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Mini-Review

Phase Behavior of Binary Mixtures of SOPC and Cholesterol

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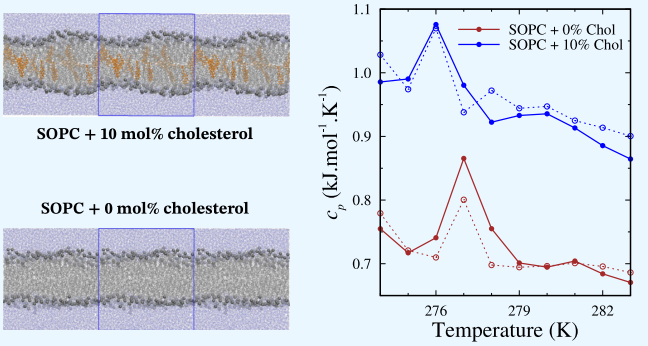
ABSTRACT: The major components of cell membranes are phospholipids. Due to their amphiphilic structure, in a solution they arrange in bilayers. The phase behavior of lipid bilayers and thus their functions, such as cellular organization, cellular transport, membrane fusion, drug delivery, and others, are susceptible to temperature changes and/or admixing with biologically active materials. A typical representative of phospholipids is SOPC (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) possessing a saturated hydrocarbon acyl chain and an unsaturated one, exhibiting a single cis double bond. Cholesterol plays a primordial role in maintaining the mechanical stability of the cell membrane. We present a comprehensive review of the phase behavior of the binary mixture of SOPC with cholesterol as a function of the temperature explored via atomistic molecular dynamics and Slipids force field in the vicinity of the experimental melting point, T_m = 279 K, corresponding to the temperature driven phase transition from the gel phase (L_β) to the liquid disordered phase (L_α). The behavior of the thermodynamic properties and structural characteristics with different concentrations of cholesterol show that the pure SOPC bilayer and its counterpart mixed with 10 mol % cholesterol exhibit phase transitions at 277 and 276 K, respectively, and that cholesterol reinforces the fluidity of the bilayer leading to the emergence of a liquid ordered phase (L_o). At cholesterol contents larger than 30 mol %, the bilayer exhibits a liquid ordered phase (L_o) at any temperature. The ensuing phase diagram is found to reproduce reasonably well its counterpart constructed experimentally.



1. INTRODUCTION

Already at the end of the 19th century, the hypothesis was put forward that biological cells possess a protective shell, that is a membrane composed of oleophilic substances dubbed "lipoids" (a historical overview can be found in ref 1). Nowadays, it is widely accepted that the membrane can be regarded as a fluid mosaic model containing phospholipids, carbohydrates, and proteins. Phospholipids are amphiphilic molecules with two nonpolar hydrophobic fatty acyl chains and a polar hydrophilic phosphate head with a glycerol backbone. Depending on the number of double bonds in the composition of the hydrocarbon tail, lipids fall into two categories: saturated and unsaturated. In a bulk aqueous solution, phospholipids spontaneously assemble in bilayers corresponding to the most thermodynamically stable molecular structure thanks to the hydrophobic effect inherent to phospholipid acyl chains, both saturated and unsaturated. The lateral distribution of phospholipids stems from the balance of attractive interactions (van der Waals, capillarity, $\pi-\pi$ stacking, hydrogen bonds, entropic forces) and repulsive ones (steric effects, related to headgroup size and Coulomb repulsion among similarly charged lipids). Understating the complex molecular processes taking place in cell membranes in the presence of bioactive materials allows us to gain useful knowledge in the role of admixtures on the physical properties and function of the membranes and to pinpoint potential applications.

Biological membranes consist of heterogeneous mixtures of phospholipids distinguishable by their headgroup structure, hydrocarbon chain length, degree of unsaturation of the acyl chains, and conformation of their hydrocarbon chains. The most common lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin.[2] Phosphatidylcholine is involved in a number of metabolic processes[3] as well as the repair of the cell membrane surface. Phosphatidylethanolamine plays a vital role in blood clotting and together with phosphatidylserines increases the rate of thrombin formation,[3b] while sphingomyelin, as well as other sphingolipids, forms rigid domains in the cell membrane.[3c] A fifth less common type of phospholipid, namely, phosphatidylinositol, also participates in the composition of membranes. Although it is almost negligible in amount compared with the other membrane components, it

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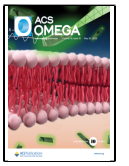

plays an important role in cell signaling. In addition, the lipid bilayer contains phosphatidic acid, phosphatidylglycerol, and sterols, such as cholesterol, that act as membrane fluidity regulators, since they react to the proximity of acyl chains. The latter lipids possess a polar hydroxyl group attached to the A ring and a nonpolar aliphatic chain.

The physical properties and functions—cellular organization, cellular transport, membrane fusion, drug delivery, and others—of lipid bilayers, are highly sensitive to temperature variations and/or admixing with different kinds of lipids and proteins.[2,4] Bilayers containing a single species of lipids exhibit qualitatively distinct phases with specific physical properties. The transition from one phase to another is induced by the variation of an intensive thermodynamic quantity, such as temperature, pressure, chemical potential, etc. At the transition point, some macroscopic physical quantities, such as the heat capacity and bilayer thickness, display abrupt changes. At high temperatures lipid bilayers melt in a liquid solution to form an isotropic liquid disordered phase (L_α) with disordered hydrocarbon chains (trans-gauche conformation). Lowering the temperature, the phospholipids begin to self-assemble, and beneath the main/melting transition temperature, say T_m, one observes the onset of either one of two distinct ordered gel phases with upright (L_β) or tilted ($L_{\beta'}$) relative to the normal of the bilayer stretched hydrocarbon chains (all-trans conformation) arranged in a compact flat bilayer. For $L_{\beta'}$, the tilt angle increases with dilution in the solvent. At even lower temperatures, the membrane solidifies in a highly ordered solid phase (S_o) with hindered mobility of the lipids. In the case of hydrated bilayers with a mixture of different phospholipid molecules, a ripple phase ($P_{\beta'}$) characterized by periodic ripples may show up between the $L_{\beta'}$ and L_α phases.

Admixing the lipid bilayer with cholesterol drastically affects the lipid bilayer's structure and function. The placement of cholesterol molecules in the bilayer obstructs the acyl chain ordering promoting the trans conformation leading to a rich phase behavior depending on the temperature and the amount of cholesterol in the bilayer. A schematic phase diagram of the lipid cholesterol mixture is depicted on Figure 1. While low concentrations of cholesterol result in the emergence of a small coexistence region around the melting temperature, T_m, at very high concentrations a liquid ordered (L_o) phase characterized by simultaneous enhancement of tails ordering (particular to the gel phase) and the heads mobility (particular to the liquid state) shows up and the melting temperature is smeared out. At intermediate cholesterol concentrations, a coexistence phase of liquid ordered (L_o) and gel phase (L_β) takes place at lower temperatures and a coexistence of liquid disordered (L_α) and liquid ordered (L_o) phases at higher temperatures. The line separating both coexistence phases is slightly (about 5 K) below the melting temperature regardless of cholesterol concentration. The phase diagram of lipid bilayers mixed with cholesterol has universal features, bearing striking similarities for all kinds of phospholipids (fully saturated, fully unsaturated, and mono-saturated); nonetheless the phase transition temperatures depend on lipid hydrocarbon chain length and degree of saturation.[5]

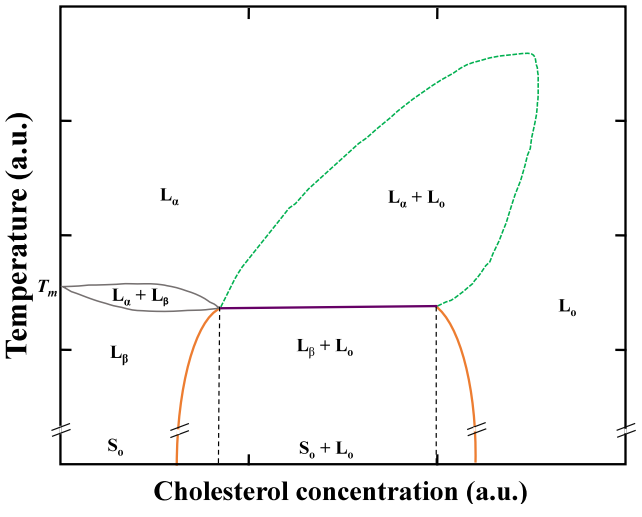

Figure 1. Schematic phase diagram of the SOPC-cholesterol binary mixture as a function of the temperature in arbitrary units. The symbols designate the homogeneous equilibrium states with S_o denoting solid ordered, L_β - gel, L_α - liquid disordered, and L_o - liquid ordered.

The qualitative behavior of the phase diagram is corroborated by experimental and theoretical studies of membranes consisting of various kinds of lipids. For example, the experiments on membranes consisting of an unsaturated lipid like DOPC (one double bond in each of the acyl chains) showed that the associated phase behavior can be described satisfactorily with the aid the typical lipid phase diagram.[6] This lipid has a higher T_m, but the tendency for the L_o state between 10 and 30 mol % cholesterol is preserved. For a binary mixture of cholesterol and the saturated DSPC lipid, the presence of both upright (L_β) and tilted ($L_{\beta'}$) was established and the main transition to L_α was found to lie above T_m = 323 K.[7] Domain formation in mixtures of the saturated DPPC or DMPC and cholesterol systems at the transition are unraveled by MD and a hexagonal arrangement of lipids has been shown.[8]

The phospholipid SOPC (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) falls into the phosphatidylcholine group (18:0/18:1) with a saturated sn-1 hydrocarbon acyl chain and an unsaturated sn-2 one hosting one cis double bond, bringing about a kink that facilitates the membrane fluidity. The phase diagram of a SOPC-cholesterol binary mixture as a function of the temperature is obtained experimentally via DSC and proton MAS NMR.[9] It is largely similar to that in Figure 1 except for the nonexistence of the gel (L_β) phase beneath T_m at an arbitrary cholesterol concentration. In other words, there is solely a transition from the solid ordered (S_o) phase to the liquid disordered (L_β) phase in SOPC without cholesterol and their coexistence with the liquid ordered (L_o) phase under the effect of cholesterol. More recent experimental studies[10] aiming at determining the structural, mechanical, and thermodynamic properties of hydrated SOPC bilayers containing different amounts of cholesterol were conducted using various techniques: DSC calorimetry, FTIR spectroscopy, and Raman scattering. DSC analysis of the thermal properties revealed that pure SOPC exhibits a temperature driven first-order phase transition from gel (L_β) to liquid disordered (L_α) phase. On the other hand, dilution of SOPC in different amounts of water drastically affects the behavior of the thermodynamic quantities due to the augmentation of hydrogen bonding of water with the C═O carbonyl or P═O phosphate groups and sn-2 chain. Different DSC heating rates were probed, and it was found that the optimal one corresponds to 278 K/min. Furthermore, the transition temperature slightly increases with the degree of hydration between 10 and 33 wt % of water leading to T_m = 279 K. DSC experiments were carried out at 5 K/min heating and

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cooling rates on systems of SOPC with different amounts of cholesterol in a water solution of 20 wt %; the obtained results revealed that the optimal concentrations to reach effective miscibility of SPOC and cholesterol lies below 30 mol % cholesterol content. In the L_β phase, increasing the cholesterol concentration in the SOPC bilayer decreases the gauche/trans population ratio leading to a liquid ordered phase (L_o). At 50 mol % cholesterol reinforces the tail ordering of the SOPC bilayer leaving the headgroups mobile suggesting a liquid ordered phase (L_o). Overall, the experimental studies carried out showed that the phase behavior of the probed systems is consistent with the schematic phase diagram depicted in Figure 1.

The present review gives a comprehensive account of our knowledge of the thermodynamic properties and structural behavior of cholesterol-containing SOPC bilayers obtained via extensive molecular dynamics over a wide range of temperatures in the vicinity of the melting temperature. We point out that such an exhaustive study of the phase diagram of mixtures of phospholipids (saturated or unsaturated) with cholesterol as a function of the temperature has not been undertaken so far. We will focus our attention on the temperature domain in the vicinity of the experimental melting temperature T_m = 279 K in the pure bilayer and the effect of admixing with various amounts of cholesterol. Furthermore, we will explore the phase diagram in the temperature−cholesterol concentration plane, depicting the boundaries of the equilibrium phases. We will complement our investigation by analyzing the behavior of the lipid-cholesterol mixture at very high temperatures, where the system is expected to exhibit a purely disordered phase. A schematic representation of the molecules of SOPC and cholesterol is shown in Figure 2.

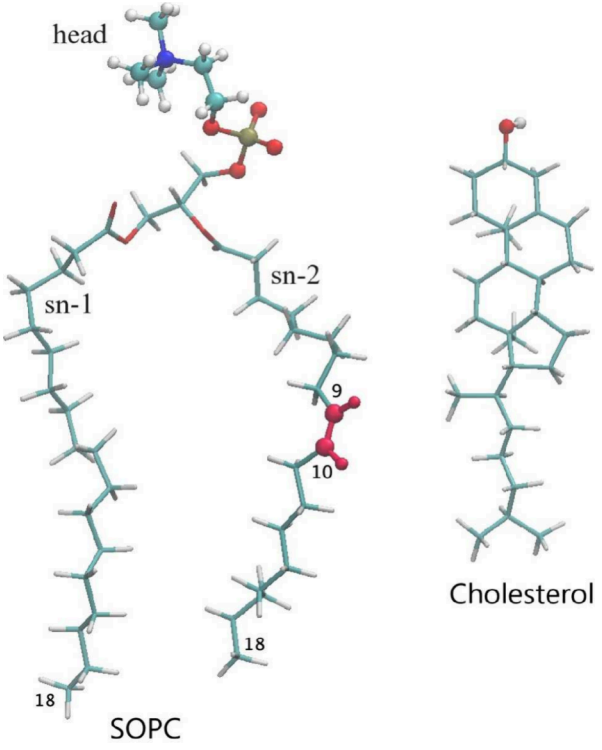

Figure 2. Structure of the molecules of SOPC lipid and cholesterol. The hydrophilic head, and the double bond in the unsaturated acyl chain and the hydroxyl group of cholesterol are emphasized.

The review is organized as follows: Section 2 is devoted to an overview of the computational methodology used to probe the physical properties of lipid membranes along with a short introduction to the different force fields. Section 3 analyzes the behavior of some thermodynamic and structural quantities and discusses the simulation results for the phase diagram in the vicinity of the experimental melting temperature. The review concludes with Section 4, where we summarize the results on molecular modeling of cholesterol-containing SOPC membranes and discuss potential future developments.

2. MOLECULAR DYNAMICS SIMULATIONS

2.1. The Force Field. Atomistic computer simulations carried out via molecular dynamics (MD) techniques allow for the theoretical investigation of complex lipid membranes by analyzing the activity of the constituent atoms over a period of time. They provide useful insights into the most probable mechanisms underlying their physical properties and biological functions. Thus, these methods are used to study the organization and dynamics of biological systems and the conformational changes taking place. Simulations are usually based on classical force fields (FFs) that have proven to be essential due to their unrivaled computational efficiency.

FFs used in MD simulations of lipid molecules must be capable of reproducing both the chemical structure and the complex physical behavior related to their characteristic phases. Another important aspect is the interaction of phospholipids with water molecules, which also has a non-negligible influence on the behavior of the membranes. To account for the multitude of interactions among all atoms, the chemical bonds between them, and the intermolecular interactions, three different formulations of FFs are available in the literature. They differ in the manner the atoms in the target lipid molecules are treated. (i) All-atom FF offers parameters for the interaction of every single atom, including the hydrogen atom. It involves more computational power, as the approximations are kept to a minimum. FFs suitable for simulating phospholipid bilayers are CHARMM, Slipids, Lipid14, and OPLS-AA. (ii) United Atom FFs do not account for nonpolar hydrogen atoms while methyl (CH_3) or methylene groups (CH_2) are grouped in a single unit. Whence, three of four atoms are collected to form one grain with associated specific parameters. GROMOS, OPLS-UA, TraPPE, and C36-UA were used for lipid simulations. (iii) Coarse-grained (CG) FFs bring about the greatest approximation to model systems. In this case, three to four heavy atoms are grouped in one grain. Thus, for example, a lipid molecule containing about 130 atoms is reduced to 12 beads. These fields offer a substantial computation speedup and allow for quite long simulation times. When choosing CG FF, one must take into account the nature of the approximations in the chemical structure and its potential impact on the studied phenomenon. Typical representatives of these fields are MARTINI, Shinoda−DeVane−Klein Force Field, and Multiscale-CG with Force Matching. A comprehensive review on biologically relevant FFs and their implementation to model different sorts of lipids with an extensive list of references can be found in ref 11. These models, although not specific to particular lipids, offer valuable insights into the general properties of binary mixtures.

For the simulations of the SOPC model systems considered in the present review, we use the Slipids (Stockholm lipids) FF[12] that involves optimized parameters for a broad range of phospholipids. Using MD simulations, the named FF is validated against experimental data of relevant physical

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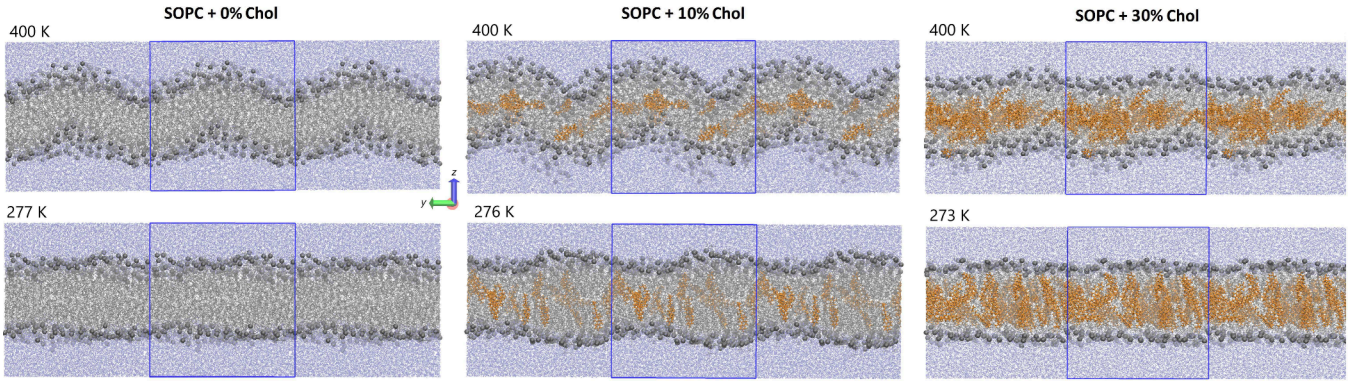

Figure 3. SOPC lipid bilayers at high temperature and after cooling for the studied concentrations (0, 10, and 30 mol % cholesterol). The periodic box is shown in blue, and phosphorus atoms are emphasized.

quantities, such as the area per lipid and the lateral diffusion coefficient for pure phospholipids and mixed lipid bilayers with different cholesterol concentrations. In general, it performs satisfactorily despite not being able to accurately describe the behavior of the hydrocarbon tails.

Most studies on SOPC lipid patches with the aid of Slipids FF were performed at relatively high temperatures. Here, we report results on the transferability of this FF to different phases at low temperatures in the vicinity of the experimental gel (L_β) to the liquid ordered (L_α) melting temperature of SOPC and its ability to reproduce to some extent the phase diagram of the binary mixture of this lipid with cholesterol. To this end, a series of studies[13] developed and explored several systems of the unsaturated SOPC lipid containing different concentrations of cholesterol and at different temperatures close to the experimental main transition temperature. We analyze the behavior of various physical quantities, such as the mass density distribution, the electrostatic potential, the heat capacity, the order parameter and the tilt angle of the lipid tails, the area per lipid and the lateral diffusion coefficient, the radial distribution function, and the hydrogen bonding. Moreover, we make comparisons to experimental and atomistic simulation data of bilayers of similar phospholipids available in the literature.

2.2. Computational Details. To test the accuracy of the Slipids FF,[12] initial studies aiming at finding the optimal initial structures of SOPC bilayer were conducted using MD. Moreover, we have used two fully prepared patches with initial coordinates provided along with the Slipids FF distribution from one hand and those generated with CHARMM-GUI[14] from the other. Both systems contain 128 SOPC molecules and 5120 water molecules interacting via the TIP3P model.[15] The systems were investigated at seven different temperatures (273, 275, 276, 277, 279, 281, and 283 K) at a pressure of 1 bar.[13] The atomistic molecular dynamics simulations were carried out in the NPT ensemble with the aid of Gromacs 4.4.7,[16] and the analysis was performed over 100 ns long trajectories. The obtained results confirmed that the newest version of Slipids is superior to the earlier one and that the initial structures built up using CHARMM-GUI are better suited to reproduce the main experimental features of SOPC.

Using the CHARMM-GUI tool, binary bilayers composed of 256 lipid molecules of SOPC and different amounts of cholesterol molecules (ranging from 10 to 50 mol % concentration) were constructed.[13c] The distribution of the molecules is symmetric in the two monolayers of the membrane. The five systems differ negligibly in periodic box sizes and water molecule saturation. The ensuing bilayers are simulated at a temperature of 273 K and a pressure of 1 bar in the NPT ensemble. The duration of the relaxation trajectory was set to 400 ns, and the physical quantities of interest were calculated over the subsequent 50 ns production run. Here, we have used the atomistic MD simulations package Gromacs 2021.3[16] in conjunction with Slipids.[12] The FF was found to perform reasonably well, and various experimental data were reproduced fairly well. Moreover, the concentration of 30 mol % cholesterol was found to be critical, as the studied properties are smooth functions of the temperature.

Extensive simulations examining the effect of hydration on the main features of the lipid bilayers were performed.[13d] A binary SOPC bilayer with 50 mol % cholesterol was examined with three different degrees of hydration (25, 40, and 50 water molecules per lipid). For the sake of comparison, the TIP4P water model more suitable for ice[17] was also used. It was concluded that the largest hydration degree does not lead to significant changes in the bilayers but increases the computational time, suggesting that an optimal value is 40 water molecules per lipid and that the water model TIP3P is the most appropriate for molecular dynamics simulations of SOPC bilayers.

Binary SOPC systems with 0, 10, and 30 mol % of cholesterol content were studied. The lipid bilayers were examined in the neighborhood of the experimentally determined main transition temperature $T_m = 279$ K.[10] Each of the three systems is relaxed at 271 K, and the final coordinates of the bilayer are used for subsequent heating by 1 K to the next temperature; the procedure is repeated until the highest temperature is reached. The duration of the trajectories at each temperature for the three bilayers is 3 μs.[13e] To ascertain that the system is indeed in the liquid ordered rather than the liquid disordered phase, additional simulations were performed at 400 K. The analyses show that the Slipids FF is able to describe the melting phase transition, and the corresponding temperatures are determined (277 K for 0 mol % and 276 K for 10 mol %).

To elucidate the transitional behavior of the binary mixture of SOPC with cholesterol, the final configurations at 400 K of the three systems were cooled to the lowest temperature in the studied interval. Thus, brand-new extensive MD simulations were conducted with a cooling process performed at a rate of 10 K/ns along with an annealing process. The thermostat used for cooling is a V-rescale in combination with a C-rescale barostat.[18] Simulations are performed in the NPT ensemble with Nose–Hoover/Parrinello–Rahman[19] at 1 bar pressure. The duration

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of the trajectories is again 3 μs, and snapshots of the initial (at 400 K) and final coordinates for the considered cholesterol concentrations are shown in Figure 3. The integration step is 2 fs with a leapfrog integrator.[20] Simulations for all systems were calculated with Gromacs 2022.4,[16] and the analyses are carried out over the last portion of the trajectory starting from 2.9 to 3.0 μs.

Due to the huge number of degrees of freedom ensuing from the large number of SOPC, cholesterol, and water molecules and the different interactions in the systems under considerations, it turns out that trajectories lasting at least 3 μs are required to obtain a correct description of some physical properties of the systems under consideration. Analysis was performed on the heat capacity at three different values simulation times: 1, 2, and 3 μs. It is found that 3 μs is the optimal value that takes into account the number of atoms in the system and the time necessary for the entire system to relax and reach thermodynamic equilibrium.[13e]

3. THE EFFECT OF CHOLESTEROL ON THE TRANSITIONAL BEHAVIOR OF SOPC

The transitional behavior of binary mixtures of SOPC with cholesterol is explored by computing thermodynamic and structural quantities. Basic characteristics of the membranes, such as the specific heat, the ordering of the acyl chains, and the mobility of the headgroup, were probed by analyzing the outcome of MD simulations. The effect of the concentration of cholesterol on the temperature driven transitional behavior is studied at concentrations as large as 50 mol % in the SOPC bilayer.

3.1. Thermodynamic Properties. Phase transitions are essentially characterized by a sharp peak in the molar heat capacity, c_p.[4] During MD simulations, c_p was calculated with the aid of the built-in Gromacs tool through enthalpy fluctuations. At low temperatures in a close vicinity of the experimental melting temperature, simulation results of the binary mixture of SOPC with cholesterol showed a rapid increase with an abrupt change in the slope at 30 mol % of c_p with the amount of cholesterol in the SOPC bilayer.[13a,c] The absence of melting at concentrations of cholesterol of 30 mol % and higher was confirmed at all temperatures,[13e] while experiments[10] showed that such a behavior takes place above 50 mol %. During heating, in the SOPC bilayer without cholesterol, the heat capacity, c_p, shows two cups at 273 and 277 K. The peak at 273 K is attributed mainly to the thermal fluctuations of the freezing temperature of the water hydrating the bilayers, while the peak at the higher temperature corresponds to the melting of SOPC at T_m = 277 K from a gel (L_β) to a liquid disordered phase (L_α). The heat capacity c_p of the SOPC bilayer mixed with 10 mol % of cholesterol exhibits a single peak at the melting temperature 276 K. To double check this behavior, we performed additional simulations by cooling down the SOPC system from 400 K according to the procedure described above. We found that the behavior of c_p during cooling is broadly similar to that obtained during heating. The heating[13e] and cooling data of the cholesterol free membrane and the one containing 10 mol % are shown in Figure 4. Notice that the melting temperature slightly decreases with an increasing amount of cholesterol in the bilayer, but for high concentration, it is smeared out.

3.2. Behavior of the Acyl Chains. Structural changes of SOPC bilayers due to admixing with cholesterol at different temperatures can be quantified through the deuterium order parameter that can be computed[13c,e] with aid of Gromacs built-in tools. The simulation results based on Slipids FF show that the order parameter values are underestimated compared to available experimental data.[21] Moreover, its profile does not depend smoothly on the temperature, reflecting the fact that L_α is not a pure state. The behavior of the order parameter for both saturated (sn-1) and unsaturated (sn-2) chains shows a strong dependence on the cholesterol concentration and temperature. In the unmixed SOPC lipid bilayer, the behavior of the order parameter reflects a high degree of ordering at low temperatures and a signature of melting at 277 K. The mixed membrane of SOPC with 10 mol % cholesterol shows a lower degree of ordering than its pure SOPC counterpart at low temperatures that might be interpreted as a coexistence phase of gel (L_β) and liquid ordered phase (L_o) and melting at 276 K toward a liquid disordered phase (L_α) coexisting with a liquid ordered phase (L_o) at high temperatures. Tilt angle analysis shows changes in both saturated and unsaturated chains at temperatures of 277 K for the unmixed bilayer and at 276 K for the one mixed with 10 mol % cholesterol. When the amount of cholesterol in the membrane reaches 30 mol %, both acyl chains are highly packed with a higher degree of disorder than both systems with lower cholesterol content. In this case, there is no indication of melting. This behavior is inherent to all SOPC bilayers with higher concentrations of cholesterol.[13c] Such a behavior may be traced back to the larger amount of cholesterol that hinders the mobility of the lipid tails.

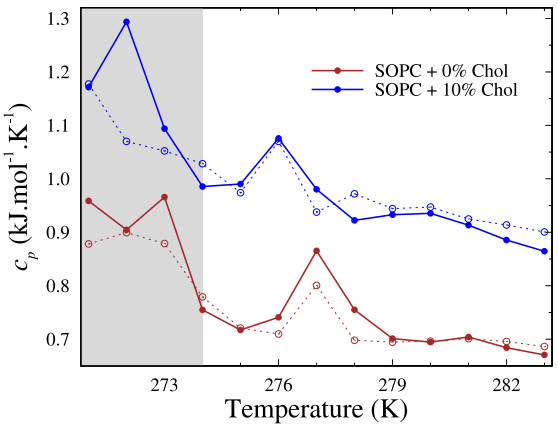

Figure 4. Molar heat capacity at different temperatures for the SOPC lipid without and with 10 mol % of cholesterol. The solid line corresponds to heating and the dotted line to cooling. The shaded area corresponds to the region where water freezing dominates the thermodynamics of the systems.

Further insights into the behavior of the lipid membranes and the effect of cholesterol may be obtained by investigating the behavior of the system during cooling at a very high temperature. At 400 K, the pure SOPC bilayer and its counterparts containing 10 and 30 mol % cholesterol are completely disordered (see e.g. Figure 3). After cooling, all systems recover their degree of ordering in agreement with the behavior of the heat capacity. Whence, the bilayer with 10 mol % cholesterol exhibits a melting transition from a low-temperature region with coexistence of a gel phase (L_β) and liquid ordered phase (L_o) to a domain where the liquid disordered (L_α) and liquid ordered (L_o) phases coexist.

The behavior of the tilt angle of the acyl chains relative to the membrane surface clearly indicates the potential melting temperatures of SOPC bilayers mixed with different amounts

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of cholesterol corroborated the outcome of the molar heat capacity.

3.3. Lipid Headgroup Mobility. Additional information on the membrane ordering can be obtained via the average headgroup area per lipid and the lateral diffusion coefficient, a measure of lipid's translation along the lipid bilayer. The former quantity, computed via Qhull[22] during cooling and heating, is found to depend on the amount of cholesterol in the SOPC bilayer within the considered temperature range. The calculated average area per lipid (Figure 5) in the presence of cholesterol is

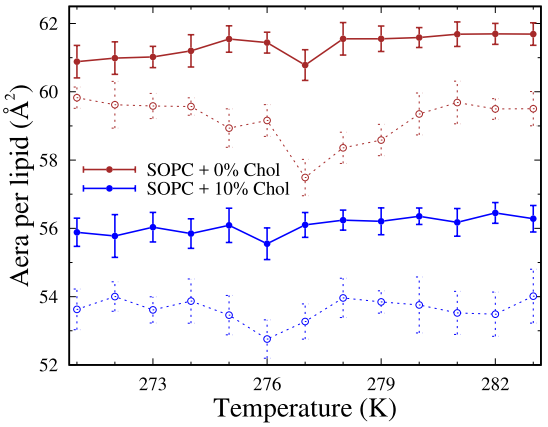

Figure 5. Area per lipid at different temperatures for the systems of only SOPC lipid and with 10 mol % of cholesterol. The solid line corresponds to heating and the dotted line to cooling.

lower than those typical for a gel phase, suggesting a more fluid phase. In the cholesterol-free bilayer and that with 10 mol % cholesterol content, shallow minima are observed at 277 and 276 K, respectively. This indicates that the peak of the heat capacity close to 0 K for both membranes may be traced back to the water molecules, while the other one is a feature of the lipid molecules. The average area per lipid of the SOPC molecules decreases from 61.38 to 49.35 Å^2 as the amount of cholesterol increases from 0 to 50 mol % in the membrane.[13c] On one hand the cholesterol molecules' area per lipid decreases monotonically from 36.50 Å^2 to 40.00 Å^2. This is evidence of the onset of higher degree of fluidity of lipid bilayer with reduced lipid mobility in agreement with experiments.[23]

The lateral diffusion coefficient of about 10^{-8} cm^2 s^{-1} depends weakly on cholesterol concentration with a slightly increasing slope. This behavior suggests a low mobility of the lipid heads in the membrane due the presence of cholesterol that is consistent with the experimental value characteristic of the liquid ordered (L_o) phase.[24] The weak influence of cholesterol on the headgroup lateral diffusion is reported experimentally with NMR spectrometry.[25] As the temperature increases, the lateral diffusion coefficient increases in line with the emergence of a higher degree of fluidity in the mixed bilayers. These findings are commensurate with the behavior of the radial distribution functions for the headgroups.

4. CONCLUSIONS

We explore the temperature-driven transitional behavior of binary mixtures of SOPC bilayers with cholesterol through atomistic molecular dynamics in conjunction with Slipids force field. This force field is known to reproduce reasonably well the experimental data of a number of thermodynamic and structural quantities. We report and analyze recent results of a thorough study of the thermodynamics and structural features on cholesterol-containing lipid bilayers composed of the SOPC molecules mixed with cholesterol in different molar amounts (ranging from 0 to 50 mol %). Simulations were performed by heating the system (water and bilayer) gradually starting from 271 up to 283 K and cooling it down from 400 to 271 K. The behavior of the molar heat capacity and the average area per lipid against temperature reveals that cholesterol affects the transitional behavior of the SOPC bilayer. Increasing the temperature of an SOPC system without cholesterol initially in a gel phase (L_β) melts at 277 K toward a liquid disordered phase (L_α). In a mixed SOPC bilayer with 10 mol % cholesterol, there is a phase transition at 276 K from a low-temperature region where two phases—the gel (L_β) and liquid ordered (L_o) phases—coexist to a high temperature with coexisting liquid disordered (L_α) and liquid ordered (L_o) phases. At 30 mol % and higher concentrations of cholesterol in the SOPC bilayer, there is no phase transition and the bilayer is always in the liquid ordered phase (L_o).

The obtained phase behavior suggests that the Slipids force field reproduces reasonably well the experimental behavior of thermodynamic quantities and the structural properties of binary mixtures of SOPC and cholesterol in the vicinity of the experimental melting temperature of pure SOPC. The structural quantities associated with the hydrophobic tails show singularities at some temperatures, while the behavior of the headgroups, except for the average areas per lipid, is weakly dependent on the amount of cholesterol. On the other hand, the Slipids force field fails to accurately predict the experimental behavior of the order parameter and the critical amount (about 35−40 mol %) of cholesterol above which no phase transition occurs in the binary mixture. Thus, to obtain accurate qualitative and quantitative predictions, the parameters inherent to this FF require further refinement.

The present review focuses on the thermodynamic and structural properties of single lipid species. As a rule of thumb, membranes are composed of a multitude of phospholipids mixed with different sorts of proteins and biologically active elements. Thus, additional clarity in the established phenomena and trends would be provided by enriching the model systems with at least two different types of lipids. Analyses up to the critical concentration of cholesterol will provide information only on the effect of the different structures of the lipid molecules in the highly mixed membrane and will not be attributed to its presence in the layer. The diversity of lipids with different degree of saturation will lead to the appearance of an additional ripple phase ($P_{\beta'}$), which also has its significant contribution to the membrane phenomena that is suitable for further analysis. In addition to the focus on one phase, interest for further consideration is the interaction of several phases realized at the same temperature, a phenomenon that will again arise from the mixing of several types of lipids. Another less considered and important aspect, especially in transport processes, is the ensuing curvature of the membrane. The bending of the bilayer is a significant effect leading to global changes in the entire system and local reorganizations between molecules. The model systems of SOPC and cholesterol presented in the current work also have a clearly noticeable curvature and although it does not exhibit a $P_{\beta'}$ phase at low temperatures, it is an interesting effect and is also worth considering in future studies.

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Notes

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