

evaluated the functional consequences of severing this molecular interaction. The resulting phenotype for these perturbations was a non-inactivating one, albeit having altered conduction properties (smaller single-channel conductance). Additionally, a similar phenotype was observed for the *Shaker* K⁺ channel when the equivalent position T442 was mutated to either Gly or Ala, suggesting that a common mechanism underlies activation coupled to C-type inactivation in K⁺ channels. Finally, the X-ray solution structure for these KcsA mutants trapped in the open state, will be presented and discussed in the context of activation coupled to C-type inactivation in K⁺ channels.

“DGG is supported by FRSQ and CIHR fellowships.”

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2)Cuello, L.G., et al. *Nature*, 2010. **466**(7303): p. 203-8.

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1978-Plat

On the Structural Basis of Modal Gating Behavior in K⁺ Channels

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¹Case Western Reserve University, Cleveland, OH, USA, ²University of California San Francisco, San Francisco, CA, USA, ³D. E Shaw Research, Hyderabad, India, ⁴D. E Shaw Research, NY, NY, USA, ⁵Texas Tech University, Lubbock, TX, USA, ⁶University of Chicago, Chicago, IL, USA. Modal-gating shifts represent an effective regulatory mechanism by which ion channels control the extent and time course of ionic fluxes. Under steady-state conditions, the K⁺ channel KcsA displays three distinct gating modes, high-*P*_o, low-*P*_o, and a high-frequency flicker mode, each with about an order of magnitude difference in their mean open times. In KcsA, the hydrogen bond network between Glu71, Asp80 and Trp67 that surrounds the selectivity filter has been shown to regulate C-type inactivation, with the Glu71-Asp80 pair having the strongest influence on selectivity filter stability. Here, we show that in the absence of C-type inactivation, mutations at the pore-helix position Glu71 unmask a series of kinetically distinct modes of gating in a side-chain-specific way which mirror those seen in wild-type channels. Results from high-resolution crystal structures along with molecular dynamic simulations suggest that specific interactions in the side-chain network surrounding the selectivity filter, in concert with ion occupancy, alter the relative stability of pre-existing conformational states of the pore. These findings highlight the key role of the selectivity filter in regulating modal gating behavior in K⁺ channels.

1979-Plat

Mode Shift of the Voltage Sensors in Shaker K⁺ Channels is Caused by Energetic Coupling to the Pore Domain

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The voltage sensors (S1-S4) of Kv channels are known to undergo a conformational change when triggered by membrane depolarisation that subsequently leads to opening of the central pore (S5-S6) and ion conduction. The electromechanical coupling between these domains is mediated by two well conserved regions: the C-terminus of the S6 segment and the S4-S5 linker. Upon change in the membrane potential, the structural rearrangements induced by the charged S4 segment can be measured as gating currents. During prolonged depolarization, voltage-gated ion channels present a behaviour called the “mode-shift”. This shift causes the QV to change towards negative potentials and essentially affects the energy required by the system to bring all sensors back to their resting state. To understand the structural cause of this process, we investigated the coupling between the pore and voltage sensor and its influence on the development of the mode shift. In our approach we used the cut-open voltage clamp fluorometry method to simultaneously measure gating or ionic currents and conformational changes of the S4 segment. We identified mutations that fully uncouple voltage sensors from the pore domain thus eliminating the mutual influence of these two domains on one another. The results show that, when eliminating the coupling between these domains, the voltage sensors require less energy to move. At the same time the mode shift occurring during prolonged depolarization is abolished. By instead preventing open state stabilization, voltage sensors are kept in the opposite mode. We also found that the mode shift is accompanied by a conformational change in the S4 segment. We propose that the pore influences the voltage sensor by the presence of a “mechanical load” and allosterically induces a conformational change in the S4.

1980-Plat

A Shaker Potassium Channel with a Miniature Engineered Voltage Sensor

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Voltage-gated ion channels sense transmembrane voltage changes via a paddle-shaped motif that includes the C-terminal part of the third transmembrane segment (S3b) and the N-terminal part of the fourth segment (S4) that harbors voltage-sensing arginines. Here, we find that residue triplets in S3b and ^{NT}S4 can be deleted individually, or even in some combinations, without compromising the channels’ basic voltage-gating capability. Thus, a high degree of complementarity between these S3b and ^{NT}S4 regions is not required for basic voltage gating per se. Remarkably, the voltage-gated Shaker K⁺ channel remains voltage gated after a 43 residue paddle sequence is replaced by a glycine triplet. Therefore, the paddle motif comprises a minimal core that suffices to confer voltage gating in the physiological voltage range, and a larger, modulatory part. Our study also shows that the hydrophobic residues between the voltage-sensing arginines help set the sensor’s characteristic chemical equilibrium between activated and deactivated states.

1981-Plat

A Converging Consensus of the Structure of a Voltage-Sensing Domain in its Resting State

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Understanding the voltage-sensing mechanism of Kv⁺ channels requires gaining knowledge of the active and resting conformations of the voltage-sensing domain (VSD). However, the lack of structural information remains an obstacle in revealing the activation pathway between conformations. Although crystallographic studies have provided atomic resolution structures of Kv⁺ channels in their putative open/inactive state, no crystal structure of a Kv⁺ channel in the resting state is currently available. In the absence of complete atomic resolution structures, experimental and computational studies have sought to complement the structural information of the channel in both conformations. Despite efforts to generate a consistent model of the resting state no consensus has been reached. In the present work, we have probed the accuracy of Khalili-Araghi’s model of a VSD in the resting state by subjecting it to experimentally-determined structural constraints. These include metal bridges between transmembrane helices S1/S4, S2/S4, S2/S3, and an interaction between S2/S4 that spatially position the transmembrane helices with respect to each other. Of particular importance, we simulated the actual mutations and interactions that were involved in the referenced experiments. The purpose of these simulations has been to address the compatibility of Khalili-Araghi’s VSD model with the available experimental data. The simulations demonstrate that a rough consensus exists on the overall conformation of the resting state of the VSD when all available experimental information is utilized.

Support: GM030376, GM062342

1982-Plat

Voltage-Gated Potassium Ion Channels are Frustrated

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It has been suggested for some time that prolines allow the S6 helices of voltage-gated potassium ion channels to move between straight and kinked conformations thereby gating the channel. The kinked conformation was subsequently observed in the x-ray crystallographic structures of Kv1.2 and the paddle chimera, both of which are open. Although closed structures of potassium ion channels with straight inner helices have been observed, none of these have the characteristic PVPV motif. When the voltage sensor moves in response to changes in transmembrane potential, it pulls on the S4-S5 linker, moving this also, which in turn presses on the S6 helix and closes the channel. What remains unclear in this mechanical model is how the channel then opens. We shall show using free energy calculations that the S6 helices of the paddle chimera prefer to be kinked and that conformations corresponding to a closed channel are not favoured. Our results suggest that when the channel is open the S6 helices adopt similar conformations to those observed in the x-ray crystallographic structures. These calculations include the pore region of the paddle chimera, a lipid bilayer and explicit water and therefore include the steric and cooperative effects introduced by moving all four S6 helices simultaneously. We conclude that the closed state of the paddle chimera is frustrated; the S6