Inward Rectifier K Channels

3623-Pos

Radiolytic Footprinting Reveals Conformational Changes During Potassium Channel Gating

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Potassium channels are dynamic proteins that undergo large conformational changes as they make transitions between their open and closed conformations to regulate cellular electrical activity. Understanding the gating mechanism of these channels therefore requires methods for probing channel structure in both their open and closed conformations. In order to address this we used radiolytic footprinting and mass spectrometry to study the gating mechanism of the inwardly-rectifying potassium channel KirBac3.1. By subjecting the purified protein in defined states to focused synchrotron X-ray beams with millisecond timescale exposures we modified solvent accessible amino acid aide chains in the membrane pore as well as in the intercellular domain. These modifications were quantified and identified using high-resolution mass spectrometry. The differences in the extent of such modifications on specific side chains between the closed and open state are used as probes to reveal local conformational changes that occur during channel gating. The data indicate that TM2, the slide-helix, G-loop and outer mouth of the pore undergo large conformational changes during channel gating. These results provide validation of a proposed gating mechanism of the Kir channel and demonstrate a novel method of probing the dynamic gating mechanism of integral membrane proteins and ion channels.

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Structural Dynamics of KirBac1.1 Probed by Fluorescent Labeling and FRET

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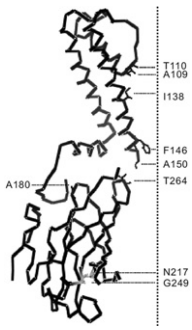
We assessed the accessibility of fluorescent probes in purified KirBac1.1 proteins in detergent (DM) solution, to probe channel structure. Introduced cysteines at pore-lining positions in the inner cavity (A109C, T110C and I138C) were inaccessible to Alexa-Fluor-488-C5 maleimide (monitored by fluorescence incorporation). However, G-loop residues (A150C, T264C, G249C), and A180C at the external wall of the cytosolic domain, were readily modified. PIP2 activates eukaryotic channels, but inhibits KirBac1.1. Addition of C8-PIP2, reduced modification rate (~15%) at A150C, T264C and G249C, as well as A180C (~30%). The data indicate a major barrier to fluorophore at the inner cavity entrance, but no barrier at the G-loop. Moreover, they suggest that during PIP2-induced closure, the cytosolic vestibule 'narrows', and there is reduced accessibility of residues at the outside face (A180C) of the cytosolic domain. To explore conformational changes during gating, we co-labeled residues with Alexa-Fluor 488 and Alexa-Fluor 568, and examined FRET in channels reconstituted into liposomes (POPE:POPG, 3:1). In 1% PIP2, FRET efficiency was unchanged at N217C and A180C, increased at A150C, and decreased at G249C, suggesting that, during closure, there is narrowing at the bundle crossing, and widening in the cytosolic vestibule.



3625-Pos

Novel Insights Into the Outer Pore Domain Structure in Inward Rectifier Channels Kir 2.3

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The Kir2 channels belong to a family of potassium selective channels with characteristic strong inward rectification but they differ substantially in their pH sensitivity. The extracellular histidine Kir2.3(H117) contributes to the pH dependence of K-channels containing Kir2.3. Here, we study the possibility of intramolecular interactions of the residue Kir2.3(H117) with conserved cysteines in close proximity to the selectivity filter. We engineered a cobalt coordination site and reduction/oxidation sensitivity in Kir2.3 by introduction of a cysteine into the putatively hydrogen bonding residue (Kir2.3(H117C)) confirming that this residue is in proximity to Kir2.3(C141). Using SCAM we determined the location of the Kir2.3(H117) and Kir2.1(E125) in the outer pore mouth and incorporated these data into a 3D model. We conclude that formation of a hydrogen bond at low pH may stabilize the outer pore domain to favour the selectivity filter in a slightly distorted conformation thus reducing ion permeation. The data presented provide molecular insight into the unique pH regulation of inward rectifier channels.

3626-Pos

A Conserved Arginine Near the Selectivity Filter of Kir1.1 Controls Rb/K Selectivity

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ROMK (Kir1.1) channels are important for K secretion and recycling in the collecting duct, connecting tubule and thick ascending limb of the mammalian nephron. We have identified a highly conserved *Arg* in the P loop of the channel near the selectivity filter that controls Rb/K selectivity. Mutation of this *Arg* to a *Tyr* (R128Y-Kir1.1b, R147Y-Kir1.1a) increased Rb/K selectivity by 11 $\pm$ 1 fold, NH4/K selectivity by 6 $\pm$1 fold, and Cs/K selectivity by 32 $\pm$ 5 fold over wild-type (wt), with no significant change in K/Na selectivity. R128Y remained susceptible to block by both external Ba and the honey bee toxin, TPNQ, although it had a 100 fold lower affinity for TPNQ than wild-type. Single-channel R128Y-Kir1.1b conductance averaged 12 $\pm$ 0.5 pS in 100mM RbCl, 0 Mg, 0 Ca solutions, compared to 18 $\pm$ 2 pS for wt-Kir1.1b in the same Rb solutions. In excised, inside-out patches (polyamine-free, 0 Mg solutions), R128Y-Kir1.1b had a non-rectifying Rb conductance of 10 $\pm$ 0.6 pS, but no visible K current, consistent with its low K selectivity. The kinetics of Rb permeation through R128Y were similar to the kinetics of K permeation through wt-Kir1.1b, but with a longer open time (245 ms vs. 19ms for wt); and two closed states (2.8ms, 25ms) yielding an average open probability (Po) of 0.7 at $-$100mV, compared to a Po $>$ 0.9 for wt-Kir1.1, which has a single closed state of 1.3ms. The observed 11 fold increase in Rb/K selectivity with no change in K/Na selectivity or rectification is consistent with R128Y-Kir1.1b causing a subtle change in the selectivity filter, perhaps by disruption of an intra-subunit salt bridge (R128-E118) near the filter.

3627-Pos

Characterization of the Liposomal Rubidium Uptake Assay

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The liposomal uptake assay is a useful technique in the study of the ensemble behavior of ion channels. For the study of potassium channels, purified channel protein is reconstituted into liposomes, in which an intra- to extra- liposomal K gradient is created. Uptake of radioactive 86Rb, added to the extra-liposomal solution, is concentrated into liposomes that have K selective channels, and is measured as a surrogate of channel activity. The assay allows one to define experimental conditions that are often difficult to control in other techniques used to study ion channels, such as membrane composition. Baseline characteristics of the assay, such as liposome integrity and K gradient stability, can influence results, and is the main focus of this presentation. Liposomes comprising a 9:1 ratio of POPE:POPG are stable over several hours, and 50% uptake capacity remained for liposomes stored at room temperature for ~48 hours. The rate of 86 Rb uptake in the presence of valinomycin, a K ionophore used in the measure of maximal uptake, was near maximal at ~0.1 mcg/ml/mg lipid, with higher concentrations resulting in liposome fragility and lower maximal uptake. The time course of uptake in the presence of valinomycin (0.1 mcg/ml/mg lipid) was on the order of minutes, with a time constant ~3 minutes. Lipid membrane composition influenced rate of uptake due to valinomycin. Liposomes formed from 100% PE had ~3 fold decreased rate of uptake compared with 9:1 POPE:POPG liposomes. Further characterizations are ongoing and will be presented.

3628-Pos

Changes in T-Tubular Potassium Revealed by Inward Rectifier I_{k1} Tail Currents in Mouse Ventricular Myocytes

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Cardiac ventricular myocytes possess an extensive t-tubule system which plays a number of important roles including facilitation of membrane potential propagation across the cell body. It has been shown that ion fluxes at a highly restricted t-tubular space may lead to significant accumulation/depletion of specific ions which in turn may affect t-tubular, as well as whole-cell, membrane potential. The extent of ion accumulation/depletion depends on the current densities and the volume/structure of t-tubules. In this study we used the whole-cell patch-clamp technique to monitor t-tubular accumulation of potassium (caused by outward potassium currents in response to 400 ms voltage