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Access Constraints and Binding Energetics of a Potassium Channel Inactivation Gate

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Voltage-activated potassium (Kv) channels open an intracellular gate in response to changes in transmembrane voltage, allowing K^+ to permeate at rates near diffusion. In some Kv channels, permeation can be interrupted by the N-terminus of the protein acting as a blocking particle, binding at the intracellular cavity of the channel and physically obstructing the permeation pathway. Despite being tetramers, Kv channels require only one bound N-terminus to inactivate. In nature, inactivation is targeted by RNA editing, which converts an isoleucine of the intracellular cavity into a valine. The functional consequence is a large increase in unbinding kinetics.

How does each ''wall'' of the cavity contribute to the increase in unbinding kinetics of a single bound N-terminus? To address this question we made concatenated Shaker heterotetramers consisting of only one free N-terminus and a single isoleucine to valine mutation at position 470 (I470V), mimicking the RNA editing site in humans and rodents. Surprisingly, the rate of unbinding was the same for individual I470V mutations in each wall; each corresponded to a decrease in binding energy of about 0.4 RT. In fact, the binding energy decreased linearly as a function of the number of I470V mutated subunits. These results suggest that despite being tethered to a particular subunit, the single inactivation particle has sufficient freedom to interact with position 470 of each subunit with roughly equal probability. Where does this freedom come from? It is expected that the inactivation gates enter the cavity through lateral 'windows', similarly to K^+. Can our single tethered inactivation gate enter through each of the four windows of the protein? We will address this question by making individual cysteine mutations at each window and testing the inactivation gate's binding kinetics after chemical modification.

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Gating Interactions between the Cytoplasmic T1 Domain and Pore of a Kv Channel

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Whereas the gating roles of the voltage-sensing and pore domains of Kv channels have been extensively investigated, much less is known about the mechanisms governing the regulation of gating by the cytoplasmic T1 domain. Previous work from this lab strongly suggests that the Kv4.1 T1 domain undergoes conformational changes directly linked to gating (J. Gen. Physiol., 127:391-400, 2006); however, the structural basis of the communication between the activation gate and the T1 domain has remained elusive. To tackle this problem, we applied cysteine scanning mutagenesis, voltage-clamp electrophysiology, double mutant cycle analysis (DMCA) and oxidizing reagents to probe specific interactions between the cytoplasmic T1 domain L4 helix, the S4-S5 linker and S6 tail. The energetic impact of the mutations on activation gating is apparently small. By comparison, the energetic impact of the mutations on inactivation gating was significantly greater. DMCA of K141C(L4)+A422C(S6) revealed a significant interaction energy (4 kcal/mol), which suggests an interaction between K141 in the L4 helix and A422 in S6. Supporting this possibility, the currents induced by K141C(L4)+A422C(S6) are inhibited (~65% of control) by an oxidizing agent (tBuHO2), whereas the single mutants are not; and the effect is reversed by the reducing agent DTT. These results indicate the formation of a disulfide bond between the interacting side chains, which is consistent with physical interaction between K141(L4) and A422(S6). Overall, we conclude that regulation of inactivation gating by the T1 domain involves a physical interaction between the T1 L4 helix and the distal segment of S6. Supported by NIH grant R01 NS032337 (MC).

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Is the Activation Gate Closed in Kv Channel Closed-State Inactivation?

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Kv4.x channel complexes including auxiliary subunits such as DPP6-S and KChIP1 undergo preferential closed-state inactivation (CSI; Baehring and Covarrubias, 2011, *J Physiol*, 589.3:461-479; Fineberg et al., 2012, *J Gen Physiol*, in press). However, the molecular basis of CSI in Kv4.x channels remains poorly understood. A key unanswered question of this problem is whether the channel's activation is actually closed, as CSI implies. We have previously proposed that CSI results when the activated channel fails to open or simply prefers to close. This hypothesis specifically suggests that the intracellular activation gate may adopt a stable closed conformation, even under depolarized conditions that would initially favor the activated ''up'' state of the voltage sensors. To test this hypothesis, we are exploiting the trapping paradigm previously investigated by Holmgren *et al*. to test the state of the intracellular activation gate (1997, *J Gen Physiol*, 109:527-535). Essentially, if the activation gate closes behind a quaternary ammonium (QA) blocker inside the pore, the blocker cannot exit the pore; effectively, it is trapped. We are expressing the ternary Kv4.1/I400C channels (this mutation favors trapping) including DPP6-S and KChIP1 in *Xenopus* oocytes. Then, in the inside-out patch-clamp configuration, we are using a computer-controlled perfusion system to deliver the QA derivative decyltrimethylammonium bromide (C_{10}) to the bath and probe the ability of the channel's activation gate to trap C_{10} in the closed, open and inactivated states. Preliminary results show that the trapping paradigm may yield an answer to the central question of this study. If the activation gate is indeed closed during CSI, C_{10} trapping will only occur when the drug is applied to the conducting channel, but no trapping of C_{10} will be observed in the closed and inactivated states. Supported by NIH grant R01 NS032337 (MC).

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Water at the Potassium Channel Gate: Quantum Calculations Show an Oscillation in Water Structure

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Quantum calculations on the open gate and the immediately surrounding region of the $K_v1.2$ voltage gated potassium channel show that water adopts two conformations there, one when the ion is present, and another with no ion present. An energy minimum at the gate with no ion allows an ion to enter from the solution; from the gate it repels an ion in the channel pore cavity into the selectivity filter (essentially the ''knock-on'' mechanism). The pore cavity minimum can then be occupied by the ion moving from the gate minimum, leaving it available for anion from bulk, so the cycle continues. The arrangement of water, and the ion hydration, defines the gate energy minimum, and this in turn depends on the highly conserved prolines, especially P407 (2A79 pdb numbering), in the PVPV sequence in the gate. Absent an energy minimum at the gate an ion entering from solution would be repelled back into solution by the ion in the cavity. Furthermore, the ion remains hydrated by 8 water molecules, at least 1 more than in bulk, as it passes through the gate, based on optimizations with the ion at three different positions: 1) at the gate position nearest P407 2) 2Å above 3) 2Å below, this position. The open gate maintains very nearly the same protein atom positions throughout. Additional calculations will be required to determine the exact interaction energy with an ion in the cavity, and the amount of water that is present in the cavity. Optimizations of the system configuration were done at HF/4-22GSP level, with 692 atoms (693 with the ion). Energy was determined using both B3LYP/6-31G* and bvp86/6-31G*, but the system was too large for free energy determination.

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Water Structure at the Potassium Channel Gate: The Importance of the Prolines in the PVPV Sequence, as Shown by Computational Mutations, and the Role of a Histidine in Gating

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Conservation of the PVPV sequence at the potassium channel gate has not been well understood. Quantum computations (optimization at HF/4-22GSP level) of the gate of the $K_v1.2$ channel that are presented here show that the proline-proline distances (ring atoms) are preserved as the ion moves through the gate. This calculation is supplemented by mutating one of the prolines to valine (P407V, 2A79 pdb numbering for all residues in this abstract) in the computation, producing a PVVV sequence. Water structure changed dramatically, as the protein backbone moved inward nearly 3Å, effectively closing the channel; the proline ring N-N distance between diagonally opposite prolines dropped from 14.3 Å (distance from optimization: X-ray distance = 15.6Å) to 11.4 Å, too small to allow passage of a hydrated K^+. This suggests that the prolines play a key structural role, modulating both protein and water structure. Furthermore, protonation of H418 may close the channel. Protonation of this histidine shows it moving toward the gate by about 3 Å, which should close the gate electrostatically, while deprotonation opens the gate. Protonation was calculated with two added protons, the other being accepted by E142 of the T1 intracellular moiety. When deprotonated, the histidine does not move as much as 1Å from the X-ray position in the optimization, and is linked to E142; this accounts for the known importance of the T1 segment in gating. E136, also of T1, could accept a third proton. There is a salt bridge structure including these residues similar to the one occupying a key position in the proton pathway in the H_v1 channel.