Ion Binding Sites and Hydration in the Selectivity Filter of the Bacterial Sodium Channel Navab

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LETTER

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Sodium Ion Binding Sites and Hydration in the Lumen of a Bacterial Ion Channel from Molecular Dynamics Simulations

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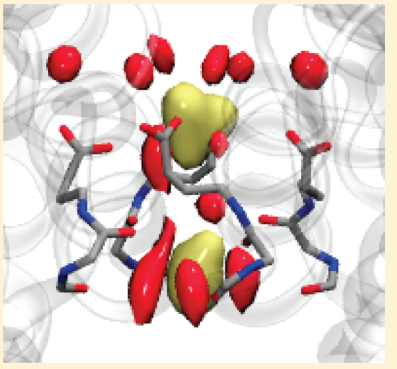
Supporting Information

ABSTRACT: Molecular dynamics (MD) calculations have been used to identify Na^+ binding sites in the lumen of a bacterial voltage-gated ion channel. MD trajectories have been carried out starting from the recently reported X-ray crystal structure of NavAb in a closed or inactivated conformation. The X-ray structure is stable on the time scale of the MD simulations when equilibrated in a fully hydrated lipid bilayer. Exposure to a 70 mM NaCl solution and a trajectory spanning ∼0.15 μs reveals two locations in the selectivity filter (SF) of the channel, with Na^+ coordinated to both water molecules and negatively charged protein residues. The nature of the Na^+ dynamic hydration environment has been explored, and surprisingly water seems to be able to permeate from bulk, via the SF, to the central cavity, whereas Na^+ ions remain near their primary SF locations, a finding that contrasts with the situation that obtains for potassium channels.



SECTION: Biophysical Chemistry

Sodium is hydrated by either five (trigonal-bipyramidal) or six (octahedral) water molecules in crystal hydrate structures, whereas K^+ is hexa-coordinated.[1,2] In room-temperature concentrated salt solutions, neutron scattering data suggest that Na^+ coordination by water ranges from 4.8 to 5.3, whereas K^+ has slightly higher values but with a much less well-defined hydration shell.[3] Molecular simulations employing classical force fields are in fair agreement with these experimental findings for bulk hydration behavior.[4] The recently published structure[5,6] of a bacterial Na^+ ion channel (NavAb) in a closed-pore conformation with activated voltage sensors raises the question as to the nature of Na^+ (compared with K^+) coordination/hydration in the nanoscale confinement that is obtained in the lumen of an channel.[7,8]

The situation for voltage-gated K^+ (Kv) channels has been elucidated by numerous ingenious experiments as well as via molecular dynamics (MD) simulation. In brief, to pass through the Kv channel, K^+ ions must transit through a selectivity filter (SF) that is selective for K^+ over Na^+ by about 1000:1.[9−11] The narrow Kv-SF allows only single-file waters or K^+ ions and thus requires significant dehydration of the transiting ion.[10,12] In the preferred "knock on" mechanism, K^+ and waters alternate occupancy of four possible SF binding sites and move coherently when nudged by a K^+ that occupies a site external to the SF.[13−17] Typically, on passing the SF, K^+ has only two waters of hydration. The new crystal structure for NavAb suggests a much wider SF pore, which is consistent with the larger flux of Na^+ through Nav channels compared with K^+ through Kv channels.[5] Moreover, the Na^+ selectivity of NavAb over K^+ is only about 8:1. It is therefore of considerable interest to identify the Na^+ binding sites and to understand the hydration structures of Na^+ in Nav channels, which is the primary goal of the present MD simulations. The recent crystal structure of NavAb[5,6] offers a rare opportunity to make significant progress for the field.

Following the initial setup, independent trajectories MD1 and MD2, each spanning more than 0.1 μs, at constant temperature (300 K) and pressure (1 atm), neutral pH, and with no applied TM electrostatic potential, were carried out on the NavAb structure inserted in a fully hydrated POPC bilayer. Specifically, MD1 contained no added salt, whereas MD2 was bathed in 70 mM NaCl. In both MD runs, the X-ray structure was found to be very stable in the membrane environment (Figure 1), with an rmsd profile for the whole TM domain converging to a plateau value of <3.5 Å, which is similar to previous MD studies on voltage-gated K^+ (Kv) channels.[18,19] In both MD1 and MD2, the SF motif preserved its starting structure, exhibiting a structural drift (∼1.5 Å) analogous to that observed for the SF of K^+ channels.[12,13] The SF of NavAb remains stable both with and without bound Na^+ on our MD simulation time scale.

Early in each of the MD runs, water molecules migrated from the bulk to occupy protein cavities, including external crevices of the voltage-sensor (VS) domain and the central lumen (CL), to ensure

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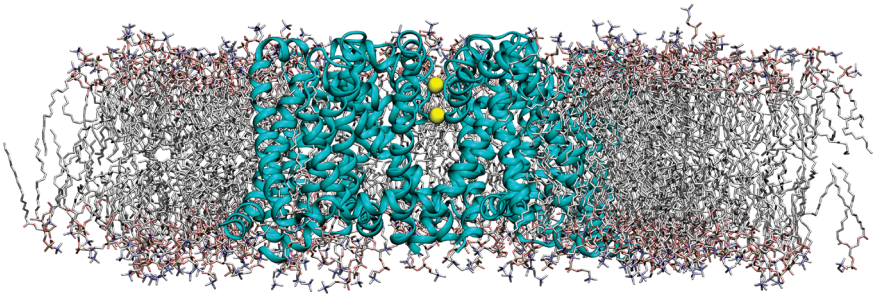

Figure 1. Snapshot taken from an MD simulation of a membrane-bound sodium channel (NavAb) in the presence of ~70 mM NaCl solution. Water and other ions have been removed for ease of viewing. Note that there are two (yellow) Na^+ in the selectivity filter. The membrane protein NavAb is rendered in a cyan ribbon format. The POPC lipids are rendered as a ball-and-stick representation.

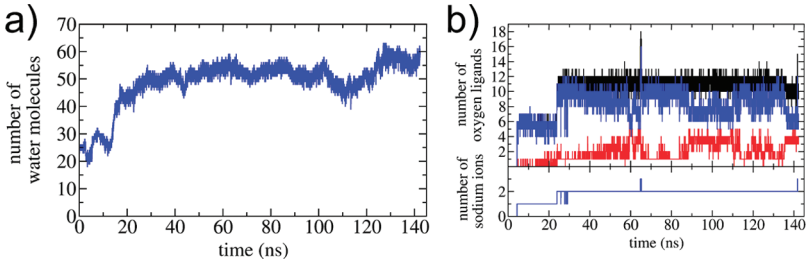

Figure 2. (a) Number of water molecules in the central cavity as a function of time. Waters fill the cavity by diffusion through the selectivity filter. (b) Lower panel is the number of Na^+ ions and the upper panel is the number of oxygen ligands from the protein (red) and waters (blue); the black curve is the total number of oxygen atoms coordinated to Na^+. The black curve is almost constant, but the two components fluctuate significantly, suggesting a rather dynamic binding mode between Na^+ and protein residues in the selectivity filter.

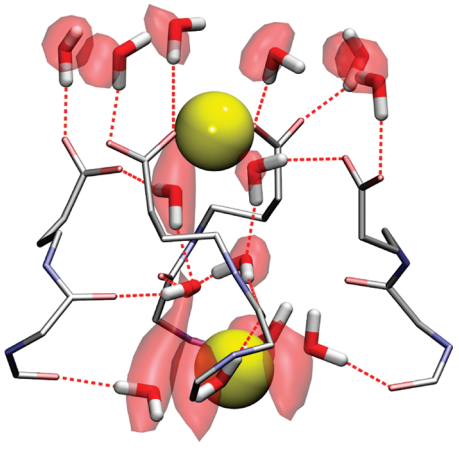

Figure 3. Oxygen density from hydration waters is superimposed on a snapshot of the selectivity filter with two bound Na^+ ions and their solvation shells.

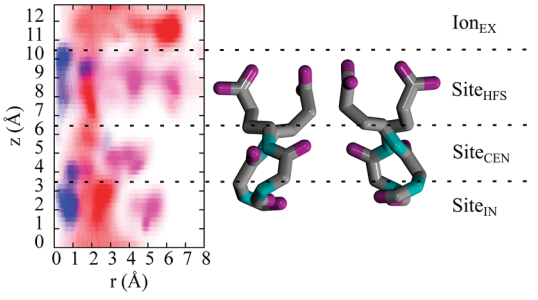

Figure 4. Radial-axial densities of key atoms in the selectivity filter from a 2D histogram in polar coordinates about the lumen axis (z) in a ball-and-stick representation: Na^+ (blue), water oxygen (red), oxygen from carbonyl, and carboxylate of protein residues (purple). The sites in the channel filter region are labeled with the notation used in ref 5. It should be noted that the upper binding site, labeled Ion-EX, is occupied exclusively by water; Site-HFS has two Na^+ conformations, one of which also has waters; Site-CEN has water and a low occupation Na^+ site ~3 Å off the central axis; and Site-IN has a Na^+ site ~1 Å off-axis and a water site ~3 Å off-axis, respectively.

full hydration of the channel embedded in the membrane; ~ 50 water molecules gained access into the CL via the external entryway of the SF (Figure 2). In MD2, two Na^+ ions spontaneously occupy the SF preferentially at two specific binding sites HFS and IN (Figures 3 and 4). The dynamical process involved first one ion binding to Site-HFS, followed by its transition to Site-CEN and then to Site-IN; this last transition was concomitant with binding of another ion to Site-HFS. This configuration remained for the ~0.15 μs so far examined.

Analysis of the number of oxygens, n_0, in the first-coordination shell (3.1 Å cutoff) of Na^+ indicates hexa-coordination in each site: $n_0 = 5.5 \pm 0.4$, being comprised of hydrating waters, $n_0^W \approx 4$ and SF ligands, and $n_0^{SF} \approx 1$. Oxygen providers at Site-HFS are the carboxyls of residues Glu^{177}. The structure of the Na^+ coordination shell in both of these sites resembles closely the octahedral geometry of crystal sodium hydrate structures (Figure 3). In detail, at Site-HFS, the ion binds directly two nonadjacent Glu^{177} carboxyls occupying the apical positions of the octahedron and with two H-bonded water molecules held in position by H-bonding to the other two Glu^{177} carboxyls. Two

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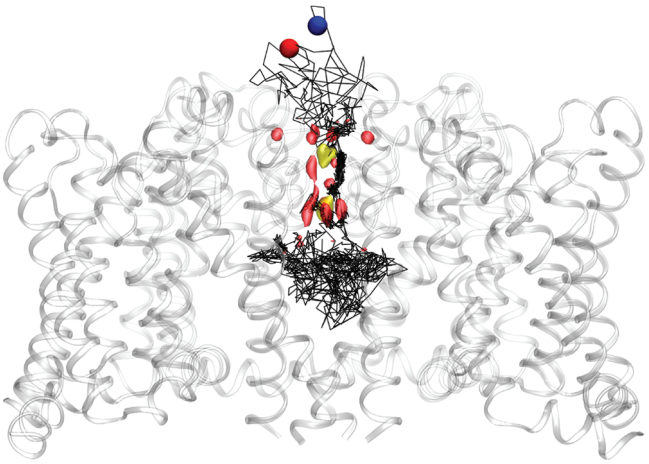

Figure 5. Cartoon representation of the trajectory of a water molecule is shown. The water enters the selectivity filter from the bulk and crosses into the cavity before returning to the bulk. The red and blue spheres mark the position of this water oxygen at ∼35 and ∼42 ns, respectively. Each segment of the black line is 4 ps long. This water molecule skims past the upper Na^+ (Site-HFS) but jumps from site to site in the solvation shell of the lower Na^+ (Site-IN).

further water molecules from the aqueous environment of the vestibule complete the coordination shell of Na^+. Beside this on-axis C2 symmetric binding mode, off-axis (asymmetric) binding can also occur with minor probability in which the ion interacts directly with only one of the four carboxyls (Figure 4).

At site-IN, Na^+ interacts with four water molecules locked in position by H-bonding interactions with the carbonyl oxygens of residue Thr^{175} and featuring an almost perfect square arrangement on a plane orthogonal to the filter axis of symmetry. The solvation shell also encompasses another water molecule lying on average above this plane in Site-CEN, where it can establish one or two H-bonds with the carbonyls of Leu^{176}. Note that while transiting between Site-HFS and Site-IN Na^+ passes through Site-CEN, binding directly to carbonyl oxygens provided by Leu^{176}.

Despite the spatial arrangement of H-bond acceptors in the SF being well-preserved throughout the MD trajectory and the solvation shell having a well-defined average structure, Na^+ binding involves highly dynamical interactions with water- and protein-oxygens (Figure 4). Indeed, a relatively diffuse density of water oxygens inside the SF provides the ions with a significant degree of mobility along the channel axis of symmetry. At Site-HFS, the ion is able to slide along the axis to explore regions as far as 3 Å away from the most populated binding spot (Site-CEN features an even larger degree of structural heterogeneity with ions and water molecules competing for the interaction with the carbonyl oxygens of Leu^{176}). Site-IN is, instead, well-ordered; the density separation for ions, waters, and carbonyl oxygens along the radial direction reflecting the square planar arrangement mentioned above. Comparison between the residence times of Na^+ coordinating waters in the SF (Site-IN: ∼102 ps, Site-HFS: ∼73 ps) and in the bulk (∼23 ps) reveals indeed relatively high mobility of SF-embedded water molecules. Moreover, individual water molecules seem to be able to permeate from bulk through the SF to the CL, whereas the two Na^+ ions remain near their primary locations in the filter region (Figure 5).

Overall, the SF of NavAb seems to mimic the first coordination shell of Na^+ in bulk water by providing two ionic binding sites that hexa-coordinate the ion, a structural principle also observed in the SF of Kv channels.[9−11,13−17] The SF of NavAb is, however, different from that of K^+ channels in many ways: (i) the NavAb SF is wider than the K^+ construct; the former can accommodate partially hydrated bound Na^+ ions in sharp contrast with the dehydrated binding of K^+ in the SF of K^+ channels; (ii) asymmetric (off-axis) Na^+−water−SF-residue configurations take place in NavAb (see Figure 4) contrasting with the single−file configuration of ions and waters in K^+ channels; and (iii) finally, exclusive to NavAb, water can permeate the SF decoupled from ionic conduction (Figure 5).

In consideration of the fact that no bound Na^+ ions were found in the SF of the NavAb X-ray structure, even though the channel was crystallized in the presence of a Na^+-containing solution, Catterall and coworkers put forward hypotheses regarding the ion-bound structure of the filter, which the present investigation helps to corroborate, provides additional insight, or both. First, the authors of ref 5 hypothesized that Na^+ binds to the site-HFS in an asymmetric fashion (off-axis). Whereas this binding mode is indeed observed, the on-axis C2 symmetric site is the one preferentially occupied in the MD simulation. Site-CEN was hypothesized to feature four H-bonded water molecules arranged in a square-planar geometry to thereby effectively solvate Na+, whereas in this central site, the MD finds a small, yet significant probability of binding directly to the carbonyl oxygen. Finally, Figure 4 unveils an oxygen density peak at the Ion-EX region, located ∼3 Å above the carboxyls of Glu^{177}, indicative of strongly structured H-bonded water molecules. It is worth mentioning that equivalent peaks in the electron density were detected in the crystals ∼2 Å above the high oxygen density found in the MD, which the authors of ref 5 tentatively attributed to Na^+. It is tempting therefore to speculate that the structured water molecules found in the present MD simulation of the membrane-bound channel provides further oxygen ligands to assist Na^+ diffusing toward the entrance of the SF.

Classical MD simulations have revealed the nature of the Na^+ binding sites in the SF of the NavAb channel. Two primary binding sites were identified, Site-HSF and Site-IN, along with other lower occupancy sites. Despite the fact that Na^+ permeability in sodium channels has been traditionally rationalized in the framework of one-ion models,[20] the present atomistic MD results are consistent with recent phenomenological descriptions of Nav conduction, suggesting multiple occupancy of the SF.[21,22] Na^+ binding was found to involve highly dynamical interactions with both water and protein oxygen ligands as well as highly mobile water molecules within the SF. Remarkably, waters were observed to cross the SF without displacing the bound Na+ ions, raising the intriguing hypothesis that transport of waters may be decoupled from that of ions. Given the latter observation, seemingly at odds with the strong coupling observed for potassium channels,[23−25] further experimental studies aimed at investigating the dynamical processes underlying the selectivity and conduction properties of NavAb are likely to be informative. We are in the process of studying an "open" NavAb structure in more detail with a goal to investigate Na^+ conduction under an applied field. As a prelude to this more extensive study, and starting from the membrane-bound structure with two Na^+ in SF region, we inserted an extra Na^+ into the CL. We then launched an MD simulation to see what happens, or rather to see if we can glean a hint of the likely conduction mechanism. A 20 ns MD trajectory reveals that the additional Na^+ in the cavity repeatedly moves toward the two ions in the filter, seemingly trying to nudge them to move synchronously and initiate a conduction event. Because no conduction was evident

from the closed cavity with one additional ion added from bulk, a second Na^+ was added to the cavity. This strong electrostatic perturbation was sufficient to initiate Na^+ diffusion out of the SF and its replacement by the fourth ion. The "nudging" collisions of cations from the central cavity with resident SF cations and the diffusion event (movie file in the Supporting Information) are consistent with the knock-on mechanism previously reported for the conduction process in K^+ channels.[17] However, the single-file transportation of ions seen in K^+ channels is not observed in NavAb, which presents a side-by-side ion configuration at the level of site-CEN. Of course, these observations are very preliminary,and much more needs to be done before we can have confidence in our findings. In particular, although the reported crystal structure is stable on our MD simulation time scale, with and without bound ions, the fluctional nature of the Nav residues of the SF, and the behavior of analogous Kv residues[26] suggests that other conformations of the SF,[27] which have not been explored, might be close in energy. Future work with metadynamics[28] will explore this free energy landscape in detail.

COMPUTATIONAL METHODS

MD simulations were performed on the NavAb channel, with the X-ray crystal structure solved at a resolution of 2.7 Å (pdb code 3RVY) used as an initial configuration. For each of the four identical polypeptide chains, the cysteine residue at position 217 was mutated into isoleucine to get a structural model of the wild-type NavAb. The channel was then embedded in a hydrated membrane containing 434 palmitoyl-oleoyl-phosphatidyl-choline (POPC) and 28 173 water molecules; in separate MD runs,ions were added to ensure charge neutrality (MD1) and to provide a solution buffer with an ionic concentration of ~0.07 M (MD2; 44 Cl^- and 36 Na^+). The simulation used the CHARMM22-CMAP force field with torsional cross-terms for the protein[29,30] and CHARMM27 for the phospholipids.[31] A united-atom representation was adopted for the acyl chains of the POPC lipid molecules.[32] The water molecules were described using the TIP3P model.[33] Periodic boundary conditions were applied throughout the MD trajectory. The electrostatic potential was solved using the particle mesh Ewald (PME) method[34] with a real space spherical cutoff of 11 Å and a fast Fourier transform (FFT) grid spacing of 1.2 Å. Lennard-Jones interactions were cut off at 11 Å, with a switching function starting from 8 Å. The equations of motion were solved with the velocity-Verlet integrator using a time step of 2.0 fs. A multiple time step integration scheme was used,[35] and the long-range component of the forces was evaluated every two time steps. The lengths of all bonds involving hydrogen atoms were constrained with the SHAKE method,[36] and the system was run at 300 K and 1 atm using Langevin themostat[37] and barostat schemes.[38] Decay times for the latter were set to 1.0 and 0.1 ps, respectively. MD simulations were performed with the code NAMD2.7.[39] The initial setup began with 300 steps of conjugate gradient energy minimization; the heavy atoms of the backbone of the protein were held at their minimized positions for the first 10 ns of simulations by applying harmonic positional restraints with a force constant of 1 kcal/mol/$Å^2$.

ASSOCIATED CONTENT

Supporting Information. Additional data and a movie file showing Na^+ diffusion. This material is available free of charge via the Internet at http://pubs.acs.org.

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