Molecular Dynamics Simulations of a Phospholipid−Detergent Mixture

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A series of four molecular dynamics computer simulations totaling more than 40 ns in length have been carried out on hydrated bilayer systems to study the interaction between a diacylphospholipid, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and an oligo(ethylene oxide) alkyl ether detergent, $C_{12}E_4$. Simulations of pure lipid bilayers, pure detergent bilayers, and a mixed system in a 5:1 lipid-to-detergent ratio, show good agreement with experimental measurements. The simulations confirm experimental observations of the membrane's ability to significantly alter the conformations of the solute detergent molecules and offer an explanation of some seemingly contradictory experimental results. In addition to providing a molecular level explanation of the changes in solute structure, the present simulations demonstrate the importance of environment on solutes incorporated into membranes and show that molecular dynamics simulations is a tool with great potential for the study of membrane−polymer interactions.

Introduction

Molecular dynamics (MD) simulation of lipid bilayer membranes is quickly gaining use as an important tool for characterizing the structure and dynamics of lipid membrane systems.[1−5] Membrane simulations have moved from simple models of tethered alkyl chains,[6] to complete descriptions of lipid and water in full atomic detail,[7] to representations of complex membranes containing small solutes,[8] cholesterol,[9] and transmembrane proteins.[10−12] In developing methods for simulating pure lipid bilayers, great care has been taken in studying the influence on simulation results of such factors as: potential energy functions and their parametrization,[13] treatment of long-range Coulombic interactions,[14] choice of statistical mechanical ensemble,[15] and finite sized simulation cells.[16] The study of membranes with heterogeneous composition, particularly those with embedded peptides or proteins, presents even greater challenge because typically only a few solute molecules are contained within the membrane, resulting in relatively poor sampling statistics. Additionally, an ever-present concern when assessing the validity of such MD simulations is whether sufficient trajectory has been generated to ensure that the system is at equilibrium.[17]

In the present study, we have undertaken simulations of a mixed membrane consisting of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with the nonionic surfactant solute, $C_{12}E_4$. This detergent consists of a dodecyl alkyl chain and a headgroup made up of four ethylene oxide segments ($O-CH_2-CH_2$), with a terminal OH group. This system has been extensively studied experimentally[18−22] both because of the importance of lipid−detergent interactions in membrane solubization and reconstitution,[23] and as a model system for studying fundamental questions such as thermodynamics[24−26] and hydration[27] of membrane systems. Modeling studies to date on this system have employed simple hard-disk representations of the lipid headgroup[28] or lattice-model representations of the chain segments.[29] The wealth of experimental data on this system makes it an attractive candidate for validating techniques for atomic-level MD simulations of complex membranes. Additionally, both the lipid and the detergent individually form bilayer structures in water. Thus parallel simulations of pure POPC and pure $C_{12}E_4$ bilayers were undertaken, allowing study of the change in both lipid and detergent upon mixing.

Recently, Klose et al. used nuclear magnetic resonance (NMR) to determine deuterium order parameter profiles for POPC and pure $C_{12}E_4$ bilayers as well as a mixed system in a 5:1 lipid-to-detergent ratio.[28] Only negligible changes in the order parameters of the palmitic chain of POPC were observed, however, the dodecyl chains of $C_{12}E_4$ were significantly ordered by the lipid environment. This would suggest that the surface area per lipid is constant upon mixing while the detergent surface area contracts. Using the formula due to Nagle,[30] relating the order parameter in the plateau region near the top of the alkyl chains to the surface area per molecule, we roughly estimate that the NMR data of Klose et al.[28] corresponds to a 3.9 Å^2 decrease in detergent surface area. In a separate set of experiments, utilizing X-ray diffraction and gravimetric techniques, König et al.[22] studied mixed membranes of POPC and $C_{12}E_4$ under identical conditions and concluded that the area per molecule of POPC decreased significantly ($\sim$10 Å^2) while the area per $C_{12}E_4$ increased slightly. Their analysis of the X-ray and gravimetric data give an area for 1 POPC and 0.2 $C_{12}E_4$ of $\sim$64 Å^2, i.e., a POPC bilayer can take the detergent into the membrane with no increase in total surface area. For comparison, ideal area additivity gives an area requirement of 72.5 Å^2 and our estimate based on the order parameter plateau gives 68.6 Å^2. Thus the gravimetric and NMR approaches suggest significantly different conclusions as to the total membrane surface area, a point that will be addressed in the present work. This extremely large uncertainty in the membrane surface area illustrates one difficulty in setting up accurate MD simulations of complex membranes.

In the following we briefly describe the protocol used in carrying out the MD simulations, followed by a comparison of simulation results with experimental data and a detailed descrip-

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tion of the changes in the atomic level structure of the solute upon incorporation in the membrane. The conclusion summarizes the most important results of this study and discusses their implications for simulations of other complex membrane systems.

Procedure

The periodic simulation cell contained 72 lipid or surfacant molecules (36 per monolayer) at hydration levels that correspond to 97% relative humidity. A partially flexible simulation cell was employed with the *z* dimension (i.e., the bilayer normal) adjusted to maintain the $P_{zz} = 1$ atm, and the *x* and *y* dimensions fixed to maintain the desired surface area. The lengths of the *x* and *y* dimensions of the simulation cell were set by the number of molecules and the surface area per molecule. The pure lipid and pure surfactant systems had areas per molecule of 64.0 and 42.6 Å^2/molecule, respectively, as estimated from experimental measurements.[22] For these systems the experimentally determined number of hydrating waters, 13.5 for POPC and 6.9 for $C_{12}E_4$, were included in each simulation. The POPC $C_{12}E_4$ mixed system was constructed from 60 POPC and 12 $C_{12}E_4$ molecules by randomly replacing lipid molecules with detergents and then scaling the lateral dimensions of the system to give the desired surface area. As mentioned in the previous section, experimental estimates of the surface requirements for the mixed system were ambiguous thus three simulations of the POPC/$C_{12}E_4$ mixture were carried out. The first simulation (to be denoted I) assumed additivity of surface areas would hold. The second simulation (denoted II) was constructed on the basis of the structural data from the combined X-ray and gravimetric approach which suggested that the surface area of the lipid and detergent was equal to that of the lipid alone.[22] The final simulation (III) was constructed by assuming that the area/POPC was unchanged upon mixing but that the $C_{12}E_4$ surface area decreased by 3.9 Å^2/molecule (as suggested by experimental NMR order parameter profiles in ref 28). Initial coordinates for the POPC molecules were taken from a previously published POPC simulation.[31] The $C_{12}E_4$ coordinates were generated by carrying out Langevin dynamics simulations of single molecules and randomly sampling the resulting configurations.

The program CHARMM, Chemistry at HARvard Molecular Mechanics[32] was used with the PARM22b4b all-atom parameter set[13] and its extension to unsaturated lipids.[33] The CHARMM potential contains terms for bond lengths, bond angles, torsional angles, and improper torsional angles. The interactions between nonbonded atoms are described by Coulombic interactions between partial point charges on the atomic centers and a Lennard-Jones (LJ) 6-12 potential. The LJ potential was switched smoothly to zero over the region from 10 to 12 Å. Electrostatic interactions were included via the particle mesh Ewald summation.[34] All bonds involving hydrogen were fixed at their equilibrium distances using the SHAKE algorithm.[35] A time step of 2 fs was employed with a modified leapfrog Verlet integration scheme. A neighbor list, used for calculating the LJ potential and the real space portion of the Ewald sum, was kept to 14 Å and updated every 20 fs. A variant of the extended system formalism, the Langevin Piston algorithm, was used to control the normal pressure.[36] The temperature was maintained at 25 °C by means of the Hoover thermostat.[37] Coordinates sets were saved every 1 ps for subsequent analysis. Simulations were carried out using 8 processors on a Beowulf-type parallel computer. Simulations ranged in length from 5 to 14 ns, with the first nanosecond taken as an equilibration period and not used in the analysis. Each nanosecond of simulation took approximately 5 days of wall time. This protocol has been successfully applied to the simulation of numerous saturated and unsaturated phosphatidylcholine bilayers in the recent past.[31,33,38,39]

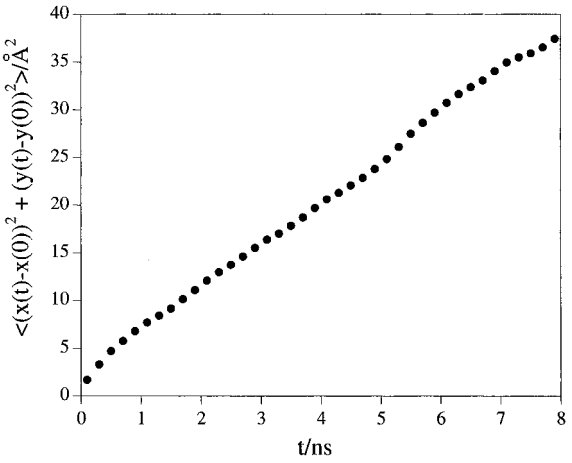

Figure 1. Lateral displacement correlation function for the detergent centers of mass as a function of time in simulation III.

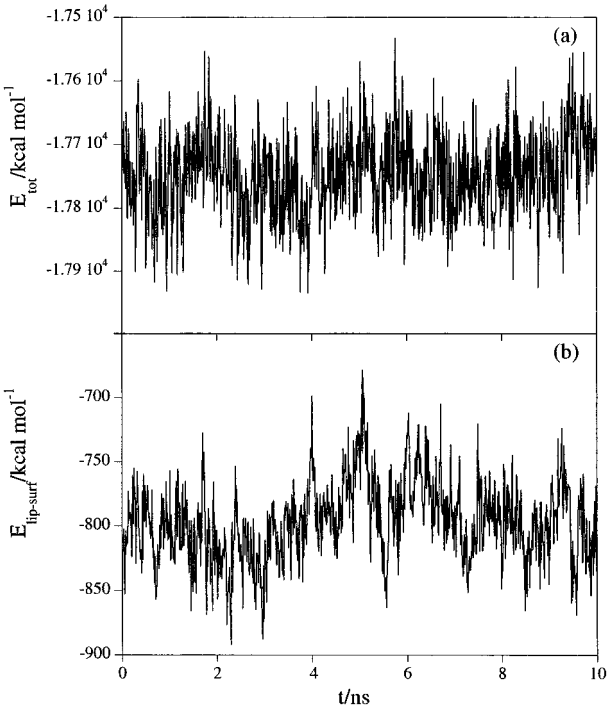

Figure 2. Interaction energy (from simulation III) as a function of time for (a) the complete lipid/detergent/water system, and (b) the lipid−detergent interactions.

Results

An important consideration in this work is the degree to which a complex membrane can equilibrate on the molecular dynamics time scale. After discarding the first nanosecond of trajectory as equilibration the lateral displacement of detergent molecules was followed as a function of time to assess the mixing of the membrane components. These data, presented in Figure 1, show that on the time scale of the simulation significant movement is observed so that each detergent has opportunity to interact with numerous lipid molecules. Further evidence that the mixed system is well equilibrated is provided in Figure 2a where the system energy is plotted as a function of time, showing no drift

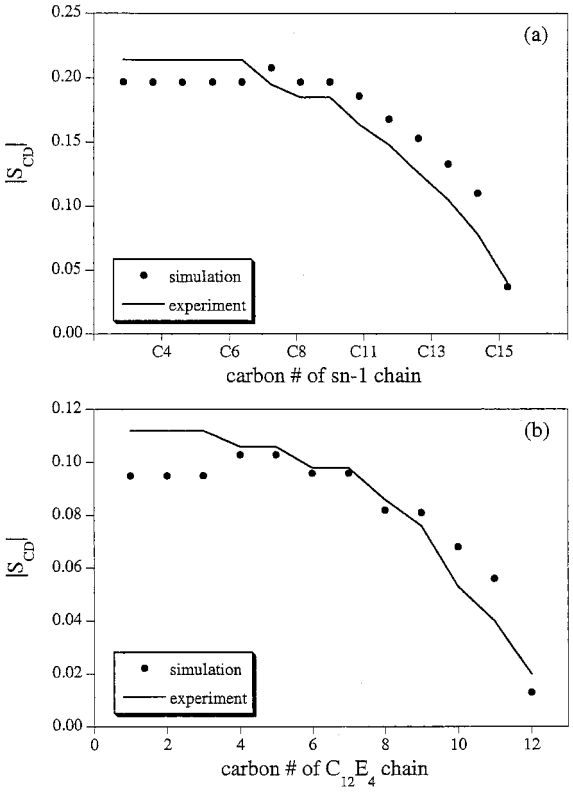

Figure 3. Deuterium order parameter profile for (a) the sn-2 chain of POPC and (b) the dodecyl chain of $C_{12}E_4$, in their pure bilayer forms. The symbols are the simulation results and the solid lines are the experimental data from ref 28.

during the production phase of the simulation. The lipid−detergent interaction energy, presumably the most difficult quantity to equilibrate from the randomly assigned initial coordinates, is given in Figure 2b, again showing a stable membrane.

In analyzing the simulations, we begin with a comparison of the deuterium order parameter profile of the alkyl chains from the pure lipid and pure detergent simulations. The experimentally measured order parameters, obtained from quadrupole splitting measurements, can be compared with those calculated from the simulation using the following relation:

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

where θ is the angle between the CH bond vector and the bilayer normal. Figure 3 shows the deuterium order parameter profile for both pure lipid and pure detergent simulations along with the experimental results of ref 28. Good agreement between simulation and experiment is obtained, especially considering that the standard error in the simulation order parameter values is on the order of 0.015. In both simulation and experiment, the order parameters for methylene segments of the lipid are roughly twice those of the surfactant. Further comparison of the POPC simulation with experiment can be made on the basis of the X-ray scattering profile shown in Figure 4. The locations of the headgroup peaks are within one Angstrom when comparing simulation and experiment. These results suggest that the simulation produces reasonable structures for the pure lipid and pure detergent systems. It has been previously shown[38] that simulation results for order parameters and scattering profiles are extremely sensitive to the choice of surface area per molecule, thus Figures 3 and 4 show that the experimental values

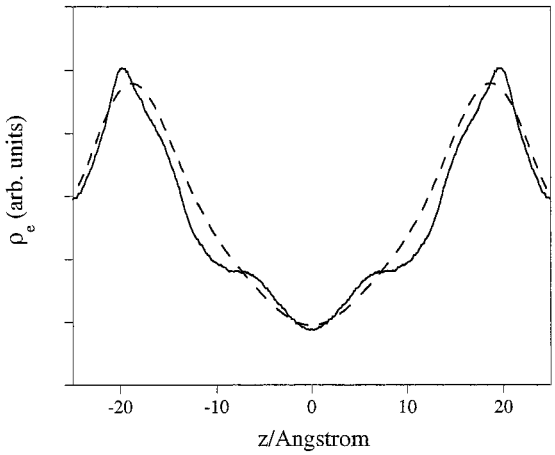

Figure 4. The electron density profile for POPC. The solid line gives the simulation result while the dashed line gives that determined from X-ray scattering. Note that the experimental result is on an arbitrary scale and thus only the locations of features are relevant, not their absolute density values.

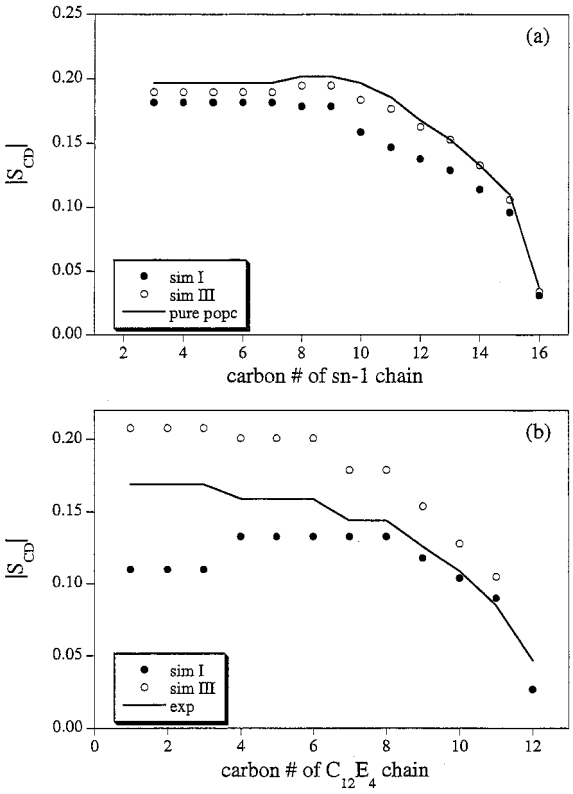

Figure 5. Deuterium order parameter profiles for (a) the sn-2 chain of POPC, and (b) the dodecyl chain of $C_{12}E_4$, in a mixed membrane.

of 64.0 and 42.6 Å^2/molecule, assumed for the POPC and $C_{12}E_4$ simulations, respectively, are reasonably accurate.

Turning to the mixed POPC/$C_{12}E_4$ membrane, the deuterium order parameter profiles will be the primary tool for deciding which of the three simulations provides the best model of the system. Simulation II with the smallest surface area was quickly seen to deviate from the experimental observations (lipid $|S_{CD}| > 0.25$) and was stopped after 5 ns. Figure 5 gives the sn-1 lipid chain and detergent dodecyl chain order parameters for simulations I and III. Both simulations reproduce the qualitative features of the experimental results, i.e., the increase in detergent order and small change in lipid order upon mixing. In simulation I the order parameters of the detergent are greater than in the pure detergent bilayer, however, they are below experimental

measurements. Similarly, the lipid order parameters in simulation I are decreased compared to their corresponding pure lipid values. Both these results suggest that simulation I has a surface area that is too low, i.e., ideal additivity of surface areas is not followed but rather the detergent has a condensing effect on the lipid membrane. In simulation III, the lipid order parameter profile is only slightly decreased (in accord with the experimental measurements) while the $C_{12}E_4$ segments undergo an approximate doubling of their order parameters. These results suggest that although ideal additivity of surface areas does not appear to hold, the condensation effect is likely on the order of 2 Å^2, rather than the 3.9 Å^2 assumed in simulation III. Clearly, the simulation results are inconsistent with the gravimetric estimates of membrane surface area. The striking difference between simulations I and III in Figure 5b emphasize the importance of membrane dimension on the results of MD simulation. The remainder of the analysis will focus on simulation III, where the membrane had the greatest effect on solute structure.

Comparison of the dodecyl chain order parameters between Figures 3b and 5b, shows the dramatic effect that membrane environment has on the conformations of the detergent molecules in simulation III. Analysis of the MD trajectory allows one to explain these conformational changes in terms of the gauche/trans population ratios in the alkyl chains and reorientation of the entire detergent molecule. The distribution of dodecyl chain dihedral angle values observed during the simulation was used to calculate a potential of mean force for rotation around these bonds. These calculations show that the gauche state of the detergent chains is destabilized by slightly more than 0.1 kcal/mol, corresponding to a reduction in the fraction gauche from 0.26 to 0.22 (the standard error is <0.01). This conformational change leads to an overall increase in dodecyl chain length from 11.84 ± 0.02 to 12.29 ± 0.09 Å. Additionally, the detergent acyl chains reorient themselves to lie more perpendicular to the membrane surface, moving from an average value of 53° (from the bilayer normal) to 38°, as calculated from the orientation of the vector connecting C1 and C12. Overall, the combination of chain lengthening and molecular reorientation increases the projection of the dodecyl chains along the bilayer normal 2.4 Å, an increase of 34%. Both of these effects contribute to explain the dramatically increased deuterium order parameters in Figure 5b.

One might imagine that the conformational changes described above are driven by the hydrophobic chain length mismatch between detergent and lipid. It seems reasonable that lengthening and straightening of the detergent chains would allow them to better fit into the hydrophobic core of the membrane. However, changes in the structure of lipid and detergent molecules, summarized by the transverse distribution of molecular fragments shown in Figure 6, suggest otherwise. The upper panel displays the POPC distributions (solid denotes pure lipid, dashed denotes mixture). The lipid hydrocarbon thickness, as measured by the location of the carbonyl groups or the location of the drop in methylene density, is very nearly identical in the pure lipid and mixed systems (in accord with the NMR order parameter analysis and the notion that lipid structure in largely unperturbed by the presence of detergent). More interesting is the lower panel that displays the distribution of $C_{12}E_4$ fragments. The ethylene oxide segments, which are found throughout the pure detergent bilayer, are essentially excluded from the center of the mixed bilayer and overall are shifted to the lipid interface. Comparison of Figures 6a and 4b shows the ethylene oxide distribution is centered on the lipid carbonyl groups with

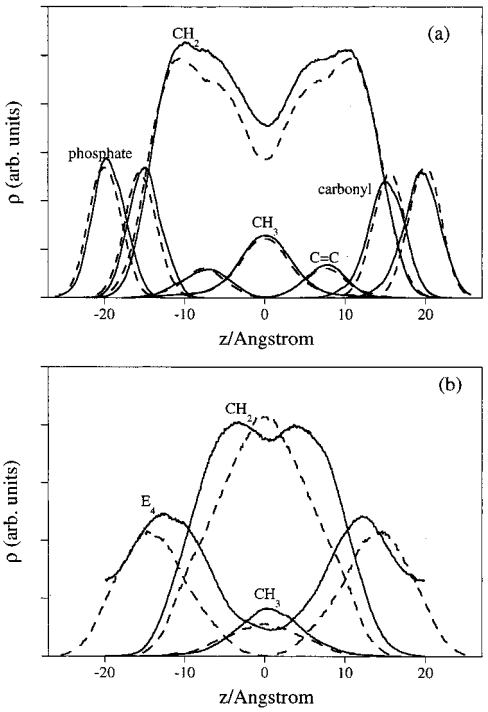

Figure 6. Electron density due to lipid and detergent molecular fragments: (a) distribution of the phosphate, carbonyl, methylene, double bond, and methyl segments of POPC, (b) distribution of the ethylene oxide headgroup, methylene, and methyl segments of $C_{12}E_4$. In each panel, the solid line gives the pure bilayer simulation results while the dashed line presents the mixed system results.

significant penetration into the hydrocarbon core. This observation is in agreement with NOESY NMR studies on the mixed system.[18] These experiments also observed NOESY cross-peaks between the ethylene oxide segments and the lipid methylene groups near the double bond, and frequent contacts between these groups is also reproduced by the simulation. The surprising result displayed in Figure 6b is the decreased width of the detergent methylene distribution. The order parameter analysis showed that the projection of each dodecyl chain along the bilayer normal increased significantly when incorporated into the membrane, however, this structural change is apparently accompanied by an interdigitation of the detergent chains. This is confirmed by calculations showing that the average projection of the C1−C12 vector along the *z* axis is greater than the distance between C1 and the bilayer center. In simulation I and the pure detergent bilayer simulation, both with low dodecyl chain order parameters, no such interdigitation is observed. We caution, however, that the order parameters in simulation III are somewhat higher than observed experimentally and it is possible that a surface area intermediate between simulations I and III would likely agree better with the experimental data and would not necessarily lead to detergent chain interdigitation.

As all the simulations were carried out at identical temperature and normal pressure, the system densities can be evaluated to compare changes in molecular volume upon mixing. Since the number of water molecules per lipid or surfactant was kept constant in all simulations, the volume of the mixed simulation should be equal to the sum of 80% of the POPC simulation cell and 20% of the $C_{12}E_4$ simulation cell if molecular volumes are conserved. In simulation I ideal mixing was observed to within the statistical precision of the simulation, however, in simulation III a 1.3% decrease in system volume was seen. This decrease may seem small, but if the entire change is attributed to detergent volume it represents 1/3 of the dodecyl chain

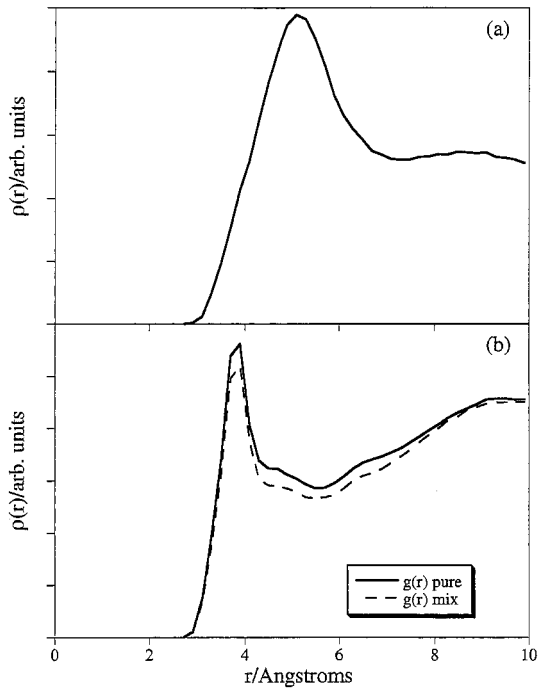

Figure 7. Radial distribution functions for (a) carbonyl carbon and ethylene oxide oxygen, (b) carbonyl carbon and water oxygen.

volume (assuming volumes of 27 and 54 Å^3 per methylene and methyl segment, respectively). Presumably this condensation occurs due to the detergent chain interdigitation discussed above.

Several experimental studies of the thermodynamics of mixing have been carried out on POPC/$C_{12}E_4$ systems. The mixing process is observed to be endothermic over all lipid/detergent ratios,[26] unfortunately the experiments are carried out with very dilute solutions of lipid and detergent so that only qualitative comparisons between the simulation (done at reduced hydration) and the calorimetry experiments are possible. The change in enthalpy upon forming the mixed system (simulation III) from the pure detergent and pure lipid states was calculated in the same manner as the volume change described in the previous paragraph. The calculated enthalpy change was 1.5 kcal/mol, i.e., when 5/6 of a mole of lipid is mixed with 1/6 of a mole of detergent, 1.5 kcal of heat is absorbed. The simulation can be further analyzed to separate the energetics into lipid−lipid, lipid−detergent, lipid−water, detergent−detergent, and detergen−water interactions. This analysis shows that the favorable lipid−detergent interactions outweigh the decreased lipid−lipid and detergent−detergent interactions upon mixing; however, both lipid and detergent interactions with water are decreased. This suggests that both lipid and detergent are partially dehydrated upon mixing and that the interactions between detergent headgroups and lipid headgroup and carbonyls segments, while favorable, are not as strong as the interactions these fragments had with water.

The dehydration of POPC upon addition of $C_{12}E_4$, suggested by the analysis of simulation energy terms, has also been inferred from changes in carbonyl chemical shifts and headgroup ^{13}C spin lattice relaxation times.[18] To quantify this dehydration, the radial distribution function for carbonyl carbons and ethylene oxide oxygens was calculated from simulation III. The results, presented in Figure 7, show a broad solvation peak at a separation of ∼5 Å. This effect is confirmed by calculation of the carbonyl−water distribution function for the pure lipid (dashed line) and mixed (solid line) simulations. Integrating the carbonyl−water distributions to 4.5 Å (the width of the solvation peak), a loss of 0.2 water molecules per carbonyl is observed upon addition of detergent. The same analysis of the carbonyl−ethylene oxide distributions shows a gain of an equal number of ethylene oxide interactions when the distribution is integrated. Additionally, the simulation results suggest the carbonyls are the primary site of dehydration, with no discernible change in choline hydration observed.

Figure 8 provides a snapshot of the mixed simulation III. Several features described in the preceding paragraph are evident from the graphic, including interdigitation of the surfactant methyl groups (represented as purple spheres), proximity between surfactant ethylene oxide groups and the lipid carbonyls (colored in red), and the relatively dehydrated nature of the ethylene oxide groups. The alignment of the surfactant chains by the lipid molecules is also seen.

An interesting result of the present study of surface areas is that although several simulation results, most notably the deuterium order parameters, are very sensitive to the choice of surface area, the lateral pressure calculated during the simulation is not. The surface tensions calculated from simulations I, II, and III were 21, 17, and 17 dyn/cm, respectively. The differences between these values are less than the error estimates in the surface tension calculation, demonstrating that simulation of these systems with a constant lateral pressure could lead to significant artifacts if the value of the surface tension was incorrectly set and/or the potential energy parameters were slightly in error.

Conclusions

We have presented the results of molecular dynamics simulations of a phospholipid−detergent mixture. Quantities calculated from the simulation describing transverse membrane structure, acyl chain conformation, intermolecular contacts, and thermodynamics of mixing, are in qualitative agreement with experimentally measured X-ray scattering data, deuterium order parameter profiles, NOESY cross-relaxation rates, and isothermal titration calorimetry. The membrane environment exhibits a powerful ordering force on the incorporated solutes, altering both the internal conformations of the molecules, their alignment relative to the bilayer surface, and their location within the membrane. Conversely, the detergent affects the membrane through a partial dehydration of the carbonyl groups. At the low detergent concentrations used here this effect is relatively small; however, if the dehydration effect was maintained on a pure detergent basis then a 1:1 lipid−detergent ratio would result in a loss of 50% of the carbonyl-bound waters.

The importance of molecular reorientations, which occur on the nanosecond time scale,[17] for achieving equilibrium suggests that long MD simulations are required to eliminate artifacts from model building of initial conditions. On account of the relatively isotropic lateral organization of the present system, little translational diffusion was required to achieve an equilibrium state. In more complex systems, e.g., a trans-membrane helix solvated by lipids, the time requirement for equilibration could be much greater if the number of solvating lipids increases or decreases via molecular translations. Recent simulations of a lipid/protein complex have in fact demonstrated that results are sensitive to initial starting positions on the one nanosecond time scale.[40] For the present study more than 40 ns of bilayer simulation were carried out on the four different systems, using all-atoms models with no truncation of Coulombic forces, demonstrating that complex membrane simulations that are both long and accurate can be carried out with currently available computer resources.

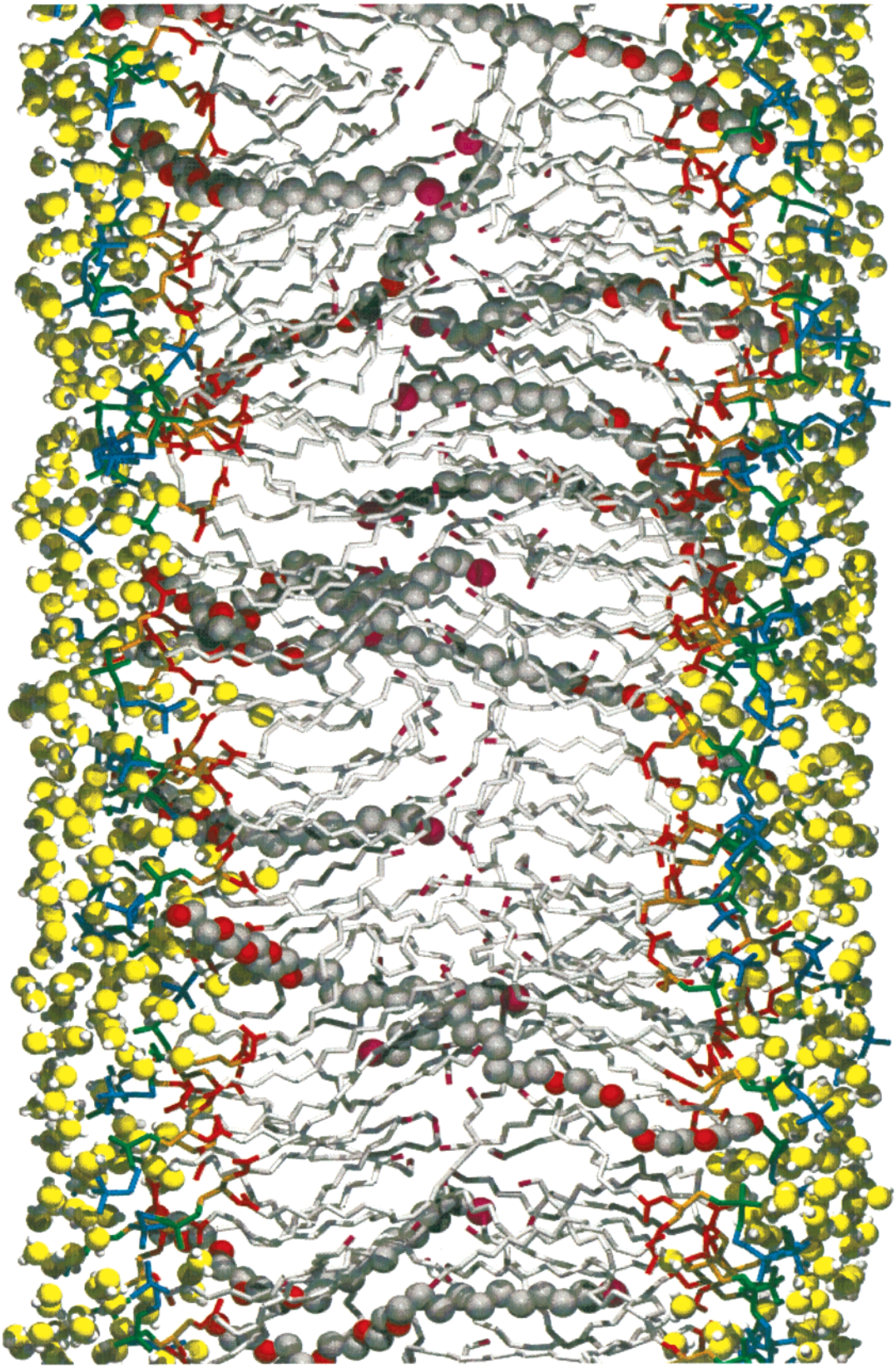

Figure 8. Snapshot of the simulation taken from system III. The water and surfactant molecules are displayed as spheres with the lipid represented by bonds. The water hydrogens, water oxygens, surfactant carbons, and surfactant oxygens are colored white, yellow, gray, and red, respectively. The lipids are colored as followed: purple, chain terminal methyl; gray, chain methylene; red, carbonyl and ester oxygen; brown, glycerol carbon; green, phosphate; blue, choline.

We have demonstrated that obtaining a quantitative picture of the membrane structure requires a precise determination of the bilayer surface area. We believe this is a general feature of MD simulations of complex membranes, emphasizing the need to test simulation results against experimental observations wherever possible. This points out the importance of experimental studies that provide accurate fundamental structural data, such as surface area requirements, for mixed membranes. As

shown by the present work, simulations can also aid in the analysis of experimental data and point out areas for further investigation. For example, an interesting result of the present study is that the simulation results agree with experiment when the gravimetrically determined molecular areas for the pure bilayers are used, but not when these same experimental methods are applied to the mixed system.

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