The Journal of Physiology

CROSSTALK

CrossTalk proposal: Proton permeation through H_V1 requires transient protonation of a conserved aspartate in the S1 transmembrane helix

Thomas E. DeCoursey
Department of Physiology and Biophysics, Rush University, Chicago, IL 60612, USA
Email: tdecours@rush.edu

Here I present evidence in support of the premise that H^+ selective permeation through the voltage gated proton channel H_V1 involves obligatory protonation/deprotonation of an aspartic acid residue (Asp^{112} in human H_V1) in the middle of the S1 transmembrane helix. An alternative view is the 'frozen water' mechanism, in which the channel traps one or more water molecules that block cation flux but permit Grotthuss-style proton hopping. I concede at the outset that the issue is not resolved and is not in fact resolvable based on current evidence.

The historical basis for evaluating H_V1 conduction mechanisms was a series of measurements of the biophysical properties of H^+ currents. For over a decade the fundamental paradigm was to compare proton fluxes through H_V1 with those through gramicidin (Table 1), known to be a cylindrical pore through which ions and water permeate in single file (Rosenberg & Finkelstein, 1978; Wallace & Ravikumar, 1988). The characteristics of H^+ current through gramicidin differ from proton conduction in bulk water, but H^+ conduction through H_V1 is radically different from both (Table 1). Hence, many papers from this era concluded that the pathway through H_V1 is more complex than a water-filled pore and includes obligatory protonation/deprotonation of at least one amino acid side-chain (DeCoursey & Cherny, 1994, 1997, 1998).

This interpretation has been questioned, most explicitly by Ramsey and colleagues (2010), although others have espoused the view that H^+ permeates H_V1 via a water wire (Wood *et al.* 2012; Pupo *et al.* 2014). What follows are six relevant properties of H_V1 currents, which to my knowledge are not in dispute, and their implications for the conduction mechanism.

Selectivity

H_V1 is extremely selective for H^+, excluding all other ions. This extraordinary selectivity can be explained by invoking obligatory protonation/deprotonation of a titratable group during permeation (Nagle & Morowitz, 1978). Just such a group was identified as a perfectly conserved (among at least 140 species in the National Centre for Biotechnology Information database) Asp in the middle of the S1 transmembrane helix. Proton selectivity is compromised if this critical Asp is mutated to a neutral amino acid, converting the channel to anion selectivity (Musset *et al.* 2011). In a reduced quantum mechanical model of the selectivity filter of H_V1, interaction of H_3O^+ with the Asp and Arg side-chains sufficed to produce H^+ selective conduction, while excluding other ions (Dudev *et al.* 2015). Similarly, obligatory protonation/deprotonation of His^{37} imparts H^+ selectivity to the M2 influenza A viral proton channel (Pinto *et al.* 1997).

Anomalous Gu^+ permeation

Despite its selectivity for H^+ (DeCoursey, 2003), at non-physiologically high pH H_V1 appears to conduct guanidinium ions, Gu^+ (DeCoursey, 2013). The anomalous permeation of this foreign ion was attributed to its ability to denature proteins by breaking hydrogen bonds and disrupting water structure (DeCoursey, 2013). H^+ selective permeation is proposed to occur when H_3O^+ protonates Asp, breaking its hydrogen bonds with Arg, leaving a neutral H_2O molecule bridging the two side-chains (Dudev *et al.* 2015). Gu^+ can break salt bridges between Glu and Arg or Lys by interacting specifically with carboxylate$^-$ groups (Meuzelaar *et al.* 2015); thus it permeates H_V1 by violating the hydrogen bonds that occlude the pore to other ions. If the H_V1 conduction pathway were a simple water wire, it is difficult to fathom why Gu^+ would permeate but smaller or larger cations would not.

Deuterium isotope effect

The deuterium isotope effect on current amplitude is 1.9 (H^+/D^+) (DeCoursey & Cherny, 1997), much greater than that for H^+ conduction in bulk water or in the water-filled pore of gramicidin (Table 1). The isotope effect for bulk H^+ mobility is 1.4 (Lewis & Doody, 1933). Other molecules whose H^+ transport pathways involve protonation/deprotonation of amino acids exhibit higher isotope effects (Blair & Berg, 1990; DeCoursey & Cherny, 1997). The isotope effect of 2 in the M2 proton channel (Mould *et al.* 2000) is consistent with His protonation/deprotonation kinetics. Because D^+ binds more tightly than H^+, the pK_a of many proteins increases in heavy water; a carboxyl group binds D^+ threefold more tightly than H^+ (Schowen, 1977). The isotope effect is consistent with protonation/deprotonation of Asp^{112} during each conduction event.

H_V1 has extraordinary temperature dependence

Both permeation and gating of H_V1 have a higher Q_{10} (the factor by which a rate increases for a 10°C increase in temperature) than almost any other ion channel, 2–3 and 6–9, respectively

Thomas E. DeCoursey's scientific path meandered through a childhood highlighted by summers spent in King's Canyon and Wind Cave National Parks, then to Cincinnati (mentor: Shirley H Bryant), Glasgow (Otto F Hutter), Irvine, California (Michael D Cahalan), and finally Rush University in Chicago where he is Professor of Physiology & Biophysics. Potassium and other channels served as 'gateway' channels leading to the discovery of voltage-gated proton channels in mammals in 1991. For the past quarter century his focus has been on all aspects of proton channels, from their critical roles in phagocytes and dinoflagellates to site-directed mutagenesis aimed at elucidating structure-function relationships.

DOI: 10.1113/JP274495

Table 1. Proton conduction through water, gramicidin, and H_V1 channels

Property	Bulk water	Gramicidin	H_V1
Selectivity (P_{H^+}/P_{Na^+})	7[1]	38–150[2]	$>10^6$–$10^{8(3)}$
Deuterium isotope effect (I_{H^+}/I_{D^+})	1.41[4]	1.35[5]	1.9[6]
Activation energy (kcal mol^{-1})	2.6[7]	4.8[8]	18–27[9]

The activation energy is for proton permeation through open channels. The temperature dependence of gramicidin includes measurements on dioxolane-linked channels. [1]Robinson & Stokes, 1959; [2]Myers & Haydon, 1972; [3]DeCoursey, 2003; [4]Lewis & Doody, 1933; [5]Akeson & Deamer, 1991; Chernyshev *et al*. 2003; [6]DeCoursey & Cherny, 1997; [7]Robinson & Stokes, 1959; [8]Akeson & Deamer, 1991; [9]DeCoursey & Cherny, 1998. This table was reprinted with minor changes from: DeCoursey TE. (2008). Voltage-gated proton channels: what's next? *J Physiol* **586**, 5305–5324.

(DeCoursey & Cherny, 1998; Kuno *et al.* 2009). The Q_{10} for permeation in other ion channels is typically 1.2–1.5 (DeCoursey & Cherny, 1998), like that for ion conductivity in bulk solution. This suggests either that diffusive entry into the pore of other channels is rate determining, or their permeation path mimics aqueous diffusion. The temperature dependence of H^+ conductivity in bulk solution is lower than that of other ions, so the high Q_{10} for permeation through H_V1 indicates an energetically difficult pathway. For example, rotation of a protonated side-chain could advance the proton across a narrow hydrophobic region. An imidazolium ring flip likely occurs during H^+ conduction in the M2 viral proton channel, which also has a high Q_{10} (Lin & Schroeder, 2001; Hu *et al.* 2010). In contrast, H^+ permeates the gramicidin water wire easily, with Q_{10} 1.35 (Table 1). In addition, the apparent mobility of H^+ inside gramicidin is not much lower than in bulk solution (Cukierman, 2000). Ion carriers and pumps exhibit high Q_{10} in the range of H_V1 (Blair & Berg, 1990; DeCoursey & Cherny, 1998). A telling example is the *Shaker* voltage sensing domain with the Arg→His mutation R365H, in which the introduced His acts as a proton carrier, shuttling protons with a Q_{10} of 2.6 (Starace *et al.* 1997).

Permeation is rate limiting

In contrast with the water-filled pore of gramicidin (Decker & Levitt, 1988), diffusion of protonated buffer to the H_V1 channel is not rate limiting because the current decreases only slightly when buffer concentration is lowered 100-fold, from 100 to 1 mM (DeCoursey & Cherny, 1996). At even lower buffer concentration, 150 μM, H^+ current through M2 channels was decreased by 89% (Mould *et al.* 2000). H^+ current through the gramicidin water wire is roughly proportional to $[H^+]$ over many orders of magnitude (DeCoursey, 2003). In contrast, the H_V1 unitary conductance increases only 3.7-fold unit^{-1} as pH_i is lowered (Cherny *et al.* 2003).

Kinetic competence

The H_V1 permeation mechanism must be capable of conducting at least 10^5 H^+ s^{-1}. This gives a mean transit time of $\leq$10 μs at pH_i 5.5 at 120 mV above E_H with a unitary conductance of 140 fS (Cherny *et al.* 2003). The gramicidin water-filled pore can conduct up to 2×10^9 H^+ s^{-1} (Cukierman, 2000); showing that a water wire is kinetically competent, if not excessively so. Nagle and Morowitz estimated that a generic hydrogen bonded chain (HBC) involving amino acid side-chains might conduct 4×10^5 H^+ s^{-1} (Nagle & Morowitz, 1978). The *Shaker* voltage sensing domain with R362H mutation acts as a hyperpolarization-activated proton channel, with the introduced His shuttling 5.6×10^4 H^+ s^{-1} (Starace & Bezanilla, 2004). Carbonic anhydrase II shuttles protons via His^{64} and is the fastest known enzyme, with a turnover rate of 10^6 s^{-1} (Rowlett & Silverman, 1982). These examples show that obligatory proton shuttling by an amino acid side-chain (including protonation, side-chain excursion, and deprotonation) can occur at appropriate rates.

Future solutions

One could measure water flux. Rapid water flux through H_V1 would argue against the frozen water model, but could be compatible with ion exclusion as occurs in aquaporins. NMR could detect the protonation state and protonation kinetics of key amino acids. High-resolution crystal structures of open and closed H_V1 channels might show the presence or absence of a fully-formed water wire capable of transferring H^+. Then again they might not.

Call for comments

Readers are invited to give their views on this and the accompanying CrossTalk articles in this issue by submitting a brief (250 word) comment. Comments may be submitted up to 6 weeks after publication of the article, at which point the discussion will close and the CrossTalk authors will be invited to submit a 'Last Word'. Please email your comment, including a title and a declaration of interest, to jphysiol@physoc.org. Comments will be moderated and accepted comments will be published online only as 'supporting information' to the original debate articles once discussion has closed.

References

Akeson M & Deamer DW (1991). Proton conductance by the gramicidin water wire. Model for proton conductance in the F_1F_0 ATPases? *Biophys J* **60**, 101–109.

Blair DF & Berg HC (1990). The MotA protein of *E. coli* is a proton-conducting component of the flagellar motor. *Cell* **60**, 439–449.

Cherny VV, Murphy R, Sokolov V, Levis RA & DeCoursey TE (2003). Properties of single voltage-gated proton channels in human eosinophils estimated by noise analysis and by direct measurement. *J Gen Physiol* **121**, 615–628.

Chernyshev A, Pomès R & Cukierman S (2003). Kinetic isotope effects of proton transfer in aqueous and methanol containing solutions, and in gramicidin A channels. *Biophys Chem* **103**, 179–190.

Cukierman S (2000). Proton mobilities in water and in different stereoisomers of covalently linked gramicidin A channels. *Biophys J* **78**, 1825–1834.

Decker ER & Levitt DG (1988). Use of weak acids to determine the bulk diffusion limitation of H^+ ion conductance through the gramicidin channel. *Biophys J* **53**, 25–32.

DeCoursey TE (2003). Voltage-gated proton channels and other proton transfer pathways. *Physiol Rev* **83**, 475–579.

DeCoursey TE (2013). Voltage-gated proton channels: molecular biology, physiology, and pathophysiology of the H_V family. *Physiol Rev* **93**, 599–652.

DeCoursey TE & Cherny VV (1994). Voltage-activated hydrogen ion currents. *J Membr Biol* **141**, 203–223.

DeCoursey TE & Cherny VV (1996). Effects of buffer concentration on voltage-gated H^+ currents: does diffusion limit the conductance? *Biophys J* **71**, 182–193.

DeCoursey TE & Cherny VV (1997). Deuterium isotope effects on permeation and gating of proton channels in rat alveolar epithelium. *J Gen Physiol* **109**, 415–434.

DeCoursey TE & Cherny VV (1998). Temperature dependence of voltage-gated H^+ currents in human neutrophils, rat alveolar epithelial cells, and mammalian phagocytes. *J Gen Physiol* **112**, 503–522.

Dudev T, Musset B, Morgan D, Cherny VV, Smith SM, Mazmanian K, DeCoursey TE & Lim C (2015). Selectivity mechanism of the voltage-gated proton channel, H_V1. *Sci Rep* **5**, 10320.

Hu F, Luo W & Hong M (2010). Mechanisms of proton conduction and gating in influenza M2 proton channels from solid-state NMR. *Science* **330**, 505–508.

Kuno M, Ando H, Morihata H, Sakai H, Mori H, Sawada M & Oiki S (2009). Temperature dependence of proton permeation through a voltage-gated proton channel. *J Gen Physiol* **134**, 191–205.

Lewis GN & Doody TC (1933). The mobility of ions in H^2H^2O. *J Am Chem Soc* **55**, 3504–3506.

Lin TI & Schroeder C (2001). Definitive assignment of proton selectivity and attoampere unitary current to the M2 ion channel protein of influenza A virus. *J Virol* **75**, 3647–3656.

Meuzelaar H, Panman MR & Woutersen S (2015). Guanidinium-induced denaturation by breaking of salt bridges. *Angew Chem Int Ed Engl* **54**, 15255–15259.

Mould JA, Li HC, Dudlak CS, Lear JD, Pekosz A, Lamb RA & Pinto LH (2000). Mechanism for proton conduction of the M_2 ion channel of influenza A virus. *J Biol Chem* **275**, 8592–8599.

Musset B, Smith SME, Rajan S, Morgan D, Cherny VV & DeCoursey TE (2011). Aspartate 112 is the selectivity filter of the human voltage-gated proton channel. *Nature* **480**, 273–277.

Myers VB & Haydon DA (1972). Ion transfer across lipid membranes in the presence of gramicidin A. II. The ion selectivity. *Biochim Biophys Acta* **274**, 313–322.

Nagle JF & Morowitz HJ (1978). Molecular mechanisms for proton transport in membranes. *Proc Natl Acad Sci USA* **75**, 298–302.

Pinto LH, Dieckmann GR, Gandhi CS, Papworth CG, Braman J, Shaughnessy MA, Lear JD, Lamb RA & DeGrado WF (1997). A functionally defined model for the M2 proton channel of influenza A virus suggests a mechanism for its ion selectivity. *Proc Natl Acad Sci USA* **94**, 11301–11306.

Pupo A, Baez-Nieto D, Martinez A, Latorre R & González C (2014). Proton channel models: filling the gap between experimental data and the structural rationale. *Channels (Austin)* **8**, 180–192.

Ramsey IS, Mokrab Y, Carvacho I, Sands ZA, Sansom MSP & Clapham DE (2010). An aqueous H^+ permeation pathway in the voltage-gated proton channel Hv1. *Nat Struct Mol Biol* **17**, 869–875.

Robinson RA & Stokes RH (1959). *Electrolyte Solutions*. Butterworths, London.

Rosenberg PA & Finkelstein A (1978). Interaction of ions and water in gramicidin A channels: streaming potentials across lipid bilayer membranes. *J Gen Physiol* **72**, 327–340.

Rowlett RS & Silverman DN (1982). Kinetics of the protonation of buffer and hydration of CO_2 catalyzed by human carbonic anhydrase II. *J Am Chem Soc* **104**, 6737–6741.

Schowen RL (1977). Solvent isotope effects on enzymic reactions. In *Isotope Effects on Enzyme-catalyzed Reactions*, eds Cleland WW, O'Leary MH & Northrop DB, pp. 64–99. University Park Press, Baltimore.

Starace DM & Bezanilla F (2004). A proton pore in a potassium channel voltage sensor reveals a focused electric field. *Nature* **427**, 548–553.

Starace DM, Stefani E & Bezanilla F (1997). Voltage-dependent proton transport by the voltage sensor of the *Shaker* K^+ channel. *Neuron* **19**, 1319–1327.

Wallace BA & Ravikumar K (1988). The gramicidin pore: crystal structure of a cesium complex. *Science* **241**, 182–187.

Wood ML, Schow EV, Freites JA, White SH, Tombola F & Tobias DJ (2012). Water wires in atomistic models of the Hv1 proton channel. *Biochim Biophys Acta* **1818**, 286–293.

Additional information

Competing interests

None declared.

Funding

Support: NIH 1R01GM102336, NSF MCB-1242985.