Mg^{2+}-dependent Regulation of BK Channels: Importance of Electrostatics

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A signature feature of large conductance, calcium- and voltage-activated K^+ channels (now usually termed "BK" channels) is their dual regulation by two physiological signals, cytosolic Ca^{2+} and membrane voltage. This dual-sensing capacity of BK channels distinguishes them from other voltage-dependent K^+ channels in terms of the physiological roles they can play, allowing their voltage-sensing function to be dynamically regulated by variations in submembrane $[Ca^{2+}]$. Furthermore, as illustrated abundantly in various past articles of this journal (Cox et al., 1997; Horrigan and Aldrich, 1999; Horrigan et al., 1999; Rothberg and Magleby, 1999; Cui and Aldrich, 2000; Rothberg and Magleby, 2000), this dual-sensing ability of BK channels has provided an almost unparalleled model for investigation of ion channel allosteric gating mechanisms. This line of investigation led to the basic idea developed robustly by Horrigan and Aldrich (2002) that the regulation of BK gating by Ca^{2+} and voltage arises from three coupled equilibria, a voltage-sensor equilibrium (J), a ligand-binding equilibrium (K), and the channel pore domain open–closed equilibrium (L). These coupled equilibria are schematized in Fig. 1 A (Horrigan and Aldrich, 2002), with the coupling between voltage sensor and gate opening given by D, coupling between ligand binding and gate opening by C, and between voltage sensor and ligand binding by E.

In order for BK channels to play their role as dual sensors of Ca^{2+} and voltage in appropriate physiological ranges, it is essential that neither signal be so strongly coupled to gate opening that it negates the role of the other sensor. As a consequence, BK channels are well suited for teasing apart interactions among multiple allosterically coupled equilibria. Furthermore, once having such equilibria quantitatively defined, it allows for detailed analysis of the structural determinants that contribute to the energetics of the equilibria and their coupling, as has been done for the analysis of the role of various charged residues in the BK S1–S4 domain in voltage sensing (Ma et al., 2006). BK channels thus differ from channels specialized to be regulated by primarily a single physiological signal, whether voltage or ligand, where the coupling between sensor and gate opening is often so tight as to negate simple separation of distinct conformational equilibrium with the channel protein.

An important reason for having a well-defined allosteric gating mechanism describing BK channel behavior is that it provides an essential tool for understanding mechanistically how other kinds of regulatory elements may influence BK channel function. In addition to Ca^{2+} and voltage, the gating behavior of BK channels can be modulated by a variety of other factors, most notably accessory β subunits (Nimigean and Magleby, 1999; Xia et al., 1999; Cox and Aldrich, 2000; Orio et al., 2006; Wang et al., 2006) and cytosolic Mg^{2+} (Shi and Cui, 2001; Zhang et al., 2001), but also including pH (Avdonin et al., 2003), heme (Horrigan et al., 2005), phosphorylation (Tian et al., 2001), and other soluble messengers. The power of having a viable allosteric model for BK channels is that the underlying mechanisms by which each of these regulatory factors alters BK gating can be determined in an energetically meaningful fashion—is a modulator affecting only voltage sensor function, modifying the Ca^{2+} regulatory mechanism, directly acting to gate or close the channel, or acting in some general fashion that influences multiple aspects of gating? By evaluating regulatory effects in terms of the defined allosteric model, this provides the promise that the structural elements and energetic basis for such regulation can be determined. Although this is the potential power of such an approach, rarely has this promise been achieved. Now two recent papers, one from Frank Horrigan's lab in this issue of the journal (see Horrigan and Ma on p. 13) and the other jointly coauthored by the labs of Jianmin Cui and Horrigan (Yang et al., 2007), apply this approach with remarkable success to investigation of the mechanism by which cytosolic Mg^{2+} activates BK channels. Together these papers not only establish that the underlying physical basis for the Mg^{2+} action involves specific repulsive electrostatic interactions between Mg^{2+} and a key basic residue in the S4 voltage sensor (Yang et al., 2007) but define the energetic consequences of these interactions on the parameters describing the BK channel gating behavior (Horrigan and Ma, 2007). Knowing both the identity of the charged loci involved in the electrostatic effects, and how they impact on allosteric behavior, has allowed

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Horrigan and Ma (2007) to propose an intriguing and simple explanation of Mg^{2+} action based on the inverse relation between the distance separating a Mg^{2+} bound to the cytosolic domain from R213 in the S4 voltage sensor and the electrostatic force between them, which tend to stabilize both the voltage sensor and the cytosolic domain (see details below) in their activated conformations. Energetically, it appears that this explanation applies, irrespective of the specific conformational positions of voltage sensor and Mg^{2+} site. Somewhat ironically, our understanding of the physical details of how Mg^{2+} regulates BK channel function, in some aspects, has vaulted ahead of our understanding of the physical details of either the voltage regulation or Ca^{2+} regulation of BK channels.

Before highlighting the key findings from these new papers, some background regarding Mg^{2+} regulation of BK channels is warranted. The story begins ~20 years ago when two separate labs (Golowasch et al., 1986; Oberhauser et al., 1988) described the differential ability of divalent cations to influence activity of BK channels. In particular, millimolar concentrations of Mg^{2+} (and other divalent cations) were observed to enhance activation of BK channels by Ca^{2+}, while having little ability to directly activate BK channels. The basis for the Mg^{2+}-dependent effects on BK channels and its physiological significance then remained dormant for more than a decade, a period during which significant progress was made in the cloning of BK channel α subunits (Butler et al., 1993) and the first identification of potential high-affinity Ca^{2+} binding sites on the BK channel α subunits (Schreiber and Salkoff, 1997). Our mechanistic understanding of BK channel gating also advanced considerably during this time with the development of allosteric gating models based on the implicit symmetry of tetrameric channels containing four voltage sensors and four Ca^{2+} binding sites (Cui et al., 1997; Horrigan and Aldrich, 1999; Horrigan et al., 1999; Rothberg and Magleby, 1999). Such models have provided a meaningful framework for analysis of the mechanistic basis of BK channel regulation by ligands and voltage.

A key advance from these studies was the recognition that voltage and Ca^{2+} each regulate BK channel activation relatively independently, formalizing the idea that a voltage sensor equilibrium and Ca^{2+}-binding equilibrium each couple via separate pathways to the closed–open conformational equilibrium of the pore domain (Fig. 1 A). These ideas culminated in the notable paper by Horrigan and Aldrich that provided explicit definition of the key allosteric constants for a full model incorporating the idea that BK gating involves these three independent allosteric equilibria (Horrigan and Aldrich, 2002). Bolstered by these advances, two laboratories revisited the question of how Mg^{2+} might regulate BK channel gating and described the ability of a range of Mg^{2+} concentrations to regulate the activation of BK macroscopic conductance

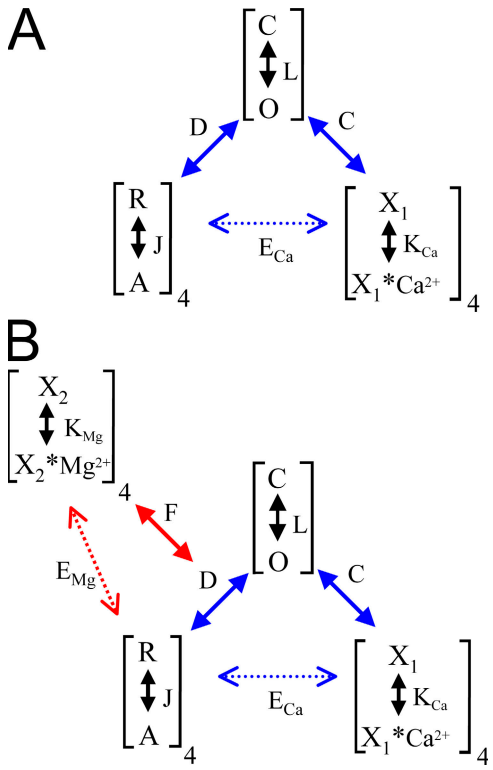

Figure 1. Diagram of basic allosteric schemes describing regulation of BK channels by voltage, Ca^{2+}, and Mg^{2+}. In A, the scheme tested by Horrigan and Aldrich to describe BK gating contains three coupled allosteric equilibria: L, the closed–open conformational equilibrium, J, the voltage sensor equilibrium, and K_{Ca}, the Ca^{2+} binding equilibrium (Horrigan and Aldrich, 2002). D describes coupling factor linking the voltage sensor equilibrium to gate opening, C, the coupling factor linking Ca^{2+} binding to channel opening, and E_{Ca}, the coupling factor linking Ca^{2+} binding to voltage sensor movement. Values for coupling factors are: D ~ 25; C ~ 8; and E_{Ca} ~ 2.4. Since D and C correspond to the contribution of a single sensor to gate opening, voltage sensor movement can shift the gate opening equilibrium up to D^4 (~400,000), while Ca^{2+} binding increase gate opening up to C^4 (~4,096). In B, the scheme developed by Ma and Horrigan to account for allosteric regulation by Mg^{2+} (Horrigan and Ma, 2007) is integrated with the scheme in A. The Mg^{2+} binding equilibrium is given by K_{Mg}, which coupled (coupling constant F) to an increase in the coupling strength between the voltage sensor equilibrium (J) and gating (L). Mg^{2+} binding also weakly influences the voltage sensor equilibrium, J, as defined by coupling constant E_{Mg}. F is determined to be ~2, which given the four Mg^{2+} binding sites and four voltage sensors, results in an overall effective coupling between J and L defined by F^4D^4 or $6.25*10^6$.

over a range of voltages and $[Ca^{2+}]$ (Shi and Cui, 2001; Zhang et al., 2001). These studies showed that Mg^{2+}-dependent effects involve a low-affinity, relatively nonselective divalent cation site that regulates BK gating independently from regulation mediated by the high-affinity sites involved in Ca^{2+}-dependent regulation. Furthermore, it was suggested that Mg^{2+} might directly regulate the channel closed–open equilibrium, perhaps in a fashion similar to regulation of the closed–open equilibrium by Ca^{2+}, although through a distinct binding site.

Around the same time, Rod MacKinnon's lab published the initial structure of a cytosolic domain involved in ligand-dependent regulation of the bacterial *Escherichia coli* 6TM K^+ channel (Jiang et al., 2001). Such so-called RCK domains (for regulator of conductance for K^+) are now recognized as a common feature of a large family of bacterial transporters and channels (Lingle, 2007). Remarkably, RCK domains share homology with a pair of similar regulatory domains on each BK channel α subunit (Jiang et al., 2001). Additional work on the RCK-containing, Ca^{2+}-regulated MthK bacterial K^+ channel by Youxing Jiang, both while in the MacKinnon laboratory (Jiang et al., 2002) and after establishing his own laboratory (Dong et al., 2005; Ye et al., 2006), showed that the MthK cytosolic domain arises from a set of four distinct homodimers assembled in an octameric gating ring. A Ca^{2+}-dependent conformational change in the gating ring structure is thought to provide the energy required for channel opening by tugging on the linkers connecting the gating ring to the pore domain inner helices (Jiang et al., 2002). This view of gating in the MthK channel now guides much of the thinking about the role of the cytosolic domain of BK channels, which is conjectured to involve a similar octamer of RCK-containing modules (see Lingle, 2007). Although the relevance of these structural ideas to BK channels still requires additional validation, the presence of putative RCK domains in BK channels immediately suggested to investigators of BK channels that such domains may contain sites involved in ligand-dependent regulation.

Following this lead, both the Cui and Lingle laboratories independently found that Mg^{2+}-dependent regulation of BK channels could be largely removed by mutation of particular residues in the putative first RCK domain (RCK1). In particular, two residues, E374 (Shi et al., 2002) and E399 (Xia et al., 2002), each independently removed essentially all regulation of BK gating by Mg^{2+} at concentrations of 10 mM and below. Furthermore, using homology modeling between the *E. coli* 6TM RCK domain and the BK channel sequence, the Cui group also generated a possible structure of a portion of the BK RCK1 domain that might coordinate Mg^{2+} binding (Shi et al., 2002). Although direct structural information demonstrating ligation of Mg^{2+} remains unavailable, this analysis suggested that E374, E399, and potentially Q397 might participate in coordination of Mg^{2+} binding. Subsequent work using a more thorough mutagenesis screen provided further support for the idea that both E374 and E399 contribute to Mg^{2+} binding, whereas the Q297 position may influence the ability of Mg^{2+} to bind at E374 and E399 (Yang et al., 2006).

Although these earlier studies establish the existence of a low-affinity Mg^{2+} regulatory site, the mechanistic basis for regulation by Mg^{2+} required further clarification. The first hint that the low-affinity Mg^{2+} site (also activated by mM Ca^{2+}) might act in a fashion distinct from the high-affinity Ca^{2+}-dependent site was the observation of a differential ability of the low-affinity Mg^{2+} sites and the high-affinity Ca^{2+} sites to increase channel open probability (Po) at negative voltages, where voltage sensors are in resting positions (Horrigan and Aldrich, 2002). The idea here is that the effects of ligands on Po at negative potentials allows specific determination of the ability of a ligand to directly modulate the C-O equilibrium (defined by the equilibrium constant L in Fig. 1 A). For BK channels, concentrations of Ca^{2+} that act at the high-affinity sites markedly increase Po at negative potentials (~4,000-fold), because the Ca^{2+} binding equilibrium directly couples to the C-O equilibrium. The inability of the low-affinity site to increase Po at negative potentials therefore suggests that the low-affinity site must be altering some other aspect of BK gating distinct from effects on L. A potential role for the involvement of the voltage sensor in Mg^{2+} effects was then given support in a subsequent paper from the Cui and Horrigan labs (Hu et al., 2003), which examined the ability of mutations in the BK S4 segment and S4–S5 linker to influence either gating regulation by either micromolar Ca^{2+} or millimolar Mg^{2+}. The results clearly demonstrated that mutations in the C-terminal half of S4 and in the S4–S5 linker had little or no effect on activation by micromolar Ca^{2+}. However, mutation of R213 completely abolished activation by mM Mg^{2+}, while other residues on the cytosolic side of position R213 had milder effects on activation by Mg^{2+}. Horrigan's lab then established that, among several basic residues in the S4 segment, R213 is the primary contributor to gating charge movements (Ma et al., 2006). These results clearly indicated that the voltage sensor is critical for Mg^{2+}-dependent activation, but left open two key questions: first, what is the mechanism by which Mg^{2+} binding might interact with the voltage-sensor and, second, how does Mg^{2+} binding lead to enhanced channel activation?

Now two recent papers (Horrigan and Ma, 2007; Yang et al., 2007), one of them in this issue, provide a major leap in our understanding. One paper shows that the energetic basis for the Mg^{2+}-dependent effects involves a specific electrostatic interaction between bound Mg^{2+} and the R213 residue in the S4 voltage sensor (Yang et al., 2007). The second paper (Horrigan and Ma, 2007) provides an insightful evaluation of the consequences of this electrostatic interaction and reveals that Mg^{2+} primarily increases the strength of coupling between voltage sensors and channel activation, an effect that arises from the ability of Mg^{2+} to favor the stabilization of channels in states with both activated voltage sensors and open gates. In both papers, a rich variety of experimental tests are employed to support the final conclusions.

What are the results supporting the idea that Mg^{2+} binding facilitates BK activation by a simple electrostatic interaction between Mg^{2+} and the voltage-sensing residue, R213 (Yang et al., 2007)? First, Mg^{2+}-dependent

activation is sensitive to ionic strength in a fashion consistent with the effect arising from an electrostatic interaction. Importantly, Ca^{2+}-dependent activation exhibits little dependence on ionic strength. Second, charge manipulations at position Q397, which has been shown to be positioned close to the site of Mg^{2+} binding (Shi et al., 2002; Yang et al., 2006), influence gating in a fashion consistent with a simple electrostatic interaction. Specifically, the Q397K and Q397R mutations mimic the ability of Mg^{2+} to shift activation to more negative potentials, whereas Q397E and Q397D shift activation to more positive potentials. Third, introduction of net positive charge on the Q397C residue by reaction with MTSET resulted in an MTSET-dependent shift in gating similar in magnitude to that produced by Mg^{2+}, and the shift in activation produced by the Q397K mutation is sensitive to ionic strength in a fashion similar to the Mg^{2+} effect. Finally, the effects of net positive charge at the Q397 position was shown to produce functional effects on gating current and channel Po similar to those produced by Mg^{2+}.

To test whether these effects involve a specific interaction of Mg^{2+} with R213, the ability of 10 mM Mg^{2+} to shift gating was examined in a series of constructs in which all the potentially cytosolically exposed charged residues in the voltage sensor domain (S1–S4 and S4–S5 linker) were mutated. Only R213 abolished the ability of Mg^{2+} to shift gating; furthermore, the R213Q mutation also abolished the ability of Q397R to shift activation. Reintroduction of positive charge by MTSEA in R213C restored the ability of 10 mM Mg^{2+} to shift activation.

Together these results compellingly argue that the energetic basis for the ability of Mg^{2+} to shift activation arises from a simple electrostatic repulsion between bound Mg^{2+} and R213 in the S4 voltage sensor. An implication of the results is that the voltage sensor and the Mg^{2+}-binding site must be sufficiently close to allow such interactions. Based on consideration of the known structures of the cytosolic domain of the MthK K^+ channel (Jiang et al., 2002) and the position of voltage sensors in the Kv1.2 channel structure (Long et al., 2005a,b), the authors point out that both the Mg^{2+} binding site and the R213 residue in BK channels may be positioned at comparable lateral distances from the axis of the permeation pathway (Yang et al., 2007).

At first glance, it might seem natural to assume that the repulsive influence from Mg^{2+} simply "pushes" on R213, thereby favoring the tendency of voltage sensors to move to an activated position. Now, as reported in this issue, Horrigan and Ma address this question in detail, by defining the specific effects of Mg^{2+} on the various allosteric constants defining activation of BK channels (Horrigan and Ma, 2007). The analysis leads to a somewhat surprising conclusion. Namely, the primary effect of Mg^{2+} is to increase the strength of coupling (allosteric constant: D) between voltage sensor movement and channel activation, whereas Mg^{2+} has only minor direct effects on the voltage sensor equilibrium (J). (The overall scheme that integrates the allosteric effects of Mg^{2+} with those of voltage- and Ca^{2+}-dependent regulation of the closed–open equilibrium is given in Fig. 1 B.) As described in more detail below, an analysis of the kinetic and equilibrium effects of Mg^{2+} leads to the conclusion that the effects of Mg^{2+} are markedly state dependent. The ability of Mg^{2+} to increase activation therefore arises from a relative stabilization of channels in which both voltage sensors and cytosolic gating ring are in activated positions. Since the electrostatic interactions between R213 and Mg^{2+} are apparently larger when voltage sensors are in resting positions or when the cytosolic domain is in a closed conformation, the effect of Mg^{2+} can be thought of as the consequence of the relative minimization of the electrostatic energy between R213 and Mg^{2+} when both voltage sensors and gating ring are in activated conformations. This general picture of Mg^{2+} action therefore corresponds to a rather novel mechanism in which electrostatic repulsion acts to modulate the effective coupling between two other coupled allosteric equilibria, in this case, voltage sensor movement and channel activation.

So what are the observations that lead to these conclusions? Within the framework of the Horrigan-Aldrich model (Fig. 1 A), the ability of Mg^{2+} to activate gating might arise in any of several mechanistically distinct ways: (a) Mg^{2+} might alter the closed–open equilibrium (L), (b) Mg^{2+} might shift the voltage sensor equilibrium (J), favoring voltage sensor activation at a given voltage, or (c) Mg^{2+} might alter the coupling (D) between voltage sensor movement (J) and the closed–open (C-O) equilibrium (L). Horrigan and Ma systemically address these possibilities to reach the conclusion that effects on D account for most of the actions of Mg^{2+}.

Possible effects of Mg^{2+} on the C-O equilibrium were considered first. Examination of the ability of Mg^{2+} to increase channel open probability at negative potentials, where voltage sensors are inactive, showed that, whereas 2 μM Ca^{2+} markedly increases Po at negative potentials, Mg^{2+} was without effect. This was consistent with the earlier results showing that the effective Po at negative potentials was not increased by Ca^{2+} concentrations acting on the low-affinity site (Horrigan and Aldrich, 2002). Furthermore, in constructs with mutations that shift the voltage sensor equilibrium either to more negative (R207Q) or more positive (R167E) voltages, the effects of Mg^{2+} are not intrinsically voltage dependent, but are coupled to the activation range of the voltage sensors. This also argues that Mg^{2+} binding is not directly coupled to regulation of the C-O equilibrium (L). Then, to test for possible effects on J, Horrigan and Ma determined the effects of Mg^{2+} on the voltage dependence of gating current movements along with determinations of the fast time constant of gating current decay (τ_{gFast}). Their results showed that the voltage

sensor equilibrium is shifted about −17 mV at 10 mM Mg^{2+}, with a small slowing of τ_{gFast} at more negative potentials and a faster τ_{gFast} at more positive potentials. Importantly, the ability of 10 mM Mg^{2+} to shift the voltage sensor equilibrium is reduced by ∼50% by mutation of E374 and E399, supporting the idea that the E374/E399 Mg^{2+} binding site accounts for the effects of Mg^{2+} on J. However, despite these effects on J, the authors calculate that the observed −17-mV shift in the gating current equilibrium would only account for a shift in activation Po of similar magnitude. This contrasts markedly with the approximately −67-mV shift in GV curves produced by 10 mM Mg^{2+} and shows that the effects of Mg^{2+} on J alone are insufficient to account for the activation by Mg^{2+}.

To examine the possible effects of Mg^{2+} on the coupling, D, between voltage sensors and channel activation, Horrigan and Ma then use a particularly clever and insightful set of experiments. In previous work, these investigators found that mutation of the specific residues, R207Q and R210C, in the S4 voltage sensor resulted in channels in which voltage sensor activation was shifted to more negative potentials (Ma et al., 2006). For such constructs, a range of voltages could be identified over which voltage sensors are essentially locked in activated positions, but channel Po is still less than maximal. Taking advantage of this opportunity to examine effects of Mg^{2+} on Po under conditions in which the voltage sensors are activated, Horrigan and Ma show that Mg^{2+} still facilitates opening, both in R207Q and R210C, when voltage sensors are constitutively activated. Since it was already established that Mg^{2+} is not directly influencing the C-O equilibrium, this argues that that the major effect of Mg^{2+} is to increase D, the effectiveness of coupling between voltage sensor movement and channel opening. An important implication of these results is that the ability of Mg^{2+} to increase Po of R210C channels clearly means that, irrespective of what the mechanism may be, the effects of Mg^{2+} must be mediated by something distinct from alteration of the voltage sensor equilibrium itself.

The idea that Mg^{2+} enhances voltage sensor/gate coupling is given further support by an examination of the voltage dependence of Po in wild-type channels, both in the absence and presence of Mg^{2+}. The key task here was to determine the relative shift in half activation of voltage sensors either for closed (V_{hc}) or open (V_{ho}) channels. Although direct measurements of the gating charge equilibrium for open channels are difficult, in previous work Horrigan has established procedures for an approximation of the open channel gating current equilibrium (Ma et al., 2006; Horrigan and Ma, 2007). Using this approach, the present work shows that 10 mM Mg^{2+} results in an approximately −50-mV additional shift in the open channel voltage sensor half-activation potential compared with the shift observed for closed channels. Whereas the small effect (−17 mV) of Mg^{2+} on the closed channel voltage sensor equilibrium reflects the small effect of Mg^{2+} on J, the larger shift observed for the shift of the open channel voltage sensor equilibrium directly reflects an effect of Mg^{2+} on D, the strength of coupling between J and L. Thus, analysis of effects of Mg^{2+} on wild-type gating confirms the results obtained with channels with constitutively activated voltage sensors. Satisfyingly, these effects of Mg^{2+} on D are abolished with mutation of E374 and E399, confirming that it is the binding site for Mg^{2+} on RCK1 that is critical for these effects.

To provide additional mechanistic insight into how the interactions between Mg^{2+} and R213 alter BK gating, Horrigan and Ma also examine effects of Mg^{2+} on kinetic properties of BK channels at negative potentials. It has been previously shown that the effects of Mg^{2+} on BK channel activation are associated with a pronounced slowing of BK channel tail currents at negative potentials, whereas activation rates at positive potentials are largely unaltered (Zhang et al., 2001; Zeng et al., 2005). Such effects might seem at odds with the result described above, that Mg^{2+} has no significant effect on BK channel Po when voltage sensors are in resting states. To address this conundrum, single channel open and closed time durations were examined in patches expressing the R167E construct, so that channels could be studied at modest voltages while voltage sensors are still in resting states. As expected, 10 mM Mg^{2+} did not change Po. However, 10 mM Mg^{2+} increased the durations of both open times and closed times, thereby accounting for the observation that Po at negative potentials is unchanged by 10 mM while deactivation rates are still slowed.

On balance, then, the results reveal that the effects of Mg^{2+} on BK activation arise largely from an increased strength of coupling (D) between the voltage sensor and gate, with a smaller contribution from direct effects on J. In addition, Mg^{2+} exerts small effects on rates of voltage sensor movement (τ_{gFAST}), while also affecting the rates of transition between open and closed states at voltages where voltage sensors are inactive. Is there a unified physical model that might account for all this distinct observations, all of which are mediated by Mg^{2+} acting at the E374/E399 site on the RCK1 domain? The explanation developed by Horrigan and Ma and presented in Fig. 8 B of their paper is based on two key ideas: first, that both voltage sensors and the cytosolic gating ring undergo conformational changes during gating and, second, that the effects of Mg^{2+} seem to involve a preferential stabilization of channels in which both voltage sensors and gating ring is activated. The basis for the dual movements of both voltage sensors and the Mg^{2+} site reasonably arises from our understanding of voltage sensor movements in KvAP and KV1.2 and gating ring expansion in the MthK channel. In the presence of Mg^{2+}, channels can exist in four possible

conformations: one with inactive voltage sensors and inactive gating ring, one with active voltage sensors and active gating ring, and two with one sensor active and the other sensor inactive. The key idea encapsulated in the Horrigan and Ma model (their Fig. 8 B) is that, as a consequence of state-dependent changes in the relative positions of Mg^{2+} and R213, the repulsive electrostatic energies are minimized when both voltage sensors and the gating ring are in activated state positions. In all other states a closer positioning between R213 and Mg^{2+} results in increasing electrostatic interactions that tend to destabilize these states. Horrigan and Ma are justifiably cautious regarding their model, particularly given the absence of specific structural information regarding the relative positioning of the Mg^{2+} binding site and the voltage sensor in different states. However, the model provides a valuable framework for thinking about how state-dependent changes in the strength of a "simple" electrostatic interaction may result in differential stabilization of particular states relative to others. A particularly important point concerning the Horrigan and Ma model is the following. Irrespective of the specific conformational changes that one might propose to account for the kinetic and Po effects, the effect of Mg^{2+} results from a state-dependent minimization of repulsive electrostatic energies between Mg^{2+} and R213, leading to stabilization of activated voltage sensors and activated gating ring.

Beyond the elucidation of the fundamental mechanism by which BK channels can be regulated by Mg^{2+}, this pair of papers establish the important general point that voltage-dependent channels can be modulated by factors that directly influence the voltage-sensing part of the channel protein. One could imagine that in other channels there may be different strategies that also regulate the coupling of voltage sensor status and channel activation in a similar fashion, whether via auxiliary subunits that might place charges in positions that influence voltage sensors or via enzymatic modifications that alter the local charge in the vicinity of voltage sensors. How local charge might influence the voltage sensor equilibrium or coupling to channel activation would depend on the specific positioning of charges relative to the positions of the voltage sensors in resting and activated states. Though such details may vary in different cases, an important contribution of these papers from the Cui and Horrigan labs is that they demonstrate the physical plausibility of a new type of mechanism by which activity of voltage-gated channels can be regulated.

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