Constant Normal Pressure, Constant Surface Tension, and Constant Temperature Molecular Dynamics Simulation of Hydrated 1,2-Dilignoceroylphosphatidylcholine Monolayer

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ABSTRACT A constant normal pressure, constant surface tension, and constant temperature ($NP_N\gamma T$) molecular dynamics (MD) simulation of the liquid condensed phase of a 1,2-dilignoceroylphosphatidylcholine (DLGPC) monolayer has been performed at 293.15 K. A DLGPC molecule has two saturated 24-carbon acyl chains, giving the hydrocarbon core thickness of the monolayer ~28 Å, which is close to the hydrocarbon core thickness of a membrane of a living system. $NP_N\gamma T$ ensemble was used to reproduce the experimental observations, such as area/lipid, because surface tension is an essential factor in determining the monolayer structure. Data analysis on DLGPC/water monolayer shows that various liquid condensed-phase properties of the monolayer have been well reproduced from the simulation, indicating that surface tension 22.9 mN/M used in the simulation is an appropriate condition for the condensed-phase $NP_N\gamma T$ simulation. The simulation results suggest that this long-chain phospholipid monolayer shares many structural characteristics with typical short-chain 1,2-diacylphosphatidylcholine systems, such as DPPC/water monolayer in the condensed phase and DPPC/water bilayer in the gel phase. Furthermore, it was found that DLGPC/water monolayer has almost completely rotationally disordered acyl chains, which have not been observed so far in short-chain 1,2-diacylphosphatidylcholine/water bilayers. This study indicates the good biological relevance of the DLGPC/water monolayer which might be useful in protein/lipid studies to reveal protein structure and protein/lipid interactions in a membrane environment.

INTRODUCTION

Two-dimensional protein arrays anchored to a lipid Langmuir monolayer at an air/water interface have become valuable alternative systems to conventional crystallography for determining molecular structure at high resolutions (Zhao et al., 2000; Kaganer et al., 1999; Gidalevitz et al., 1999). In Langmuir monolayer systems, the water surface provides an ideally smooth (uncorrugated) substrate, and two thermodynamic variables, temperature and surface pressure, can be directly controlled. Phospholipids, which are one of the most important components of a biological membrane, can form stable Langmuir monolayers at the air/water interface because of their ideal amphiphilic properties (Garrett and Grisham 1995). A variety of experimental techniques can be applied for phospholipid monolayers to study their structures and properties, such as optical fluorescence microscopy, atomic force microscopy, infrared spectroscopy, x-ray reflection, neutron reflection and isotherms, etc. A biological membrane can be considered as two weakly coupled monolayers. Therefore, phospholipid monolayers are often used as an excellent model for studying membrane protein structure and protein/membrane interactions.

1,2-dilignoceroylphosphatidylcholine (DLGPC) has two saturated 24-carbon hydrocarbon chains. It has the same polar head group as 1,2-dipalmitoylphosphatidylcholine (DPPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) have, which are used extensively in lipid bilayer studies (Helm et al., 1987; Nagle et al., 1996). Based on the similarity between the monolayer and bilayer, it can be estimated that DLGPC/water monolayer in the condensed phase has approximately the same tilt angle, 30°, as the DPPC/water bilayer in the gel phase has. Thus DLGPC Langmuir monolayer roughly gives the membrane hydrocarbon core thickness ~25 Å, which is close to the usual hydrocarbon core thickness, 25~30 Å, of a membrane of a living system (White and Wimley, 1999). Thus the hydrophobic mismatch problem between the membrane protein and the lipid may be reduced in DLGPC Langmuir monolayer. However, this problem can arise when short-chain 1,2-diacylphosphatidylcholine monolayers, such as DPPC or POPC, are applied in studying protein/membrane interactions whose hydrocarbon core thickness is usually <18 Å (Helm et al., 1987; Wiener, 1989). Although this longest-chain commercially available phospholipid Langmuir monolayer has advantages in protein/membrane studies, molecular-level detailed structural information of this monolayer is still relatively limited from both the current experiment and theory. Molecular dynamics (MD) investigation in this paper can give insights into the structure and dynamics of the monolayer.

1,2-diacylphosphatidylcholines with long acyl chains ($n \geq 20$) share many phase and structure characteristics with short-chain 1,2-diacylphosphatidylcholines ($n < 20$) but also exhibit other unique properties in different experiments. The Comparison of the phase transition behavior of multilammellar liposomes of 1,2-diacylphosphatidylcholines with the chain length ranging from 12 to 24 was made by high-sensitivity differential scanning calorimetry (Lipka

Submitted 25 October 2001 and accepted for publication 10 December 2001.

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0006-3495/02/05/2511/09 $2.00

et al., 1984). The results have revealed that the number of H_2O molecules per lipid is similar for all the lipids in L_β or gel phase ($19H_2O$/lipid) but it increases with increasing chain length in L_α or liquid crystal phase. Main transition temperature also increases with the increasing chain length due to the increased attraction between the hydrocarbon chains. X-ray scattering and infrared spectroscopic studies have been performed on saturated 1,2-diacylphosphatidylcholine bilayers with even chain lengths varying from 16 to 24 (Sun et al., 1996a, b; Snyder et al., 1996). It was found that, in the normal gel phase, area/lipid is almost the same for all the bilayers, although the long-chain bilayers have more densely packed chains, i.e., smaller area/chain due to larger tilt angle. Anomalous phase behavior of long-chain 1,2-diacylphosphatidylcholine bilayers has also been discovered in these experiments. In the low-temperature regime, $T < 40°$, the long-chain bilayers can exist in a more closely packed phase with smaller tilt angle compared with the usual gel phase, and the stability of the new phase increases with the acyl chain length. In the high-temperature regime, $T > 45°$, another new ripple phase coexists with the normal gel phase in the long-chain bilayer systems, and this new hexagonal ripple phase also has smaller chain tilt angle than the ripple phase of the short-chain bilayers. Infrared spectroscopy studies on splitting of the methylene scissors band revealed the gel state miscibility in the lipid mixtures, such as $diC_{18}PC/diC_{20}PC$, $diC_{18}PC/diC_{22}PC$, and $diC_{18}PC/diC_{24}PC$. It is known that a chain-length difference of 2 leads to the near-ideal mixing, whereas a difference of 6, such as the $diC_{18}PC/diC_{24}PC$ mixture, leads to nearly completely phase separation, with each phase containing only one type of lipid (Mendelsohn et al., 1995).

A number of MD simulation studies on short-chain 1,2-diacylphosphatidylcholine bilayers, such as DPPC and DMPC, were carried out to reveal atomic-level structural and dynamic information (Tu et al., 1995; Venable et al., 2000; Takaoka et al., 2000; Shepherd et al., 2001). Those MD simulations have usually well reproduced many important experimental observations, such as area/lipid, chain packing, acyl chain tilt and conformation, head group orientation, electron density profiles, etc. Although the lipid bilayers have narrow range of area/lipid, the monolayers tend to spread to fill the available surface area with the area/lipid ranging from hundreds of square angstroms to tens of square angstroms, depending on the surface pressure applied. So, surface tension needs to be well controlled in the monolayer simulation to characterize the structure and properties of the studied system. This novel DLGPC/water monolayer constant normal pressure, constant surface tension, and constant temperature ($NP_N\gamma T$) MD study will supply detailed information about the characteristics of hydrocarbon chain packing, chain tilting, head group orientation, hydration state, etc., of this long-chain system. The simulation results will help to apply this monolayer in the protein/lipid studies because two-dimensional protein/membrane systems are used extensively in various experiments.

In this paper, detailed results of the $NP_N\gamma T$ MD investigation on DLGPC/water monolayer are given. Also the comparison is made between this MD result and a wide variety of experimental data and related other simulation results. Unique aspects of DLGPC/water monolayer revealed from the MD simulation are presented as well.

METHODS

DLGPC was constructed using InsightII (Molecular Simulations Inc., San Diego, CA) based on the x-ray crystal structure of DMPC, which has the same head group as DLGPC, but with 14 carbon atoms in each acyl chain (Pearson and Pascher, 1979). Ten carbons were added to each acyl chain of DMPC to generate a DLGPC molecule. The acyl chains were set to be vertical to the monolayer surface initially. The initial area per lipid was set to 48 $Å^2$ to be close to 47 $Å^2$/lipid for DPPC bilayer in the gel phase obtained from x-ray measurements (Nagle et al., 1996). The total number of lipids used in the simulation is 56, which comprise a monolayer surface with area 52×52 Å ≈ 2700 $Å^2$.

MD simulations were carried out using CHARMM27 code and CHARMM27 all-atom topologies and force fields, including a rigid TIP3P water (Brooks et al., 1983; Mackerell et al., 1998). For constant volume, constant temperature (NVT) MD simulation, the Nosé–Hoover method was used to control the temperature of the system, with the thermal inertial parameter set to be 50 (Nosé, 1984; Hoover, 1985). The algorithms for performing simulation of interfacial systems under constant normal pressure and surface tension as used in the $NP_N\gamma T$ ensemble have been described by Zhang et al., (1995). Three dimensional boundary conditions were used, and the length of the simulation cell normal to the monolayer was set to be 250 Å, large enough to ensure that the interactions between periodic replicas in this direction are negligible. Particle-mesh ewald was used to calculate the electrostatic energies and forces with ~1-Å grid spacing for three-dimensional fast Fourier transform. The van der Waals interactions were calculated with the simple truncation at 12 Å using a shifting function. The SHAKE algorithm was applied to constrain the lengths of bonds involving hydrogen atoms.

The initial DLGPC configuration was first subjected to ~10-ps NVT MD simulation at low temperature, and then, this unhydrated monolayer underwent another ~50-ps NVT MD simulation with 1-fs time step at 20°C. During the simulation, the vertical acyl chains of the monolayer tilted to ~30° with respect to the monolayer normal. Next, a slab of water about 30-Å thick with the same surface area as that of the monolayer was placed beneath the lipids. Then, NVT simulation was applied for ~500-ps for the hydrated system to evolve the monolayer to more stable configuration. Finally, the $NP_N\gamma T$ algorithm with 2-fs time step was used for another ~1.2-ns simulation to let the hydrated monolayer reach equilibrium area/lipid. The normal pressure was set to be 1 atom and the temperature 293.15 K. The surface tension, γ, used is 22.9 mN/M so the surface pressure on this Langmuir monolayer is ~45 mN/M, which is the often-used experimental condition. The surface pressure is calculated as

$$\pi = \gamma_0 - \gamma_1, \tag{1}$$

where π is the surface pressure and γ_0 is the water/air surface tension, which was measured to be 67.9 mN/M at room temperature (Feller et al., 1995). The analysis was performed in the final 250-ps $NP_N\gamma T$ simulation.

RESULTS AND DISCUSSION

Figure 1 is a snapshot of the fully equilibrated DLGPC/water Langmuir monolayer after 1.2-ns $NP_N\gamma T$ simulation.

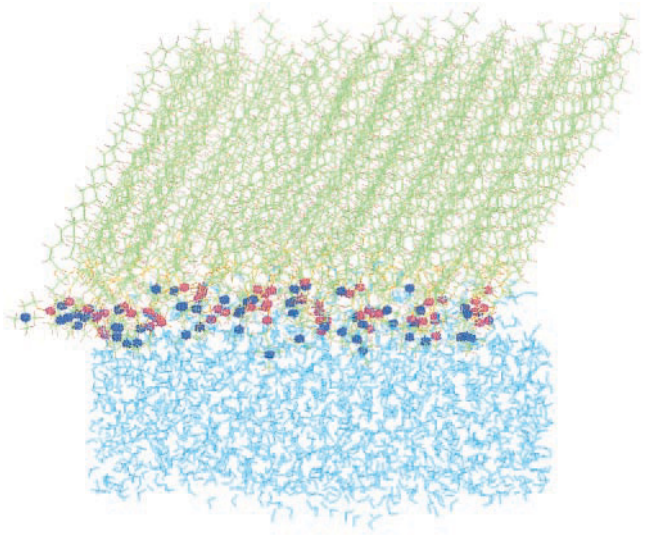

FIGURE 1 An instantaneous configuration of DLGPC/water monolayer after ~1.2-ns $NP_N\gamma T$ MD simulation. Coloring scheme: C, green; H, pink; N, blue; O, orange; P, magenta; water, light blue.

The data analysis was organized as follows. First, the area/lipid and tilt angle of the DLGPC/water monolayer were investigated to give the basic structure of the monolayer. Then, detailed studies on the conformation of the acyl chains, including order parameter, S_{CD}, profiles, dihedral angle distributions, and rotational order parameter distribution, were carried out to better characterize the monolayer hydrocarbon core. Then the head group conformation was analyzed to reveal the hydration state and P–N vector distribution in the monolayer. Next, the motional correlation and rotational correlation between the acyl chains within ~20-ps were studied to yield the dynamic information of the system. Finally, the electron density profiles along the monolayer normal were obtained to further compare with other monolayer and bilayer systems.

Surface area

The average area/lipid of DLGPC/water monolayer after $NP_N\gamma T$ simulation is 46.1 ± 0.3 Å^2. Under the studied condition, DLGPC/water monolayer has the same area/lipid as does DPPC/water monolayer, which was measured to be ~46 Å^2/lipid from the isotherm studies (Cadenhead et al., 1980; Albrecht et al., 1978). The area/lipid of the monolayers is also close to that of DPPC bilayer in the gel phase, which is 47.20 ± 0.5 Å^2 (Nagle et al., 1996). This similarity between monolayer and bilayer implies that the phase formed in DLGPC/water monolayer under the studied conditions has bilayer in the gel phase characteristics.

Acyl chain tilt

The tilt angle is defined as the angle between monolayer normal and a tilt vector, t_i, drawn from the fourth to the twenty-second carbon in an acyl chain of a DLGPC molecule. After the simulation, tilt angles of two branches of acyl chains, Sn1 and Sn2, are almost the same, 29.2 ± 0.6° and 29.6 ± 0.6°, respectively. The average tilt angle for DLGPC/water monolayer is 29.4 ± 0.6°. The tilt angle obtained from this system is almost identical to the tilt angle of DPPC/water monolayer, which is 30° measured from x-ray reflectivity (Helm et al., 1987). DLGPC tilt angle is also close to the tilt angle 31.6° for DPPC bilayer in the gel phase measured from x-ray experiments (Sun et al., 1996). Both DLGPC/water and DPPC/water monolayers adopt the common values of area/lipid and tilt angle, satisfying the equation (Hauser et al., 1981):

$$S \cos \theta_t = n\Sigma, \tag{2}$$

where S is the molecular area of a lipid molecule on the monolayer surface, Σ is the cross-section area of the hydrocarbon chain perpendicular to the long axis, θ_t is the chain tilt angle, and n is the number of hydrocarbon chains per lipid molecule. From this equation, the cross-section area of each hydrocarbon chain is calculated to be ~20.1 Å^2. DLGPC and DPPC monolayers have similar values of area/lipid and tilt angle, suggesting that it is the bulky head group that induces the chain tilting, and, the same bulky head group results in the same tilt angle irrespective of the length of the acyl chain in the condensed phase of 1,2-diacylphosphatidylcholine/water monolayers.

Acyl chain conformation

The order parameter, S_{CD}, is a measure of the motional disorder of the methylene group in a hydrocarbon chain. It is calculated from the simulation trajectory files using

$$S_{CD} = \tfrac{1}{2}\,|3\langle \cos^2\alpha(t)\rangle - 1|, \tag{3}$$

where $\alpha(t)$ is the instantaneous angle between the direction of the C–D bond in the acyl chain and the monolayer normal at time t, and the angle brackets represent an average over the length of the simulation.

The order parameter profiles, S_{CD}, calculated from the MD simulation are shown in Fig. 2. It is observed that the segment C_{i2} in the Sn2 chain of DLGPC has an order parameter of 0.14, which is smaller than that in the Sn1 chain, which is 0.31. It means that C_{i1}–C_{i2} bond is more parallel to the monolayer surface in the Sn2 chain. Therefore, Sn1 and Sn2 chains have different orientations near the head group region, and this can be found in Fig. 3 for two typical DLGPC molecules in the monolayer. The plateau in S_{CD} profile of the Sn1 chain includes segments from C_{32} to C_{322} and those methylene groups' motion is restricted. Compared with Sn1 chain, Sn2 chain's restricted methylene segments extend one more methylene group to C_{223}, indicating that Sn2 chain is more buried in the monolayer. Different S_{CD} distributions for two chains in one lipid

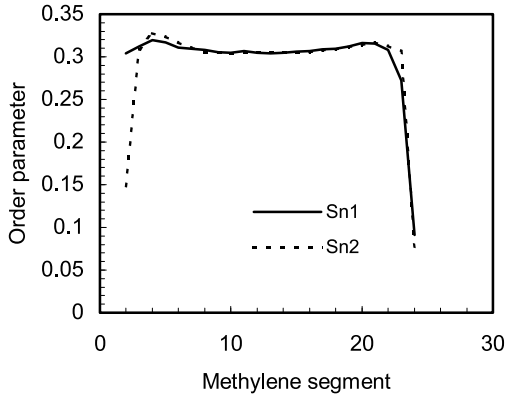

FIGURE 2 The calculated order-parameter profile from C_{i2} to C_{i24} in DLGPC/water system. Sn1 chain, $i = 3$; Sn2 chain, $i = 2$.

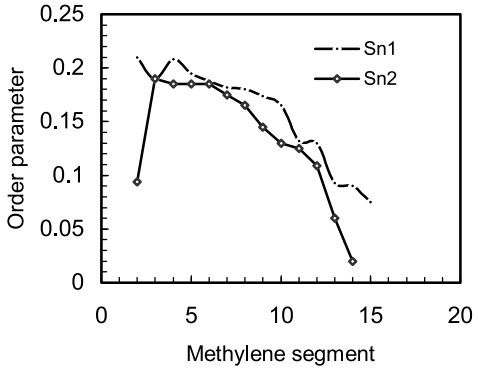

FIGURE 4 Experimental order parameter profiles from NMR quadripolar splittings for Sn1 chain of DPPC (Seelig and Seelig, 1974) and Sn2 chain of DMPC (Oldfield et al., 1978; Rice and Oldfield, 1979).

molecule show the chain inequivalence in this monolayer, and this has also been observed in lipid bilayer (Zaccai et al., 1979). Compare the S_{CD} profiles for DLGPC/water monolayer with the experimental S_{CD} profiles for Sn1 of DPPC and Sn2 of DMPC bilayers in the L_α phase obtained from NMR quadripolar splitting (Fig. 4). It can be concluded that, in all these systems, Sn2 chain always starts from a small-order parameter at C_{22}, whereas Sn1 chain reaches the plateau from the beginning C_{32}. The plateaus in DPPC and DMPC bilayers are less planar and have smaller values than in DLGPC monolayer. Different plateau characteristics between long-chain and short-chain lipid systems imply that long-chain 1,2-diacylphosphatidylcholines can have uniquely more ordered phases as various experiments revealed.

A dihedral angle φ_i is defined by four consecutive carbon atoms ($C_iC_{i+1}C_{i+2}C_{i+3}$) in the lipid acyl chain, and it describes the rotation about $C_{i+1}C_{i+2}$ bond. A dihedral angle can transit among three stable states, the *trans* state (corresponding to the potential minimum at 180°), the *gauche+* state (corresponding to the potential minimum at +60°) and the *gauche−* state (corresponding to the potential minimum at −60°). Figure 5 shows that, in the condensed phase of DLGPC/water monolayer, the lipid hydrocarbon chains are essentially in an all-*trans* fully extended conformation.

The two chains have similar *trans* fraction from segments C_{i3} to C_{i19}. At segment C_{i2}, the Sn2 chain has less *trans* fraction than the Sn1 chain has, whereas, from segments C_{i19} to C_{i22}, the Sn2 chain has more *trans* fraction. In accordance with previous S_{CD} results, *trans/gauche* distributions also indicate that Sn2 chain is more bent toward the monolayer surface near the head group region and Sn2's acyl-chain tail region is more restricted than Sn1's.

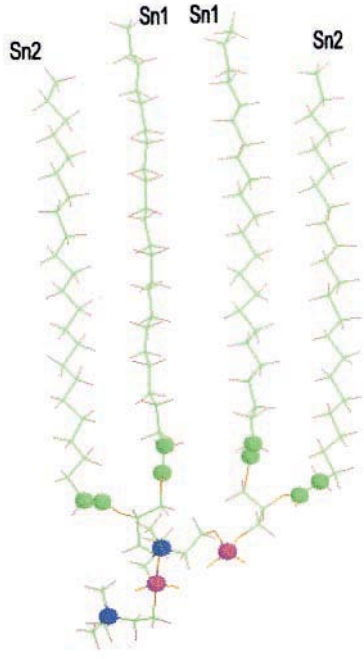

FIGURE 3 Two typical orientations of DLGPC molecules in the monolayer. The coloring scheme is the same used in Fig. 1.

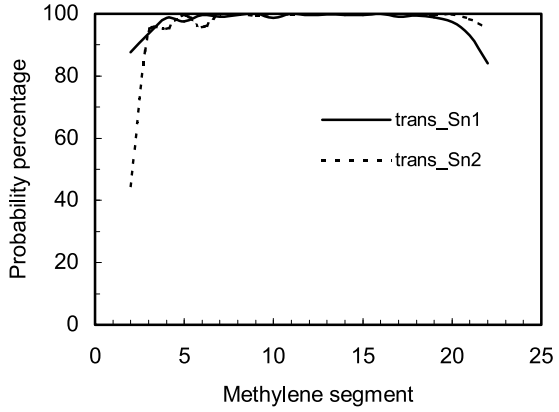

FIGURE 5 Fractions of *trans* bond of two acyl chains as a function of methylene segment in DLGPC/water monolayer.

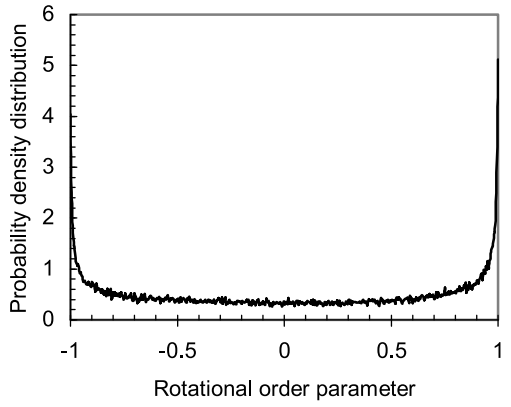

FIGURE 6 Probability density distribution of the rotational order parameter g_{12}.

For the average number of *gauche* states per chain of lipid, this monolayer MD simulation gives ~0.5 *gauche* states per chain compared with ~0.4 for DPPC/water bilayer in the gel phase and ~3 for DPPC/water bilayer in the liquid crystal phase from various experiments and MD simulations (Seelig and Seelig, 1974; Mendelsohn et al., 1989; Tu et al., 1995; Venable et al., 2000). Therefore, the property of hydrocarbon chain in DLGPC/water monolayer at the studied surface pressure and temperature is closer to that of the short-chain bilayer in the gel phase than in the liquid crystal phase.

Rotational order parameter, g, describes the ordering of the orientation of the zig-zag hydrocarbon chain planes relative to each other. g is defined as

$$g = \langle 2\cos^2\omega - 1\rangle, \tag{4}$$

where ω is the azimuthal angle of hydrocarbon chain relative to their long axis. $g = \pm 1$ corresponds to the complete order, and $g = 0$ to complete disorder (Nagle, 1993).

The average rotational order parameters are 0.042 and 0.018 for Sn1 and Sn2 chain respectively, giving the average rotational order parameter of the system 0.03 (Fig. 6). Different values of rotational order parameter are almost equally populated and they are close to 0, indicating almost complete disorder in the chain rotational orientation. Therefore, each hydrocarbon chain in DLGPC/water monolayer can independently rotate around its long axis, and those hydrocarbon chains can be treated as the cylindrical rods from the rotational point of view. This independent rotational behavior is similar to that in aliphatic Langmuir monolayers (Kaganer et al., 1999).

The disordered rotational orientation in DLGPC monolayer is different from that in DPPC bialyer in the gel phase studied by the experiment and MD simulation with the experimental g value for two acyl chains around -0.3 (Nagle, 1993; Tu et al., 1995). Based on these results, the short-chain DPPC bilayer in the gel phase is more rotationally ordered than the long-chain DLGPC/water monolayer.

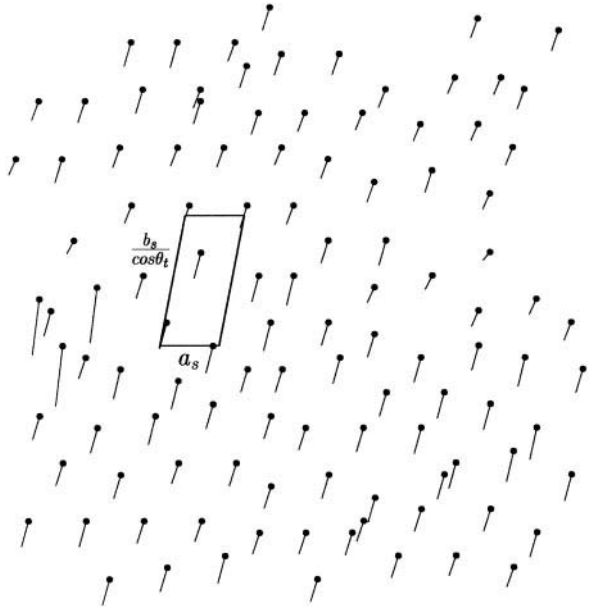

FIGURE 7 Projection of hydrocarbon chain C4–C22 (*dot*) vectors onto the monolayer surface of DLGPC/water system.

Rotationally flexible acyl chains in DLGPC/water monolayer might facilitate the adsorption of membrane protein into the monolayer because conformational change usually occurs in the lipid acyl chains that surround the adsorpted protein (Krol et al., 2000; Wu et al., 2001; Bos and Nylander, 1996).

The projection of hydrocarbon chain axes of DLGPC molecules onto the monolayer surface is shown in Fig. 7. The hydrocarbon chains in this monolayer system are packed primarily in a distorted hexagonal pattern with the nearest-neighbor tilt direction. Subcell parameters for the hydrocarbon chains are $a_s = 4.8$ Å and $b_s = 8.4$ Å. The chain-packing behavior of this monolayer is similar to that of the short-chain bilayers in the gel phase (Ruocco and Shipley, 1982; Abrahamsson et al., 1978).

Head group conformation

Unlike hydrocarbon chains, which exhibit ordered packing, the head groups appear to be disordered in this monolayer. Radial distribution functions are calculated for P–N, N–N, and P–P pairs (Fig. 8). The head groups are closely packed in this monolayer with an average distance of 4.2 Å between phosphorus and nitrogen atoms in the first-neighbor shell. The first-neighbor shell contains ~2.5 atoms. The second-neighbor shell of phosphorus atoms contains ~5 phosphorus atoms in the range of 6~7 Å. N–N distribution function gives a very broad peak for its nitrogen-atom shell, with the distance varying from 6 to 10 Å, indicating more disordered distribution for the nitrogen atoms. Radial distributions of water oxygen atoms around phosphorus, nitrogen, and car-

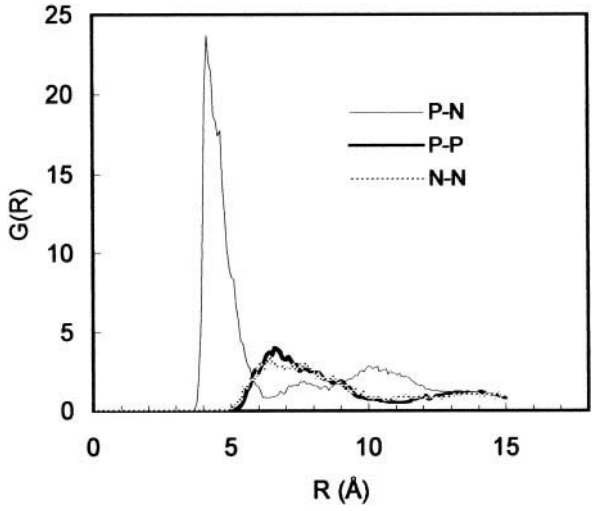

FIGURE 8 Phosphorus–nitrogen, phosphorus–phosphorus, and nitrogen–nitrogen radial distribution functions in DLGPC/water system. The integrations over the first peaks of P–N, P–P, and N–N distributions give 2.5, 4.9, and 4.6, respectively, as the number of the nearest neighbors.

bonyl carbons in Sn1 and Sn2 chains show the hydration states of these atoms. Integration of these distribution functions gives 3.4, 8.8, 0.56, and 0.18 as the number of water molecules surrounding the above atoms, respectively (Fig. 9). Nitrogen atoms are more hydrated than phosphorus atoms. Therefore, nitrogen atoms extend more into the water layer. Carbonyl carbon in Sn2 chain is more hydrated than that in Sn1 chain, because Sn2 chain is more bent to the monolayer surface near the head group region. Only a small number of water molecules surround the carbonyl carbon in Sn1 chain, indicating that hydration is probably up to this atom. Similarly, MD simulation on DPPC bilayer in the gel

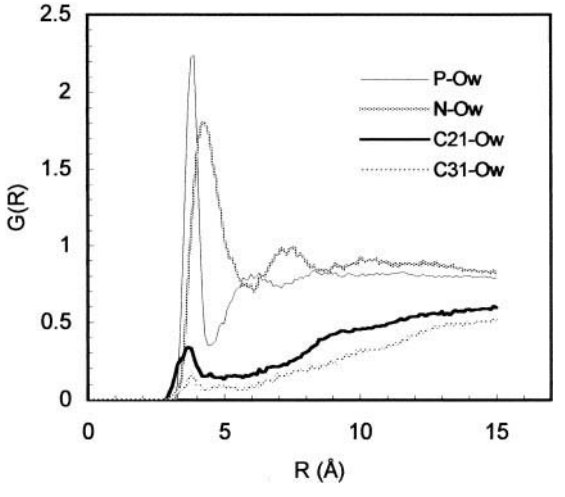

FIGURE 9 Radial distributions of water oxygen atoms around phosphorus, nitrogen, carbonyl carbon C21 and C31 atoms. The integrations over the first peaks of those curves give 3.4, 8.8, 0.56, and 0.18, respectively, as the number of the nearest-neighbor water molecules.

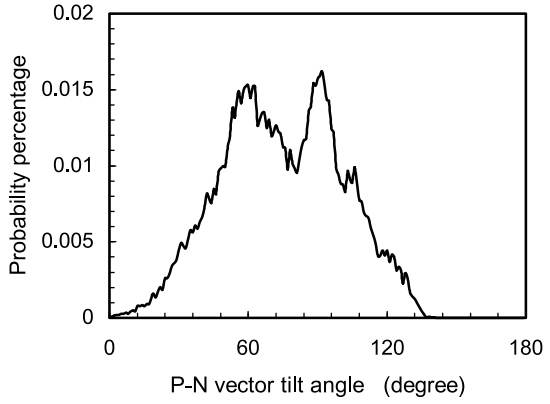

FIGURE 10 Probability density distribution of the P–N vector tilt angle.

phase also gives more ordered distribution for phosphorus atoms than for nitrogen atoms and much less hydrated carbonyl region (Tu et al., 1995; Venable et al., 2000).

P–N orientation

The P–N tilt angle is defined as the angle P–N vector makes with the monolayer normal. The average P–N vector tilt angle calculated from the simulation is 74.6 ± 25.8°. From the P–N vector tilt angle distribution (Fig. 10), it is known that there are two main P–N orientations toward the monolayer, one with the average tilt angle 61° and the other 91°. The crystal structure of DMPC bilayer also gives two P–N orientations, with P–N tilt angle 63° and 73°, respectively. Neutron reflection study on the head group orientations in DPPC/water monolayer revealed that P–N vector tends to be parallel to the monolayer surface in the liquid expanded phase and perpendicular in the condensed phase. Thus P–N tilt angle 91° in DLGPC/water monolayer indicates the expanded phase characteristic, and 61° indicates the condensed phase characteristic of the system. The simulation on DPPC bilayer in the gel phase gives the average P–N tilt angle 70° containing also two roughly equally populated orientations, of 41° and 84°, respectively (Pearson and Pascher, 1979; Brumm et al., 1994; Tu et al., 1995). According to the above DLGPC/water Langmuir monolayer structural analysis, the phase of DLGPC/water monolayer under the studied conditions can be classified as the liquid condensed, close-packed heads and disordered backbone plane L_{2d} phase, according to Kaganer et al., (1999).

Correlation between acyl chains

To measure the correlation between motions of the hydrocarbon chains, a vector $\Delta\mathbf{r}_i$ (t) which is the difference between the tilt vector t_i (t) at time t and the time average of this tilt vector, is defined. The motional correlation $C_M(r)$ between two hydrocarbon chains, i and j, with a separation

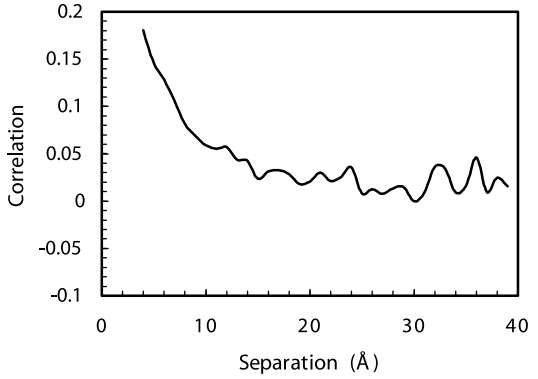

FIGURE 11 The average motional correlation of lipid chains as a function of distance over 20-ps period.

r is a dot product calculated using Eq. 5 over 20-ps simulation time (Robinson et al., 1994),

$$C_{\mathrm{M}}(r)dr = \left\langle \frac{\Delta \mathbf{r}_{\mathrm{i}}(t) \cdot \Delta \mathbf{r}_{\mathrm{j}}(t)}{|\Delta \mathbf{r}_{\mathrm{i}}(t)||\Delta \mathbf{r}_{\mathrm{j}}(t)|} \right\rangle dr. \quad (5)$$

According to the $C_{\mathrm{M}}(r)$ in Fig. 11, the motion of the chains is correlated at separations about 4~7 Å (corresponding to the nearest neighbors). This result is similar to that from the simulation on DMPC bilayer L_α phase.

The correlation between the rotational motions of the hydrocarbon chains is defined as

$$C_{\mathrm{R}}(r)dr = \langle \Delta \cos \omega_{\mathrm{i}}(t) \cdot \Delta \cos \omega_{\mathrm{j}}(t) \rangle dr, \quad (6)$$

where $\Delta \cos \omega_{\mathrm{i}}(t)$ is the difference between $\cos \omega_i$ at time t and the time average of $\cos \omega_i$ for the ith hydrocarbon chain. From Fig. 12, it is known that $C_{\mathrm{R}}(r)$ values are basically around zero for all the distances. There is no rotational motion correlation between the hydrocarbon chains even for the nearest neighbors. This result may provide a reason why DLGPC/water monolayer has almost completely rotational disorder in the acyl chains.

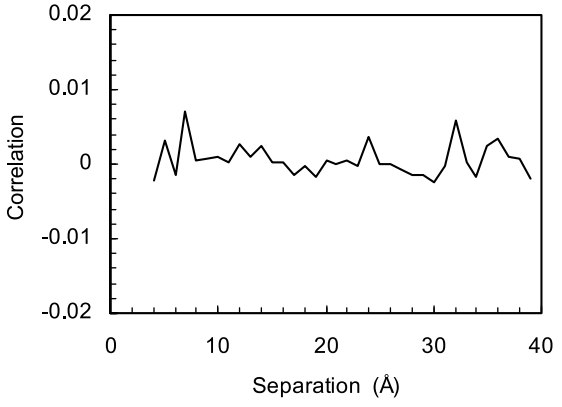

FIGURE 12 The average rotational correlation of lipid chains as a function of distance over 20-ps period.

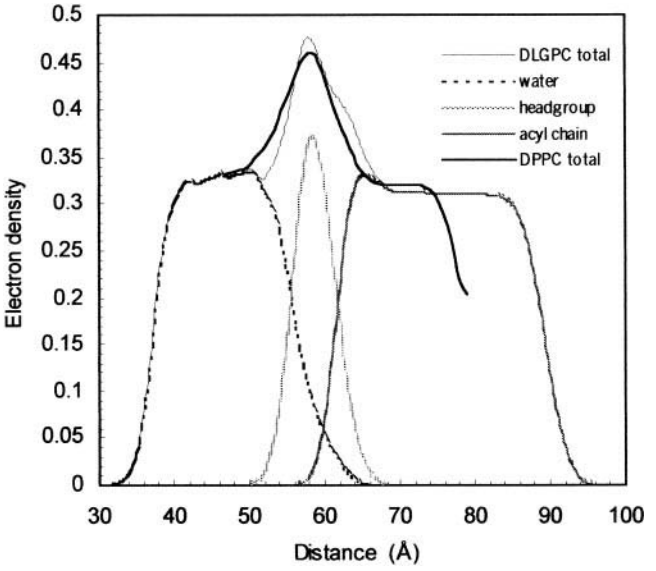

FIGURE 13 Electron density distribution profiles along the monolayer normal averaged over the simulation of DLGPC/water system. Separate contributions from different parts in DLGPC molecule are shown by the corresponding curves. Electron density profile for one leaflet of DPPC bilayer in gel phase obtained from x-ray measurement is also shown in solid line. The origin of the distance is arbitrary.

Electron density profiles

Electron density profiles of DLGPC/water monolayer were calculated from the simulation data by placing a Gaussian distribution of electrons on each atomic center with a variance equal to the van der Waals radius for each configuration and averaging over all the configurations (Fig. 13). For comparison, the electron density profile for one leaflet of DPPC/water bilayer in the gel phase measured from x-ray experiment is also shown in Fig. 13 (Wiener et al., 1989). The length of the head group region in DLGPC/water monolayer from the simulation is ~8 Å and it is close to 7.9 Å in DPPC/water monolayer from the best-fit x-ray data (Helm et al., 1987). The acyl-chain length of this monolayer is ~28 Å from the simulation, which is in the range of hydrocarbons core thickness of a membrane of a living system. From the separate contributions from different regions of the monolayer, it is clear that water molecules penetrate into the monolayer for ~10 Å, and some acyl chains also diffuse into the head group region and the water layer. All these regions contribute to the maximum electron density of the system. The maximum electron density is 0.47 for this monolayer, and it is close to the experimentally obtained 0.46 for DPPC bilayer in the gel phase. The electron densities of the plateaus for the acyl chains in DLGPC monolayer and DPPC bilayer are 0.31 and 0.32, respectively, but there is an electron density minimum in the middle of the bilayer.

From the above analysis, it is known that DLGPC/water monolayer in the condensed phase has 46.1 $Å^2$/lipid and

acyl-chain tilt angle 29.4°. Similar values have been obtained for DPPC/water monolayer and DPPC bilayer in the gel phase. The acyl chains in DLGPC monolayer are essentially in an all-*trans* fully extended conformation with 0.5 *gauche* states per chain. It is close to 0.4 *gauche* states per chain for DPPC bilayer in the gel phase. As in DPPC bilayer, two acyl chains in DLGPC monolayer are inequivalent, revealed by different S_{CD} distributions. But DLGPC monolayer has larger S_{CD} values than does DPPC bilayer in the liquid crystal phase. In contrast to DPPC bilayer in the gel phase, which has partially rotationally ordered acyl chains, DLGPC monolayer has almost zero rotational order parameter, indicating complete disorder in the chain rotational orientation. The hydration state of the head group and bimodal distribution of P–N vector orientation in DLGPC monolayer are comparable to those observed in DPPC bilayer in the gel phase. The motion of the acyl chains is correlated for the neareset neighbors and there is no rotational correlation between the acyl chains in DLGPC monolayer. Electron density profiles reveal similarities between DLGPC monolayer and DPPC bilayer in the gel phase, except that DPPC bilayer has an electron density minimum in the center of the hydrocarbon core, whereas the monolayer has an almost constant electron density within the hydrocarbon core.

CONCLUSION

$NP_N\gamma T$ MD simulation was performed on DLGPC/water Langmuir monolayer. First, the initial unhydrated crystalline DLGPC monolayer underwent ~50-ps NVT simulation, then the hydrated monolayer was subjected to ~500-ps NVT MD simulation to transit to the liquid condensed phase. The subsequent 1.2-ns $NP_N\gamma T$ simulation evolved the system to the equilibrium as area/lipid decreased from initial 48 $Å^2$/lipid to final 46.1 $Å^2$/lipid. The $NP_N\gamma T$ simulation well reproduced a number of experimental observations at surface tension 22.9 mN/M condition. Many similarities between this long-chain monolayer and usual short-chain systems have been revealed, such as hydrocarbon chain conformation, head group's orientation, pair correlation functions, hydration state, inequivalency of two acyl chains, electron density profiles, etc. These similarities indicate that, in the condensed phase, it is the head group, not the hydrocarbon chain region of 1,2-diacylphosphatidylcholine, that is the major factor in determining the packing properties of the lipids. Like short-chain systems, the motion of the hydrocarbon chains is correlated for the nearest neighbors in this system. However, unlike short-chain bilayer systems, this long-chain monolayer has rotationally disordered hydrocarbon chains as observed from this MD study. DLGPC/water monolayer has hydrocarbon core thickness ~28 Å, comparable to that of a membrane of a living system, as revealed from the calculated electron density profile of the system. DLGPC/water monolayer has head group and hydrocarbon core close to those of DPPC bilayer in the gel phase, and various experimental techniques are readily available to the monolayers instead of the bilayers. As a result, DLGPC/water monolayer in the liquid condensed phase has the advantage in the membrane/protein studies, and this long-chain Langmuir monolayer can act as a good model system for membrane biophysics when two-dimensional studies are desired. More MD investigation can also be performed on DLGPC/protein/water monolayers to yield complementary structural information on protein structure and protein/lipid interaction at the atomic level, which might be difficult to obtain from the experiment.

This work was supported by National Institute of General Medical Science Program Project Grant PO1 GM56538.

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