Dynamics of K^+ Ion Conduction through Kv1.2

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ABSTRACT The crystallographic structure of a potassium channel, Kv1.2, in an open state makes it feasible to simulate entire K^+ ion permeation events driven by a voltage bias and, thereby, elucidate the mechanism underlying ion conduction and selectivity of this type of channel. This Letter demonstrates that molecular dynamics simulations can provide movies of the overall conduction of K^+ ions through Kv1.2. As suggested earlier, the conduction is concerted in the selectivity filter, involving 2–3 ions residing mainly at sites identified previously by crystallography and modeling. The simulations reveal, however, the jumps of ions between these sites and identify the sequence of multi-ion configurations involved in permeation.

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Potassium channels are integral membrane proteins that allow rapid (10^8 ions/s) and selective conduction of K^+ ions across the cell membrane. Four identical segments of the highly conserved sequence of amino acids, TVGYG, comprise the selectivity filter of the channel and provide four binding sites for K^+ ions. K^+ ions in each of these binding sites are surrounded by two layers of oxygen atoms provided by backbone carbonyl groups and side chains of threonine residues. Under physiological conditions, the selectivity filter is occupied by two or three K^+ ions.

Based on the crystal structure of KcsA (1), a bacterial potassium channel, in the closed state, and modeling (2), mechanisms for K^+ conduction through the selectivity filter have been proposed. These mechanisms involve concerted transition of K^+ ions in the selectivity filter between two equally occupied two-ion states, in which the K^+ ions are located at sites 1 and 3, separated by a water molecule at site 2 (denoted here as the [1, 3] state), or sites 2 and 4 with a water molecule at site 3 ([2, 4] state). The mechanisms also include three-ion states as intermediates, e.g., state [0, 2, 4], which involves an extra binding site for K^+ ions labeled 0, which was observed in higher resolution diffraction. The simulated system in state [0, 2, 4] is shown in Fig. 1.

The availability of the 2.9 Å resolution crystal structure of Kv1.2 (3), a voltage-gated potassium channel, in the open state made it possible to carry out molecular dynamics (MD) simulations of K^+ permeation through the channel driven by a voltage bias, without a priori assumptions on the conduction mechanism. Here we report, to our knowledge, the first such simulation that tests the existing notion of K^+ conduction (1, 2, 4).

The simulations are based on the crystal structure of Kv1.2, from which only the pore domain, residues 312–421, had been simulated in a solvated POPE lipid bilayer (Fig. 1). The system was equilibrated for 10 ns (Sim0), followed by a series of three simulations with an applied electric field (voltage bias) Sim1, Sim2, and Sim3, for a total time of 25 ns, using NAMD (5). A K^+ was placed near the intracellular mouth of the protein, at the level of the lipid-water interface, at the beginning of each of these simulations to eliminate the need for K^+ to diffuse to this site, a process that would unnecessarily extend the simulation time. The potential bias was emulated by applying a constant force to every charged atom and corresponds to a value of 1 V across the lipid bilayer (with a length of 30 Å). The voltage bias was chosen so high to expedite ion conduction for better sampling, but this may be altering the ion conduction mechanism, although no evidence for this emerged; protein and lipid bilayer proved stable under the 1 V voltage bias during all simulations reported.

In the simulation, the backbone dihedral angles of residues 374–378 in the selectivity filter were constrained to the values in the crystallographic structure. A harmonic constraint with a small force constant of 1.5 kcal/mol.rad^2, corresponding to a thermal root mean-square deviation of 51°, was used to prevent large spontaneous reorientation or ''flipping'' of the carbonyl groups of the selectivity filter. Such spontaneous flipping events are observed in unconstrained simulations of the Kv1.2 pore domain, and had also been reported in the MD simulations of KcsA (6), Kir6.2 (7), and KirBac1.1 (8). These flipping events may correspond to inactivation of the channel (9).

The equilibrium simulation (Sim0) assumed two K^+ ions at sites 2 and 4, with two water molecules located at sites 1 and 3, corresponding to state [2, 4]. A third external K^+ entered site 0 within 200 ps. The ions in the selectivity filter remained in state [0, 2, 4] for the rest of Sim0, as shown in Fig. 1. The root mean-square deviation of the protein backbone from the crystal structure does not exceed 2 Å, which is typical for simulations of membrane proteins, indicating stability of the transmembrane domain of Kv1.2 with the voltage-sensors eliminated.

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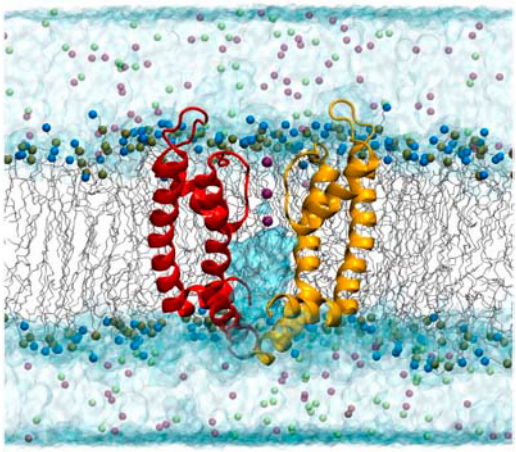

FIGURE 1 Pore domain of Kv1.2, embedded in a hydrated POPE lipid bilayer. Two of the four protein subunits are shown in cartoon representation, with three K^+ ions inside the selectivity filter. Water molecules are shown in transparent blue surface representation. Lipid molecules are represented by lines with their nitrogen, and phosphorus atoms in van der Waals representation. K^+ and Cl^- ions are shown in purple and green, respectively.

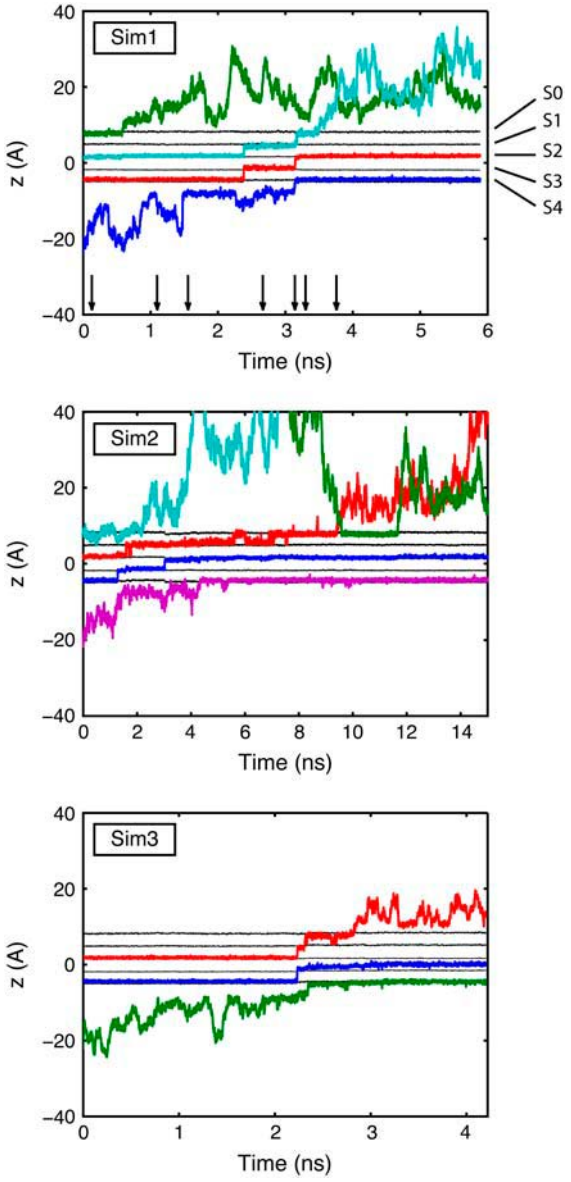

FIGURE 2 Trajectories of K^+ ions, projected onto the symmetry axis of the channel. The position of five binding sites, 0–4, defined as the geometric center of two adjacent oxygen rings (from residue 374 to 378) lining the selectivity filter, are shown as thin black lines. Results are shown for three simulations with an applied voltage bias of 1 V. For simulation Sim1, we indicate through arrows the moments when snapshots, shown in Fig. 3, were taken.

The K^+ trajectories of three simulations with voltage bias Sim1, Sim2, and Sim3 are depicted in Fig. 2. The three-ion state, [0, 2, 4], although stable in a 10 ns equilibrium simulation, has now a shorter lifetime. During Sim1, this state quickly, i.e., within 700 ps, changes to a two-ion state, [2, 4], by dissociation of the outermost ion from site 0. The [2, 4] state is stable until a third K^+ approaches the filter from the central cavity. The third ion pushes the two K^+ ions in the filter to the [1, 3] state. The concerted transition of ions is followed by entrance of a water molecule and a K^+ to occupy sites 3 and 4, respectively, thereby translocating the two K^+ ions in the filter to sites 0 and 2, resulting in the [0, 2, 4] state. Snapshots of Sim1 are shown in Fig. 3.

In Sim2, state [0, 2, 4] is maintained for $\sim$2 ns. As the central cavity ion approaches the filter, the two K^+ ions at sites 2 and 4 are pushed to the [1, 3] state. During this transition, for brief periods of time (400 ps), the K^+ ions are located at adjacent sites 2 and 3. This transition state is also observed in Sim1, however, only transiently for $<$50 ps. The water molecule located between two K^+ ions, originally at site 3, is pushed away from the conduction pathway and eventually exits the selectivity filter. Entrance of a water molecule and a K^+ at sites 3 and 4 results in the K^+ ions to occupy state [1, 2, 4]. The K^+ at site 1 fluctuates between sites 1 and 0, until eventually it exits the filter on the extracellular side.

In Sim3, starting from state [2, 4], a transition to state [1, 3] is observed within the first 3 ns. State [1, 3] is followed by a quick transition, after 150 ps, to a state in which a water molecule and a K^+ enter the filter from the central cavity, pushing the outermost K^+ to site 0. This K^+ becomes hydrated and exits the channel on the extracellular side. Upon exit of the K^+ from site 0, the selectivity filter is occupied by two K^+ ions separated by a water molecule. Different from Sim1 and Sim2, in Sim3, no sharp transition between states [1, 3] and [0, 2, 4] is observed. One K^+ is located at site 4; however, the second K^+ is positioned halfway between sites 3 and 2. Four carbonyl groups of Val-375, located between sites 3 and 2, coordinate the K^+ in this position. Movies of Sim1-Sim3 are supplied as Supplementary Material.

The states [2, 4] and [1, 3] had been suggested in (4) to have similar stability (third ion discounted); a positive bias

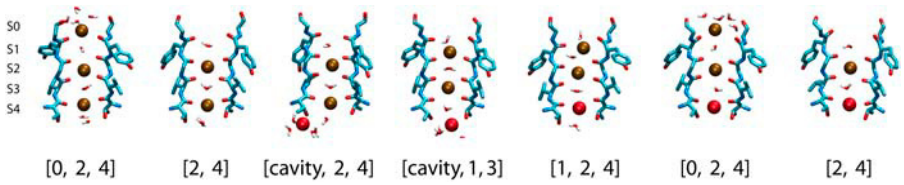

FIGURE 3 Snapshots of the selectivity filter of Kv1.2 from Sim1. K^+ ions and water molecules inside the selectivity filter are shown. For clarity, only two of the four protein subunits are presented. Five binding sites are labeled according to the crystallographic structure; the snapshots are labeled by the ion states corresponding to the ion occupancies shown.

should favor the [1, 3] state. The potential of mean force obtained from free energy perturbation (10) and umbrella sampling MD simulations (11) suggest a low energy barrier of 2–5 kcal/mol between these states at equilibrium. However, in our simulations, the [2, 4] → [1, 3] transition is initiated only when a third K^+ in the central cavity approaches the selectivity filter, suggesting a lower energy for the [2, 4] state. This ion lingered around the C-terminus of one of the four reentrant pore helices, located behind the selectivity filter. K^+ in this position is coordinated by 3–4 water molecules as well as the side-chain oxygen atom of Thr-374 and the backbone oxygen atom of Thr-373 of the pore helices.

For brief periods of time, during the [2, 4] → [1, 3] transition, in Sim1 and Sim2, two K^+ ions were located at two adjacent sites, 3 and 2 (see Fig. 2.) During the transition, the carbonyl group of Val-375, between sites 2 and 3, in one of the subunits becomes significantly reoriented, moving its backbone carbonyl away from the filter. Although the backbone dihedral angles of the selectivity filter residues were constrained in our simulations, because of the choice of weak constraints, such flipping events are not prohibited. These transient states could be due to the large voltage bias (1 V), but could also arise from the flexibility of the selectivity filter, manifested by glycine residues at every other position in the filter. The observed flexibility might be important for ion permeation, and does not have to compromise ion selectivity (12).

Several three-ion states, in which K^+ ions occupy two adjacent binding sites, arise in Sim1 and Sim2. Indeed, state [0, 2, 3] arising transiently in Sim1 and Sim2, and state [1, 2, 4] arising in Sim2, exhibit free energies comparable to that of state [1, 3] when a third K^+ is present in the cavity (10). Simultaneous occupation of adjacent sites by K^+ ions has also been observed in Brownian dynamics simulations (2). Permeation pathways including [0, 2, 3] and [1, 2, 4], although induced here by the large voltage bias, most likely contribute to K^+ permeation. At higher concentration of K^+, for which the selectivity filter has a higher probability to be occupied by three instead of two K^+ ions, conduction pathways including states [0, 2, 3] and [1, 2, 4] should open new permeation mechanisms and increase permeation rates.

Our simulated conduction is consistent with the ''knock-on'' mechanism put forward by Hodgkin and Keynes (13), and is in general agreement with crystallographic (1) and simulation results (2, 11). With the availability of the structure of an open potassium channel, the mechanism of ion permeation and selectivity of K^+ over Na^+ can be revealed further through MD simulations.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

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