Commentary

The BK_{Ca} Channel's Ca^{2+}-binding Sites, Multiple Sites, Multiple Ions

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In this issue, Zeng et al. report results from a series of elegant experiments that, among other contributions, provide new evidence in support of the hypothesis that the BK_{Ca} channel contains two types of high-affinity Ca^{2+}-binding sites. Those that work with this channel will quickly recognize the significance of these data. To the less well initiated, however, it might seem surprising that the number of Ca^{2+}-binding sites the BK_{Ca} channel contains has yet to be firmly established. It has been thirteen years since the BK_{Ca} channel's pore-forming α subunit was cloned (Atkinson et al., 1991), and its Ca^{2+}-sensing mechanism has been studied for over twenty. The truth is, however, that although a good deal of evidence has been produced that supports the notion that the BK_{Ca} channel has two types of high-affinity Ca^{2+}-binding sites (and at least one type of low-affinity site), it is this study from the Lingle group that puts the final buttress firmly in place under the two-site theory. Before discussing the present contribution, however, some background is in order.

Although well known for its Ca^{2+}-sensing abilities, the BK_{Ca} channel is at its core a voltage-gated K^+ channel, complete with an amphipathic S4 helix and a K^+ channel pore sequence (Atkinson et al., 1991). Different from other K^+ channels, however, the BK_{Ca} channel has a very large single-channel conductance. It has an extra transmembrane domain that places its NH_2 terminus in the extracellular space (Meera et al., 1997). It has its own brand of β subunits that dramatically influence gating (Knaus et al., 1994; McManus et al., 1995; Wallner et al., 1999; Brenner et al., 2000; Zeng et al., 2001), and it has evolved a very large (800 amino acid) COOH-terminal cytoplasmic domain, presumably for the purpose of Ca^{2+} sensing. I say presumably, however, because this COOH-terminal domain contains no established Ca^{2+} binding motifs. Where are the Ca^{2+} sensors?

The first successful assault on this question came from the Salkoff laboratory, who showed, with domain-swapping experiments across species, that the Ca^{2+}-sensing properties of the BK_{Ca} channel segregate with the channel's cytoplasmic domain, not its integral membrane domain (Wei et al., 1994), and that there is an acidic region in this cytoplasmic domain where mutations alter Ca^{2+} sensing (Schreiber and Salkoff, 1997). They called this 28–amino acid region the "Ca^{2+} bowl" and proposed that it forms a Ca^{2+}-binding site. Surprisingly, however, mutations in the Ca^{2+} bowl do not eliminate Ca^{2+} sensing. They only reduce the ability of Ca^{2+} to shift the channel's conductance–voltage curve by at most half (Bao et al., 2002). Where does the remaining Ca^{2+} sensitivity come from?

There seemed to be two possibilities. One was that the Ca^{2+} bowl mutations were only partially disabling a Ca^{2+}-binding site, such that the remaining Ca^{2+} sensitivity was still coming from the Ca^{2+} bowl. The other was that there is a second Ca^{2+}-binding site, unrelated to the Ca^{2+} bowl, that provides the remaining Ca^{2+} sensitivity. In support of the second site hypothesis, Schreiber and Salkoff (1997) offered the following observation. Ca^{2+} bowl mutations alter Ca^{2+} sensing, but they do not alter the ability of Cd^{2+} to activate the BK_{Ca} channel. Thus, they proposed that Cd^{2+} was selectively binding to the second site.

Further support for the two site hypothesis came more recently from the Cox and Lingle groups. Both showed that although Ca^{2+} bowl mutations do not eliminate Ca^{2+} sensing on their own, they severely limit (Bao et al., 2002) or eliminate (Xia et al., 2002) Ca^{2+} sensing up to 100 μM Ca^{2+}, when combined with either of two mutations far upstream of the Ca^{2+} bowl, M513I or D362A/D367A. Moreover, since the effects of M513I and D362A/D367A combined additively with those at the Ca^{2+} bowl, it was concluded that M513I and D362A/D367A very likely knock out a second Ca^{2+}-binding site (Bao et al., 2002; Xia et al., 2002).

Of course this conclusion is not iron clad, as it could just happen that mutations at D362/D367 or M513 and those in the Ca^{2+} bowl disrupt a single binding site in a roughly additive manner, and in truth there is only one type of high-affinity Ca^{2+}-binding site. This seemed a particularly important possibility, given that neither D362/D367 nor M513 have neighboring sequences that would suggest a Ca^{2+}-coordinating region. Thus, in the absence of a crystal structure or real binding data, whether the BK_{Ca} channel contains one or two

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types of high-affinity Ca^{2+}-binding sites seemed like a difficult issue to definitively resolve.

Zeng et al. (2005), however, have cleverly done so here by returning to the observation of Schreiber and Salkoff (1997): the second site, if it exists, should bind Cd^{2+}, while that associated with the Ca^{2+} bowl should not. Therefore, under the two site hypothesis, the mutation D362A/D367A would be expected to eliminate the ability of Cd^{2+} to activate the BK_{Ca} channel, while the Ca^{2+} bowl mutation, D5N5, should have no effect. Remarkably, these predictions were exactly born out in Zeng et al.'s data. Thus D362A/D367A and D5N5 must be acting on different binding sites, and it seems inescapable that the BK_{Ca} channel has two types of high-affinity Ca^{2+}-binding sites, each with a different selectivity for divalent cations, and each disabled by site-specific mutations.

It is remarkable that a binding site can select strictly for Ca^{2+} over Cd^{2+}. Both ions carry the same charge and their ionic radii differ by only 0.02 Å. The basis of this selectivity must, therefore, arise from the occupancies of these ion's electron orbitals. In fact, as Zeng et al. point out, the coordination chemistry of Cd^{2+} is such that it prefers soft ligands such as sulfur, and to a lesser extent the imidazole nitrogen, while Ca^{2+} prefers hard ligands such as oxygen (Ho, 1975). Thus, in searching for Ca^{2+}-coordinating residues at the second site, it may be fruitful to consider methionine, cysteine, and histidine residues as potential ligand donors. Indeed, although we previously considered it unlikely that M513 coordinates Ca^{2+} at the second site (Bao et al., 2002), this conclusion may need to be reexamined.

In addition to examining Ca^{2+} and Cd^{2+}, Zeng et al., in their typical thorough fashion, also examined the activation of the BK_{Ca} channel and its mutants by Mn^{2+} Co^{2+}, Ni^{2+}, Mg^{2+}, and Sr^{2+}, and in so doing they came to another important conclusion. The smaller of these ions, Mn^{2+}, Co^{2+}, Mg^{2+}, and Ni^{2+}, activate the BK_{Ca} channel by binding to a third low-affinity Ca^{2+}-binding site, one that had been identified previously as the site of Mg^{2+} action (Shi and Cui, 2001; Zhang et al., 2001; Shi et al., 2002; Xia et al., 2002). This site can be specifically eliminated by a mutation at E399 (Shi et al., 2002; Xia et al., 2002). Thus, the observation that Oberhauser et al. (1988) made fifteen years ago that Ca^{2+}, Cd^{2+}, Sr^{2+}, Mn^{2+}, and Co^{2+} can activate the BK_{Ca} channel and in that order of efficacy, we can now understand was due not to the selectivity of a single binding site, but rather to a combination of three binding sites, each with a distinct selectivity. In addition, because even a combination of mutations at all three sites cannot eliminate all of the activating effects of Ni^{2+}, Mg^{2+}, Co^{2+}, and very high concentrations of Ca^{2+}, Zeng et al. suggest that there is yet another low-affinity Ca^{2+}-binding site waiting to be structurally identified.

As if this were not enough, Zeng et al. also contribute another fascinating observation. It has been known for many years that BK_{Ca} channel kinetics are Ca^{2+} dependent. Raising Ca^{2+} speeds activation in response to voltage steps and slows deactivation (DiChiara and Reinhart, 1995; Cui et al., 1997). One might reasonably ask how these kinetic changes come about. A great deal of evidence indicates that the kinetics of BK_{Ca} channel opening and closing are governed by a single concerted conformational change whose forward and reverse rates are each modified allosterically by Ca^{2+} binding (Cox et al., 1997; Rothberg and Magleby, 2000; Horrigan and Aldrich, 2002). But how are they modified and why?

Here, through careful study of binding site–specific mutants, Zeng et al. report that, if we restrict our attention to Ca^{2+} concentrations up to 10 μM, the BK_{Ca} channel's Ca^{2+}-dependent activation rate is due primarily to Ca^{2+} binding to the Ca^{2+} bowl, while the Ca^{2+} dependence of its deactivation rate is due to Ca^{2+} binding to the second high-affinity site. That is, broadly speaking, one site governs the channel's activation rate (at least up to 10 μM Ca^{2+}) and the other the deactivation rate. What an unexpected result. What seemed so simple upon first examination actually relies on the binding properties of two distinct types of high-affinity Ca^{2+}-binding sites.

In a physical sense, though, what could be going on to create such a circumstance? What is it about one site that makes it affect activation and the other deactivation? If one accepts that the BK_{Ca} channel's kinetics are determined by a single conformational change, and one recalls that the affinity of each Ca^{2+}-binding site must necessarily increase as the channel opens, then interpreting these results in terms of transition state theory, they would seem to indicate that in the transition state of the closed-to-open conformational change, the Ca^{2+} bowl–related site has already changed to its higher-affinity structure, while at the second site the structural change to higher affinity has not yet occurred (Auerbach, 2003). One day, when we have movies of this channel in action, it will be interesting to see if this is in fact the case. Spoiling this simple interpretation, however, at higher Ca^{2+} it appears that Ca^{2+} binding to the second site also speeds activation, a result that seems hard to explain unless the D362A/D367A mutation is acting on more than one site. It will be interesting to stayed tuned, particularly to work from the Lingle group, to see how this conundrum is resolved and as well for more insights into Ca^{2+} sensing by this the king of ion channels.

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