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

Carlos Gonzalez, Hans P. Koch, Ben M. Drum, Hans Peter Larsson.
University of Miami, Miami, FL, USA.

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

Luis G. Cuello[1], D. Marien Cortes[1], Eduardo Perozo[2].
[1]Texas Tech University Health Science Center, Department of Cell Physiology and Molecular Biophysics, Lubbock, TX, USA, [2]The University of Chicago, Department of Biochemistry and Molecular Biology, Chicago, IL, USA.

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

H. Raghuraman, Eduardo Perozo.
Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL, USA.

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

Luis G. Cuello[1], D. Marien Cortes[1], Eduardo Perozo[2].
[1]Texas Tech University Health Science Center, Department of Cell Physiology and Molecular Biophysics, Lubbock, TX, USA, [2]The University of Chicago, Department of Biochemistry and Molecular Biology, Chicago, IL, USA.

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

Matsuki Yuka, Masayuki Iwamoto, Hirofumi Shimizu, Shigetoshi Oiki.
University of Fukui, Fukui, Japan.

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

John A. Cieslak, Adrian Gross.
Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

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,

we set out to determine the interaction footprint of the VSD on the PD using ESEEM spectroscopy. We have previously demonstrated that deuterium ESEEM is well suited to investigate the interaction of membrane proteins with their surrounding environment. In the present study, we determine the water accessibility profile of the KvAP PD in the presence and absence of the VSD. We show that a region of the PD near the monomer interface demonstrates decreased deuterium coupling in the presence of the VSD compared to what would be expected based on residue immersion depth. Furthermore, the observed deuterium coupling at this region increases to expected levels upon removal of the VSD. We conclude that the protected region of the PD represents the interaction footprint of the VSD on the surface of the pore.

1637-Pos

Down-State Model of the KvAP Full Channel

Eric V. Schow, Alexandr Nizkorodov, J. Alfredo Freites, Stephen H. White, Douglas J. Tobias.
University of California at Irvine, Irvine, CA, USA.

Voltage-gated potassium (Kv) channels have at least two distinct conformations, an ''up'' state, corresponding to the open/activated state of the channel, and a ''down'' state, corresponding to the resting state of the channel. Kv channels are tetramers that consist of a central pore domain (PD) and four peripheral voltage-sensing domains (VSDs) that respond to changes in the transmembrane (TM) potential. The PD opens and closes via mechanical coupling to the VSDs, which undergo large conformational changes as the TM potential changes. The molecular mechanism of these changes is poorly understood, because the Kv crystal structures reported to date are exclusively in the up state. We have recently reported a down-state model of the isolated VSD of KvAP that is consistent with existing experimental data. Based on this down-state model, we have now generated a down-state model for the KvAP full channel using targeted molecular dynamics. We used the end point of an equilibrated simulation of the KvAP full channel in the up state as a starting point and four symmetrically arranged down-state VSDs as targets. The PD was unconstrained during the simulation. Preliminary results suggest that, as expected, steric interactions between the S4-S5 linker and the intracellular half of S6 result in a measurable narrowing of the pore. We compare our model to the closed-state structure of the KcSA channel (2001, Science 280: 69), which consists of a Kv-homologous PD but no VSD, and to the Kv1.2 mammalian channel down-state model of Pathak et al (2007, Neuron 56: 124). This work is supported by NIH grants GM74637 and GM86685 and NSF grant CHE-0750175, and we are grateful for the allocation of computer time on the NSF-supported Teragrid resources provided by the Texas Advanced Computing Center.

1638-Pos

Interactions Between Lipids and Voltage Sensor Paddles Detected with Tarantula Toxins

Mirela Milescu, Frank Bosmans, Kenton J. Swartz.
NIH/NINDS, Bethesda, MD, USA.

Studies on voltage-activated potassium (Kv) channels show that modification of the surrounding lipids can alter channel function, raising the possibility that lipids interact directly with specific regions of Kv channels. We explored the interaction of lipids with S1-S4 voltage-sensing domains from different voltage-activated ion channels and voltage-sensing proteins, and used tarantula toxins that bind to S3b-S4 paddle motifs within the membrane to detect lipid-paddle interactions. We found that the conversion of sphingomyelin to ceramide-1-phosphate alters the gating and pharmacology of voltage-activated channels, and that the paddle motif determines the effects of lipid modification. We also found that mutations in two defined regions of the paddle motif weaken toxin binding to the paddle by disrupting lipid-paddle interactions. Our results show that lipids bind to voltage sensors and demonstrate that the pharmacological sensitivities of voltage-activated ion channels are influenced by the surrounding lipid membrane.

1639-Pos

Conformational Changes in Potassium Channel Voltage-Sensing Domains Reconstituted into Different Lipids

Dmitriy Krepkiy, Kenton J. Swartz.
NIH, Bethesda, MD, USA.

In the voltage-activated potassium channels, S1-S4 voltage-sensing domains control opening and closing of an associated pore domain. The S3b-S4 paddle motif within these domains moves at the protein-lipid interface to drive channel activation in response to changes in voltage. Electrophysiology experiments show that changes in the lipid composition significantly alters the energetics of voltage activation (Ramu, Y., 2006; Schmidt, D., 2006, 2009; Milescu, M., 2009). In particular, interactions between S4 Arginines and lipid phosphodiester groups have been proposed to be crucial for activation of voltage sensors; in the absence of the phosphodiesters, voltage sensors appear to be confined to the resting state. In order to define the structural basis of these interactions we purified the S1-S4 domain from KvAP and homogeneously reconstituted it in either a POPC:POPG (1:1) lipid mixture or DOTAP, a lipid without a phosphate group. Although the α-helical secondary structure is identical in these lipids as observed by circular dichroism spectroscopy, the fluorescence properties of single Trp70 in the middle of S2 helix is quenched when the S1-S4 domain is reconstituted into DOTAP. We investigated the chemical environment of the S3b-S4 paddle in different lipids by labeling a residue in the tip of the S3b-S4 paddle (Ala111Cys) with the fluorophore Bimane. The fluorescent properties of Bimane are significantly different upon reconstitution in DOTAP as compared to POPC:POPG lipid mixture. The relative fluorescence intensity of Bimane is two-fold higher in DOTAP compared to POPC:POPG, and in DOTAP, Bimane exhibits significant (~80 nm) Red Edge Excitation shift. These data suggest that there are changes in structure of S1-S4 reconstituted in different lipids, which might correspond to the conformational changes between resting and activated states. The work is in progress to characterize these changes by structural biology techniques.

1640-Pos

Divalent Cations are Antagonists of the Sodium-Dependent Potassium Channel Slo2.2

Gonzalo Budelli, Celia Santi, Alice Butler, Lawrence Salkoff.
Washington University in St. Louis, St Louis, MO, USA.

We studied the effect of different divalent cations on the Na^+-dependent potassium channel Slo2.2 (Slack) using inside-out patches from *Xenopus* oocytes and a Slack-HEK cell line heterologously expressing Slack channels. In the presence of intracellular sodium, the addition of divalent cations reduces significantly Slack currents recorded in macropatches. Among the divalent cations studied, the most effective in reducing channel activity was Cd^{++} followed by Ni^{++}, Ca^{++} and Mg^{++}. Several results suggest that this effect is not caused by blocking the pore of the channel. First, the decrease in currents is not voltage dependent as expected for a cation blocking the channel pore. Second, single channel recordings show a decrease in open probability but not a significant reduction in single channel conductance. Third, some of these cations activate rather than block the Ca^{++}-dependent channel Slo1, which is likely to have a pore structure similar to Slo2.2. Outside-out patches have been used to show that divalent cations do not have an inhibitory effect when applied to the extracellular side of the channel. We propose that the divalent cations may be competing with Na^+ for the Na^+ binding site. To test this hypothesis, we will examine the effect of divalent cations on several channel mutants and chimeras. These experiments also may help to reveal the structure and localization of the Na^+ binding site.

1641-Pos

Four and a Half Lim Domains (FHL) Genes Reduce Conductivity of the KCNA5 Channel

Ivana Poparic[1], Astrid Gorischek[2], Valerie Wagner[2], Klaus Wagner[1], Christian Windpassinger[1], Wolfgang Schreibmayer[2].
[1]Institute of Human Genetics, Medical University of Graz, Graz, Austria, [2]Department of Biophysics, Medical University of Graz, Graz, Austria.

Myopathies are inherited muscle disorders characterized by weakness and atrophy of voluntary skeletal muscles, sometimes including the cardiac muscle. A phenotypically distinct, X-linked myopathy with postural muscle atrophy, termed XMPMA, has been recently described and linked to mutations in the *FHL1* gene. FHL1, a member of LIM-only proteins, is expressed in skeletal and cardiac muscle and suggested to play a role in sarcomere synthesis and assembly. Three splice variants (A, B and C) exist, which differ in expression pattern, binding partners and subcellular localization. A mutation found in a large XMPMA family (C224W) affects only isoforms A and B.

Aim of our study is to functionally characterize mutated FHL1 isoforms and their interaction with the voltage-gated potassium channel (KCNA5 or Kv1.5), which is involved in cardiac excitability. These interactions may partly explain the cardiac involvement within the clinical spectrum of XMPMA patients.

K^+ currents were recorded in Xenopus leavis oocytes injected with KCNA5 mRNA with or without coexpression of $FHL1A^{WT}$, $FHL1A^{C224W}$ or FHL1C. Upon coexpression of all three FHL1 proteins, K^+ current density was differently decreased, when compared to oocytes expressing KCNA5 alone. Kinetics of the channel was not affected. These results support the role of FHL1 as a key molecular component in regulation of expression of KCNA5. Future experiments will concentrate on colocalization and molecular interaction of FHL1 and KCNA5 in mammalian *in vitro* systems (HL1 cells).

Support by Graz Medical University ***Ph.D. programme*** and ***County of Styria*** (GZ: A3-16.R-10/2009-112).