Cholesterol Effects on the Phosphatidylcholine Bilayer Nonpolar Region: A Molecular Simulation Study

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ABSTRACT A 15-ns molecular dynamics (MD) simulation of the fully hydrated dimyristoylphosphatidylcholine-cholesterol (DMPC-Chol) bilayer in the liquid-crystalline state was carried out to investigate the effect of Chol on the hydrocarbon chain region of the bilayer. The last 8-ns fragment of the generated trajectory was used for analyses. As a reference system, a pure DMPC bilayer (M. Pasenkiewicz-Gierula, Y. Takaoka, H. Miyagawa, K. Kitamura, and A. Kusumi, 1999, *Biophys. J.* 76:1228–1240) simulated for 14 ns was used. The study shows that a Chol-induced increase of the bulk molecular order parameter along both β- and γ-chain is mainly caused by a decrease of the average tilt of the chains, because the bulk average number of *gauche* rotamers/myristoyl chain is not significantly changed by Chol. Nevertheless, for DMPCs located near Chol molecules both the number of *gauche* rotamers/chain and the chain tilt are decreased. The magnitude of the Chol effect on the PC alkyl chains depends, in addition to the PC-Chol distance, on the side of the Chol molecule (α- or β-face) that the chains are in contact with. This study provides some new insight into the properties of the coexistence region of the partial phase diagram for DMPC-Chol bilayers.

INTRODUCTION

Cholesterol (Chol) is an important constituent of eukaryotic cell membranes where it accounts for up to 50 mol % of the membrane lipids (Sackmann, 1995). The biological roles of Chol involve maintenance of proper fluidity (Kusumi et al., 1983; Mouritsen and Jørgensen, 1994), formation of glycosphingolipid-Chol-enriched raft domains (Simons and Ikonen, 1997), reduction of passive permeability (Bittman et al., 1984; Subczynski et al., 1989, 1994), and increasing mechanical strength (El-Sayed et al., 1986; Bloom et al., 1991; Bloom and Mouritsen, 1995) of the membrane. Because Chol plays such important roles in the membrane, phospholipid-Chol interactions have been studied extensively (for recent reviews see Bittman, 1997; McMullen and McElhaney, 1996).

Experimental studies of phosphatidylcholine (PC)-Chol membranes have revealed that on the molecular level, Chol increases both the order of the alkyl chains (ordering effect) (Trouard et al., 1999; Urbina et al., 1995, 1998; Sankaram and Thompson, 1990; Pasenkiewicz-Gierula et al., 1990; Kusumi and Pasenkiewicz-Gierula, 1988; Oldfield et al., 1978) and the membrane surface density (condensing effect) (Smaby et al., 1997; Marsh and Smith, 1972), whereas it practically leaves PC dynamics unchanged (Morrison and Bloom, 1994; Lindblom et al., 1981).

The effect of Chol on saturated PC bilayers depends on its concentration, as show partial phase diagrams for PC/Chol mixtures (Bloom and Mouritsen, 1995, and papers cited therein). At concentrations above 22 mol %, Chol promotes a liquid-ordered (*lo*) phase of the PC-Chol bilayer (Bloom and Mouritsen, 1995; Vist and Davis, 1990; Ipsen et al., 1987). At concentrations below ~10 mol % and temperatures above the main phase transition temperature for the pure PC bilayer (T_c), Chol does not affect ordering of PC alkyl chains in a measurable way and a PC-Chol bilayer is in the same liquid-disorder (liquid-crystalline) (*ld*) phase as a pure PC bilayer (Vist and Davis, 1990). For intermediate Chol concentrations, i.e., between ~10 and ~22 mol % and temperatures above T_c, in PC-Chol bilayers *lo* phase coexists with *ld* phase (two-phase *ld-lo* region) (Bloom and Mouritsen, 1995; Vist and Davis, 1990; Sankaram and Thompson, 1990; Ipsen et al., 1987; Recktenwald and McConnell, 1981). The *lo* and *ld* regions of the phase diagram have been extensively studied by experimental methods both on thermodynamic and molecular levels (Vist and Davis, 1990; for a recent review see McMullen and McElhaney, 1996) as well as by theoretical methods (Ipsen et al., 1987) and are well characterized. The *ld-lo* region is not accessible to NMR studies due to fast exchange of molecules between the two coexisting domains; thus, molecular level information about this region remains inadequate (Vist and Davis, 1990; Bloom and Mouritsen, 1995).

Based on the results of infrared (IR) spectroscopy, a Chol-induced increase of the orientational order of PC hydrocarbon chains was explained as a result of a decrease in the number of *gauche* rotamers/PC chain (Ipsen et al., 1987). Recently, using Fourier transform (FT)-IR technique, Mendelsohn and Snyder (1996) obtained up to fivefold decrease in the *gauche* rotamer formation at some carbon positions in the PC chain when Chol content in a dipalmitoylphosphatidylcholine (DPPC) bilayer was increased from 0 to 50 mol %.

The planar tetracyclic ring system of Chol is not symmetric about the ring plane. The sterol ring has a flat side with no substituents (α-face) and a rough side with two

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methyl substituents (β-face) (cf. Fig. 9). Comparison of the ordering effect of Chol with that of lanosterol (two methyl substituents projecting from the α-face of the sterol ring) (Urbina et al., 1995; Yeagle, 1985; Dahl, 1981) indicated that the smooth face of the ring promotes higher ordering of PC alkyl chains than the rough face.

The ordering effect of Chol revealed experimentally has been reproduced in computer simulations of PC-Chol bilayers (Scott and Kalaskar, 1989; Scott, 1991; Edholm and Nyberg, 1992; Robinson et al., 1995; Tu et al., 1998; Smondyrev and Berkowitz, 1999). Monte Carlo (MC) calculations of Scott and Kalaskar (1989) and Scott (1991) showed that Chol significantly decreased *trans-gauche* isomerization of PC chains, particularly those that were in the vicinity of more than one Chol molecule. However, even these chains did not assume all-*trans* conformations. MC simulation additionally confirmed a stronger ordering effect of the Chol α-face than the β-face (Scott, 1991). Molecular dynamics (MD) simulations of Smondyrev and Berkowitz (1999) and Tu et al. (1998) clearly demonstrated that the Chol effect on the bulk membrane properties depends on the molar content of Chol and its distribution in the membrane. The order parameter profile of PC alkyl chains as well as the number of *gauche* rotamers per chain in PC bilayers containing 11–12.5 mol % Chol (Smondyrev and Berkowitz, 1999; Tu et al., 1998), were practically the same as in pure PC bilayers. However, in DPPC bilayer containing 50 mol % Chol (Smondyrev and Berkowitz, 1999), the order parameter profile was significantly shifted upwards, compared with that in a pure bilayer, and the number of *gauche* rotamers decreased from 7.0 in the pure bilayer to 5.1 and 4.3, depending on the Chol distribution. The study of Robinson et al. (1995) showed that Chol influences PC molecules in its vicinity differently from those further away.

Our previous MD simulation study of a dimyristoylphosphatidylcholine (DMPC)-Chol bilayer concerned the effect of Chol on the membrane/water interface organization (Pasenkiewicz-Gierula et al., 2000a,b). It showed that at the interface, polar groups of DMPC, Chol, and water interact via hydrogen (H) bonds and charge pairs. These interactions form an extended network that links 96% of DMPC and 70% of Chol molecules at any instant.

In this paper, we concentrate on the effect of Chol on the hydrocarbon chain region in the DMPC-Chol bilayer membrane containing ~22 mol % Chol and simulated for 15 ns. This bilayer represents a region of the partial phase diagram where pure PC and PC-Chol domains coexist (*ld-lo* region) (Vist and Davis, 1990) and enables us to study the effect of Chol on the nearby lipids and those further away. Analyses of the last 8-ns fragment of the trajectory generated in the simulation show that the magnitude of the alkyl chain ordering by Chol depends both on the DMPC-Chol distance and on the side (face) of the Chol molecule that a DMPC molecule is in contact with. Basic mechanisms responsible for the ordering effect of Chol are shown.

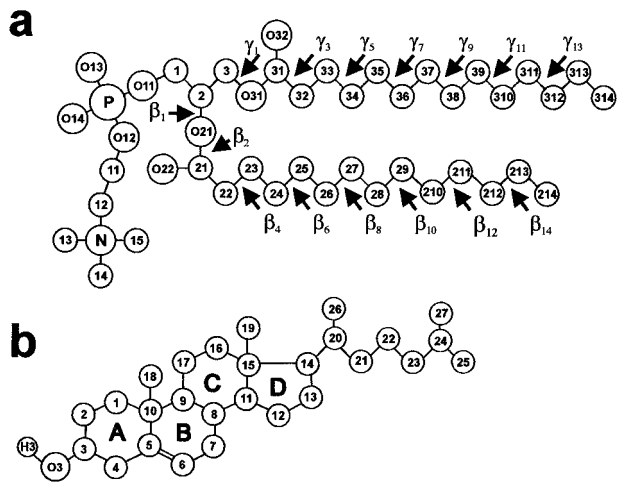

FIGURE 1 Molecular structure with numbering of atoms and torsion angles of DMPC (*a*), and Chol (*b*) (chemical symbol for carbon atoms, C, is omitted). Torsion angles in the DMPC β- and γ-chain are indicated. The Chol rings are labeled A, B, C, and D.

METHOD

Simulation systems

A DMPC-Chol bilayer membrane used in this study consisted of 56 DMPC, 16 Chol (~22 mol % Chol), and 1622 water molecules. Details concerning construction and the first 4.3 ns of simulation of this bilayer are described in Pasenkiewicz-Gierula et al. (2000b). A MD simulation of the bilayer was carried out further, and after 5 ns all chiral centers in Chol molecules were checked. Apparently, due to the united-atom approximation, in half of Chol molecules chirality at the C14 chiral center (cf. Fig. 1 *b*) spontaneously inverted. The chirality in these molecules was corrected and since 5 ns it has been controlled by appropriate improper torsion terms in the potential function. The simulation was carried out for the total time of 15 ns. As a reference system, a pure DMPC bilayer simulated for 14 ns was used. Details concerning construction and equilibration of this bilayer were described in Pasenkiewicz-Gierula et al. (1997, 1999). Fig. 1 shows the structure and numbering of atoms and torsion angles in DMPC and Chol molecules.

Simulation parameters

DMPC-Chol and pure DMPC bilayers were simulated using AMBER 4.0 (Pearlman et al., 1991). For DMPC and Chol, optimized potentials for liquid simulations (OPLS) parameters (Jorgensen and Tirado-Rives, 1988) were used. The procedure for supplementing the original OPLS base with the missing parameters for DMPC was described by Pasenkiewicz-Gierula et al. (1999) and for Chol by Pasenkiewicz-Gierula et al. (2000b). For water, TIP3P parameters (Jorgensen et al., 1983) were used. The united-atom approximation was applied to CH, CH_2, and CH_3 groups of

DMPC and Chol. The hydroxyl group of Chol was treated with full atomic details. The atomic charges of the DMPC molecule were taken from Charifson et al. (1990) (a detailed explanation is given in Pasenkiewicz-Gierula et al., 1999). The atomic charges of the Chol are given in (Pasenkiewicz-Gierula et al., 2000b).

Simulation conditions

Three-dimensional periodic boundary conditions with the usual minimum image convention were used. The SHAKE algorithm (Ryckaert et al., 1977) was used to preserve the bond length of the water molecule and the hydroxyl group of Chol, and the time step was set at 2 fs (Egberts et al., 1994). For nonbonded interactions, a residue-based cutoff was employed with a cutoff distance of 12 Å. Our test calculations indicated that in the case of bilayers built of neutral lipids, cutoff and full electrostatic simulations give similar conformations and ordering of lipids. Therefore, to reduce calculation time and to compare this system with the reference system (Pasenkiewicz-Gierula et al., 1997), the cutoff simulation was chosen in this study. To further reduce calculation time, each DMPC molecule was divided into six residues (Pasenkiewicz-Gierula et al., 1997), and each Chol molecule was divided into three residues (Pasenkiewicz-Gierula et al., 2000b). The list of nonbonded pairs was updated every 25 steps.

Simulation was carried out at a constant temperature of 310 K (37°C), which is above the main phase transition temperature for a pure DMPC bilayer (~23°C), and a constant pressure (1 atm). Temperatures of the solute and solvent were controlled independently. Both the temperature and pressure of the system were controlled by the Berendsen method (Berendsen et al., 1984). The relaxation times for temperatures and pressure were set at 0.4 and 0.6 ps, respectively. Applied pressure was controlled anisotropically, where each direction was treated independently and the trace of the pressure tensor was kept constant (1 atm).

Group criteria

To study the effect of Chol on nearby and further away PCs, DMPC molecules in the DMPC-Chol bilayer were classified into three groups. The selection proceeded in three steps: 1) pairs of DMPC and Chol molecules from the same leaflet, for which the distance between their centers of mass (CM) was smaller than 5 Å, were selected; 2) for each pair, distances between the β- and γ-chain methylene groups and the ring atoms of the Chol were checked (when at least three methylene groups in one of the DMPC chains were closer than 5 Å from any three atoms in the Chol ring, the DMPC and Chol were picked as nearest neighbors); and 3) DMPCs from the nearest-neighbor pairs, located on the α-face side (a flat face with no substituents) of Chol molecules, comprised the first group, and DMPCs located on the β-face side (a rough face, with two methyl substituents) of Chol molecules, comprised the second group. The third group comprised DMPCs whose methylene groups of the β- and γ-chain were further than 7 Å from any of the Chol ring atoms. Each group contained not less than 10 DMPC molecules.

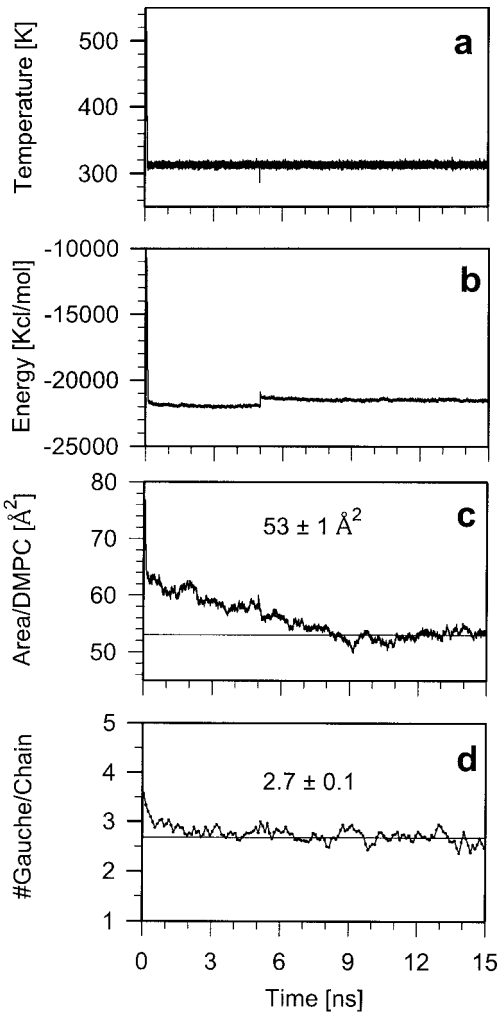

FIGURE 2 Diagrams showing the time development of the temperature (*a*), potential energy (*b*), surface area per DMPC (*c*) (the equilibrium average surface area is 53 ± 1 Å^2), and number of *gauche* bonds per chain (*d*) (the equilibrium average number is 2.7 ± 0.1). The errors are standard deviations. Thin lines in *c* and *d* indicate average values of the parameters in the time range between 7 and 15 ns.

RESULTS

Characterization of the membrane system

Time development of the system temperature (Fig. 2 *a*), system potential energy (Fig. 2 *b*), surface area/DMPC (Fig. 2 *c*), and number of *gauche* conformations/myristoyl chain (Fig. 2 *d*) was monitored from the onset of simulation until 15 ns. The surface area/DMPC in the DMPC-Chol mem-

TABLE 1 Average values of the molecular order parameter, S_{mol}, chain tilt angle, number of *gauche*/myristoyl chain, and lifetimes of *trans* and *gauche* conformations for the β- and γ-chain of DMPCs from the first, second, and third groups in the DMPC-Chol bilayer and those for the β- and γ-chain of all DMPCs in DMPC-Chol and pure DMPC bilayers

	S_{mol}		Tilt (°)		Number of *gauche*		Lifetime (ps) *trans*/*gauche*	
Group/Membrane	β-chain	γ-chain	β-chain	γ-chain	β-chain	γ-chain	β-chain	γ-chain
First	0.53 ± 0.01	0.50 ± 0.01	19 ± 1	19 ± 1	2.2 ± 0.1	2.1 ± 0.1		
Second	0.48 ± 0.01	0.49 ± 0.01	19 ± 1	19 ± 1	2.2 ± 0.1	2.0 ± 0.1		
Third	0.43 ± 0.01	0.26 ± 0.01	20 ± 1	28 ± 1	2.6 ± 0.1	2.6 ± 0.1		
DMPC	0.30 ± 0.01	0.31 ± 0.01	27 ± 0.5	28 ± 0.5	2.4 ± 0.1	2.4 ± 0.1	180 ± 4	190 ± 4
							55 ± 2	55 ± 2
DMPC-Chol								
PC	0.45 ± 0.01	0.40 ± 0.01	20 ± 0.5	22 ± 0.5	2.3 ± 0.1	2.2 ± 0.1	215 ± 4	230 ± 4
Chol			17* ± 2				60 ± 2	60 ± 2

The errors are standard errors.
*Tilt of the sterol ring.

brane was obtained by subtracting the cross-sectional area of eight Chol molecules (8 × 39 Å^2) from the total surface area of the membrane and then dividing it by 28 DMPC molecules present in each leaflet. The mean surface area of the Chol molecule of 39 Å^2 was determined by Hyslop et al. (1990) in a Chol monolayer. According to data shown by Smaby et al. (1997), the surface area of Chol in a PC-Chol bilayer should not differ much from that in the monolayer. As can be seen in Fig. 2 *c*, the surface area/DMPC decreased between 0 and 2.3 ns and between 5 and 7 ns of MD simulation. Between 2.3 and 5 ns and between 7 and 15 ns, the surface area/DMPC had stable average values of 58.4 ± 1 Å^2 and 53 ± 1 Å^2, respectively. The potential energy, after initial decrease, was stable between 1.5 and 5 ns, but at 5 ns it increased to reach a higher stable value within the next 1 ns. Discontinuity in values of the surface area and potential energy at 5 ns and necessity of the second equilibration of the system resulted from the imposition of proper chirality at the inverted C14 chiral center in several Chol molecules (cf. Methods) and addition of improper torsion terms for all chiral centers in the Chol molecule to the potential energy function. The latter prevented chirality inversion in all Chol molecules. The number of *gauche* conformations/myristoyl chain reached the value of 2.7 ± 0.1 within the first 2 ns and remained unchanged afterwards. Therefore, it was concluded that the DMPC-Chol bilayer membrane reached its second thermal equilibrium at 7 ns of simulation. Results described in this paper were obtained from an 8-ns trajectory generated between 7 and 15 ns of MD simulation. Reported average values are time and ensemble averages. Errors of the surface area/PC, S_{mol}, number of *gauche* rotamers/chain, are given in standard deviations as in our previous paper (Pasenkiewicz-Gierula et al., 2000b), whereas errors of values in Table 1 and in Figs. 7, 8, 10, and 12 are given in standard errors to significantly compare calculated average values. Fig. 3 is a snapshot of the pure DMPC bilayer at 12 ns (Fig. 3 *a*) and the DMPC-Chol bilayer membrane at 10 ns (Fig. 3 *b*).

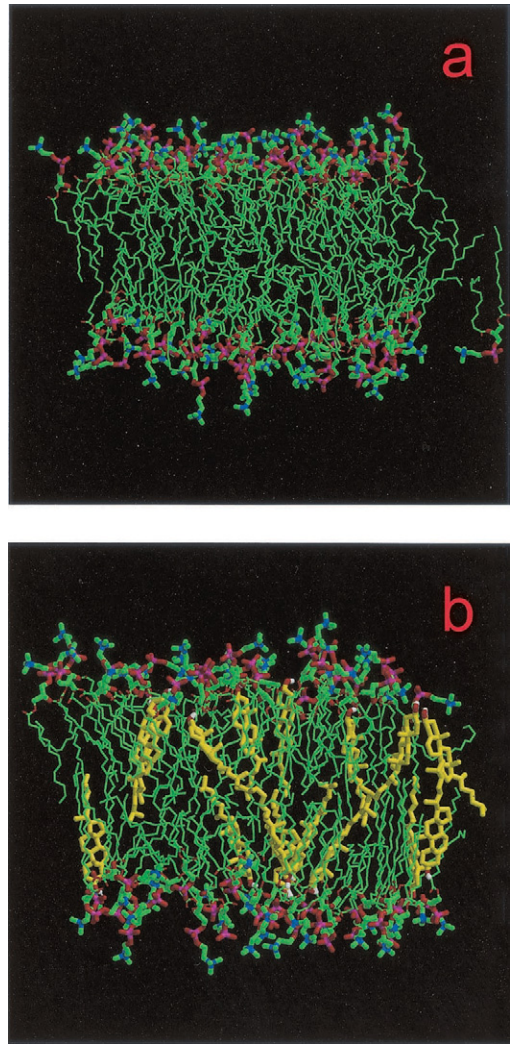

FIGURE 3 Snapshots of the equilibrated DMPC bilayer after 12 ns of MD simulation (*a*) and DMPC-Chol bilayer after 10 ns of MD simulation (*b*). The Chol molecules and DMPC headgroups are in the stick representation. The Chol molecules are yellow, and all other atoms, including the OH group of Chol, are in standard colors. Water is removed to better show bilayer surfaces.

In our previous paper on the DMPC-Chol bilayer, a 2-ns trajectory generated between 2.3 and 4.3 ns of MD simulation was analyzed to determine the properties of the membrane/water interface (Pasenkiewicz-Gierula et al., 2000b). Initially, it was judged that the bilayer reached thermal equilibrium after 2.3 ns, as average values of the representative parameters did not depend on time between 2.3 and 4.3 ns (cf. Fig. 2). However, correction of inverted chirality at the 5th ns required additional 2-ns equilibration. Within this time the mean surface area/DMPC decreased to 53 ± 1 Å^2 and was 7 Å^2 smaller than in the pure DMPC membrane. A decrease of the surface area/PC of a similar magnitude was observed in DMPC-Chol monolayer (Smaby et al., 1997). After the first 2.3-ns equilibration, the average area/DMPC was 58.4 ± 1 Å^2 (Pasenkiewicz-Gierula et al., 2000b). A decrease of the surface area/DMPC during the second equilibration by an additional ~5 Å^2 affected the properties of the membrane/water interface in a predictable way. As Murzyn et al. (2001) showed, in the interfacial region of a PC bilayer, numbers of PC-water and PC-PC interactions are correlated with the surface area available to the PC headgroup. An increase of the area/PC from 40 to 90 Å^2 resulted in ~30% increase of the PC hydration and over twofold decrease in the number of PC-PC links/PC (Murzyn et al., 2001). In accordance with these results, in the DMPC-Chol bilayer a decrease of the surface area/DMPC from 58.4 to 53.0 Å^2 practically did not change the average number of water molecules hydrating a PC (11.3 H_2O/PC) and a Chol (1.0 H_2O/Chol), whereas it increased the average number of PC-Chol links from 1.2 to 1.8/Chol and PC-PC links from 2.2 to 2.4/PC.

Although in the equilibrated DMPC-Chol bilayer the mean surface area/DMPC is by 7 Å^2 smaller than that in the pure DMPC bilayer, the average number of *gauche* rotamers/myristoyl chain is the same and equal to 2.7 ± 0.1. A similar effect of Chol intercalation into a PC bilayer was observed experimentally (Hyslop at al., 1990). Profiles of the molecular order parameter (S_{mol}) along the DMPC β- and γ-chain in DMPC and DMPC-Chol bilayers are shown in Fig. 4. In agreement with experimental (Sankaram and Thompson, 1990; Urbina et al., 1995, 1998) and other MD simulation (Smondyrev and Berkowitz, 1999) data, Chol increased S_{mol} of the alkyl chains at all depths in the membrane.

Average P-P and N-N spacings (distance between average positions of P (N) atoms in two leaflets of the bilayer) are over 2 Å larger in the DMPC bilayer containing 22 mol % Chol than in the pure DMPC bilayer. A Chol-induced increase of the PC bilayer thickness was observed experimentally (Sankaram and Thompson, 1990; Leonard and Dufourc, 1991) and in MD simulations (Smondyrev and Berkowitz, 1999).

The discussed above trends in the effect of Chol on PCs in PC-Chol bilayers observed experimentally were visible in a shorter (2-ns) simulation; however, they were much better reproduced when the productive simulation was carried out for 8 ns.

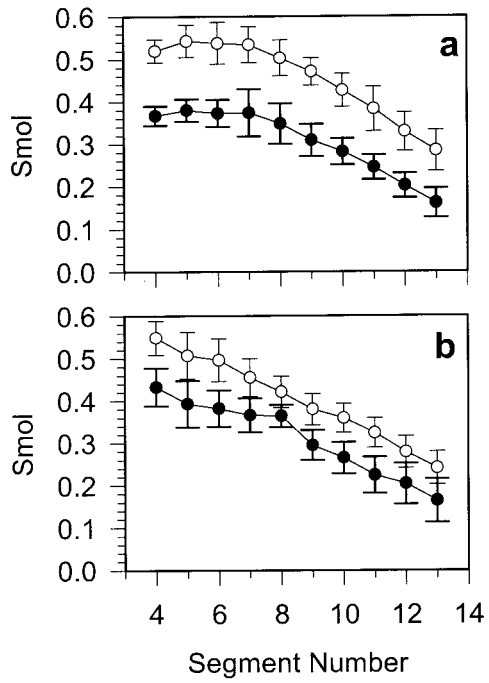

FIGURE 4 The molecular order parameter (S_{mol}) profiles calculated for the DMPC β-chain (*a*), and γ-chain (*b*) in pure DMPC (●) and DMPC-Chol (○) bilayers at 310 K. The errors bars are standard deviations. Average values of S_{mol} are given in Table 1.

Cholesterol effect on the bulk order and isomerization of DMPC alkyl chains

In the analyses below, the values of parameters were obtained by averaging relevant quantities over all PC molecules in the membrane (and time), irrespective of their distance from Chol molecules. These average values are called membrane, or bulk, averages. Averaging conformation-related quantities, only torsion angles 4–14 were taken into account, because, as Fig. 5 indicates, neither in pure DMPC nor in DMPC-Chol bilayers are β3 and γ3 in well defined, stable conformations (*trans* or *gauche*). However, in the section above, average numbers of *gauche* rotamers/alkyl chain included these torsion angles to enable the comparison of the present results with those obtained by us previously and found in the literature.

Molecular order parameter of PC alkyl chains

The order parameter for the *n*th segment of an alkyl chain, S_{mol}, was calculated from (Hubbell and McConnell, 1971)

$$S_{mol} = \frac{1}{2}\langle 3\cos^2\theta_n - 1\rangle,$$

where θ_n is an instantaneous angle between the *n*th segmental vector, i.e., (C_{n-1}, C_{n+1}) vector linking $n - 1$ and $n + 1$ carbon atoms in the alkyl chain and the bilayer normal; $\langle\ \rangle$ denotes both the ensemble and the time average. S_{mol} pro-

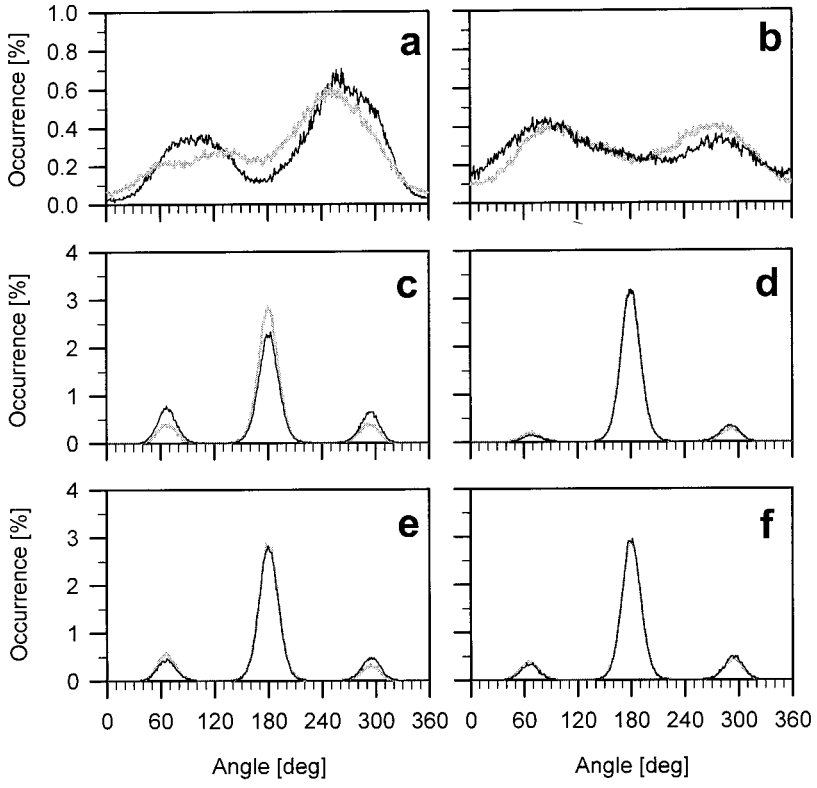

FIGURE 5 Populations of torsion angles in the beginning of β- and γ-chain in pure DMPC (*gray*) and DMPC-Chol (*black*) bilayers at 310 K: β3 (*a*), γ3(*b*), β4 (*c*), γ4 (*d*), β5 (*e*), and γ5 (*f*). Angles with values 60° ± 30°, 180° ± 30°, and 300° ± 30° correspond to *gauche*$^+$, *trans*, and *gauche*$^-$ conformations, respectively.

files along the β- and γ-chain in DMPC (Pasenkiewicz-Gierula and Róg, 1997) and DMPC-Chol bilayers are shown in Fig. 4. Mean values (averages over segments 4–14) of the order parameter for the β- and γ-chain are given in Table 1. As can be seen from Fig. 4 and Table 1, the effect of Chol is stronger on the β- than γ-chain.

Tilt of the PC alkyl chains

A tilt angle of a PC chain was calculated from $\cos^2$ of the angle between the bilayer normal and the average segmental vector (averaged over segmental vectors 4–14). As can be read from Table 1, Chol decreased the membrane average tilt of both β- and γ-chain by ~7°. Distributions of tilt angles of the β- and γ-chain in DMPC and DMPC-Chol bilayers are shown in Fig. 6. For the DMPC-Chol bilayer, the distributions are narrower and shifted toward lower values compared with those for the DMPC bilayer.

Tilt of the Chol ring

Chol tilt was defined as an angle between the (C3, C15) vector (cf. Fig. 1 *b*) and the membrane normal. The Chol average tilt, calculated based on the cone angle formalism, is 17° (Table 1).

Conformation of PC alkyl chains

Probability profiles of the *gauche* conformation along the β- and γ-chain in DMPC and DMPC-Chol bilayers are shown

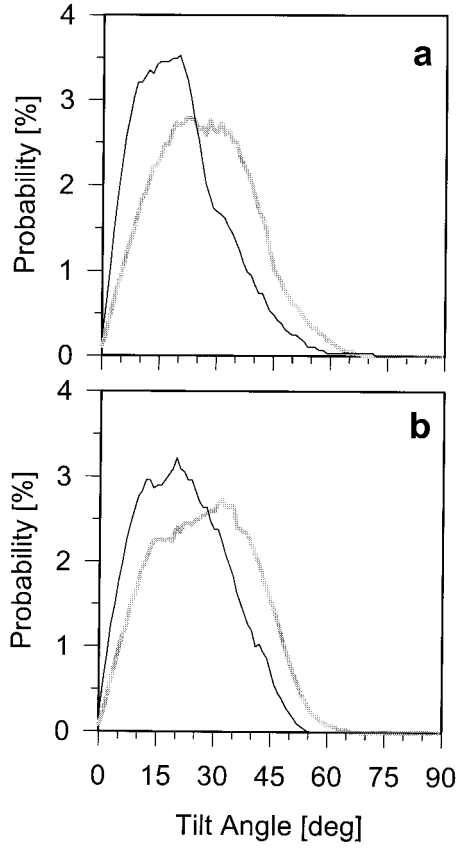

FIGURE 6 Distributions of tilt angles for β-chain (*a*), and γ-chain (*b*) in pure DMPC (*gray*) and DMPC-Chol (*black*) bilayers. Average tilt angles are given in Table 1.

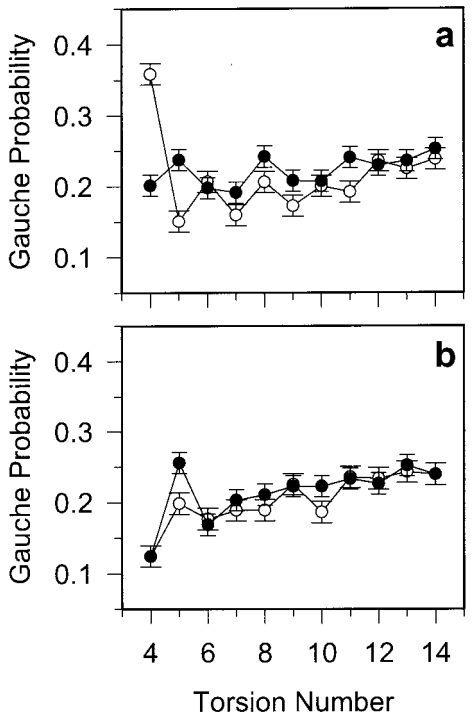

FIGURE 7 Probabilities of *gauche* conformations along β-chain (*a*) and γ-chain (*b*) in pure DMPC (●) and DMPC-Chol (○) bilayers. The errors bars are standard errors. Average numbers of *gauche* conformations/alkyl chain are given in Table 1.

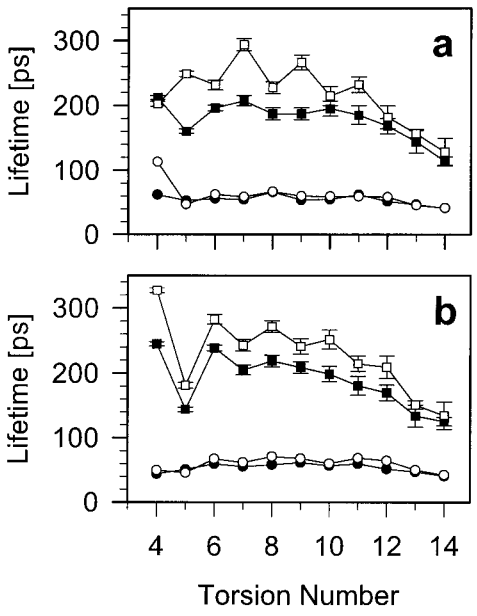

FIGURE 8 Profiles of lifetimes of the *trans* (■ and □), and *gauche* (● and ○) conformations along β-chain (*a*) and γ-chain (*b*) in pure DMPC (■ and ●) and DMPC-Chol (□ and ■) bilayers. The errors bars are standard errors; for the *gauche* conformation the errors are less than the size of the symbols. Average lifetimes are given in Table 1.

in Fig. 7. The Chol effect on the β-chain is to significantly increase the *gauche* probability for torsion angle β4, decrease the probability for β5-β11, and leave it unchanged for β12-β14. Chol has little effect on the probability of *gauche* rotamers in the γ-chain. Small differences between membrane average numbers of *gauche* rotamers per chain in DMPC and DMPC-Chol bilayers (Table 1) result from a redistribution of probabilities along the β-chain and similar distribution of probabilities along the γ-chain in the DMPC-Chol bilayer compared with the DMPC bilayer.

Lifetimes of conformational states

Rotational freedom about C-C bonds in the alkyl chain allows transitions between conformational states. Lifetime profiles of *gauche* and *trans* conformations along the β- and γ-chain in DMPC and DMPC-Chol bilayers are shown in Fig. 8. Average lifetimes (average over torsion angles 4–14) are given in Table 1. In the DMPC-Chol bilayer, the lifetimes of *gauche* conformations along both β- and γ-chain are practically the same as in the DMPC bilayer, whereas those of *trans* conformations are for most torsion angles higher; however, the increase is not large. Thus, Chol slightly stabilizes *trans* conformation and leaves *gauche* almost unchanged, except for β4.

Cholesterol effect on the order and isomerization of alkyl chains of DMPCs from selected groups

To recognize the effect of the Chol α- and β-face as well as the distance from Chol on the conformation of DMPC alkyl chains in the DMPC-Chol bilayer, three distinct groups of DMPC molecules were selected and analyzed (cf. Method). First group consisted of DMPCs that were neighbors of the Chol α-face; second, of DMPCs that were neighbors of the Chol β-face; third, of DMPCs that were not in contact with any Chol molecule. To account for the diffusion processes in the bilayer, the 8-ns trajectory was divided into four 2-ns fragments, and analyses were conducted independently for each fragment. The reported results were averages over the fragments. A DMPC molecule qualified for one of the groups if it fulfilled the group criteria for at least 1.5 ns of the 2-ns trajectory (e.g., it belonged to the first group if it was a near neighbor of the α-face of Chol for at least 1.5 ns). Molecules, which were not in well defined states, were not assigned to any group and were not analyzed. Each group consisted of 11–14 DMPC molecules; thus, 35–40 of 56 DMPC molecules present in the bilayer were analyzed for each time fragment. Because the number of molecules belonging to the groups was limited, long MD simulation was required to obtain statistically significant results.

It is interesting to note that in most cases, both β- and γ-chain were simultaneously in contact with the Chol α-face, whereas either β- or γ-chain was in contact with the Chol β-face. There was no apparent preference for the β- or

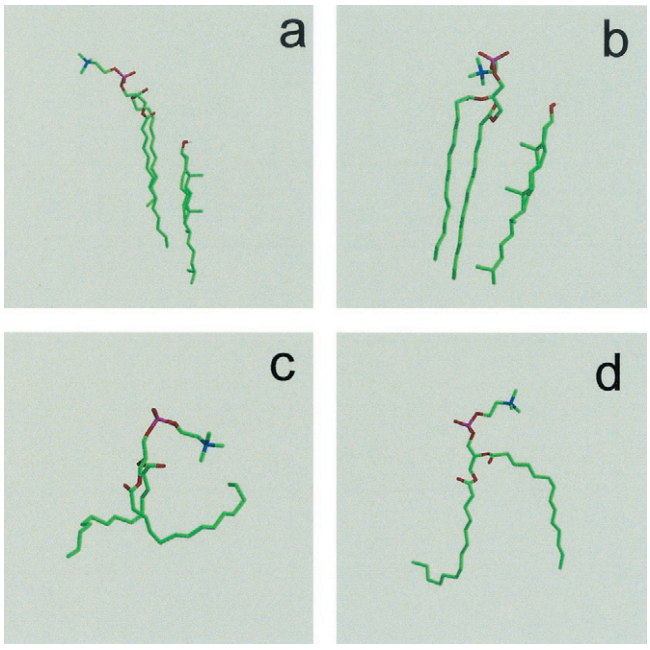

FIGURE 9 Examples of DMPC-Chol pairs (*a* and *b*) and DMPC molecules (*c* and *d*) in the DMPC-Chol membrane. (*a*) DMPC from the first group; (*b*) DMPC from the second group; (*c* and *d*) DMPCs from the third group. All atoms are in standard colors.

γ-chain to interact with Chol (either α- or β-face), and both chains made contacts with Chol equally often.

In Fig. 9, examples of DMPCs neighboring the Chol α-face (Fig. 9 *a*), β-face (Fig. 9 *b*), and those from the third group (Fig. 9, *c* and *d*) are shown.

Molecular order parameter of PC alkyl chains

S_{mol} profiles for DMPCs from the first, second, and third groups in the DMPC-Chol bilayer are shown in Fig. 10, *a* (β-chain) and *b* (γ-chain). Profiles of S_{mol} along β- and γ-chains for DMPCs from the first group are compared in Fig. 10 *c*, and those for DMPCs from the second group in Fig. 10 *d*. Fig. 10, *a*—*d*, demonstrates that the ordering effect of the Chol α-face is stronger than that of the β-face, and β- and γ-chains are similarly affected by Chol. In Fig. 10, *e* and *f*, the profiles for DMPCs from the third group are compared with those for the pure DMPC bilayer (Pasenkiewicz-Gierula and Róg, 1997). It is apparent that Chol differently affects β- and γ-chains: ordering of the β-chain is higher than that in the pure DMPC bilayer, whereas that of the γ-chain is for most segments similar. It is interesting to note that S_{mol} values for the last four segments of the β-chain are practically the same for all three groups of DMPCs. Furthermore, ordering of γ-chains of DMPCs from the first and second groups differs only for segments 4–6 (carbon atoms from these segments are most frequently the

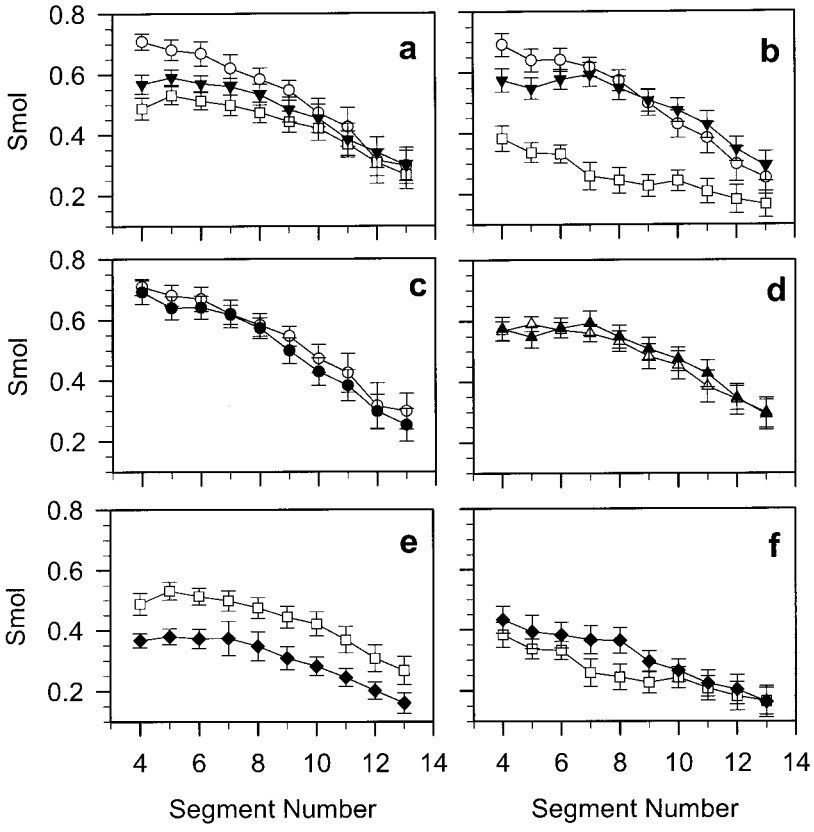

FIGURE 10 The molecular order parameter (S_{mol}) profiles for DMPCs from selected groups in DMPC-Chol membrane. (*a* and *b*) β-chain (*a*), and γ-chain (*b*) of DMPCs from the first (○), second (▼), and third (□) groups; (*c*) β-chain (●), and γ-chain (○) of DMPCs from the first group; (*d*) β-chain (▲) and γ-chain (△) of DMPCs from the second group; (*e*) β-chain of DMPCs from the third group (□) and β-chain of DMPCs from pure DMPC bilayer (♦); (*f*) γ-chain of DMPCs from the third group (□) and γ-chain of DMPCs from pure DMPC bilayer (♦). The errors bars are standard errors. Average values of S_{mol} are given in Table 1.

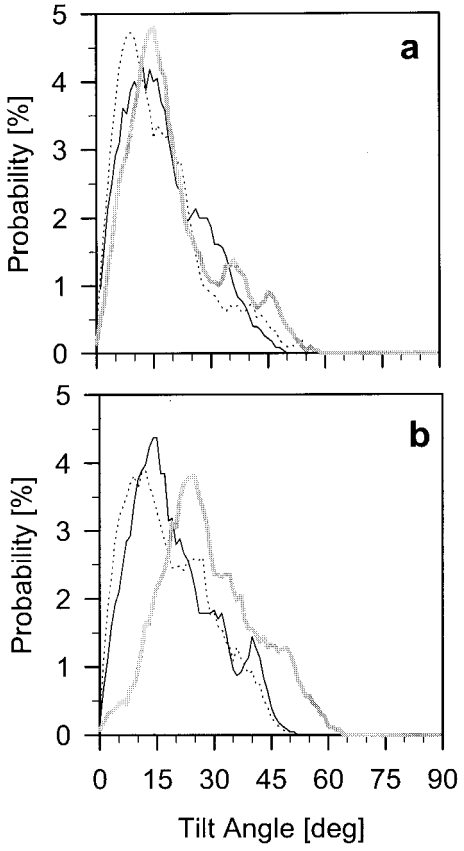

FIGURE 11 Distributions of tilt angles for β-chain (*a*) and γ-chain (*b*) of DMPCs from the first (*dotted*), second (*black*), and third (*gray*) groups in DMPC-Chol bilayer. Average tilt angles are given in Table 1.

nearest neighbors of the methyl groups of the Chol β-face) and is much higher than that of DMPCs from the third group. Average values of S_{mol} for the selected groups of DMPCs are compared in Table 1.

Tilt of PC alkyl chains

Distributions of tilt angles for hydrocarbon chains of DMPCs from the first, second, and third groups are shown in Fig. 11. Average values of the angles are given in Table 1. Both distributions and average values of tilt are practically the same for DMPCs from the first and second groups and are similar for the β- and γ-chain. The distributions are narrower and shifted to lower values compared with those in the pure DMPC bilayer (Figs. 11 and 6). For DMPCs from the third group, the distribution and average value of the β-chain tilt are similar to those for the DMPCs from the first and second groups; however, the average tilt of the γ-chain is substantially larger (Table 1) and its distribution is shifted to larger values (Fig. 11). On average, the γ-chain tilt is similar to that for the pure DMPC bilayer (Table 1); however, the tilt distribution is narrower and does not cover small angles (Figs. 11 and 6). A larger value of an average tilt is consistent with a lower value of the average S_{mol} for the γ-chain of DMPCs from the third group.

Conformation of PC alkyl chains

Probabilities of *gauche* conformation along the β- and γ-chain of DMPCs from the first, second, and third groups are shown in Fig. 12, *a* and *b*. The highest probabilities are

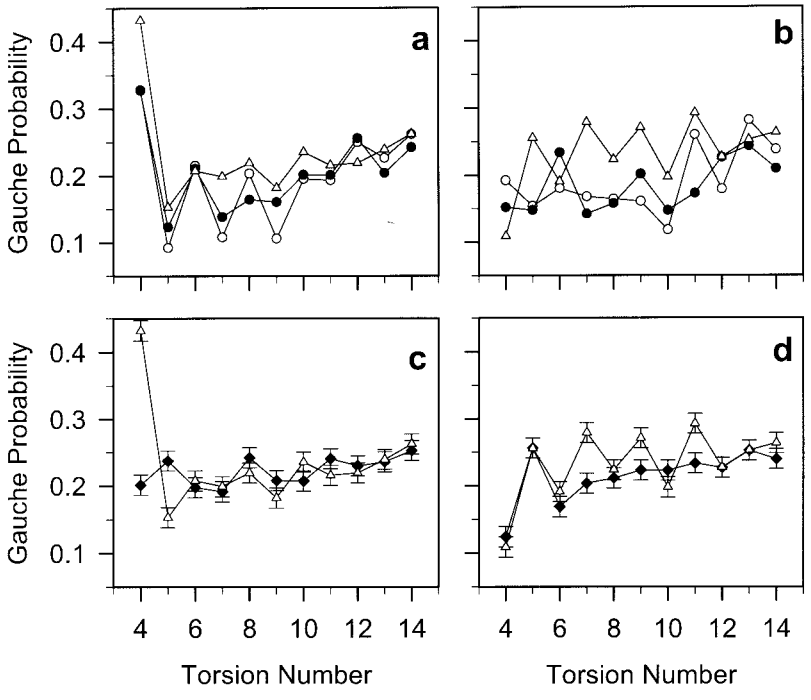

FIGURE 12 Probabilities of *gauche* conformations along alkyl chains. (*a* and *b*) β-chain (*a*) and γ-chain (*b*) of DMPCs from the first (●), second (○), and third (△) groups in DMPC-Chol bilayer; (*c*) β-chain of DMPCs from the third group (△) and from pure DMPC bilayer (◆); (*d*) γ-chain of DMPCs from the third group (△) and from pure DMPC bilayer (◆). The errors bars in *c* and *d* are standard errors. In *a* and *b*, due to a large number of data points, the error bars were not assigned to each point; however, the average standard error is 0.015. Average numbers of *gauche* conformations/alkyl chain are given in Table 1.

for the third group, except γ5, for which the probability is the smallest. Toward the end of the β-chain, differences among groups disappear; for the γ-chain, the effect is weaker. In Fig. 12, *c* and *d*, *gauche* probability profiles for DMPCs from the third group are compared with those from the pure DMPC bilayer. For the β-chain, the profiles differ only for β4 and β5; for the γ-chain, the profiles differ for γ7, γ9, and γ11. Average numbers of *gauche* rotamers/chain for the groups and the pure DMPC bilayer are given in Table 1. Because β4, γ7, γ9, and γ11 for DMPCs from the third group have higher probability of *gauche* than the corresponding torsion angles in the pure DMPC bilayer, average numbers of *gauche* conformers per chain are larger. For DMPCs from the first and second groups the numbers are smaller than those in the pure DMPC bilayer. This is the reason why the membrane average number of *gauche* rotamers/chain in DMPC-Chol bilayer is similar to that in the pure DMPC bilayer (Table 1).

Lifetimes of conformational states

Because of inadequate statistics, we were not able to obtain meaningful values of lifetimes for the three groups of DMPCs in the DMPC-Chol bilayer.

DISCUSSION

Results presented in this paper were obtained from the last 8-ns fragment of the 15-ns trajectory generated in MD simulation of the DMPC-Chol bilayer containing 22 mol % Chol. At this Chol content (PC:Chol ratio is 7:2), there are PCs in direct contact with Chol and those far from any Chol molecule, so the effect of Chol on the properties of an average DMPC and that from a selected group could be analyzed. The long productive simulation time of 8 ns enabled a proper statistical evaluation of the data. The results of this simulation were compared with those obtained from 14-ns MD simulation of the pure DMPC bilayer. The main focus of this work was to determine the atomic level details of the Chol ordering effect on the PC alkyl chains.

Average DMPC

The intercalation of Chol into a PC bilayer promoted higher ordering of PC alkyl chains. This effect may result from 1) reduced formation of *gauche* rotamers along PC chains and 2) reduced tilting of PC chains. Separation of these two mechanisms by any method is difficult because they are interrelated; however, using FT-IR spectroscopy, a significant Chol-induced decrease in the formation of *gauche* rotamers was observed (Mendelsohn and Snyder, 1996). Similar reduction was reported by Morrison and Bloom (1994) who used the ^{2}H NMR spin relaxation method. Therefore, Chol-promoted ordering of alkyl chains has been most often attributed to a decreased probability of *gauche* conformation along PC chains.

In accord with experimental results (Urbina et al., 1998; Sankaram and Thompson, 1990) and other computer simulations (Smondyrev and Berkowitz, 1999; Robinson et al., 1995) in the simulated DMPC-Chol bilayer, S_{mol} values for PC chains averaged over all membrane DMPCs were considerably higher than those in the pure DMPC bilayer (Fig. 4). However, Chol only moderately affected both the probabilities of *gauche* conformation along the chains and the lifetimes of the conformational states, except for β4 (Figs. 7 and 8). Instead, Chol promoted a large decrease of an average tilt of alkyl chains (Table 1; Fig. 6). These results strongly suggest that higher ordering of alkyl chains in the DMPC-Chol bilayer mainly results from a more parallel to the bilayer normal orientation of the chains. These chains, particularly γ-chains, exhibited similar internal flexibility as more tilted (less ordered) chains in the pure DMPC bilayer. This result is at apparent variance with conclusions drawn from FT-IR (Mendelsohn and Snyder, 1996) and ^{2}H NMR relaxation time (Morrison and Bloom, 1994) measurements. However, in those experiments, different PCs and different Chol concentrations were used.

The Chol effect on the β-chain differed from that on the γ-chain. In the β-chain, Chol promoted both a redistribution of the *gauche* probability along the chain (Fig. 7 *a*) and a decrease of the chain tilt (Fig. 6 *a*), whereas only γ-chain tilt was affected by Chol (Figs. 7 *b* and 6 *b*). These may be the reason why Chol-induced ordering of the β-chain is stronger than that of the γ-chain (Fig. 4). In agreement with observations of Tu et al. (1998), average numbers of *gauche* rotamers/chain were similar in DMPC-Chol and pure DMPC bilayers.

DMPCs from selected groups

To estimate how Chol-induced ordering of a PC chain depends on the distance between the chain and the nearest Chol as well as on the side of the Chol in contact with the chain, DMPC molecules in the bilayer were segregated into three groups, i.e., those with chains 1) near the Chol α-face (first group), 2) near the Chol β-face (second group), and 3) away from any Chol molecule (third group) (cf. Group criteria and Results sections). There was no apparent preference for the β- or γ-chain to interact with Chol (either α- or β-face). In general agreement with experimental results (Urbina et al., 1995; Yeagle, 1985) and MC simulation (Scott, 1991), alkyl chains of DMPCs from the first group were either more ordered (upper parts) than or similarly ordered (lower parts) to those from the second group (Fig. 10, *a* and *b*). The molecular basis for higher ordering of DMPCs from the first group than the second group is not very apparent as the difference neither in distributions of tilt angles (Fig. 11) nor in distributions of *gauche* probabilities

(Fig. 12) was very pronounced. Most likely, higher ordering is due to a slightly larger number of β- and γ-chains with small tilt angles in the first than the second group (Fig. 11). Both in the first and second group, Chol similarly affected the DMPC β- and γ-chain (Fig. 10, *c* and *d*). For DMPCs from the third group, orientational order of β-chains considerably differed form that of γ-chains (Fig. 10, *e* and *f*, and Table 1). γ-Chains of DMPCs not in contact with Chol were similarly ordered to γ-chains of DMPCs in the pure bilayer, whereas β-chains were evidently affected by Chol.

For all three groups, like for the bulk membrane, β4 had higher and most of the remaining torsion angles had lower probability of *gauche* than the corresponding torsion angles in the pure DMPC bilayer. An increased probability of *gauche* conformation for β4 in a PC-Chol bilayer was reported by Smondyrev and Berkowitz (1999). They explained this effect by Chol-induced restriction in the motion of carbon atoms in the beginning of the β-chain. In line with these results, our simulation indicated that for bulk membrane the lifetime of *trans* conformation of β4 was similar in DMPC-Chol and DMPC bilayers whereas that of the *gauche* conformation was greater (Fig. 8). A direct influence of Chol on the *gauche* conformation of β4 is not very likely, as the probability of *gauche* was the highest for β4 of DMPCs from the third group (Fig. 12). However, higher surface density in a DMPC-Chol bilayer may impose restrictions on transitions between conformational states of certain torsion angles, and the bent region of the β-chain seems to be most susceptible to a change in the PC packing. Paradoxically, as can be concluded from Figs. 7 and 12, in all three groups, high probability of *gauche* for β4 induced smaller probability of *gauche* for other torsion angles along β-chains, particularly β5, and also smaller tilt of these chains. For the γ-chain, higher probability of *gauche* for γ4 and lower probability of *gauche* for γ5 than the corresponding probabilities in the pure DMPC bilayer were observed in the first and second groups only, and the effect was much less pronounced than in the β-chain. These might explain the difference in the Chol-induced ordering of β- and γ-chains in DMPCs from the third group and, in general, higher bulk ordering of β- than γ-chains in DMPC-Chol bilayer.

An average tilt of the Chol ring was 17° (Table 1) and fell into a range of values (16–19°) obtained experimentally (Oldfield et al., 1978, Dahl, 1981; Dufourc et al., 1984; Murari et al., 1986). As Murari et al. (1986) observed, such a tilt facilitates hydration of the Chol hydroxyl group. An average tilt of β- and γ-chains of DMPCs in the vicinity of Chol molecules was 19° (Table 1); thus, these chains were aligned almost in parallel with the Chol ring. This alignment propagated on the β-chains of DMPCs away from Chol molecules but, as discussed above, not on the γ-chains (Table 1). As the intercalation of Chol into a PC bilayer causes a reduction in the tilt of PC alkyl chains, it is more rational to assume that Chol aligns PC chains and not vice versa. Most likely, the transfused, tetracyclic ring system of Chol acts as a rigid surface that reduces the angular amplitude of PC chain reorientation. The amplitude is to a large extent controlled by conformations of torsion angles in the beginning of PC alkyl chains. Thus, specific conformations of the fourth and fifth torsion angles in β- and γ-chains most likely optimize the spatial arrangement of Chol molecules and surrounding alkyl chains. In effect, ordering of PC chains in a PC bilayer containing Chol is higher than in a pure PC bilayer.

As was shown in many experiments, in the DMPC-Chol bilayer the probability of *gauche* increased toward the end of the alkyl chain. The effect was more pronounced for the β-chain (Fig. 12) but was not very large.

It was difficult to establish the lifetime of a contact between Chol and PC alkyl chains from this MD simulation. Nevertheless, we were able to estimate that the exchange of DMPCs between the third and either first or second group is slower than 4 ns. The exchange between the first and second group is faster and of the order of 1.5 ns. Thus, DMPCs that are neighboring Chol molecules remain as neighbors for a relatively long time. During this time, neighboring DMPC molecules may, most likely due to Chol rotation, face different sides of the Chol molecule they are in contact with. This indicates that Chol does not form a stable complex with surrounding DMPC molecules. This conclusion is in line with our results (Róg and Pasenkiewicz-Gierula, 2001) and those of Hyslop et al. (1990) that Chol does not strongly interact with PC alkyl chains; instead it promotes stronger van der Waals interactions among chains.

This study demonstrates that in the DMPC-Chol bilayer containing ~22 mol % Chol, pure PC and PC-Chol domains coexist. Hydrocarbon chains of DMPCs from the pure PC domain are less ordered, more tilted, and have higher probability of the *gauche* conformation than those from the PC-Chol domain. The pure PC domain contains approximately six DMPC molecules in each leaflet. Preliminary analysis indicates that this domain is surrounded by PC-Chol microdomains, each of which contains one Chol molecule and about five alkyl chains. The exchange between the domains is slow on the MD simulation time scale (slower than 4 ns); however, on the NMR time scale it is fast. Precise determination of the size, distribution, and lifetime of PC-Chol microdomains requires further analyses and will be a subject of another publication.

CONCLUSION

A computer model of the fully hydrated liquid-crystalline DMPC-Chol bilayer membrane that is stable for 15 ns was constructed using MD simulations. Results obtained for this bilayer were compared with those for pure DMPC bilayer simulated for 14 ns.

The average surface area/DMPC in the DMPC-Chol bilayer was 7 Å smaller than that in the pure DMPC bilayer.

When bulk DMPC-Chol bilayer properties are analyzed, Chol significantly increased molecular order parameter values along alkyl chains relative to those in the pure DMPC bilayer. This increase was mainly due to a decrease of an average tilt of PC alkyl chains as the average number of *gauche* conformations/chain was similar in both bilayers.

When properties of DMPCs from selected groups are analyzed, the Chol α-face promoted a stronger ordering effect on PC alkyl chains than the β-face. Neither of the Chol faces had a preference to interact with either β- or γ-chain. Ordering of β- and γ-chains of DMPCs in the vicinity of the Chol α- and β-face was similar. The Chol effect on the ordering of β-chains of DMPCs away from Chol was stronger than that of γ-chains.

Higher ordering of DMPC alkyl chains in the DMPC-Chol bilayer (except for γ-chains of DMPCs from the third group) might be attributed to, respectively, increased and decreased probability of *gauche* conformation for the fourth and fifth torsion angles in β- and γ-chains relative to probabilities of the corresponding torsion angles in the pure DMPC bilayer.

A Chol molecule and surrounding alkyl chains stay in contact for a time longer than 4 ns; however, exchange between DMPCs from the first and second group is of the order of 1.5 ns. Thus, in the DMPC-Chol bilayer containing ~22 mol % Chol a pure DMPC domain coexists with a DMPC-Chol domain.

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