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Ca^{2+}–dimethylphosphate complex formation: Providing insight into Ca^{2+}-mediated local dehydration and membrane fusion in cells

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Abstract

Earlier studies using X-ray diffraction, light scattering, photon correlation spectroscopy, and atomic force microscopy, strongly suggest that SNARE-induced membrane fusion in cells proceeds as a result of calcium bridging opposing bilayers. The bridging of phospholipid heads groups in the opposing bilayers by calcium leads to the release of water from hydrated Ca^{2+} ions as well as the loosely coordinated water at PO-lipid head groups. Local dehydration of phospholipid head groups and the calcium, bridging opposing bilayers, then leads to destabilization of the lipid bilayers and membrane fusion. This hypothesis was tested in the current study by atomistic molecular dynamic simulations in the isobaric–isothermal ensemble using hydrated dimethylphosphate anions (DMP^-) and calcium cations. Results from the study demonstrate, formation of DMP–Ca^{2+} complexes and the consequent removal of water, supporting the hypothesis. Our study further demonstrates that as a result of Ca^{2+}–DMP self-assembly, the distance between anionic oxygens between the two DMP molecules is reduced to 2.92 Å, which is in close agreement with the 2.8 Å SNARE-induced apposition established between opposing bilayers, reported earlier from X-ray diffraction measurements.
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Keywords: Membrane fusion; SNAREs; Calcium bridging; Opposing bilayers; Local dehydration

1. Introduction

In cells, specialized proteins called SNAREs (t-SNAREs in the target membrane, and v-SNARE in the vesicle membrane) overcome repulsive forces to bring opposing membranes close to within a distance of ~2.8 Å, imparting specificity and regulation to membrane fusion (Jeremic et al., 2004a,b). Studies using light scattering and X-ray diffraction measurements of SNARE-reconstituted liposomes demonstrate that fusion proceeds only when Ca^{2+} ions are available between the t- and v-SNARE-apposed membranes. Studies show that no fusion between vesicles occurs when t-SNARE-vesicles and v-SNARE-vesicles are first mixed, prior to the addition of calcium (Jeremic et al., 2004b). The result suggests that hydrated calcium $[Ca(H_2O)_6]^{2+}$, measuring ~6 Å, is too large to fit between the ~2.8 Å t- and v-SNARE-apposed membranes space (Jeremic et al., 2004b), hence unable to bridge the opposing membrane bilayers.

Calcium ion is essential to a number of life processes and is present in every living cell. It participates in diverse cellular activities, such as metabolism, secretion, proliferation, muscle contraction, cell adhesion, learning, and memory. Although calcium is abundantly present within the cell, it is well sequestered and is available only on demand. Upon certain stimulus, Ca^{2+} concentrations in specific nano environments of the cell are elevated by several orders of magnitude within a brief period (<1 ms). This prompt mobilization of Ca^{2+} is essential for numerous physiological functions, such as membrane fusion, leading to the release of neurotransmitters, enzymes, or hormones. Experimental evidence strongly suggests calcium bridging of opposing bilayers, leading to the release of water from hydrated Ca^{2+} ion as well as the loosely coordinated water at the PO-lipid head group. Local dehydration of phospholipid

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head groups is expected to destabilize bilayers, leading to membrane fusion (Jeremic et al., 2004a,b). This hypothesis was tested in the current study by atomistic molecular dynamics simulations in the isobaric–isothermal ensemble, using hydrated dimethylphosphate anions (DMP^-) and calcium cations. Results from the study demonstrate formation of $DMP–Ca^{2+}$ complexes and the consequent removal of water, supporting the hypothesis.

2. Methods

2.1. Simulation

NPT molecular dynamics simulations were performed at 298 K and 101.325 kPa on a system containing 2000 water molecules, 11 Ca^{2+} cations and 22 dimethylphosphate anions (DMP^-). Periodic boundary conditions and the minimum image convention were applied in all three dimensions. Electrostatic interactions were calculated using particle-mesh Ewald. Lennard–Jones interactions were truncated after 14 Å. The CHARMM force field version 27 was used to model all interactions between molecules, including water (Foloppe and MacKerell, 2000). Simulations were initiated from a random initial configuration created by Packmol (Martinez and Martinez, 2003). NAMD version 2.6 was used for all simulations (Phillips et al., 2005), while visualization of molecular configurations and other data analyses were performed with VMD version 1.8.6 (Humphrey et al., 1996). Simulations were performed for 20 ns (16 ns equilibration, 4 ns production) using a 2 fs time step. The volume of the system reached its average value of 61.4 nm^3 ($r = 1.06$ g/cm^3) within 200 ps. Simulations were run for an additional 10 ns beyond the 20 ns reported in this work and no significant changes were observed in the Ca^{2+}–O(water) RDF after a 16–20 ns period.

3. Results and discussion

Molecular dynamics simulations in the isobaric-isothermal ensemble at 298 K and 101.325 kPa was used to determine whether Ca^{2+} was capable of bridging opposing phospholipid head groups in the early stages of the membrane fusion process. As previously utilized in the study of nucleic acids (Guan et al., 1994, 1995; Schneider et al., 1996), DMP^- was chosen for the current study since it represented the smallest molecular fragment of typical membrane phospholipids that retained properties of the phospholipid head group, while providing a significant reduction in the computational complexity, and hence enhance accuracy of the study. Furthermore, the strategy of using the DMP^-, rather than full phospholipids, in these simulations facilitates the search for spontaneously formed Ca^{2+}–phospholipid structures, which may bridge the head groups of opposing phospholipid bilayers. The DMP^- fragments can undergo free translational and rotational diffusion, as well as rapid internal conformation changes, so that structure formation with Ca^{2+} is explored rapidly and comprehensively, whereas full membrane phospholipids can exist only in self-assembled bilayers that restrain the motion of individual phospholipids molecules and greatly lengthen the time scale for structure formation. For example, in the phospholipid bilayer simulations of Bockmann and Grubmuller (2004), sequential binding of Ca^{2+} to phospholipids carbonyl oxygens within a single bilayer was incomplete even after 150 ns of simulation time, and those simulations did not include mechanisms for Ca^{2+} bridging of opposing bilayers. However, we have to remain aware of the possibility that both the initiation of membrane fusion due to Ca^{2+} bridging of opposing bilayers and the entire membrane fusion reaction occur within different time frames and different kinetics. It is the initiation of membrane fusion of fusion via calcium and the organization of participating atoms in this process with which we have been concerned in this report.

In Fig. 1, the volume fluctuations for the first 4 ns of simulations are presented, illustrating the rapid equilibration of the system with respect to density. While the volume of the system was stable after 200 ps, significant changes in the microstructure were observed between 0.2 and 8 ns following initiation of simulation (Fig. 2). Snapshots of molecular configurations observed during the course of the simulations are shown in Fig. 2. Radial distribution functions (RDF) were calculated at various points during the course of the simulation, to quantify the local order in the vicinity of Ca^{2+} and the anionic oxygens of DMP^-. In Fig. 3, the radial distribution function for Ca^{2+} interacting with the oxygen of water is shown as a function of time step. In the early stages of the simulation, all calcium ions are found associated with water. However, as the simulation progresses, preferential association of Ca^{2+} with the anionic oxygen of DMP^- limits the number of water molecules that may associate with Ca^{2+}. The result is a significant reduction in the height of the Ca^{2+}–O(water) peak of the RDF over time. After 8 ns of simulation, the emergence of ring structures involving two Ca^{2+} and two DMP^- was observed. An example of such a ring complex observed after 17 ns of simulation is shown in Fig. 4. The average distances between Ca^{2+} and the anionic oxygens of DMP^-, and the resultant bond angles formed by O–Ca–O were determined by taking

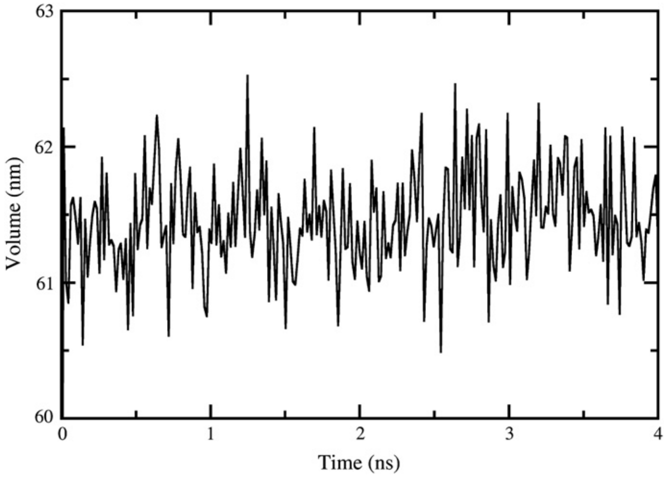

Fig. 1. Volume as a function of time, for NPT molecular dynamics simulations at 298 K and 101.3 kPa.