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A Molecular Dynamics Study of DMPC Lipid Bilayers Interacting with Dimethylsulfoxide–Water Mixtures

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Abstract The diffusion process of dimethylsulfoxide (DMSO) through zwitterionic dimyristoylphosphatidylcholine (DMPC) lipid bilayer was studied by means of molecular dynamics (MD) simulations. To account for the cryoprotectant concentration difference between the inside and the outside of the cell, dual DMPC lipid bilayers which separate two aqueous reservoirs with and without DMSO were modeled. The initial configuration of the simulation model had DMSO molecules present in one of the aqueous phases (outside the cell) at two different concentrations of ~ 3 and ~ 6 mol%. MD simulations were performed on the systems for 50 ns at 323 K and 1 bar. Although the simulation time considered in the study was insufficient for the DMSO molecules to reach the other aqueous phase and equilibrium, early stages of the diffusion process indicated that DMSO molecules had a tendency to diffuse towards the other aqueous phase. The effects of DMSO on bilayer structural characteristics during the diffusion process were investigated. Simulations were analyzed to correlate the following properties of lipid bilayers in the presence of two different aqueous phases: area per lipid, lipid thickness, mass density profiles, lipid tail order parameter and water dipole orientation. Area per lipid calculated for the leaflet facing the aqueous DMSO–water mixture did not show any significant difference compared to area per lipid for the DMSO-free pure DMPC bilayer. Mass density profiles revealed that DMSO molecules had a strong tendency to diffuse toward the aqueous phase with pure water. The lipid tail order parameter calculated for the sn-1 tail of the leaflet facing the aqueous DMSO–water mixture showed that the ordering of lipid tails decreased compared to the leaflet exposed to pure water. However, the ordering of lipid tails in a system where a single bilayer is hydrated by an aqueous DMSO–water mixture is far lower.

Keywords Atomistic study · Structural characteristics · Area per lipid · Mass density profile · Tail order parameter

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Introduction

In a typical cryopreservation protocol, the biological sample to be preserved is first equilibrated with chemical additives or cryoprotective agents (CPAs) to alleviate cell damage from either dehydration or intracellular ice formation due to the subsequent freezing process. Understanding the interactions of CPAs with cell membranes (idealized as lipid bilayers) on the microscopic level is one of the great challenges in cryobiology. Detailed experimental interactions and the structural organization of hydrated lipid–CPA systems have so far been rather well studied (Chang and Dea 2001; Feller et al. 2002; Gordeliy et al. 1998; Kiselev et al. 1999; Merz and Roux 1996; Mou et al. 1994; Scheidt and Huster 2008; Schlichter et al. 2001; Shashkov et al. 1999; Tristram-Nagle et al. 1998; Yamashita et al. 2000; Yu and Quinn 1995, 1998a, b, 2000). Although the experimental approach is still the cornerstone of membrane research, the fundamental properties of lipid membranes have as yet defied explanation. Recent development of new algorithms and revolutionary advances in molecular modeling have provided unique capabilities for analyzing the properties of biological systems from an atomistic perspective with a great deal of insight that is not accessible by current experimental techniques (Merz and Roux 1996; Saiz et al. 2002).

Consequently, computational methods such as molecular dynamics (MD) simulations have become established tools for detailed analysis of the interactions between phospholipid bilayers and various CPAs (Devireddy 2010).

Dimethylsulfoxide (DMSO), an aprotic solvent, is one of the most widely used CPAs in cryopreservation. The cryoprotective property of DMSO was discovered more than 50 years ago by Lovelock and Bishop (1959), and concentrations up to 40 % are used in the cryopreservation of a wide variety of cells (Devireddy and Bischof 1998; Kardak et al. 2007). DMSO is an amphiphilic, polyfunctional molecule with a polar sulfoxide (S=O) group and two hydrophobic methyl (CH_3) groups. Aqueous DMSO induces cell fusion (Ahkong et al. 1975) and cell differentiation (Lyman et al. 1976) and increases cell membrane permeability (Anchordoguy et al. 1992). In most cases DMSO penetrates cell membranes in order to exert its protective ability; this has led to numerous studies and hypotheses about its properties and interactions with biological membranes. However, due to the limitations of current experimental techniques, the intriguing molecular details involved in the transportation of DMSO and water through the cell membranes cannot be revealed. Thus, MD came into play to study the detailed molecular mechanisms involved in the interactions of DMSO with lipid bilayers.

Previously, MD simulations focused on understanding the interactions of a single lipid bilayer with pure DMSO solvent has been studied (Gurtovenko and Anwar 2007; Moldovan et al. 2007; Paci and Marchi 1994; Smondyrev and Berkowitz 1999; Sum and de Pablo 2003). It is reported that a DMSO concentration >9 mol% results in pore formation even in the absence of any externally applied stresses (Gurtovenko and Anwar 2007; Moldovan et al. 2007). However, realistically, in cryopreservation the composition of the cryoprotectant between the intracellular and extracellular environments (the cell membrane being the barrier) is quite different and, therefore, the effects of DMSO on the outer and inner leaflets of a lipid bilayer differ significantly. One approach to this problem is to explicitly consider a dual lipid bilayer membrane wherein an individual leaflet is exposed to different concentrations of DMSO, corresponding to the extra- and intracellular environments. Dual lipid bilayer setups had been previously modeled to perform MD simulations. Sachs et al. (2004) was one of the early ones to establish a transmembrane potential gradient across a double DMPC lipid bilayer configuration and investigate the penetration of Na^+ and Cl^- ions through the bilayer. Later, Gurtovenko (2005) studied the interactions between salt (NaCl) ions and DMPC lipid components using a double lipid bilayer configuration. They also performed MD simulations on single lipid bilayers (with and without NaCl) to compare the properties of the leaflets of the resulting asymmetrical lipid bilayer and those of symmetrical bilayers. In another study, Leekumjorn and Sum (2006) performed MD simulation employing a double DPPC lipid bilayer configuration to study the diffusion process of 5 wt% DMSO molecules. After performing MD simulation for 95 ns at 350 K, they concluded that only about 10 % of DMSO molecules penetrated from an aqueous phase corresponding to the extracellular region (with DMSO molecules) and crossed the lipid bilayer to reach the other aqueous phase (with no DMSO molecules) corresponding to the intracellular region of the membrane. Based on a phenomenological model applying Fick's law, they approximated that after a simulation time of 2,000 ns equilibrium in terms of DMSO concentration would be reached between the two aqueous phases (with and without DMSO).

In the present study MD simulations were performed on a dual DMPC lipid bilayer system in the presence of 3 and 6 mol% DMSO (9 mol% concentration of DMSO started nucleating pores within the lipid bilayers). The dual lipid bilayer simulation model used for MD simulations is shown in Fig. 1. As a reference, single DMPC lipid bilayer systems in the presence (at concentrations of 3, 6 and 9 mol%) and absence of DMSO were also simulated. The structural properties of the DMPC lipid leaflets exposed to a concentration gradient (0 mol% DMSO as intracellular region and 3 or 6 mol% DMSO as extracellular region) are compared to leaflets exposed to systems without any gradient. The structural properties involved were area per lipid, mass density profiles, tail order parameters and water orientation profiles.

Simulation Model and Methodology

MD simulations were performed with the GROMACS MD package (Lindahl et al. 2001). Several sets of MD simulations were carried out on zwitterionic dimyristoylphosphatidylcholine (DMPC), single lipid bilayers immersed in pure water and DMSO–water solution at three concentrations and a dual lipid bilayer configuration, establishing a concentration gradient across the lipid bilayer (see Fig. 1). For the dual lipid bilayer system concentrations of DMSO considered were 3 and 6 mol%. Systems at DMSO concentrations of 3 mol% (5,422 water molecules and 163 DMSO molecules), 6 mol% (5,422 water molecules and 326 DMSO molecules) and 9 mol% (5,422 water molecules and 489 DMSO molecules) were thoroughly investigated with the lipid bilayer in fluid phase and in equilibrium at 323 K. The choice of 323 K for simulation might seem counterintuitive given that cryopreservation protocols require temperatures below 273 K. However, 323 K represents the characteristic temperature for the liquid–crystalline phase in DMPC lipids; thus, DMPC most

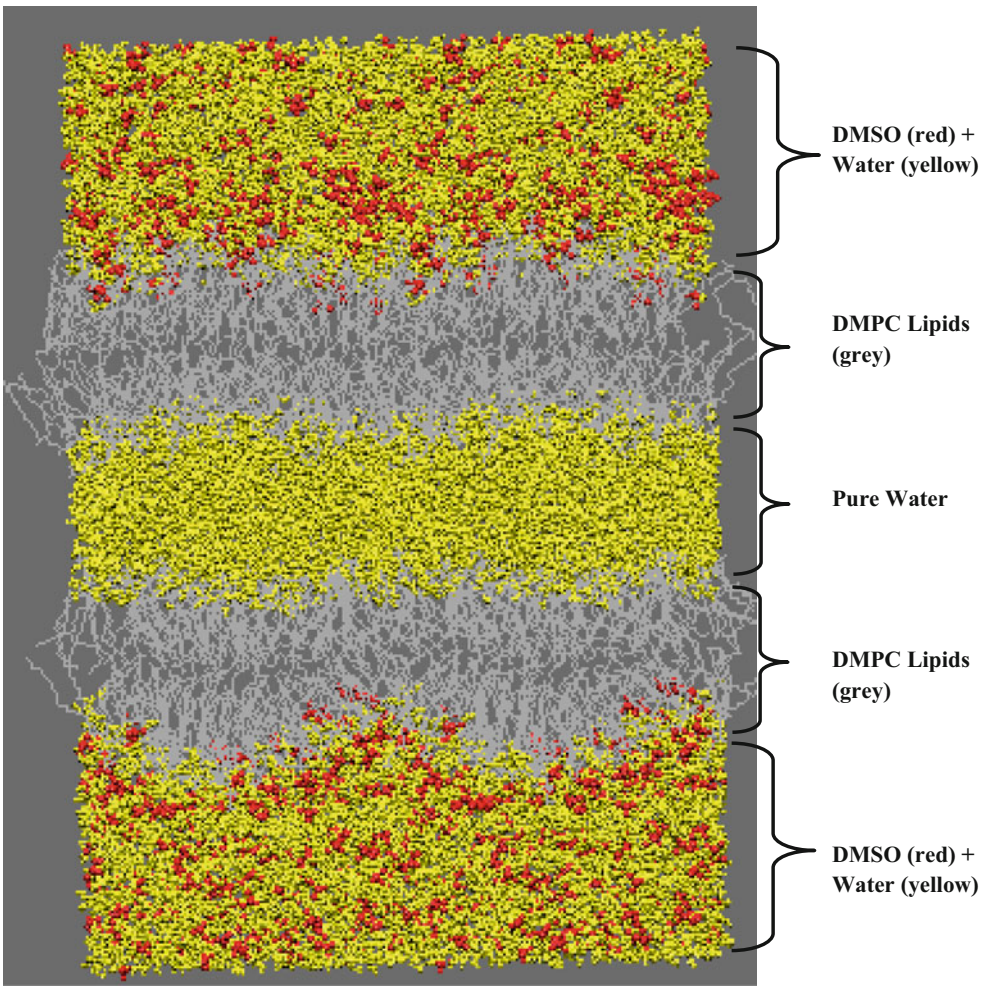

Fig. 1 Snapshot of the dual DMPC lipid bilayer (asymmetric) system at 50 ns of simulation. In the snapshot DMSO molecules are denoted in *red*, water in *yellow* and lipids in *gray*. Note that in the single lipid bilayer system both sides of the lipid will be DMSO–water mixtures (not shown), as opposed to the dual lipid bilayer system where the lipids are exposed to water on one side and the other side is exposed to DMSO–water mixture (Color figure online)

closely mimics the behavior of phospholipids in mammalian cell membranes at this temperature. Hence, our simulations were performed at a bath temperature of 323 K. In all simulations the DMPC bilayer comprised 96 lipid molecules (48 DMPC lipid molecules on each leaftlet).

The force-field parameters for both bonded and nonbonded interactions were taken from Berger et al. (1997), while the partial charges were taken from Saiz and Klein (2001). DMSO molecule interactions were modeled using a GROMACS force field (Lindahl et al. 2001). The simple point charge (SPC) model (Berendsen et al. 1981) was used for water. The bilayer system lies in the *xy* plane, with the bilayer normal parallel to the *z* axis. Periodic boundary conditions were applied along the three spatial dimensions. In the DMPC bilayer system, lipid, water and DMSO molecules were weakly coupled separately to a temperature bath using the Berendsen thermostat (Berendsen et al. 1984) with a coupling time constant of 0.1 ps. The bath temperature was 323 K (characteristic temperature for the fluid phase of the bilayer system). Pressure was maintained at 1 atm using the semi-isotropic pressure coupling to a Berendsen barostat (Hess et al. 1997) with a time constant of 1.0 ps. Accordingly, the height of the simulation box (*z* direction) and the cross-sectional area (*xy* plane) were allowed to vary independently of each other, thereby allowing the area of the bilayer and the distance between the interfaces to fluctuate independently. All bond lengths were kept constant to their equilibrium values by the LINCS algorithm (Hess et al. 1997). The nonbonded Lennard-Jones interactions were cut off at a distance of 1.0 nm, and the simulation time step was set to 2 fs. Long-range electrostatics were updated every 10 time steps and handled by particle-mesh Ewald (PME) algorithm (Essmann et al. 1995). An energy minimization procedure based on the steepest descent algorithm was initially applied to the initial structure prior to the actual MD run.

For all simulations the atomic coordinates were saved every 2 ps for analysis. The DMPC lipid bilayer system was simulated for a total of ~50 ns (for dual lipid bilayer system) and ~100 ns (for single lipid bilayer system immersed in pure water and DMSO–water solution). For the dual lipid bilayer system the last 10 ns of the simulation results were used for data collection/analysis, whereas for the single lipid bilayer system the last 50 ns of the simulation results were used for data collection/analysis. Various structural and ordering parameters characterizing the DMPC lipid bilayers in the presence of different concentrations of DMSO were investigated. For simplicity in the case of the dual lipid bilayer system, the leaflet facing the aqueous phase without any DMSO is denoted as the inner leaflet (facing toward the intracellular region) and the leaflet facing the aqueous phase with DMSO is denoted as the outer leaflet (facing toward the extracellular region).

Results and Discussion

To better understand the structural effects on the cell membranes due to the diffusion process of DMSO between the extra- and intracellular domains, a dual lipid bilayer configuration setup was simulated (Fig. 1). In a typical cryopreservation experiment a transmembrane concentration gradient of DMSO is established across the bilayer. Note that our simulation system closely resembles the physical scenario that a cell experiences during a cryopreservation protocol; i.e., the intracellular space is devoid of DMSO, while the extracellular space is a mixture of DMSO and water. Thus, the dual lipid bilayer closely mimics the exposure of cells to DMSO solution. Although the simulation time considered in the study is not sufficient to observe an equilibrium state, it is significant enough to estimate the structural characteristics of cell membranes.

Figure 1 depicts a snapshot of the dual lipid bilayer simulation system with a concentration gradient between 0 mol% DMSO (pure water, denoting intracellular region) and 3 or 6 mol% DMSO (denoting extracellular region), established across the bilayer after a running time of ~50 ns. It is evident from the snapshot that there is no penetration of DMSO molecules into the aqueous phase representing the intracellular region. The two most important structural characteristics of the lipid bilayers affected during the diffusional process of DMSO are area per lipid molecule and bilayer thickness.

The area per lipid molecule is obtained by dividing the area of the simulation box in the *xy* plane by the total number of lipid molecules in a single leaflet. Figure 2 depicts the time variation of the area per lipid in the outer leaflet of the dual lipid bilayer setup in the presence of 3 or 6 mol% DMSO–water mixture over 50 ns simulation time. For reference and comparison, the corresponding time variations of the area per lipid for bilayer systems in pure water and single lipid bilayer with 3 or 6 mol% DMSO–water solution are also given. The simulation results suggest that the increase in area per lipid of the outer leaflets in the presence of 3 or 6 mol% DMSO was not significant compared to the area per lipid in the pure water system. The increase in area per lipid was quite significant in the single lipid bilayer system in the presence of 3 or 6 mol% DMSO. This result suggests that the concentration gradient of DMSO established across the bilayer in the dual lipid bilayer configuration has an effect over the packing of the lipid molecules in the leaflet. Bilayer thickness is calculated based on the methodology described previously (Pinisetty et al. 2006). Table 1 lists the number of molecules, area per lipid and corresponding lipid bilayer thickness values for the pure water system, the single lipid bilayer system with 6 mol% DMSO and the dual lipid bilayer system with 3 or 6 mol% DMSO. It can be clearly seen that the thickness of the bilayer was reduced with an increase in DMSO concentration. It can also be observed that bilayer thickness in the dual lipid bilayer system was reduced by ~19 and ~28 % for 3 and 6 mol% DMSO, respectively, compared to the pure water system (i.e., 3.6 nm). In the single lipid bilayer system, bilayer thickness was reduced by ~33 and 44 % for 3 and 6 mol% DMSO, respectively. The increase in area per lipid and the corresponding decrease in bilayer thickness (see Table 1) can be correlated such that volume per lipid does not change significantly.

Additional information about the structural effects can be obtained from the mass density profiles for the system including the regions that correspond to the two lipid bilayers, the two aqueous phases and various atomic groups. The mass density profiles are determined by dividing the simulation box along the direction normal to the membrane into a number of thin slices of equal thickness and subsequently determining the total number of atoms located in each slice. The data are then time-averaged over a large number of snapshots evenly distributed over the last 10 of the 50-ns simulated time interval. The density profiles have been shifted for clarity such that the center of the system is always located at $z = 0$. The overall mass density profiles of DMPC lipid, water and DMSO are shown in Fig. 3 across the double lipid bilayer configuration setup for 3 mol% (Fig. 3a) and 6 mol% (Fig. 3b) concentrations of DMSO. In Fig. 3a, b the region for $|z| < 0.5$ nm corresponds to the pure water aqueous phase, which also is the center of the double lipid bilayer setup.

At locations $1.9 < |z| < 2.1$ nm and $3.2 < |z| < 3.5$ nm one can identify the inner leaflet–pure water interface region and the outer leaflet–water/DMSO interface region, respectively, in which the lipid density profile exceeds the solvent density. The location $2.5 < |z| < 3$ nm corresponds to a region where the alkyl chains of the two lipid leaflets meet. It

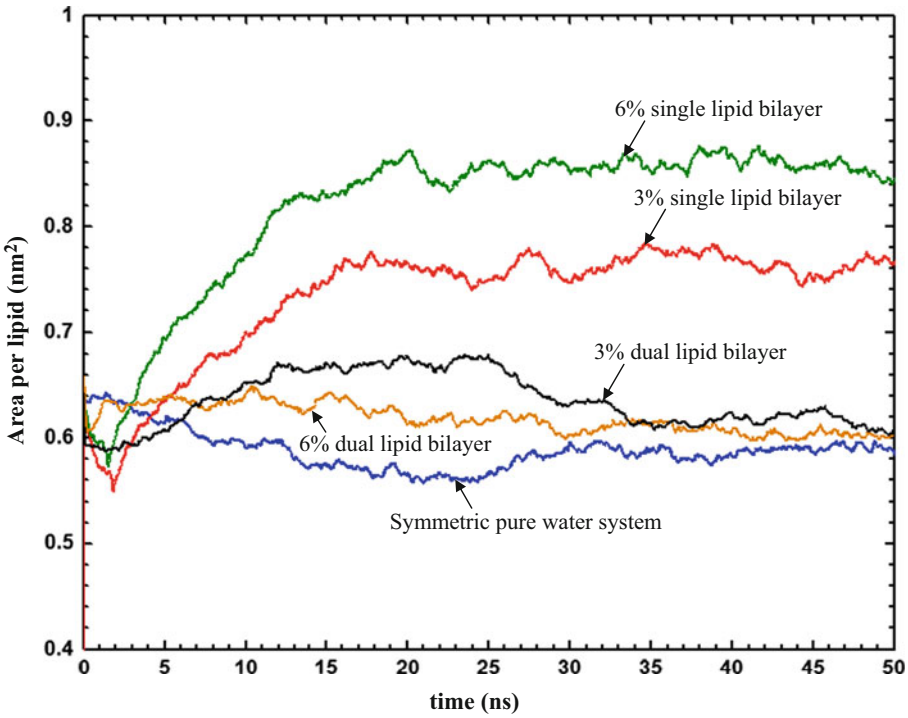

Fig. 2 Time dependence of the area per lipid for the single lipid bilayer system (symmetric system) and the outer leaflet of the dual lipid bilayer system (asymmetric system)

Table 1 Summary of simulation characteristics for dual (asymmetric) and single (symmetric) lipid bilayer configurations

System	Mol% DMSO	Number of atoms	Number of lipids	Number of water molecules	Number of DMSO molecules	⟨Lipid area⟩ (nm^2)	Bilayer thickness (nm)
Asymmetric (dual)	3	33,231	192	8,133	156	0.6	2.9
	6	34,479	192	8,133	312	0.61	2.6
Symmetric (single)	0	20,662	96	5,422	0	0.59	3.6
	3	21,286	96	5,422	156	0.78	2.4
	6	21,910	96	5,422	312	0.82	2.0

can be clearly seen in Fig. 3 that there is no penetration of either water or DMSO into the tail region of the lipids. The separation of the two peaks in the mass density profile of DMPC lipids corresponds to the average position of the lipid headgroups in the two leaflets. This separation measures the effective thickness of the lipid bilayers. By comparing the results from the present study with simulation results of a DMPC single lipid bilayer in pure water, we inferred that the actual thickness of the bilayer was reduced for both 3 and 6 mol% DMSO concentrations. These results corroborated the results of bilayer thickness calculations given in Table 1.

To gain insight into the structural changes of the dual lipid bilayer configuration, the mass density profiles of the two charged groups, specifically the phosphate (P) and choline (N) groups, are plotted for 3 mol% (Fig. 4a) and 6 mol% (Fig. 4b) DMSO concentrations. (The presence of DMSO in Fig. 3a at $0 < |z| < 2$ seems to be an artifact of the statistical analysis since this phenomenon is not observed in either Figs. 3b or 4b. Note that Figs. 3b and 4b correspond to higher concentrations of DMSO [6% vs. 3%] than in Figs. 3a and 4a, and one would expect that, if true, the permeation of DMSO would be more pronounced in the higher-concentration study in comparison to the lower-concentration study.) It can be noted from Fig. 3a, b that DMSO has a tendency to accumulate below the outer leaflet–water/DMSO interface and below the headgroup regions. It is also observed that the peaks of nitrogen and phosphorus for the inner leaflet come closer (they nearly coincide) compared to the peaks in the single DMPC lipid bilayer–pure water system (data not shown). Although in the dual lipid bilayer system there was a reduction in peaks for the inner and outer leaflets compared to the leaflets of their corresponding single lipid bilayer systems, the order of the peaks was maintained such that the nitrogen peak was located slightly outside the peak of phosphorus density.

In addition to the mass density profiles, the deuterium order parameter (S_{CD}) measures the alignment of the phospholipid tails in the bilayer and gives valuable information regarding the structure of the bilayer (Tieleman et al. 1997). The deuterium order parameter typically

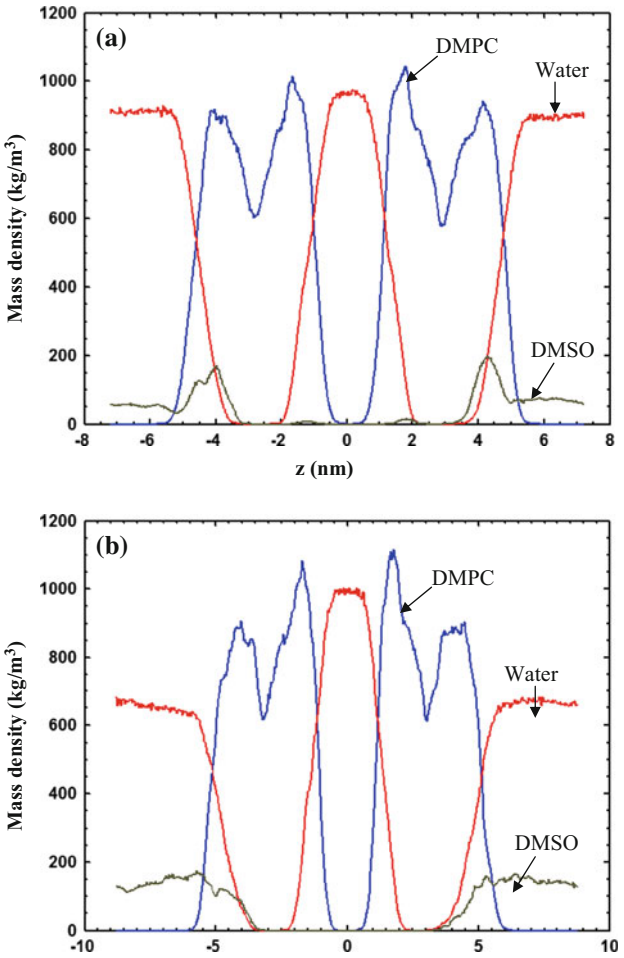

Fig. 3 Mass density profiles of DMPC lipid, water and DMSO in a dual lipid bilayer system at DMSO concentrations of (**a**) 3 mol% and (**b**) 6 mol%

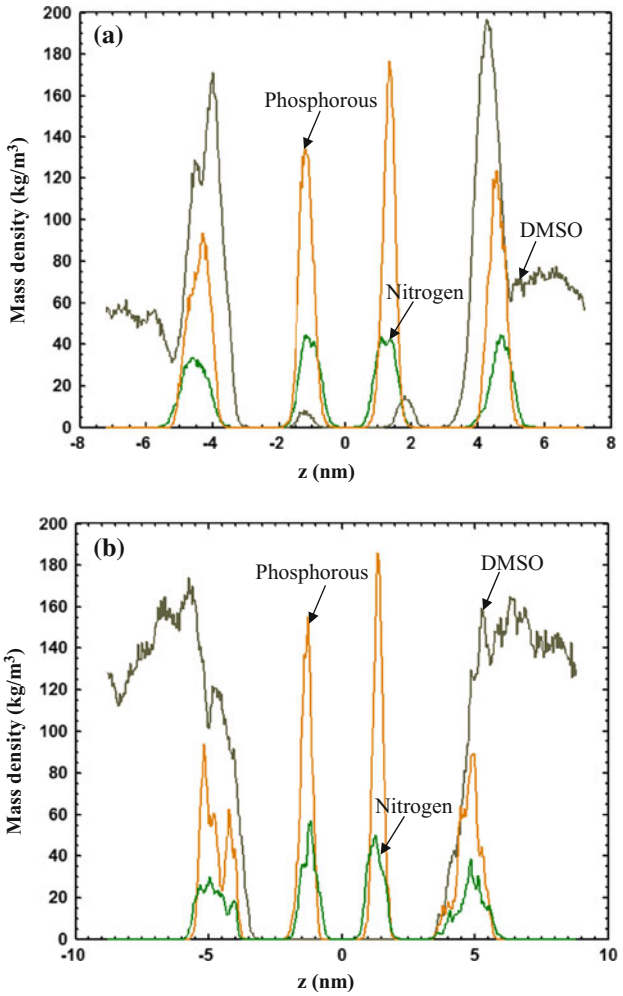

Fig. 4 Mass density profiles of DMSO, phosphorus atom and nitrogen atom in a dual lipid bilayer system at DMSO concentrations of (**a**) 3 mol% and (**b**) 6 mol%

ranges from 0 to 0.5. A value of 0.5 is associated with an all-*trans* conformational state, and a value of 0 is associated with the isotropically disordered state. Figure 5a, b depicts the order parameter profiles for a 2 * S_{CD}(sn-1) chain of the outer leaflets with 3 and 6 mol% concentrations of DMSO, respectively. For comparison, the order parameter profile of a 2 * S_{CD}(sn-1) chain for the single lipid bilayer system with pure water and DMSO is also given. It can be seen in Fig. 5a, b that the ordering of tails in the inner leaflet is decreased compared to the ordering of tails in the single lipid bilayer system in the presence of pure water. In Fig. 5a it can also be seen that the ordering of tails in the single lipid bilayer system in the presence of 3 mol% DMSO is decreased compared to the ordering of tails in the outer leaflet. But in Fig. 5b it is found that the ordering of tails in the outer leaflet closely matches the ordering of tails in the single lipid bilayer system in the presence of 6 mol% DMSO, except for the atoms near the headgroup region and deep in the tail region. These results suggest that the exposure of leaflets to different aqueous solutions has an impact on the ordering of tails compared to the exposure of leaflets to similar aqueous solution. Also, variation in DMSO concentration has an impact over the ordering of lipid tails.

The water ordering in the vicinity of the bilayer–water interface (hydration layer) can be obtained from studying the mean cosine value, $\langle \cos \theta \rangle$, of the angle between the dipolar moment and the bilayer normal vector. The mean cosine value is obtained by averaging over dipolar orientations of all water molecules present in the system over the last 10 ns of the simulation time. When generating this figure the normal unit vector parallel to the z-axis was considered to have the same orientation for the solvent on both sides of the leaflets in the bilayer. Consequently, by symmetry, the cosine average has the opposite sign on either side of the pure water, which happens to be the center of the dual lipid bilayer setup. Figure 6 shows the results from our MD simulations for the ordering of water in the vicinity of the outer leaflet in the presence of 3 and

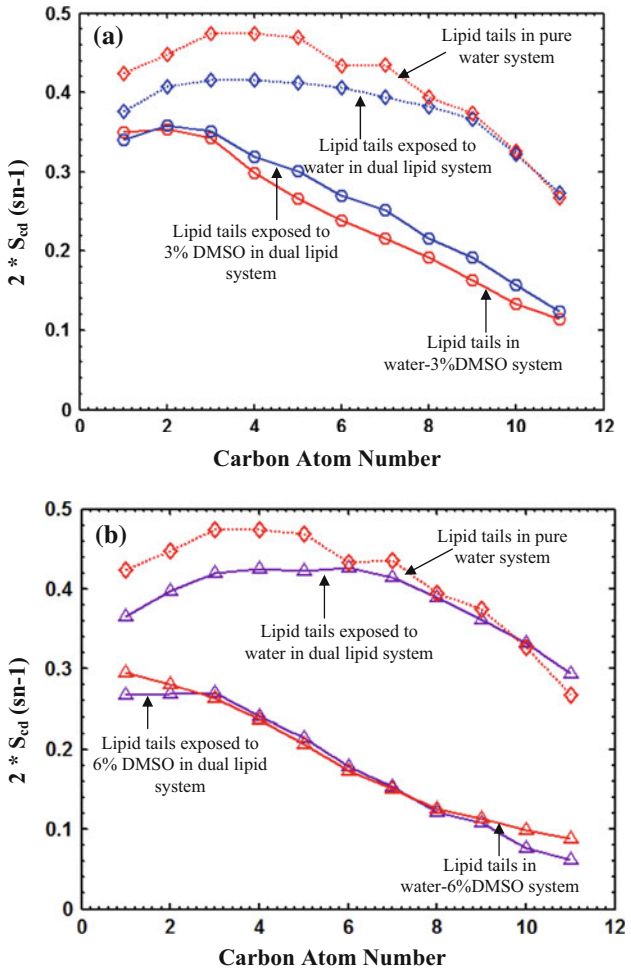

Fig. 5 Dueterium-order parameter of sn-1 chain of a DMPC lipid for an inner leaflet (compared with order parameter of lipids within a single lipid bilayer system hydrated by pure water) and outer leaflet (compared with order parameter of lipids within a single lipid bilayer system hydrated by water–DMSO mixture) at DMSO concentrations of (**a**) 3 mol% and (**b**) 6 mol%

6 mol% concentrations of DMSO. As seen in Fig. 6 there is a decrease in peak heights and separation distance between the peaks in the dual lipid bilayer system with 3 and 6 mol% DMSO compared to the single lipid bilayer/pure water system (Devireddy 2010). By assuming a negligible change of the hydration layer thicknesses due to the presence of DMSO, the change in the separation of the hydration layers between the outer leaflet/water–DMSO solvent and the inner leaflet–pure water can be attributed entirely to the change in bilayer thickness. This hypothesis seems to corroborate quantitatively and qualitatively with bilayer thickness results obtained from the mass density profiles and the values presented in Table 1. The observed decrease in membrane thickness in the presence of DMSO is also correlated with no significant variation of the area per lipid, as shown in Table 1, leading to variations in the value of cell membrane permeability. This variation in

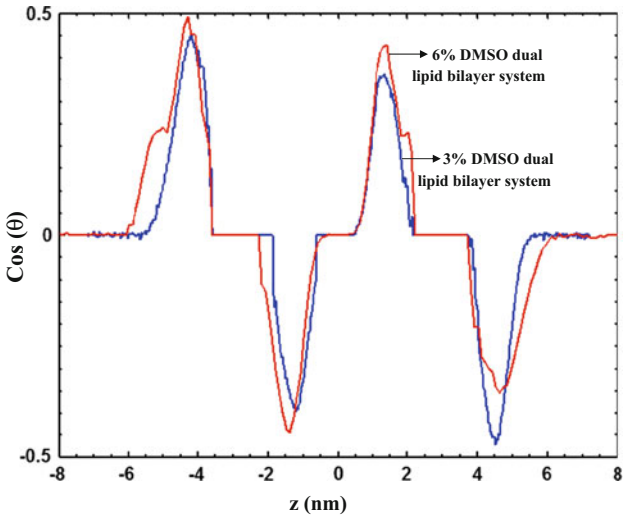

Fig. 6 Water orientation profiles in a dual lipid bilayer system configuration at 3 and 6 mol% DMSO concentrations

membrane permeability and the structural changes within the membrane might be contributing to our ability to successfully cryopreserve biological systems in the presence of chemicals (like DMSO) compared to their absence (Pinisetty et al. 2006; Kardak et al. 2007; Devireddy 2010).

Conclusions

The aim of this study was to investigate the interactions and the effects on the structural properties of DMPC lipid bilayers during the diffusion process of DMSO in the presence of a concentration gradient using a dual lipid bilayer system configuration. The simulations show that (1) the DMSO molecules have a tendency to accumulate in a layer below the membrane–water interface (just below the phosphate and choline groups of the outer leaflet), (2) the ordering of the lipid tails of the molecules present within the outer leaflet is also lowered by the presence of DMSO and (3) in the presence of 3 and 6 mol% DMSO there is no substantial increase in the area per lipid of the outer leaflet.

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