subunits undergo a conformational change that precedes opening. This is surprising, because each monomer is thought to contain a separate conduction pathway. Monomeric channel gating had twice weaker temperature dependence than dimeric channels, consistent with a more complex gating mechanism in the dimer. Finally, monomeric channels opened 6.6 times faster than dimeric channels. Combined, these observations suggest that the native proton channel is a dimer in which the two monomers are closely apposed and interact during a cooperative gating process.

1631-Pos

Strong Negative Cooperativity Between Subunits in Voltage-Gated Proton Channels

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Voltage-activated proton (H_V) channels are essential components in the innate immune response. Hv channels are dimeric channels with one proton permeation pathway per subunit. It is not known how Hv channels are activated by voltage and whether there is any cooperativity between subunits during voltage activation. Using cysteine accessibility measurements and voltage clamp fluorometry, we show that the fourth transmembrane segment S4 functions as the voltage sensor in H_V channels from *Ciona Intestinalis*. Surprisingly, in a dimeric H_V channel, the S4s in both subunits have to move to activate the two proton permeation pathways. In contrast, if H_V subunits are prevented from dimerizing, then the movement of a single S4 is sufficient to activate the proton permeation pathway in a subunit. These results suggest a strong negative cooperativity between subunits in dimeric H_V channels.

1632-Pos

High-Resolution Crystallographic Analysis of the Kcsa Gating Cycle from Cysteine-Trapped Open Channels

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The K+ channel pore domain contains all the elements necessary to catalyze the selective permeation of K+ ions, in addition to regulate events underlying activation and inactivation gating. In KcsA, an inactivation process related to C-type inactivation in eukaryotic channels has been attributed to putative conformational changes at the selectivity filter (SF)[1]. Previously, we have provided crystallographic evidence for the conformational changes associated to C-type inactivation, albeit at relatively low-resolution [2]. Here, we have taken advantage of a cysteine-bridged locked open KcsA-mutant to study the structural changes at the selectivity filter when the activation gate (AG) is open and the filter transitions between its conductive and non-conductive conformations. We report the structures of KcsA for the non-inactivating mutant E71A at 2.1 Å; the fully inactivated mutant Y82A at 2.32 Å; and the non-inactivating mutant F103A at 2.64 Å, where the allosteric coupling between the two gates (AG and SF) has been impaired. This set of high-resolution structures for different KcsA kinetic states represent a sharp improvement over the resolution of non-cysteine trapped mutants and will be interpreted in relation to their complementary functional characterization.

1. Cordero-Morales, J.F., et al., Molecular determinants of gating at the potassium-channel selectivity filter. Nat Struct Mol Biol, 2006. 13(4): p. 311-8.
2. Cuello, L.G., et al. (2008). Structural basis of K+ channel C-type inactivation: Crystal structure of KcsA in the Open/C-type inactivated conformation. 52nd Annual meeting of the Biophysical Society. Mini-symposium

1633-Pos

Gating-Related Conformational Changes in the Outer Vestibule of KcsA: a Fluorescence and Pulsed-Epr Analysis

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In K^+ channels, the selectivity filter and surrounding structures play a crucial role in inactivation gating and flicker. KcsA is a pH-gated K^+ channel and its gating is modulated by transmembrane voltage. In this work, we monitored the gating-related structural dynamics in the outer vestibule of KcsA using site-directed NBD fluorescence and pulsed-EPR analysis. Fluorescence polarization results show that in KcsA, the dynamics of the outer vestibule is substantially different when comparing inactivating (wild type) and non-inactivating (E71A) forms of the channel. In addition, the rate of solvent relaxation (dynamics of hydration) is found to be faster in non-inactivating form of KcsA upon gating as determined by red edge excitation shift (REES) analysis. This increased rate of solvent relaxation correlates well with the increased rotational mobility of the outer vestibule residues in the open, non-inactivating state (E71A at pH 4). To gain further insight on the dynamic properties of these conformational fluctuations in the outer vestibule of KcsA during gating, four pulse Double-Electron-Electron Resonance (DEER) EPR spectroscopy is being currently used. This approach allows for the determination of inter-subunit distances between 20-60 Å, directly informing on the overall distance distribution. We have used tandem dimer constructs of spin-labeled KcsA for the residues corresponding to the outer vestibule of KcsA to determine average distances and distance distributions at low pH, under conditions that stabilize the inactivated (wild-type filter) and the non-inactivating (E71A) states. The results will be discussed in terms of the conformational transitions in the outer vestibule during activation and inactivation gating.

1634-Pos

An Engineered Cysteine-Bridge Locks KcsA Inner Bundle Gate in Its Open Conformation

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Ion channels undergo conformational changes that allow them to transition along defined kinetic states. Previously, we have carried out an extensive crystallographic characterization of the key kinetic states that form the K+ channel gating cycle. Key among them is the structure of KcsA with the inner bundle gate in its open conformation and the selectivity filter in its inactivated (non conductive) form. Aiming to obtain high-resolution structural information of these trapped states, we have engineered a series of cysteine-bridges in the activation gate of a constitutively open KcsA mutant based on the structural properties of the open gate. We reasoned that restricting the conformational freedom of the activation gate, by locking it in the open conformation, would lead to a significant improvement in the resolution of the crystallographic data. This was carried out through a series of cysteine mutants in both TM1 and TM2 which generated covalently concatenated channels, even in the absence of an external oxidative agent. Biochemical and functional analyses suggested that channels were covalently locked open and that the crosslinked channel was stable under a variety of conditions, highly thermally resistant and was monodisperse under gel filtration chromatography. This new approach should help obtain high-resolution structural information of KcsA mutants trapped in different kinetic states and provide additional correlation to the functional characterization of each kinetic state.

1635-Pos

pH-Dependent Gating of KcsA Potassium Channel

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KcsA potassium channel is a pH-dependent channel and is activated when cytoplasmic side becomes acidic. In KcsA channel there are two gates in series along the permeation pathway: the filter gate and the helix gate. Recently crystal structure of the full-length KcsA channel was revealed and the pH-sensitive domain was identified. It is crucial to elucidate functional properties of the helix gate in relation to the pH sensing. However, complicated behavior of the filter gate makes the single-channel analysis difficult for the wild-type KcsA channel. Here we examined pH-dependence of the helix gate and its gating kinetics using an inactivation-free mutant, E71A. The E71A channel was reconstituted into the planar lipid bilayer membrane and the gating behavior was recorded during stepwise changes in cytoplasmic pH. In contrast to the wild-type KcsA, the open probability was almost 100% at pH 3.0. Flickery gating was observed in the negative potentials. As the pH approached to neutral the channel became closed and recovered when pH was returned to acidic. We found that the pH dependency of E71 channel was shifted towards neutral compared to that of the wild-type channel. Frequent transitions between open and closed states were observed around the pKa, from which kinetic properties of the helix gate were analyzed. The mechanism underlying the shift of the pH-sensitivity will be discussed.

1636-Pos

Electron Spin Echo Envelope Modulation (ESEEM) Reveals the Footprint of the Voltage Sensor on the KvAP Pore Domain

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In voltage gated potassium channels, two interfaces between the central pore domain (PD) and the peripheral voltage sensor domain (VSD) must exist for the efficient transduction of membrane potential changes into mechanical opening of the gate. The first interface, located between the S4-S5 linker (VSD) and the S6 helix (PD), couples VSD motion to PD motion. Additionally, a strong secondary interface is mechanistically required to act as an anchor point between the domains so that force can be efficiently transduced to the PD. However, no such interface is apparent in any current crystal structure. As multiple studies have identified the S1 helix as the likely point of anchoring of the VSD,