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Voltage-gated K Channels - Gating II

2480-Pos Board B450

Gate Opening Remotely Controls the Interaction between the Voltage Sensor and the Cytosolic Domain in BK Channels

Huanghe Yang, Jingyi Shi, Jianmin Cui.
Washington University, St. Louis, MO, USA.

In voltage- and ligand-gated ion channels the voltage sensor domain (VSD) and the ligand sensor change conformation upon stimulation, which then triggers the activation gate to open. However, the retrograde control of the conformation of the sensors by the activation gate has not been well studied. Recently, we reported that Mg2+ binds to the interface between the cytosolic domain and the membrane-spanning VSD of BK type Ca2+-activated K+ channels and activates the voltage sensor through an electrostatic interaction (Yang et al., 2007; Yang et al., 2008). Here we show that the interaction between Mg2+ and the voltage sensor is controlled by the opening of the activation gate. A mutation (F315A) in the middle of the pore-lining S6 segment altered channel opening such that the channels did not open even though the voltage sensor was fully active. The lock of the channel at the closed conformation also abolished the electrostatic interaction between Mg2+ and the VSD. 100 μm [Ca2+]i opened the activation gate of the mutant channel and reestablished the electrostatic interaction. Therefore, the activation gate and the sensory domains in BK channels are allosterically coupled and undergo concerted movements during channel gating.

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2481-Pos Board B451

Cooperativity Between Voltage-sensing Domains in the Human BK Channel Revealed by Voltage-clamp Fluorometry.

Antonios Pantazis, Vadym Gudzenko, Nicoletta Savalli, Azadeh Kohanteb, Daniel Sigg, Riccardo Olcese.
UCLA, Dept. of Anesthesiology, Los Angeles, CA, USA.

Like other members of the voltage-gated K+ channel superfamily, BK channels are thought to derive voltage sensitivity from charge-possessing transmembrane segments S2-S4. Particularly in BK, S2 is thought to have a direct role in voltage sensing (Ma et al., JGP2006). We combined cut-open oocyte voltage-clamp with fluorometry, after labeling unique cysteines introduced in cysteine-less BK channels (hSlo) with TMRM (Savalli et al., PNAS2006 and JGP2007), to resolve voltage-dependent conformational rearrangements near the extracellular side of S2. The intensity of fluorescence emission (ΔF) was strongly voltage-dependent (FV_{half}=-92 ± 2.7mV, Fz=0.95 ± 0.07, n=8), reporting protein rearrangements. To investigate voltage sensor function, we targeted two putative voltage-sensing residues: D153 (S2) and R213 (the single voltage-sensing residue in S4 -Ma et al., JGP2006). Neutralizing D153 in S2-labeled channels abolished voltage-evoked fluorescence deflections, strongly supporting the role of D153 in voltage activation of S2. Neutralizing R213 in the S4-labeled channel gave rise to a detectable but weakly voltage-dependent ΔF (Fz<0.2, n=2), perhaps arising from the S3 charge (D186, Ma et al., 2006). Cooperativity amongst voltage sensing transmembrane segments was evaluated by investigating ionic currents and ΔF from an S4 charge mutant labeled in the S2 and *vice versa*. In both cases, protein rearrangements were detected, albeit less voltage-dependent (Fz=0.21-0.23, n=5-6 respectively). These findings revealed that strong S2-S4 cooperativity underlies voltage sensing in the intact channel. In contrast to fluorescence experiments, the change in voltage sensitivity of ionic currents was smaller (Gz=0.70-0.86), supporting the view that the pore has intrinsic voltage dependence. An allosteric model of gating composed of two types of voltage sensing tetramers surrounding a single pore was used to provide a global fit of the experimental results.

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Structural and Functional Analysis of the Purified Cytosolic C-Terminus of the Human BK Channel

Anoosh Javaherian[1], Taleh Yusifov[1], Debora Nicoll[2], Chris Gandhi[3], Riccardo Olcese[1].
[1]UCLA, Dept. of Anesthesiology, Los Angeles, CA, USA, [2]UCLA, Dept. of Physiology, Los Angeles, CA, USA, [3]Caltech-Howard Hughes Medical Institute Research Laboratory, Pasadena, CA, USA.

The large-conductance voltage- and Ca^{2+}-activated K^+ channels (BK) are activated by membrane depolarizations and intracellular Ca^{2+}. Two consecutive intracellular RCK (Regulators of K^+ Conductance) domains, RCK1 and RCK2, are responsible for calcium sensitivity and together form the majority of the C-terminus.

Information about the structural and functional properties of the individual RCK1 and RCK2 domains has been reported by our group (Yusifov, et. al. PNAS 2008). However, the characterization of the full C-terminus remains unresolved. Here we report the initial structural and functional characterization of a high-purity protein expressed and purified from *E. coli* corresponding to the human (hSlo) BK channel C-terminus. The expressed C-terminus includes 684aa, starting from the S6-RCK1 linker and encompassing RCK1, RCK2, and the interconnecting 92aa RCK1-RCK2 linker.

The calcium-binding activity of the C-terminus (10ug), loaded on a nitrocellulose membrane, was probed by dot blot analysis of $^{45}Ca^{2+}$-binding. The C-terminus displayed a strong calcium-binding property when compared to Albumin.

The organization of the secondary structure of the C-terminus was investigated using Circular Dichroism (CD) spectroscopy. Far-UV CD spectra (190-260nm) of the C-terminus, analyzed with CONTIN/LL algorithm from the CDpro suite (SMP56 protein reference set), gave a secondary structure consisting of 29% α-helix, 20% β-strand, 22% turn, and 29% unordered.

The quaternary structure of C-terminus was investigated using size-exclusion chromatography with a Superdex 200 10/300 column. The C-terminus eluted in a single peak at a molecular weight of 330kDa corresponding to the theoretical tetrameric C-terminus complex (310kDa). In denaturating condition (SDS-gel electrophoresis), the C-terminus migrated as a monomeric 74kDa band (expected 77.6kDa).

In conclusion, we have successfully purified the functional human BK channel C-terminus domain, which allows for further investigation of the properties of the mammalian BK ''Gating ring,'' encompassing eight RCK domains.

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Calcium Sensing Properties of the RCK1 Domain of the Human BK Channel: Effects of the D362/367A Mutation

Taleh Yusifov[1], Anoosh Javaherian[1], Debora Nicoll[2], Chris Gandhi[3], Riccardo Olcese[1].
[1]UCLA, Dept. of Anesthesiology, Los Angeles, CA, USA, [2]UCLA, Dept. of Physiology, Los Angeles, CA, USA, [3]Caltech-Howard Hughes Medical Institute Research Laboratory, Pasadena, CA, USA.

Recent studies suggest that Ca^{2+}-dependent activation of the large-conductance voltage- and Ca^{2+}-activated K^+ (BK) channel is controlled by two RCK (Regulators of K^+ Conductance) domains located in the C-terminus, encompassing high affinity Ca^{2+}-sites D362/367 and M513 in RCK1, and a Ca^{2+}-bowl (D894-898) in RCK2 (Schreiber, et al, 1999, Lingle et al. 2002, Bao et al. 2004). Previously, we characterized Ca^{2+}-induced conformational changes and the role of the Ca^{2+} bowl in the hSloRCK2 domain using solution-based analysis (Yusifov et al., PNAS 2008).

Using a similar approach, we are now investigating the Ca^{2+}-dependent properties of the WT and D362/367A mutations of a purified protein corresponding to the amino acid sequence (322*IIE¼ DPL*667) that forms the human BK channel's RCK1 domain.

The calcium-binding activity of purified RCK1 (10ug), loaded on a nitrocellulose membrane, was directly probed by dot blot analysis of $^{45}Ca^{2+}$-binding. Albumin and Troponin were used as negative and positive controls, respectively. RCK1 showed remarkable calcium-binding ability when compared to Albumin.

Circular Dichroism (CD) analysis of the WT-RCK1 revealed a calcium-dependent spectral change, corresponding to an increased β-strand content of ~9% as the free [Ca^{2+}] was increased from 0.015 to 31.2μM. This change was paralleled by a similar decrease in α-helix content, while the turns and unordered fractions remained practically unchanged.

On the other hand, the CD spectra of RCK1-D362/367A mutant displays ~7-8% increased beta content, similar to the calcium-bound form of WT-RCK1. The Far-UV CD spectra obtained of hSloRCK1-D362/367A mutant in increasing free Ca^{2+} displayed no changes, suggesting a lack of substantial Ca^{2+}-dependent structural changes. Based on these findings, we propose that the D362/367A mutation in hSloRCK1 may lead to a conformational state of hSloRCK1 that is unable to translate Ca^{2+}-binding to channel gating.

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Cholesterol-ethanol Interactions On Vascular Myocyte BK Channels: Contribution To Alcohol-induced Cerebrovascular Constriction

Thirumalini Vaithianathan, Anna Bukiya, Jianxi Liu, Alejandro M. Dopico.
University Of Tennessee Health Science Center, Memphis, TN, USA.

The activity of large conductance, calcium- and voltage-gated potassium (BK) channels critically limits the degree of vascular smooth muscle contraction, favoring cerebrovascular dilation (Brayden and Nelson, 1992). Ethanol at levels reached in circulation after moderate binge drinking (50 mM) inhibits the activity of cerebrovascular myocyte BK channels, leading to endothelium-