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Bead-like passage of chloride ions through ClC chloride channels

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Abstract

The ClC chloride channels control the ionic composition of the cytoplasm and the volume of cells, and regulate electrical excitability. Recently, it has been proposed that prokaryotic ClC channels are H^+-Cl^- exchange transporter. Although X-ray and molecular dynamics (MD) studies of bacterial ClC channels have investigated the filter open–close and ion permeation mechanism of channels, details have remained unclear. We performed MD simulations of ClC channels involving H^+, Na^+, K^+, or H_3O^+ in the intracellular region to elucidate the open–close mechanism, and to clarify the role of H^+ ion an H^+-Cl^- exchange transporter. Our simulations revealed that H^+ and Na^+ caused channel opening and the passage of Cl^- ions. Na^+ induced a bead-like string of $Cl^--Na^+-Cl^--Na^+-Cl^-$ ions to form and permeate through ClC channels to the intracellular side with the widening of the channel pathway.

Keywords: ClC channels; Chloride channels; Molecular dynamics; Conformational change; Selectivity filter; Ion permeation

1. Introduction

The ClC chloride channels, which are ubiquitous channel proteins found in both prokaryotic and eukaryotic cells, play a crucial role in controlling the ionic composition of the cytoplasm and the volume of cells. In skeletal muscle, ClC chloride channels stabilize the resting membrane potential and regulate electrical excitability, thus, the ClC channels are the key enzyme in homeostasis of electrolytes [1–3]. Several structures of the ClC channels have been solved [4,5] and these structural analyses revealed that the ClC channels are homo-dimers in which each of the two identical subunits contains its own independently gated pore. The published X-ray structure of the bacterial ClC channel is a gate-closed state [4] whereas its mutation (E148A) is in a constitutively gate-open state [5]. Several theoretical studies [6–8] have attempted to clarify the molecular mechanism of ClC channels. Recently it has been proposed that prokaryotic ClC channels are H^+-Cl^- exchange transporters [9]. To survive in the acidic stomach, bacteria must extrude H^+ accumulated on the intracellular side in the extremely acidic environment. Bacteria use ClC channels as H^+-Cl^- exchange transporters in which intracellular H^+ moves toward the extracellular side, while extracellular Cl^- passes through to the intracellular side. Despite the availabilities of these experimental and theoretical data about the ClC channels, three fundamental questions remain; 1) what is the open–close mechanism of the gate? 2) how does the H^+ ion take part in the H^+-Cl^- transport mechanism? 3) why does each chain show independent conductance? To answer these questions, we performed molecular dynamics (MD) simulations of the ClC channels, and investigated the dynamics of not only H^+ but also other positive ions, Na^+, K^+ or H_3O^+ on the intracellular side.

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Only when H^+ or Na^+ resided in the intracellular interior of the channel protein, did we observe Cl^- passage to the intracellular side and the reverse movement of H^+ or Na^+ to the extracellular side. Both intracellular H^+ and Na^+ induced the Cl^- passage to the intracellular side, but intracellular Na^+ induced the formation of a bead-like string of Cl^-–Na^+–Cl^-–Na^+–Cl^- ions and the permeation of the bead-like string through ClC channels to the intracellular side. In this paper, we described Cl^- permeation which was driven mainly by intracellular Na^+.

2. Methods

2.1. Simulation methodology

MD simulation is a widely used and effective method to investigate the protein dynamics and its physical properties [10–13]. X-ray structures of ClC from *Salmonella serovar typhimurium* (StClC) (Protein Data Bank (PDB) ID: 1KPL), from *Escherichia coli* (EcClC)(PDB ID: 1KPK) [4], and those of mutant^{E148A} (1OTT) and mutant^{E148Q} (1OTU) from *E. coli* [5] were used as the initial structures for our simulations. The coordinates of the entire N-terminal segment for one of the two monomers (residues 12–32 in chain A) were absent in the X-ray structure, 1KPL. We have modeled this segment by duplicating it from the chain B and splicing it into chain A, because it would play an important role in its function. Each ClC was then placed in a DMPC membrane, which was modified with partial charge calculated using RHF/6–31*G single-point calculation with Gaussian 98 (Gaussian Inc.) and the restrained electrostatic potential method, and was solvated with TIP3P water molecules [14]. After the neutralization of the system by the random positioning of Na^+ and Cl^- (intracellular region for Na^+ and extracellular region for Cl^-), the solvent and counter ions except for ions in the pore were optimized by a 5000 steps of energy minimization while the position of ions in the pore and solute were fixed. Next, the solute and ions in the pore were optimized while the position of the counter ions and solvent atoms were kept frozen. All MD simulations were carried out using Amber ver. 7 [15] on personal computers (Pentium III 933 MHz × 32). The bond length involving hydrogen atoms was constrained to equilibrium length by the SHAKE method [16] and the time step was set at 1 fs. Amber parm96, a parameter set for molecular mechanical force fields used for simulations of biomolecules, was adopted [17]. The systems (including ~100,000 atoms) were heated to 300 K for 0.05 ns and equilibrated for 1.95 ns (total of 2.0 ns) in the NPT ensemble, with periodic boundary conditions and particle mesh Ewald method. The temperature and pressure were kept constant at 300 K (with a time constant of 1.0 ps) and 1 atm (with a relaxation time of 0.2 ps), respectively. In some simulations, during the equilibration, a voltage of 100 mV was applied across the pore (direction from the inside to the outside of the cell) to mimic the membrane potential and enhance the ion permeation [10].

2.2. Simulation systems

We examined the following systems (Fig. 1C): P0: 1KPL was made to hold Cl^- in the channel protein interior without 8 intracellular Na^+. P1: Eight Na^+ were located in the deep

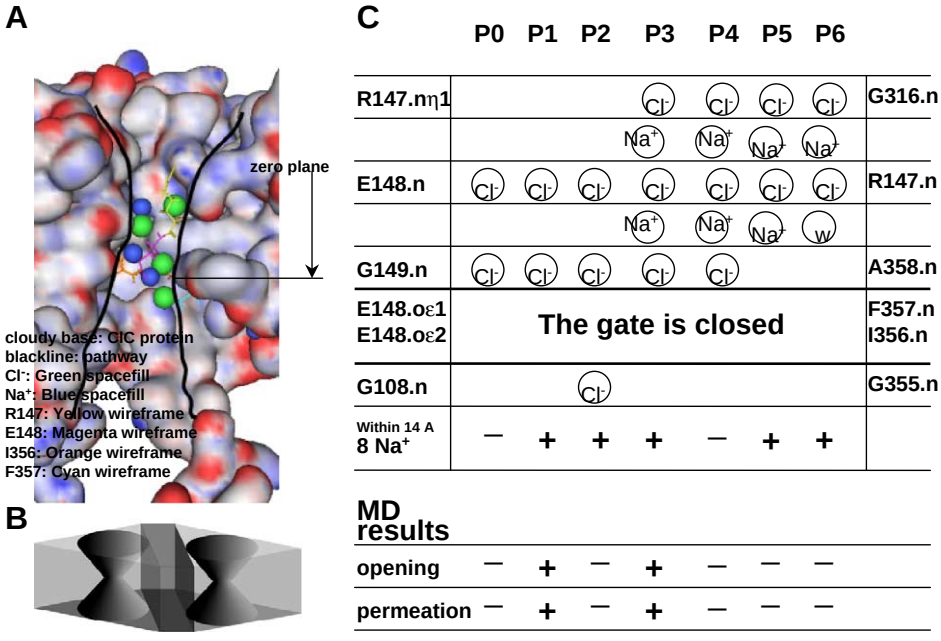

Fig. 1. Simulation systems. Channel pathway of the StClC (chain A). The ClC channel is represented by surface model which is colored according to electrostatic potential (the color is shaded red to blue corresponding to negatively to positively charge). Cl^- is shown light green spacefill model; Na^+, blue spacefill model; R147a, yellow wireframe; E148a, magenta wireframe; I356a, orange wireframe; F357a, cyan wireframe. The level of zero plane is shown (see Numerical calculation). B. Illustration of the ClC channel with the narrowest part (gate). The channel has two independent hourglass-shaped pores. C. The initial structure of each system is illustrated. The interface among E148.Oε1, E148.Oε2, I356.N, and F357.N forms the gate of the selectivity filter (neck of the hourglass-shaped pore). The gate is closed in initial structures. Other both side atoms are nitrogen atoms which bound with Cl^- ions in StClC channel simulations.