Cooperative interactions between subunits regulate gating in holo-proton conductive channels

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Voltage-gated, proton-selective ion channels (Hv or VSOP channels) have been described in a variety of cell types in vertebrates, including leukocytes, pulmonary epithelium and striated muscle. Recently identified cDNAs that give rise to Hv currents encode for an integral membrane protein with strong similarity to the S1-S4 transmembrane segments of voltage-gated Na⁺, K⁺ and Ca²⁺ channel subunits. This "voltage sensor domain" protein appears to represent the main Hv channel subunit, which further dimerizes to form holo-Hv channels. Similar to aquaporin channels, each Hv channel subunit contains its own permeation pathway, so that a holo-Hv channel contains two operational proton conductive pores. In the present study, the authors have investigated whether the charge-containing S4 segment functions as the major voltage-sensing element in the Hv channel subunit, similar to its role in other voltage-gated cation channels.

To examine the physical re-arrangement of the S4 transmembrane segment in response to depolarizing voltage pulses, the authors expressed Hv channel cDNA cloned from the sea squirt Ciona intestinalis in Xenopus laevis oocytes and utilized two distinct analytical strategies: 1) assessing the state-dependent accessibility/modification of substituted cysteine residues in S4 by bulky methanethiosulfate derivatives, such as MTSET and MTSES, and 2) specifically labeling the S4 segment with a fluorescent probe and then measuring fluorescent signals associated with gating of the channel between closed and open states (i.e. voltage clamp fluorometry). Voltage clamp techniques were also utilized to estimate the movement of charged residues (i.e. equivalent gating charge, eₒ) within the membrane electric field associated with channel activation. The authors also constructed monomeric Hv channels by deleting the N- and C-termini of each subunit, thereby preventing subunit dimerization. This strategy allowed the authors to directly examine the functional and mechanistic consequences of subunit interaction in native Hv channel homo-dimers. Most of these approaches have been successfully used previously to examine the biophysical and mechanistic properties of gating in voltage-activated K⁺ and Na⁺ channels.

In Hv channels containing an Ile to Cys substitution in the S4 segment at position 248 (near the external membrane surface), activation of the channel by depolarization promoted modification of the substituted Cys by externally applied MTSET, whereas no modification was observed under hyperpolarizing conditions (i.e. channel remained in the closed state). Modification of a Cys residue substituted at Ala246 by MTSES also occurred in a state-dependent manner, and similar results were obtained for a Cys substitution at Val252. These results thus suggest that Ala246, Ile248 and Val252 become accessible to the bulk aqueous solution upon channel activation, possibly as a result of an outward movement of the S4 segment or rearrangement of the structural environment around these individual amino acid positions. In contrast, similar Cys substitutions at locations predicted to lie closer to the intracellular end of the S4 segment (i.e. Ile262 and Asn264) were found to be modified at hyperpolarized potentials following internal application of MTSET, and the rate of...
modification was slowed with membrane depolarization. These data suggest that Ile262 and Asn264 are more accessible to the cytoplasmic solution in the closed state compared to the open state of the channel. Collectively, these observations appear comparable to results reported in the S4 segments of K\textsubscript{\textbf{a}} channels based on crystallographic and substituted Cys accessibility data, and further suggested that an equivalent of 2–3 gating charges associated with S4 may move in a single Hv subunit. To evaluate the kinetics of S4 movement associated with gating, the authors utilized voltage clamp fluorometry, in which changes in measured fluorescence are assumed to reflect conformational changes in the physical environment surrounding the amino acid position containing the introduced fluorescent probe. By labeling the outer end of the S4 segment with Alexa488-maleimide in Ser242Cys-substituted channels, the authors observed that re-arrangement of the S4 segment preceded the onset of ionic current in response to a depolarizing pulse, and the observed fluorescence signal was consistent with the concerted movement of two S4 segments (i.e. one in each channel subunit). In Hv channels constructed to be monomeric by deletion of the N- and C-terminus in each subunit, the S4-associated fluorescence signal overlapped the ionic current observed during channel activation, suggesting that movement of the single S4 segment is sufficient to activate the ion conduction pathway. As a further test of their predicted activation mechanism, the authors carried out electrophysiological measurements to evaluate the effective gating charge ($e_0$) in both dimeric and monomeric Hv channels. It was reasoned that if the S4 segment in each subunit of a dimeric channel must move in order for the two separate conduction pores to open upon depolarization, then one would expect the measured gating charge to be twice as large in a dimeric versus monomeric Hv channel. However, if activation of an individual conduction pore requires only the movement of the S4 segment in the same subunit, and occurs independently of the S4 segment in the second subunit of a pair, then the effective gating charge coupled with proton permeation should be similar in dimeric and monomeric channels. Analyses of electrophysiological recordings revealed that 2–3 effective gating charges were associated with the activation of monomeric channels, whereas it 4–6 gating charges were observed with the opening of dimeric channels. The observed doubling of the effective gating charge in dimeric versus monomeric Hv channels thus suggests a considerable amount of gating cooperativity between subunit pairs, such that neither conduction pore in the complex may open unless both S4 segments have been activated. Monomeric channels would not experience such prohibitive inter-subunit interactions, so opening of the conduction pore is dependent upon the movement of only a single S4 segment.

In summary, the authors have demonstrated that the S4 segment of Hv channels behaves as a voltage-sensitive element coupled to channel activation. Moreover, the opening of the proton conduction pores in a dimeric channel occurs in a strongly cooperative manner, consistent with the likelihood that both S4 segments must activate in order for either conduction to open. Our understanding of the physical mechanism underlying such gating control will undoubtedly be enhanced with more detailed structural information describing subunit contacts in dimeric Hv channels. Finally, it is noteworthy that a closely related, independent study by Ehud Isacoff and colleagues, published in the same issue of the journal, describes several aspects of the cooperative gating mechanism in dimeric Hv1 channels that complement and support the data presented by Gonzalez et al. Together, these two studies provide compelling evidence for a novel type of subunit interaction that tightly regulates gating of the conduction pore in a voltage-activated ion channel.

Note
See also: Tombola F, Ulbrich MH, Kohout SC, Isacoff EY. The opening of the two pores of the Hv1 voltage-gated proton channel is tuned by cooperativity. Nat Struct Mol Biol 2010; 17:44-50; PMID: 20023640; DOI: 10.1038/nsmb.1738.