The Journal of General Physiology

Commentary

How S4 Segments Move Charge. Let Me Count the Ways

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The four "charged particles" predicted to underlie the voltage-dependent gating of some ion channels (Hodgkin and Huxley, 1952) are now known to be the four S4 segments, putative α-helices in which every third residue (in many, but not all, S4 segments) is either arginine or lysine. These charged structures must reside at least partly within the membrane electric field and move some of their positive charges across this electric field in response to changes of membrane potential. There are several classes of movement that could accomplish this feat (for some examples, see Yellen, 1998), including a collapse of the electric field around the S4 segment, a novel mechanism suggested in a paper in this issue of the *Journal of General Physiology* (Bell et al., 2004).

The electrophoretic task of the voltage sensor is to move charge down a gradient of membrane potential. In response to a depolarization (inside more positive) the charged side-chains of S4 segments either must move outward through the membrane's electric field or else the field must move inward past these side-chains (Yang et al., 1996). Thermodynamically and topologically these electrostatic alternatives are equivalent, involving a relative movement between the basic S4 residues and the electric field. To evaluate the many candidates that have been proposed for the actual charge transfer it is important to consider whether the S4 segment maintains its secondary structure during charge movement. Although the voltage-dependent unwinding of the putative S4 helix within the hydrophobic interior of the membrane has been considered (Durell and Guy, 1992; Sigworth, 1994; Aggarwal and MacKinnon, 1996), the energetic cost of breaking so many hydrogen bonds within the lipid (10 s of kcal/mol; Ben-Tal et al., 1996) makes this an unlikely possibility. Therefore, I will assume for the moment that S4 is an α-helix and that it maintains its helical structure during charge movement.

The environment around the S4 segment is critical for its ability to participate in charge movement. Each of the four subunits of voltage-gated potassium channels (Kv), or four tethered domains of sodium or calcium channels, comprises segments S1–S6, generally believed to be transmembrane α-helices. Only the S4 segment is significantly charged, with up to eight basic residues. A large body of experimental work has led to the notion that the S4 segment has a transmembrane orientation and, except for a short hydrophobic corset, also known as the gating pore, around its waist, is surrounded by aqueous crevices or vestibules (Larsson et al., 1996; Yang et al., 1996; Yusaf et al., 1996; Baker et al., 1998; Wang et al., 1999; Schönherr et al., 2002). This concept of the milieu surrounding the S4 segment derives mainly from cysteine accessibility scanning using hydrophilic cysteine reagents. These structural features suggest that the electric field drops primarily across the gating pore and that charge movement involves a sliding motion of the S4 segment through this hydrophobic gasket.

Uncertainties remain, but the above picture has evolved and guided the experimental and conceptual development of most current gating models—to the point where it can be regarded as the canonical model for charge movement. By stark contrast, the recent crystal structure of a bacterial Kv channel, KvAP (Jiang et al., 2003a), implies that S4 segments are largely embedded in lipid, which leads to a very different model for voltage sensor movement. A helix-loop-helix "voltage sensor paddle," comprised of the proximal ends of the S3 and S4 segments and the short S3-S4 linker, is proposed to move as a unit across the hydrocarbon core of the bilayer during charge movement (Jiang et al., 2003b). This radically different concept of charge movement is at odds both with the prevailing notion that S4 segments are largely surrounded by water at all membrane potentials, and also with the results reported in two papers in this issue of the journal (Bell et al., 2004; Vemana et al., 2004) in studies on hyperpolarization-activated HCN channels. Both of these papers show that the NH_2-terminal end of the S4 segment of the mammalian HCN channel is accessible to the extracellular aqueous space at all membrane potentials, whereas the paddle model predicts that this region of the S4 segment is close to the intracellular aspect of the lipid bilayer at hyperpolarized voltages (Jiang et al., 2003b). Although the interpretation of cysteine labeling has its limitations (Karlin and Akabas, 1998), the conflict between these two models of charge movement is real, as also pointed out in other recent papers that evaluate the voltage-sensor paddle model (Ahern and

J. GEN. PHYSIOL. © The Rockefeller University Press • 0022-1295/2004/01/1/4 $8.00
Volume 123 January 2004 1–4
http://www.jgp.org/cgi/doi/10.1085/jgp.200308975

Horn, 2003; Broomand et al., 2003; Cohen et al., 2003; Gandhi et al., 2003; Lainé et al., 2003; Lee et al., 2003; Miller, 2003).

Before discussing the Bell et al. (2004) and Vemana et al. (2004) articles in more detail, I would like to focus briefly on interpretational uncertainties that may result from the detailed potential profile across membranes and membrane proteins. The electric field is normal to the plane of the membrane in a featureless low-dielectric bilayer, but the shape of the field in the vicinity of the S4 segment is likely to be much more complicated, due in part to the aqueous crevices that penetrate the protein from both sides of the membrane. If the electric field remains approximately normal to the membrane, as assumed in several recent models (Glauner et al., 1999; Gandhi and Isacoff, 2002; Horn, 2002; Lecar et al., 2003), then charge movement can be achieved by simply sliding the S4 helix outward through the gating pore in response to a depolarization. Indeed, some S4 residues appear to move from one side of the membrane to the other during changes of membrane potential (Yang et al., 1996; Starace et al., 1997; Starace and Bezanilla, 2001). If, on the other hand, the aqueous crevices reshape the electric field so that it lies more parallel to the membrane, then a pure rotation of the S4 segment can transfer charge without a translational movement of the helix (Cha et al., 1999; Bezanilla, 2000, 2002; Horn, 2002). These alternative S4 movements, translation and rotation, are not mutually exclusive. In the “helical screw model” depolarization causes each charged residue along the S4 segment to follow the same path through the hydrophobic core of the protein (Catterall, 1986; Guy and Seetharamulu, 1986; Glauner et al., 1999; Keynes and Elinder, 1999; Gandhi and Isacoff, 2002; Lecar et al., 2003). Because these charged residues are oriented in a left-handed spiral around the helix, outward charge movement through a fixed gating pore is induced when an S4 segment follows this spiral itinerary.

HCN channels are structurally homologous to Kv channels, having S1–S6 segments, including a positively charged S4 segment with the characteristic pattern of several basic residues spaced three residues apart. The location of the HCN activation gate, at the cytoplasmic convergence of the four S6 segments, is also shared with potassium channels (Del Camino et al., 2000; Del Camino and Yellen, 2001; Shin et al., 2001; Rothberg et al., 2002). In spite of these structural similarities HCN channels, unlike Kv channels, open when hyperpolarized. One apparently remote possibility, that S4 segments of HCN move inward during a depolarization, was convincingly excluded by cysteine accessibility scanning of nonvertebrate HCN isoforms (Männikkö et al., 2002; Latorre et al., 2003; Sesti et al., 2003). The data from these three studies are consistent with the conventional model for S4 movement, in that S4 segments are largely surrounded by aqueous vestibules and move outward through a short gating pore when depolarized. Thus, the fundamental biophysical difference between Kv and HCN channels is that the coupling between S4 position and the activation gate has the opposite polarity. Interestingly, however, and indicative of the remaining uncertainties, Bell et al. (2004) and Vemana et al. (2004) propose distinctly different ways that the S4 segments could move charge, based on cysteine accessibility scanning.

There are two significant differences between the voltage-dependent accessibilities of S4 residues of mHCN1 and Kv channels. The first concerns the outer four basic residues of the S4 segments. These residues carry most of the charge in Kv channels (Aggarwal and MacKinnon, 1996; Seoh et al., 1996) and accordingly show the largest voltage-dependent changes of accessibility, compared with other basic residues in the S4 segment. In mHCN1, by contrast, the outer four basic residues of the S4 segment remain permanently accessible from the extracellular solution at all voltages. The second significant difference concerns the length of the inaccessible gating pore. In Kv channels depolarization makes extracellular S4 residues emerge outside while intracellular residues disappear into the gating pore. The overall effect is topologically simple - outward translation of the S4 segment through an inaccessible barrier of roughly fixed dimensions (but see Larsson et al., 1996). In mHCN1, however, depolarization causes intracellular S4 residues to disappear without a comparable emergence of residues on the outside. If the S4 segment retains its secondary structure, this means either that the intracellular aqueous vestibule collapses upon depolarization (Fig. 6 B of Bell et al., 2004), i.e., the gating pore becomes ~20 Å longer, or that the bottom of the S4 segment swings out of this vestibule into a hydrophobic region adjacent to it. Two other possibilities involving changes in secondary structure were considered, either a voltage-dependent kinking of the S4 segment (Fig. 6 A in Bell et al., 2004), or a partial unraveling of the S4 segment at hyperpolarized voltages (Fig. 7 in Vemana et al., 2004). Depending on the yet-unknown morphology of the electric field around the S4 segment, any of these conformational changes could be accompanied by outward charge movement.

The available experimental results on mHCN1 cannot distinguish among the aforementioned possibilities, but they open the playing field to new contestants for models of charge movement. The distinctive feature of this new class of models is in the dynamic roles of the participants. Instead of positively charged, rigid rods moving stiffly through a static landscape of aqueous vestibules and a gating pore, voltage changes could induce dramatic metamorphoses of the S4 segment it-

self and its environment. The notion that membrane-spanning α-helices are rigid rods may need to be revised. Furthermore, the possibility of an important role of malleable vestibules and crevices is a particularly intriguing concept. The ability of water and protons to penetrate proteins is well known (Ernst et al., 1995; Luecke et al., 1998); this infiltration leads to a significant increase in the dielectric constant of the protein interior (Garcia-Moreno et al., 1997; Dwyer et al., 2000). Such a network of crevices will reshape the electric field (Sansom et al., 1997; Islas and Sigworth, 2001). Moreover, changes in transmembrane potential can move charge, as suggested here (Bell et al., 2004), by reshaping aqueous crevices around charged residues (see also Nguyen and Horn, 2002). Finally, the strongly hydrophilic arginine and lysine residues that spiral around the S4 segment may play a role in shaping and maintaining hydrophilic crevices, which in turn may enhance the flexibility of the voltage-sensing domain by reducing hydrophobic forces tending to adhere adjacent transmembrane α helices. Crevice sculpting and preservation may also be an explanatory factor in the strong conservation of some of the S4 basic residues that do not themselves carry charge.

Olaf S. Andersen served as editor.

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