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

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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

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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

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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

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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

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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).

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