Molecular Dynamics Simulation of DPPC Bilayer in DMSO

Alexander M. Smondyrev and Max L. Berkowitz
Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

ABSTRACT We performed molecular dynamics simulations on dipalmitoylphosphatidylcholine (DPPC)/dimethylsulfoxide (DMSO) system that has the same lipid:solvent weight ratio as in our previous simulation done on DPPC/water. We did not observe a large change in the size of DPPC membrane when the solvent was changed from water to DMSO. Also, we did not observe that a large number of DMSO molecules is permeating into the membrane, as it was suggested to explain the observed change in the bilayer repeat period. We found that the surface potential reverses its sign when water is replaced by DMSO. Based on the results from our simulations, we propose that the repulsion force acting between membranes is reduced when DMSO is added to solvent water and therefore membrane surfaces approach closer to each other and the extra solvent is removed into excess solution.

INTRODUCTION

Dimethylsulfoxide (DMSO) and its aqueous solutions are among the most widely used solvents in organic chemistry, chemical technology, and cell biology. DMSO ($(CH_3)_2SO$) is a polyfunctional molecule with a polar S = O group and two hydrophobic groups CH_3. Its structure enables DMSO to solubilize a wide variety of compounds. DMSO has many important biological properties. It is a widely used cryoprotectant for biological structures such as cells, tissues, and organs. DMSO is also able to induce cell fusion (Ahkong et al., 1975) cell differentiation (Lyman et al., 1976), to increase permeability across membranes (Anchordoguy et al., 1992), and to change the properties of proteins (Arakawa et al., 1990). Other uses of DMSO include anesthesia (Jacob and Herschler, 1986), anti-inflammation effect, antiviral and antibacterial activity and radioprotection abilities (Milligan and Ward, 1994). Although the effects of DMSO are well known and studied, the molecular mechanisms involved are still unknown. They are often explained by modifications of membrane structure and stability. Recent experimental studies using X-ray diffraction and differential scanning calorimetry methods provided more information about the properties of phosphatidylcholines in aqueous DMSO (for review, see Yu and Quinn, 1998a). It was found that, in phospholipid bilayers, DMSO can produce new phases (Tristram-Nagle et al., 1998) and change their stability (Yu and Quinn, 1995). DMSO also has a significant effect on the repeat spacing distance (Yu and Quinn, 1998b) and modifies hydration forces (Yu and Quinn, 1995).

Properties of DMSO/water mixtures were modeled extensively using molecular dynamics methods (Rao and Singh, 1990; Luzar and Chandler, 1993; Liu et al., 1995; Vaisman and Berkowitz, 1992). More recently, effects of DMSO on the structure of enzyme subtilisin (Zheng and Ornstein, 1996) and Leu-Enkephalin (van der Spoel and Berendsen, 1997) were investigated in molecular dynamics simulations. Although a number of experiments studied the properties of phospholipid bilayers in DMSO/water solutions, only one simulation study of the effects of DMSO on bilayer properties, done by Paci and Marchi (1994), is known to us. The main goal of their work was to study the permeability of glycerolipid bilayer to a polar molecule (DMSO). Given the limited amount of molecular detailed information on the DMSO/phospholipid system, we decided to investigate the properties of this system using molecular dynamics computer simulation technique. We present here the results of a constant pressure simulation of a dipalmitoylphosphatidylcholine (DPPC) bilayer in pure DMSO solution at $T = 323$ K. Our goal is to compare the structures of DPPC bilayers in DMSO and water.

Our simulations of the DPPC/DMSO system were done at the same temperature and same lipid:solvent weight ratio as in the case of DPPC/water system. Thus, we excluded any possible effects caused by the presence of water molecules in the system and focused only on the effects of DMSO.

METHODS

To prepare the initial configuration, we used the final configuration from our previous simulation of the DPPC/water system (Smondyrev and Berkowitz, 1999). We kept coordinates of 64 DPPC molecules unchanged and removed all water molecules. After that, we added DMSO molecules on both sides of the bilayer. The length of the simulation cell in z-direction was adjusted to accommodate 312 DMSO molecules. Thus, the lipid-to-solvent weight ratio was the same as in the simulations of the DPPC/water system. With phosphorus atoms held fixed, we gradually decreased the length of the simulation cell in z-direction to 59 Å in a series of 2-ps constant volume simulations. The final value of the interlamellar spacing was estimated by taking the area per lipid headgroup of 62 $Å^2$ and the volumes of DPPC and DMSO of 1230 $Å^3$ and 118 $Å^3$, respectively. At this point, we performed a 50-ps constant volume simulation at $T = 323$ K with unconstrained phosphorus atoms. After equilibrating the system at constant volume, we carried a 2-ns molecular dynamics simulation at constant

Received for publication 21 October 1998 and in final form 18 February 1999.

Address reprint requests to Dr. Max L. Berkowitz, Department of Chemistry, University of North Carolina, Venable & Kenan Laboratories CB 3290, Chapel Hill, N.C. 27599-3290. Tel.: 919-962-1218; Fax: 919-962-2388; E-mail: maxb@gibbs.oit.unc.edu.

0006-3495/99/05/2472/07 $2.00

pressure $P = 0$ atm and temperature $T = 323$ K with periodic boundary conditions. We kept angles of the simulation cell fixed and varied the dimensions of the cell using Hoover barostat. Thermostat and barostat relaxation times were 0.2 ps and 0.5 ps, respectively. We used the OPLS model for DMSO [Jorgensen, 1996 (unpublished. See Ref. 18 of Y.-J. Zheng and R. L. Ornstein, *J. Am. Chem. Soc.* 118:4175–4180.)]. The molecular geometries of DMSO molecules were kept rigid during the simulation. Initial coordinates of atoms in DMSO molecules were taken from the crystal structure (Thomas et al., 1966). For lipid molecules, we used the same united atom potential as in our recent simulations of the DPPC/water system (Smondyrev and Berkowitz, 1998). All bond lengths of DPPC molecules were held fixed using SHAKE algorithm with tolerance 10^{-4}, allowing us to use the time step of 0.002 ps. The Ewald summation technique was used to calculate electrostatic contributions with tolerance 10^{-4}. The real space part of the Ewald sum and van der Waals interactions were cut off at 10 Å. Calculations were performed on an SGI Origin 2000 at the University of North Carolina using DL_POLY simulation package, version 2.8, developed in Daresbury Laboratory, England (Smith and Forester, 1996).

RESULTS

After the first 500 ps of simulation, configurational energy (see Fig. 1) and volume of the simulation cell were converged. Thus, we used the last 1500 ps for data analysis. In Fig. 2, we show the area per headgroup and lamellar spacing as a function of time during the entire run. The values of the area per headgroup and lamellar spacing calculated over the last 1500 ps are 60.4 ± 0.6 Å^2 and 58.7 ± 0.6 Å, respectively. Although the average repeat distance did not change significantly compared to our simulation of the DPPC/water system, the average area per headgroup became slightly lower. (The area per headgroup and repeat distance in the simulation of DPPC bilayer surrounded by water were 61.6 ± 0.6 Å^2 and 59 ± 1 Å, respectively). The change in the geometry of the membrane had little effect on the chain ordering. We calculated the deuterium order parameter using the expression (Egberts and Berendsen, 1988)

$$-S_{CD} = \tfrac{2}{3}S_{xx} + \tfrac{1}{3}S_{yy} \quad (1)$$

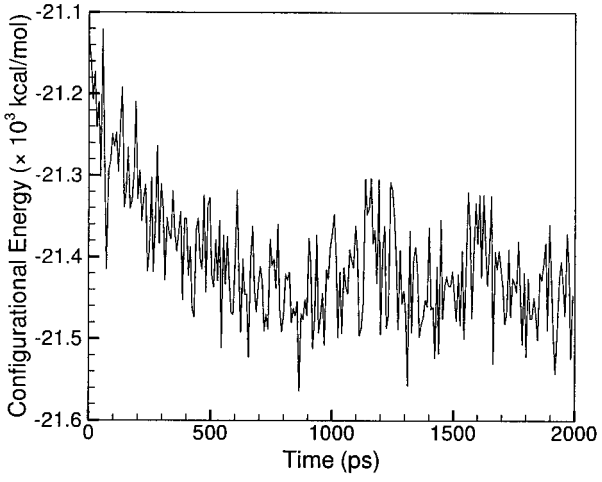

FIGURE 1 Time evolution of the total configurational energy for the DPPC bilayer in DMSO.

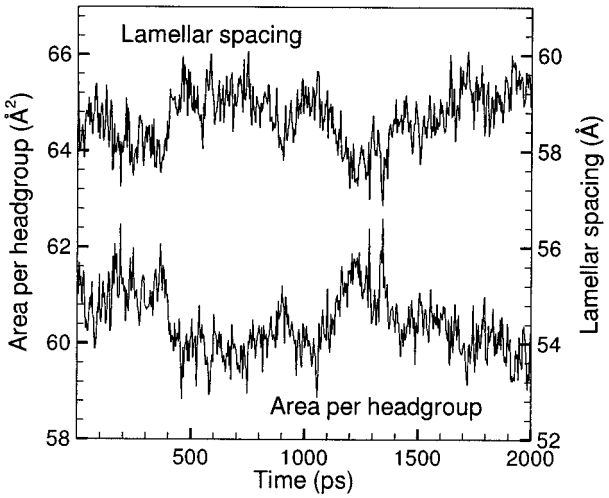

FIGURE 2 Time evolution of the area per headgroup and lamellar spacing for the DPPC bilayer in DMSO.

where $S_{ij} = \langle 1.5 \cos \theta_i \cos \theta_j - 0.5\delta_{ij} \rangle$; θ_i is the angle between the ith molecular axis and the bilayer normal (z-axis). In Fig. 3, we compare $|S_{CD}|$ values for the Sn-2 chain from our simulations of DPPC/water (Smondyrev and Berkowitz, 1999) and DPPC/DMSO systems. The order parameter profiles obtained in two simulations are very close to each other, indicating that no major structural changes occurred in lipid tails. The average numbers of gauche defects (about 7 per DPPC molecule) were equal, within the error margin, for both systems. To find the difference in the structures of DPPC membranes in water and DMSO, we calculated the average distances from the bilayer center to different carbon atoms in DPPC (see Table 1). Interestingly, the distances to carbon atoms in hydrocar-

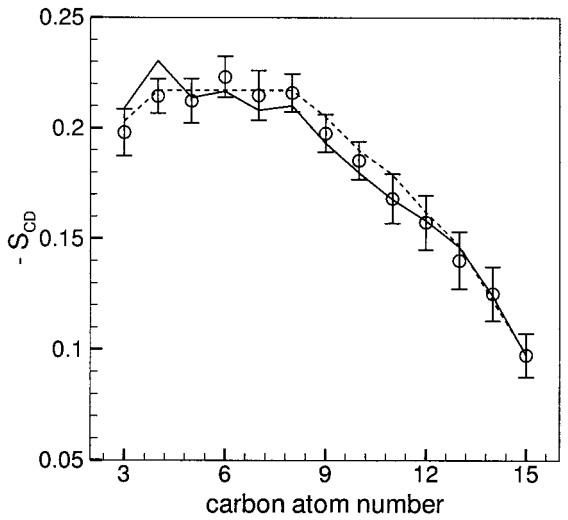

FIGURE 3 Deuterium order parameter S_{CD} for the Sn-2 hydrocarbon chain for DPPC bilayer in DMSO (*empty circles*). Error bars were obtained by dividing the run into five 300-ps blocks. We also show the S_{CD} order parameters for the DPPC bilayer in water obtained from simulation (*solid line*) and experiment (*dashed line*) (Douliez et al., 1995).

TABLE 1 Distances from Bilayer Center (Å)

Atom	DPPC/Water	DPPC/DMSO
P	19.00 ± 2.00	18.87 ± 2.57
C_γ	19.53 ± 3.61	18.43 ± 3.32
C_α	19.47 ± 2.48	18.92 ± 2.88
C_β	19.39 ± 2.92	18.70 ± 3.02
CG-3	17.19 ± 2.03	17.11 ± 2.69
C4	11.75 ± 1.93	11.89 ± 2.52
C5	10.85 ± 1.92	11.06 ± 2.52
C9	7.14 ± 1.88	7.36 ± 2.39
C14	3.21 ± 1.74	3.23 ± 1.97
C15	2.06 ± 2.40	1.98 ± 2.60

bon chains and phosphorus atoms remained almost unchanged. At the same time, the distances to carbons in the headgroup became smaller by ~0.5 Å for α and β carbons and by ~1.0 Å for γ carbons. Thus, the average distances to α, β, and γ carbon atoms become smaller than the distance to phosphorus atoms. This suggests that vectors connecting phosphorus and nitrogen atoms become more parallel to the membrane surface when DPPC bilayer is solvated in DMSO. In Fig. 4, we show the distributions of cosines of the angle between the P–N vector and bilayer normal for DPPC/water and DPPC/DMSO systems. The probability of conformations corresponding to the case when the P–N vector rises above the plane of the membrane becomes lower when water is replaced by DMSO. Accordingly, the P–N vector has a higher probability to orient parallel to the membrane surface and even point inside the bilayer for a system containing DMSO. The average value of the angle between the P–N vector and bilayer normal is 81° for DPPC bilayer in water and 94° for DPPC bilayer in DMSO. These results can also be expressed in terms of the angle between the P–N vector and the bilayer plane. In water, the P–N vector points into the solvent layer and makes an angle +9° with the membrane plane. In DMSO, the inclination of the P–N vector toward the bilayer plane is −4°, which indicates that, on average, the P–N vector points toward bilayer interior. These results agree with the data for the positions of carbon atoms in the headgroup relative to the bilayer center. Additional information about the structure of the DPPC bilayer can be obtained from radial distribution functions. In Fig. 5, we show the P–P and N–N radial distribution functions for DPPC bilayers in water and in DMSO. Although the N–N radial distribution function profile was almost structureless in the DPPC/water system, we observed an appearance of a distinct peak in the presence of DMSO. This indicates that the repulsion between choline groups is reduced, which can also lead to an increase in the interaction between DPPC molecules in the presence of DMSO. Also, for the DMSO-containing system, the position of the first peak in the P–P radial distribution function is shifted by about 0.3 Å toward larger values when compared to its position in the DPPC/water system. The change in the average area per headgroup cannot account for this difference. On the contrary, one would expect that, for the DPPC/DMSO system, which has the lower average area per headgroup, the lateral projection of the distance between two phosphorus atoms should become smaller. One possible explanation is that, in the system with the DMSO, phosphorus atoms shift up and down along the bilayer normal, which results in the increase in the most probable P–P distance. Our data for the distance from the bilayer center indicate that, although the average values for the phosphorus atoms are very close for DMSO and water-containing systems, the distribution of distances in DMSO is slightly wider than in water. In Fig. 6, we show the electron density profiles obtained from the simulations. The contributions of DPPC molecules are matched very closely, whereas the total elec-

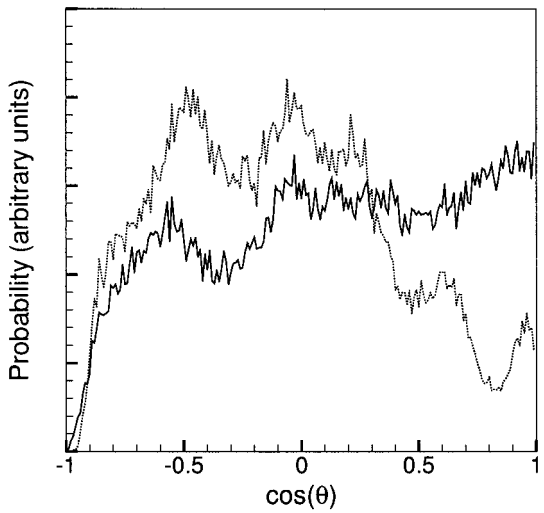

FIGURE 4 Distributions of cosines of the angle between the P–N vector and bilayer normal for the DPPC/water system (*solid line*) and DPPC/DMSO system (*dotted line*). When the cosine is positive, the P–N vector points into the solvent layer.

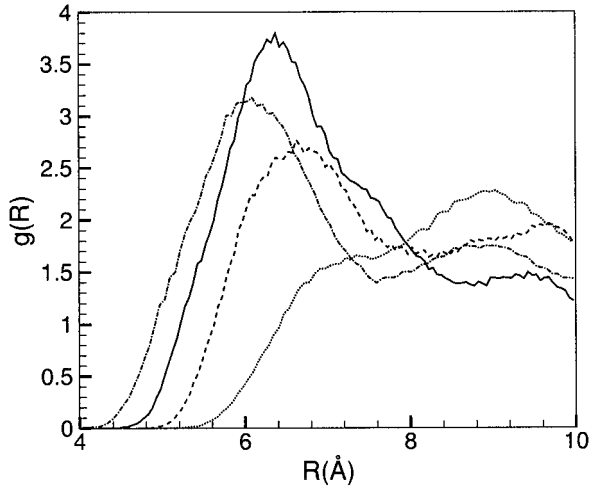

FIGURE 5 Radial distribution functions: *solid line,* phosphorus–phosphorus for the DPPC/DMSO system; *dash-dotted line,* phosphorus–phosphorus for the DPPC/water system; *dashed line,* nitrogen–nitrogen for the DPPC/DMSO system; *dotted line,* nitrogen–nitrogen for the DPPC/water system.

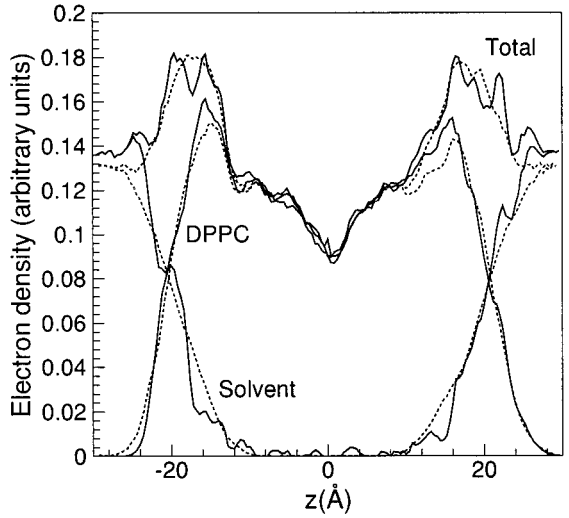

FIGURE 6 Electron density profiles across the bilayer for DPPC/DMSO (*solid lines*) and DPPC/water (*dashed lines*) systems. Separate contributions from DPPC and solvent molecules are also shown on this figure.

tron density profiles are slightly different. For the DPPC/DMSO system, the profile is not as smooth as for the DPPC/water system and shows two peaks. This is probably because DMSO contributes differently into the electron density profile.

To see how the conformational changes in the membrane headgroup and change of the solvent affected the electrostatic properties of the bilayer, we calculated the variation of the electrostatic potential $\psi(z)$ across the bilayer

$$\psi(z) - \psi(0) = -\int_0^z dz' \int_0^{z'} \rho(z'')\, dz'', \qquad (2)$$

where $\rho(z)$ is the local excess charge density. The total potential and separate contributions due to lipid and solvent molecules for bilayers in water and DMSO are shown in Fig. 7, *A* and *B*. The part of the potential due to the DPPC molecules is larger when the bilayer is surrounded by water molecules. To determine how changes in headgroup orientation affect the electrostatic potential caused by DPPC molecules, we divided it into components by DPPC headgroups and two ester groups. We plotted these data in Fig. 7 *C* for bilayers in water and in DMSO. Curves representing contributions to the DPPC electrostatic potential due to two ester groups for bilayers in water and DMSO almost overlapped. At the same time, a drastic difference is seen in the part of the electrostatic potential due to DPPC headgroups. For DPPC bilayer in water, this part is positive, and its amplitude is very similar to the one due to ester groups. When the bilayer is solvated in DMSO, its headgroups are orienting more parallel to the membrane surface and even point toward the membrane interior as indicated by the sign of the average angle between P–N vector and bilayer plane (−4° for DPPC bilayer in DMSO). As a result, the headgroup component of the DPPC electrostatic potential be-

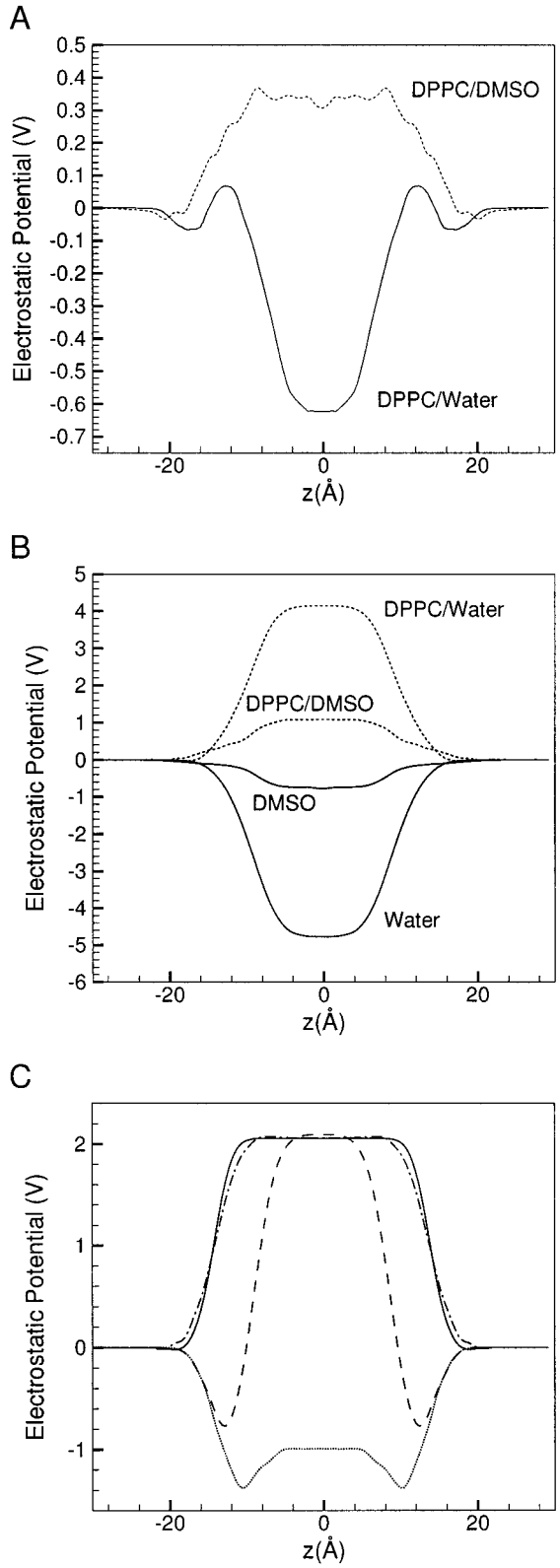

FIGURE 7 Electrostatic potentials along the bilayer normal for DPPC/DMSO and DPPC/water systems. *A,* Total potentials; *B,* separate contributions due to DMSO (or water) (*solid lines*) and lipid (*dashed lines*); *C,* contributions to the lipid potential due to ester groups: *solid line,* in DPPC/water system; *dash-dotted line,* in DPPC/DMSO system and due to headgroups: *dashed line,* in DPPC/water system; *dotted line* in DPPC/DMSO system. Notice the difference in scale on three figures.

comes negative, whereas its absolute value is smaller than for a bilayer in water. This is consistent with the observation that the absolute value of the P–N vector tilt, with respect to the membrane plane, is larger when bilayer is solvated in water. The amplitude of the potential due to DMSO also decreased compared to that in water. Interestingly, for the DPPC/DMSO system, the total potential (chosen to be zero inside the bilayer) increases to a value of +350 mV. The absolute value of this potential is smaller than the value obtained for the DPPC/water system (−600 mV). As we can see, total potentials for the DPPC/DMSO and DPPC/water systems have opposite signs. This result may have dramatic effects on the protein–membrane interaction and the permeability of water molecules and ions across membranes. Our simulations suggest that adding DMSO to water surrounding lipid membrane might lower the total membrane potential, and, at some concentration, cause it to change its sign.

One of the possible factors that can affect the change in the electrostatic potential is the distribution of DMSO molecules around the DPPC headgroups. Damodaran and Mertz (1993) and Essmann et al. (1995) showed that peaks in the radial distribution functions of water oxygens and hydrogens around nitrogen atoms in DPPC molecules are located at the same distances. In Fig. 8, we show pair distribution functions for distances between DPPC and DMSO atoms. From this figure, we conclude that the orientation of DMSO molecules strongly depends on the local charge density. DMSO molecules are oriented with their positively charged atoms close to the phosphate group, whereas the S–O bond points away. In the proximity of the choline group, the situation is reversed. The distribution functions indicate that oxygens of DMSO are the closest to nitrogens, whereas the positively charged atoms are further away. Double bonded oxygens of the ester group also have a strong effect on the orientations of DMSO molecules, whereas single bonded oxygens do not impose any preferential orientation.

Another interesting issue discussed in the literature is whether DMSO molecules penetrate deep inside the bilayer interior (Yu and Quinn, 1998a). Based on the data obtained from our simulations, we conclude that there was no noticeable increase in the solute density in the bilayer interior. The distance from the bilayer center, where density of DMSO drops to zero, is very similar to the distance observed in simulations with water. At the same time, few DMSO molecules were able to penetrate up to the middle of bilayer (see Fig. 6). In Fig. 9, we display the trajectories of several molecules, which, at certain time during the simulation, were at distances less then 12 Å from the bilayer center. As we can see from this figure, two of the DMSO molecules were able to penetrate as far as the center of the membrane and one of them continued to move across the bilayer. We can also see that, at certain times, the position of the DMSO molecules relative to the bilayer center was changing rapidly, probably the result of the jump-like motion between some cavities formed by the hydrocarbon tails. Interestingly, similar data collected for water molecules indicate that the number of distinct water molecules selected

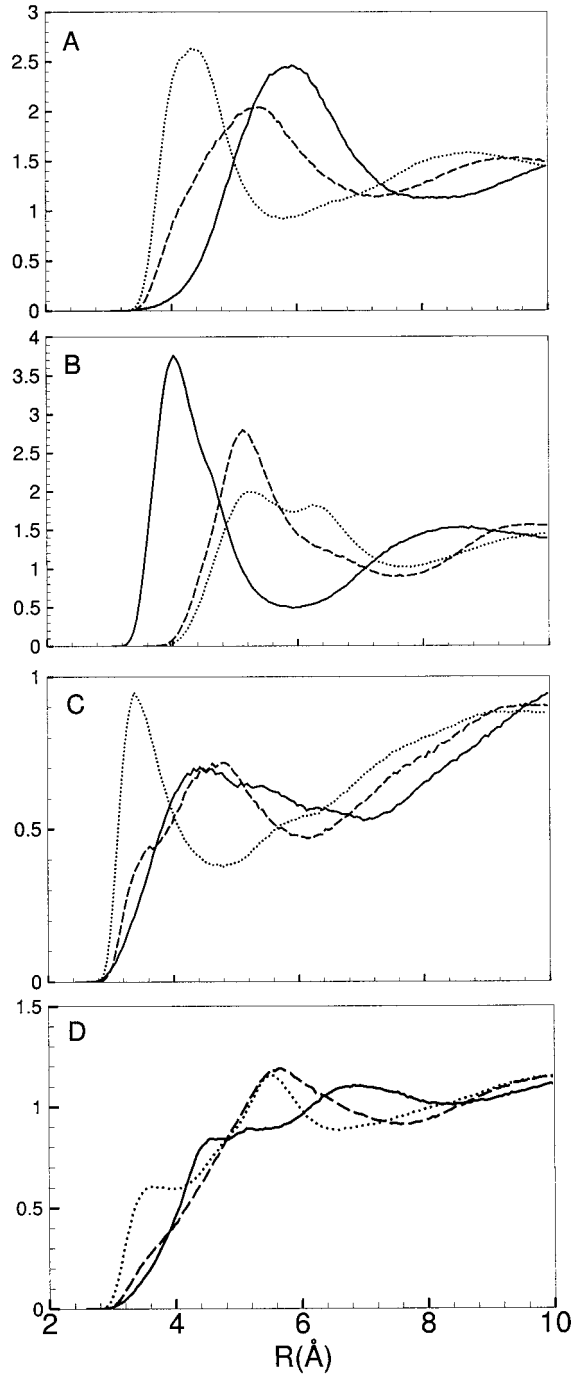

FIGURE 8 Pair distribution functions between DMSO atoms and DPPC atoms. Atoms of DPPC: *A*, phosphorus; *B*, nitrogen; *C*, carbonyl oxygens; and *D*, ester oxygens. DMSO atoms: oxygen (*solid line*), sulfur (*dashed line*) and carbons (*dotted line*).

on the basis of the criterion mentioned above (depth of penetration) was larger by a factor of 10. We found that most of these water molecules were moving freely between the interior of the membrane and the region of bulk water, whereas DMSO molecules that reached below the DPPC headgroups remained there. Recent simulation of Paci and Marchi (1994) showed that the DMSO molecule is expelled

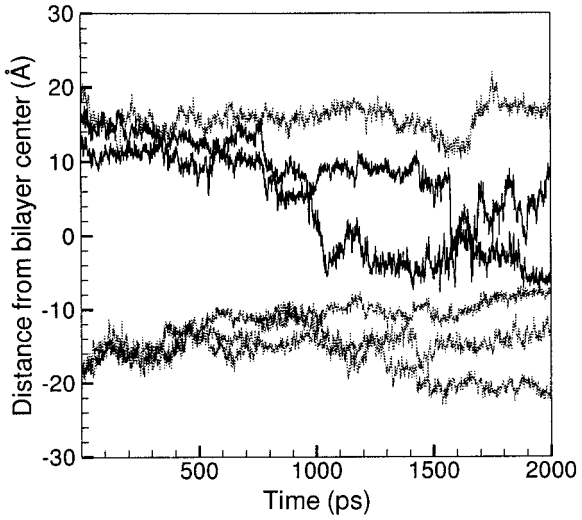

FIGURE 9 Trajectories of centers of mass of DMSO molecules along the bilayer normal. Solid lines show the trajectories of the molecules that reached the bilayer center.

from the bilayer interior after 200 to 600 ps, depending on its initial location. Our simulation shows that DMSO molecules can remain inside the lipid bilayer over longer periods of time.

DISCUSSION AND CONCLUSIONS

Recent experiments of Yu and Quinn (1998b) showed that bilayer thickness decreases when DMSO concentration in solvent increases. They argued that the decrease in the bilayer thickness is accompanied by an increase in the average area per lipid headgroup. Our simulations did not provide any evidence to support this model. The area per headgroup did not change significantly when water surrounding lipid bilayer was replaced by pure DMSO. Although time scales available in our simulations might not be sufficient to observe noticeable changes in membrane geometry, we did not see any trends suggesting that the area per headgroup is increasing. We found that DMSO does not penetrate extensively into the hydrophobic region of the lipid bilayer (as was suggested by Anchordoguy et al., 1992), and this observation is in agreement with the electron density data (Yu and Quinn, 1998b). Based on the results of our simulations, we suggest that addition of DMSO to water solvent decreases the distance between membrane surfaces expelling extra solvent. This explanation is consistent with experimental results of Tristram-Nagle et al. (1998), who showed that, upon addition of DMSO to water (up to $X = 0.2$), the thickness of membrane does not change, whereas the solvent distance decreases. The decrease in solvent spacing is consistent with the observation that the strength of the repulsive forces acting between membranes becomes smaller upon addition of DMSO into the solution (Yu and Quinn, 1998a). As was shown by McIntosh and Simon (1994) the repulsive forces acting between phospholipid membranes in water can be separated into three components: undulation, hydration, and steric. The undulation component resulting from large scale fluctuations of the entire membrane is the most prominent one when the distance between membrane surfaces is above 1 nm. The hydration component is the dominant one when membrane separations are between ~0.4 nm and ~0.8 nm and is the result of solvation of headgroups by water (McIntosh and Simon, 1994). The steric component, which is dominant at distances between bilayer surfaces below 0.4 nm, is caused by small-scale protrusions of individual molecules or changes in headgroup conformations. The appearance of a distinct peak in nitrogen–nitrogen pair distribution function (Fig. 5) for bilayers in DMSO indicates that the order in headgroups is increasing. As a result, interactions between headgroups become stronger and membrane rigidity increases, which leads to a decrease in undulation force. The increase in the strength of headgroup interactions is also indicated by the increase of the phase transition temperatures for membranes when DMSO is added to solvent (Yu and Quinn, 1998b). The hydration component of the force is also diminished, because DMSO changes the hydrogen-bonding network of water (Vaisman and Berkowitz, 1992). We propose that, when DMSO is added to water, it destroys the clathrate structures of water around DPPC headgroups. Such structures were found in recent simulations, where it was also assumed that water bridges between clathrates are needed to stabilize the membrane (Essmann et al., 1995). Finally, based on the distribution of the angle between the P–N vector and the bilayer normal observed in our simulation, we conclude that DMSO reduces the probability of small-scale protrusions of the headgroups. This should decrease the steric repulsion when two membranes are brought closer together.

Data from our simulations suggest that addition of DMSO to water solvent reduces all three components of the repulsive force. As a result, membrane surfaces move closer to each other and the lamellar spacing decreases. Closer approach of two bilayers is the first step in membrane fusion, which is enhanced when DMSO is added to the interbilayer solvent. We propose that extra solvent is removed into the excess solution and does not penetrate into the membrane, therefore the geometry of the membrane (thickness and area per headgroup) does not change substantially. We also observe that the magnitude of the bilayer electrostatic potential is reduced when water solvent is replaced with pure DMSO. According to Cevc and Marsh (1985), hydration force is proportional to the square of the electrostatic potential, and therefore, it is smaller for membranes in DMSO compared to membranes in water. Moreover, the sign of the potential changes, which suggests that, at some DMSO/water concentration, the potential is zero. In this case, the hydration force is minimal. Experimental studies of lipid bilayers in DMSO/water solvent can be used to further check the relationship between electrostatic potential and hydration forces. It is also evident that further simulations of lipid bilayers surrounded by DMSO/water

solution may explain why and how DMSO changes the properties of phospholipid membranes.

After this work was submitted for publication, we learned about the work of Gordeliy et al. (1998), who studied the structure of DPPC membranes in DMSO/water mixture using the X-ray diffraction technique. According to this work, the DPPC membrane in pure DMSO is undergoing a phase transition from interdigitated gel phase to liquid crystal phase at 77 ± 1°C. Our simulations were performed on a liquid crystal phase membrane in pure DMSO at 50°C. The main difference between the conditions in the experiment and our simulation is in the amount of solvent. In experiment (which is done in excess solvent) the amount of solvent between the bilayers adjusts to thermodynamic conditions. In our simulations, we have chosen the constant amount of solvent so that the mass ratio of lipid to DMSO is the same as in the simulations of the lipid/water system. Moreover, we also set the temperature at the same value (as in the lipid/water simulation) to study only the effects caused by solvent change. It is possible that our simulations explore a metastable state of the system, but often it is an advantage of a simulation that one can study thermodynamic states that are hard or impossible to prepare in experiment. We want to emphasize here that our conclusion: repulsive forces acting between membranes in DMSO are reduced compared to the forces acting between membranes in water, is in agreement with the conclusion from the work of Gordeliy et al. (1998).

The studies reported in this paper were supported by the National Science Foundation under grant MCB9604585.

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