Uncooperative Voltage Sensors

Richard Horn

Department of Molecular Physiology and Biophysics, Institute of Hyperexcitability, Jefferson Medical College, Philadelphia, PA 19107

A typical voltage-gated potassium (Kv) or sodium channel is exquisitely sensitive to small changes of membrane potential, so much so that a depolarization of <5 mV can increase its open probability by an order of magnitude. The high sensitivity is crucial for the rapid generation and propagation of action potentials in excitable cells. Optimizing sensitivity to membrane potential requires some careful design considerations, as I will discuss below. One important factor is that these proteins are either true tetramers, like Kv channels and the bacterial sodium channel NaChBac, or are pseudotetramers composed of homologous concatenated domains, as in eukaryotic sodium channels. Each sodium channel domain, or each Kv channel subunit, consists of six transmembrane segments, the fourth of which (S4) is a mélange of hydrophobic and basic amino acids. Every third residue of an S4 segment is an arginine or lysine. Typically, the four subunits of a Kv channel are identical, and the four sodium channel domains differ from one another. The canonical Kv channel *Shaker* has seven basic residues in its S4 segment, whereas a typical sodium channel has from four to eight positively charged side chains in each of its four S4 segments. The "voltage-sensing domain" is comprised of everything from the cytoplasmic 5′ end of the S1 segment through the intracellular 3′ end of the S4 segment. The remainder of the transmembrane segments (S5 and S6), and the loops between them, are typically called the "pore domain." The pathway for ion permeation is the water-filled central axis formed at the convergence of the four pore domains, one from each subunit or homologous domain. The activation gate that opens upon channel depolarization is formed by the intracellular ends of the four S6 segments (Doyle et al., 1998; Long et al., 2007).

Many of the essential features of the voltage-sensing mechanism are known. The primary voltage sensors are the cationic side chains of basic amino acids in the S4 segments. In the *Shaker* channel, the outermost four basic residues, all arginines, do the vast majority of the heavy lifting. Depolarization moves each of these side chains, along with its charges, almost completely across the membrane electric field, accounting for the bulk of charge movement during channel activation (Aggarwal and MacKinnon, 1996; Seoh et al., 1996). A further consensus is that, as in the original Hodgkin-Huxley model (Hodgkin and Huxley, 1952), all four of the channel's S4 segments must be in an activated conformation at a depolarized voltage before the channel can open. Part of the evidence for this assertion is that preventing the outward movement of only one S4 segment, by photocrosslinking it to a neighboring region, prevents *Shaker*'s activation gate from opening (Horn et al., 2000). Therefore, channel opening requires the participation of all four voltage sensors. This conclusion is furthered advanced by an article in this issue (see p. 467) by Gagnon and Bezanilla (2009). These authors propose, and provide evidence, that if three of the voltage sensors in a channel are in a permanently activated conformation, the fourth voltage sensor can open and close the channel by itself.

Design optimization

Imagine an intelligent designer presented with the goal of maximizing the sensitivity of an ion channel to changes of membrane potential. How would she approach this task? She would presumably begin by accompanying gate opening with as much charge movement as possible, knowing that the steepness of voltage-dependent opening correlates with the absolute amount of charge moved in response to a change of membrane potential. Second, she would make the gating process as simple as possible. A two-state closed-open transition with voltage-dependent forward and backward rate constants produces the steepest voltage dependence of gating, steeper, for example, than that obtained by any multistate sequential model of activation (Almers, 1978; Sigworth, 1994; Sigg and Bezanilla, 1997). Neither of these design criteria is ideal, however, for voltage-gated ion channels embedded in a bilayer membrane. The price paid for excess gating charge is that any depolarizing current, injected, for example, during action potential propagation, is diverted from charging the linear capacitance of the membrane (i.e., changing the membrane potential) to moving the excess gating charge of the voltage sensors. In effect, the charge

Correspondence to Richard Horn: Richard.Horn@Jefferson.edu

Abbreviation used in this paper: Kv, voltage-gated potassium.

The Rockefeller University Press $30.00
J. Gen. Physiol. Vol. 133 No. 5 463–466
www.jgp.org/cgi/doi/10.1085/jgp.200910236

that underlies channel gating increases the nonlinear capacitance of the membrane (Hodgkin, 1975). Any extra capacitative load from supercharged voltage sensors would slow the rate of action potential propagation. Moreover, creating simplicity out of a multimeric protein with four separate voltage sensors is a tall order. Movement of each voltage sensor is a kinetic transition, so that channel opening will require at least four conformational changes, not one.

Cooperativity

One solution to the simplicity challenge is to use cooperativity. Just as positive cooperativity in binding increases sensitivity to ligand concentration (Perutz, 1989), cooperativity in gating transitions enhances the steepness of the voltage-dependent opening of ion channels (Sigworth, 1994; Zagotta et al., 1994; Sigg and Bezanilla, 1997). There are two ways that such cooperativity might be introduced into a channel's gating mechanism, by including it in either voltage sensor movement or gate opening.

The first approach would be to create a positive cooperativity in S4 movement, so that outward movement of one voltage sensor, for example, would decrease the activation energy for outward movement of another voltage sensor. In the extreme, the S4 segments would be coupled so tightly that they would move back and forth in lock-step response to changes of membrane potential; i.e., S4 movement would be concerted. Although this would work conceptually to help maximize voltage sensitivity, there are two inherent difficulties with this design strategy. The first is that a negative, not positive, cooperativity in S4 movement is predicted if the S4 segments are close enough to interact electrostatically. The outward movement of one S4 segment with its positive charges would introduce a repulsive electrostatic energy to the outward movement of a neighboring S4 segment. In fact, one study of *Shaker* channel gating reported a slightly negative cooperativity in charge movement along the activation pathway (Zagotta et al., 1994; but see Schoppa and Sigworth, 1998). The other obstacle to the creation of highly cooperative S4 movement is revealed in the crystal structure of a eukaryotic Kv channel, where the four voltage-sensing domains are situated peripherally, like the leaves of a four-leaf clover, around the central pore domains (Fig. 1) (Long et al., 2007). The disjoint locations of the voltage-sensing domains do not obviate allosteric communication, of course, because there is extensive intersubunit contact; but the S4 segments themselves (the black helices in Fig. 1) are well separated. Moreover, the large distance between S4 segments would tend to minimize electrostatic interactions between them.

Hypothetical considerations aside, however, detailed electrophysiological studies, some of which are accompanied by fluorescence measurements from labeled voltage sensors, show that independent movement of the individual S4 segments underlies the bulk of the charge movement coupled to channel opening (for review see Sigworth, 1994; Yifrach, 2004; Tombola et al., 2006).

So how can steep voltage dependence be introduced into a quartet of uncooperative voltage sensors? In *Shaker* channels, strongly positive cooperativity can be found, not so much in voltage sensor movement, but in the final opening steps at the depolarized end of the activation pathway. The four S6 segments appear to move in a concerted manner, rather than individually, to either open or close the activation gate, and this gate movement is very tightly coupled to the position of the S4 segments. In a wild-type channel, all four of the S4 segments must be in a fully activated state for the channel to be open, and when the S4's are all activated, the equilibrium constant of gate movement strongly favors the open state. This introduces a positive cooperativity to the entire gating scheme, which in turn enhances the voltage dependence of activation gating (Sigworth, 1994; Zagotta et al., 1994; Sigg and Bezanilla, 1997). It is worth noting that the level of cooperativity of gating processes in *Shaker* falls on a continuum, with activation gating at one extreme, ball-and-chain inactivation at the other (the four balls bind independently; MacKinnon et al., 1993), and C/P-type inactivation in between (Ogielska et al., 1995; Panyi et al., 1995).

Gating controlled by a single voltage sensor

The above considerations make a prediction that is tested by Gagnon and Bezanilla (2009), namely that a single voltage sensor could force the *Shaker* activation gate open and closed, if the other three voltage sensors are all in a constitutively activated conformation. Gagnon and Bezanilla achieved this feat by using concatenated tandem tetramers, a single polypeptide containing four subunits, each of which can be mutated individually. This technique, previously used to explore subunit cooperativity (Tytgat and Hess, 1992; Zheng and Sigworth, 1998; Mannuzzu and Isacoff, 2000; Zandany et al., 2008), was used here to create a tetramer with one wild-type subunit, and three identical mutant subunits in which the outermost four S4 arginines were replaced by neutral polar residues, either glutamine or asparagine. I will refer to this 3:1 heterotetramer as the mutant construct. Gagnon and Bezanilla argue convincingly that the mutant subunits are largely insensitive to membrane potential and therefore cannot contribute to voltage sensing. In the absence of a membrane electric field, i.e., at zero mV, *Shaker*'s wild-type S4 segments are in their outward depolarized conformation (Patlak, 1999). However, it is not obvious a priori whether neutralizing the primary charge-carrying residues would leave the S4 segment in a fully activated conformation, a necessary prerequisite for the 3:1 mutant construct to be functional and responsive to changes of membrane potential.

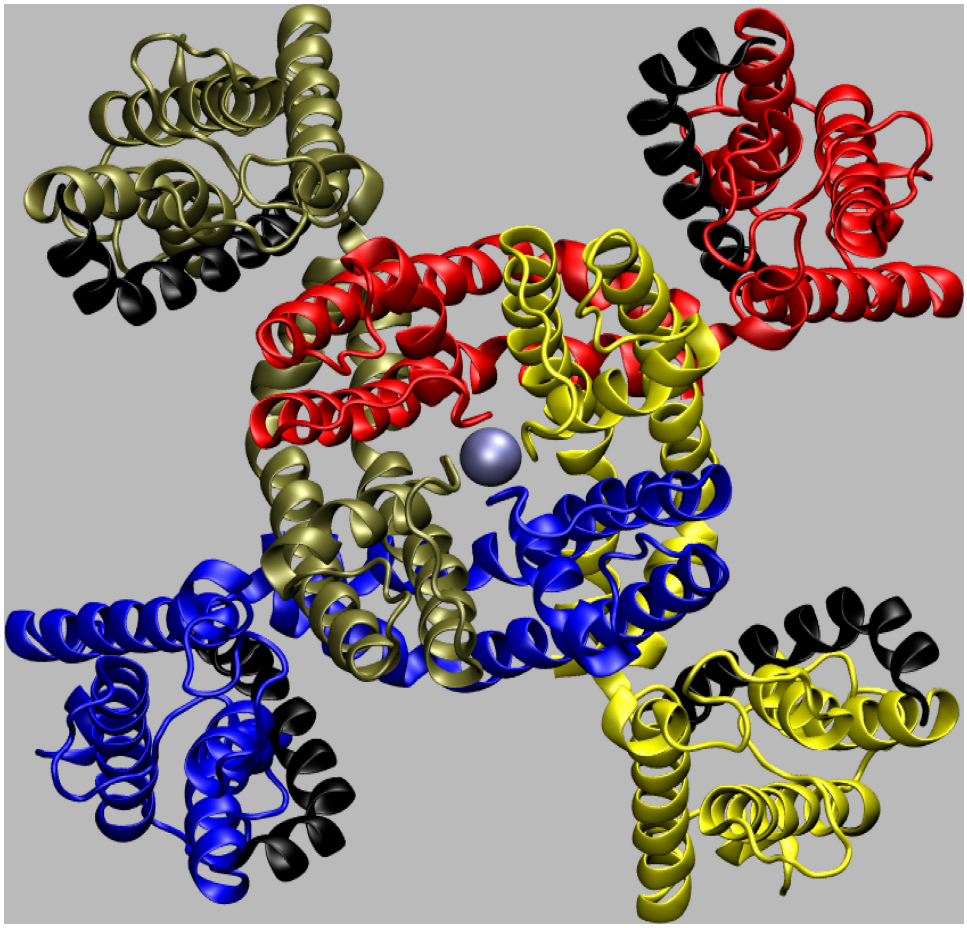

Figure 1. Bird's eye view of a Kv channel, based on its crystal structure (Long et al., 2007). Each subunit has a different color (red, blue, tan, and yellow), a K^+ ion in the selectivity filter is cyan, and the S4 segments of each subunit are black. The VMD program was used for graphics (http://www.ks.uiuc.edu/Research/vmd/).

Nevertheless, the data in this paper strongly suggest that the neutralized voltage sensors in the mutant construct are in a constitutively activated conformation, waiting patiently for the single fully charged S4 segment to join them and allow the channel to open.

As evidence for this scenario, the conductance–voltage relationships are left-shifted and have a shallow voltage dependence in the mutant construct. This is expected if the single charged S4 segment pays little attention to the three neutral S4 segments, and opening occurs when the one charged S4 segment is in its activated conformation. Another satisfied prediction of this proposal is that the usual sigmoidicity, or delay, preceding opening in response to a step depolarization should be reduced because only one S4 segment has to move to open the channel; i.e., there are fewer closed states to traverse in the mutant construct. Finally, the kinetics of deactivation are slowed in the mutant construct, as expected because a wild-type homotetramer can be closed when any of its four voltage sensors deactivate, which for probabilistic reasons would occur more rapidly by chance than the deactivation of a single voltage sensor.

One of the intriguing findings in this study is that, at fully activated voltages, the rate of slow C/P-type inactivation is almost oblivious to the presence of subunits with a neutral S4 segment. This supports the idea that slow inactivation is accompanied by voltage-dependent movement of *Shaker*'s charged S4 segments (Loots and Isacoff, 2000), and that the neutral S4 segments are unresponsive to changes in membrane potential. However, the nature of the coupling between S4 movement and slow inactivation remains a mystery.

Although the ability of a single S4 segment to pilot voltage-dependent gating in a Kv channel is a novel observation, controlling activation gating with a reduced arsenal of S4 segments has been observed previously in naturally concatenated subunits, i.e., in a sodium channel. The S4 segment of the second homologous domain (D2) can apparently be trapped in its activated conformation by the binding of β–scorpion toxin (Cestèle et al., 2001). As in the study of Gagnon and Bezanilla, immobilizing this S4 segment shifted the channel activation to more hyperpolarized potentials and slowed the rate of deactivation.

The resurgence in the use of concatenated tetramers as a tool to study cooperativity among potassium channel subunits (Zandany et al., 2008; Gagnon and Bezanilla, 2009) has been joined recently by a complementary technique. Bosmans et al. (2008) have transplanted a selected

region from one of the four sodium channel domains into a Kv channel, producing a channel with four identical subunits, thus allowing them to study one homologous domain at a time. Selected use of these two approaches promises to unmask some of the previously inscrutable features of the allosteric communication among entwined Kv subunits and between the homologous domains of sodium channels.

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