Cholesterol in model membranes

A molecular dynamics simulation

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ABSTRACT Molecular dynamics simulations of a model membrane with inserted cholesterol molecules have been performed to study the perturbing influence of cholesterol. In the fluid phase of a lipid bilayer at 13 mol% concentration of cholesterol, local ordering of the hydrocarbon chains is induced. This perturbation decays with the distance from the cholesterol, and the effect extends 1.25 nm. It can be monitored in several ways, e.g., by an order parameter corresponding to deuterium nuclear magnetic resonance quadrupolar splittings, by the fraction of gauche bonds, or by the local bilayer thickness. At constant surface density, the local ordering is accompanied by disordering of the bulk phase, and, consequently, the net ordering effect is small. After compressing the system laterally in accordance with experimentally known surface areas, the bulk order parameters agree with those of a pure system, and the average order parameters are in accordance with experimental data. The necessity for this lateral compression is supported by calculated lateral pressures. At lower cholesterol concentration (3%), no direct perturbing effect is observed. A smaller lateral pressure than in a pure system indicates that the system with cholesterol is expected to have a smaller surface area, which would result in an increase of the order parameters, thus accounting for the experimental observations. The lack of spatial variation is, however, puzzling and may indicate a cooperative ordering effect.

INTRODUCTION

Experimental investigations of model membranes and real plasma membranes with cholesterol incorporated have been performed for about 20 years. The state of the art for the dipalmitoyl phosphatidyl choline (DPPC)/cholesterol system is well explored and described in a recent paper (1), in which the phase diagram as a function of temperature and cholesterol content is constructed. The techniques used are deuterium nuclear magnetic resonance (NMR) and differential scanning calorimetry. A new phase, the β-phase, appears at high cholesterol concentrations in addition to the gel and liquid crystalline phases. In this article, we consider moderate cholesterol concentrations and high temperatures, and the fluid liquid crystalline phase is stable. In that phase, cholesterol increases the molecular order (1–7) and membrane thickness (8–10). An intuitive explanation is that the stiffness of the cholesterol molecules brings the neighboring chains into more extended states with a lower fraction of gauche bonds and an orientation more perpendicular to the membrane.

In contrast to the vast experimental activity, there have been few theoretical efforts to understand the properties of membranes containing cholesterol. Most of these studies have been based on a lattice model for the lipid bilayer (11 and references therein). The conformational states of the chains are grouped together into a small number of states ($\sim$10) with different shape factors and degeneracies. The interaction between the molecules is described on a two-dimensional lattice by a model Hamiltonian, in which the interaction energy is proportional to the shape factors of each lipid in a pair. In the vicinity of a cholesterol molecule, the chain-chain interactions are decoupled and the van der Waals coupling to the cholesterol molecule increases with the order of the chain. The parameter values are chosen ad hoc, and the model is evaluated either by Monte Carlo simulation methods or in a mean field approximation. A quantitative description of experimental findings is not expected, but the model qualitatively describes several phenomena, e.g., thermal anomalities, order parameter changes, thickness, and, to some extent, phase behavior.

A more detailed model in which the interaction energy is calculated from three-dimensional conformations and positions of the chains has been studied by Scott and co-workers (12, 13) by Monte Carlo methods. The hydrocarbon chains are allowed to move in the membrane plane, but they are confined in the perpendicular direction. This treatment is more realistic than the two-dimensional lattice model, but it still fails to account for two important properties of the real system: the chains need not be oriented with the all-trans conformation perpendicular to the membrane plane and one need not restrict the conformations to the discrete states given by the minima of the dihedral potential. In fact, the order parameters obtained by Scott and co-workers are almost twice as large as the experimental results, indicating that their model is too restrictive. To compensate for this flaw, their values were divided with a factor, which is justified by the need to account for the lack of tilt in the model.

We investigate here an even more detailed model of the lipid/cholesterol system in which we allow for tilt of the chains and for continuous variation of the dihedral angles subject to a dihedral potential. As in Scott's model, a detailed description of headgroups and the headgroup-water interactions is lacking. Molecular dynamics simulations of this system were performed. The order parameters obtained from a simulation of a system

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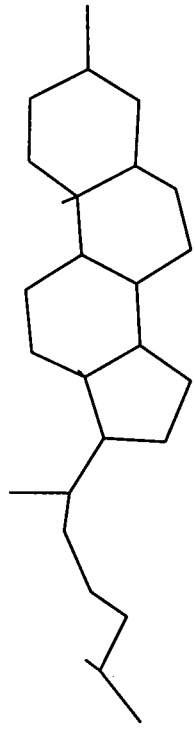

FIGURE 1 The cholesterol molecule after equilibration.

without cholesterol agree well with experimental values (without introduction of correction factors). The purpose of this paper is to study the perturbing effect of cholesterol on the lipid chains.

METHODS

The model and method are similar to those used by van der Ploeg and Berendsen (14, 15) in a study of a bilayer of 10 unit chains modeling a decanol/decanoate system. A detailed description is made of the hydrocarbon part of the system but not of the headgroup/water region. The bilayer is stabilized by a harmonic potential that keeps the headgroups in a plane. The strength of this potential (25 kJ nm^{-2} mol^{-1}) was chosen to provide a reasonable roughness of the membrane surface (i.e., mean square deviation of the head groups positions). A reason for taking this simplified approach is that our main interest is the perturbing influence of the cholesterol on the hydrocarbon region, and van der Ploeg and Berendsen (14, 15) show that many of the properties of this region can be well described without using a more complete and more time-consuming model.

The head groups as well as CH_2 and CH_3 groups are treated as unified atoms interacting through a Lennard-Jones potential, with parameters that are taken from van der Ploeg and Berendsen (14). Electrostatic interactions are not included. The ring system of the cholesterol molecules is modeled as rigid with bond lengths and angles fixed (see Fig. 1). The same Lennard-Jones potential is used for the hydrocarbon groups of cholesterol as for the CH_2 groups of the chains. The dihedral potential for the chains is the Ryckaert-Bellemans potential (16). For the cholesterol tail, a symmetric cosine-potential is used, and Lennard-Jones interactions are included between atoms three bonds apart to model the difference between trans and gauche states.

A Lennard-Jones potential with parameters from Ryckaert and Bellemans simulation (16) of liquid butane were also used. For the interaction between two CH_2 groups, this potential has a much deeper well than in our final choice of the potential. The first set of parameters were found unsuitable for modeling the lipid bilayer for reasons that will be discussed later. The potential parameters used here (14) are not identical, but quite similar, to those (taken from Jorgensen [17]) used in Scott and co-worker's (12, 13) MC-simulations.

It was found necessary to use a larger force constant for the headgroup of the cholesterol and, in addition, to include a harmonic force acting on one atom at the other end of the ring system to bring the molecule perpendicular to the membrane surface. If this was not done, the cholesterol molecule was pushed out of the lipid bilayer after some time.

For the molecular dynamics, the GROMOS program was used with a time step of 5 fs for the integration of the equations of motion. This may be compared with the time steps used in van der Ploeg and Berendsen (14) and Ryckaert and Bellemans (16), which were 8 and 3.6 fs. We tested whether this time step was appropriate by conducting shorter simulations with a time step of 2 fs and found no essential difference between the results. The energy drift in short runs without the use of temperature scaling was small and was not significantly reduced by using a smaller time step. The bond lengths were kept fixed using the SHAKE algorithm (18). The nonbonded interactions were discontinuously (no smooth switching function) cut off at 0.80 nm (i.e., about twice the Lennard-Jones diameter). Periodic boundary conditions were invoked in the two lateral dimensions, which means that the surface density was kept fixed. In the third dimension, the system size (the bilayer thickness) adjusted automatically. The temperature was kept at 330 K by scaling the velocities with a time constant of 100 fs.

Initially, two monolayers consisting of 64 all-*trans* 16 unit chains on a square lattice of the appropriate surface density (0.29 nm^2 per chain in most cases) were generated. In one layer, the coordinates were displaced one-half lattice spacing in both the lateral directions, and the layers were positioned as close as possible without causing them to repel each other. The initial thickness was 34 Å. Each inserted cholesterol molecule replaced two lipid chains. The cholesterol coordinates were taken from a crystal structure (19) and were equilibrated in a vacuum molecular dynamics simulation with the force field later used in the full simulation, including the lipid chains.

Starting from all-*trans* chains on a square lattice (Fig. 2 *a*), the system gradually transformed into the disordered high temperature phase (Fig. 2 *b*). Equilibrium was reached after 100 ps of simulation as indicated by the time development of the fraction of gauche bonds (Fig. 3). A typical time between successive trans/gauche isomerizations in a bond was found to be 20 ps from the simulations. Thus, there was ample time in simulations that extended over 300 ps to obtain a reasonable yield of representative conformations.

Several surface densities were studied, but most of our simulations corresponded to an area of 0.29 nm^2 per chain in agreement with experimental surface areas for a pure lecithin system, which vary between 0.29 and 0.30 nm^2, depending on water content and the nature of the head groups (e.g., 20, 21). Four systems were simulated as follows: a pure system without cholesterol consisting of 128 chains at a surface area of 0.29 nm^2 per chain; systems with two and eight cholesterol molecules replacing 4 and 16 chains, respectively, at the same total surface area (these mixtures correspond to molar fractions of cholesterol in a lecithin system of 3.2 and 13.3%); and a system with two cholesterol molecules and 124 chains with a surface area of 0.26 nm^2 per chain. The surface area will be discussed in relation to the results in the next section.

RESULTS AND DISCUSSION

The main aim of this study is to investigate the ordering effect of cholesterol on the hydrocarbon chains of a lipid bilayer. To quantify this behavior, a number of different measures of the order can be used. The order parameter S_{CD} is directly accessible from experiment by deuterium NMR. It is a measure of the orientation of the C—H

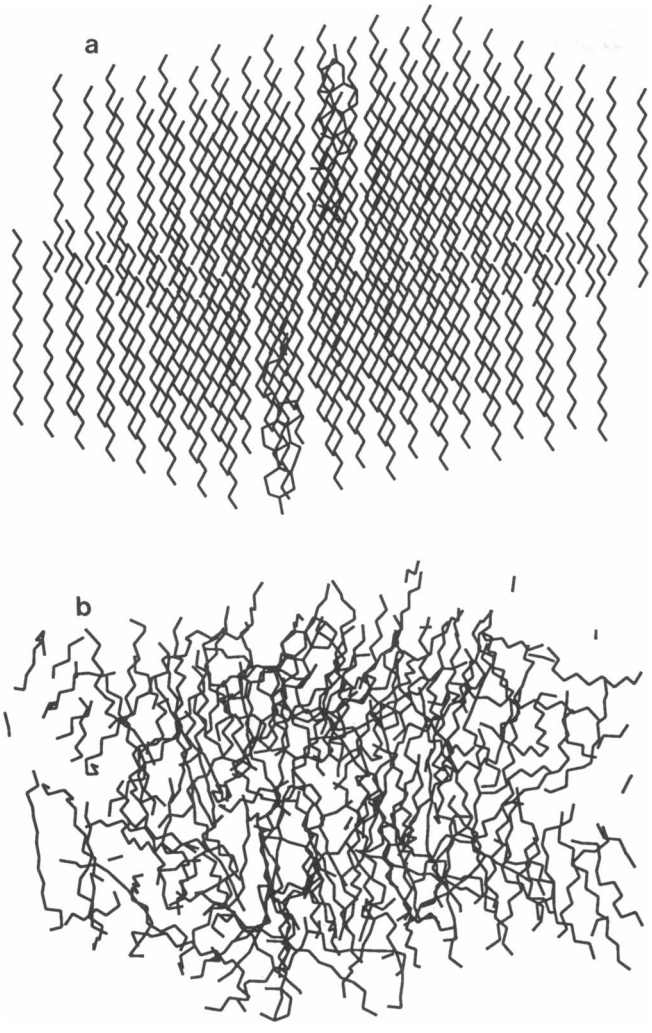

FIGURE 2 (*a*) The initial conformation of the system. (*b*) The conformation after equilibration.

(C—D) bonds of the hydrocarbon groups and may be expressed as an average over the angle θ, a bond forms with the membrane normal

$$S_{CD} = \tfrac{1}{2}(3\langle\cos^2\theta\rangle - 1).$$

S_{CD} varies from −0.5, when the chains are ordered and perpendicular to the membrane surface, to zero for a completely disordered system. In Fig. 4, *a* and *b*, the calculated values of this order parameter are shown as a function of chain position for various systems. The values are averaged over all chains. For comparison, the experimental S_{CD} order parameters for a pure DPPC system (22) are also shown. At 0.29 nm^2 surface area per chain, there is not much difference between the simulated systems, without cholesterol, with two and eight cholesterols, and experimental data for a system without cholesterol. There are small deviations from the experimental curve that will be commented on later. We do, however, see a substantially increased order in the system at the higher surface density (Fig. 4 *b*). The main conclusion from these curves is that at constant surface density the effect of cholesterol on the average order parameter is small. The accuracy of the calculated order parameters is ~0.005 as estimated from the standard deviation of subaverages.

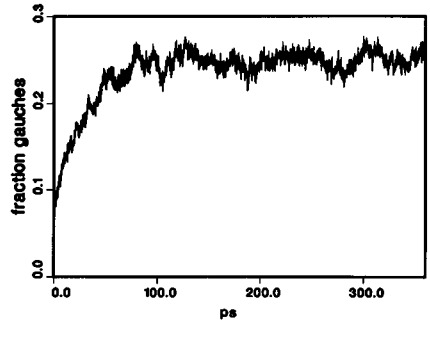

FIGURE 3 The fraction of gauche bonds as a function of time.

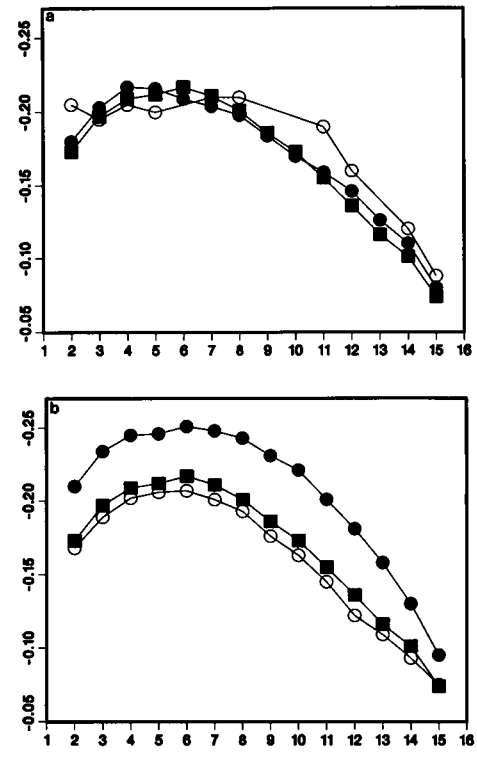

FIGURE 4 The average order parameters as a function of chain position. (*a*) Filled circles are calculated for the system without cholesterol at 0.29 nm^2 surface area per chains, the filled squares for the system with two cholesterols at the same surface density, whereas the open circles show experimental S_{CD} order parameters from a pure DPPC system (20). (*b*) Filled circles are calculated at the higher surface density (0.26 nm^2 per chain) and the filled squares and open circles at 0.29 nm^2 with two and eight cholesterols, respectively.

The spatial variation of the order parameters can be resolved in our simulation, but the same experimental information is not available. In Fig. 5, the order parameters are shown as averages over the whole chain and as a function of the distance from the closest cholesterol molecule. There is an ordering effect of the cholesterols that

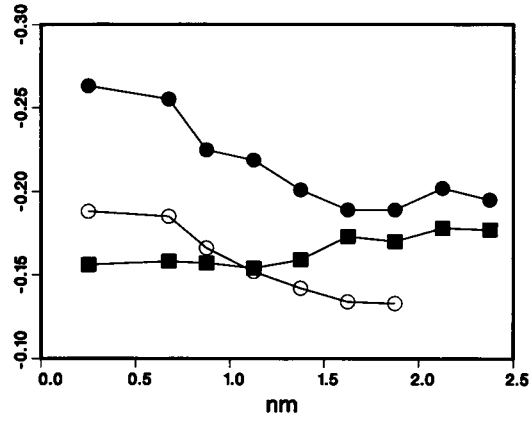

FIGURE 5 The order parameters (averaged over all chain atoms) as a function of the distance to the closest cholesterol. Filled circles are at 0.26 nm^2 per chain, the filled squares at 0.29 nm^2 per chain, and the open circles at the same density but with eight instead of two cholesterols.

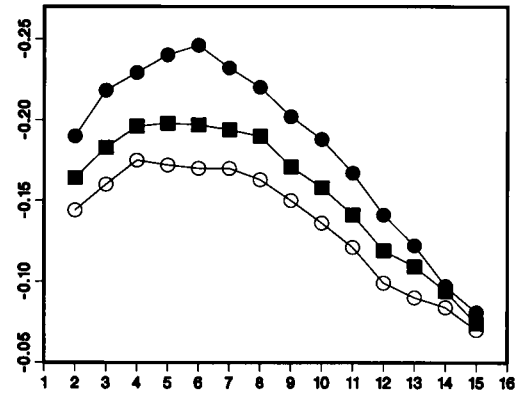

FIGURE 7 The order parameter S_{CD} as a function of chain position for the system with eight cholesterols. Filled circles are for chains closer than 0.75 nm from a cholesterol molecule, filled squares for chains with the nearest cholesterol at a distance between 0.75 nm and 1.25 nm, and the open circles for chains with > 1.25 nm to the closest cholesterol.

extends ~1.25 nm both for the system at high surface density and the system with eight cholesterols at normal surface density. For the system with two cholesterols at normal surface density, no ordering close to the cholesterol can be discerned. In Fig. 6, another measure of the order, the fraction of *trans* bonds, is shown as a function of the distance from the closest cholesterol. There is an increase close to the cholesterol for the system with eight cholesterols and for that with two cholesterols at high surface density, but no effect is found in the latter system at normal surface density. In Fig. 7, the order parameters at high cholesterol concentration are plotted as a function of chain position for chains in three zones, closer than 0.75, 0.75–1.25, and farther out than 1.25 nm from the closest cholesterol. We observe a clear effect of the cholesterol in the two closest zones. The ordering decreases toward the end of the chain, and it is most significant in the region of the chains that are at the same depth in the membrane as the ring system of the cholesterol. Finally, the order parameters in the outermost zone are substantially smaller (15–20%) than the order parameters of a pure system (either simulated or experimental) given in Fig. 4 *a*. This is due to that the simulations has been performed at constant surface area. In reality, the surface area per lipid decreases on inclusion of cholesterol. This will be discussed and corrected for later.

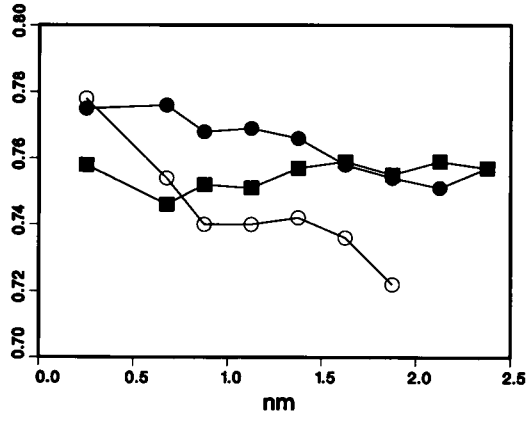

FIGURE 6 The fraction of trans bonds as a function of the distance from the cholesterol. Filled circles are at 0.26 nm^2 per chain, the filled squares at 0.29 nm^2 per chain, and the open circles at the same density but with eight instead of two cholesterols.

Although there is generally good agreement between the experimental and simulated order parameter profiles for the system without cholesterol, two discrepancies remain. First, there is a slight drop in the calculated order parameters in the head group region instead of the experimental plateau. Second, the calculated order parameters decrease faster in the middle of the chain. The first fact is probably due to the simplified description of the headgroup region and the neglect of solvent water. We have chosen to use a weak potential to restrain the headgroups in the direction perpendicular to the membrane plane, giving a root mean square (rms) spread of ±0.25 nm. It is not clear from experiments how rough the membrane surface is. We can compare our results with full simulations, including water and head groups (23, 24). These simulations indicate a rms spread of the headgroups of ±0.30 nm. The result depends, however, on the model description of headgroups, water, and electrostatics. In our model, a more plateau-like behavior of the order parameters close to the headgroup and a less rough membrane surface would be expected if a stronger restraining potential was used to keep the head groups in a plane.

In Fig. 8, we see that cholesterol causes local thickening of the monolayer with 0.1–0.15 nm (7–10%) for all three systems. The effect is smallest for the system with two cholesterols at 0.29 nm^2 per chain. This observation

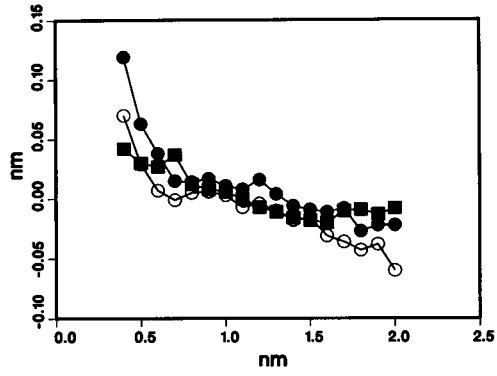

FIGURE 8 The deviation from the average thickness of half the bilayer as a function of distance from the closest cholesterol molecule. Filled circles for the system at 0.26 nm^2 per chain, filled squares at 0.29 nm^2 per chain, and open circles for the system with eight cholesterols.

contradicts the behavior of the fraction of *trans* bonds and the S_{CD} order parameters that do not indicate any local ordering at the lower cholesterol concentration and low surface density.

We will now consider the effect of cholesterol on the surface area per lipid. For this, we first review the experimental situation. Surface area and membrane thickness as a function of the cholesterol content in a membrane have been studied experimentally in Schwartz et al. (8), Needham et al. (9), and McIntosh (10). Since the volume density can, approximately, be considered constant (the volume change during the main phase transition is only 1.4% [25] or 4% [26], whereas the surface density change is 20%), the surface area and thickness are simply related. The experimental results obtained for lecithins with different headgroups using different methods are very similar. The experimental data (8–10) indicates that the surface area per lipid decreases linearly with cholesterol content for low cholesterol concentrations (<20%) and is reduced to 98.6 and 94.1% of the surface area of that of the pure system at the two cholesterol concentrations considered by us. When comparing calculated average order parameters with the experimental ones, it is essential to have used the correct surface density in the simulations.

The surface area ought to be corrected for two reasons. First, we have replaced two chains with one cholesterol when introducing the cholesterols into the system, which is equivalent to assuming that the area of the cholesterol molecule is 2×0.29 nm^2. The area of a cholesterol molecule is 0.38 nm^2 (19) in the x-ray structure. If the extra 0.2 nm^2 per cholesterol is distributed among the chains, the surface area per chain becomes larger in the systems containing cholesterol than in the pure system. The figures are 0.003 and 0.014 nm^2, respectively, for the systems with two and eight cholesterols. The difference explains why the system with eight cholesterols is slightly less ordered than the system with two cholesterol molecules (Fig. 4 *b*). In addition, we know from experiments that cholesterol induces a thickening of the bilayer and an increased surface density. Using the experimental figures discussed in the last paragraph, we find that the area needs to be reduced by 0.003 and 0.014 nm^2, respectively, at the two cholesterol concentrations considered. This means that lateral compression with 0.006 and 0.028 nm^2 or 2.1 and 9.7%, respectively, is necessary to obtain the proper surface densities. We know from the simulation at higher surface density that a reduction of the surface area per lipid from 0.29 to 0.26 nm^2 causes an increase of the order parameter by 0.033 or 19.2%. Assuming that the average order parameter varies linearly with surface density, we find for the two cholesterol concentrations considered that the average order parameters are −0.175 and −0.176, respectively, at constant surface density. Allowing for the experimentally known surface density change, we instead obtain −0.178 and −0.192. For the pure system, we have an order parameter of −0.171. The effect is very small when the surface density is kept constant, whereas it is more pronounced when the surface density is changed to its proper value.

The experimental order parameters (1–7) come from different systems, and there is some variation between them. In the study by Stockton and Smith (3), which contains the most complete data, the order parameters are reported for deuterated distearoyl diphosphatidyl choline dissolved in a DPPC bilayer at 330K. The order parameters increase linearly with cholesterol concentration. The experimental figures correspond to an increase of the average order parameter of 4.6 and 19.6%, respectively, at the two concentrations of cholesterol we have used. On the other hand, Jacobs and Oldfield (5) reported on dimyristoyl diphosphatidyl choline, an increase in the order parameters that is only about half the value of these in Stockton and Smith (3) and nonlinear in the cholesterol concentration with a smaller effect at low concentrations. Our figures after correction of the surface area are 4.1 and 12.3%. Comparing with Stockton and Smith (3), the ordering is weaker in our simulation at the higher surface density. We conclude that the order parameters fall within the range of different experimental results, when the known surface density change is allowed for. At constant surface density, the changes of order parameters induced by cholesterol are much smaller than the experimental figures.

Two other results indicate that a surface density change is induced by cholesterol. First, the bulk order parameters (Fig. 7) in the system at high cholesterol concentration are only 80% of those of the pure system (experimental or from our simulations; Fig. 4 *a*). The ordering and increase in surface density close to the cholesterol molecules have occurred at the cost of disordering and decreased surface density far away from the cholesterols. Second, the lateral pressure calculated from the virial theorem and given in Table 1 is significantly smaller (accuracy ±1 MN m^{-2}) for the cholesterol containing systems. It would be favorable for the system to

TABLE 1 **Data for the simulations**

	1	2	3	4
Number of chains	128	124	124	112
Number of cholesterols	0	2	2	8
Area (nm^2)	18.56	16.64	18.56	18.56
Area/chain (nm^2)	0.290	0.262	0.293	0.304
Temperature (K)	330	330	330	330
Lateral pressure (MN m^{-2})	62.7	66.7	54.6	52.1
Normal pressure (MN m^{-2})	42.0	41.3	35.7	37.4
Potential energy (kJ/mol system)	−223	−404	−105	+301
Thickness (nm)	2.92	3.21	2.93	2.87
Volume (nm^3)	54.13	53.38	54.30	53.24
Fraction gauche bonds (%)	25.2	24.0	24.8	27.1
Average order parameter (S_{CD})	−0.171	−0.205	−0.172	−0.161
Average tilt angle of lipid chain (degrees)	30	26	28	32
Average tilt angle of cholesterol (degrees)	—	10	16	12
Average instantaneous collective tilt (degrees)	5	6	6	7
Lateral diffusion constant of chains (m^2/s)$*10^9$	1.4	0.9	1.6	1.8
Lateral diffusion constant of cholesterol	—	0.7	1.2	1.0

contract in the lateral direction to bring the bulk order parameters and lateral pressure into agreement with those of a pure system at this temperature. Regarding pressure, one should keep in mind that we have simulated a membrane without head groups and water. In a real system, the lateral pressure should be smaller than the normal and give a positive surface tension, due to the system minimizing the lipid/water interface and decreasing the hydrophobic energy. The effect cannot be seen in a simulations in vacuum, and other simulations of this type (15, 27) result in larger lateral than normal pressure.

It still remains to explain the lack of ordering close to the cholesterol at 3 mol% concentration. According to experiments, an increase in surface density exists. It is consistent with the lateral pressure, and it explains the average order parameters. However, it is difficult to understand the surface density increase without any local perturbation close to the cholesterols. It is possible that this result is due to poor statistics. The accuracy of the average order parameter at a given chain position is ±0.005 as estimated from subaverages. When the spatial variation around a cholesterol molecule is considered, the accuracy will be less since fewer chains contribute. We can improve the statistics by considering the average over all chain positions. The order parameters are 0.04 larger close to the cholesterol compared with the bulk in the system with eight cholesterols. If there is a similar local effect in the system with two cholesterols, the statistics should, according to these considerations, be good enough to observe the ordering. A problem may be that there are only two cholesterols in the system and hence few neighbor chains. Some of these may be trapped in disordered states that will take a long time to leave. What further indicates that the statistics may not be good is that there is ordering around the cholesterol at 0.26 nm^2 area per chain but not at 0.29 nm^2. We cannot find a physical reason for this difference.

If the absence of ordering at low cholesterol concentration is a real effect, it reflects cooperativity of some kind. At the low cholesterol concentration, the closest cholesterols are two periodic copies at a distance of >4 nm, and, consequently, there are no chains that feel the presence of more than one cholesterol. At the higher concentration, a typical distance between cholesterols is ~2 nm, which leaves space for two to four chains. Thus, there are chains that feel the presence of more than one cholesterol, which could make the ordering influence much stronger. A cooperative effect also has been suggested by Scott (13) based on results from Monte Carlo simulations at cholesterol concentrations of 2, 13, and 23 mol%. In his simulations, the cholesterol molecules cannot move at all, which creates a static and ordered structure at high cholesterol concentrations and may explain the nonlinear dependence of ordering on concentration.

Several definitions of the tilt angle can be found in the literature. One example is based on the eigenvector corresponding to the smallest eigenvalue in the moment of inertia tensor (27) and another on the width of the membrane in relation to the length of the lipid chains (27). We use the following definition of the tilt angle θ for the hydrocarbon chains

$$\cos\theta = \frac{1}{N}\sum_{k=1}^{N}\frac{|\mathbf{u}_k \times \mathbf{n}|}{|\mathbf{u}_k||\mathbf{n}|},$$

where $\mathbf{n}$ is the membrane normal, $\mathbf{u}_k$ the vector from the last hydrocarbon group to the headgroup, and N is the number of molecules. We found an average tilt of ~30 degrees (average in time). The tilt angle depends on the surface density and it is correlated between chains, but the correlation extends only to the first layer of neighbors as seen from Fig. 9. The instantaneous collective tilt for the whole system

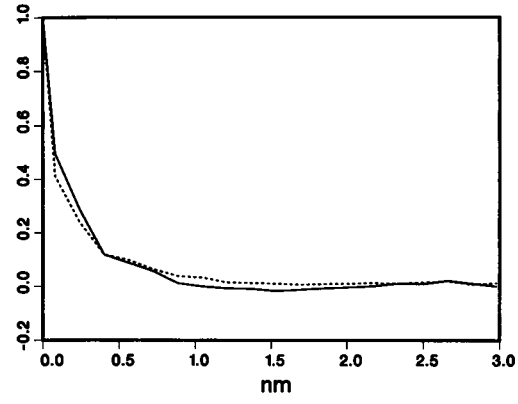

FIGURE 9 The spatial autocorrelation of the chain tilt.

$$\cos\theta^{\text{coll}} = \frac{1}{N}\left|\sum_{k=1}^{N}\frac{\mathbf{u}_k \times \mathbf{n}}{|\mathbf{u}_k||\mathbf{n}|}\right|,$$

is 5–7 degrees (shown in Table 1), which is small enough to be explained as a statistical artifact due to the finite size of the system. The collective tilt has a correlation time of the order of 25 ps, and when studied on time scales of hundreds of picoseconds, the time average of the tilt will certainly be negligible. The ring system of cholesterol molecules has a smaller tilt of 10–16 degrees, which is expected due to the restraining potential used to keep them perpendicular.

Comparing our results with those of Scott and Kalaskar (12) and Scott (13), we first note that their order parameters are a factor of two larger than the experimental parameters as well as those from our simulations. Scott et al. (12, 13) adjust their values by dividing by a factor of two that is supposed to compensate for the absence of local tilt. We allow for tilt in our simulations, and the order parameters we calculate agree with experiments without multiplication by arbitrary factors. The local tilt found from our simulations is 30 degrees, which corresponds to a factor 1.6 in the order parameters. Therefore, the deviation between the order parameters in Scott et al. (12, 13) and the experimental ones could not be explained by the local tilt exclusively. We believe that the restriction of the dihedral angles to three discrete states also restrains the system and contributes to the increased order parameters.

The results above show that the chains are more ordered close to cholesterol molecules. What happens to dynamic quantities such as diffusion constants? If the motion can be regarded as two dimensional diffusion, the relation

$$\langle r^2 \rangle = 4Dt$$

should hold. The symbol r denotes the distance traveled during time t, and D is the lateral diffusion constant. The bracket denotes time averaging. In Fig. 10, $\langle r^2 \rangle$ is plotted against time. The behavior is not purely diffusive, as the "constant" D would be larger for shorter times. The average lateral diffusion constants calculated from the long time behavior (the linear part in Fig. 10) are given in Table 1. These lateral diffusion constants are of the order 10^{-9} m^2 s^{-1} and in agreement with similar simulations in van der Ploeg and Berendsen (14, 15). They are one order of magnitude larger than experimental lateral diffusion constants. The reason for the discrepancy is that headgroup-headgroup and headgroup-water interactions are essential for slowing down the diffusion process. Therefore, proper modeling of this region is necessary to describe the lateral diffusion correctly. We may, however, still compare the different simulations. We find that the system with higher surface density has a diffusion constant a factor 2 smaller than the other systems. On the other hand, cholesterol does not have any significant effect on the lateral diffusion of the chains. The latter observation is in agreement with NMR studies (25).

Finally, we discuss the simulations in which we used the stronger Lennard-Jones potential. In that case, we had to increase the temperature to go from our start configuration to a disordered system. When the temperature was lowered to 330K, a collective tilt was successively built up and the content of gauche bonds decreased. This process is slow with a time scale that is longer than the molecular dynamics simulations. It took 1 ns before an ordered and tilted phase with 5% gauche bonds showed up. Our interpretation is that the model with stronger Lennard-Jones potentials has a higher phase transition temperature so that the liquid crystalline phase is not stable at 330K. This behavior is expected because when the well of the Lennard-Jones potential is made deeper, the other potential parameters are unchanged. The mechanism of the phase transition is essentially competition between entropy caused by chain disorder and the cohesive Lennard-Jones energy. If the latter term is increased by changing the potential, the phase transition temperature will be lowered. It is, however, not obvious that ordering will occur at constant surface density, whereas the higher surface density allows for a considerably lower Lennard-Jones energy in the ordered phase. An impor-

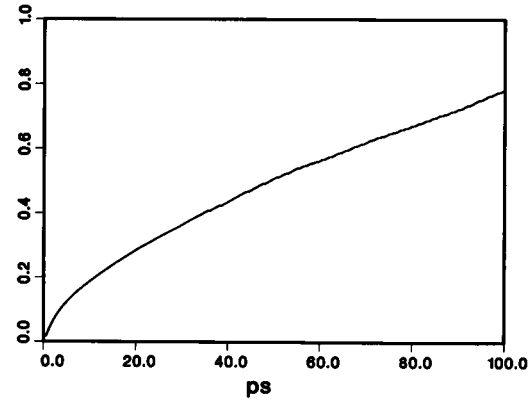

FIGURE 10 The square of the average lateral displacement of the chains in nm^2 as a function of time in a system without cholesterol.

tant observation from the simulation with the Lennard-Jones parameters of Ryckaert and Bellemans (16) is that we can obtain an ordered phase even at the surface area of the high temperature phase. In this low temperature phase, a collective tilt plays an important role, and we see an ordering effect of cholesterol that is due to a substantial decrease in the collective tilt close to a cholesterol molecule. Thus, the main effect of the cholesterol is to hinder the tilt and not to order the chains. The ordinary gel phase has a smaller surface area than this low temperature phase and would have much less tilt (at least in our model).

SUMMARY

At high (13 mol%) cholesterol concentrations, a distinct ordering of the hydrocarbon chains is found close to the cholesterol molecules. The effect is seen in order parameters as well as trans-gauche fractions and local bilayer thickness. The ordering extends beyond the nearest neighbors to a radius of ~1.25 nm. In the first neighbor shell (radius of 0.75 nm), the S_{CD}-order parameter is increased by >30%. Outside that region and to 1.25 nm, an effect of 10–15% is seen. The effect is pronounced in the upper and middle parts of the chain and falls off closer to the end of the chain. At a lower cholesterol concentration (3 mol%), there is a slightly increased thickness of the membrane close to the cholesterol but no increase in order parameters or *trans* content of the chains. An explanation for the observed behavior is that the influence of cholesterol on lipid chains is cooperative, but we cannot completely exclude that it is a statistical artifact due to the limited timespan of the simulations.

The ordering close to the cholesterol at the high concentration is compensated for by disordering of the bulk phase. The lateral pressures are lower in the systems containing cholesterol than in a pure system, which suggests that the incorporation of cholesterol is accompanied by an increased surface density (in agreement with experiments in references 8–10). The increased surface density would explain why almost no difference is seen in average order parameters between a pure system and one containing cholesterol at the same surface density. We suggest that the main effect of cholesterol on the average order comes from the increased surface density needed to keep the ordered lipid chains close to the cholesterol in equilibrium with the bulk chains.

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