Structural basis for gating charge movement in the voltage sensor of a sodium channel

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AUTHOR SUMMARY

Voltage-gated sodium (Na_V) channels are responsible for initiation and propagation of action potentials in nerve, muscle, and other electrically excitable cells (1). These channels permit the movement of charged particles (ions) across cell membranes, and thereby, they rapidly change the electrical potential across the membrane and generate action potentials. Here, we used a combination of structural modeling, bioinformatics analysis, and 3D structures of related Na_V and potassium (K_V) channels to analyze the molecular mechanism by which a bacterial Na_V channel responds to changes in membrane potential. We show that sequential state-dependent interactions between the positive gating charges in S4 segments and their negatively charged partners in nearby membrane segments of the channel serve to move the gating charges out across the membrane as the membrane potential becomes more positive. Our results define the interactions of the gating charges in the resting and activated states of the voltage-sensing component of the channel, the voltage-sensing domain, and provide experimental confirmation of those models by structure function studies.

Mammalian Na_V and calcium (Ca_V) channels consist of four homologous domains (I–IV), each containing six segments (S1–S6) that cross the cell membrane and a pore loop that reenters the membrane between the S5 and S6 segments (2). Segments S1–S4 form the voltage-sensing domain, whereas the pore consists of segments S5 and S6 and the pore loop. The hallmark feature of the voltage-gated ion channels is the steep dependence of their activation on the voltage across the cell membrane. This property derives from the voltage-driven outward movement of gating charges in response to depolarization (that is, in response to making the membrane potential more positive inside the cell) (3). The S4 transmembrane segment in the voltage-sensing domain has four to eight arginine residues spaced at intervals of 3 aa, which serve as gating charges. These gating charges are pulled in by the internally negative transmembrane electric field in the resting state of the cell and are released to move out on depolarization. Outward movement of the gating charges must be catalyzed by the voltage sensor because of the large thermodynamic barrier against the movement of electrically charged amino acid residues across the membrane. The simpler bacterial Na_V channels (4) consist of four small, identical subunits, and each is similar in structure and function to one domain of mammalian Na_V and K_V channels. We studied the ancestral Na_V channel NaChBac to focus on the essential structural elements required for voltage sensing.

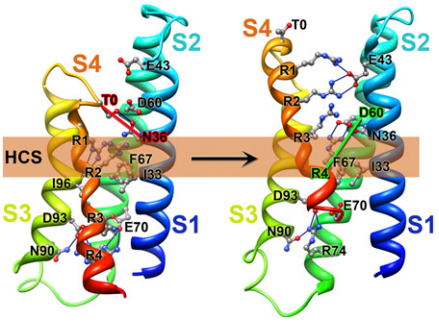

Fig. P1. Model of the voltage-sensing domain of NaChBac. Transmembrane view of the lowest-energy models of the voltage-sensing domain channel in Resting State 1 (*Left*) and Activated State 3 (*Right*). Side chains of the gating charge-carrying arginines in S4 and key residues in S1, S2, and S3 segments are shown in stick representation and labeled. Most models of Resting State 1 predict that R1 forms hydrogen bonds with a portion of S3. R3 makes ionic interactions with the intracellular negatively charged cluster, and R4 forms an ion pair with D93 (in S3). Activated State models predict various interactions among R1, R2, R3, R4, and the surrounding regions.

The voltage sensors of ion channels move the gating charges out across the membrane permeability barrier. As a landmark in our structures, we highlight a hydrophobic constriction site, which has been conserved throughout evolution (Fig. P1, orange). It lines the narrowest part of the pathway for gating charge movement and likely forms a hydrophobic seal that prevents ion movement (Fig. P1). We modeled three activated states of the voltage-sensing domain of NaChBac based on structural constraints derived from disulfide-locking studies of cysteine residues that were substituted for the gating charges and their interacting partners. In the most activated state (Activated State 3) (Fig. P1), a network of hydrogen bonds stabilizes the voltage sensor, and the side chains of gating charges R1–R4 interact with nearby negatively charged and hydrophilic amino acid side chains in the extracellular negative cluster (Fig. P1). Our models of these three activated states illustrate progressive outward movement of the S4 gating charges as they exchange hydrogen-bonding and ion-pair partners along their path. These interactions catalyze the outward movement of the gating charges from interaction with the intracellular negative charge cluster through the hydrophobic constriction site to interaction with the extracellular negative cluster.

Because there is no electrical potential in a protein crystal, X-ray crystallography cannot be used to determine the structure of the resting states of ion channels; the resting state requires a negative membrane potential in the range of −80 mV. We used the disulfide-locking method to map interactions of the gating

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The authors declare no conflict of interest.

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charges and the intervening hydrophobic amino acid residues with the key ion pair partner, D60 in the S2 segment, in the resting states of NaChBac. The results support a model in which T0, R1, and R2 plus intervening hydrophobic residues interact with D60 in the resting state. The interaction of L112, which precedes R2, with D60 takes place near the transition to the activated states. Mutant cycle analysis showed that interactions of D60 with T0 and R1 oppose activation, whereas D60 interactions with R2 favor activation. The exchange of interactions of D60 from R1 to R2 may represent the transition point to the activated states.

Based on these well-defined molecular interactions in the resting states, our structural analysis characterized the structures of three resting states in which the gating charges are positioned progressively more on the intracellular side of the membrane and interact with the intracellular negative charge cluster (Fig. P1). In the deepest resting state of the voltage-sensing domain (Resting State 1), R3 and R4 are located on the intracellular side of the hydrophobic constriction site, where they interact with the intracellular negative charge cluster, and R1 and R2 are positioned within the narrow gating pore region, where they can prevent ion leak and respond rapidly to changes in electrical potential (Fig. P1).

A critical test of our model is to predict the results of disulfide-locking experiments with the hydrophobic residues that are located between the gating charges in the S4 segments. Our structural models of resting states predict an α-helical conformation at the extracellular end of the S4 segment but a 3_{10} helical conformation from R2 to R4. This prediction was confirmed by the disulfide-locking studies. Comparison of our structural models for resting states vs. activated states suggests several probable features of the voltage-sensing mechanism. (*i*) Hydrophobic side chains create the hydrophobic constriction site (Fig. P1, orange band). (*ii*) S4 moves out, rotates, and tilts as it passes through the hydrophobic constriction site (Fig. P1). (*iii*) On the extracellular side, the movement of S4 creates a larger cleft between the S1–S2 and S3–S4 loops in activated states (Fig. P1). (*iv*) On the intracellular side, the combination of S4 motions imposes a sideways gating movement of the S4–S5 linker parallel to the plane of the inner surface of the membrane. This movement may cause the entire voltage-sensing domain to rotate relative to the axis of the pore and induce pore opening. (*v*) A conformational change within the S4 segment of the voltage-sensing domain allows the R1–R3 region of S4 to carry gating charges through a narrow groove formed by the S1, S2, and S3 segments (Fig. P1). These movements of the voltage-sensing domain define the structural basis for electrical signaling by voltage-gated ion channels (Movies S1, S2, and S3 show these movements of the voltage-sensing domain more clearly).

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