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COMMENTARY

Interrogating the gating motions of the NaK channel

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Previous crystallographic studies depicted a physical gate of the NaK channel localized at a bundle crossing of pore-lining helices, but solution NMR studies in the current issue of *JGP* suggest otherwise.

The past two decades have ushered in an explosion of structural studies of ion channels, broadening the perspective of potential mechanisms to describe ion channel function, in terms of the physical conformations of states that underlie ion conduction. For canonical tetrameric potassium channels and their relatives, it once seemed to many that the "trap-door" mechanism, as described in the seminal work of Armstrong and Hille (1972), might be the norm. Initial crystal structures of potassium channels largely corroborated this view; the prokaryotic channel KcsA, captured by x-ray crystallography in the closed state, provided the first direct structural evidence that channels might gate via a bundle crossing of pore-lining helices, whereas the crystal structure of another prokaryotic K channel, MthK, illustrated that these pore-lining helices could be splayed apart to reveal an unimpeded ion conduction pathway (Doyle et al., 1998; Jiang et al., 2002a; Jiang et al., 2002b). The closed KcsA and open MthK structures were followed by crystal structures of another bacterial channel, called NaK, used in the study by Kurauskas et al. (2022) in this issue of the *Journal of General Physiology*.

The NaK channel earned its name because in Rb-flux assays, this channel was found to be both Na^+ and K^+ permeable (Shi et al., 2006). The full-length NaK channel (NaK FL) was initially crystallized in a conformation resembling the KcsA crystal structure, with the pore-lining helical bundle-crossing (HBC) pinched closed to occlude the conduction pathway (Fig. 1). As with KcsA, each of the four subunits contain two transmembrane helices, although NaK has an unusual selectivity filter (SF) sequence, consisting of TVG***D***G, instead of the canonical TVG***Y***G found in most K^+ selective channels; this substitution appears to account for the weak cation selectivity of NaK. Also, NaK was found to contain an additional helix, M0, which in the NaK FL crystal structure forms a "collar" around the perimeter of the HBC.

The NaK FL structure suggested the possibility that the collar of M0 helices may interact with the pore-lining helices to stabilize the HBC-closed conformation of NaK, in a mechanism analogous to the interaction between the S4–S5 linkers and the pore-lining S6 helices in domain-swapped voltage-gated K^+ channels (Long et al., 2005). Subsequently, Alam and Jiang (2009) constructed and crystallized a truncated mutant, NaKNΔ19, with its 19-residue M0 helix deleted. This resulted in a NaK structure with the HBC in a splayed-apart conformation, strikingly similar to the open MthK structure (Fig. 1). Thus, with the crystal structures of NaK FL and NaKNΔ19, NaK became the first of the canonical tetrameric channel superfamily to be crystallized with the HBC in two distinct conformations, in what seemed to be a confirmation of the idea that these channels could gate via steric opening and closing at the HBC.

Although the picture of NaK channel gating seemed to be developing in a straightforward manner, it was complicated by the observation that NaK FL and NaKNΔ19 did not yield measurable single-channel currents when reconstituted in planar lipid bilayers. In earlier studies, it was observed that Rb-flux through NaKNΔ19 channels was greater than that of NaK FL, consistent with the HBC-gate interpretation (Shi et al., 2006). However, it was further observed that a pore mutation, F92A, increased Rb flux through NaKNΔ19 by several-fold compared to NaKNΔ19 without the F92 mutation, and that the F92A mutation appears to be critical to measure unitary currents through these channels by electrophysiology (Fig. 1; Alam and Jiang, 2009; Derebe et al., 2011). Together these observations suggest that the NΔ19 truncation on its own may not necessarily be a determinant of ion conduction, leaving the relation between the NΔ19 truncation, splaying of the HBC, and the functional gating states of the NaK conformations to be unclear.

In this issue of *JGP*, Kurauskas et al. (2022) address the discrepancy between NaK FL and NaKNΔ19 x-ray structures and function using solution NMR spectroscopy. While crystal structures of these two NaK variants have yielded high-resolution snapshots of two distinct HBC conformations, NMR spectra provides complementary data on the structures of the channel in lipid bicelles. Although obtaining well-resolved

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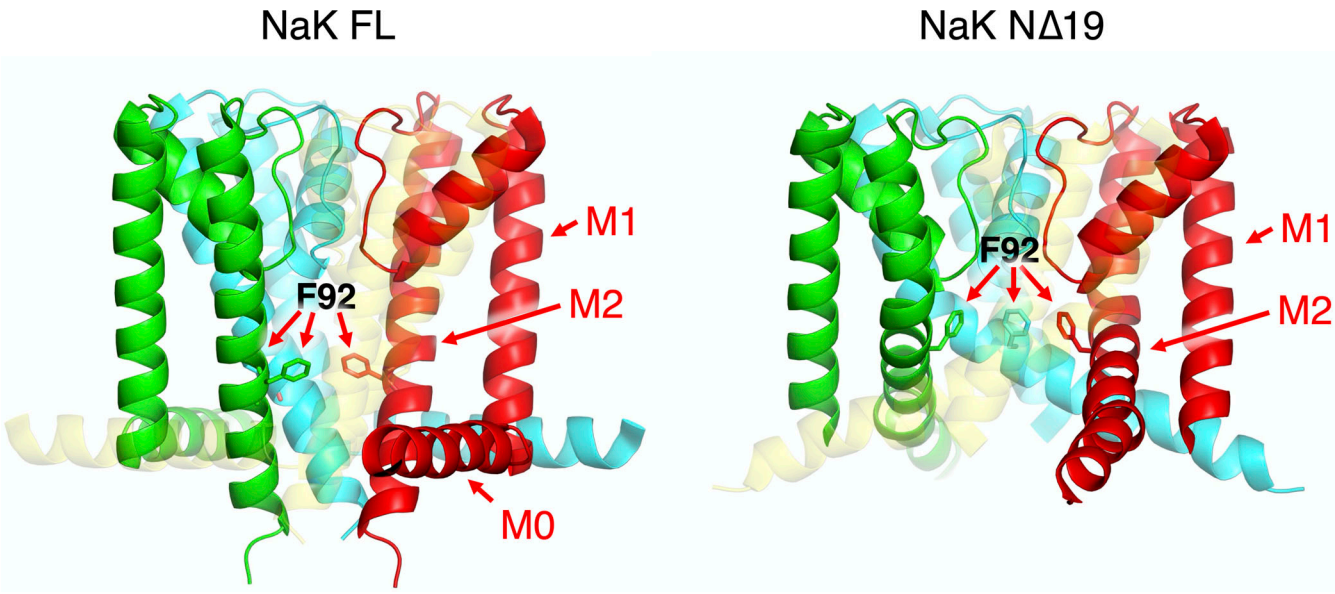

Figure 1. **Crystal structures of NaK FL and NaK NΔ19.** Four subunits comprise the channel. Three of subunits are shown as solid green, cyan, and red; the fourth subunit is shown as transparent yellow to enable visualization of the ion conduction pathway. Left: NaK FL contains helices M0, M1, and M2, with the M2 helices in the HBC-closed conformation. In the crystal structure, the M0 helices from each subunit together form a collar around the HBC, and the side chain of F92, on the pore-lining M2 helix, is rotated to narrow the conduction pathway. Right: NaK NΔ19 is a truncated variant with the M0 helix deleted. In this structure, the M2 helices are splayed apart in the HBC-open conformation, and F92 is rotated to widen the conduction pathway.

spectra for a tetrameric channel can pose technical challenges, here the authors have used a combination of backbone and methyl resonances to measure chemical shift perturbation and site-specific distance restraints. Methyl resonances provide sensitive indices of structure and dynamics even for large proteins and complexes (Tugarinov and Kay, 2005; Tugarinov et al., 2005), and these were advantageous to study NaK channels, in which Ile, Leu, and Val are fairly uniformly distributed through the sequence and comprise ∼30% of the residues. Using methyl-NOESY spectra, it was possible for the authors to obtain 250–325 distance constraints among methyl groups in each of the channel variants, which in turn could be compared between the two variants and also interpreted in the context of previous crystal structures.

Kurauskas et al. (2022) found that their truncated variant (NaK Δ18, which was less prone to aggregation) yielded spectra that were consistent with the HBC-open conformation of NaKNΔ19. However, remarkably, they further observed that spectra from the NaK FL channel were consistent with the same HBC-open conformation. Distance constraints provided by methyl resonances from several key residues provide strong support for this interpretation. An explanation for this paradox may be rooted in the observation that the M0 helix underlies crystal contacts among neighboring NaK tetramers in the crystal lattice, as noted previously (Alam and Jiang, 2009). By either eliminating the M0 helix (as with the NaKNΔ19 variant) or eliminating crystal packing, the channel assumes the HBC-open conformation. Kurauskas et al. (2022) further explored the possibility that NaK FL may visit the HBC-closed conformation used NMR spectra collected at lowered temperature, but found no indication that the HBC-closed conformation was visited substantially under these conditions.

If the HBC-open conformation is the apparent default, then how might this conformation be impacted by the F92A mutation, which increases NaK channel conduction in electrophysiological and Rb-flux experiments? Based on methyl HMQC and methyl NOESY experiments, the authors show that the most salient changes in the F92A mutant correspond to residues in the SF and pore helix region, as well as residues in the M2 helix directly adjacent to this region. For example, chemical shift perturbations (CSPs) are observed for methyl groups from SF residue V64, pore helix residues V59 and L61, and M2 residues I84, V91, and I95. These observations are consistent with the idea that the functional effects of the F92A mutation on ion conduction may arise from alterations in SF conformation, although the nature of this change is not yet clear. CSPs in this region of the pore could also be attributed to increased water and ion accessibility that would result from the much smaller alanine side chain in the F92A conduction pathway.

This new interrogation of channel gating supports the idea that there may be more to channel gating than the opening and closing of a helical bundle crossing. Indeed, recent structures of the human BK channel seem consistent with the idea that the gate of the channel lies beyond the HBC region (Jia et al., 2018; Tao and MacKinnon, 2019). It is certainly worth noting that the physiological basis for NaK channel gating is not known, and perhaps discovery of an activator of NaK channels will enable further detailed analysis of gating mechanisms relevant to wide variety of channels in this family.

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