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Effects of ion interactions with a cholesterol-rich bilayer

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ABSTRACT

Previous molecular dynamics (MD) simulations of ion-lipid interactions have focused on pure phospholipid bilayers. Many functional microdomains in membranes have a complex composition of cholesterol and phospholipids. Here, we reveal the distinctiveness of the interactions and the effects of the ions on a cholesterol-rich bilayer by performing MD simulations of a cholesterol-rich bilayer with a Na^+/K^+ mixture or a $Na^+/K^+/Ca^{2+}/Mg^{2+}$ mixture. The simulations reveal that Ca^{2+} maintains its dominant role in the interaction with the cholesterol-rich bilayer, but the binding affinity of Mg^{2+} to the cholesterol-rich bilayer is even weaker than the affinities of Na^+ and K^+, whereas its interaction with pure phospholipid bilayers is strong and is only slightly weaker than that of Ca^{2+}. Additionally, it was found that the presence of additional divalent cations induces the headgroups of phospholipids to be more perpendicular to the membrane surface, reducing the lateral movement of lipids and slightly altering the ordering and packing of the cholesterol-rich bilayer, different from divalent cations, which strongly influence that ordering and packing of pure phospholipid bilayers. Therefore, this study indicates that cholesterol in the membrane could affect the interactions between membrane and cations. The findings could be helpful in understanding the biological processes relevant to regulation of cations in cholesterol-rich regions.

1. Introduction

Biological membranes in cells provide a barrier between the inside and outside environments and are involved in a variety of cellular functions such as signal transduction, substance exchange, and cell–cell communication and recognition [1]. Biological membranes are complex assembled structures that consist mainly of phospholipids, proteins and cholesterol, their compositions and distributions regulated differently for different membranes [2]. In physiological environments, biological membranes are immersed in solutions of salt ions mainly including Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- that are considered the most important biologically relevant salt ions. Simplified lipid bilayer models are often used to investigate the interactions and the influence of salt ions on the membranes. The simplest case of a membrane based on pure phospholipid bilayers has been extensively studied [3–13]. Lipid mixtures were also investigated to extend the understanding of the specificity of ionic binding [14–21]. These studies have revealed that various membrane properties such as bilayer thickness, order parameters, lipid mobility, and surface charge density can be influenced by ion binding.

Molecular dynamics (MD) simulation, a powerful tool for investigating the specificity of ion binding, has been widely and successfully used to probe the effects of ions on the lipid bilayers. The effects of ions on the properties of mixed lipid bilayers have been experimentally measured and numerically simulated [19–21]. However, cholesterol is also a key component of biological membranes and is known to modify membrane properties in a number of important ways [22]. The incorporation of certain levels of cholesterol in the membrane increases the packing density of phospholipids and reduces the mobility of lipids [22–24]. As a result, cholesterol may affect the extent of ion absorption and thus the strength of the influence on the membrane. Recently, Magarkar el al [25]. carried out MD simulations of interactions between Na^+

Abbreviations: POPE, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine; POPS, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylserine; Chol, Cholesterol.

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ions and phospholipid bilayers with increasing levels of cholesterol and showed that increasing the level of cholesterol decreases Na^+ binding. However, the understanding of the interactions and effects of ions on the cholesterol-rich bilayer has not yet been fully elucidated.

Here, we performed MD simulations to study the structures and dynamics of cholesterol-rich bilayers interacting with Na^+, K^+, Ca^{2+}, and Mg^{2+} ions. A bilayer model consisting of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylserine (POPS), and cholesterol (Chol) was designed. For clarity, this designed bilayer model was denoted as the PPC bilayer. Based on the PPC bilayer model, we first constructed a simulation system with a mixture of Na^+, K^+, Ca^{2+}, and Mg^{2+} ions. Considering that divalent cations may play a dominant role in altering membrane properties [9], another system containing a mixture of Na^+ and K^+ ions was also generated as a control for comparison.

2. Materials and methods

To investigate the interactions and effects of cations on the cholesterol-rich bilayers, we constructed a PPC bilayer model composed of 62 POPE (~50 mol%), 22 POPS (~17 mol%), and 44 Chol (~33 mol%). Because the physiological concentrations of Na^+ and K^+ ions are much higher than those of Ca^{2+} and Mg^{2+} ions, we constructed one system ($PPC_{NaKCaMg}$) of cholesterol-rich bilayers with 80 Na^+, 80 K^+, 16 Ca^{2+}, and 16 Mg^{2+} ions. To investigate whether divalent cations play the same dominant role in altering cholesterol-rich bilayers properties as they do in pure phospholipid bilayers reported in our previous studies [9,13], another system (PPC_{NaK}) with 80 Na^+, 80 K^+ was constructed as a control for comparison with the $PPC_{NaKCaMg}$ system. Each system was solvated with ~28000 TIP3P [26] water molecules, and Cl^- ions were added to net-neutralize the systems. More details of the two simulation systems are shown in Fig. S1. Simulation parameters used in the present study are similar to our previous published simulations [13] and are given in the Supplementary Material.

3. Results

3.1. Cation-lipid interactions

The effects of the cations on the structural and dynamic properties of a bilayer are directly associated with the cation-lipid interactions. Therefore, we start analysing the cation-lipid interactions for both systems by performing calculations of bound ions and ion-lipid bonds. The computed radial distribution functions (RDFs) of the lipid oxygen atoms relative to the cation position were used to define ion binding. An ion within the cut-off distance from the oxygen atoms was considered a bound ion. The cut-off distances for Na^+, K^+, Ca^{2+} and Mg^{2+} ions are 0.31, 0.36, 0.30, and 0.24 Å, respectively [10]. Fig. 1 shows the time evolution of fractions of different bound ions. It was found that fractions of the bound Na^+ and K^+ ions reached equilibrium quickly, whereas an increasing number of Ca^{2+} ions were observed to bind to lipid oxygen atoms before 700 ns in our simulation systems, indicating that a long time is necessary for the MD simulations of the divalent ions interacting with the cholesterol-rich bilayers. At the same time, the fluctuation of the numbers of bound Na^+ and K^+ ions are much greater than that of Ca^{2+} ions, suggesting that the mean lifetime for a calcium-lipid complex is longer than that for the other cation-lipid complexes. Moreover, while the average percentage of bound Ca^{2+} ions in the last 200 ns of simulation for the $PPC_{NaKCaMg}$ system is ~100%, it was found that less than ~5% of K^+ and Na^+ ions bind to lipids in all simulation systems, and all the Mg^{2+} ions are distributed in aqueous solution in the $PPC_{NaKCaMg}$ system.

To clearly elucidate the composition of the bound ions, Table S1 presents statistics for the bound ions in different systems based on the last 200 ns of MD trajectories. In the PPC_{NaK} system, ~3.6 of 80 Na^+ ions and ~4.0 of 80 K^+ ions are bound to the lipid bilayer. After divalent Mg^{2+} and Ca^{2+} ions were added into the bilayers, less bound Na^+ and K^+ ions (~1.5 Na^+ ions and ~1.5 K^+ ions in $PPC_{NaKCaMg}$ system) were found, demonstrating that the binding of Na^+, K^+ ions with cholesterol-rich lipids can be affected by the bound divalent ions. All 16 Ca^{2+} ions bind the negatively charged PPC bilayer. However, no bound Mg^{2+} ion was observed. Therefore, of the four studied ions, Ca^{2+} ions are always the predominantly bound ions and should display dominant effects on the structure of the cholesterol-rich bilayers.

We investigated the cation-lipid interaction modes that reflect the intrinsic properties of the binding between cations and lipids. We first obtained the statistics for the numbers of ions that interact with one, two, three, and four lipid molecules. While the major bound Na^+ and K^+ ions coordinate with either one or two lipids, most of the bound Ca^{2+} ions interact with three or four lipids (Fig. 2A–B). To further elucidate which lipid oxygen atoms are usually involved in each ion-lipid interaction, the numbers of different ion-oxygen coordinated pairs were calculated. As shown in Fig. 2D, Ca^{2+} ions interact with both POPE and POPS oxygen atoms in the $PPC_{NaKCaMg}$ system. With respect to the specific location of bound oxygen atoms, Ca^{2+} ions interact with phosphate oxygen atoms of POPE and carboxyl oxygen atoms of POPS (Fig. 2D). For bound Na^+ and K^+ ions, all four modes (ion-phosphatidyl, ion-carbonyl, ion-hydroxy, and ion-carboxyl), among which the ion-hydroxy bonds are the least observed, contribute to the ion-lipid interaction (Fig. 2C–D). At the same time, it should be noticed that monovalent cations can coordinate with cholesterol by an ion-hydroxy bond in the cholesterol-rich bilayers (Fig. 2C–D).

3.2. Effects of ion mixtures on the PPC bilayer

The work described above elucidated different cation-lipid interactions for two simulation systems with different ion mixtures. To reveal the influences of ion mixtures on the structure of cholesterol-rich bilayers, we calculate six structural and dynamical parameters, i.e., headgroup orientation, bilayer thickness, area per lipid, ordering of hydrophobic tails, diffusion coefficient, and charge density distributions, as described below.

The interaction of the cations with the membrane may alter the orientation of lipid headgroups. The orientation of a lipid headgroup was defined as the angle (θ) between the P→N vector (from the phosphorus atom to the nitrogen atom in the headgroup) and the outward normal axis of the bilayer (Fig. 3A). This angle specifies whether the lipid headgroups are parallel or perpendicular to the membrane surface. Fig. 3A shows the distribution probability of θ in the PPC bilayer for the two systems with different ion mixtures. The peak of the profiles for POPE and POPS molecules in the PPC_{NaK} system are ~82.4° and ~72.7°, with almost identical average values of θ (~77.1° and ~77.6°). Interestingly, in the $PPC_{NaKCaMg}$ system, two peaks of the angle probability distribution profiles for both POPE and POPS could be found. The two peaks of the profiles for POPE and POPS in the $PPC_{NaKCaMg}$ system are ~68.2°, ~87.1° and ~32.8° and ~52.9°, with the average θ values of ~67.5° and ~58.2°, respectively. These results indicate that the presence of additional divalent cations results in a significant reorientation of the lipid headgroups. The angle probability distributions for both POPE and POPS were broadened and shifted toward lower angles, especially for anionic POPS lipids, suggesting that the headgroups of POPE and POPS in the $PPC_{NaKCaMg}$ system are more perpendicular to the membrane surface than those in the PPC_{NaK} system. This result is