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ELUCIDATING THE MOLECULAR MECHANISM OF C-TYPE INACTIVATION IN POTASSIUM CHANNELS

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EXECUTIVE SUMMARY

C-type inactivation of K^+ channels is a molecular process of great physiological significance that affects the firing patterns of neurons in the central nervous system and the repolarization of cardiac cells in the heart. Despite years of studies, recent experimental results with semi-synthetic channels inserting unnatural amino acids in the structure suggest that a constricted filter conformation may not correspond to the C-type inactivated state. To resolve this issue, molecular dynamics simulations and free energy computations based on atomic models of the KcsA potassium channel were carried out. The computational results support the notion that the constricted conformation of the selectivity filter correspond to the functional C-type inactivated state of the KcsA channel. Our previous studies have put us on a strong path to execute this computational research [1–3].

RESEARCH CHALLENGE

Identifying the structural features associated with C-type inactivation in K^+ channels is a very important goal and, in this regard, the bacterial KcsA channel has played a critical role. Although C-type inactivation has traditionally been associated with a functional behavior of voltage-gated channels such as Shaker, all the known functional markers of C-type inactivation are experimentally recapitulated by KcsA, providing a coherent view of the molecular determinants that affect inactivation. In addition, because the TTVGYGD canonical selectivity filter sequence is highly conserved, it is expected that the accessible conformational states of the selectivity filter, i.e., conductive and inactivated, ought to be fairly similar throughout most of the K^+ channel family.

METHODS & CODES

We have carried out molecular dynamics simulations based on atomic models of the channels with the program NAMD using the CHARMM force field PARAM27.

RESULTS & IMPACT

Molecular dynamics simulations and free energy calculations were used to investigate the molecular mechanism of C-type inactivation. The impact of different inner-gate openings on the conformation and dynamics of the selectivity filter was systematically studied, and—for the first time—our 2D free energy calculation quantitatively characterized the conformational preferences of the selectivity filter in which a fully open gate (~23 angstroms) highly favors a constricted filter, whereas a partially open gate (~16 angstroms) notably prefers to maintain a conductive filter. The spontaneous, rapid, and consistent conductive-to-constricted transition observed in simulations with a fully open gate reveals a hydrogen-bond network controlling the cooperactivity for the tetramer constriction. By contrast, the partially-to-fully open transition for the inner gate is much slower, and the rearrangement of inner helices demonstrates considerable conformational heterogeneity. Analysis based on the simulations provides comprehensive details of the long-range allosteric gating coupling, and, more importantly, reveals the molecular basis for the delayed kinetics of C-type inactivation. These recent results are described in manuscripts that are currently under review.

WHY BLUE WATERS

Blue Waters offers the ability to carry out extensive umbrella sampling computations with Hamiltonian replica-exchange molecular dynamics simulations (i.e., window swapping) using multiple copies of the system.

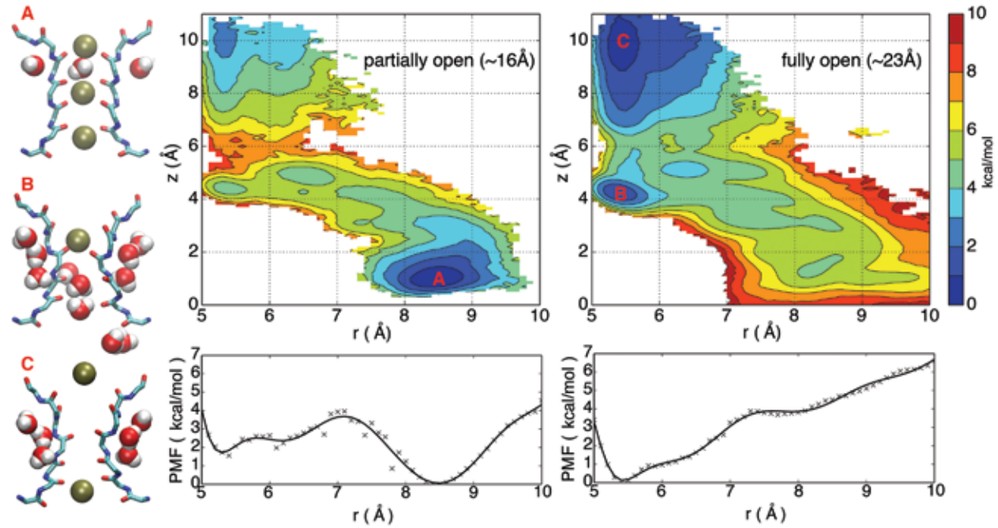

Figure 1: 2D PMF for the conductive-to-constricted transitions for the selectivity filter with different opening degrees of inner gate. The horizontal reaction coordinate r describes the width of the selectivity filter and is defined as the average cross-subunit pinching distance between the Cα atoms of G77. The vertical reaction coordinate z indicates the position of the external K^+ ion along Z axis relative to the center of the selectivity filter. The lower panel is the one dimension PMF along horizontal reaction coordinate r, with integration of the vertical reaction coordinate z. The typical conformations for three free energy basin (left) are shown in stick for protein and VDW representation for both water and K^+ ion.