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Multi-ion free energy landscapes underscore the microscopic mechanism of ion selectivity in the KcsA channel☆

David Medovoy, Eduardo Perozo, Benoît Roux *

Biochemistry and Molecular Biology, Institute for Biophysical Dynamics, Gordon Center for Integrative Science, The University of Chicago, 929 E 57th Street, Chicago, IL 60637, USA

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ABSTRACT

Potassium (K^+) channels are transmembrane proteins that passively and selectively allow K^+ ions to flow through them, after opening in response to an external stimulus. One of the most critical functional aspects of their function is their ability to remain very selective for K^+ over Na^+ while allowing high-throughput ion conduction at a rate close to the diffusion limit. Classically, it is assumed that the free energy difference between K^+ and Na^+ in the pore relative to the bulk solution is the critical quantity at the origin of selectivity. This is the thermodynamic view of ion selectivity. An alternative view assumes that kinetic factors play the dominant role. Recent results from a number of studies have also highlighted the great importance of the multi-ion single file on the selectivity of K^+ channels. The data indicate that having multiple K^+ ions bound simultaneously is required for selective K^+ conduction, and that a reduction in the number of bound K^+ ions destroys the multi-ion selectivity mechanism utilized by K^+ channels. In the present study, multi-ion potential of mean force molecular dynamics computations are carried out to clarify the mechanism of ion selectivity in the KcsA channel. The computations show that the multi-ion character of the permeation process is a critical element for establishing the selective ion conductivity through K^+-channels. This article is part of a Special Issue entitled: Membrane Proteins edited by J.C. Gumbart and Sergei Noskov.

1. Introduction

Potassium (K^+) channels are a ubiquitous class of transmembrane proteins that passively and selectively allow K^+ ions to flow through them, after opening in response to an external stimulus [1]. These channels are perhaps best known for being a key component of the action potential in excitable cells — but are found in a variety of different cell types and organisms, in all domains of life, and can be activated by a variety of different stimuli. Their functionality gives them a key role, in the cell, in regulating the cytoplasmic K^+ concentration and the electrochemical potential across the cell membrane. Potassium channels have been implicated in human cardiac disorders, such as long-QT syndrome, and in cancers. They are a common target of natural peptide toxins, and are an important target for therapeutic pharmacology.

One of the most critical functional aspects of K^+ channels is their ability to remain very selective for K^+ over Na^+ while allowing high-throughput ion conduction at a rate close to the diffusion limit [2–4]. In practice, however, the concept of selectivity for an ion channel can mean different things depending on whether the system is probed experimentally via equilibrium binding assays or non-equilibrium ionic current measurements. Classically, ion channel selectivity has been characterized by determining the permeability ratio from the reversal potential (zero net current) under bionic conditions. However, measurements of reversal potentials become difficult for highly selective channels such as the K^+ channels. In this case, alternate methods such as Ba^{2+} blockade relief [5,6] or Na^+ punchthrough [7], provide more effective methods to characterize selectivity with quantitative accuracy. Each method reports on different aspects of the system, e.g., Ba^{2+} block is more sensitive to the depth of the free energy minima of the binding sites (i.e., equilibrium binding), while Na^+ punchthrough is more sensitive to the height of free energy barriers (i.e., non-equilibrium rates).

The classic explanation of selectivity posits that the free energy difference between K^+ and Na^+ in the pore relative to the bulk solution is the critical quantity at the origin of selectivity [3,4]. This "thermodynamic" view is strongly supported by the Ba^{2+} blockade experiments of Miller and co-workers [5,6,8], and by the ITC microcalorimetry measurements of Lockless et al. [9], which offer experimental evidence of equilibrium binding site selectivity in the KcsA channel. Because the blocks by Ba^{2+} last for a very long time, the experiment allows an estimate of the quasi-equilibrium dissociation constant of Na^+ or K^+ for a binding site called the "external lock-in site". While the basic concepts of the thermodynamic view are well established, additional studies

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* Corresponding author at: 929 E 57th Street, Room W323B, Chicago, IL 60637, USA.
E-mail address: roux@uchicago.edu (B. Roux).

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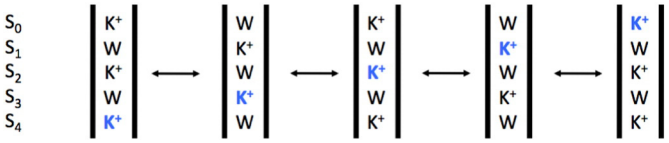
demonstrated that additional factors can play an important role. For instance, Nimigean and Allen have clarified kinetic aspects of selectivity based on studies of Na^+ blocks in the bacterial KcsA channel [10–12].

More recently, results from a number of studies have highlighted the remarkable importance of the multi-ion single file on the selectivity of K^+ channels. By examining the properties of MthK [13] and NaK [14,15] mutants, Jiang and co-workers showed that the channel becomes selective only if four consecutive binding sites exist along the narrow selectivity filter. This has culminated more recently with studies of two engineered mutants of the NaK channel, referred to as "NaK2K" and "NaK2CNG" [16,17]. According to reversal potential measurements from single-channel electrophysiology, the NaK2K construct is K^+-selective and the NaK2CNG construct is non-selective. Remarkably, despite being non-selective in ion permeation, the NaK2CNG filter display equilibrium binding preference for K^+ over Na^+, as indicated by measurements with isothermal titration calorimetry (ITC) and concentration-dependent ion replacement within the filter observed through crystallographic titration experiments. The K^+-selective channels bind two or more K^+ ions in the narrow filter, whereas the non-selective channels bind fewer ions. Based on the crystallographic titration experiments, the NaK2K construct has two high-affinity K^+ sites while the NaK2CNG construct has only one K^+-selective site. These experiments show that both K^+-selective and non-selective channels select K^+ over Na^+ ions at equilibrium, implying that equilibrium selectivity is insufficient to determine the selectivity of ion permeation [16,17]. The data indicate that having multiple K^+ ions bound simultaneously is required for selective K^+ conduction, and that a reduction in the number of bound K^+ ions destroys the multi-ion selectivity mechanism utilized by K^+ channels. To explain K^+ channel selectivity, a simple kinetic model with two K^+ high-affinity sites and a double-barrier was proposed [17]. However, while these experimental results are intriguing, the underlying microscopic mechanism remains unclear. Fundamental questions remain about the mechanism of selective ion conduction and whether it is supported by some multi-site/multi-ion kinetic processes, or by near-equilibrium binding site thermodynamic processes, is unclear. Obviously, selectivity reported by ion permeation reflects the non-equilibrium dynamic process of multi-ion translocation across the filter and this needs to be taken into account in the discussion.

While the thermodynamic/kinetic debate can be resolved in terms of classic arguments (the mechanism is controlled by free energy differences at the bottom of the binding wells versus the top of the activation barriers), the critical multi-ion character of the selectivity revealed by the recent studies forces us to reformulate these classic arguments in a broader context. The implication is that the multi-ion character of the permeation process must, somehow, be a critical element for establishing the selective ion conductive through K^+-channels. How this mechanism is encoded into the three-dimensional molecular structure of the protein is an unresolved question.

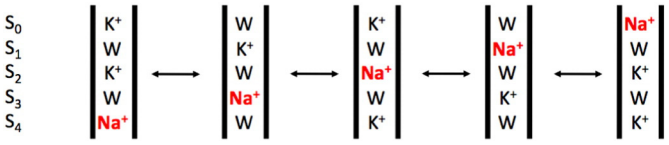
Our goal is to use molecular dynamics (MD) computations to quantitatively outline the step-by-step multi-ion processes through the K^+ channel selectivity filter that underlies selective permeation. We choose to base our computations on the well-characterized prokaryotic potassium channel KcsA from *Streptomyces lividans* [18]. This was the first selective biological ion channel for which a crystal structure was determined to atomic resolution [19], and it has ever since proven to be a tremendously useful model system for computational studies of the different functional aspects of K^+ channels, including permeation [20–23], selectivity [10,11,24], and C-type inactivation [25–29]. Most of the previous MD simulation studies of ion permeation through the selectivity filter of the KcsA channel have relied on the high-resolution crystallographic structure in complex with a Fab antibody (PDB ID 1K4C) [30], which has its intracellular activation gate in the closed state. Recently, Luis Cuello and collaborators have obtained a high-resolution structure of the KcsA channel with an open intracellular activation gate and a selectivity filter in the conductive conformation (private communication). The main advantage of using a structure with an open intracellular gate is that we include configurations with an ion in the cavity and one ion in the site S_4, which are energetically unfavorable [21].

Conceptually, the permeation process of K^+ ions through the KcsA channel is believed to proceed predominantly as a "knock-on" mechanism in which the single-file translocation of 2–3 K^+ ions interspersed by water molecules through the narrow selectivity filter [21,31],



Scheme 1.

Logically, under conditions corresponding to high K^+ concentration, the permeation of one isolated Na^+ "defect" is likely to be chaperoned by the predominant K^+ ions fore and aft, and proceeds according to the following steps,



Scheme 2.

The new X-ray structure (Luis Cuello, private communication) provides a unique opportunity to examine the problem of ion permeation through a channel in its functional "open-conductive" state.

To characterize the process of selective permeation through the KcsA channel in the open-conductive state according to Schemes 1 and 2 above, we compute the multi-ion potential of mean force (PMF) that underlies the multi-ion permeation process using umbrella sampling MD simulations. To ascertain the selectivity of the channel, we compare the multi-ion PMF of K^+ ions moving as a K^+/K^+/K^+ single-file through the filter (Scheme 1) with that of a K^+/Na^+/K^+ single-file (Scheme 2). The umbrella sampling computations allow us to map the free energy landscape for the complete multi-ion translocation event of the single file of multiple ions across the entire filter. We then use the string method [32–34] to analyze and visualize the multi-ion transition in terms of an optimal pathway along the free energy landscape.

2. Methods and computational details

The high-resolution X-ray crystallographic structure of open-conductive KcsA was inserted into a previously equilibrated solvated bilayer simulation system based on the moderate resolution (3.2 Å) X-ray structure (PDB ID 3F5W) [27]. The simulation system comprises a bilayer of 112 DPPC molecules, surrounded by a 150 mM KCl aqueous salt solution to ensure electrical neutrality, for a total of ~43 k atoms [28]. The glutamic acid at position 71 was protonated (Berneche and Roux [21]) and the termini were patched with standard N- and C-terminal capping groups. Engineered disulfide bonds between positions 28 and 118, necessary to lock the channel into the open-state in the high-resolution X-ray structure, were maintained. The protein was modeled after the CHARMM force-field; CHARMM22 with CMAP corrections for protein [35] and CHARMM36 for lipids [36]. The water model is TIP3P [37], the K^+ ion is from Ref. [38], and the Na^+ is from Ref. [24]. Following Noskov et al. [24], adjusted non-bonded parameters were used for interactions between protein carbonyl oxygens and cations. An additional artificial dihedral restraint was added to keep the carbonyls of Val76 in their crystallographic orientation and prevent them from 'flipping', as

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