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TOPICAL REVIEW

BK-type calcium-activated potassium channels: coupling of metal ions and voltage sensing

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Ion channels and lipid phosphatases adopt a transmembrane voltage sensor domain (VSD) that moves in response to physiological variations of the membrane potential to control their activities. However, the VSD movements and coupling to the channel or phosphatase activities may differ depending on various interactions between the VSD and its host molecules. BK-type voltage, Ca^{2+} and Mg^{2+} activated K^+ channels contain the VSD and a large cytosolic domain (CTD) that binds $Ca^{2+}$and Mg^{2+}. VSD movements are coupled to BK channel opening with a unique allosteric mechanism and are modulated by Ca^{2+} and Mg^{2+} binding via the interactions among the channel pore, VSD and CTD. These properties are energetically advantageous for the pore to be controlled by multiple stimuli, revealing the adaptability of the VSD to its host molecules and showing the potential for intracellular signals to affect the VSD in order to modulate the function of its host molecules.

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Introduction

BK channels are large conductance voltage and Ca^{2+} activated K^+ channels encoded by the *Slo1* gene (Atkinson *et al.* 1991). Similar to other K^+ channels, BK channels contain a central ionic pore comprising transmembrane α helices S5 and S6 from four Slo1 subunits and the selectivity filter for K^+ permeation (Atkinson *et al.* 1991; Adelman *et al.* 1992; Butler *et al.* 1993; Shen *et al.* 1994; Tseng-Crank *et al.* 1994; Doyle *et al.* 1998). The opening of BK channels repolarizes the membrane potential and reduces Ca^{2+} entry into the cell by closing voltage-dependent Ca^{2+} channels, which regulate various physiological processes including neurotransmitter release in synapses (Robitaille & Charlton, 1992; Raffaelli *et al.* 2004; Wang, 2008), contraction of smooth muscle cells in airway and blood vessels (Brayden & Nelson, 1992; Kotlikoff, 1993; Brenner *et al.* 2000), circadian pacemaker output in central nerve systems (Meredith *et al.* 2006; Pitts *et al.* 2006; Kent & Meridith, 2008) and electric tuning by hair cells in vertebrates (Art & Fettiplace, 1987; Fuchs *et al.* 1988; Hudspeth & Lewis, 1988). Each Slo1 subunit has a voltage sensor domain (VSD) formed by transmembrane segments S1–S4 and a large cytosolic domain (CTD) containing Ca^{2+} binding sites (Fig. 1). Voltage sensor movements in response to depolarization of the membrane potential and the binding of intracellular Ca^{2+} are separately coupled to the pore to activate the channel.

Similar molecular mechanisms may underlie the coupling between voltage sensor movements and pore opening in voltage gated K^+ (K_V) channels and BK channels (Cui *et al.* 2009). However, while in some K_V channels, such as Shaker, the coupling between the voltage sensor and pore seems to be tight or even obligatory, i.e. the pore opens when and only when all the voltage sensors are activated, the coupling in BK channels is allosteric, i.e. the pore can open when the voltage sensor is either activated or at rest but the opening is favoured when the voltage sensor is activated. In addition, in BK channels the CTD is located close to the membrane-spanning voltage

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sensor and pore domains. This structural feature allows intracellular Mg^{2+} to bind closely to the cytosolic side of the voltage sensor and directly affect voltage sensor movements via an electrostatic interaction (Yang *et al.* 2007). This article will review the special properties of voltage sensing and the influence by Ca^{2+} and Mg^{2+} ions in BK channels.

Allosteric mechanisms of voltage- and Ca^{2+}-dependent activation of BK channels

The VSDs of BK channels contain charged amino acid residues that are highly conserved among K_V channels (Fig. 1*A*). The arginine residues at positions 207, 210 and 213 in the S4 segment (all residue numbers are based

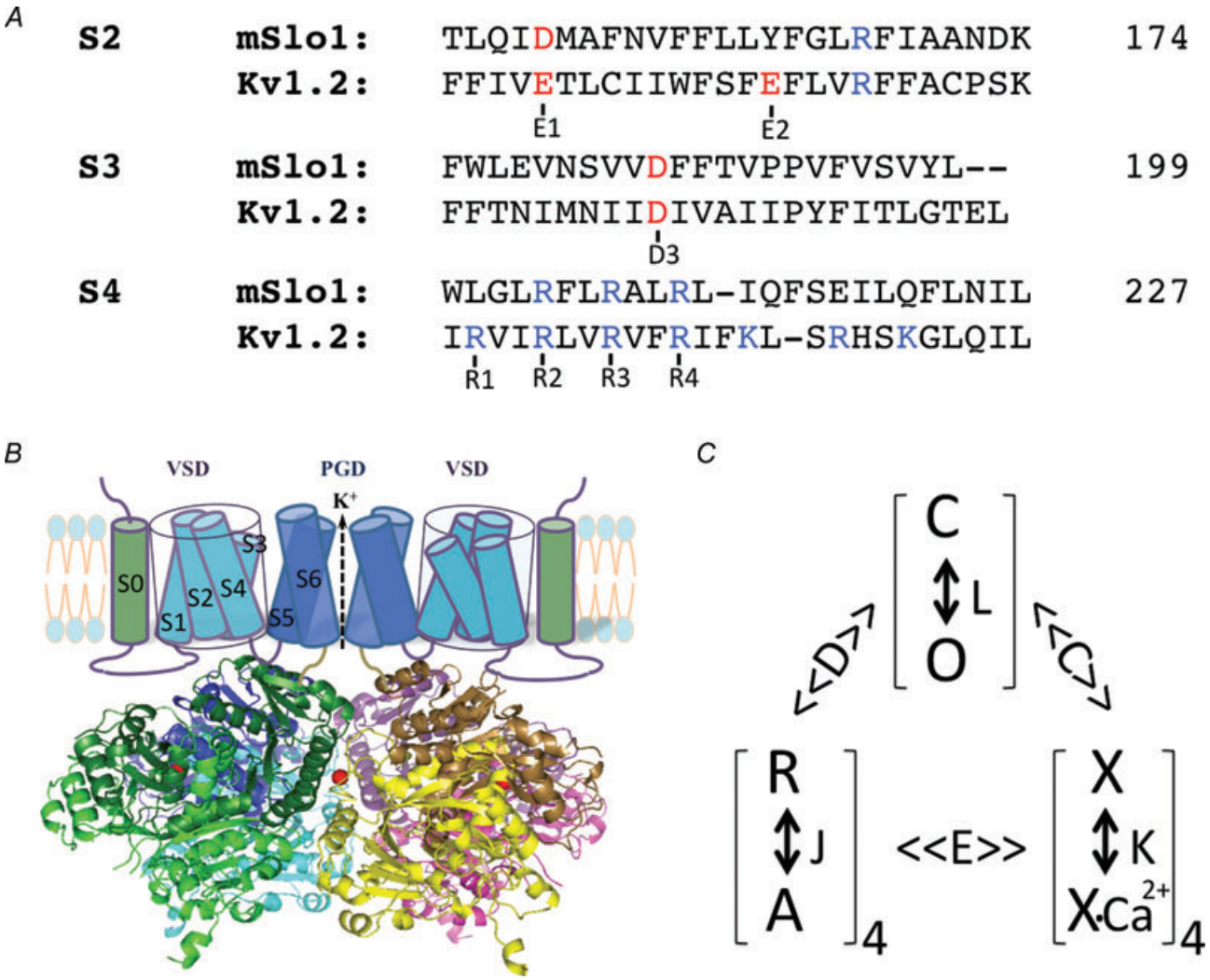

Figure 1. Voltage- and Ca^{2+}-dependent activation of BK channels
A, sequence alignment of S2–S4 in the voltage sensor domain of mSlo1 and K_V1.2 channels, highlighting the conserved charged residues (red and blue) that are important in voltage-dependent activation of both BK and K_V channels. The position of the last amino acid in each segment of mSlo1 is given (numbers). *B*, structure model of BK channels. The cartoon of the membrane-spanning domains only shows two subunits for clarity. The cytosolic domain is the tetrameric Slo1 structure (Yuan *et al.* 2010). The subunits are represented by different colours and the dark and light shades of each colour show the RCK1 and RCK2 structural domains within the same subunit (RCK: regulator for conductance of K^+). A bound Ca^{2+} ion to the Ca^{2+} bowl in each subunit is shown as a red sphere. *C*, model of allosteric gating of BK channels by voltage and Ca^{2+}. The channel can open and close (O and C) with an equilibrium constant L, which is modulated by the activation of four voltage sensors (R and A, with an equilibrium constant J) and the binding of four Ca^{2+} ions (a simplification from actual binding of eight Ca^{2+} ions, X and X-Ca^{2+}, with a dissociation constant K) with the allosteric factor D and C, respectively. The voltage sensor activation and Ca^{2+} binding also affect each other with a factor E. The model was proposed by Horrigan & Aldrich (2002).

on the mbr5 sequence of mouse Slo1 (mSlo1) subunit, GenBank accession number, GI: 347143) (Butler *et al.* 1993) correspond to R2, R3 and R4 in K_V channels (Gandhi & Isacoff, 2002), Asp153 in S2 corresponds to E1/D1 in Kv channels (Silverman *et al.* 2003; Wu *et al.* 2010), and Asp186 in S3 corresponds to D3 in K_V channels (Fig. 1*A*). The S2 segment does not contain the conserved E2 residue of K_V channels at the cytosolic side, but instead a positively charged Arg167 that is located four residues below the E2 position. Although the mutations of each of the three arginine residues in S4 alter voltage dependence of BK channel opening (Diaz *et al.* 1998; Cui & Aldrich, 2000), it was shown that only Arg213 contributes to voltage sensing (Ma *et al.* 2006). In addition, the charged residues Asp153 and Arg167 in S2 and Asp186 in S3 also contribute to voltage sensing. Neutralization of each of these residues in the four mSlo1 subunits reduced effective gating charge by 1.20, 0.92, 0.48 and 0.88, respectively, from 2.32 of the WT channel (Ma *et al.* 2006). The role of these charges is somewhat different from that of the equivalent charged residues in K_V channels, where the arginine residues in S4 serve as the primary gating charges, while the negatively charged residues in S2 and S3 interact with S4 arginines to stabilize channel protein (Papazian *et al.* 1995; Tiwari-Woodruff *et al.* 1997; Long *et al.* 2005) and steer S4 movements during activation (Wu *et al.* 2010); only E2 in the S2 segment of the Shaker K^+ channel had been shown to serve as a gating charge (Seoh *et al.* 1996). Since in BK channels the charged residues in S2, S3 and S4 contribute similarly to voltage sensing, the movements of the VSD in BK and K_V channels may differ during activation. A model proposes that in BK channels the VSD undergoes a global conformation change that results in a repacking of all membrane-spanning segments in the VSD during activation, as opposed to a large movement of S4 alone in K_V channels (Ma *et al.* 2006; Pantazis *et al.* 2010).

In BK channels, each Slo1 subunit contains an additional transmembrane segment S0 at the N-terminus of the VSD (Wallner *et al.* 1996) (Fig. 1*B*). In the BK channel structure, S0 is located at the periphery of VSD close to S2 (Liu *et al.* 2008; Wang & Sigworth, 2009). S0 is important for modulation of BK channels by auxiliary β subunits; it is also essential for the function of the Slo1 protein since the truncated Slo1 lacking S0 could not express any currents but the coexpression of the truncated Slo1 with a separate S0 segment restored channel function (Wallner *et al.* 1996). Mutations of S0 affect voltage-dependent activation of BK channels (Koval *et al.* 2007). Nevertheless, it is not clear how S0 contributes to the movements of the VSD during voltage dependent activation.

Voltage sensor activation in BK channels facilitates channel opening. In the absence of intracellular Ca^{2+}, the channel can be activated by voltage; the macroscopic conductance and activation rate increase with depolarization (Meera *et al.* 1996; Cui *et al.* 1997). Likewise, the open probability of single BK channels increases with voltage (Nimigean & Magleby, 2000; Rothberg & Magleby, 2000; Talukder & Aldrich, 2000). Voltage sensor movements can be measured by gating currents (Stefani *et al.* 1997; Horrigan & Aldrich, 1999) and fluorescence changes from the fluorophore labelling the S3–S4 linker (Savalli *et al.* 2006) prior to channel opening. Mutations of charged amino acid residues in the VSD reduce the steepness of the voltage dependence of channel opening (Diaz *et al.* 1998; Cui & Aldrich, 2000; Ma *et al.* 2006). Finally, intracellular Mg^{2+} (Yang *et al.* 2007) and the auxiliary β subunits of BK channels (Bao & Cox, 2005; Orio & Latorre, 2005; Savalli *et al.* 2007; Yang *et al.* 2008*b*) modulate channel opening by altering voltage sensor function.

While it is clear that BK channels are activated by voltage, the channel can open even when the voltage sensors are not activated; conversely, the channel may remain closed when the voltage sensors are activated. For an ion channel that opens only after the voltage sensors are activated, the probability of channel opening (P_o) depends on voltage with an exponential function at negative voltages, where the voltage sensors are at the resting state and the probability of their traverse to the activated state is low (Almers, 1978; Sigworth, 1994; Sigg & Bezanilla, 1997). For instance, the Shaker K^+ channel shows such a P_o–voltage relation at negative voltages where P_o is as low as 10^{-7}, suggesting the tight or even obligatory relation between voltage sensor activation and channel opening (Islas & Sigworth, 1999). However, for BK channels, the P_o–V relation becomes flat as voltage decreases below 0 mV, deviating from the exponential relationship at $P_o \approx 10^{-5}$ and is much shallower at $P_o \leq 10^{-6}$ (Horrigan *et al.* 1999; Cui & Aldrich, 2000; Yang *et al.* 2010). This result indicates that BK channels can open spontaneously and independently of voltage sensor movements at negative voltages. Another piece of evidence for the non-obligatory relation between voltage sensor movements and channel opening is that the On-gating current elicited by a depolarizing voltage pulse from a negative voltage has a much faster time course than channel opening. The ionic current does not turn on until the On-gating current completes relaxation to 0 (Fig. 2*B*) (Horrigan & Aldrich, 1999), suggesting that the gating charges have moved to the activated state before the channels open. Thus, the voltage sensors can activate while the channel is closed and the activation of the voltage sensors facilitates channel opening. Based on these results and the voltage dependence of ionic and gating currents, an allosteric model was proposed for voltage-dependent activation of BK channels (Horrigan *et al.* 1999). In this model the voltage sensors can activate when the channel is either at the closed or open state but in open channels the activation of the voltage sensors is more favoured;

reciprocally, the activation of the voltage sensors promotes channel opening (Fig. 1*C*).

The allosteric coupling between voltage sensor movements and channel opening in BK channels is particularly suitable for Ca^{2+}-dependent activation. At voltages below – 150 mV, where the voltage sensors are at the resting state, an increase of intracellular Ca^{2+} concentration enhances P_o by ~10^4 (Horrigan & Aldrich, 2002; Sweet & Cox, 2008; Yang *et al.* 2010). Therefore, Ca^{2+} binding can open the channel without voltage sensor activation, which is energetically advantageous for Ca^{2+} to activate the channel, especially at negative voltages.

Two Ca^{2+} binding sites have been identified in the CTD of the Slo1 subunit by functional studies and mutagenesis. One site is located in the Ca^{2+} bowl that contains consecutive Asp residues at positions 895–901 (Moss *et al.* 1996*a*,*b*; Schreiber & Salkoff, 1997; Schreiber *et al.* 1999; Bian *et al.* 2001; Braun & Sy, 2001; Bao *et al.* 2004), and the other resides at Asp367 (Xia *et al.* 2002). Recently, an X-ray crystallographic structure of the CTD of Slo1 shows a Ca^{2+} ion binding to the Ca^{2+} bowl that is coordinated by the side chains from Asp898 and Asp900 and main chain carbonyl from Gln892 and Asp895 (Yuan *et al.* 2010), but the second Ca^{2+} binding site was not identified in the structure. Ca^{2+}-dependent activation of BK channels can also be described by an allosteric model (McManus & Magleby, 1991; Cox *et al.* 1997), in which Ca^{2+} can bind to the channel in both the closed and open states but with a higher affinity when the channel is open, and therefore Ca^{2+} binding shifts the equilibrium between the closed and open states toward open (Fig. 1*C*). Because both voltage and Ca^{2+} promote BK channel opening and because both voltage sensor activation and Ca^{2+} binding are favoured when the channel is open, Ca^{2+} binding shifts voltage dependence of channel opening toward less positive voltages, while depolarization enhances the apparent affinity and cooperativity of Ca^{2+} binding (Cox *et al.* 1997; Cui *et al.* 1997). Thus, voltage sensor movements are modulated by Ca^{2+}, and vice versa, through an allosteric connection via channel opening. In addition, Ca^{2+}-dependent activation and voltage sensor movements also affect each other more directly (Horrigan & Aldrich, 2002; Sweet & Cox, 2008), possibly via the interaction between the VSD and CTD (see below).

Mg^{2+} activates BK channels through an electrostatic interaction with the VSD

In addition to the sites at the Ca^{2+} bowl and Asp367 that bind Ca^{2+} with affinities in the micromolar range, BK channels contain another site that binds both Ca^{2+} and Mg^{2+} with affinities in the millimolar range (Golowasch *et al.* 1986; Oberhauser *et al.* 1988; Shi & Cui, 2001; Zhang *et al.* 2001). Because this site binds metal ions at the physiological concentration of Mg^{2+}, it is known as the Mg^{2+} binding site. This site is unique in that Mg^{2+} is coordinated by residues from both the CTD (Glu374 and Glu399) (Shi *et al.* 2002; Xia *et al.* 2002) and the VSD (Asp99 and Asn172) (Yang *et al.* 2008*a*) (Fig. 2*A*). It shows that the CTD in the BK channel is spatially very close to the VSD. Consistent with this result, it was shown that two Cys mutations that substitute for Asp99 in the VSD and Gln397 in the CTD can form a disulfide bond (Yang *et al.* 2008*a*) that is 2.9–4.6 Å in distance Cβ–Cβ

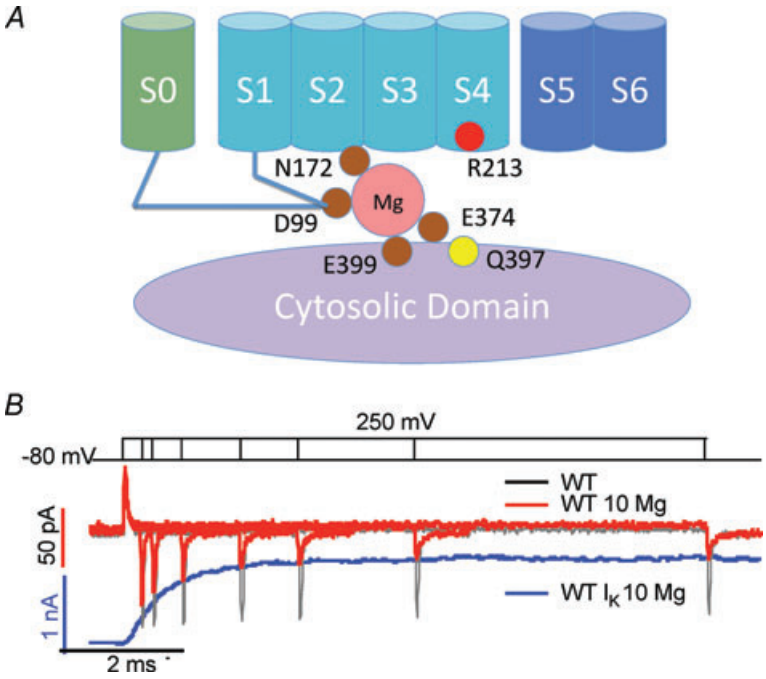

Figure 2. Mg^{2+} modulation of the voltage sensor function in BK channels
A, cartoon showing amino acid residues involved in Mg^{2+} binding and interaction with the voltage sensor domain. *B*, gating currents in the absence and presence of 10 mM Mg^{2+} (black and red traces) in response to various lengths of voltage pulses from −80 mV to +250 mV (top). The ionic current (blue trace) in response to a −80 to +250 mV voltage pulse in 10 mM Mg^{2+} is aligned with gating currents to show the correlation in time courses between channel opening and the decay in Off-gating current amplitude in 10 mM Mg^{2+}. Adapted from Yang *et al.* (2007), ©2007 National Academy of Sciences of the USA.

of the two Cys residues (Hazes & Dijkstra, 1988). The close association between the VSD and CTD was also demonstrated in the electron cryomicroscopy structure of BK channels (Wang & Sigworth, 2009). Recently, it was shown that X-ray crystallographic structure of the Slo1 CTD fits well with the cytosolic face of the K_V channel VSD (Yuan *et al.* 2010).

The Mg^{2+} ion bound to the BK channel is positioned close to the VSD, allowing it to affect the VSD through an electrostatic interaction. Evidence for the involvement of the VSD in Mg^{2+}-dependent activation of BK channels was first shown in a study in which the mutation of Arg213 to Gln (R213Q) abolished Mg^{2+} sensitivity of BK channel activation (Hu *et al.* 2003). Since Arg213 is the only residue in S4 contributing to gating charge (Ma *et al.* 2006), this result suggested that Mg^{2+} might alter voltage sensor movements by an electrostatic interaction. The evidence supporting this idea was shown in a number of experiments (Yang *et al.* 2007). First, Mg^{2+}-dependent activation depends on the ionic strength of the intracellular solution, and the effect of Mg^{2+} diminishes with increasing ionic strength, consistent with the idea that Mg^{2+} activates the channel with an electrostatic interaction. Second, a positive charge at residue 213 is necessary and sufficient for Mg^{2+}-dependent activation. Neutralization of the charge of Arg213 with mutations R213Q or R213C abolishes Mg^{2+}-dependent activation, while a covalent modification of R213C by a positively charged [2-(trimethylammonium)ethyl]methane thiosulfonate ($MTSET^+$) restores Mg^{2+} sensitivity. This result suggests that Arg213 is the target of the electrostatic interaction with Mg^{2+}. The third experiment supporting the electrostatic interaction is that positive charges added to Gln397 by either mutation or chemical modification can mimic the effect of Mg^{2+} to activate the channel. Gln397 is located close to the Mg^{2+} binding site, only two residues away from one of the Mg^{2+} coordinator Glu399 (Shi *et al.* 2002; Xia *et al.* 2002), and mutations of Gln397 to charged residues alter Mg^{2+} binding by electrostatic interactions (Yang *et al.* 2007). These results indicate that the electric field of the charges at 397 overlaps with that of the bound Mg^{2+} ion. Therefore, both charges can interact with Arg213 through the electric fields.

The electrostatic repulsion between Mg^{2+} and Arg213 in S4 slows down the return of the voltage sensor from the activated state to the resting state. This is supported by measuring the effect of Mg^{2+} on gating currents of BK channels (Yang *et al.* 2007). Interestingly, the effect of Mg^{2+} on gating currents depends on whether the channel is in the closed or open state. In BK channels, the voltage sensor is allosterically coupled to the activation gate and can activate in both the closed and open state (Horrigan & Aldrich, 1999). The On-gating current in response to a depolarization pulse decays rapidly before the onset of the ionic current (Fig. 2*B*), representing voltage sensor activation in the closed state. It was found that Mg^{2+} has little effect on the On-gating current but prolongs and reduces the amplitude of the Off-gating current upon membrane repolarization (Yang *et al.* 2007; Horrigan & Ma, 2008). The changes in the Off-gating current by Mg^{2+} are increasingly apparent with longer depolarization pulses and the time course matches with the onset of the ionic current elicited by the same voltage (Fig. 2*B*), indicating that the returning of the voltage sensor to the resting state as measured by the Off-gating current is slowed down by Mg^{2+} more when the channel is open than when the channel is closed. These results suggest that channel opening enhances the interaction between Mg^{2+} and S4, possibly by a conformational change that moves the bound Mg^{2+} closer to S4.

Summary

BK channels are activated by voltage and intracellular Ca^{2+} and Mg^{2+}. The BK channel contains the transmembrane VSD as well as Ca^{2+} and Mg^{2+} binding sites in a large CTD. The voltage and metal sensors all control the opening of the same ionic pore in response to various physiological signals. While the architecture of the VSD in BK channels is similar to that of other voltage-dependent channels, the movements of the VSD during channel activation and the coupling between the VSD and the activation gate in BK channels are unique, and as such the charged residues in S2, S3 and S4 make similar contributions to voltage sensing and the VSD activation promotes channel opening through an allosteric mechanism. These properties are well suited to the function of BK channels to sense multiple stimuli and may be derived from the interactions of the VSD not only with the pore but also with the unique S0 segment and the CTD in BK channels. These interactions among different structural domains also mediate the modulation of VSD movements by Ca^{2+} via allosteric mechanisms and by Mg^{2+} via electrostatic interactions.

The movements of the VSD in response to depolarization can either open or close ion channels (Perozo *et al.* 1992; Mannikko *et al.* 2002) and can activate lipid phosphatases (Murata *et al.* 2005; Hossain *et al.* 2008), indicating that the VSD has the ability to adapt to various environments in its host protein molecules and functional requirements. The VSD may also be modulated by intracellular factors such as ligand binding, post-translational modification, and the interactions with cytosolic domains, accessory proteins or subunits to alter the function of voltage-dependent ion channels (Perozo & Bezanilla, 1990; Jones *et al.* 1997; Terlau *et al.* 1997; Bao & Cox, 2005). The study of voltage-dependent activation of BK channels may provide insights into the principles of the VSD versatility and modulation that affect the function of VSD containing molecules.

References

Adelman JP, Shen KZ, Kavanaugh MP, Warren RA, Wu YN, Lagrutta A, Bond CT & North RA (1992). Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron* **9**, 209–216.

Almers W (1978). Gating currents and charge movements in excitable membranes. *Rev Physiol Biochem Pharmacol* **82**, 96–190.

Art JJ & Fettiplace R (1987). Variation of membrane properties in hair cells isolated from the turtle ccochlea. *J Physiol* **385**, 207–242.

Atkinson NS, Robertson GA & Ganetzky B (1991). A component of calcium-activated potassium channels encoded by the *Drosophila slo* locus. *Science* **253**, 551–555.

Bao L & Cox DH (2005). Gating and ionic currents reveal how the BKCa channel's Ca^{2+} sensitivity is enhanced by its β1 subunit. *J Gen Physiol* **126**, 393–412.

Bao L, Kaldany C, Holmstrand EC & Cox DH (2004). Mapping the BKCa channel's "Ca^{2+} bowl": side-chains essential for Ca^{2+} sensing. *J Gen Physiol* **123**, 475–489.

Bian S, Favre I & Moczydlowski E (2001). Ca^{2+}-binding activity of a COOH-terminal fragment of the *Drosophila* BK channel involved in Ca^{2+}-dependent activation. *Proc Natl Acad Sci U S A* **98**, 4776–4781.

Braun AP & Sy L (2001). Contribution of potential EF hand motifs to the calcium-dependent gating of a mouse brain large conductance, calcium-sensitive K^+ channel. *J Physiol* **533**, 681–695.

Brayden JE & Nelson MT (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* **256**, 532–535.

Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT & Aldrich RW (2000). Vasoregulation by the β1 subunit of the calcium-activated potassium channel. *Nature* **407**, 870–876.

Butler A, Tsunoda S, McCobb DP, Wei A & Salkoff L (1993). mSlo, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science* **261**, 221–224.

Cox DH, Cui J & Aldrich RW (1997). Allosteric gating of a large conductance Ca- activated K^+ channel. *J Gen Physiol* **110**, 257–281.

Cui J & Aldrich RW (2000). Allosteric linkage between voltage and Ca^{2+}-dependent activation of BK-type mslo1 K^+ channels. *Biochemistry* **39**, 15612–15619.

Cui J, Cox DH & Aldrich RW (1997). Intrinsic voltage dependence and Ca^{2+} regulation of mslo large conductance Ca^{2+}-activated K^+ channels. *J Gen Physiol* **109**, 647–673.

Cui J, Yang H & Lee US (2009). Molecular mechanisms of BK channel activation. *Cell Mol Life Sci* **66**, 852–875.

Diaz L, Meera P, Amigo J, Stefani E, Alvarez O, Toro L & Latorre R (1998). Role of the S4 segment in a voltage-dependent calcium-sensitive potassium (hSlo) channel. *J Biol Chem* **273**, 32430–32436.

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT & MacKinnon R (1998). The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* **280**, 69–77.

Fuchs PA, Nagai R & Evans MG (1988). Electrical tuning in hair cells isolated from the chick cochlea. *J Neurosci* **8**, 2460–2467.

Gandhi CS & Isacoff EY (2002). Molecular models of voltage sensing. *J Gen Physiol* **120**, 455–463.

Golowasch J, Kirkwood A & Miller C (1986). Allosteric effects of Mg^{2+} on the gating of Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. *J Exp Biol* **124**, 5–13.

Hazes B & Dijkstra BW (1988). Model building of disulfide bonds in proteins with known three-dimensional structure. *Protein Eng* **2**, 119–125.

Horrigan FT & Aldrich RW (1999). Allosteric voltage gating of potassium channels II. Mslo channel gating charge movement in the absence of Ca^{2+}. *J Gen Physiol* **114**, 305–336.

Horrigan FT & Aldrich RW (2002). Coupling between voltage sensor activation, Ca^{2+} binding and channel opening in large conductance (BK) potassium channels. *J Gen Physiol* **120**, 267–305.

Horrigan FT, Cui J & Aldrich RW (1999). Allosteric voltage gating of potassium channels I. Mslo ionic currents in the absence of Ca^{2+}. *J Gen Physiol* **114**, 277–304.

Horrigan FT & Ma Z (2008). Mg^{2+} enhances voltage sensor/gate coupling in BK channels. *J Gen Physiol* **131**, 13–32.

Hossain MI, Iwasaki H, Okochi Y, Chahine M, Higashijima S, Nagayama K & Okamura Y (2008). Enzyme domain affects the movement of the voltage sensor in ascidian and zebrafish voltage-sensing phosphatases. *J Biol Chem* **283**, 18248–18259.

Hu L, Shi J, Ma Z, Krishnamoorthy G, Sieling F, Zhang G, Horrigan FT & Cui J (2003). Participation of the S4 voltage sensor in the Mg^{2+}-dependent activation of large conductance (BK) K^+ channels. *Proc Natl Acad Sci U S A* **100**, 10488–10493.

Hudspeth AJ & Lewis RS (1988). Kinetic analysis of voltage- and ion-dependent conductances in saccular hair cells of the bull-frog, *Rana catesbeiana*. *J Physiol* **400**, 237–274.

Islas LD & Sigworth FJ (1999). Voltage sensitivity and gating charge in Shaker and Shab family potassium channels. *J Gen Physiol* **114**, 723–742.

Jones LP, Patil PG, Snutch TP & Yue DT (1997). G-protein modulation of N-type calcium channel gating current in human embryonic kidney cells (HEK 293). *J Physiol* **498**, 601–610.

Kent J & Meridith AL (2008). BK channels regulate spontaneous action potential rhythmicity in the suprachiasmatic nucleus. *PLoS One* **3**, e3884.

Kotlikoff MI (1993). Potassium channels in airway smooth muscle: a tale of two channels. *Pharmacol Ther* **58**, 1–12.

Koval OM, Fan Y & Rothberg BS (2007). A role for the S0 transmembrane segment in voltage-dependent gating of BK channels. *J Gen Physiol* **129**, 209–220.

Liu G, Zakharov SI, Yang L, Deng SX, Landry DW, Karlin A & Marx SO (2008). Position and role of the BK channel α subunit S0 helix inferred from disulfide crosslinking. *J Gen Physiol* **131**, 537–548.

Long SB, Campbell EB & MacKinnon R (2005). Voltage sensor of K_V1.2: structural basis of electromechanical coupling. *Science* **309**, 903–908.

Ma Z, Lou XJ & Horrigan FT (2006). Role of charged residues in the S1–S4 voltage sensor of BK channels. *J Gen Physiol* **127**, 309–328.

McManus OB & Magleby KL (1991). Accounting for the Ca^{2+}-dependent kinetics of single large-conductance Ca^{2+}-activated K^+ channels in rat skeletal muscle. *J Physiol* **443**,739–777.

Mannikko R, Elinder F & Larsson HP (2002). Voltage-sensing mechanism is conserved among ion channels gated by opposite voltages. *Nature* **419**, 837–841.

Meera P, Wallner M, Jiang Z & Toro L (1996). A calcium switch for the functional coupling between alpha (hslo) and β subunits (K_V, Ca β) of maxi K channels. *FEBS Lett* **382**, 84–88.

Meredith AL, Wiler SW, Miller BH, Takahashi JS, Fodor AA, Ruby NF & Aldrich RW (2006). BK calcium-activated potassium channels regulate circadian behavioral rhythms and pacemaker output. *Nat Neurosci* **9**, 1041–1049.

Moss GW, Marshall J & Moczydlowski E (1996*a*). Hypothesis for a serine proteinase-like domain at the COOH terminus of Slowpoke calcium-activated potassium channels. *J Gen Physiol* **108**, 473–484.

Moss GW, Marshal J, Morabito M, Howe JR & Moczydlowski E (1996*b*). An evolutionarily conserved binding site for serine proteinase inhibitors in large conductance calcium-activated potassium channels. *Biochemistry* **35**, 16024–16035.

Murata Y, Iwasaki H, Sasaki M, Inaba K & Okamura Y (2005). Phosphoinositide phosphatase activity coupled to an intrinsic voltage sensor. *Nature* **435**, 1239–1243.

Nimigean CM & Magleby KL (2000). Functional coupling of the β_1 subunit to the large conductance Ca^{2+}-activated K^+ channel in the absence of Ca^{2+}. Increased Ca^{2+} sensitivity from a Ca^{2+}-independent mechanism. *J Gen Physiol* **115**, 719–736.

Oberhauser A, Alvarez O & Latorre R (1988). Activation by divalent cations of a Ca^{2+}-activated K^+ channel from skeletal muscle membrane. *J Gen Physiol* **92**, 67–86.

Orio P & Latorre R (2005). Different effects of β1 and β2 subunits on BK channel activity. *J Gen Physiol* **125**, 395–411.

Pantazis A, Gundenko V, Savalli N, Sigg D & Olcese R (2010). Operation of the voltage sensor of a human voltage- and Ca^{2+}-activated K^+ channel. *Proc Natl Acad Sci U S A* **107**, 4459–4464.

Papazian DM, Shao XM, Seoh SA, Mock AF, Huang Y & Wainstock DH (1995). Electrostatic interacions of S4 voltage sensor in Shaker K^+ channel. *Neuron* **14**, 1293–1301.

Perozo E & Bezanilla F (1990). Phosphorylation affects voltage gating of the delayed rectifier K^+ channel by electrostatic interactions. *Neuron* **5**, 685–690.

Perozo E, Papazian DM, Stefani E & Bezanilla F (1992). Gating currents in Shaker K^+ channels. Implications for activation and inactivation models. *Biophys J* **62**, 160–168; discussion 169–171.

Pitts GR, Ohta H & McMahon DG (2006). Daily rhythmicity of large-conductance Ca^{2+}-activated K^+ currents in suprachiasmatic nucleus neurons. *Brain Res* **1071**, 54–62.

Raffaelli G, Saviane C, Mohajerani MH, Pedarzani P & Cherubini E (2004). BK potassium channels control transmitter release at CA3-CA3 synapses in the rat hippocampus. *J Gen Physiol* **557**, 147–157.

Robitaille R & Charlton MP (1992). Presynaptic calcium signals and transmitter release are modulated by calcium-activated potassium channels. *J Neurosci* **12**, 297–305.

Rothberg BS & Magleby KL (2000). Voltage and Ca^{2+} activation of single large-conductance Ca^{2+}-activated K^+ channels described by a two-tiered allosteric gating mechanism. *J Gen Physiol* **116**, 75–99.

Savalli N, Kondratiev A, de Quintana SB, Toro L & Olcese R (2007). Modes of operation of the BK_{Ca} channel β2 subunit. *J Gen Physiol* **130**, 117–131.

Savalli N, Kondratiev A, Toro L & Olcese R (2006). Voltage-dependent conformational changes in human Ca^{2+}- and voltage-activated K^+ channel, revealed by voltage-clamp fluorometry. *Proc Natl Acad Sci U S A* **103**, 12619–12624.

Schreiber M & Salkoff L (1997). A novel calcium-sensing domain in the BK channel. *Biophys J* **73**, 1344–1363.

Schreiber M, Yuan A & Salkoff L (1999). Transplantable sites confer calcium sensitivity to BK channels. *Nat Neurosci* **2**, 416–421.

Seoh SA, Sigg D, Papazian DM & Bezanilla F (1996). Voltage-sensing residues in the S2 and S4 segments of the Shaker K^+ channel. *Neuron* **16**, 1159–1167.

Shen KZ, Lagrutta A, Davies NW, Standen NB, Adelman JP & North RA (1994). Tetraethylammonium block of Slowpoke calcium-activated potassium channels expressed in *Xenopus* oocytes: evidence for tetrameric channel formation. *Pflugers Arch* **426**, 440–445.

Shi J & Cui J (2001). Intracellular Mg^{2+} enhances the function of BK-type Ca^{2+}-activated K^+ channels. *J Gen Physiol* **118**, 589–606.

Shi J, Krishnamoorthy G, Yang Y, Hu L, Chaturvedi N, Harilal D, Qin J & Cui J (2002). Mechanism of magnesium activation of calcium-activated potassium channels. *Nature* **418**, 876–880.

Sigg D & Bezanilla F (1997). Total charge movement per channel. The relation between gating charge displacement and the voltage sensitivity of activation. *J Gen Physiol* **109**, 27–39.

Sigworth FJ (1994). Voltage gating of ion channels. *Q Rev Biophys* **27**, 1–40.

Silverman WR, Roux B & Papazian DM (2003). Structural basis of two-stage voltage-dependent activation in K^+ channels. *Proc Natl Acad Sci U S A* **100**, 2935–2940.

Stefani E, Ottolia M, Noceti F, Olcese R, Wallner M, Latorre R & Toro L (1997). Voltage-controlled gating in a large conductance Ca^{2+}-sensitive K^+ channel (hslo). *Proc Natl Acad Sci U S A* **94**, 5427–5431.

Sweet TB & Cox DH (2008). Measurements of the BK_{Ca} channel's high-affinity Ca^{2+} binding constants: effects of membrane voltage. *J Gen Physiol* **132**, 491–505.

Talukder G & Aldrich RW (2000). Complex voltage-dependent behavior of single unliganded calcium-sensitive potassium channels. *Biophys J* **78**, 761–772.

Terlau H, Heinemann SH, Stuhmer W, Pongs O & Ludwig J (1997). Amino terminal-dependent gating of the potassium channel rag eag is compensated by a mutation in the S4 segment. *J Gen Physiol* **502**, 537–543.

Tiwari-Woodruff SK, Schulteis CT, Mock AF & Papazian DM (1997). Electrostatic interactions between transmembrane segments meditate folding of Shaker K^+ channel subunits. *Biophys J* **72**, 1489–1500.

Tseng-Crank J, Foster CD, Krause JD, Mertz R, Godinot N, Dichiara TJ & Reinhart PH (1994). Cloning, expression, and distribution of functionally distant Ca^{2+}- activated K^+ channel isoforms from human brain. *Neuron* **13**, 1315–1330.

Wallner M, Meera P & Toro L (1996). Determinant for β-subunit regulation in high-conductance voltage-activated and Ca^{2+}-sensitive K^+ channels: an additional transmembrane region at the N terminus. *Proc Natl Acad Sci U S A* **93**, 14922–14927.

Wang L & Sigworth FJ (2009). Structure of BK potassium channel in a lipid membrane from electron cryomicroscopy. *Nature* **461**, 292–295.

Wang ZW (2008). Regulation of synaptic transmission by presynaptic CaMKII and BK channels. *Mol Neurobiol* **38**, 153–166.

Wu D, Delaloye K, Zaydman MA, Nekouzadeh A, Rudy Y & Cui J (2010). State-dependent electrostatic interactions of S4 arginines with E1 in S2 during $K_V7.1$ activation. *J Gen Physiol* **135**, 595–606.

Xia XM, Zeng X & Lingle CJ (2002). Multiple regulatory sites in large-conductance calcium-activated potassium channels. *Nature* **418**, 880–884.

Yang H, Hu L, Shi J, Delaloye K, Horrigan FT & Cui J (2007). Mg^{2+} meditates interaction between the voltage sensor and cytosolic domain to activate BK channels. *Proc Natl Acad Sci U S A* **104**, 18270–18275.

Yang H, Shi J, Zhan G, Yang J, Delaloye K & Cui J (2008*a*). Activation of Slo1 BK channels by Mg^{2+} coordinated between the voltage sensor and RCK1 domains. *Nat Struct Mol Biol* **15**,1152–1159.

Yang H, Zhang G, Shi J, Lee US, Delaloye K & Cui J (2008*b*). Subunit-specific effect of the voltage sensor domain on Ca^{2+} sensitivity of BK channels. *Biophys J* **94**, 4678–4687.

Yang J, Krishnamoorthy G, Saxena A, Zhang G, Shi J, Yang H, Delaloye K, Sept D & Cui J (2010). An eplilepsy/dyskinesia-associated mutation enhances BK channel activation by potentiating Ca^{2+} sensing. *Neuron* **66**, 871–883.

Yuan P, Leonetti MD, Pico AR, Hsiung Y & Mackinnon R (2010). Structure of the human BK channel Ca^{2+}-activation apparatus at 3.0 Å resolution. *Science* **329**, 182–186.

Zhang X, Solaro CR & Lingle CJ (2001). Allosteric regulation of BK channel gating by Ca^{2+} and Mg^{2+} through a nonselective, low affinity divalent cation site. *J Gen Physiol* **118**, 607–636.

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