molecules in the vicinity of the selectivity filter. Overall, our results show that the conformational dynamics associated with the selectivity filters of collapsed (Low K^+) and open-inactivated KcsA are significantly different despite showing essentially identical conformations as derived from crystal structures. This highlights the importance of hydration dynamics in gating of K^+ channels.

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Role of the S6-PxP Motif in U-Type Inactivation of Heterotetrameric Kv2.1/Kv6.4 Channels
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Voltage-gated K^+ (Kv) channels assemble as tetramers of α-subunits that each consist of 6 trans-membrane segments (S1-S6) and cytoplasmic N- and C-termini. The S1-S4 segments form the voltage-sensing domains while the four S5-S6 segments form the central pore. A conserved PxP motif within S6 provides flexibility to the bottom half of S6 and regulates channel gating. Subunits of the Kv5-6 and Kv8-9 subfamilies - also known as silent Kv subfamilies (KvS) - require co-assembly with Kv2 subunits to be functionally expressed. KvS subunits alter the biophysical properties of these heterotetramers compared to Kv2; e.g. Kv6.4 induces a 40 mV hyperpolarizing shift in the voltage-dependence of inactivation and potentiates the U-type inactivation. KvS subunits lack the 2nd proline of the PxP motif which has been implicated in the Kv9.3-induced effects on Kv2.1 gating. To test the effect on U-type inactivation we exchanged the Kv2.1 PIP and Kv6.4 PAT sequences. The U-type inactivation of the mutant homomeric Kv2.1(PAT) channels was strongly potentiated resulting in less than 20% of the channels in an inactivated state above +50 mV. In addition, the voltage-dependence of activation and inactivation displayed hyperpolarizing shifts of 25 mV. Conversely, Kv6.4(PIP) subunits decreased the U-type inactivation of Kv2.1/Kv6.4(PIP) heterotetramers to a level intermediate between Kv2.1 homotetramers and Kv2.1/Kv6.4 heterotetramers and induced a 10 mV hyperpolarizing shift in the voltage dependence of inactivation. These results indicate that the absence of a full PxP motif contributes to Kv6.4-induced potentiation of the Kv2.1 U-type inactivation U-type inactivation. (Supported by FWO fellowships to JS and EB & grant FWO-G.0449.11N to DJS).

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Voltage Dependence of BK Channels Gating Ring Motion Studied by State Dependent FRET
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Large conductance voltage- and calcium-dependent potassium channels (BK) open probability is regulated by changes in transmembrane voltage and intracellular divalent ions (Ca^{2+} and Mg^{2+}) concentration. The gating ring, formed by the two RCK (Regulator of Conductance of Potassium) domains present in the C-terminal of each subunit of the tetramer, contains the divalent ion binding sites. We previously have showed that large rearrangements of the gating ring occur in a Ca^{2+} and voltage dependent manner. This movement of the gating ring is not directly related with the opening of the channel. It appears that a change in the voltage dependence of the open probability of the channel does not modify the voltage dependence of the movement of the gating ring. However, the voltage dependence of the movement of the gating ring is shifted toward a more negative potential if we modify the voltage dependence of the voltage sensor by co-expression of the BK alpha subunit with the Beta1 subunit or by mutations. These results indicate that the voltage dependent movement of the gating ring appears to be related to the voltage dependence of the voltage sensor and not directly related with the opening of the gate.

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Quantitative Mapping of Interactions in the Voltage-Sensor Pore Interface of the Shaker Potassium Channel
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Changes in the membrane potential of an excitable cell induce movement of voltage-sensing units within voltage-gated ion channels (VGICs). The energy associated with this movement is transmitted to the channel gate, ultimately promoting its opening. Numerous proposals have been made as to how this signal is relayed from the voltage-sensors to the channel gate, such as side chain interactions and backbone movement; however, it has been difficult to ascertain the precise molecular mechanisms underlying energy transduction. Recently, it was shown that median voltage estimates from the charge-voltage relationship of VGICs can be used to derive the net free energy change (ΔGnet) associated with their voltage-dependent activation. By combining this approach with mutant cycle analysis, it is possible to identify residues that are energetically coupled and contribute to the activation process. Here, we have undertaken a systematic analysis of contact pairs at the interface between the voltage-sensor and pore domains of Shaker potassium channel in order to gain insight into how an initial signal can propagate from one region of the channel to another and trigger the opening of the channel gate. Our results will be discussed in the context of overall molecular mechanism of electromechanical coupling in voltage-gated ion channels.

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Binding of Quaternary Ammonium Ions to a Potassium Channel
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We have used stopped flow spectrophotometry to measure the binding affinities of quaternary ammonium (QA) ions to the KcsA potassium channel. KcsA is a pH sensitive channel that activates at low pH and then inactivates rapidly. In membranes containing POPC:POPG at a 4:1 ratio, approximately half of the channels inactivate within one second. Stopped flow spectrophotometry allows rapid mixing of reagents and measurement of fluorescence on a millisecond time scale. Channel activity was measured by mixing a suspension of 100 nm proteoliposomes at neutral pH with a thallium containing buffer at a low pH, and monitoring the flux of thallium through open potassium channels via the quenching of a trapped fluorophore. Using this method, channel inhibition was measured with several species of QAs. Due to the hydrophobic nature of the drugs used, solubility of the long chain QAs became a limiting factor in our measurements. We found that binding affinity increased with alkyl-chain length from four to eight carbon atoms. Tetrabutylammonium (TBA) was measured to have an inhibition constant of ~120μM. The solubility limits of tetrahexylammonium (THA) and tetraoctylammonium (TOA) did not permit the measurement of a complete inhibition curve. At the solubility limits of THA (2μM) and TOA (100nM) the measured inhibition was ~70% and ~30%, respectively.

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Structure and Dynamics of the Mthk K^+ Channel Selectivity Filter during Gating
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Potassium channel inactivation is essential for regulating the duration and frequency of neuronal action potentials, yet the associated structural changes involving the selectivity filter are not well understood. We have investigated the influence of ionic conditions on the structure and dynamics of the MthK K^+ channel pore using X-ray crystallography and molecular dynamics (MD) simulations. Crystals of the MthK pore were grown in a range of [K^+]. Electron density maps showed that as [K^+] is lowered, ion binding to the S2 site within the selectivity filter is eliminated without altering the conductive conformation. In order to investigate how ion binding may influence the behavior of the selectivity filter in the absence of crystal lattice constraints, we performed MD simulations of the MthK pore in different ionic conditions. We found that K^+ binding exerts a significant influence on the structural dynamics of the selectivity filter. With two K^+ ions bound at sites S2/S4, the filter carbonyl groups remained directed towards the conduction pathway with only occasional, unstable outward rotations. Binding of two water molecules behind the selectivity filter, corresponding to those observed in the crystal structures, further stabilized the conductive carbonyl positions. In contrast, with no K^+ ions bound, carbonyl rotations, perturbed protein interactions behind the filter, and a constricted pore were observed, similar to the collapsed low [K^+] KcsA crystal structure. This likely non-conductive MthK conformation is of similar energetic stability with the conductive conformation during these simulations, which may explain why crystallization of the conductive conformation is possible in low [K^+]. From these results we conclude that closure of the MthK selectivity filter is a viable gating mechanism and that interactions between protein, ions, and water govern the dynamic behavior of the filter.