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Hydration valve controlled non-selective conduction of Na^+ and K^+ in the NaK channel

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ABSTRACT

The Na^+ and K^+ channels are essential to neural signaling, but our current knowledge at the atomic level is mainly limited to the conducting mechanism of K^+. Unlike a K^+ channel having four equivalent K^+-binding sites in its selectivity filter, a NaK channel has a vestibule in the middle part of its selectivity filter, and can conduct both Na^+ and K^+ ions. However, the underlying mechanism for non-selective ion conduction in NaK remains elusive. Here we find four small grottos connecting with the vestibule of the NaK selectivity filter, which form a vestibule-grotto complex perpendicular to the filter pore with a few water molecules within it. It is shown that two or more of the water molecules coming to the vestibule to coordinate the cation are necessary for conducting both Na^+ and K^+ ions, while only one water molecule in the vestibule will obstruct ion permeation. Thus, the complex with the aid of interior water movement forms a dynamic hydration valve which is flexible in conveying different cations through the vestibule. Similar exquisite hydration valve mechanisms are expected to be utilized by other non-selective cation channels, and the results should shed new light on the importance of water in neural signaling.

1. Introduction

Fifty-five years ago, Hodgkin and Huxley found that Na^+ and K^+ permeation through cell membranes is the root of neuron signaling [1]. In the following decades our basic understanding of ion channels has been enriched by extensive electrophysiological researches, especially with the aid of patch-clamp techniques, but it is the finding of the K^+ channel KcsA crystal structure that shows us for the first time the atomic mechanism of how ions are selected and conducted in a K^+ channel. K^+ channels have a highly conserved selectivity filter sequence of TVGYG and form a simple one-dimensional filter pore consisting of four equivalent K^+-binding sites numbered S1~S4 from the extracellular to the intracellular sides for selectively conducting K^+ ions [2]. Under physiological conditions, two K^+ ions are proposed to bind in the selectivity filter in the 1, 3 or 2, 4 configuration, referring to two K^+ ions occupy sites S1, S3 or sites S2, S4, respectively, with a water molecule residing between them. It is suggested that the K^+ conduction mechanism involves a concerted transition of K^+ ions in the selectivity filter between these two equally occupied configurations [3–6]. An alternative K^+ conduction mechanism has also been proposed recently where site vacancies are involved and K^+ ions are shown to be able to occupy adjacent binding sites [7].

The newly presented NaK channel from *Bacillus cereus* conducting both K^+ and Na^+ ions [8–11] has a selectivity filter sequence of $_{63}$TVGDG$_{67}$, which is different from that of a potassium channel but similar to the specific sequence TIGET of most cyclic nucleotide-gated (CNG) non-selective cation channels working in our photoreceptors and olfactory cells [12,13], as acidic residues aspartate D and glutamate E are both negatively charged and have similar structures. Thus, the structure of the NaK channel gives us a unique opportunity to unveil the secret of permeation of ions through non-selective cation channels. In NaK, the region near residues $_{65}$GD$_{66}$, corresponding to ion binding sites S1 and S2 of a K^+ channel selectivity filter, becomes a vestibule (Fig. 1A) with no specific ion binding site [8]. Therefore, to determine how ions can be conducted through the selectivity filter of NaK weakened by the vestibule becomes a challenge and important to understand non-selective ion permeating mechanisms. Recently, Vora et al. studied the conduction rates of K^+ and Na^+ ions through a modified model of the NaK channel, with the M0 helices being removed and the radii of the intracellular gate and the selectivity filter being increased [14]. However, the mechanism controlling ion conduction through the selectivity filter of NaK remains unclear. Here we find an intrinsic structural feature of four small grottos connecting with the vestibule in both the open and closed structures of the NaK channel [8,10]. An aqueous vestibule-grotto (V-G) complex is thus formed in a plane perpendicular to the central ion conducting pore. The overall profiles of the V-G complex are shown in Figs. 1 and S1, which were obtained by probing the interior surface of the channel with the program HOLE [15]. Each grotto is formed from two adjacent filter loops at Gly65-Asp66 and their p-loop helices at Tyr55-Thr60. It is demonstrated by extensive molecular dynamics (MD) simulations and free energy analyses that the water filled V-G complex acts as a

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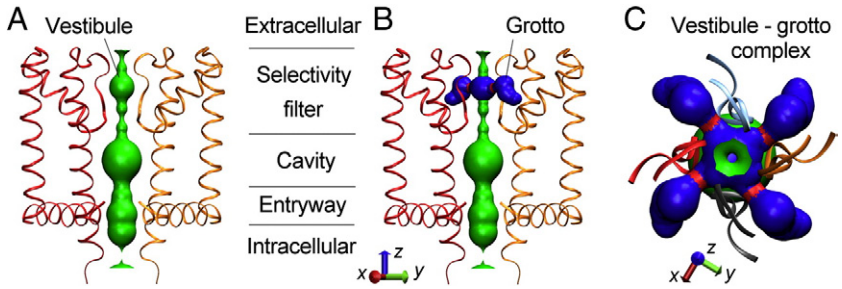

Fig. 1. The structure feature of the NaK channel. (A) The central pore. (B) Side view of two grottos from the pore. (C) Top view of the vestibule-grotto complex. The red belts between the grottos and the vestibule show the narrow necks with radius less than 1.15 Å. For clarity, only two monomer subunits are shown in panels A and B.

dynamic hydration valve, which is flexible to adjust itself to conduct K^+ and Na^+ ions in distinct mechanisms to enable the non-selective ion permeation and maintain the stability of the channel simultaneously. The newly reported high-resolution structures of the NaK channel may also provide experimental supports for the existence of the hydration valve: a few water molecules in the grottos and the vestibule have been found in the high-resolution crystal structures (PDB IDs 3E8H, 3E89, 3E8F, etc.) [10,11], as shown in Fig. S1.

2. Methods

The basic MD model, generated with VMD [16], consists of the 2.8 Å resolution structure (PDB ID 2AHZ) of the K^+-bound NaK [8] embedded in a fully hydrated dimyristoylphosphatidylcholine (DMPC) lipid bilayer, surrounded by a 200 mM KCl aqueous salt solution box. It contains totally 42,871 atoms, including 6900 protein atoms, 127 DMPC lipid molecules (67 and 60 in the extracellular and intracellular sides, respectively) with 14,986 atoms, 20,955 water atoms, 3 K^+ ions in the pore, at sites S1, S3 and in the cavity, respectively, and 6 K^+ and 21 Cl^- ions in the bulk solution added randomly using the autoionize plugin of VMD. The cavity is filled with 38 water molecules initially. Ionizable residues are set to their default ionization state, i.e., Glu and Asp with a negative charge, Lys and Arg with a positive charge, and all other residues with zero charge, according to pK_a calculations using the program PEOPKA [17,18]. The whole system is then in an electro-neutral state. The modeling methodology is in reference to [19,20].

Initially, the basic model was firstly minimized for 5000 steps and equilibrated for 200 ps with the protein and the ions in the pore being fixed to fill the gap between the protein and the lipids. In the remaining 800 ps equilibration stage, all other ion configurations (Table S1) were constructed, and gradually decreasing harmonic restraints were applied to the protein and the ions in the pore. Then, a 3 ns production run simulation was performed for each system with no restraints. On the base of these results, 6 additional configurations (Table S2) were constructed and simulated using the above protocol, with the water molecules in the grottos being harmonically restrained (with a force constant of 2 kcal/mol/Å^2) during the 1 ns equilibration period. Based on the equilibrated stable configurations, SMD simulations were implemented to induce entire ion permeation events by applying an upward constant electric field or force to each ion in the pore. In order to prevent global drift of the system, the C_α atoms of Glu23 residues at the intracellular end of M1 helices, far away from the selectivity filter, were restrained with a harmonic potential of 1 kcal/mol/Å^2 (see Tables S3 and S4 for details) [19].

All simulations were performed using the program NAMD2 [21]. The CHARMM27 force field was used for the protein and phospholipids [22]. The TIP3P model was used for water [23]. Multiple sets of the Lennard-Jones parameters for K^+ and Na^+ ions were used to yield reasonable solvation free energies in bulk water and liquid *N*-methylacetamide [24]. Periodic boundary conditions were applied in all directions. Electrostatic forces were calculated without cutoff, using the particle mesh Ewald (PME) method [25] with a grid density of at least 1 /Å^3. Smooth (8–10 Å) switching off was applied for the van der Waals interactions. All simulations were carried out with a time step of 1 fs. The Langevin dynamics was employed to control the temperature at 310 K, and the Nose-Hoover Langevin piston method was used to maintain the pressure at 1 atm [26,27].

Potentials of mean force (PMFs) for water diffusion between the vestibule and the grottos and ion conduction along the selectivity filter were calculated using the umbrella sampling simulations [28]. For water diffusion, the last frame of the SMD simulation with 3 ions in the pore was used as the starting point. The reaction coordinate was defined as the distance between the center of mass of the carboxyl carbon atoms of Val64–Asp66 and that of the water in the vestibule, projected on the (x, y) plane. The reaction pathway extended to 8 Å and the width of each window is 0.25 Å. During the umbrella sampling simulations, a biasing harmonic potential of 5 kcal/mol/Å^2 was applied on the oxygen atom of the water molecule along the reaction coordinate, and the ions in the selectivity filter were constrained with a harmonic potential of 20 kcal/mol/Å^2. All simulations were carried out for 500 ps with the first 100 ps for equilibration.

For the two-dimensional PMF $W(Z_{ion1},Z_{ion2})$ calculations, the starting configurations were selected from the SMD simulations with four ions, numbered ion-1 to ion-4 from the extracellular to the intracellular sides, in the pore. Harmonic potentials [5,29] acting on the z coordinates of ion-1 and ion-2, with 0.5 Å increments, were implemented for each umbrella window with a force constant of 20 kcal/mol/Å^2, while ion-3 and ion-4 were constrained along the pore axis with a force constant of 20 kcal/mol/Å^2, at 5.5 Å and 1.5 Å for K^+ and 3.5 Å and 0.7 Å for Na^+, respectively. In each window, the system was initially equilibrated for 100 ps followed by a 400 ps production run. During simulations with one water molecule in the vestibule, the grotto water molecules were prevented from entering the vestibule to hydrate the ions, while the simulation protocol was similar to that used above. Umbrella histograms were then unbiased and combined using the weighted histogram analysis method (WHAM) [30] (The code was obtained by courtesy of A. Grossfield http://dasher.wustl.edu/alan/ for WHAM calculations).

3. Results

3.1. Stable ion binding configurations

Four potential ion binding sites were revealed in the high-resolution structures of the NaK channel: the extracellular entrance, the vestibule, and sites S3 and S4 [11]. Consistent with the recent computational studies of NaK [14,31–33], the electron density maps also showed that K^+ ions prefer to bind within the ion binding sites, while Na^+ ions are inclined to bind to a single plane of four oxygen atoms from the selectivity filter [11]. However, detailed ion binding configurations in the NaK selectivity filter, without Ca^{2+} blocking at