Single particle imaging of a mammalian BK channel
Extending primary sequence to tertiary structure

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Structure/function analyses of membrane ion channels and transporters have benefited enormously from experimental strategies combining the precise manipulation of site-directed mutagenesis with the high sensitivity and kinetic resolution of patch clamp technology. More recently, three-dimensional, structural determinations of these proteins-of-interest have proven invaluable in the interpretation of such mutation-based functional analyses, by allowing the mechanistic insights to be considered in the context of an actual, rather than an imagined, structural framework. The tremendous advances made in our understanding of the molecular workings of K+-selective ion channels highlight the value that these two complementary approaches can bring. The large conductance, calcium-activated K+ channel (BK) is a member of the voltage-gated K+ channel family, but has a few notable features that distinguishes it from the more typical family members (i.e. Kv1–4 subclasses). Structurally, the BK channel pore-forming α subunit contains an additional transmembrane segment (S0) at its N-terminus, which positions this region outside of the cell. There is also a long intracellular C-terminal domain that comprises approximately two-thirds of the primary sequence and contains several regulatory regions, including two predicted RCK domains and a high affinity, calcium-binding site termed the “Ca2+ bowl”. Functionally, BK channels are independently gated by membrane voltage and elevations in cytosolic free calcium. Similar to their Kv channel cousins, BK channels have a bona fide voltage sensor domain (i.e. transmembrane segments S1–S4) that underlies depolarization-induced activation. The proximal RCK domain and distal Ca2+ bowl, both present in the long C-terminal tail, impart gating sensitivity to micromolar levels of cytosolic calcium. Although recent crystallographic information from a simpler, calcium-activated K+ channel (MthK) from bacteria has aided our understanding of RCK-mediated gating in mammalian BK channels, the overall picture of channel activation and regulation remains incomplete, due in large part to the extra structural complexity of the mammalian channel.

In an important first step towards obtaining a detailed molecular picture of the mammalian BK channel, Wang and Sigworth have now described a low resolution structure of functional human BK channels reconstituted in a lipid membrane environment. In doing so, this group has provided a basic structural framework that orients and sets the physical boundaries for the key functional domains of the channel complex (i.e. voltage sensor domain, gating ring composed of RCK domains, Ca2+ bowl, S0 segment, conduction pathway, etc.). To accomplish this feat, human BK channel α subunits expressed in HEK 293 cells were affinity purified via an N-terminal epitope tag and reconstituted into 1-palmitoyl, 2-oleoyl phosphocholine liposomes. Activity of reconstituted channels was confirmed by a cationic fluorescent dye (JC-1) that reported changes in membrane potential induced by variations in external [K+], and this activity could be blocked by the presence of iberiotoxin and barium (interestingly, the degree of block by each agent individually further indicated the proportion of channels inserted either inside-out

Key words: BK channel, proteoliposome, electron microscopy, voltage sensor, RCK domain

Submitted: 10/06/09
Accepted: 10/06/09
Previously published online: www.landesbioscience.com/journals/channels/article/10269
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Commentary to: Wang L and Sigworth FJ. Structure of the BK potassium channel in a lipid membrane from electron cryomicroscopy. Nature. 2009; 461:292–5; PMID: 19718020; DOI: 10.1038/nature08291.
or outside-out following liposome reconstitution). For imaging purposes, reconstituted channels were maintained primarily in a closed conformation (i.e. nanomolar free calcium and membrane potential near 0) and image generation was performed by single particle reconstruction following electron microscopy of cryogenically frozen samples (cryo-EM). To optimize the collection of data and reduce the number of single particle images and preparations required for faithful reconstruction, the authors generated uniform, high density arrays of BK channel proteoliposomes by introducing a small amount of biotin-labeled phospholipid into their liposomes and then binding the channel-containing vesicles onto two-dimensional streptavidin crystals. Because variability in liposome size affected the dimensions of the lipid bilayer and prevented precise three-dimensional reconstruction of imaged particles from different proteoliposomes, the authors developed the method of "random spherically constrained" single particle reconstruction. In this procedure, the phospholipid membrane contribution to each imaged particle was first modeled and then subtracted, thereby allowing reconstruction of the protein particle itself without interference from electron densities associated with the membrane. Using this approach, final projections were prepared from a collection of ~8400 particle images, obtained from 644 specimen micrographs. The three-dimensional resolution of reconstructed particles was estimated to be 1.7–2 nm.

So, what did the authors see? Looking perpendicular to the lipid bilayer, the reconstructed holo-channel appears somewhat like an inverted pear. There is a clear transmembrane region, a central ion conduction pathway, and extracellular surface protrusions corresponding to the turret region of the pore and the S2 segment of the voltage sensor domain. The N-terminal S0 segment is also apparent at the periphery of the transmembrane region. It is likely that the large protein mass visible below the transmembrane region corresponds to the channel’s long intracellular C-terminus. By comparing their reconstructed image of the closed BK channel with the structural features of the six-transmembrane (6TM) Kᵥ1.2 channel and a bacterial 6TM cyclic-nucleotide-gated channel (MlotiK), the authors noted strong similarities amongst the transmembrane regions of the three channels and that the voltage sensor domains of Kᵥ1.2 and MlotiK could be readily accommodated within the volume of the BK channel complex. Similarly, mapping the known structure of the gating ring, derived from the MthK channel, onto the reconstructed BK channel structure indicated that this region formed by RCK domains most likely resides directly beneath the transmembrane core, adjacent to the inner leaflet of the membrane bilayer. This position would also agree well with functional studies describing divalent metal ion-dependent interactions between amino acids in the first RCK domain and the transmembrane helices and linkers of the BK channel. Finally, the remaining channel density extending below the putative RCK domain/gating ring structure likely represents the Ca²⁺ bowl region and extreme C-terminus of the channel complex, although this assignment remains speculative.

In summary, the authors have provided the first three-dimensional model of a functionally competent, mammalian BK channel, in which the voltage-sensor domain appears to be grossly similar to those described in higher resolution KV1.2 and MlotiK channel structures determined by X-ray crystallography. The experimental advances made in this study will undoubtedly allow investigators to obtain increasingly higher resolution images of the BK channel structure, and it now seems only a matter of time before an atomic-scale image is achieved. Given the wealth of functional data and strong interest that surrounds BK channel gating and its regulation, such structural information is certainly long-awaited in the field.