Mechanisms of Voltage Sensing and Gating

3748-Pos Board B476

Generalized Interaction Energy Analysis of Intersubunit Linkage in Shaker Potassium Channels

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Voltage-dependent potassium channels are crucial for electrical excitability and cellular signaling; however, the molecular machinery that the channel employs, to relay the state of the voltage sensor to the pore, is not well understood. To gain insight into this voltage-transduction pathway, interacting networks need to be reliably mapped. Here we present a methodology to estimate the strength of site-specific interactions called the Generalized Interaction-energy Analysis (or GIA). Our approach involves combining thermodynamic cycle analysis with information from the gating charge versus voltage curves of putative interactors. This methodology was benchmarked against well-established kinetic models of Shaker potassium channels and BK channels using Monte Carlo like sampling. Our simulations show that GIA can provide free energy estimates in a self-consistent manner that will be useful to identify site-specific interactors that contribute to gating transitions. Implementing this approach on the Shaker potassium channel, we identify a cluster of highly conserved residues, located in the intracellular side of the channel pore, by the gate, that are energetically coupled. Specifically, it appears that tyrosine 485, on the S6 helix, is critical for maintaining an intracellular side of the channel pore, by the gate, that are energetically coupled. A novel picture of how the voltage sensor moves and how these movements simultaneously with ionic current to monitor PGD opening, we were able to gauge PIP2 effects on PGD opening and VSD activation. We detected coupling by performing VCF on locked-open channel mutants, allowing us to directly observe the impact of pore-opening on VSD activation through the VSD-PGD coupling. Here we demonstrate the ability of our methodology to provide mechanistic insights into drug effects using the potent Kv7.1 channel activator ML277 (Matta 2012). As reported previously, 10 μM ML277 caused a mild right-shifts VSD activation in WT channels. This result contradicts pore-opener behavior, which would have left-shifted the fluorescence-voltage (FV) relationship. In contrast we see a dramatic left-shift in the FV relationship of the lock-open mutant, leading to the conclusion that ML277 strengthens the coupling process. Consistently, we saw a higher apparent affinity for ML277 in Kv380 mutants, which have been shown to exhibit increased PIP2 affinity. This study shows the utility of our methodology to analyze drug effects on voltage-sensitive gating.

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Single Molecule Fluorescence of an S4-Based Voltage Sensor

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Single-channel recordings revolutionized our understanding of voltage-gated ion channels by allowing observation of behaviors that are obscured by large ensemble averages. Yet despite its power, single-channel recordings only allow indirect inference about the motions of the voltage sensor since only transitions between "open" and "closed" states of the channel can be seen; most transitions between states remain hidden. For this reason, direct measurement of the motion of a single voltage sensor has long been a goal to understand the details of voltage sensing; unfortunately, at present the elementary charge transition is below experimental resolution. We report here observation of fluorescence from single voltage sensors conjugated to fluorescent proteins. These recordings respond to voltage and are able to recapitulate macroscopic recordings when averaged together. The protein we used is the "Archlight" voltage sensor (Jin, L. et al. Neuron, 2012.), along with derivatives thereof. This sensor consists of the voltage sensing domain from Ci-VSP coupled to a GFP derivative, and it generates robust changes in fluorescence that are voltage sensitive. Our recordings are taken from oocyte membranes using total internal reflection microscopy at a frame rate of 500 hertz and at a temperature of approximately 13 degrees Celsius. This combination of low temperature and fast acquisition allows detection of residues of the protein at distinct fluorescence levels with stochastic movement between these levels being biased by voltage. Presumably these distinct fluorescence levels correspond to distinct states of the voltage sensor. The transitions between these states can be analyzed and modeled, producing a novel picture of how the voltage sensor moves and how these movements are influenced by membrane potential. Support: NIH GM030376.

3752-Pos Board B480

Effects of Decreased Hydrophobicity above R1 in S4-Based Voltage Sensors

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Membrane proteins containing S4-based voltage sensors domains (VSD) respond to changes in the membrane potential by transferring electrically charged side chains (gating charges), mainly located in the S4 segment, across the membrane electric field. The region just above the first charge (R1) is generally thought to control the VSD voltage-dependence. Here, we decreased residue hydrophobicity between N353 to L361 just above R1 in the Shaker Kv channels and found that for the two positions L358 and L361, less hydrophobic residues dramatically shifted the Charge vs. Voltage (Q-V) curve to more negative voltages, up to —132 mV. Screening L358 with several amino acids revealed a linear correlation between the side chain hydrophobicity and the Q-V curve midpoint. Interestingly, structural models of the VSD place the L358 and L361 side chains towards the hydrophobic core of the lipid bilayer in a resting state and towards the aqueous solvent in the active state, suggesting that less hydrophobic mutations may destabilize the resting state and/or stabilize the active state. To explore the origins of these phenotypes, we co-mutated some hydrophilic point mutations at position 358 or 361 together with the ILT mutation, which uncouples VSD movement from pore opening. Our results indicate that most double mutants shifts further the Q-V curves compared to the point mutations, but did not shift the G-V curves compared to the ILT mutant, suggesting that hydrophobic substitutions at positions 358 and 361 most likely destabilize the VSD resting state. Support: NIH GM030376.

3753-Pos Board B481

Genetically-Encoded Fluorescent Voltage Sensors Capable of Resolving a 6MV Depolarization

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Genetically encoded fluorescent sensors of membrane potentials utilize the voltage sensing domain of the voltage-sensing phosphatase (VSPs) gene family. Probes based on the Danio rerio voltage-sensing domain exhibit fast optical responses of around 5 μsec. Here we developed several potential optical sensors by mutating the voltage-sensing domain which consists of four transmembrane segments (S1–S4). Mutations to S4 changed the voltage response resulting in a...
Voltage gated potassium (Kv) channels are trans-membrane proteins that open and close their ion selective pore in response to changes in membrane voltage. Three negatively charged residues in the S2 and S3 helices are conserved in most voltage-gated channels and form an electrostatic interaction network with positively charged residues of the S4, voltage sensing, helix. Previous studies with mouse Kv1.2 have shown that the length and composition of the S3-S4 linker are important in shaping the voltage response of Kv channels. To analyze how this S3-S4 loop effect has been adapted in a different lineage of the channel, we have studied the Kv1-family channel jShak1 from the jellyfish Polyorchis penicillatus. In jShak1 the conserved negatively charged glutamate residue in the S2 helix (position 227) is replaced by neutral asparagine. jShak1 has one less positively charged motif in the S4 helix and a very short S3-S4 linker, consisting of only five amino acid residues. Previous studies with jShak1 indicate that these factors might be responsible for constraining the interactions between the charged residues of the voltage sensing domain by limiting the movement of the S4 helix. We have replaced the natural short loop of jShak1 with different homo-polymers, varying both in length and charge, and have counted the last intrinsically charged residue (K) in both natural and synthetic homo-loops. N227D and N227E mutations were combined with all synthetic loops to make double mutant channels. The homo-loops without a negatively charged residue at 227 do not shift the equilibrium significantly, but that the nature of the C-terminal residue alone can affect the V50 and both the V50 and the Boltzmann slope factor, when combined with a negatively charged residue at 227.

Voltage-gated cation (e.g. Na\(^{+}\)) channels are central to neurological signal transmission. The mechanism of voltage gating, namely the coupling of conformational changes in the voltage sensing domain (VSD) in response to depolarizing potentials with respect to the resting transmembrane electric potential, to opening the pore domain (PD) resulting in transmembrane ionic current, remains unresolved. We report the direct measurement of changes in the scattering-length density (SLD) profile of the VSD protein, vectorially-oriented within a reconstituted phospholipid bilayer membrane, as a function of the transmembrane electric potential by time-resolved x-ray and neutron interferometry. The changes in the experimental SLD profiles are predicted by molecular dynamics simulations, thus providing an interpretation in terms of the VSD’s atomic-level 3-D structure.

The human Ether-a-go-go Related Gene (hERG) K\(^{+}\) channel plays a central role in cardiac action potential repolarization. Compared to other Kv channels where fast activation is due to rapid voltage sensor (S4) movement, hERG activation is slow and the mechanisms remain under debate. Previous studies have shown that charges in the voltage sensing domain contribute to determining the rate of channel activation, which includes transition of the S4 and downstream events in S4-pore coupling. The functional role of individual charges in S4 movement alone is still unclear in hERG channels. We have investigated the effect of neutralization of positive charges (K525-S538) on the voltage dependence of S4 equilibrium by examining the voltage and time-dependent accessibility of cysteine substituted I521 in the tip of the S4. At -120 mV, S521C is almost inaccessible to MTSET for R531Q, R537Q and K538Q, but is accessible for R528Q, R534Q and K525Q. This suggests that at -120 mV, position 521 in the R531Q and R537Q, K538Q mutants is mostly buried, whereas it is extruded in the other mutants. The slopes of the S4 equilibrium curves of K538Q and R531Q are greatly altered, indicating that K538Q favors the transition of S4 from the resting to activated states, while R531Q favors the reverse transitions. To extract the kinetics of S4 movement, we examined the dependence of the MTSET modification rate on pulse duration. We found that time constants of S4 equilibrium for R528:521C, R537:531C, R537:531C and R531Q:521C are slower than control, with the largest effect seen for R531Q:521C. Our data suggest that K525, R528, and R534 normally play a role in stabilizing S4 in the resting state; while R528, R537 and R531 facilitate the transition of S4 from the resting to the activated state.