



A Course-Grain Molecular Dynamics Simulation Study on the effects of varying amount of NaCl on *dioleoyl-phosphatidylcholine* (DOPC) and *dipalmitoyl-phosphatidylcholine* (DPPC) Lipid Bilayers

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Abstract: The structural and physical changes of adding small amount of NaCl ions on dioleoyl-phosphatidylcholine (DOPC) and dipalmitoyl-phosphatidylcholine (DPPC) Lipid Bilayers is presented in this study. Eight systems of 128 DOPC and DPPC lipids solvated in water and equilibrated at 323 K and 1 atm pressure were prepared with varying amounts of NaCl (0, 10, 20, and 30). Each system was simulated using MARTINI course grain force field and the GRoningen MACHine for Chemical Simulations (GROMACS). The physical properties of each lipid bilayer system such as the area per lipid, order parameter, bilayer thickness and lateral diffusion coefficients were calculated. For the DOPC lipid-systems with NaCl, the calculated area per lipid and lateral diffusion coefficients are ranging from 0.621 nm² to 0.624 nm² and 0.522 ± 0.049 μm²s⁻¹ to 0.526 ± 0.049 μm²s⁻¹, respectively. For the DPPC lipid-systems with NaCl, the calculated area per lipid and lateral diffusion coefficients are inversely proportional to the number of NaCl. The area per lipid and lateral diffusion coefficients are ranging from 0.573 nm² down to 0.568 nm², and 0.800 ± 0.037 μm²s⁻¹ down to 0.629 ± 0.0014 μm²s⁻¹, respectively. Addition of low amounts of NaCl on DOPC and DPPC lipids minimally changes the bilayer thickness and order parameter of the lipid systems. The results of this study indicate that addition of low amounts of NaCl ions minimally changes the physical properties of DPPC and DOPC lipids.

Key words: dioleoyl-phosphatidylcholine (DOPC); dipalmitoyl-phosphatidylcholine (DPPC); GROMACS and MARTINI Force field; Course –grain Molecular dynamics; NaCl ions

1. INTRODUCTION

Lipid bilayers are important components of biological cells and play an important role in different cell membrane dynamics. It provides a mechanism to regulate the passage of nutrients, wastes, and small molecules into the membrane. Majority of these membranes are zwitterionic or electrostatically neutral which comprises about 90% of all lipid membranes types (Brockmann et al, 2003). Most of the uncharged lipids belong to the

phosphatidylcholines (PC) groups, a class of phospholipids that is often found in the *Chlorella vulgaris* algae. This alga has recently gained considerable attention on biofuels research because of its high oleic acid content and on its potential application as an alternative source of energy (Manrique et al, 2014).

Biological membranes are normally found in aqueous solution containing different ions (eg. Na⁺, Ca²⁺, Cl⁻, I⁻) of varying concentration. The



interaction of these ions with the molecular components of the cell is important in different membrane processes (Bockman, et al 2003). Several studies indicate that salt induces different structural and physico-chemical changes in the lipid bilayer. These include, but not limited to, reduction of area/lipid, changes in bilayer thickness and order parameter, and the reduction of the lateral diffusion coefficient (Bockman, et al., 2003, Pandit, et al 2003, Filippov, 2009, and Garcia and Rodrigues, 2011). The addition of ions also alters the electrostatic properties of the lipid bilayer which cause the enlargement of water spaces, weakening of the Van der Waals forces, and swelling of the lipid membrane (Petrache, et al, 2006 and Aroti, et al 2006).

The compelling evidences of the salt-induced effects on lipid bilayer properties could have a possible application on the on-going search for a cost-effective mechanism of removing water from the *Chlorella Vulgaris*. One of the important issues in using algae as an alternative source of biofuel is its high water content. To minimize the growth of microorganisms and to increase the fuel yield from *Chlorella Vulgaris*, the water content should be reduced to at least 10% (Becker 1994 and Viswanathan, et al, 2011 cited by Manrique, et al, 2014). There are several methods employed in removing water from the algae but majority of these are technology intensive and require high energy input (Culaba et al, 2013 cited by Manrique, et al 2014). To further understand the mechanism of water removal from *Chlorella Vulgaris*, Manrique, et al (2014) studied the behaviour of water in DOPC in the molecular scale. Using course grain molecular dynamics simulation, they show that physical parameters such as temperature and osmotic pressure can induce lipid property changes. They have shown successfully that a minimum osmotic pressure of 69.2 MPa is enough to facilitate water removal in the membrane, an important result in view of finding a cost effective method in algae drying process. Altering the physico-chemical structure of the lipid membrane, through the addition of ionic salts might offer an additional solution in lipid dehydration.

This work presents a part of an on-going study that investigates the effect of different ionic salts on lipid bilayer properties. This study particularly explores the effect of small amounts of NaCl ions on the properties of zwitterionic lipid dioleoyl-phosphatidylcholine (DOPC) and dipalmitoyl-phosphatidylcholine (DPPC) membranes. It specifically aims to determine the changes on the physical properties, such as area/lipid, order parameter, and lateral diffusion coefficient, of neutral lipids in the low salt concentration regime.

2. METHODOLOGY

To determine the physical changes brought about by the addition of small amount of NaCl ions, the study utilized course grain molecular dynamics simulation (CG- molecular dynamics) using the MARTINI force – field. The MARTINI uses a 4:1 mapping scheme, where on average, four atoms are represented by a single interaction center (Marrink, et al, 2007). MARTINI considers only four types of interaction sites to simplify the model. The DPPC lipid, for instance, is represented as charged choline (NC3) and phosphate (PO4) groups, non-polar glycerol (GL) and apolar hydrocarbon tails (C) (Marrink, et al., 2007, Bennun, et al., 2009). Compared with an all atom models, course graining of molecules in MARTINI results to a computationally more efficient model and significantly reduces the simulation time. For the starting structure, pre-equilibrated 128 DOPC and DPPC lipids and solvated were used with 1500 and 2000 course grain water, respectively, giving an effective hydration of 11 – 16 waters/lipid. The lipid systems were arranged in a periodic box with dimensions 7.02505 nm x 6.27717 nm x 15.25120 nm.

Four systems with varying amount of Na and Cl ions were prepared for each lipid types. The ions were initially placed above the lipid system before the simulation to model the from-the-top distribution of the ions on the lipid. The first simulation system was composed of pure DOPC and water only; the second consisted of 10 ions (5 Na and 5 Cl); the third had 10 Na and 10 Cl ions; and the fourth contained 15 Na and 15 Cl ions. The same procedure was repeated for the DPPC lipid system. All the simulations were run for 500 ns with 0.02 ps time-step and were conducted at constant 323 K temperature and 1 atm pressure. The coulomb type interaction was changed to reaction field, the pressure coupling was set off and semi isotropic pressure coupling type was chosen. All the calculations were conducted using the open source GROMACS 5.01 simulation suite (Spoel D et al., 2005).

3. RESULTS AND DISCUSSION

3.1 Area per lipid and order parameter

The effect of varying ion concentrations on the area per lipid and order parameters of DOPC and DPPC lipids were analyzed. The area per lipid is one of the important properties of lipids bilayers as it describes the asymmetric distribution of lipids head groups in the simulation box (Manrique et al, 2014). The area per lipid is computed from the area of the x-y plane of the simulation box divided by half of the

total number of the lipid head groups. The lipid system has fully equilibrated within 100 ns (see figure 1).

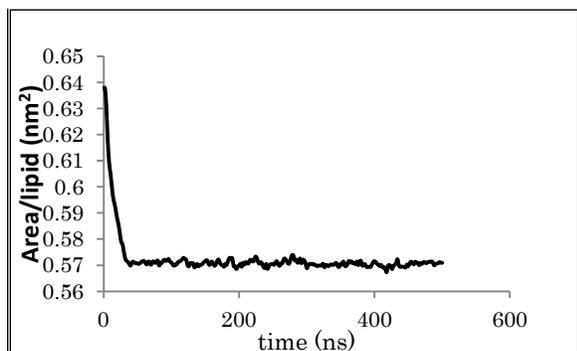


Fig 1. Equilibration of the area per lipid

Figure 2 shows the area/lipid of both DPPC and DOPC for varying amounts of Na and Cl ions. For salt – free DOPC, a value of 0.621 nm² was obtained, a little lower than 0.623, 0.623, and 0.624 nm² for DOPC with 10, 20, and 30 NaCl ions, respectively. The values obtained in this study were in close agreement with the results obtained in the MD studies of Gurtovenko (2006) (0.652 ± 0.002 nm²) and Bockman (2003) (0.655 and 0.606 nm²) involving the effect of NaCl ions on POPC lipids. The area per lipid of the salt-free DPPC is 0.573 nm², but this increased to 0.623 and 0.624 nm² for DPPC with 10 and 20 NaCl ions, respectively. The obtained values decreased to 0.568 nm² when number of ions was increased to 30. The results in this study agree with the wide range of values for PC lipids found in the literature that addition of NaCl minimally decreases the area/lipid of PC membranes (T.B. Stanishneva-Konvalova, O.S. Sokolova, 2014, P. Jurkiewicz et al., 2012, López Cascales, 2006, Pandit, 2003, Jorge R. Rodriguez and Angel E. García 2012, Bockman et al. 2003, Gurtovenko et al. 2006, Pandit et al. 2003). The binding of the ions to the lipid head groups might explain the increasing compaction of the lipid resulting to a reduction of the area/lipid (Pandit et al. 2003).

The ordering of the lipid hydrocarbon tails can be determined through the calculation of the order parameter. The order parameter is defined as

$$S_{CD} = \left\langle \frac{3}{2} \cos^2(\theta) - \frac{1}{2} \right\rangle \quad (\text{Eq. 1})$$

where:

S_{CD} = Order parameter

θ = angle between the CD bond and the bilayer normal

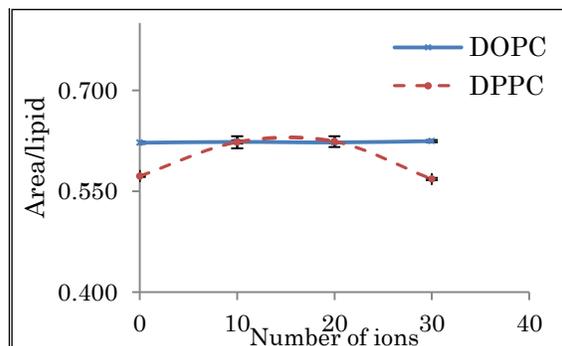


Fig. 2. The calculated area/lipid of the DOPC and DPPC for varying amounts of NaCl.

In the simulations, it was observed that the order parameter for DOPC minimally changed from 0.315 (NaCl-free) to 0.316 (with NaCl) upon addition of NaCl ions. It was worth noting that for DOPC, increasing the amount of NaCl in the system did not significantly change the ordering of the hydrocarbon tails. On the other hand, the addition of NaCl ions on DPPC, resulted to a very minimal increase of the order parameter from 0.537 (salt-free) to 0.539 (DPPC with 30 ions). The slight increase of the order parameter in the DPPC is reflected on the slight reduction of the area/lipid. This reduction caused the hydrocarbon tails to slightly change their orientation with respect to the bilayer normal.

3.2 Bilayer Thickness

Figures 3 and 4 show the number density profiles of the phosphate heads of the NaCl-free lipids and DPPC and DOPC lipids, respectively. The difference between the two corresponding peaks is a measure of the bilayer thickness. The density profiles of the PO₄ heads for both lipids (with and without NaCl) were almost identical except for the relative change in position of the peaks. The addition of NaCl ions slightly shifted the position of the phosphate heads along the z-axis but does not significantly change the thickness of the bilayer. For both lipids and varying amount of ions, the bilayer thickness remained significantly unchanged (4.2703 nm). This result implies that for the lipid system considered in this study, the addition of Na and Cl ions do not significantly altered the thickness of the bilayer.

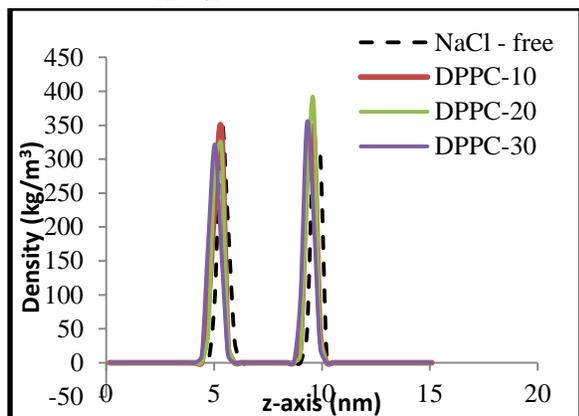


Fig 3. DPPC with varying NaCl ions

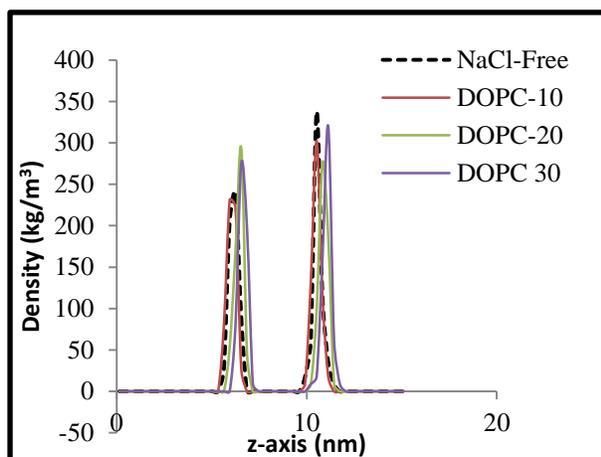


Fig 4. DOPC with varying NaCl ions.

This observation agrees with the results obtained by Petrache et al (2006), where addition of potassium bromide (KBr) and potassium chloride (KCl) did not significantly alter the bilayer thickness of 1,2-dilauroyl-sn-glycero-phosphatidylcholine (DLPC). All-atom molecular dynamic studies of Bockman et al (2003) and Pandit et al, (2003), on the other hand, showed that increasing the number of NaCl on POPC increased the bilayer thickness because of the electrostatic repulsion of the ions located at the phosphate heads. Gurtovenko et al (2008) observed membrane compression upon addition of NaCl to POPC lipid. The difference in the results obtained in this study with those found in the literature (Bockman, et al 2003, Pandit et. al 2003, and Gurtovenko et al. 2008) could be attributed to the difference in the simulation parameters (force

field, ensemble, simulation time, and the lipid-type) used in the respective studies.

The number density profiles of Na⁺, Cl⁻ and PO₄ with respect to the z – axis, for both DPPC and DOPC, were plotted and shown in figures 5 and 6, respectively. A close inspection of the graphs reveals that Na⁺ ions peak at the phosphate head region while Cl⁻ accumulates around the membrane surface close to Na⁺. Figure 5 shows the molecular dynamics visualization of the ion screening at the membrane surface. The electrostatic attraction between the positive sodium ion and the anionic phosphate head could explain this observation (ion-pairing) (Vacha et al. 2009). The density profile of Cl peaks only at the surface close to the position of Na⁺, a clear manifestation of the counter-ion effect of chlorine (Vacha et al., 2009). It is worth noting that the ions were unable to penetrate beyond the phosphate head group region (screening effect). In a related work, Sach et al (2004), using all-atom MD, observed that Na and Cl ions have limited capability to deeply bind into the bilayer because of their smaller sizes. Smaller ions are screened at the membrane surface because they are easily attracted towards the oppositely charge component at membrane's surface (Vacha et al. 2009). In contrast, larger ions like iodide and perchlorate, due to their size, polarizability and ion-pairing with the choline group, are easier absorbed into the membrane (Vacha, et al. 2009).

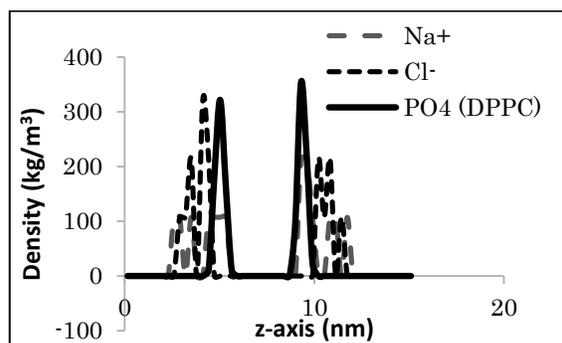


Fig. 6. Number density profile of Na⁺ Cl⁻ and PO₄ for DPPC

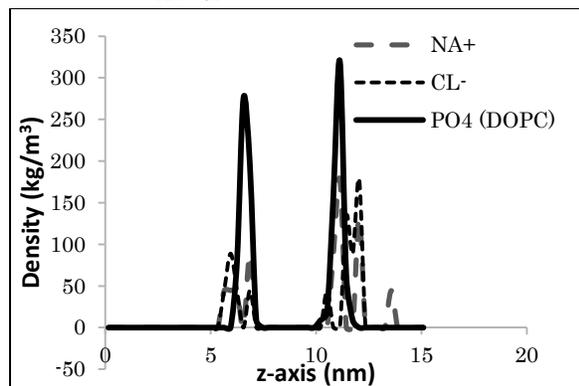


Fig. 6. Number density profile of Na⁺ Cl⁻ and PO₄ for DOPC

3.3 Lateral Diffusion

The addition of NaCl ions affecting the mobility of lipid head groups during the bilayer formation was also investigated. The lateral diffusion coefficients (DL) of DPPC and DOPC were measured for varying NaCl ion concentrations. The lateral diffusion coefficient was measured by calculating the slope of the linear region of the lipid mean square displacement (MSD) curve. To improve the accuracy of the result, the effect of the lipid's center of mass was removed in each simulation. The values and standard error of the calculated lateral diffusion coefficient are shown in figure 7.

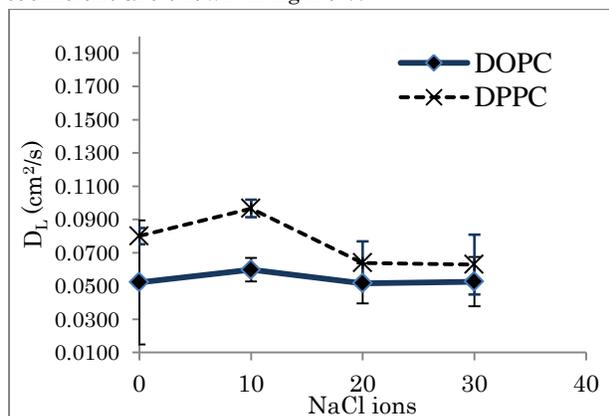


Fig. 7. The lateral diffusion coefficients of DPPC and DOPC lipids with varying amounts of NaCl ions.

For the DOPC, DL ranges from $0.522 \pm 0.0049 \mu\text{cm}^2\text{s}^{-1}$ for NaCl – free system and $0.526 \pm 0.018 \mu\text{cm}^2\text{s}^{-1}$ for the system with 30 ions. Similarly, DPPC's lateral diffusion ranges from 0.800 ± 0.037

$\mu\text{cm}^2\text{s}^{-1}$ down to $0.629 \pm 0.0014 \mu\text{cm}^2\text{s}^{-1}$. The overlapping standard error of the mean for all the systems imply that the effect of varying amount of NaCl ions did not significantly alter the mobility of the lipids. This result is consistent with the non-invasive pulse field NMR measurement of DL for DOPC bathed in NaCl and CaCl₂ (Filippov, et al 2009). Fillipov et al (2009) work shows that the diffusion coefficient of DOPC is unaffected by the addition of salt. The values computed in this study were much higher than the values obtained using the all-atom simulation found in the literature (Bockman, et al. 2003, and Gurtovenko, et al. 2008) but comparable to results of Karjiban et. al (2013) using MARTINI course grain molecular dynamics.

4. Conclusion

DOPC and DPPC lipid bilayers were successfully simulated in the presence of varying amount of NaCl ions. The physical properties of the lipid membrane, such as area per lipid, bilayer thickness, order parameter, and lateral diffusion coefficient were comparable to the wide range of values obtained in the literature. This study was able to show, using the course grain molecular dynamics simulation, that addition of low amount of NaCl ions induce minimal effects on lipid bilayer properties. Screening of ions at the lipid membrane was observed. Sodium ions, in particular, coordinated with the anionic phosphate heads while chlorine ions converged at the membrane surface close to the Na⁺ ions, a manifestation of counter-ion effect.

5. Recommendation

This study is part of a preliminary work that explores the effect of different types of ionic salts on the physico-chemical properties of zwitterionic lipid bilayers. Further investigations can be made by changing the ion types and the physical parameters of the system. The effect of temperature, pressure, ion type, and large ion concentration on lipid bilayer properties, particularly on compression and dehydration should be considered in the future.

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