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Selectivity and Permeation of Alkali Metal Ions in K^+-channels

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Ion conduction in K^+-channels is usually described in terms of concerted movements of K^+ progressing in a single file through a narrow pore. Permeation is driven by an incoming ion knocking on those ions already inside the protein. A fine-tuned balance between high-affinity binding and electrostatic repulsive forces between permeant ions is needed to achieve efficient conduction. While K^+-channels are known to be highly selective for K^+ over Na^+, some K^+ channels conduct Na^+ in the absence of K^+. Other ions are known to permeate K^+-channels with a more moderate preference and unusual conduction features. We describe an extensive computational study on ion conduction in K^+-channels rendering free energy profiles for the translocation of three different alkali ions and some of their mixtures. The free energy maps for Rb^+ translocation show at atomic level why experimental Rb^+ conductance is slightly lower than that of K^+. In contrast to K^+ or Rb^+, external Na^+ block K^+ currents, and the sites where Na^+ transport is hindered are characterized. Translocation of K^+/Na^+ mixtures is energetically unfavorable owing to the absence of equally spaced ion-binding sites for Na^+, excluding Na^+ from a channel already loaded with K^+.

Introduction

Under physiological conditions, K^+-channels allow the passive diffusion of K^+ down their electrochemical gradient.[1] They distinguish ions according to electric charge and they show substantial selectivity among ions of the same valence; e.g. selectivity ranges from 1000-fold to 10-fold preference for K^+ over Na^+.[2,3] Besides K^+, four other ions are known to permeate K^+-channels with a more moderate tendency, and unusual conduction features; Rb^+, Tl^+, NH_4^+ and Cs^+.[4,5] However, when added to the internal medium, Cs^+ as well as Na^+ and Li^+ can block outward delayed K^+ currents.[2] Cs^+ is the most powerful blocker of these three monovalent cations,[6] the sequence being $Cs^+ > Na^+ > Li^+$. Neither Na^+ nor Li^+ blocks the K^+ current with fast kinetics from the extracellular side.[7]

The region of the protein responsible for the selective conduction is the selectivity filter (SF),[8] which is a narrow pore 12 Å long, formed by a tetrameric arrangement of the highly conserved amino acid sequence TVGYG.[9,10] The carbonyl oxygen atoms of TVGYG, together with the side chain oxygen atoms of T, define five binding sites for ions, named S0–S4 (Fig. 1). Ions in solution must be dehydratated before entering the SF. Once in the SF, a K^+ is coordinated by up to eight oxygen atoms from the protein. Free energy calculations of the relative binding affinities for K^+ and Na^+ at binding sites S1–S4 revealed that these positions are selective for K^+ over Na^+, with the highest selectivity achieved at binding site S2.[11–13] Reflecting their ionic radii, the hydrated alkali metal ions display coordination numbers and configurations expected to be octahedral (6 coordination) in the case of Na^+

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Abbreviations used: SF, selectivity filter; MD, molecular dynamics; PMF, potential of mean force.

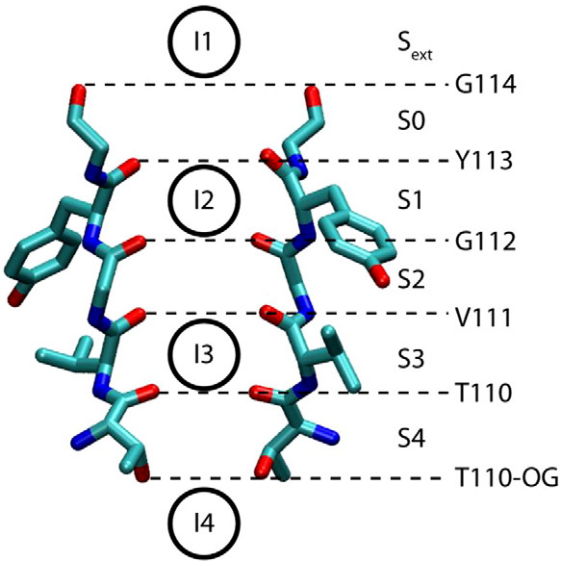

Fig. 1. Selectivity filter of K^+-channels. Amino acids T110–G114 of two out of the four opposite subunits of the KirBac1.1 SF are shown in liquorice representation. Horizontal lines highlight the positions of the carbonyl oxygen atoms of amino acids T110–G114, and the side chain oxygen atoms of amino acid T110 (T110 OG). The average positions of these atoms are used to define the boundaries between the K^+ binding sites S0–S4. Free energy maps are calculated for a multi-ion conduction mechanism with ions I1–I4, numbered from the extracellular side.

and square antiprismatic (8 coordination) in the case of K^+.[14,15] In this respect, therefore, binding sites S1–S4 should be optimal for K^+ but not for Na^+. However, Na^+ is still capable of occupying certain positions in the SF of K^+-channels. The nature of the putative Na^+ binding sites in K^+-channels was first described by computational work.[11,16] It was observed that Na^+ resides in-plane with the four carbonyl oxygen atoms of the T or the first G of TVGYG, with two water molecules completing its coordination shell. Crystallographic data supported the existence of these sites, whereby Na^+ binds, for instance between sites S4 and S3, in the plane of the backbone carbonyls of the T of the TVGYG motif.[17] Binding of Na^+ to this so-called B site was described to require the absence of K^+ at both S4 and S3 sites. However, no information has been reported concerning the nature of the potential binding sites when Na^+ enters the SF from the extracellular side.

In order to understand how selectivity emerges, it is important to consider how the channel performs its primary function, i.e. the conduction of K^+. Several conduction mechanisms have been proposed to describe how K^+ moves along the SF;[12,13,18] the energy landscapes are characterized by having energy minima at selected binding sites (S0–S4) and low-energy barriers for the concerted motions of the ions. During the conduction events, the SF is always occupied by at least two potassium ions and the presence of these ions is required to maintain the SF of the KcsA K^+-channel in a conductive state.[9,19] In contrast, in low concentrations of K^+,[10] the SF of this channel adopts a distinctive configuration in which the pore is physically occluded and impermeable to ions.[10,19,20] Experimentally, it has been possible to lock the SF of KcsA in the conductive state by mutating the Gly residue delimiting S2 and S1 to the unnatural amino acid D-alanine.[21] In the absence of K^+, the SF locked in the conductive state is permeable to Na^+, with a conductance lower than that of K^+. In the presence of K^+/Na^+ mixtures, the SF locked in the conductive state is highly selective for K^+, which means that Na^+ is unlikely to enter an SF occupied by K^+.[22] It was reported recently that, in the absence of K^+, the MthK K^+-channel can also conduct Na^+.[23] Crystallographic data revealed that Na^+ can bind in this K^+-channel without any structural rearrangements of the SF, even if the S2 and S3 sites provide a perfect chelating environment for K^+ but not for Na^+. All these experimental observations suggest that the preference for K^+ over Na^+ at S1–S4 binding sites accounts only partially for the selectivity characteristics in K^+-channels. Considering that Na^+ can still bind inside the SF, only at different positions, and that they can permeate the filter in the absence of K^+, the selectivity for K^+ over Na^+ arises, at least in part, as a result of the exclusion of Na^+ from an SF already occupied by K^+. Consequently, it is important to analyze the behavior of mixed configurations of Na^+ and K^+ inside the SF.[17,24] Furthermore, the calculation of free energy differences at specific positions along the SF is not enough to comprehend how and where the selectivity character is realized. In order to understand why K^+ and not Na^+, or indeed other species, travel across the SF, it is necessary to compare the free energy profiles of multi-ion conduction mechanisms of different ions.

Access to an SF occupied by K^+ by a sodium ion that approaches the SF from the intracellular cavity was studied by Thompson *et al.*[17] Crystallographic structures, electrophysiological experiments and free energy calculations support the hypothesis that in the presence of two potassium ions in the SF, a sodium ion can bind to a site above S4 and in line with the carbonyl oxygen atoms of the T residue. However, additional progress of Na^+ toward the extracellular side of the SF is unlikely. Unidimensional free-energy profiles for Na^+ moving from the intracellular cavity to site S4 with two potassium ions placed and restrained at specific positions in the SF have been described.[17] However, because the electrostatic interactions between the ions are crucial for the description of the energetics of ion permeation, it is more appropriate to calculate