COMMENTARY

Structural revelations of the human proton channel

Thomas E. DeCoursey[1]

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

The voltage-gated proton channel, H_V1, 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_V1. 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^+, Na^+, Ca^{2+}, and H^+ 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_V1 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_V1) and *Ciona intestinalis* (CiH_V1) gene products were dubbed VSOP or "voltage sensor-only protein" (6). H_V1 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, chimerae are produced: whatever it takes to get a good crystal. H_V1 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_V1 appeared. Well, not quite H_V1, but a chimera of the mouse proton channel, mH_V1, 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 section 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_V1 is closed, although with no pH gradient, the channel begins to open within 20 mV (10). H_V1 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_V1 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_2 accessibility (which indicates proximity to lipid), and NiEDDA (Ni^{2+} ethylenediaminediacetic acid) accessibility, which reports aqueous exposure. Both mobility and O_2 accessibility are larger for hH_V1 than for other VSD-containing molecules, revealing a dynamic molecule, deficient in tertiary contacts,

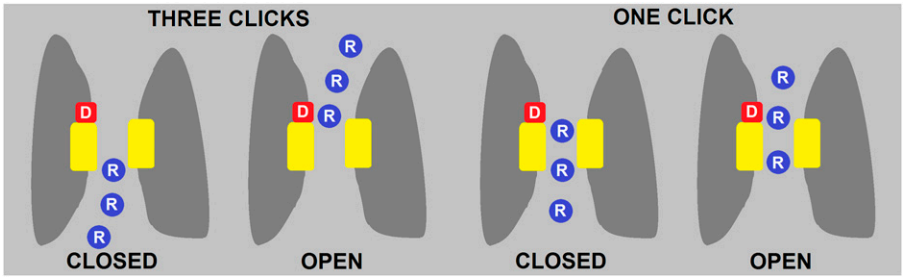

Fig. 1. The "three-click" model (*Left*) is a logical extrapolation to H_V1 of S4 movement during K^+ or Na^+ channel opening. The three Arg (R) in the S4 helix all move outward past the yellow hydrophobic gasket, from intracellular to extracellular aqueous vestibules. In the new "one-click" model (*Right*), Li et al. (3) propose that S4 moves only one turn of the helix upwards.

Author contributions: T.E.D. wrote the paper.

The author declares no conflict of interest.

See companion article on page E5926.

[1]Email: tdecours@rush.edu.

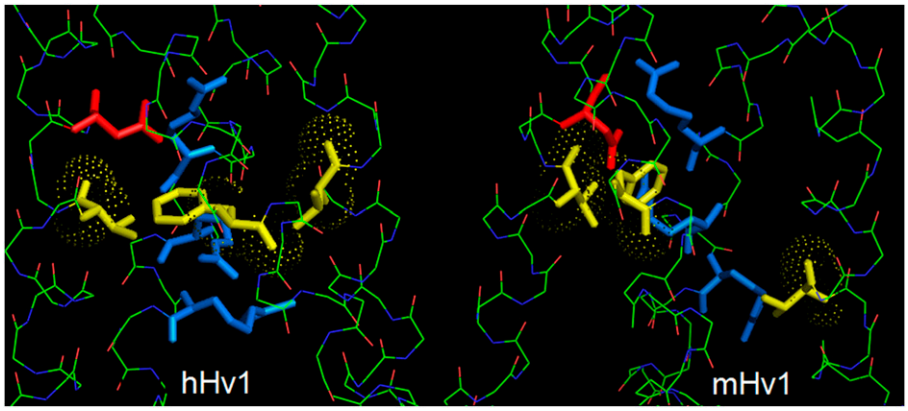

Fig. 2. The hydrophobic gasket (three yellow amino acids) is aligned in the new hH_V1 model based on the CiVSP crystal structure (*Left*), but not in the chimeric mH_V1 crystal structure (*Right*). In each closed channel viewed from the side, the three S4 Arg are blue and the Asp in S1 that produces proton selectivity is red. The apparent misalignment in mH_V1 could reflect a disturbance due to the spliced-in S2–S3 segment from CiVSP, which ends two positions below V174, the anomalous residue in mH_V1. Alternatively, a different amino acid might complete the gasket in mH_V1.

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^+ and Na^+ 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 hH_V1 (16) and CiH_V1 (17) indicate a ∼10 Å long hydrophobic region that in hH_V1 includes the selectivity filter Asp^{112} (18) and Phe^{150} that is conserved universally in VSD-containing molecules (19).

Mammalian H_V1 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 hH_V1, which lacks the C terminus, spontaneously associated as a dimer with K_d 3 μM.

Previous homology models of H_V1 used VSDs from crystal structures of other channels as templates. Li et al. (3) generated models based on structures of K_VAP, $K_V1.2$, Na_VAb, or CiVSP, and tuned these using molecular dynamics. EPR solvent accessibility data from hH_V1 favored the CiVSP-based model. This is reasonable, because phylogenetically, H_V1 are related more closely to VSP than to other channels (24). Although the closed hH_V1 model agrees well with the structure of the closed mH_V1 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 hH_V1 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 hH_V1 (16). However, in the mH_V1 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 mH_V1 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 H_V1 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 H_V1 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^{2+} even when the channel was open, arguing that S4 movement was far more restricted in hH_V1 than in other VSDs (16). Given overall agreement of the EPR data with the mH_V1 structure, the fact that CiVSP is the only VSD with structures of both down and up conformations, and the phylogenetic proximity of CiVSP and hH_V1 (24), Li et al. (3) propose a gating model for hH_V1 (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.

ACKNOWLEDGMENTS. This work was supported by National Science Foundation Award MCB-0943362 and National Institutes of Health Grant R01-GM087507.

1 DeCoursey TE (2015) The voltage-gated proton channel: A riddle, wrapped in a mystery, inside an enigma. *Biochemistry* 54(21): 3250–3268.
2 DeCoursey TE (2013) Voltage-gated proton channels: Molecular biology, physiology, and pathophysiology of the H_V family. *Physiol Rev* 93(2):599–652.
3 Li Q, et al. (2015) Resting state of the human proton channel dimer in a lipid bilayer. *Proc Natl Acad Sci USA* 112:E5926–E5935.
4 Li Q, et al. (2014) Structural mechanism of voltage-dependent gating in an isolated voltage-sensing domain. *Nat Struct Mol Biol* 21(3):244–252.
5 Ramsey IS, Moran MM, Chong JA, Clapham DE (2006) A voltage-gated proton-selective channel lacking the pore domain. *Nature* 440(7088):1213–1216.
6 Sasaki M, Takagi M, Okamura Y (2006) A voltage sensor-domain protein is a voltage-gated proton channel. *Science* 312(5773): 589–592.
7 Lee SY, Letts JA, MacKinnon R (2009) Functional reconstitution of purified human Hv1 H^+ channels. *J Mol Biol* 387(5):1055–1060.
8 Li SJ, et al. (2010) The role and structure of the carboxyl-terminal domain of the human voltage-gated proton channel Hv1. *J Biol Chem* 285(16):12047–12054.
9 Fujiwara Y, Takeshita K, Nakagawa A, Okamura Y (2013) Structural characteristics of the redox-sensing coiled coil in the voltage-gated H^+ channel. *J Biol Chem* 288(25):17968–17975.
10 Cherny VV, Markin VS, DeCoursey TE (1995) The voltage-activated hydrogen ion conductance in rat alveolar epithelial cells is determined by the pH gradient. *J Gen Physiol* 105(6):861–896.
11 DeCoursey TE, Cherny VV (1994) Voltage-activated hydrogen ion currents. *J Membr Biol* 141(3):203–223.
12 Villalba-Galea CA (2014) Hv1 proton channel opening is preceded by a voltage-independent transition. *Biophys J* 107(7): 1564–1572.
13 Yang N, George AL, Jr, Horn R (1996) Molecular basis of charge movement in voltage-gated sodium channels. *Neuron* 16(1): 113–122.
14 Starace DM, Bezanilla F (2001) Histidine scanning mutagenesis of basic residues of the S4 segment of the *Shaker* K^+ channel. *J Gen Physiol* 117(5):469–490.
15 Starace DM, Bezanilla F (2004) A proton pore in a potassium channel voltage sensor reveals a focused electric field. *Nature* 427(6974):548–553.
16 Kulleperuma K, et al. (2013) Construction and validation of a homology model of the human voltage-gated proton channel hH_V1. *J Gen Physiol* 141(4):445–465.
17 Chamberlin A, Qiu F, Wang Y, Noskov SY, Larsson HP (2015) Mapping the gating and permeation pathways in the voltage-gated proton channel Hv1. *J Mol Biol* 427(1):131–145.
18 Musset B, et al. (2011) Aspartate 112 is the selectivity filter of the human voltage-gated proton channel. *Nature* 480(7376):273–277.
19 Tao X, Lee A, Limapichat W, Dougherty DA, MacKinnon R (2010) A gating charge transfer center in voltage sensors. *Science* 328(5974):67–73.
20 Koch HP, et al. (2008) Multimeric nature of voltage-gated proton channels. *Proc Natl Acad Sci USA* 105(26):9111–9116.
21 Tombola F, Ulbrich MH, Isacoff EY (2008) The voltage-gated proton channel Hv1 has two pores, each controlled by one voltage sensor. *Neuron* 58(4):546–556.
22 Lee SY, Letts JA, Mackinnon R (2008) Dimeric subunit stoichiometry of the human voltage-dependent proton channel Hv1. *Proc Natl Acad Sci USA* 105(22):7692–7695.
23 Musset B, et al. (2010) Zinc inhibition of monomeric and dimeric proton channels suggests cooperative gating. *J Physiol* 588(Pt 9): 1435–1449.
24 Smith SME, et al. (2011) Voltage-gated proton channel in a dinoflagellate. *Proc Natl Acad Sci USA* 108(44):18162–18167.