Behavior of Cholesterol and Its Effect on Head Group and Chain Conformations in Lipid Bilayers: A Molecular Dynamics Study

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ABSTRACT Cholesterol molecules were put into a computer-modeled hydrated bilayer of dimyristoyl phosphatidyl choline molecules, and molecular dynamics simulations were run to characterize the effect of this important molecule on membrane structure and dynamics. The effect was judged by observing differences in order parameters, tilt angles, and the fraction of *gauche* bonds along the hydrocarbon chains between lipids adjacent to cholesterol molecules and comparing them with those further away. It was observed that cholesterol causes an increase in the fraction of *trans* dihedrals and motional ordering of chains close to the rigid steroid ring system with a decrease in the kink population. The hydrogen-bonding interactions between cholesterol and lipid molecules were determined from radial distribution calculations and showed the cholesterol hydroxyl groups either solvated by water, or forming hydrogen bond contacts with the oxygens of lipid carbonyl and phosphate groups. The dynamics and conformation of the cholesterol molecules were investigated and it was seen that they had a smaller tilt with respect to the bilayer normal than the lipid chains and furthermore that the hydrocarbon tail of the cholesterol was conformationally flexible.

INTRODUCTION

There have been a number of molecular dynamics computer simulations of lipid bilayers. Excluding the statistical mechanical, Brownian, and mean field methods, these include van der Ploeg and Berendsen (1982), van der Ploeg and Berendsen, (1983), Edholm et al. (1983), Marrink et al. (1993), Stouch et al. (1991), Stouch (1993), Stouch et al. (1994), Damodaran et al. (1992), Damodaran and Merz (1993), Venable et al. (1993), Xiang (1993), Biswas and Schurmann (1991), Khalatur and Pavlov (1987), Fukada et al. (1993), Heller et al. (1993), and Robinson et al. (1994). There have been fewer molecular dynamics simulations of bilayers with solutes incorporated (Edholm and Johansson, 1987; Edholm and Nyberg, 1992; Bassolino-Klimas et al., 1993). Cholesterol is an important solute to study because its physical effect on lipid bilayers has been studied extensively experimentally. It is also an uncomplicated molecule composed of a rigid ring system, a flexible hydrocarbon chain tail and a β-hydroxyl group. Its interaction and effect on lipid molecules should arise predominantly from physical noncovalent interactions, and it appears not to complex strongly with phospholipids as ^{13}C experiments show the rate of cholesterol rotation to be faster than phospholipid rotation (Yeagle, 1980).

Cholesterol has a large and important effect on biological membranes that leads to a reduced permeability of the membrane, an increase in molecular order, condensation of the lipid molecules, and a small increase in membrane thickness (for a review see Yeagle (1985) or Bloom et al. (1991)). This is believed to arise from the rigidity of the steroid ring system causing *trans - gauche* isomerizations in surrounding lipid chains to become less probable (Stockton and Smith, 1976; Oldfield et al., 1978) and an increase in the fraction of *trans* dihedrals in lipid chains around the rigid ring system that may lead to a small increase in bilayer width (McIntosh, 1978). However, inasmuch as the average number of *trans* dihedrals in a phospholipid does not increase (Pink et al., 1981; Bush et al., 1980), it follows that the lower part of the lipid hydrocarbon chain must then have an increased number of *gauche* conformations. Thus the torsional and translational motion of a lipid is reduced and consequently there is an increase in lipid molecular order (Lindblom et al., 1981; Cornell and Keniry, 1983).

Cholesterol appears to cause little change in properties at the center of the bilayer (Oldfield et al., 1978; Oldfield et al., 1971). It has been proposed that the tail of cholesterol is much more conformationally flexible than its ring system but that the tail shows less motional freedom than the hydrocarbon chains around it (Dufourc et al., 1984; Opella et al., 1976; Kroon et al., 1975).

Monte Carlo simulation techniques have been used to calculate the effect of cholesterol on molecular order parameter profiles and showed a reduction in the ability of lipid chains adjacent to cholesterol to undergo torsional isomerization (Scott and Kalaskar, 1989; Scott, 1991; Scott and McCullough, 1993). Edholm and Nyberg (1992) conducted a molecular dynamics simulation of cholesterol molecules in a model membrane. This model included neither head groups nor water molecules but provided important insights into the effect of cholesterol on lipid chain dynamics and conformation. They concluded that the effect of a cholesterol molecule extended for a radius of 1.25 nm, leading to greater order as measured by molecular order parameters as well as increases

Received for publication 9 March 1994 and in final form 13 September 1994.

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0006-3495/95/01/164/07 $2.00

in the fraction of *trans* bonds. These two effects were more pronounced in the upper and middle parts of the chain than at the terminus. An increase in bilayer thickness around the cholesterol was also reported.

Here we report the results of an analysis of 400 picoseconds of molecular dynamics from a computer simulation of 4 cholesterol molecules in a bilayer of 36 dimyristoyl phosphatidyl choline lipid molecules, which is equivalent to a cholesterol concentration of ~11% cholesterol per mole of lipid. The effects of cholesterol on the lipid head groups and hydrocarbon chains as well as the behavior of the cholesterol was investigated.

MATERIALS AND METHODS

The simulation package AMBER 3.1 (Singh et al., 1988) was used with nonbonded parameters based on the OPLS parameters of Jorgenssen and Tirado-Rives (1988). The other parameters are from the standard AMBER force field apart from those parameters for ester groups taken from the work of Charifson and co-workers (1990).

The system was run under the same conditions as earlier successful bilayer simulations (Robinson et al., 1994) with a residue-based cutoff of 9 Å and the nonbonded pair list updated every 50 femtoseconds. In the dihedral energy term, the van der Waals contribution was divided by 8 and the electrostatic energy by 2 as recommended when using AMBER united atoms with OPLS parameters (Jorgenssen and Tirado-Rives 1988). The system temperature was kept at 323 K by using the Berendsen algorithm (Berendsen et al., 1984) with a relaxation time of 100 femtoseconds. To maximize the time step, the SHAKE algorithm was used (Ryckaert et al., 1977).

The lipid molecules have the same conformations described in our earlier work, and coordinates for the cholesterol molecule were taken from the crystal structure (Pascher and Sundell, 1982) with parameters from the standard AMBER force field.

Model building was done with the aid of the QUANTA program (Molecular Simulations, Inc., 1992) and involved constructing a bilayer from two monolayers, as described in Robinson et al. (1994), and then replacing two lipid molecules in each layer with two cholesterol molecules. Because cholesterol causes a condensation of lipid layers (Reiber, 1978), the head group surface area of a lipid at the cholesterol concentration modeled should be approximately 62 $Å^2$ (McIntosh, 1978) rather than the value of 66 $Å^2$ observed for a pure lipid bilayer. Thus, a pure lipid monolayer of 20 lipids was built such that when two lipid molecules were replaced by two cholesterol molecules it had a total surface area equivalent to 18 lipids with surface area of 62 $Å^2$ plus two cholesterol molecules with a surface area of 38 $Å^2$. The cholesterols were placed in the lipid lattice so that under periodic boundary conditions they were in equivalent sites. Fig. 1 is a schematic view of the surface of the monolayer illustrating the positioning of the two cholesterols, and Fig. 2 shows the unhydrated starting structure. This bilayer was then hydrated with TIP3P waters (Jorgenssen et al., 1983) that penetrated down to the carbonyl oxygens of the lipids.

Within the bilayer starting structure, the closest contact between cholesterol atoms in opposite halves of the bilayer was 13 Å. Within a layer their closest contact was 23 Å.

Although all four cholesterols had their long axis parallel to the bilayer normal, they were rotated about this axis to give different orientations of the cholesterol with respect to the surrounding lipids and to introduce some variation between sites. All cholesterols were placed so that their hydroxyl group was in the same plane and oriented toward a carbonyl oxygen of lipid ester groups as suggested by ^{13}C nmr (Huang, 1976) and shown by x ray and neutron diffraction (Franks, 1976; Worcester and Franks, 1976). ^{31}P nuclear magnetic resonance (nmr) supports the fact that cholesterol is not present in the head group region (Yeagle et al., 1975).

Because of the ordered nature of the starting structure, great care was taken in the equilibration stage. Energy refinement on the waters alone

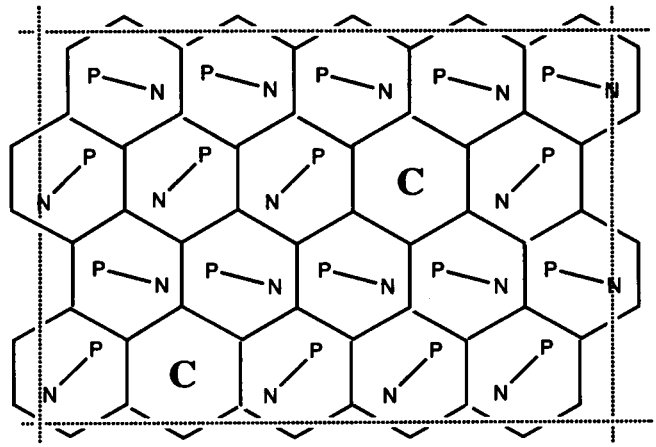

FIGURE 1 Schematic diagram of the surface of a monolayer showing the placing of the cholesterol molecules (C) with respect to the hexagonally packed lipids. Dashed lines mark the edges of the periodic box.

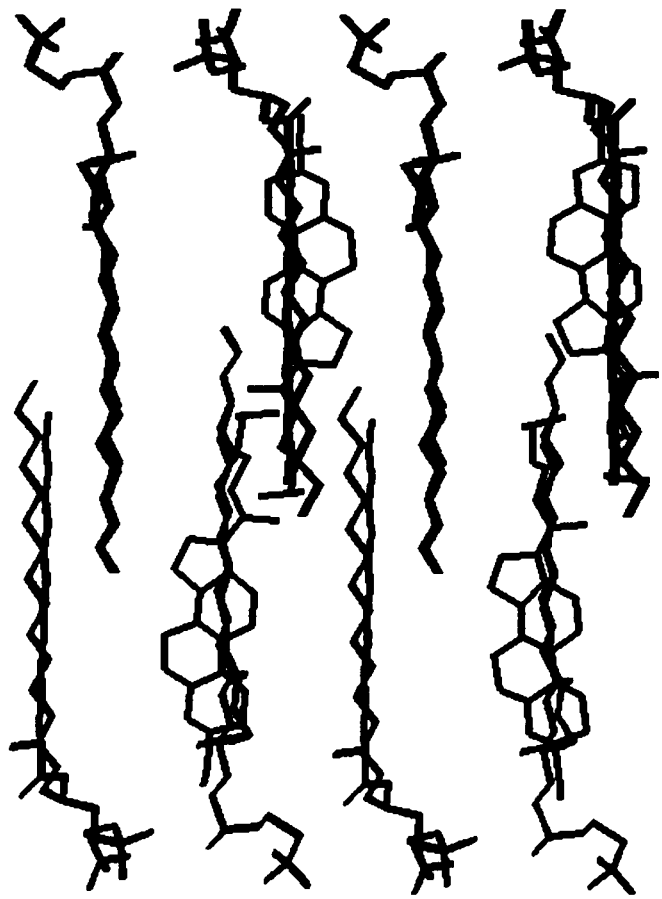

FIGURE 2 Side view of the unhydrated lipid bilayer starting structure with cholesterols.

followed by 20 picoseconds of molecular dynamics was done to equilibrate their positions around the lipid. The lipid molecules alone and then with the waters were energy refined followed by a further 20 picoseconds of molecular dynamics on the waters alone. Finally, molecular dynamics on the hydrocarbon chains alone were run to introduce disorder into the chains. The chains in the present system showed as much disorder after 70 picoseconds as did those of the pure lipid bilayer after nearly 200 picoseconds of

molecular dynamics (Robinson et al., 1994), i.e., the crystal-like starting structure broke down more quickly in the presence of cholesterol despite a reduction in volume per lipid. With the chains melted, 150 picoseconds of molecular dynamics were run with the waters, lipid molecules, and hydrocarbon tail of the cholesterol free to move, but the position of the cholesterol molecules was held fixed with all bond angles and torsions of the ring system held rigid. In the next 100 picoseconds the cholesterols were free to diffuse, but their ring systems were still held rigid by constraints. Finally all atoms were allowed to move freely with an equilibration sequence of 100 picoseconds before a production run of 400 picoseconds that was analyzed. In conclusion, equilibration involved 350 picoseconds of molecular dynamics simulation on the lipid molecules before the production run of 400 picoseconds. A snapshot from the simulation, without waters, 200 picoseconds into the production run is shown in Fig. 3.

Analysis of the behavior of the cholesterol and lipid molecules during this final 400 picoseconds was accomplished by using a program originally written by Essex (1992). Lipids were divided into those that had extensive contact with a cholesterol and those with little contact. Lipids with extensive contact with cholesterol were those that were within 5 Å of a cholesterol for greater than 80% of the simulation time, whereas a lipid was classed as having little contact if it spent less than 30% of the production simulation in contact with a cholesterol. Cholesterol behavior was determined in terms of the tilt of the long axis of cholesterol with respect to the bilayer normal, the conformations of the flexible hydrocarbon chain, and the behavior of the β-hydroxyl group.

RESULTS AND DISCUSSION

From experiments by Dufourc et al. (1984) and Taylor et al. (1981), the long axis of cholesterol is, on average, oriented perpendicular to the bilayer surface. However, experiments by Oldfield et al. (1978) indicate that the most probable tilt of a cholesterol molecule is 16° and that the cholesterol sweeps out a cone leading to the observed time-averaged tilt of 0°. The angle between the bilayer normal and a vector connecting the hydroxyl hydrogen with the final methylene of the tail of the cholesterols was evaluated from the simulation, and the distribution is shown in Fig. 4. Over the production simulation the cholesterol molecules show a nonzero tilt with a most probable tilt angle of 14° and 90% possessing a tilt of less than 24°. This is very close to the value reported by Oldfield and co-workers (1978) and, as is shown later, is less than the tilt measured in the surrounding lipid chains during the simulation.

The conformation of the cholesterol chain is described by five dihedral torsions, τ_1 to τ_5 as illustrated in Fig. 5. The crystal structure of cholesterol shows the tail to possess six conformations (Duax et al., 1980). An analysis of the populations of cholesterol tail conformations during the 400-picosecond molecular dynamics production simulation showed main tail conformations as defined by (τ_3, τ_4) with 72% (*trans, trans*), 23% (*trans, gauche*$^+$), and 3% (*gauche*$^+$, *trans*). (The dihedrals τ_1 and τ_2 are both at least 99% *trans*.) These conformations are some of those observed in the crystal structure where the (*trans, trans*) conformation is observed to occur for 72% of the cholesterol molecules and both (*trans, gauche*$^+$) and (*gauche*$^+$, *trans*) for 8% of the population.

The behavior of the lipid can be described by a variety of parameters such as the tilt of the hydrocarbon chains defined as the average angle a vector connecting two atoms makes with the bilayer normal (see van der Ploeg and Berendsen, 1983), the population of kink defects, the number of *trans* bonds in lipid chains, or the distribution of *trans* bonds as a function of chain position. Where appropriate these properties were considered for the Sn1 and Sn2 chains separately as the two chains are not equivalent due to their different attachments to the glycerol backbone. Three vectors were defined for each hydrocarbon chain corresponding to the upper half (nearer the head group), the lower half (nearer the bilayer center), and the whole chain. Furthermore, that both

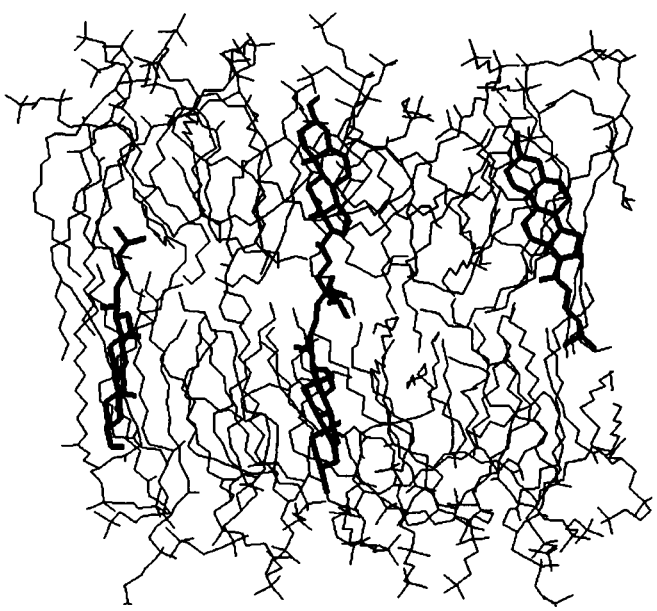

FIGURE 3 Side view of the unhydrated lipid bilayer structure 200 picoseconds into the production simulation. The cholesterol molecules are shown in bold.

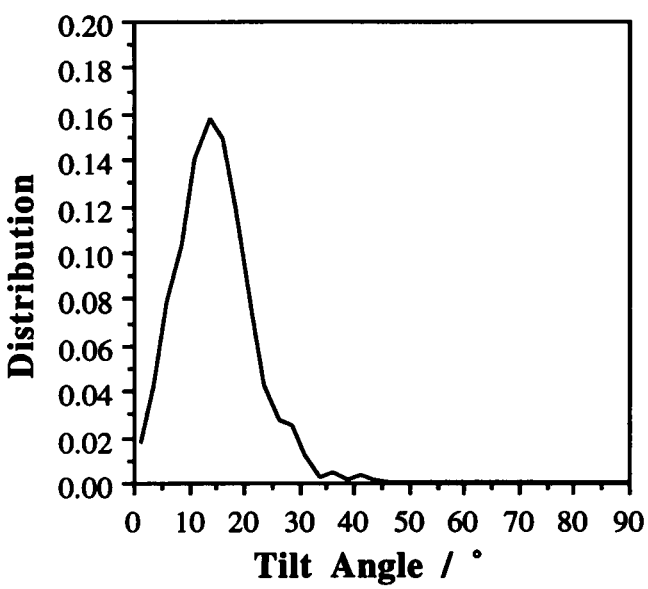

FIGURE 4 Diagram of the flexible hydrocarbon chain of cholesterol defining the dihedrals τ_1 to τ_5.

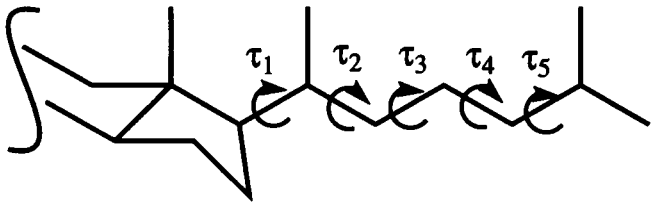

FIGURE 5 Tilt angle distribution of the cholesterol molecule with respect to the bilayer normal.

hydrocarbon chains of a lipid may not necessarily be in contact with a cholesterol molecule was accounted for.

It is believed that the diffusion of small molecules through a membrane is aided by *gauche* conformations that form kinks in the surrounding chains providing a cage in which the solute resides (Yeagle, 1987). Because cholesterol is believed to reduce the probability of kink formation by suppressing *gauche* formation in lipid chains adjacent to the rigid steroid ring, its presence should lead to a decrease in permeability. We examined the kink population in chains as well as the distribution of *trans* bonds as a function of chain position and the total *trans* dihedral population for those lipid chains adjacent to cholesterol compared with those further away for the 400 picoseconds of molecular dynamics production simulation.

To determine the effect of cholesterol on lipid chain kink population, the Sn1 and Sn2 chains were considered separately and divided into two halves. For lipids neighboring cholesterol, the upper half will be in contact with the main rigid steroid ring whereas the lower part will mainly be in contact with the more flexible cholesterol tail. Table 1 lists the results for 1–200 and 201–400 picoseconds. It is observed that in the current model, the cholesterol has a larger effect on the Sn2 chains than the Sn1 chains. Those Sn2 chains distant from a cholesterol molecule show a larger population of kinks in the upper half of the chain than do those lipid chains in extensive contact with cholesterol molecules. For the lower half of the chains it is observed there is little difference between the two sets for the occurrence of kinks. Thus for the Sn2 chain it appears that during the course of the simulation the cholesterol causes a decrease in the population of kinks within the upper half of a lipid chain where a solute would enter the membrane and hence acts as a barrier to diffusion through the membrane. In the lower half of the lipid chain, the flexible tail of cholesterol has little effect on kink population.

With regard to the total population of *trans* dihedral angles in Sn1 and Sn2 chains, the difference between chains next to cholesterol and those further away is ~2–3%, and the fractional population is ~78% for the Sn1 chains and 77% for the Sn2 chains over the 400 picoseconds of the production simulation. This absence of a difference is the same result as reported by Pink et al. (1981) and Bush et al. (1980).

To investigate further the effect of cholesterol on the number of dihedral torsions in the Sn1 and Sn2 chains, the lipid chains were divided in two and the average fraction of *trans* torsions per dihedral for these two regions compared. The results for 1–200 and 201–400 picoseconds are given in Table 2. The simulation results show that the upper half of Sn1 chains associated with cholesterol have a much larger fraction of the dihedrals being *trans* than those that are not. Thus the rigid steroid ring favors dihedrals to be *trans* in lipid chains in contact with it. The lower half of the lipid chain that is in contact with the flexible cholesterol tail has a lower fraction of *trans* torsions. The Sn2 chain does not show this behavior clearly.

The tilt of the lipid chains during the simulation was measured by calculating the angle a vector connecting two ends of a chain makes with the bilayer normal. Fig. 6 shows the plots for the Sn2 chain over 400 picoseconds and shows that chains not in contact with cholesterol have a wider distribution of tilt angles, i.e., the cholesterol may limit the extent a lipid chain may tilt. This may arise from the promotion of *trans* dihedrals in the upper part of the lipid chains next to cholesterol as single *gauche* dihedrals in a lipid chain cause large deflections from the bilayer normal (Cevc and Marsh, 1987). In comparison with Fig. 4, we see that the lipid chains show a larger tilt than the cholesterol. The non-zero tilt of the chains is consistent with experimental evidence as outlined previously (Robinson et al., 1994).

Chain order parameters measure the extent of motional order of atoms in the hydrocarbon chains and may be calculated from molecular dynamics simulations as well as measured by ^{2}H nmr (Seelig and Seelig, 1974; Davis, 1983). Contributions to deuterium order parameters include inter- and intramolecular motions, isomerization between *trans* and *gauche* isomers, the rate of *trans* - *gauche* isomerization,

TABLE 1 The fractional population of kinks in the Sn1 and Sn2 chains: comparison of those chains closely associated with a cholesterol with those that are not

Chain	Cholesterol associated with chain	Simulation time (ps)	Population of kinks in whole chain	Population of kinks in upper half of chain	Population of kinks in lower half of chain
Sn1	Yes	1–200	2.63%	1.89%	3.36%
	No	1–200	2.69%	1.76%	3.63%
	Yes	201–400	3.01%	2.57%	3.46%
	No	201–400	2.75%	2.58%	2.89%
Sn2	Yes	1–200	1.98%	1.44%	2.52%
	No	1–200	2.50%	3.01%	1.99%
	Yes	201–400	3.22%	2.28%	4.14%
	No	201–400	3.76%	3.25%	4.27%

TABLE 2 The fractional population of trans torsions in the Sn1 and Sn2 chains: comparison of those chains closely associated with a cholesterol with those that are not

Chain	Cholesterol associated with chain	Simulation time (ps)	Fraction of *trans* torsions in upper half of chain	Fraction of *trans* torsions in lower half of chain
Sn1	Yes	1–200	82.4%	76.6%
	No	1–200	75.6%	79.1%
	Yes	201–400	82.8%	80.2%
	No	201–400	76.4%	77.7%
Sn2	Yes	1–200	76.8%	76.6%
	No	1–200	76.0%	78.4%
	Yes	201–400	78.8%	81.1%
	No	201–400	80.2%	71.6%

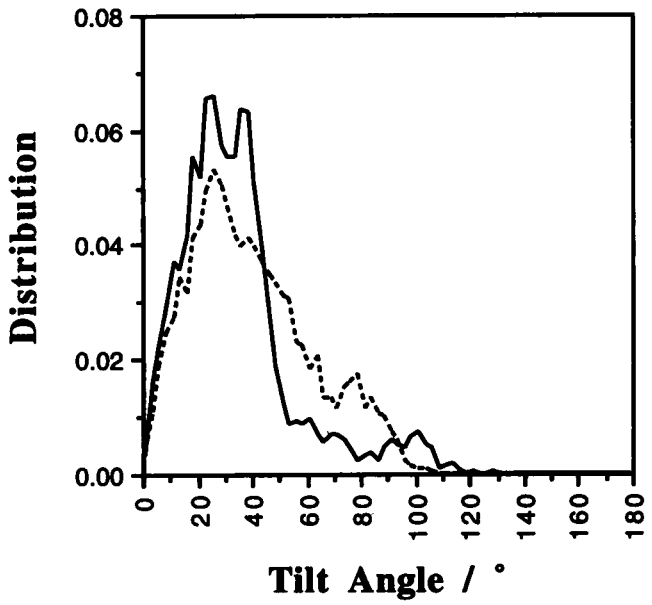

FIGURE 6 Comparison of the tilt angle distributions of the lipid Sn2 chains for those lipids associated with a cholesterol (——) with those that are not (- - - - -).

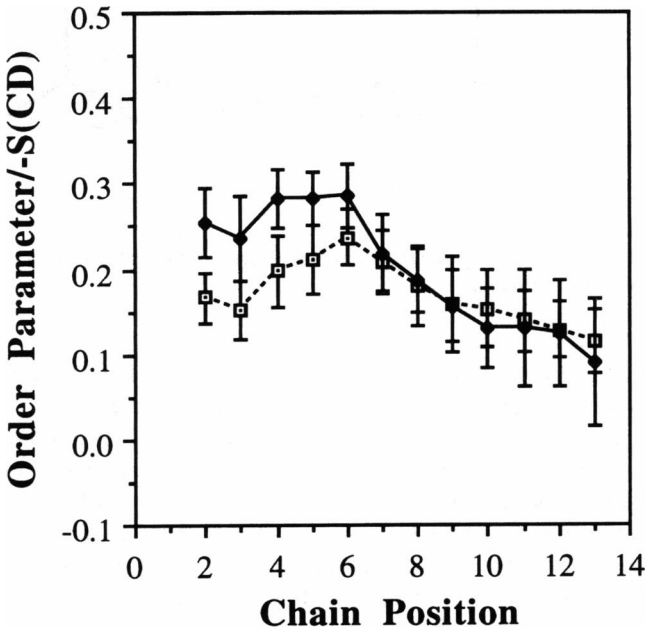

FIGURE 7 The deuterium order parameters of the Sn1 lipid chains for those lipids in contact with a cholesterol (——) compared with those that are not (- - - - -).

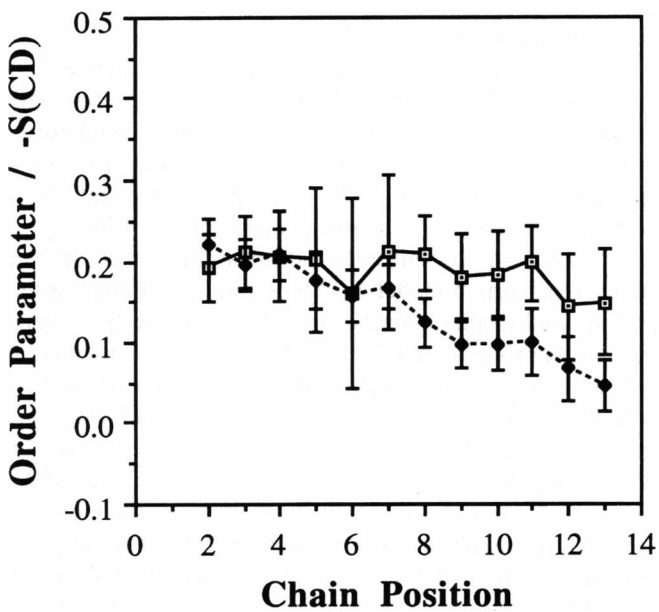

FIGURE 8 The deuterium order parameters of the Sn2 lipid chains for those lipids in contact with a cholesterol (——) compared with those that are not (- - - - -).

and the reorientation of the lipid molecule as a whole (Mayer et al., 1988). An increase in molecular order is observed in experiments (Stockton and Smith, 1976; Oldfield et al., 1971; Oldfield et al., 1978; Cornell and Keniry, 1983; Dufourc et al., 1984; Vist and Davis, 1990) and is interpreted as those chains associated with the cholesterols having less freedom of motion and less ability to undergo *trans - gauche* isomerizations. As discussed previously (Robinson et al., 1994), the comparison of order parameter profiles from nmr that average over long time scales with those from presently much shorter molecular dynamics simulations is not ideal. In Fig. 7 and 8 the deuterium chain order parameters calculated from 400 picoseconds of molecular dynamics are presented as a function of chain position for the Sn1 and Sn2 chains, respectively, with error bars being the standard deviation of the data. Although both chains show those chains nearer the cholesterols to have a larger order parameter, the Sn1 and Sn2 chains behave differently. For the Sn1 chain, the upper part of the lipid chain in contact with the rigid steroid ring is seen to have the greater motional order whereas for the Sn2 chain it is the lower half of the chain that has the greater order parameter. However, it should be noted that for the Sn2 chain the standard deviation is large for atoms in the upper part of the chain; thus it may be that the chains are close but not always interacting fully with the cholesterol molecule. The different behavior of the Sn1 and Sn2 chains during the course of the simulation may also arise from the inequiva-

lence of these two chains that leads to their being at different relative depths and the lower half of an Sn2 chain adjacent to a cholesterol molecule having restricted motion arising from the cholesterol tail not being able to pack well in the bilayer (Yeagle, 1985).

The interaction of the β-hydroxyl group of cholesterol with the ester groups of lipids was discussed in Materials and Methods. Radial distribution functions were calculated to determine the interactions during the molecular dynamics production simulations of the β-hydroxyl group of cholesterol with the water molecules and with the lipid molecules. On average, a cholesterol hydroxyl was hydrogen bonded to either 4 or 1.5 waters. The former corresponds to a hydrated cholesterol hydroxyl group and the latter to a cholesterol that has close contacts with a lipid molecule. In addition, radial distribution functions were calculated for the interaction of the cholesterol β-hydroxyl with phosphate and carbonyl groups and showed that hydrogen bonding does occur between the cholesterol and the oxygens of the phosphate in the head group and the glycerol carbonyls of the lipid. Thus the cholesterol hydroxyl can reside in two positions: deep in the membrane adjacent to the lipid carbonyls or nearer to the surface and interacting with the head groups or waters.

CONCLUSION

The main physical effects of cholesterol have been successfully reproduced by using molecular dynamics calculations on a fully hydrated, united atom model of a bilayer. These include an increase in motional order of those lipid atoms adjacent to the cholesterol as measured by the order parameter, an increase in the proportion of *trans* torsions in those dihedrals of the hydrocarbon chain next to the rigid steroid ring system, and a decrease in the population of kinks in the upper part of lipid chains in contact with a cholesterol molecule. The cholesterol was also shown to have an effect on the tilt of hydrocarbon chains with respect to the bilayer normal with those chains adjacent to cholesterol not able to tilt as far as others. The increase in fraction of *trans* dihedrals and decrease in population of kinks in the upper part of the lipid chain will have an important effect on the ability of solutes to diffuse through the membrane and supports previous ideas on how cholesterol reduces the permeability of membranes.

The cholesterol is shown to be able to hydrogen bond to the oxygens of the phosphate in the head group, to carbonyl oxygens, and to water molecules. This illustrates that cholesterol is relatively mobile within its site in the lipid bilayer and not strongly associated with the lipids.

In conclusion, the reproduction of observed experimental results provides the confidence to extend our model to other more complex systems and phenomena. Furthermore, we plan to increase the size of our model systems and simulation lengths by using parallel computers and also include a better treatment of the long-range electrostatic forces.

A.J.R. thanks Adrian Elcock for his much appreciated advice and help. Alan J. Robinson is supported by a SERC-CASE studentship with Glaxo Research and Development.

REFERENCES

Bassolino-Klimas, D., H. E. Alper, and T. R. Stouch. 1993. Solute diffusion in lipid bilayer membranes: an atomic level study by molecular dynamics simulations. *Biochemistry.* 32:12624–12637.

Berendsen, H. J. C., J. P. M. Postma, W. F. van Gunsteren, A. DiNola, and J. R. Haak. 1984. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* 81:3684–3690.

Biswas, A., and B. L. Schurmann. 1991. Molecular dynamics simulation of a dense model bilayer of chain molecules with fixed head groups. *J. Chem. Phys.* 95:5377–5386.

Bloom, M., E. Evans, and O. G. Mouritsen. 1991. Physical properties of the fluid lipid-bilayer component of cell membranes: a perspective. *Q. Rev. Biophys.* 24:293–397.

Bush, S. F., R. G. Adams, and I. W. Levin. 1980. Structural reorganisation in lipid bilayer systems: effect of hydration and sterol addition on raman spectra of dipalmitoylphosphatidylcholine multilayers. *Biochemistry.* 19: 4429–4436.

Cevc, G., and D. Marsh. 1987. Phospholipid Bilayers: Physical Properties and Models. John Wiley and Sons, Inc. New York.

Charifson, P. S., R. G. Hiskey, and L. G. Pederson. 1990. Construction and molecular modelling of phospholipid surfaces. *J. Comp. Chem.* 11:1181–1186.

Cornell, B. A., and M. Keniry. 1983. The effect of cholesterol and gramicidin A′ on the carbonyl groups of dimyristoylphosphatidlycholine dispersions. *Biochim. Biophys. Acta.* 732:705–710.

Cornell, B. A., and M. Keniry. 1983. The effect of cholesterol and gramicidin A′ on the carbonyl groups of dimyristoylphosphatidylcholine dispersions. *Biochim. Biophys. Acta.* 732:705–710.

Damodaran, K. V., and K. M. Merz. 1993. Head group-water interactions in lipid bilayers: a comparison between DMPC- and DLPE-based lipid bilayers. *Langmuir.* 9:1179–1183.

Damodaran, K. V., K. M. Merz, and B. P. Gaber. 1992. Structure and dynamics of the dilauroylphosphatidylethanolamine lipid bilayer. *Biochemistry.* 31:7656–7664.

Davis, J. H. 1983. The description of membrane lipid conformation, order and dynamics by ^{2}H nmr. *Biochim. Biophys. Acta.* 737:117–171.

Duax, W. L., J. F. Griffin, and D. C. Rohrer. 1980. Conformational analysis of sterols: comparison of X-ray crystallographic observations with data from other sources. *Lipids.* 15:783–792.

Dufourc, E. J., E. J. Parish, S. Chitrakorn, and I. C. P. Smith. 1984. Structural and dynamical details of cholesterol lipid interaction as revealed by deuterium nmr. *Biochemistry.* 23:6062–6071.

Edholm, O., H. J. C. Berendsen, and P. van der Ploeg. 1983. Conformational entropy of a bilayer membrane derived from a molecular dynamics simulation. *Mol. Phys.* 48:379–388.

Edholm, O., and J. Johansson. 1987. Lipid bilayer polypeptide interactions studied by molecular dynamics simulation. *Eur. Biophys. J.* 14:203–209.

Edholm, O., and A. M. Nyberg. 1992. Cholesterol in model membranes: a molecular dynamics simulation. *Biophys. J.* 63:1081–1089.

Essex, J. W. 1992. Free-energy calculations in molecular biology. D. Phil thesis, Oxford University, Oxford.

Franks, N. P. 1976. Structural analysis of hydrated egg lecithin and cholesterol bilayers. *J. Mol. Biol.* 100:345–358.

Fukada, T., S. Okazaki, and I. Okada. 1993. Molecular dynamics study of the lauryl alcohol-laurate model bilayer. *Biophys. J.* 64:1344–1353.

Heller, H., M. Schaefer, and K. Schulten. 1993. Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid crystalline phase. *J. Phys. Chem.* 97:8343–8360.

Huang, C. 1976. Roles of carbonyl oxygens at the bilayer interface in phospholipid-sterol interaction. *Nature.* 259:242–244.

Jorgensen, W. L., J. Chandrasekhar, J. Madura, R. W. Impey, and M. L. Klein. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 79:926–935.

Jorgenssen, W. L., and J. Tirado-Rives. 1988. The OPLS potential functions for proteins: energy minimizations for crystals of cyclic peptides and crambin. *J. Am. Chem. Soc.* 110:1657–1666.

Khalatur, P. G., and A. S. Pavlov. 1987. Molecular motions in a liquid-crystalline lipid bilayer: molecular dynamics simulation. *Makromol. Chem.* 188:3029–3040.

Kroon, P. A., M. Kainosho, and S. I. Chan. 1975. State of molecular motion in lecithin bilayers. *Nature.* 256:582–584.

Lindblom, G., L. Johansson, and G. Arvidson. 1981. Effect of cholesterol in membranes: pulsed nuclear magnetic resonance measurements of lipid lateral diffusion. *Biochemistry.* 20:2204–2207.

Marrink, S., M. Berkowitz, and H. J. C. Berendsen. 1993. Molecular dynamics simulations of a membrane/water interface: the ordering of water and its relation to the hydration force. *Langmuir.* 9:3122–3131.

Mayer, C., K. Muller, K. Weisz, and G. Kothe. 1988. Deuteron N.M.R relaxation studies of phospholipid membranes. *Liq. Cryst.* 3:797–806.

McIntosh, T. J. 1978. The effect of cholesterol on phosphatidylcholine bilayers. *Biochim. Biophys. Acta.* 513:43–58.

Molecular Simulations Inc., QUANTA, Version 3.3. Waltham, MA, 1992.

Oldfield, E., D. Chapman, and W. Derbyshire. 1971. Deuteron resonance: a novel approach to the study of hydrocarbon chain mobility in membrane systems. *FEBS Lett.* 16:102–104.

Oldfield, E., M. Meadows, D. Rice, and R. Jacobs. 1978. Spectroscopic studies of specifically deuterium labelled membrane systems: nuclear magnetic resonance investigation of the effects of cholesterol in model systems. *Biochemistry.* 17:2727–2740.

Opella, S. J., J. P. Yesinowski, and J. S. Waugh. 1976. Nuclear magnetic resonance description of molecular motion and phase separations of cholesterol in lecithin dispersions. *Proc. Natl. Acad. Sci. USA.* 73: 3812–3815.

Pascher, I., and S. Sundell. 1982. The crystal structure of cholesterol dihydrogen phosphate. *Chem. Phys. Lipids.* 31:129–143.

Pink, D. A., T. J. Green, and D. Chapman. 1981. Raman scattering in bilayers of saturated phosphatidylcholines and cholesterol: experiment and theory. *Biochemistry.* 20:6692–6698.

van der Ploeg, P., and H. J. C. Berendson. 1982. Molecular dynamics simulation of a bilayer membrane. *J. Chem. Phys.* 76:3271–3276.

van der Ploeg, P., and H. J. C. Berendson. 1983. Molecular dynamics of a bilayer membrane. *Mol. Phys.* 49:223–248.

Reiber, H. 1978. Cholesterol-lipid interactions in membranes: the saturation concentration of cholesterol in bilayers of various lipids. *Biochim. Biophys. Acta.* 512:72–83.

Robinson, A. J., W. G. Richards, P. J. Thomas, and M. M. Hann. Head group and chain conformations in biological membranes: a molecular dynamics computer simulation. *Biophys. J.* In press.

Ryckaert, J. P., G. Ciccotti, and H. J. C. Berendson. 1977. Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. *J. Comp. Phys.* 23:327–341.

Scott, H. L. 1991. Lipid cholesterol interactions: Monte Carlo simulations and theory. *Biophys. J.* 59:445–455.

Scott, H. L., and S. Kalaskar. 1989. Lipid chains and cholesterol in model membranes: a Monte Carlo study. *Biochemistry.* 28:3687–3692.

Scott, H. L., and W. S. McCullough. 1993. Lipid-cholesterol interactions in the P_{β}' phase: application of a statistical mechanical model. *Biophys. J.* 64:1398–1404.

Seelig, J., and A. Seelig. 1974. The dynamic structure of fatty acyl chains in a phospholipid bilayer measured by deuterium nuclear magnetic resonance. *Biochemistry.* 13:4839–4845.

Singh, U. C., P. K. Weiner, J. W. Caldwell, and P. A. Kollman. 1988. AMBER. Department of Pharmaceutical Chemistry, University of California, San Francisco.

Stockton, G. W., and I. C. P. Smith. 1976. A deuterium magnetic resonance study of the condensing effect of cholesterol on egg phosphotidylcholine bilayer membranes. *Chem. Phys. Lipids.* 17:251–263.

Stouch, T. R. 1993. Lipid membrane structure and dynamics studied by all-atom molecular dynamics simulations of hydrated phospholipid bilayers. *Mol. Sim.* 10:335–362.

Stouch, T. R., H. E. Alper, and D. Bassolino. Supercomputing studies of biomembranes. *Int. J. Supercomp. Appl.* 8:6–23.

Stouch, T. R., K. B. Ward, A. Altieri, and A. T. Hagler. 1991. Simulations of lipid crystals: characterisation of potential energy functions and parameters for lecithin molecules. *J. Comp. Chem.* 12:1033–1046.

Taylor, M. G., T. Akiyama, and I. C. P. Smith. 1981. The molecular dynamics of cholesterol in bilayer membranes: a deuterium NMR study. *Chem. Phys. Lipids.* 29:327–339.

Venable, R. M., Y. Zhang, B. J. Hardy, and R. W. Pastor. 1993. Molecular dynamics simulations of a lipid bilayer and of hexadecane: an investigation of membrane fluidity. *Science.* 262:223–226.

Vist, M. R., and J. H. Davis. 1990. Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: ^{2}H nuclear magnetic resonance and differential scanning calorimetry. *Biochemistry.* 29:451–464.

Worcester, D. L., and N. P. Franks. 1976. Structural analysis of hydrated egg lecithin and cholesterol bilayers. II. Neutron diffraction. *J. Mol. Biol.* 100:359–378.

Xiang, T. 1993. A computer simulation of free-volume distributions and related structural properties in a model lipid bilayer. *Biophys. J.* 65:1108–1120.

Yeagle, P. L. 1980. Cholesterol rotation in phospholipid vesicles as observed by ^{13}C-nmr. *Biochim. Biophys. Acta.* 640:263–273.

Yeagle, P. L. 1985. Cholesterol and the cell membrane. *Biochim. Biophys. Acta.* 822:267–287.

Yeagle, P. L. 1987. The Membranes of Cells. Academic Press, Orlando Florida.

Yeagle, P. L., W. C. Hutton, C. Huang, and R. B. Martin. 1975. Head group conformation and lipid - cholesterol association in phosphatidylcholine vesicles: a $^{31}P[^{1}H]$ nuclear Overhauser effect study. *Proc. Natl. Acad. Sci. USA.* 72:3477–3481.