Furthermore, these simulations have also enabled us to study the deformation of the bilayer induced by the protein- we have compared this to the bilayer deformation induced by barrel-shaped outer membrane proteins of a similar size. Thus we present a multi-scale study of the putative model of PorB- one of only two bacterial outer membrane proteins known to have a helical topology.

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Gating-related Structural Dynamics in the Outer Vestibule of KcsA: A Functional and Spectroscopic Analysis

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KcsA is activated by intracellular protons through a large conformational change in the inner helical-bundle gate. Further, the selectivity filter and surrounding structures play a crucial role as an inactivation gate in ion conduction. Here, we monitored the conformational changes in the outer-vestibule of KcsA during gating using electrophysiological and EPR measurements to gain insight on dynamic properties of these conformational fluctuations. pH-jump measurements show that Cd^{2+} facilitates the rate of inactivation of outer-vestibule mutant Y82C-KcsA, however, there is no effect of Cd^{2+} on the non-inactivating E71A/Y82C-KcsA. This suggests that the outer-vestibule has different conformations in the inactivated and non-inactivated states of KcsA. EPR mobility results show that upon opening the lower gate, Y82C undergoes a significant conformational change only in the E71A (non-inactivating) background and not in inactivated state (wild-type). This conformational change is also evident in tandem dimer Y82C constructs, even when the hydrogen-bond network at the selectivity filter is partially perturbed. Distance measurements using cw-EPR at low temperatures show that the diagonal distance between spin labels bound to tandem-dimer Y82C and E71A/Y82C-KcsA is ~12 Å when the lower gate is closed at pH7. Interestingly, the distance between the spin labels bound to Y82C is found to be 8 and 19 Å upon gating in inactivating and non-inactivating forms of KcsA. The change in the Y82C position could be related to the effects of Cd^{2+} on the rate of inactivation in different functional forms of KcsA. The diagonal inter-spin in the closed state is in excellent agreement with the distance seen in the crystal structure of the spin label linked to Y82C-KcsA in the closed state. These results are interpreted in terms of the conformational transitions in the outer-vestibule during activation and inactivation gating.