Membrane localization and conformational dynamics of KirBac1.1 slide helix during lipid-dependent activation

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Inward-rectifier potassium (Kir) channels are essential for regulating various physiological processes and are implicated in several life-threatening diseases, making them key drug targets. KirBac1.1, a well-characterized prokaryotic homolog of Kir channels, is known to undergo anionic lipid-dependent gating. Although the slide helix is an important structural component in the gating mechanism of KirBac1.1, its structural dynamics associated with the anionic lipid-driven activation is not well-understood. Here, we have reconstituted KirBac1.1 in zwitterionic POPC and anionic POPC/POPG membranes to stabilize the inactive and active conformations of the channel, respectively. Our liposome K^+ flux assay results show that all the slide helix single-cysteine mutants display PG-driven gating, and increasing the PG from 25 to 40 mol% does not have any linear dependency on both the activation and K^+ flux rates. Site-directed NBD fluorescence results suggest that the structural dynamics of the slide helix is significantly altered upon PG-induced activation. For instance, we observe significant changes in hydration dynamics and rotational mobility of slide helix residues between functional states. MEM-based lifetime distribution analysis suggests that the conformational heterogeneity of the slide helix is functional-state dependent. Importantly, membrane penetration depth measurements reveal that the slide helix in the active KirBac1.1 is located ~3 Å deeper within the membrane interface, well supported by increased fluorescence lifetimes. Notably, the non-linear relationship between structural dynamics and PG content highlights the critical role of lipid-protein interactions and membrane surface charge in PG-mediated KirBac1.1 activation. These findings provide valuable insights into Kir channel gating mechanisms, and lipid-dependent gating of other channels.