Molecular Dynamics Simulation of the Structure of Dimyristoylphosphatidylcholine Bilayers with Cholesterol, Ergosterol, and Lanosterol

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ABSTRACT Five molecular dynamics computer simulations were performed on different phospholipid:sterol membrane systems in order to study the influence of sterol structure on membrane properties. Three of these simulated bilayer systems were composed of a 1:8 sterol:phospholipid ratio, each of which employed one of the sterol molecules: cholesterol, ergosterol, and lanosterol. The two other simulations were of a bilayer with a 1:1 sterol:phospholipid ratio. These simulations employed cholesterol and lanosterol, respectively, as their sterol components. The observed differences in simulations with cholesterol and lanosterol may have their implication on the form of the phospholipid/sterol phase diagram.

INTRODUCTION

Cholesterol and closely related sterols play an important role in the function of plasma membranes in most eukaryotic cells. The incorporation of cholesterol into the phospholipid membrane usually: a) broadens and eventually eliminates gel to liquid-crystalline phase transition of phospholipid bilayers b) decreases (increases) the area per molecule of the liquid-crystalline (gel) phase of monolayers c) increases (decreases) the orientational ordering of the hydrocarbon chains in liquid-crystalline (gel) phase of bilayers (although, as was shown recently, for some lipids, such as dimyristoylphosphatidylcholine (DMPC), cholesterol induces an increase in the orientational order both in the gel and liquid crystalline phases (McMullen et al., 1994)) and d) decreases (increases) the passive permeability of the bilayer above (below) the main transition temperature. These effects were investigated using different physicochemical techniques (for recent reviews of cholesterol in membranes see Finegold (1993) and McMullen and McElhaney (1996)) and computer simulations (Scott, 1991; Robinson et al., 1995; Tu et al., 1998; Smondyrev and Berkowitz, 1999c; Nielsen et al., 1999; Pasenkiewicz-Gierula et al., 2000). Changes and alternations in structure of the cholesterol molecule are responsible for some loss of the ability to produce the above-mentioned effects. As a result, the physical properties of the bilayer are modified. Thus, it was suggested that a change in physical properties of the bilayer due to the replacement of cholesterol in the membrane by another sterol-ergosterol (this sterol can be found in the membranes of fungi, yeasts, and protozoans) may be responsible for the difference in the interaction between membrane and an antibiotic such as amphotericin B (Bolard, 1986; Brajtburg et al., 1990). The structures of cholesterol and ergosterol are displayed in Fig. 1 and, as one can see, they are not that different from each other. If there is a difference in the specific antibiotic/membrane interaction when sterol structure is modified, it would be of interest to understand its physical origin.

In Fig. 1, the structure of another sterol molecule, lanosterol, is also displayed. It is very close in its structure to both cholesterol and ergosterol. Contrary to the situation with cholesterol and ergosterol, lanosterol is not present in naturally occurring membranes, but it is a common precursor of cholesterol and ergosterol in the evolutionary pathway. The question is therefore: why has nature spent thousands of years to convert from lanosterol to other two sterols? It was suggested (Bloom and Mouritsen, 1988) that cholesterol is designed to optimize thermodynamic and mechanical properties of phospholipid membranes. Apparently cholesterol can do this, but not lanosterol. The difference in thermodynamics of cholesterol/phospholipid and lanosterol/phospholipid mixtures can be understood on the basis of the phase diagram of the sterol/phospholipid mixture. The phase diagram of a dipalmitoylphosphatidylcholine (DPPC)/cholesterol mixture in the presence of water was inferred by Vist and Davis (1990). According to their phase diagram a new phase, which is called liquid ordered (lo), appears when the concentration of cholesterol is high ($X_{chol} > 25\%$). This phase is characterized by a simultaneous presence of a high degree of conformational order of phospholipid molecules and lateral disorder. At low cholesterol concentration ($X_{chol} < 10\%$) and temperatures above the main transition temperature, the membrane is in a phase where both conformational and lateral degrees of freedom are disordered. This phase is called the liquid disordered phase (ld). At temperatures below main transition the membrane is in a gel phase where both translational and conformational order is high, such a phase is called the solid ordered (so) phase. The presence of the cholesterol in small amounts only slightly reduces the temperature of the main phase transition. Recent computer modeling data (Nielsen et al., 2000) indicate that

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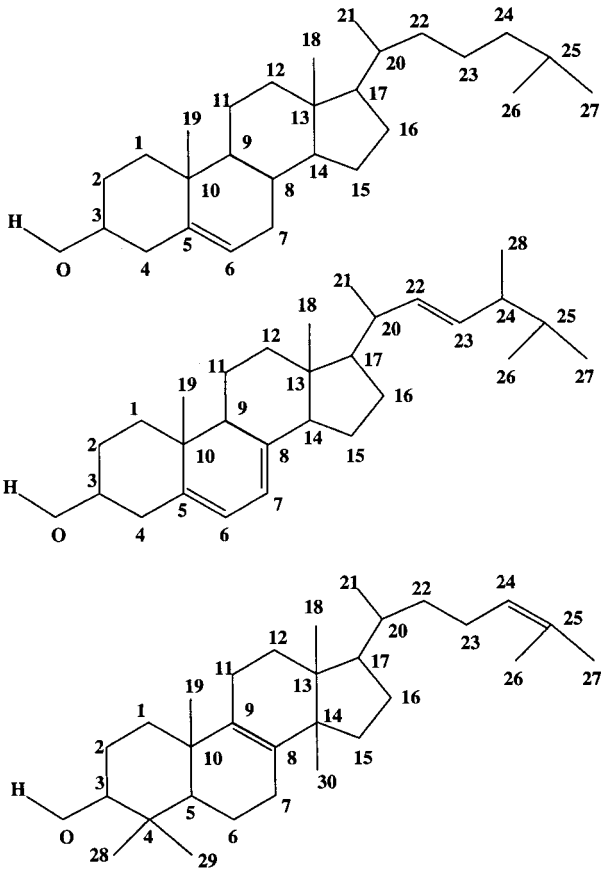

FIGURE 1 Structures of cholesterol, ergosterol, and lanosterol molecules (*top* to *bottom*). Carbon atoms are labeled with numbers, and hydrogen atoms, with the exception of the hydrogen of the OH group, are not shown.

the phase diagram of DPPC/lanosterol is very different. By studying the behavior of the NMR order parameter, Nielsen et al. (2000) concluded that the most significant characteristics of the phospholipid/cholesterol phase diagram is a stable coexistence region between the lo and ld phases, while such a region is absent from the phase diagram of lipid/lanosterol system. Based on NMR measurements and theoretical modeling, Nielsen et al. concluded that the difference in the phase diagrams is due to the difference in the interaction strength between different sterols (cholesterol vs lanosterol) and lipids. Since cholesterol and lanosterol have different molecular shape, Nielsen et al. proposed that molecular smoothness is the determining factor in the sterol/lipid interaction.

In this paper we report on a molecular dynamics computer simulation study performed on membranes containing phospholipid (DMPC) and sterols at low and high content. We study how the substitution of cholesterol with ergosterol and lanosterol influences the structural and dynamical properties of membranes with low sterol content (8:1 phospholipid:sterol ratio). In order to study the sterol/phospholipid interactions more accurately we want to perform simulations where sterol molecules are far from each other. At the same time the number of sterol molecules should be sufficiently large in order to obtain statistically significant results. This justified our choice in performing simulations at a 8:1 ratio. At high sterol content where the ratio of phospholipid to sterol was chosen as 1:1, we studied membranes with cholesterol and lanosterol.

METHODS

At low sterol concentrations (11 mol %) the lipid membrane used in our simulations consisted of 64 DMPC and 8 sterol molecules. We performed three simulations of lipid bilayer with different sterols: cholesterol, ergosterol and lanosterol. To set up the simulation system we used coordinates of DMPC molecules determined by Vanderkooi (1991). Coordinates of cholesterol molecules (Shieh et al., 1981) and for ergosterol (Hull and Woolfson, 1976) were taken from the crystal structure. Lanosterol was created from similar compound found in the Cambridge Structural Database (CSD) using model builder in Spartan software package. Initial configuration and equilibration protocol was the same as in our recent simulation of DPPC:Cholesterol bilayer at 11 mol % sterol. Initially four sterol molecules were distributed in each leaflet of the membrane so that the distance between them was at its maximum. We used the united atom force field for DMPC molecules that we employed in our previous simulations of various DPPC membranes (Smondyrev and Berkowitz, 1999a,c,d). Lipid membranes were surrounded by 1476 TIP3P (Jorgensen et al., 1983) water molecules, which corresponds to 20.5 waters per lipid molecule. Membranes with high sterol concentrations (50 mol %) were composed of 32 DMPC and 32 sterol molecules surrounded by 1312 water molecules. Initially lipid and sterol molecules were placed in regular arrays, which corresponds to structure A in our recent simulations of DPPC:Cholesterol membranes (Smondyrev and Berkowitz, 1999c). Parameters for the sterol molecules were taken from the united atom AMBER force field (Weiner et al., 1984). Partial atomic charges were calculated using the Gaussian 98 program at the 6-31G(d) basis set level and the Milliken population analysis (Frisch et al., 1998). It was found that hydroxyl group atoms and C3 carbon atom had the largest charges. For cholesterol molecule these charges are: 0.343e (in electron units) on the hydroxyl hydrogen, −0.694e on the hydroxyl oxygen, and 0.347e on C3 carbon atom. Similar charge distributions were found for ergosterol (0.333e, −0.673e, and 0.361e) and lanosterol (0.340e, −0.675e, and 0.350e). Charges on other carbon atoms in sterol rings and tails were close to zero.

After initial equilibration we performed simulations on nanosecond time scale at constant pressure ($P = 0$ atm) and temperature ($T = 308$ K) with periodic boundary conditions. Dimensions of the rectangular simulation cell were

controlled using the Hoover barostat. Thermostat and barostat relaxation times were 0.2 and 0.5 ps, respectively. All bond lengths were constrained using the SHAKE algorithm with a tolerance of 10^{-4}, allowing for the use of 0.002 ps time step. The Ewald summation technique was employed in the calculation of electrostatic contributions with a tolerance of 10^{-4}. The real space part of the Ewald sum and van der Waals interactions were cut off at 10 Å. Calculations were performed on a Cray-T3E computer at the Texas Advanced Computing Center and an IBM-SP computer at the North Carolina Supercomputer Center using the DL_POLY simulation package, version 2.8, developed in Daresbury Laboratory, England (Smith and Forester, 1996).

TABLE 1 Distances (in Å) from the bilayer center to DMPC and sterol atoms

Atom	DMPC	DMPC: Chol	DMPC: Ergo	DMPC: Lano
P	17.1 ± 1.9	18.2 ± 2.2	18.0 ± 2.2	18.3 ± 1.9
C_γ	17.9 ± 3.4	19.0 ± 3.6	19.1 ± 3.6	19.2 ± 3.4
C_α	17.6 ± 2.3	18.9 ± 2.7	18.8 ± 2.8	18.8 ± 2.3
C_β	17.7 ± 2.7	18.7 ± 3.0	18.7 ± 3.1	18.9 ± 2.7
C_{G3}	15.2 ± 2.0	16.4 ± 2.2	16.2 ± 2.2	16.4 ± 1.9
C_4	9.9 ± 1.7	10.9 ± 2.0	10.7 ± 2.0	11.0 ± 1.7
C_5	9.0 ± 1.7	9.9 ± 1.9	9.8 ± 2.0	10.1 ± 1.6
C_9	5.5 ± 1.7	6.1 ± 1.8	6.0 ± 1.9	6.2 ± 1.6
C_{13}	3.0 ± 1.6	3.1 ± 1.7	3.2 ± 1.8	3.0 ± 1.6
C_{14}	1.8 ± 2.2	2.1 ± 2.1	2.1 ± 2.4	2.0 ± 1.9
HO		13.5 ± 0.5	13.4 ± 0.5	12.8 ± 0.4
C_3		12.0 ± 0.5	11.9 ± 0.4	11.5 ± 0.3
C_{18}		4.3 ± 0.6	4.4 ± 0.4	4.5 ± 0.3

RESULTS

Simulations at small sterol concentrations

Membrane geometry

Each leaflet of the membrane in our simulation contained 32 phospholipid molecules and 4 sterol molecules. In order to determine the average area per phospholipid and sterol molecule we employed the following strategy. The total volume of the membrane V is given by $V = n_pV_p + n_sV_s$ where n_p is the number of phospholipid molecules, n_s is the number of sterol molecules, and V_p and V_s are the volumes per phospholipid and sterol molecules. We assume that the volume of a sterol molecule does not depend on the composition of the membrane, since the sterol molecule is rather rigid. The volume of the phospholipid molecule is composition dependent. For the areas we can write a similar relationship $A = n_pA_p + n_sA_s$ where the area of each compound is obtained from the relationship $A = 2V/L$; L is the effective thickness of the membrane. The membrane thickness can be estimated by taking the distance between phosphorus atoms in the opposite leaflets. This distance depends on the temperature, pressure, and composition of the membrane. Note that in the case of a pure DPPC membrane this kind of an estimate for the area per phospholipid results in a value of 64.8 Å^2 ($V = 1232$ Å^3 (Nagle and Weiner, 1988) and $L = 38$ Å) which is in a reasonable agreement with the experimentally determined value 62.9 Å^2 (Nagle et al., 1996) and the value of 61.6 Å^2 obtained directly from simulations (Smondyrev and Berkowitz, 1999d). In simulations with sterols, we assume that the volume of the sterol molecule is the same as the molecular volume of cholesterol which can be found from the crystal structure (Shieh et al., 1981) or (CSD) and is equal to 612 Å^3. The effective membrane thickness (distance between P atoms) of DMPC:Cholesterol membrane obtained from our simulations is 36.4 Å (Table 1). Therefore, the effective area per sterol molecule is 33.6 Å^2. This value is close to the value we used in our previous simulations of DPPC bilayers with cholesterol. It is also similar to the area per cholesterol molecule (32.4 Å^2) obtained in recent simulations of the DPPC/Cholesterol bilayer (Tu et al., 1998). Using the estimated area per sterol molecule we can now calculate the average area per DMPC molecule. Fig. 2 shows the time evolution of the average areas per DMPC molecule in membranes with cholesterol, ergosterol, and lanosterol. The average values determined from the last 3.0 ns of simulations are: 57.9 ± 0.9 Å^2 for the membrane with cholesterol, 58.4 ± 1.1 Å^2 for the membrane with ergosterol, and 57.3 ± 1.2 Å^2 for the membrane with lanosterol. We observe that these three values are very close to each other. It should be emphasized that the method used to obtain this

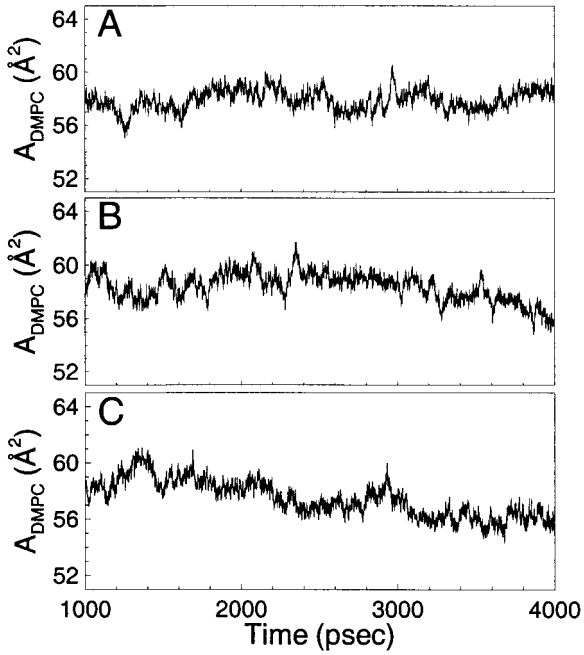

FIGURE 2 Time evolution of the area per DPPC molecule in membranes with cholesterol (*A*), ergosterol (*B*), and lanosterol (*C*).

estimate is not unique. Other methods of estimating the area per cholesterol molecule in lipid bilayers will be described in greater details elsewhere (A. M. Smondyrev and M. L. Berkowitz, manuscript in preparation). Thus the values of the area per DMPC molecule should be viewed as an estimate only.

Structure of lipid bilayers

We calculated the average distances from the bilayer center to different DMPC and sterol atoms (Table 1) in order to characterize the structural properties of lipid bilayers with various sterols. These values are compared to the data for the pure DMPC membrane (Smondyrev and Berkowitz, 1999b). It is clear that inclusion of sterol molecules into membrane results in the increase of the membrane thickness (as evident by examining the distance from the bilayer center to phosphorus atoms). Such an increase represents a main manifestation of the sterol condensing effect on the membranes. The effects of three different sterols on the membrane structure were similar. The electron density profiles calculated for lipid bilayers with cholesterol, ergosterol, and lanosterol (not shown here) were almost identical at 11 mol % sterol concentration. At the same time we found that the lanosterol hydroxyl group is located closer to the bilayer center compared to cholesterol and ergosterol. We also measured the average tilt of sterol molecules, which was defined as the angle between the bilayer normal and the vector connecting carbon atoms C_3 and C_{17} in sterol rings. The average cholesterol tilt in DMPC membrane (22.2°) is slightly higher than the one observed in membrane composed of DPPC and cholesterol (20°) (Smondyrev and Berkowitz, 1999c). DMPC membrane is thinner than the one composed of DPPC, so that cholesterol is more tilted in order to adjust its length to match the DMPC hydrophobic thickness. Interestingly the average sterol tilt in DMPC bilayer becomes higher for ergosterol (25.2°) and for lanosterol (30.1°). The distributions of the tilt angles for three different sterols in DMPC membrane are shown in Fig. 3. In Figs. 4, 5, and 6 we show the time evolutions of the tilt angle for individual sterol molecules. It is evident that lanosterol and, to a lesser extent ergosterol can tilt significantly with respect to the bilayer normal and such an orientation may be stable for several hundred picoseconds. For example, as we can observe in the Fig. 6, one of the lanosterol molecules can reorient itself in the bilayer, so that the lanosterol angle tilt is close to (90°) and therefore the sterol molecule finds itself in a plane parallel to the membrane surface. Such an arrangement of lanosterol can be seen in Fig. 7, where we display a snapshot from a simulation of a DMPC/lanosterol bilayer. As a result of the reorientations of ergosterol and lanosterol molecules the distributions of tilt angles for these sterols broadens compared to the one for cholesterol. In addition, the peaks of

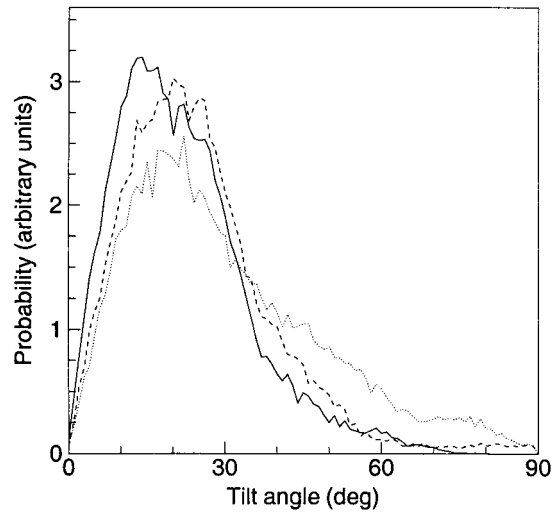

FIGURE 3 Distributions of the sterol tilt angle in bilayers with cholesterol (*solid line*), ergosterol (*dashed line*), and lanosterol (*dotted line*). When the tilt angle is zero, the sterol molecule is aligned parallel to the bilayer normal.

lanosterol and ergosterol distributions are shifted with respect to the cholesterol distribution (Fig. 3).

Incorporation of cholesterol into phospholipid membranes results in the ordering of lipid hydrocarbon tails. The degree of lipid chain order depends on sterol concentration and molecular structures of lipid molecules (Vist and Davis, 1990; Urbina et al., 1995). The ordering of hydrocarbon tails is usually characterized by the deuterium order parameter S_{CD} for selectively deuterated carbon atoms, which can

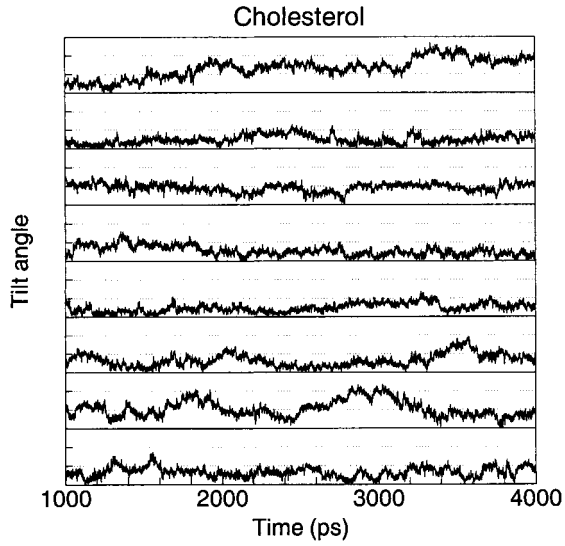

FIGURE 4 Tilt angles of the individual sterol molecules as a function of time. Dotted gridlines are drawn with 30° intervals. Solid lines correspond to the zero level.

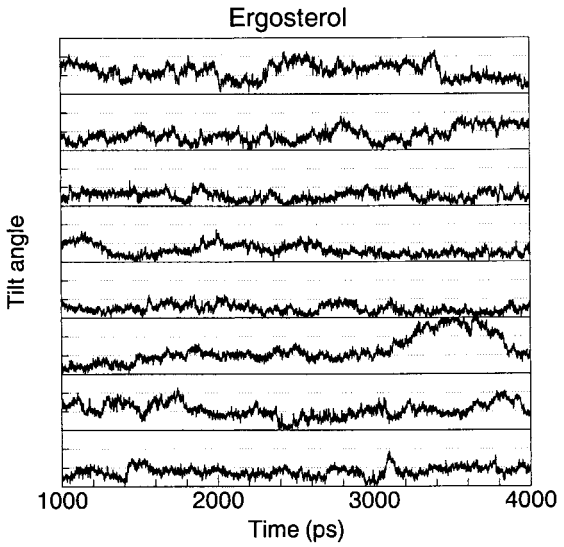

FIGURE 5 Time evolution of the tilt angles of each ergosterol molecule.

be measured using the NMR technique, or by its average quantity $\langle S_{CD} \rangle$, which is directly related to the first moment M_1 of the NMR spectrum. In DMPC/sterol membranes at 30 mol % sterol, the ordering effects of different sterols increased in the following progression: lanosterol-cholesterol-ergosterol (Urbina et al., 1995). Experimental (Nielsen et al., 2000) and theoretical (Polson et al., 2000) studies of DMPC membranes with lanosterol and cholesterol indicate that DMPC tails become more ordered in bilayers with cholesterol compared to the ones with lanosterol at all concentrations. At the same time this effect is more pronounced at high sterol concentrations and more subtle at low sterol levels. In computer simulations the deuterium order parameter S_{CD} can be defined using the following expression (Egberts and Berendsen, 1988):

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

where $S_{ij} = \langle 1.5 \cos \theta_i \cos \theta_j - 0.5 \delta_{ij} \rangle$; θ_{ij} is the angle between the ith molecular axis and the bilayer normal (z-axis). In Fig. 8 we compare $-S_{CD}$ values for the Sn-2 chain from our simulations of DMPC (Smondyrev and Berkowitz, 1999d), DMPC-Cholesterol, DMPC-Ergosterol and DMPC-Lanosterol (11 mol % sterol). As we can see the effects of three different sterols on the ordering of DMPC tails are very similar. Similar results were also obtained for the average number of gauche defects per lipid tail in bilayers with cholesterol (2.8), ergosterol (2.9), and lanosterol (2.8).

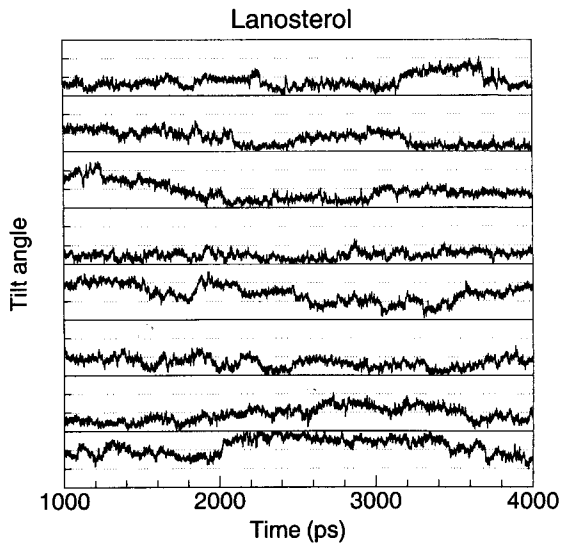

FIGURE 6 Time evolution of the tilt angles of each lanosterol molecule.

Sterol-lipid interactions

Nielsen et al. (2000) proposed that cholesterol, which has a smoother molecular surface compared to lanosterol, stabilizes the phospholipid membranes more effectively. Therefore, in their Monte Carlo simulations, Nielsen et al. employed a simple model where the depth of a potential well that describes the interaction of a sterol molecule with the phospholipid molecule was larger for cholesterol. To verify thus assumption we calculated the sterol-phospholipid interaction energy. Since this energy depends on the location of the sterol molecule in the bilayer we calculated this interaction energy as a function of the center of mass position of sterol molecules and their tilt. While the average energies have only a weak dependence on the sterol tilt, they depend on the position of the sterol molecule relative to the bilayer center. The plots for the total energies as a function of a distance between the sterol center of mass and the center of the bilayer for the three sterols are presented in Fig. 9. As we can see, the total energy becomes more negative when sterol molecules move closer to the headgroup region. Although the energies are close to each other for a given distance, nevertheless we observe that lanosterol/DMPC interaction has the lowest energy at any given distance. Therefore, as Fig. 9 illustrates to be consistent with the assumption of Nielsen et al. lanosterol molecule should be, on the average, closer to the center of the bilayer compared to cholesterol molecule.

Hydrogen bonding

The largest contribution to the sterol-lipid interaction energy comes from the van der Waals forces as indicated by the energy minimization studies of Vanderkooi (1994). However, interactions between polar groups of sterol and phospholipid molecules may also play an important role. Experimental studies of membranes with cholesterol and

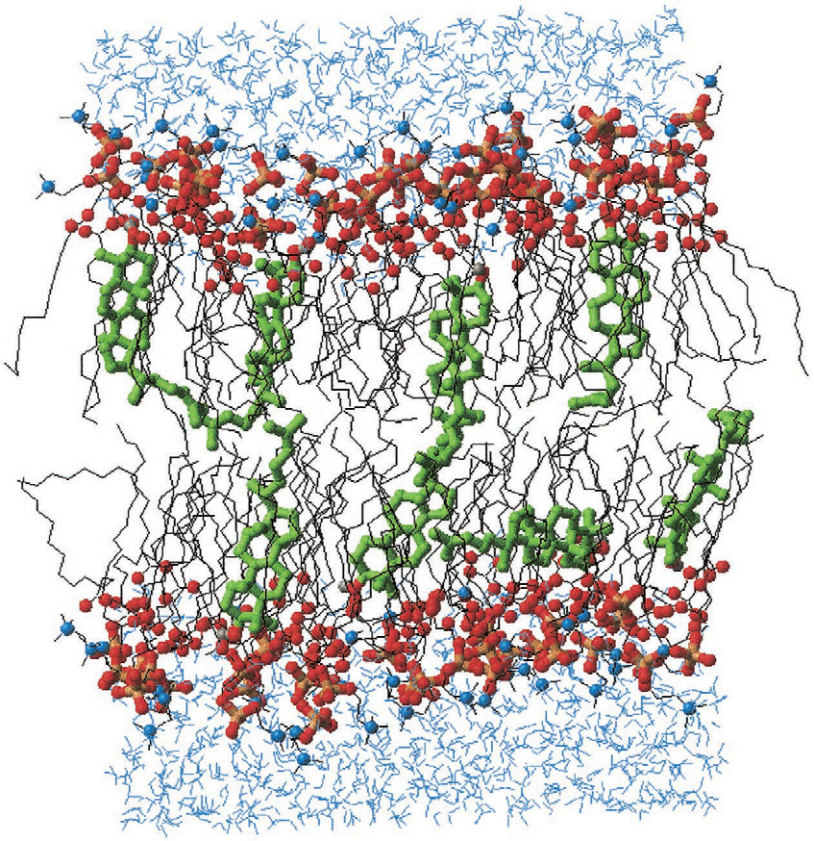

FIGURE 7 Snapshot of the lipid bilayer with lanosterol.

epicholesterol indicate that the configuration of the cholesterol hydroxyl group is important for the sterol-phospholipid interactions (Murari et al., 1986). An energy minimization simulation of DMPC:Cholesterol bilayer at 1:1 molar ratio predicted that cholesterol binds to the DMPC Sn-2 carbonyl group via a hydrogen bond (Vanderkooi, 1994). Several MD simulation studies also showed the formation of hydrogen bonds between cholesterol hydroxyl group, phospholipid oxygen atoms, and water (Robinson et al.,

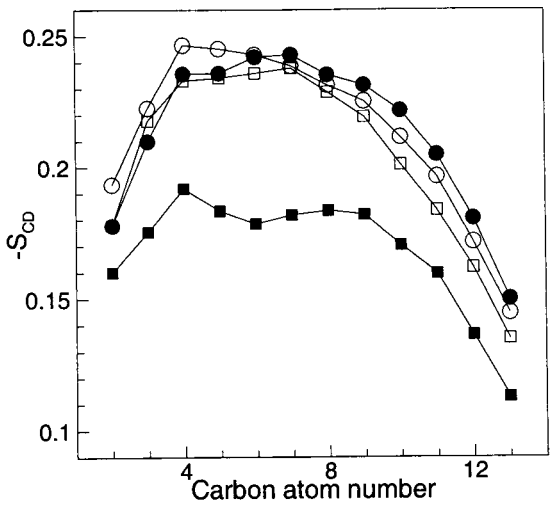

FIGURE 8 Deuterium order parameter $-S_{CD}$ in DMPC Sn-2 tails for pure DMPC (*solid square*), DMPC:cholesterol (*open circle*), DMPC:ergosterol (*open square*), and DMPC:lanosterol (*solid circle*) membranes.

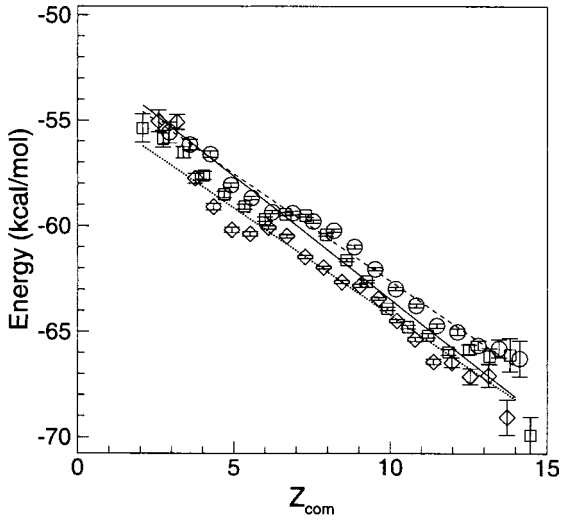

FIGURE 9 Average interaction energies between sterol molecules and membranes as a function of the sterol center of mass position with respect to bilayer center: cholesterol (*squares*), ergosterol (*circles*), and lanosterol (*diamonds*).

1995; Tu et al., 1998; Smondyrev and Berkowitz, 1999c; Pasenkiewicz-Gierula et al., 2000). Existence of the hydrogen bond between cholesterol and DMPC molecules was also predicted experimentally using a combination of cross-polarization and NMR techniques (Guerneve and Auger, 1995). Recent MD simulation of DMPC/Cholesterol bilayer at 22 mol % cholesterol showed that cholesterol can bind to phospholipid molecules via a water bridge (Pasenkiewicz-Gierula et al., 2000). In the present study we compared the hydrogen bonding of cholesterol, ergosterol and lanosterol molecules in DMPC bilayers. The hydrogen bond is defined using the geometric criteria (Pasenkiewicz-Gierula et al., 1997): the distance between water or cholesterol oxygen and the DPPC oxygen is shorter than 3.25 Å and the angle between the vector linking DPPC oxygen with water (or cholesterol) oxygen and H-O bond of the water (or cholesterol) is less than 35° as proposed by Raghavan et al. (1992). Cholesterol can either form a direct hydrogen bond with DMPC oxygen atoms or bind to them through a water bridge (Pasenkiewicz-Gierula et al., 2000). The water bridge between sterol and DMPC molecules is formed when a water molecule forms hydrogen bonds with both sterol and DMPC molecules. The average number of sterol molecules linked to different oxygen atoms of DMPC via direct hydrogen bonds or water bridges are listed in Table 2. Although cholesterol and ergosterol behave somewhat similarly, cholesterol has a slightly higher tendency to hydrogen bond to DMPC compared to ergosterol. Lanosterol forms hydrogen bonds differently compared to the other two sterols. First, it forms fewer hydrogen bonds with phosphate oxygen O_{11}, which is probably due to the fact that lanosterol is positioned closer to the bilayer center. Second, lanosterol forms more hydrogen bonds with the carbonyl oxygen O_{32} (these oxygens are the closest to the bilayer center) via water bridges.

Sterol dynamics

Different interactions between sterols and DMPC may be also linked to variations of dynamical properties of membrane lipids. Diffusion coefficients were obtained from molecular dynamics simulations using the following equations:

$$D = \lim_{t\to\infty} \frac{\langle |r(t + t_0) - r(t_0)|^2 \rangle}{2nt}$$

where n is the number of translational degrees of freedom, $r(t)$ are the coordinates of the sterol center of mass and $\langle \ldots \rangle$ indicates averaging over initial times t_0. In Fig. 10 we show the mean square displacements along the bilayer normal and in the plane of membrane for three different sterols in DMPC membranes. By fitting straight lines to these curves (in the interval from 1 ns to 2.5 ns) we obtained the following values for the lateral diffusion coefficients: $D_{lat} =$ 1.5, 3.0, and 3.2 $\times$ 10^{-7} cm^2/s for cholesterol, ergosterol, and lanosterol respectively. These values are similar to the diffusion coefficients of lipids in pure DPPC membranes determined from computer simulations (Essmann and Berkowitz, 1999) and experiments (Sackmann, 1995). The lanosterol diffusion coefficient is higher than the one for cholesterol. This was also observed in computer simulations based on the minimal model (Polson et al., 2001). The motions of three sterols in the direction along the normal to the bilayer are different. In the case of ergosterol the mean square displacement saturates to a constant value of ~4 $Å^2$ after approximately 500 ps. In the case of lanosterol and cholesterol, the fit to a straight line produced the values of D_t = 0.9 $\times$ 10^{-7} cm^2/s and 1.25 $\times$ 10^{-7} cm^2/s for the corresponding transverse diffusion coefficients. One should understand that the values of the diffusion coefficients given above are rather approximate due to a small number of sterol molecules sampled and limited time of the runs.

Simulations at large sterol concentrations

In lieu of some of the observed differences in the properties of DMPC membranes with different sterols such as cholesterol and lanosterol at sterol:phospholipid ratio 1:8, it would be interesting to find out whether or not such differences exist when membranes are rich in sterol. For this purpose we performed two more simulations: first on a membrane containing cholesterol and DMPC molecules at 1:1 ratio,

TABLE 2 Average number of hydrogen bonds per DMPC or sterol molecule

	DMPC	DMPC-Chol		DMPC-Ergo		DMPC-Lano	
	Water	Water	Chol	Water	Ergo	Water	Lano
O_{12}	0.54	0.61	0 (0.01)	0.61	0 (0.01)	0.52	0 (0)
O_{11}	0.19	0.22	0.10 (0.03)	0.23	0.09 (0.03)	0.22	0.04 (0.03)
O_{14}	1.67	1.63	0.01 (0.08)	1.66	0.01 (0.06)	1.66	0 (0.08)
O_{13}	1.68	1.68	0 (0.02)	1.66	0 (0.02)	1.65	0 (0.01)
O_{22}	1.40	1.44	0.21 (0.40)	1.45	0.10 (0.26)	1.41	0.15 (0.34)
O_{32}	0.55	0.63	0.11 (0.17)	0.61	0.06 (0.12)	0.56	0.11 (0.31)

Bonds formed with water and cholesterol molecules as indicated. In the case of sterol the first number gives the average number of direct hydrogen bonds with DMPC atoms and the second number (in parentheses) gives the number of bonds formed via water bridges.

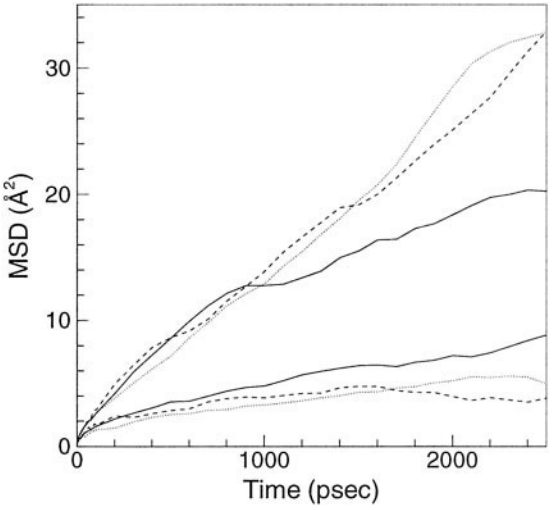

FIGURE 10 The mean square displacements in the bilayer plane (*top 3 curves*) and in the direction parallel to the bilayer normal (*bottom 3 curves*) for cholesterol (*solid line*), ergosterol (*dashed line*), and lanosterol (*dotted line*) molecules.

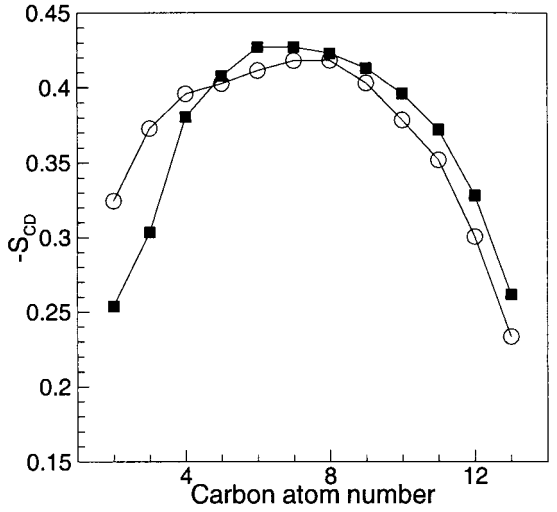

FIGURE 12 Deuterium order parameter $-S_{CD}$ averaged over two DMPC hydrocarbon chains for DMPC:Cholesterol (*solid squares*) and DMPC:Lanosterol (*open circles*) membranes at 50 mol % sterol.

and the second, on a membrane containing lanosterol and DMPC molecules in the same 1:1 ratio. The simulation of cholesterol:DMPC was done for 2 ns, while the simulation of lanosterol:DMPC was performed for 1.5 ns. This choice of the time lengths was dictated by the time required for the membrane area to stabilize. As we can see from Fig. 11 the area of the cholesterol:DMPC dimer stabilized after ~1.0 ns, while the area of the lanosterol:DMPC dimer stabilized after ~0.5 ns. The data analysis for both systems was performed for the last 1.0 ns of the two simulations. We found that the area per cholesterol:DMPC heterodimer is 77.5 ± 0.6 Å^2, while the area of the lanosterol:DMPC heterodimer is 82.6 ± 0.6 Å^2. This may be due to the fact that lanosterol molecule is bulkier than cholesterol. In Fig. 12 we show the S_{CD} order parameter averaged over two DMPC chains. As the figure shows, lanosterol has a stronger effect on the ordering of chain molecules towards the headgroups. This can be attributed to the fact that the lanosterol molecule is not as flat as cholesterol, especially toward its head due to the presence of two extra CH_3 groups. The S_{CD} order parameter for carbon atoms towards the middle of the bilayer is larger for phospholipids in membranes with cholesterol. Overall, the condensing effect of lanosterol is weaker compared to cholesterol. The membrane thickness in the presence of cholesterol is 1 Å larger than the one with lanosterol, and therefore the condensing effect of cholesterol is stronger. In Fig. 13 we show the distributions for the angle between the sterol and bilayer normal. No substantial difference is observed between the two curves on the figure. The average angle between cholesterol and the bilayer normal 10.6° is the same within the experimental error as the angle for lanosterol 10.0°. No indication that lanosterol turns perpendicular to the bilayer normal is given by the angular distribution curve and such configurations are not seen in any snapshots obtained from simulations with a high content of lanosterol.

CONCLUSIONS

We performed three molecular dynamics simulations of DMPC membranes with sterol at 8:1 ratio. Each simulation was done with a different sterol: the first was done with cholesterol, the second with ergosterol, and the third with lanosterol. Although we did not observe any major differences in the structure of the DMPC membranes with different sterols or in the energetics of the sterol-phospholipid interaction, still there were some differences between the behavior of lanosterol and other sterols. We observed that lanosterol on the average was closer to the center of the bilayer. We also found that the angle between the molecular axis and the bilayer normal was larger for lanosterol than for

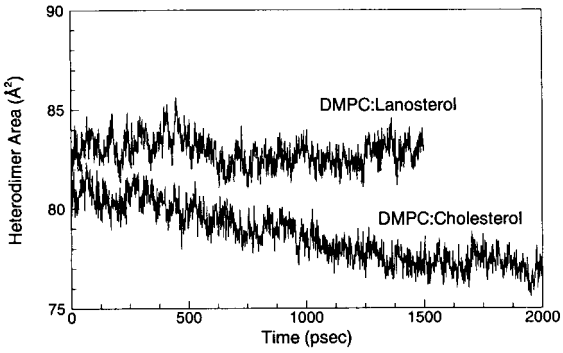

FIGURE 11 The average areas per DMPC:sterol heterodimers in membranes with cholesterol and lanosterol (50 mol % sterol) as a function time.

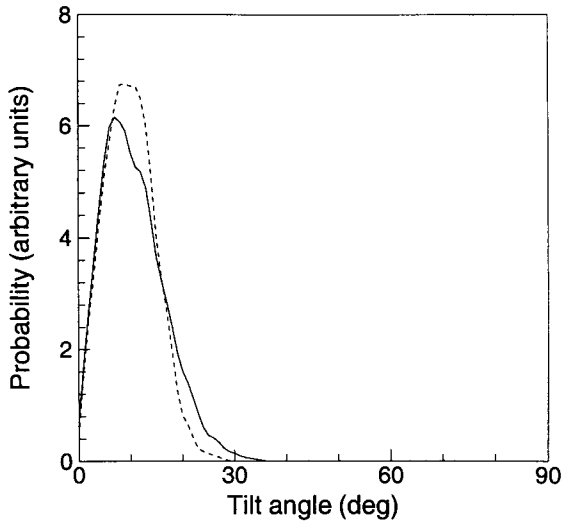

FIGURE 13 Distributions of the sterol tilt angles in membranes with 50 mol % cholesterol (*solid line*) and lanosterol (*dashed line*).

two other sterols, and we observed that one of the lanosterol molecules reorients and spends ~1 ns in a plane that is parallel to the bilayer surface. We also observed a difference in the hydrogen bonding pattern between lanosterol and DMPC. Thus, in the case of lanosterol, the hydrogen bonding between the sterol hydroxyl group and phosphate oxygen is diminished while the number of hydrogen bonds with carbonyl oxygens is increased in a manner consistent with the observation that lanosterol is (on the average) located closer to the membrane center. What can be the reason for this small difference in the location of lanosterol and other two sterols? As the structures of sterols show, lanosterol has two methyl groups attached to carbon 4, which makes the first ring of lanosterol larger in size. To fit more comfortably into the bilayer, lanosterol slides somewhat towards the bilayer center and as a result becomes more mobile (compared to cholesterol) in its orientational and lateral motion. How these differences in sterol location and mobility influence the phase diagram is a very interesting question that remains to be investigated.

We also performed two simulations with high concentration of sterol where the ratio of sterol to phospholipid was 1:1. One of the simulations was done with cholesterol and another with lanosterol. In this case we observed that cholesterol had slightly stronger condensing effect on the membrane compared to lanosterol, although the ordering of phospholipid chains close to headgroups was larger for membranes with lanosterol. We did not observe any major differences in orientational properties of sterols when membranes contained large amounts of sterols. Our simulations together with simulations performed with simpler potential models (Nielsen et al., 2000) indicate that indeed there are differences in the physical properties of membranes with different sterols. Such differences may be very important when additional molecules are present in the membranes, such as proteins. Our present results also show that more experimental and simulation work is needed in order to understand how the change in sterol structure affects the properties of membranes.

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