Helical motion of an S4 voltage sensor revealed by gating pore currents

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The mechanism by which the voltage sensors of voltage-gated ion channels move gating charge during the activation process is a subject of active debate. In this issue of Channels, Gamal El-Din et al.1 probe the movements of the S4 voltage sensor of Shaker K+ channels through clever use of omega gating pore currents generated by paired gating-charge mutations. Their results provide strong support for a sliding helix or helical screw mechanism of gating charge movement.

Models for Gating Charge Movement

In their landmark papers on voltage clamp analysis of sodium currents in the squid giant axon, Hodgkin and Huxley predicted that the activation of the sodium conductance must involve the movement of charged particles through the membrane electrical field.2 The gating charge movements they predicted have been directly measured, and current estimates indicate movement of 12–16 positive charges outward across the electric field during gating of Na+ or K+ channels.3 How do these gating charges move across the membrane?

The gating current is primarily carried by a set of four highly conserved positively charged amino acid residues (usually arginines) spaced at three-residue intervals in the S4 segments of the principal subunits of voltage-gated ion channels. S4 resides in a four-helix bundle of S1-S4 segments in each of four domains or subunits of voltage-gated Na+ or K+ channels (Fig. 1). The three-dimensional structure of the activated state of Kv1.2 channels revealed the structure of the S1-S4 segments and showed that the four arginine gating charges (R1-R4) are in an outward position relative to two highly conserved negative charges in the neighboring S2 segments(Fig. 1B, Activated).4 However, the structure of the resting state remained unknown. Structural modeling with the Rosetta Membrane algorithm revealed resting state structures in which the S4 segment was in an inward position, with the R1 and R2 gating charges interacting with the outermost negative charge in S2 (Fig. 1A, Resting).5,6 In the sliding helix or helical screw models of voltage sensor function, the S4 segments remain in a transmembrane position in the resting state, and move from resting to activated positions by a helical motion through the protein structure.7,8 A structural version of this model shows that outward movement of the S4 segment is catalyzed by stepwise exchange of ion pair partners.5,6 In contrast, in the paddle model based on the structure of the bacterial K,AP channel,9 the S3-S4 helical hairpin lies separate from the rest of the voltage sensor near the intracellular surface in the resting state and moves like a paddle through the lipid bilayer during activation (Fig. 1C and D). These two models make very different predictions concerning the molecular interactions experienced by the S4 segment during activation—primarily protein interactions in the sliding helix or helical screw models versus primarily lipid interactions in the paddle model.

Gating Pore Current as a Structural Probe of Voltage Sensor Function

Mutations of the S4 gating charges to smaller, uncharged residues induce a leak current through the mutant voltage sensor,

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Figure 1. Structural representations of the sliding helix/helical screw and paddle models of voltage-dependent gating. The K$_{1.2}$ channel was modeled using the ROSETTA Membrane method, based on previous work.\(^6\) (A) Rosetta-Membrane resting state model of K$_{1.2}$ channel.\(^6\) A single subunit of the voltage-sensing domain is shown attached to three subunits of the pore-forming domain for clarity. Transmembrane segments S3b and S4 are colored red and all other transmembrane segments are colored blue. Positions of the Cβ atoms of gating charge-carrying arginines in S4 (labeled as R1 through R4 and colored in cyan) and negatively charged residues in S2 (labeled E1 and E2 and colored in orange) are shown in sphere representation. (B) Rosetta-Membrane activated state model of K$_{1.2}$ channel.\(^6\) The model is colored and labeled as in (A). (C) The resting state model of K$_{AP}$ based on biotin and avidin measurements of the S3-S4 motion and closed state structure of KcsA.\(^9\) (D) The activated state model of K$_{AP}$ based on biotin and avidin measurements of the S3-S4 motion and open state structure of the pore-forming domain of K$_{AP}$.\(^9\)

In the Shaker K$^+$ channel, substitution of the single R1 gating charge caused gating pore current in the resting state.\(^10,11\) In the brain Na$_{1.2}$ channel, mutation of both R1 and R2 gating charges to glutamine was required to observe gating pore current in the resting state.\(^12\) In both cases, depolarization of the membrane to cause activation of the voltage sensor blocked the gating pore current, consistent with the model that outward movement of the voltage sensor moves the defective R1 and R2 gating charges out of the gating pore and blocks the leak. The ability of mutations of the R1 and R2 gating charges to induce a voltage-dependent ionic leak through the voltage sensor is compatible with the sliding helix or helical screw models of voltage sensor function, because the S4 segment is in a transmembrane position in the resting state (Fig. 1A) and removal of the positively charged arginine side chains might be expected create a pore through which an ionic leak could be conducted. On the other hand, the position of the S3-S4 helical hairpin on the intracellular side of the membrane in the resting state in the paddle model (Fig. 1C and D) would not allow a transmembrane ionic leak due to mutation of gating charges.

For Na$_{1.2}$ channels, conversion of the R2 and R3 gating charges to glutamine induces gating pore current in the activated state, which is blocked by repolarization to return the channel to the resting state.\(^12\) These results indicate that outward movement of the defective R2 and R3 gating charges into the gating pore upon activation causes an ionic leak through the voltage sensor. These results require that the S4 segment have a transmembrane position in both resting and activated states and move outward into the gating pore upon membrane depolarization.

Gamal El-Din et al.\(^1\) carry the analysis of S4 movement by measurement of gating pore currents an important additional step forward. They report that paired mutations of two arginine gating charges in Shaker K$^+$ channels to smaller residues are generally required to induce gating pore current. The previously observed gating pore current measured for mutations of the R1 gating charge alone depends on the location of a small, uncharged amino acid residue (alanine) in the -3 position in the Shaker amino acid sequence.\(^1\) Importantly, Gamal El-Din et al.\(^1\) report that paired small residues at positions R0-R1, R1-R2 and R2-R3 all induce gating pore current. Moreover, they find that progressively stronger depolarizations are required to generate gating pore current from the three paired mutants—more inward mutations require stronger depolarization to induce gating pore current. Their results argue persuasively that depolarization forces the S4 segment to move outward along a helical pathway, sequentially placing paired mutant gating charges in the gating pore and generating gating pore current. Only an outward helical motion of the S4 segment through the gating pore in the protein can easily accommodate these experimental results.\(^1\)

**Complementary Support for Helical Movement of S4 Voltage Sensors from Disulfide-Locking Experiments**

Recent work from a different experimental approach has provided strong, complementary support for helical movement of the S4 voltage sensor. Disulfide bond formation between substituted cysteine residues requires the sulfur atoms in the cysteine side chains to approach within 2 Å, providing a high-resolution method of analysis of protein interactions, and this method has been employed extensively in studies of protein folding. Using this method, Broomand and Elinder showed that the S3 segment must rotate with respect to the S4 segment during activation of the Shaker K$^+$ channel.\(^15\) Campos et al. showed that the R1 gating charge of the Shaker K$^+$ channel can be disulfide-crosslinked to amino acid residues near the extracellular end of the S2 and S3 segments.\(^14\) Moreover, DeCaen et al.\(^15,16\) have demonstrated sequential formation of ion pairs between the R3 and R4 gating charges and the two conserved...
negative charges in the inner and outer halves of the S2 segment during activation of the voltage sensor of the bacterial sodium channel NaChBac. All of these sets of results require a helical outward movement of the S4 segment to place the substituted cysteine residues in position to form disulfide bonds.

How Many Gating Charges Reside in the Constriction in the Gating Pore Simultaneously?

Based on the requirement for paired substitution of small amino acid residues in order to induce gating pore current, Gamal El-Din et al. propose that two gating charges occupy the gating pore simultaneously during activation of the voltage sensor and place a lower limit on the length of the narrow constriction in the gating pore of 9 Å. Previous work also revealed that sor and place a lower limit on the length that these results, single mutations of R1 or R2 gating charges in the voltage sensor in domain II of skeletal muscle NaV1.4 channels is sufficient to induce voltage-dependent cation gating pore current that leads to Hypokalemic Periodic Paralysis, and single mutations of the R3 gating charge induce gating pore current in the activated and slow-inactivated states that cause Normokalemic Periodic Paralysis. These results might suggest that only a single gating charge occupies a shorter gating pore in domain II of NaV1.4 channels. However, the size of the gating pore current caused by these single disease mutations (approximately 1.25% of the central pore current) is much smaller than the gating pore current reported for Shaker K+ channels, which can be as large as the alpha central pore current. Therefore, it may rather be that mutations of single amino acid residues generate inefficient gating pore conductance in comparison to paired mutations because only one of the two gating charges in the constriction in the gating pore is mutated. Considered together, the results of Gamal El-Din et al. and the results of disulfide-locking experiments are most consistent with the model of two gating charges simultaneously in position in the narrow segment of the gating pore, interacting with the two conserved negatively charged residues in the S2 segment whose positions define the gating pore structure. Mutation of a single gating charge can yield a small gating pore current, at least in NaV1.4 channels, whereas paired mutations are required for large gating pore currents. The placement of two gating charges within the gating pore may serve to catalyze the outward movement of the voltage sensor by stabilizing two gating charges simultaneously through ion pair interactions in the gating pore.

References
1. Gamal El-Din TM, Heldstab H, Lehmann C, Greeff N. Double gaps along Shaker S4 demonstrate omega currents at three different closed states. Channels 2009; 3.
2. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 1952; 117:500-44.
3. Bezanilla F. The voltage sensor in voltage-dependent ion channels. Physiol Rev 2000; 80:555-92.
4. Long SB, Campbell EB, MacKinnon R. Crystal structure of a mammalian voltage-dependent Shaker family potassium channel. Science 2005; 309:897-903.
5. Yarov-Yarovoy V, Baker D, Carterall WA. Voltage sensor conformations in the open and closed states in ROSETTA structural models of potassium channels. Proc Natl Acad Sci USA 2006; 103:7292-7.
6. Parkhak MM, Yarov-Yarovoy V, Agarwal G, Roux B, Barth P, Kolour S, et al. Closing in on the resting state of the Shaker potassium channel. Neuron 2007; 56:40.
7. Carterall WA. Molecular properties of voltage-sensitive sodium channels. Ann Rev Biochem 1986; 55:953-85.
8. Gay HR, Seeraramulu P. Molecular model of the action potential sodium channel. Proc Natl Acad Sci USA 1986; 83:508-12.
9. Jiang Y, Ruta V, Chen J, Lee A, MacKinnon R. The principle of gating charge movement in a voltage-dependent potassium channel. Nature 2003; 423:42-8.
10. Tombola F, Parkhak MM, Isacoff EY. Voltage-sensing arginines in a potassium channel permeate and occlude cation-selective pores. Neuron 2005; 45:379-88.
11. Tombola F, Parkhak MM, Goriotizia P, Isacoff EY. The twisted ion-permeation pathway of a resting voltage-sensing domain. Nature 2007; 445:546-9.
12. Sokolov S, Scheuer T, Carterall WA. Ion permeation through a voltage-sensitive gating pore in brain sodium channels having voltage sensor mutations. Neuron 2005; 47:183-9.
13. Boonmann A, Elinder F. Large-scale movement within the voltage sensor paddle of a potassium channel-suggest for a helical screw motion. Neuron 2008; 59:270-7.
14. Campos FV, Chanda B, Roux B, Bezanilla F. Two atomic constraints unambiguously position the S4 segment relative to S1 and S2 segments in the closed state of Shaker K channel. Proc Natl Acad Sci USA 2007; 104:7904-9.
15. DeCaen PG, Yarov-Yarovoy Y, Zhao Y, Scheuer T, Carterall WA. Disulfide locking a sodium channel voltage sensor reveals ion pair formation during activation. Proc Natl Acad Sci USA 2008; 105:15142-7.
16. DeCaen PG, Yarov-Yarovoy Y, Sharp EM, Scheuer T, Carterall WA. Sequential formation of ion pairs during activation of a sodium channel voltage sensor. Proc Natl Acad Sci USA 2009; 106:22498-503.
17. Sokolov S, Scheuer T, Carterall WA. Gating pore current in an inherited ion channelopathy. Nature 2007; 446:76-8.
18. Sokolov S, Scheuer T, Carterall WA. Depolarization-activated gating pore current conducted by mutant sodium channels in potassium-sensitive normokalemic periodic paralysis. Proc Natl Acad USA 2008; 105:19980-5.