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Distinct interactions of Na^+ and Ca^{2+} ions with the selectivity filter of the bacterial sodium channel Na_VAb

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ABSTRACT

Rapid and selective ion transport is essential for the generation and regulation of electrical signaling pathways in living organisms. In this study, we use molecular dynamics simulations and free energy calculations to investigate how the bacterial sodium channel Na_VAb (*Arcobacter butzleri*) differentiates between Na^+ and Ca^{2+} ions. Multiple nanosecond molecular dynamics simulations revealed distinct binding patterns for these two cations in the selectivity filter and suggested a high affinity calcium binding site formed by backbone atoms of residues Leu-176 and Thr-175 (S_{CEN}) in the sodium channel selectivity filter.

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1. Introduction

Na^+ flux is vital for initiating action potentials in the membranes of most electrically excitable cells [1]. Recent homotetrameric crystal structures of bacterial Na_V channels [2–5] provide new possibilities for investigating the molecular basis of ion selectivity and transport in these channels. The mechanisms of how these channels discriminate between different ion types are poorly understood. Recent molecular dynamics investigations [6,7] provide insights into Na^+ versus K^+ selectivity; however Ca^{2+} discrimination was not analyzed in atomistic detail so far. In this study, we investigate how bacterial sodium channels discriminate between Na^+ and Ca^{2+}, a process essential for biological function.

Na_V channels are composed of four membrane spanning subunits, containing six helices per subunit. The pore module consists of helices S5, P1 segments, a selectivity filter (SF) region, P2 segments and S6 helices, lining the inner pore cavity. Remarkably, the SF of many bacterial sodium channels contains four highly conserved glutamates (EEEE locus), which is more reminiscent of calcium channels than mammalian sodium channels [8–11]. Despite this high sequence similarity, bacterial Na_V channels distinguish between sodium and calcium ions with permeability ratios P_{Ca}/P_{Na} of 0.08–0.15 [12,13]. The three dimensional architecture of this motif is revealed by X-ray structures of bacterial Na_V channels from three different species [2–5]. How these unusual sodium channels discriminate between different ion types and how ions permeate the pore are not well understood yet.

In this study, we performed molecular dynamics (MD) simulations and single ion potential of mean force (PMF) calculations to investigate Ca^{2+} interactions with the bacterial sodium channel Na_VAb.

2. Materials and methods

2.1. Molecular dynamics simulations

MD simulations were performed with Gromacs version 4.5.4 [14]. The coordinates of Na_VAb (PDB Entry: 3RVY; resolution: 2.7 Å) with a closed pore gate were used in all simulations [2]. Cysteine residues at position 217 were mutated back to isoleucine to obtain the wild-type structure of Na_VAb, and all charged residues were treated keeping their charge states at physiological pH 7.4. Simulations were carried out with the AMBER99sb [15] all atom force field in dioleoylphospathidylcholine (DOPC) lipids [16] with the TIP3P water model [17].

All covalent bonds were constrained using the LINCS algorithm [18], allowing for an integration time step of 2 fs. A 10 Å cutoff was adopted for calculating short-range electrostatic interactions and the Particle Mesh Ewald [19] summation was used for calculating long-range electrostatic interactions. The corrected Lennard-Jones parameters for the amber forcefield [20] were implemented in this

Abbreviations: Na_VAb, bacterial sodium channel (*Arcobacter butzleri*); Na_VRh, bacterial sodium channel (*Rickettsiales* sp. *HIMB114*); MD simulation, molecular dynamics simulation; PMF, potential of mean force; SF, selectivity filter; DOPC, dioleoylphospathidylcholine; PDB, protein data bank; RMSD, root-mean-square deviation.

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study and the vdW interactions were calculated with a cutoff of 10 Å. The Nose–Hoover thermostat [21,22] and the semi-isotropic Parrinello-Rahman barostat algorithm [23] was used to maintain simulation temperature and pressure constantly at 310 K and 1 bar, respectively. Prior to MD simulations, 3000 conjugate gradient energy-minimization steps were performed, followed by 5 ns equilibration in order to fully solvate mobile water and lipids around a protein restrained with a force constant of 1000 kJ/mol/nm^2 on all heavy atoms.

2.2. PMF

One ion PMF calculations were performed by umbrella sampling [24]. Prior to this simulation, a test ion was pulled with a force constant of 2090 kJ/mol/nm^2 (5 kcal/mol/$Å^2$) along the filter, from a heavily restrained (force constant: 10,000 kJ/mol/nm^2) reference ion (placed ∼15 Å away on top of the extracellular side of the filter) [25]. This procedure resulted in 20 windows to explore the ion conductance route along the filter (total length ∼10 Å, as shown in Fig 1B inset) at 0.5 Å intervals. The test ion was initially held fixed for a 100 ps equilibration, followed by a 2 ns PMF simulations with the first 0.5 ns removed for equilibration [7]. In each umbrella sampling simulation, the probing ion was restrained harmonically with a force constant of 4180 kJ/mol/nm^2 (10 kcal/mol/$Å^2$) along the *z*-axis. A 4.18 kJ/mol/nm^2 (0.01 kcal/mol/$Å^2$) force constant was exerted on the Cα atoms of the protein as a center of mass restraint during simulations, except for the SF residues (residues 174–183) [7]. The free energy profile was calculated with the g_wham tool implemented in Gromacs. Error analysis was performed calculating 200 bootstrap iterations [26].

3. Results and discussions

Two 100 ns MD simulations with 100 mM NaCl and 100 mM $CaCl_2$ concentrations each were performed. A third simulation with either NaCl or $CaCl_2$ was extended to 150 ns for analysis. To get further insight into the different behavior of Na^+ and Ca^{2+} ions in the

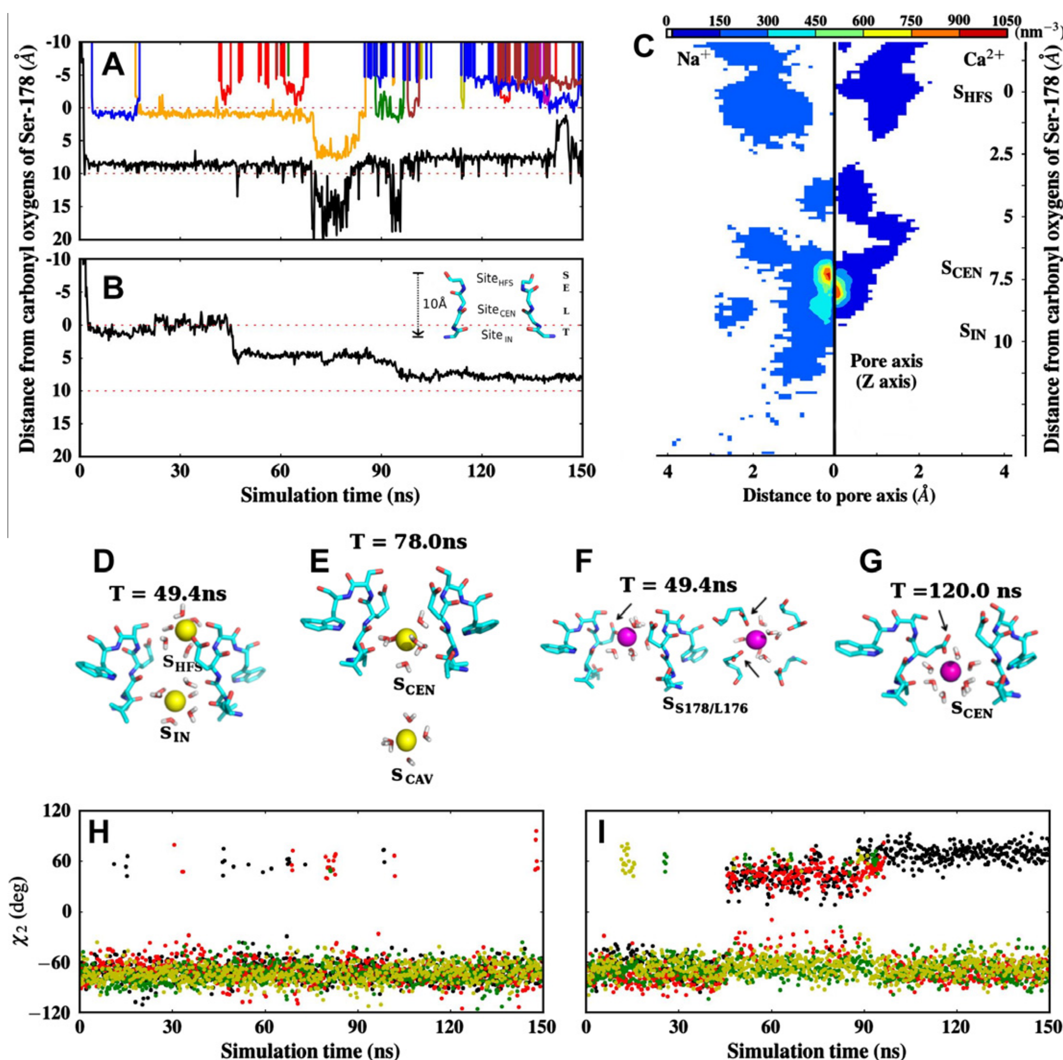

Fig. 1. Na^+ and Ca^{2+} binding patterns inside the Na_VAb SF. (A) Binding patterns of Na^+ ions as a function of time (snapshots from every 200 ps), the *y*-axis depicts the distances along the pore axis (*z*-axis) to the entrance of the SF (carbonyl oxygen atoms of Ser-178) from the extracellular solution; two red dotted lines highlight the entrance and the exit of the SF (backbone nitrogen atom of Thr-175); the inset shows the binding sites suggested by Payandeh et al. [2]. (B) Binding pattern of Ca^{2+} as a function of time (snapshots from every 200 ps). (C) Ion distribution maps of Na^+ s and Ca^{2+} inside the selectivity filter (snapshots from every 10 ps). (D) Filter snapshot of Na^+ ions at 49.4 ns; the Na^+ ions are shown as yellow spheres. (E) Filter snapshot depicting Na^+ ion positions at 78.0 ns. (F) Ca^{2+} snapshots (side and top view) taken at 49.4 ns, with Ca^{2+} shown in magenta; the black arrows indicate the side chain conformational change of Glu-177. (G) Filter snapshot taken at 120.0 ns. (H) Analysis of the χ_2 angle of Glu-177 as of function of time for NaCl (snapshots from every 200 ps); Side chains of the different subunits are colored black, red, yellow and green respectively. (I) Changes of Glu-177 χ_2 angles induced by Ca^{2+} binding as of function of time (snapshots from every 200 ps).

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