

# Structural revelations of the human proton channel

Thomas E. DeCoursey<sup>1</sup>

Department of Molecular Biophysics and Physiology, Rush University, Chicago, IL 60612

The voltage-gated proton channel, H<sub>V</sub>1, is notoriously unique among ion channels (1), and plays key roles in the health and disease of diverse tissues and species (2). Li et al. (3) combine biochemical, computational, and electron paramagnetic resonance (EPR) spectroscopic approaches to shed light on structural aspects of the human proton channel, hH<sub>V</sub>1. Their results advance the field in several key areas, culminating in a bold new model for gating.

The voltage-sensing domain (VSD) is the part of voltage-gated ion channels that senses the electrical potential across the cell membrane where the channel resides. Most such channels open upon membrane depolarization, which is accomplished mainly by cationic amino acids located in the fourth transmembrane helical segment (S4) moving outward when the inside of the cell is made more positive. This movement is transduced from the VSD (S1–S4) to the pore region (S5–S6), opening a conduction pathway. In addition to biologically important voltage-gated K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and H<sup>+</sup> channels, other classes of membrane proteins with VSDs exist that are not channels at all. One example is a voltage-sensing phosphatase (VSP), an enzyme whose activity is regulated by membrane potential. In a landmark study in 2014 (4), the Li-Perozo group reported crystal structures for CiVSP in both “down” and “up” conformations, the first VSD-containing molecule to have structures determined in both states. Voltage-gated ion channels are

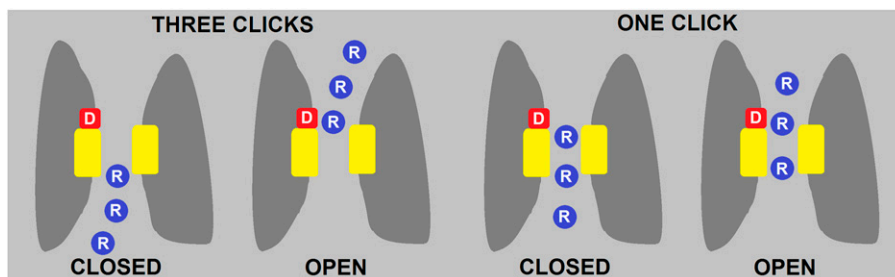
closed at negative voltages, and open upon depolarization as the S4 helix moves “up” through the membrane electrical field. Because the VSP is not a channel, “closed” and “open” become “down” and “up.” The gene for hH<sub>V</sub>1 was identified only in 2006 (5). To the astonishment of everyone, the gene product bore a striking resemblance to the VSD of other voltage-gated ion channels, so much so that the simultaneously identified mouse (mH<sub>V</sub>1) and *Ciona intestinalis* (CiH<sub>V</sub>1) gene products were dubbed VSOP or “voltage sensor-only protein” (6). H<sub>V</sub>1 has only four membrane-spanning helices (S1–S4); these sense voltage but also contain the proton conduction pathway (7).

Crystal structures are great, up to a point. They provide tremendously detailed information about molecules, but they have limitations that are sometimes overlooked. They tell us about structure, but only the structure of whatever exists in the crystal. Proteins have many conformations, and one must determine or guess which one was captured during crystallization, and hope it is a native conformation and not a broken one. Forming crystals of membrane proteins is challenging, and often the protein is modified to facilitate crystallization. Ligands or chaperone-like proteins are included, parts of the molecule are truncated, chimeras are produced: whatever it takes to get a good crystal. H<sub>V</sub>1 has not been successfully crystallized in its entirety. First came crystal structures of the C terminus alone that

lacked the entire transmembrane region (8, 9). Then in 2014, the first exciting glimpse of H<sub>V</sub>1 appeared. Well, not quite H<sub>V</sub>1, but a chimera of the mouse proton channel, mH<sub>V</sub>1, with the C terminus replaced by a leucine zipper transcriptional activator GCN4 from *Saccharomyces cerevisiae*, and with the cytoplasmic ends of S2–S3 replaced by the corresponding ends of CiVSP. Nevertheless, this three-species chimera functions as a proton channel and thus retains essential features. A protein in a crystal senses no membrane potential, and is assumed to be in a state occupied at 0 mV. This means H<sub>V</sub>1 is closed, although with no pH gradient, the channel begins to open within 20 mV (10). H<sub>V</sub>1 exhibits complex gating kinetics (10–12), suggesting it has multiple closed states. Because 0 mV is close to the “threshold” voltage where channels first open, the crystal may have captured a shallow closed state.

EPR provides useful information not available from other approaches, but has its own limitations. Significantly, the protein could be studied *in situ* in its native environment, a lipid bilayer, in contrast to a crystal in which interactions with the membrane are lost. The greatest limitation may be the necessity to introduce a bulky spin label whose presence inside the protein may perturb the native structure at least locally. Li et al. (3) replaced the lone native cysteine (Cys) of hH<sub>V</sub>1 and then introduced Cys at each of 149 locations encompassing the entire VSD. A spin label was attached to each construct and the molecules reconstituted into liposomes. Both the full-length 273-amino acid protein and a VSD-only construct were purified and shown to mediate proton conduction in liposomes; VSD-only constructs were used in all EPR measurements.

Three parameters obtained from EPR are mobility, O<sub>2</sub> accessibility (which indicates proximity to lipid), and NiEDDA (Ni<sup>2+</sup> ethylenediaminediacetic acid) accessibility, which reports aqueous exposure. Both mobility and O<sub>2</sub> accessibility are larger for hH<sub>V</sub>1 than for other VSD-containing molecules, revealing a dynamic molecule, deficient in tertiary contacts,



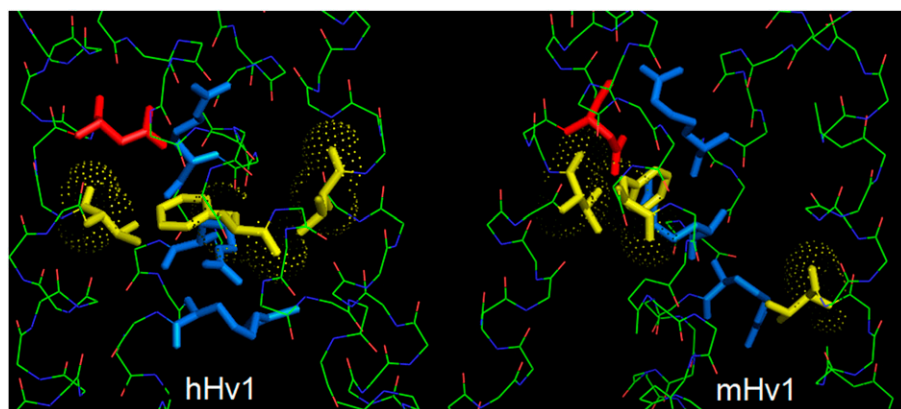
**Fig. 1.** The “three-click” model (Left) is a logical extrapolation to H<sub>V</sub>1 of S4 movement during K<sup>+</sup> or Na<sup>+</sup> channel opening. The three Arg (R) in the S4 helix all move outward past the yellow hydrophobic gasket, from intracellular to extracellular aqueous vestibules. In the new “one-click” model (Right), Li et al. (3) propose that S4 moves only one turn of the helix upwards.

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<sup>1</sup>Email: tdecours@rush.edu.



**Fig. 2.** The hydrophobic gasket (three yellow amino acids) is aligned in the new hHv<sub>1</sub> model based on the CiVSP crystal structure (*Left*), but not in the chimeric mHv<sub>1</sub> crystal structure (*Right*). In each closed channel viewed from the side, the three S4 Arg are blue and the Asp in S1 that produces proton selectivity is red. The apparent misalignment in mHv<sub>1</sub> could reflect a disturbance due to the spliced-in S2–S3 segment from CiVSP, which ends two positions below V174, the anomalous residue in mHv<sub>1</sub>. Alternatively, a different amino acid might complete the gasket in mHv<sub>1</sub>.

that historically has resisted all attempts to obtain a crystal structure. NiEDDA accessibility defines the boundaries of the transmembrane regions (S1–S4), and reveals that the channel pore has a short isthmus between two aqueous vestibules, reminiscent of the VSDs of K<sup>+</sup> and Na<sup>+</sup> channels, which also focus the electric field over a short distance (13–15). This “hydrophobic gasket” forms a dielectric barrier to water and ion permeation and separates “in” from “out.” The hydrophobic region (yellow in Fig. 1) encompasses the outer two arginines of the S4 helix (R1 and R2), but the third (R3) is accessible internally. Homology models of hHv<sub>1</sub> (16) and CiHv<sub>1</sub> (17) indicate a ~10 Å long hydrophobic region that in hHv<sub>1</sub> includes the selectivity filter Asp<sup>112</sup> (18) and Phe<sup>150</sup> that is conserved universally in VSD-containing molecules (19).

Mammalian Hv<sub>1</sub> are dimers, although each protomer has its own conduction pathway and can function independently (20, 21). EPR measurements at each of 149 positions reveal the distance between each pair in the dimer, unambiguously defining the dimer interface and resolving internecine disputes on this point (22, 23). The EPR data resoundingly establish the dimer interface to be at the top (extracellular end) of S1 and the lower part of S4. Dimerization was thought to result mainly from extensive coiled-coil interactions between the C termini (20–22). Surprisingly, Li et al. (3) found that their VSD-only construct of hHv<sub>1</sub>, which lacks the C terminus, spontaneously associated as a dimer with K<sub>d</sub> 3 μM.

Previous homology models of Hv<sub>1</sub> used VSDs from crystal structures of other channels as templates. Li et al. (3) generated models based on structures of K<sub>v</sub>AP, K<sub>v</sub>1.2, Na<sub>v</sub>Ab, or CiVSP, and tuned these using molecular dynamics. EPR solvent accessibility data from hHv<sub>1</sub> favored the CiVSP-based model. This is reasonable, because phylogenetically, Hv<sub>1</sub> are

related more closely to VSP than to other channels (24). Although the closed hHv<sub>1</sub> model agrees well with the structure of the closed mHv<sub>1</sub> chimera, parts of S2 and S3 from the CiVSP-based model must be shifted up or down to match the crystal structure. A consequence of this mismatch is seen in Fig. 2. The residues in hHv<sub>1</sub> corresponding to those forming the hydrophobic gasket in CiVSP (4), V109, F150, and V178 (yellow in Fig. 2), align horizontally in CiVSP and in our homology model of hHv<sub>1</sub> (16). However, in the mHv<sub>1</sub> structure, V174 in the S3 helix is too low. Perhaps CiVSP is not an ideal template, but it seems more than coincidental that parts of

both S2 and S3 were replaced in the chimera. Shifting S3 of mHv<sub>1</sub> up by one “click” to match CiVSP would align the gasket nicely.

The most far-reaching conclusion of Li et al. (3) is that S4 moves much less during gating than most envisioned. Seduced by the surprising resemblance of Hv<sub>1</sub> to other VSDs, everyone initially assumed that its S4 would move just like it does in other channels: basic amino acids spaced every three positions along S4 move past the hydrophobic gasket. In other channels, three to four charges move past the gasket during opening, so it was expected that all three Arg in S4 of Hv<sub>1</sub> would move from the intracellular to the extracellular side (Fig. 1). However, replacing the innermost Arg with histidine (R211H) resulted in inhibition by internal Zn<sup>2+</sup> even when the channel was open, arguing that S4 movement was far more restricted in hHv<sub>1</sub> than in other VSDs (16). Given overall agreement of the EPR data with the mHv<sub>1</sub> structure, the fact that CiVSP is the only VSD with structures of both down and up conformations, and the phylogenetic proximity of CiVSP and hHv<sub>1</sub> (24), Li et al. (3) propose a gating model for hHv<sub>1</sub> (Fig. 1) based on their “one-click” model for CiVSP (4). The S4 segment moves up just one turn of the helix, in contrast to the three or four clicks of other VSDs. If this model survives the test of time, it will add one more distinctive feature to an already unique channel.

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