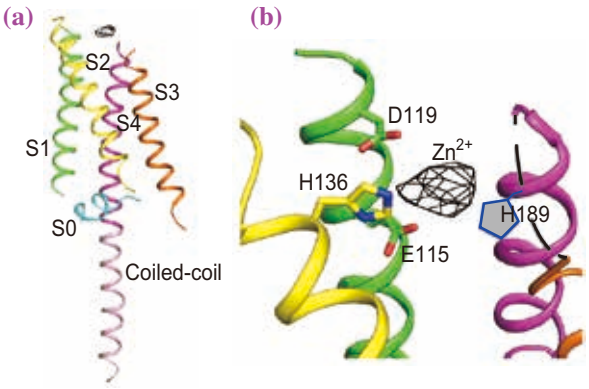

Fig. 2. Crystal structure and Zn^{2+} binding site of mHv1cc. **(a)** Four transmembrane helices are distinguished by color: S1 (green), S2 (yellow), S3 (orange), and S4 (magenta) connected to cytoplasmic coiled-coil region (pink). N-terminal cytoplasmic helix (S0) is cyan. **(b)** Black mesh shows the Zn^{2+}-anomalous difference map contoured at the 5.0σ level.

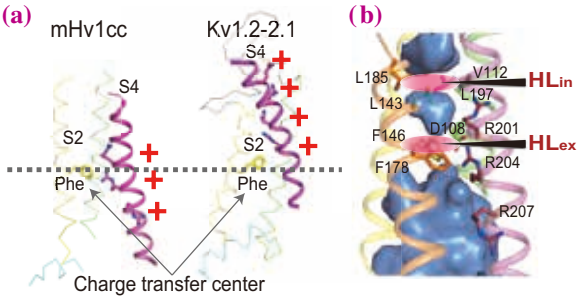

Fig. 3. Comparison of the S4 positional relationship corresponding to the charge transfer center between the mHv1cc and Kv1.2–2.1 (PDB ID: 2R9R) chimeric channel and intermolecular double hydrophobic barriers of mHv1cc. **(a)** Yellow stick model depicts these highly conserved hydrophobic residues (phenylalanine) and the charge transfer center on S2. **(b)** Double hydrophobic layers consist of the hydrophobic residues, which are shown by a stick model. These water-accessible regions are drawn using a Connolly surface (probe radius = 1.4 Å, blue surface).

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