

Return of the Electric Binding Site

Brad S. Rothberg

Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140

The BK-type Ca^{2+} -activated K^+ channel has been the subject of increasingly detailed mechanistic study since the first recordings of this channel were obtained more than 25 years ago (Pallotta et al., 1981; Latorre et al., 1982; Magleby, 2003; and references therein). But if you thought that our understanding of the gating of the BK channel and its allosteric regulation by Ca^{2+} and transmembrane voltage had reached its summit, then once again you would be wrong. In a remarkable display of patch-clamping and kinetic analysis appearing in this issue, the bear is poked once again, with this iteration of analysis constrained by high-resolution Ca^{2+} dose-response curves, containing data at 22 (!) different $[\text{Ca}^{2+}]$. Here, Sweet and Cox (see p. 491) analyze the two high-affinity Ca^{2+} binding sites of the BK channel using mutations to selectively disable each site, and obtain definitive results on how the sites behave in isolation and how they might interact with one another in the intact BK channel. A surprising finding is that binding to one of the two binding sites is modulated by transmembrane voltage. This intriguing twist opens possibilities for identifying a structural basis for interactions between the voltage sensor and one of the principal Ca^{2+} activation sites of the channel.

Mysteries of Ca^{2+} Activation

Despite the functional simplicity of the BK channel, which is activated principally (for the purpose of this Commentary) by cytoplasmic Ca^{2+} and depolarization, and our high level of understanding of the mechanisms underlying its activation, there are still many intricacies of this channel that are not well understood. And these details are not trivial; because BK channels serve as cytoplasmic Ca^{2+} detectors that can rapidly respond to and modulate transmembrane voltage, they are critical in controlling action potential firing in some neurons, and they are also important in the feedback loop controlling smooth muscle contractility (Brayden and Nelson, 1992; Brenner et al., 2000, 2005). So the mechanism by which BK channels sense and respond to cytoplasmic $[\text{Ca}^{2+}]$ is of interest.

Although many of the side chains that are functionally important in Ca^{2+} sensing have been revealed by mutagenesis combined with careful electrophysiological measurement (Bao et al., 2002; Xia et al., 2002; Zeng

et al., 2005), we must acknowledge that we do not yet know which residues coordinate Ca^{2+} in the BK channels, nor do we know the structure of the proposed binding sites. Because of the rapid dissociation rates and potentially complex interactions among the binding sites and other functional domains of the channel, it is extremely difficult to extract useful kinetic information on Ca^{2+} binding to the channel from a conventional ligand binding assay; yet, information on dissociation constants would be useful in gaining further knowledge on the binding mechanism and, consequently, the gating mechanism.

Dissecting the Dissociation Constants

In this issue, Sweet and Cox (2008) set out to extract estimates of dissociation constants for the two postulated high-affinity Ca^{2+} binding sites, using patch-clamp electrophysiology combined with mutations aimed at disabling the sites. Importantly, they focused specifically on determining the K_d s in the absence of complicating factors such as voltage-sensor movement, which can also modulate gating. To do this, they recorded channel activity at negative voltages (-80 mV) where, over the range of $[\text{Ca}^{2+}]$ used in their experiments, voltage-sensor movement should contribute minimally to channel activation. This is similar to the approach used by Horrigan and Aldrich (2002), but here it is combined with the molecular blunting of the low-affinity binding site by the E399N mutation (called “ ΔE ”), along with similar blunting of high-affinity sites by mutations in the RCK1 segment of the cytoplasmic domain, D367A (called “ ΔR ”), and in the “calcium bowl,” D897N/D898N/D899N/D900N/D901N (called “ $\Delta B_{(D5N5)}$ ”).

By recording open probabilities from BK channels (with the low-affinity site disabled) in the near absence of voltage-sensor activation, it is possible to effectively isolate the allosteric action of Ca^{2+} on BK channel gating, simplifying analysis of the effect of Ca^{2+} on gating to a model that can be described by four parameters describing the two high-affinity binding sites: K_{C1} and K_{C2} , and K_{O1} and K_{O2} (the dissociation constants for each binding site in the closed and open channel, respectively). Because these two high-affinity binding sites might have

Correspondence to Brad S. Rothberg: rothberg@temple.edu

had K_{ds} that were similar to one another in the closed or open states, it was critical to constrain the estimates of these parameters by obtaining data at many different $[Ca^{2+}]$'s. The dose-response curve (Fig. 3 in Sweet and Cox, 2008) suggests that at -80 mV, one of the sites has a higher apparent affinity than the other. With both high-affinity sites present, the estimated K_c and K_o for one site is 3.7 and 0.7 μM , and for the other site is 51 and 21 μM . Despite the uncertainty in the values of these fitted parameters, the information gained by this effort is clear; the dose-response curve is too shallow to be described by either a single high-affinity site or two sites with the same K_{ds} .

The next important step was to estimate the K_{ds} for each high-affinity site in isolation. After disabling the high-affinity RCK1 site, it was possible to estimate the K_c and K_o for the Ca^{2+} bowl site, which was 3.1 and 0.9 μM , in remarkable agreement with the estimate for one of the sites determined from channels with both high-affinity sites present. Disabling the Ca^{2+} bowl site with the $\Delta B_{(D2N2)}$ mutation yielded K_c and K_o estimates for the RCK1 site of 27 and 5.6 μM . Cox's group (Bao et al., 2004) had previously discovered that most of the effect of Ca^{2+} bowl could be eliminated by the mutations D898A/D900A (called " $\Delta B_{(D2N2)}$ "), and using this mutation to disable the Ca^{2+} bowl yielded similar K_c and K_o estimates of 23 and 4.9 μM . Thus, by these measures, the Ca^{2+} bowl site does have a higher apparent affinity than the RCK1 site when voltage sensors are deactivated. The authors then go on to determine whether the parameters for two sites measured in isolation could combine additively to yield the effect observed experimentally when both sites are functional; they do not, consistent with the idea that the two sites may negatively influence one another, either through effects on binding or gating.

A Test of the Gating Model, and the Return of the Electric Binding Site

Although voltage and Ca^{2+} are thought to act largely independent of one another in BK channel activation, it was known from previous work that description of BK gating over a very wide range of V_m and Ca^{2+} is improved by assuming some interaction between V_m and Ca^{2+} (Horrigan and Aldrich, 2002). But previous tests of the Horrigan-Aldrich model have mainly assumed only four Ca^{2+} binding sites per channel. Could additional Ca^{2+} binding sites, with strongly constrained K_c and K_o estimates, account for the apparent interactions? To test this, Sweet and Cox (2008) predict a series of G-V relations obtained at different Ca^{2+} , in the context of the Horrigan-Aldrich model, and found that the parameter to describe interaction between Ca^{2+} activation and voltage-sensor activation (the allosteric coupling factor, " E ") was still necessary. To further explore the mechanistic basis for this interaction, the authors again performed experiments on the binding site mutants. But this time,

instead of holding the voltage at -80 mV, where voltage sensors are at rest, they held the voltage for these new measurements at 0 mV, where they estimate that $\sim 35\%$ of the voltage sensors are activated.

For the Ca^{2+} bowl site, the data obtained at -80 and 0 mV superimpose, consistent with voltage having essentially no effect on Ca^{2+} activation through this site. On the other hand, voltage had a substantial effect on Ca^{2+} activation through the RCK1 site; in the $\Delta B_{(D2N2)}$ mutant, K_c and K_o decreased from 23 and 4.9 μM at -80 mV to 16 and 2.1 μM at 0 mV. These changes in affinity yield an increase in the coupling between Ca^{2+} binding and opening by a factor of 1.8 . Thus, at 0 mV, it seems that the coupling between Ca^{2+} binding to the RCK1 site and opening becomes stronger than the coupling between Ca^{2+} binding to the Ca^{2+} bowl site and opening. This interaction between voltage and apparent Ca^{2+} affinity could have implications for BK channel function in excitable cells; near the resting potential, Ca^{2+} activation of the channel is governed more strongly by the Ca^{2+} bowl site, whereas near the peak of an action potential, the RCK1 site may become a more important determinant of BK channel activity.

The On-again, Off-again Relationship between Voltage and Ca^{2+}

Of course, voltage and Ca^{2+} have met at the BK channel before. It was among the early studies on BK channel gating that Moczydowski and Latorre (1983) observed, in single-channel bilayer recordings, that the time constants of openings over a wide range of voltages (from -60 to $+50$ mV) increased with $[Ca^{2+}]$, and the time constants of closing over this range of voltages decreased with $[Ca^{2+}]$. Based on this and the observation that the P_o versus $[Ca^{2+}]$ relation could be described with a Hill coefficient of ~ 2 , they developed an economic model that described BK gating over a range of voltage and $[Ca^{2+}]$ by assuming a minimum of two Ca^{2+} binding steps, with these steps being voltage dependent (other equally detailed analyses of the time did not incorporate the effects of voltage; Magleby and Pallotta, 1983b, 1983a). So how might one physically explain a Ca^{2+} binding step that is voltage dependent? Moczydowski and Latorre (1983) offered three hypotheses: (1) that the Ca^{2+} binding sites lie within the electric field, possibly near the mouth of the channel; (2) that the sites lie within an "activation cleft," which might exist between a cytoplasmic domain of the channel and the cytoplasmic face of the lipid bilayer, and that membrane surface charges might participate in open state stabilization; or (3) that the binding rates themselves are voltage independent, but that they are coupled to a dipole that causes the affinity of the binding site to change with voltage.

Now it's important to note that this model was developed intentionally to be parsimonious, and a less economic model with voltage dependence separate from

the Ca^{2+} binding steps might also have sufficed. Years later, the amino acid sequence of the BK channel was revealed to be similar to that of Kv channels, with a charged S4 segment (Butler et al., 1993; Adelman et al., 1992; Atkinson et al., 1991). Subsequently, it was shown that BK channels can be activated by strong depolarization in the nominal absence of Ca^{2+} , and later, through systematic mutagenesis, it was found that the BK channel's sensitivity to cytoplasmic Ca^{2+} could be eliminated while its voltage-dependent activation remained intact (Cui et al., 1997; Bao et al., 2002; Xia et al., 2002). Based on these and other experimental results, it seemed clear that models in which all of voltage dependence of opening lies within the Ca^{2+} binding steps would not suffice, and that the majority of voltage-dependent gating likely arose from activation of the channel's intrinsic voltage sensor (Horrigan et al., 1999; Rothberg and Magleby, 2000; Horrigan and Aldrich, 2002).

With the work of Sweet and Cox (2008), however, Ca^{2+} once again meets voltage. So what might be the physical basis for voltage-dependent modulation of apparent affinities at the RCK1 site? The authors suggest that because the RCK1 domain lies near the membrane and possibly near the BK channel's voltage sensor, when the voltage sensor is activated, it may alter the structure of the RCK1 site to increase its affinity for Ca^{2+} , an idea similar to one of those presented by Moczydlowski and Latorre (1983). The idea of electrostatic interaction between the voltage sensor and RCK1 domain already has experimental support (Yang et al., 2007). Perhaps it will be important to explore further, and discover the full extent of interactions between these domains, in terms of both function and structure.

REFERENCES

Adelman, J.P., K.Z. Shen, M.P. Kavanaugh, R.A. Warren, Y.N. Wu, A. Lagrutta, C.T. Bond, and R.A. North. 1992. Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron*. 9:209–216.

Atkinson, N.S., G.A. Robertson, and B. Ganetzky. 1991. A component of calcium-activated potassium channels encoded by the *Drosophila slo* locus. *Science*. 253:551–555.

Bao, L., A.M. Rapin, E.C. Holmstrand, and D.H. Cox. 2002. Elimination of the BK(Ca) channel's high-affinity Ca^{2+} sensitivity. *J. Gen. Physiol.* 120:173–189.

Bao, L., C. Kaldany, E.C. Holmstrand, and D.H. Cox. 2004. Mapping the BKCa channel's "Ca $^{2+}$ bowl": side-chains essential for Ca $^{2+}$ sensing. *J. Gen. Physiol.* 123:475–489.

Brayden, J.E., and M.T. Nelson. 1992. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science*. 256:532–535.

Brenner, R., G.J. Perez, A.D. Bonev, D.M. Eckman, J.C. Kosek, S.W. Wiler, A.J. Patterson, M.T. Nelson, and R.W. Aldrich. 2000. Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. *Nature*. 407:870–876.

Brenner, R., Q.H. Chen, A. Vilaythong, G.M. Toney, J.L. Noebels, and R.W. Aldrich. 2005. BK channel beta4 subunit reduces dentate gyrus excitability and protects against temporal lobe seizures. *Nat. Neurosci.* 8:1752–1759.

Butler, A., S. Tsunoda, D.P. McCobb, A. Wei, and L. Salkoff. 1993. mSlo, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science*. 261:221–224.

Cui, J., D.H. Cox, and R.W. Aldrich. 1997. Intrinsic voltage dependence and Ca^{2+} regulation of mslo large conductance Ca-activated K+ channels. *J. Gen. Physiol.* 109:647–673.

Horrigan, F.T., and R.W. Aldrich. 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, F.T., J. Cui, and R.W. Aldrich. 1999. Allosteric voltage gating of potassium channels I. Mslo ionic currents in the absence of Ca^{2+} . *J. Gen. Physiol.* 114:277–304.

Latorre, R., C. Vergara, and C. Hidalgo. 1982. Reconstitution in planar lipid bilayers of a Ca^{2+} -dependent K+ channel from transverse tubule membranes isolated from rabbit skeletal muscle. *Proc. Natl. Acad. Sci. USA*. 79:805–809.

Magleby, K.L. 2003. Gating mechanism of BK (Slo1) channels: so near, yet so far. *J. Gen. Physiol.* 121:81–96.

Magleby, K.L., and B.S. Pallotta. 1983a. Burst kinetics of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol.* 344:605–623.

Magleby, K.L., and B.S. Pallotta. 1983b. Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. *J. Physiol.* 344:585–604.

Moczydlowski, E., and R. Latorre. 1983. Gating kinetics of Ca^{2+} -activated K+ channels from rat muscle incorporated into planar lipid bilayers: evidence for two voltage-dependent Ca^{2+} binding reactions. *J. Gen. Physiol.* 82:511–542.

Pallotta, B.S., K.L. Magleby, and J.N. Barrett. 1981. Single channel recordings of Ca^{2+} -activated K+ currents in rat muscle cell culture. *Nature*. 293:471–474.

Rothberg, B.S., and K.L. Magleby. 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.

Sweet, T.B., and D.H. Cox. 2008. Measurements of the BK_{Ca} channel's high-affinity Ca^{2+} binding constants: effects of membrane voltage. *J. Gen. Physiol.* 132:491–505.

Xia, X.M., X. Zeng, and C.J. Lingle. 2002. Multiple regulatory sites in large-conductance calcium-activated potassium channels. *Nature*. 418:880–884.

Yang, H., L. Hu, J. Shi, K. Delaloye, F.T. Horrigan, and J. Cui. 2007. Mg $^{2+}$ mediates interaction between the voltage sensor and cytosolic domain to activate BK channels. *Proc. Natl. Acad. Sci. USA*. 104:18270–18275.

Zeng, X.H., X.M. Xia, and C.J. Lingle. 2005. Divalent cation sensitivity of BK channel activation supports the existence of three distinct binding sites. *J. Gen. Physiol.* 125:273–286.