Eur Biophys J (2005) 35: 79–88
DOI 10.1007/s00249-005-0011-0

ARTICLE

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Lateral pressure profiles in cholesterol–DPPC bilayers

Received: 19 April 2005 / Revised: 22 June 2005 / Accepted: 18 July 2005 / Published online: 5 October 2005

Abstract By means of atomistic molecular dynamics simulations, we study cholesterol–DPPC (dipalmitoyl phosphatidylcholine) bilayers of different composition, from pure DPPC bilayers to a 1:1 mixture of DPPC and cholesterol. The lateral pressure profiles through the bilayers are computed and separated into contributions from the different components. We find that the pressure inside the bilayer changes qualitatively for cholesterol concentrations of about 20% or higher. The pressure profile in the inside of the bilayer then turns from a rather flat shape into an alternating sequence of regions with large positive and negative lateral pressure. The changes in the lateral pressure profile are so characteristic that specific interaction between cholesterol and molecules such as membrane proteins mediated solely via the lateral pressure profile might become possible.

Keywords Lipid bilayer · Energetics · Ion channel regulation · Thermodynamics

Introduction

From a macroscopic point of view a planar lipid membrane in equilibrium has, by definition, a vanishing surface tension. However, on a microscopic level there is, within the membrane, local lateral pressure, i.e. pressure tangential to the interface. Only when all of the local contributions are summed and averaged along the bilayer normal, the net pressure vanishes. The local lateral pressure arises from different structural components of the lipid bilayer (Marsh 1996) and, almost counter-intuitively, each of these contributions can be of the order of several hundreds of bars.

The importance of lateral pressure profiles has been discussed in several recent reviews (Ben-Shaul 1995; Marsh 1996; Kinnunen 2000; Bezrukov 2000; Eckenhoff 2001). The increased interest in understanding lateral pressure profiles is easily understood as lateral pressure has been proposed to have an important role in, for example, general anaesthesia (Cantor 1997a; Eckenhoff 2001) or inhibition and regulation of protein function (de Kruijff 1997) (see Refs. 25–50 in van den Brink-van der Laan et al. (2004) for a list of proteins for which a relation to lateral pressure has been suggested).

Due to the absence of good probes for lateral pressure, direct experimental measurements are difficult and only a single experimental study exists at present (Templer et al. 1998). In contrast, computer simulations have for the past 10 years been able to supply direct, yet not straightforward, access to study pressure profiles and their response to changes in the membrane. Calculations of lateral pressure profiles are a delicate matter as already the introduction of small simplifications to the system can render the results questionable. For example, the reported results for pure lipid bilayer systems from coarse-grained simulations (Goetz and Lipowsky 1998; Harries and Ben-Shaul 1997; Shillcock and Lipowsky 2002) disagree with their counterparts from atomistic simulations (Lindahl and Edholm 2000; Gullingsrud and Schulten 2004).

In this paper, we study the effects of cholesterol on lateral pressure. Cholesterol is an essential component of all Eukaryotic cell membranes where it plays a crucial role for both static structure and dynamics (Yeagle 1985; Simons and Ikonen 2000). In particular, it regulates the fluidity of the cell membrane (McMullen and McElhaney 1996). All of this goes along with changes in the lateral pressure profile. Indirect evidence for the importance of cholesterol on the lateral pressure comes from studies of membrane channels which are highly sensitive to the pressure of their environment (Sukharev et al. 1997;

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Hamill and Martinac 2001). One such channel is the nicotinic acetylcholine receptor which ceases to function in the absence of cholesterol (Rankin et al. 1997).

Apart from the fundamental importance of cholesterol and its effect on lateral pressure, the study of cholesterol is interesting for a second reason: cholesterol is a highly specific molecule. Already small modifications of its sterol structure lead to significant changes of the membrane properties (Endress et al. 2002; Scheidt et al. 2003). Generic theories of lateral pressure describe a molecule basically only by its volume and its rigidity. When such theories are applied to cholesterol (Cantor 1999b), they thus inevitably fail to capture many essential features of cholesterol, and even predict pressure changes of the wrong sign in some parts of the bilayer.

In this article, we thus use atomistic molecular dynamics simulations to study lateral pressure profiles for six different systems, ranging from a pure dipalmitoyl phosphatidylcholine (DPPC) bilayer to a bilayer consisting of a 1:1 mixture of DPPC and cholesterol. This paper is, to the author's knowledge, the first detailed atomistic computational study addressing the effect of cholesterol or other small molecules on the build-up of the lateral pressure profile in phospholipid membranes.

Theory

The pressure tensor p can be computed as

$$\mathsf{p} = 2\mathsf{E} - \Sigma, \tag{1}$$

where E is the kinetic energy density tensor and Σ is the configuration stress tensor. Both the latter quantities can be expressed in terms of atomistic positions, velocities and forces as

$$\mathsf{E} = \frac{1}{2}\sum_i m_i \boldsymbol{v}_i \otimes \boldsymbol{v}_i, \tag{2}$$

$$\Sigma = \frac{1}{V}\sum_{i<j} \boldsymbol{F}_{ij} \otimes \boldsymbol{r}_{ij}, \tag{3}$$

and are thus accessible in an MD simulation. While the above expressions are, strictly speaking, defined only if the summations are extended over the entire simulation volume (Heinz et al. 2004), in practise it is possible to divide the different contributions into slices according to the positions of the involved atoms (Lindahl and Edholm 2000).

This implies, however, that the force $\boldsymbol{F}_{ij}$ between particles i and j is known explicitly. This is not the case if a multipole or lattice based method [such as particle-mesh Ewald (PME)] is used to evaluate electrostatic interaction. In the first reported atomistic computation of pressure profiles (Lindahl and Edholm 2000) electrostatics was therefore truncated a distance of 1.8 nm. As it is known by now, however, using abrupt truncation, especially at such a short distance, introduces significant artefacts into bilayer systems (Patra et al. 2003, 2004a; Patra et al. 2004b; Anézo et al. 2003) and thus needs to be avoided. We thus use the reaction-field technique that has been shown to give results consistent with the application of long-range electrostatics (Patra et al. 2004b) while at the same time employing explicit expressions for $\boldsymbol{F}_{ij}$.

The global pressure is equal to the average of the local pressures, and any condition on the global pressure thus translates onto the pressure profile. Since the outside of a bilayer is at equilibrium with the environment (i.e., approximately 1 bar in most cases), the average local pressure has to be equal to that value. If the global pressure is different, the system will react by shrinking or expanding, and thus would not be in equilibrium.

Still, there is a nonvanishing local pressure even in equilibrium. The existence of an interface between the water and the lipid goes along with an energy penalty which could be lowered by packing the bilayer more densely, thereby decreasing the area per lipid. Steric constraints between the lipid tails prevent this from happening. The equilibrium value of the area per lipid is thus a compromise between the "wishes" of the headgroups and the tails. This is directly reflected in the lateral pressure profile. Since the interface region prefers a further reduction of the area per lipid, the local lateral pressure there is negative (pointing inwards) whereas it is positive in the tail region (pointing outwards).

Actually, the important factor describing the tension inside a lipid bilayer is not the lateral pressure itself but rather the surface tension, i.e. the difference between lateral and normal pressure. For this reason, all results presented in this paper actually are surface tension profiles but we will continue to refer to them as lateral pressure profiles. The latter naming convention is much more widely used as it describes the physics background much better. The lateral pressure profile in equilibrium is a direct reflection of the inhomogeneity of the bilayer along the bilayer normal. In contrast, the bilayer is homogeneous, parallel to the bilayer interface, and the normal pressure thus is (up to the numerical noise) constant and equal to the applied external pressure of 1 bar. All relevant physics contained in surface tension profiles thus is purely due to the lateral pressure profile.

Materials and methods

We study lipid bilayers comprised of 128 molecules (64 per leaflet), at various ratios of DPPC and cholesterol, hydrated by 3,655 water molecules. DPPC molecules are described by the model of Tieleman and Berendsen (1996), which utilises the description of lipids from Berger et al. (1997). Cholesterol was described by the model of Höltje et al. (2001) and the SPC model (Berendsen et al. 1981) was used to describe water. The simulations were performed using the Gromacs package, both in the standard release (Lindahl et al. 2001) and in

an adapted version that allows the computation of local pressures (Lindahl and Edholm 2000).

For computing electrostatic interactions, we employed a twin-range setup (Bishop et al. 1997) in which the interactions within a distance $r_{list} = 1.0$ nm were evaluated at every integration step, and those between r_{list} and $r_{cut} = 2.0$ nm only were evaluated at every tenth integration time step. A reaction-field approach (Tironi et al. 1995) was used to account for the interaction outside of r_{cut} by assuming a homogeneous dielectric with $\epsilon = 80$. The Lennard-Jones interaction was truncated at 1.0 nm.

DPPC, cholesterol and water molecules were separately coupled to a heat bath at a temperature $T = 323$ K, and the pressure was kept at 1 bar, both using the Berendsen algorithms (Berendsen et al. 1984). The size of the simulation box in the plane of the bilayer (x–y plane) was allowed to fluctuate independently of its height.

As initial configurations for all simulations we used the final configurations of 100 ns simulations (Falck et al. 2004b) where electrostatics were treated by PME (Essmann et al. 1995; Frenkel and Smit 2002). The actual simulation procedure was divided into two steps. First, 50 ns trajectories were generated from these structures for all cholesterol concentrations using the standard version of Gromacs. Electrostatics was treated by the reaction-field technique (Tironi et al. 1995), and the generated configurations were saved every 10 ps. The bond lengths of the DPPC molecules were constrained by the LINCS algorithm (Hess et al. 1997) and water molecules were kept rigid by the SETTLE algorithm (Miyamoto and Kollman 1992), such that an integrator time step of 2.0 fs could be used. In the second step, the pressure profiles were generated from this trajectory using an adapted version of Gromacs (see below for details). In these runs, the SHAKE algorithm (Ryckaert et al. 1977) was used to constrain bond lengths

Resolving the pressure spatially makes the simulations about one order of magnitude slower than a normal simulation. The pressure profiles were thus computed in a second step, based on the saved configurations. Starting at every saved configuration, a 4-ps simulation was run to compute the pressure profile. The first 2 ps were ignored to rule out effects of finite precision of the saved configurations. Unless mentioned otherwise, only the final 30 ns of each simulation were included in the analysis.

The instantaneous pressure p, computed from (1), fluctuates quickly in time. Even when the instantaneous spatial average over the entire simulation box is considered, the pressure easily changes by several hundred bars within a single integration time step. Computing a statistically relevant pressure profile thus is numerically challenging since one has to sample a large number of configurations. We evaluated the pressure profile for a number of simulation frames that is far larger than in previous studies where pressure profiles of bilayers were computed (Lindahl and Edholm 2000; Gullingsrud and Schulten 2004). This allowed us to divide the simulation box into 150 bins for computing the pressure profile, with the result having only negligible numerical noise. The remaining uncertainty in the pressure profile is mainly due to the temporal change of the area per lipid. To arrive at this data quality, a total of approximately 25000 h of cpu time was needed.

In previous MD studies on cholesterol–DPPC bilayers, electrostatics were handled either by plain cutoff (Tu et al. 1998) or PME (Hofsäß et al. 2003; Falck et al. 2004b). In our simulations we used the reaction-field technique, motivated by the mutual consistency between the reaction-field technique and PME found for pure DPPC systems (Patra et al. 2004b). From our simulations we found that the results with the reaction-field technique and PME are almost identical also for mixed cholesterol–DPPC bilayers. We thus refrain from reproducing the entire standard set of quantities that are used to characterise a bilayer. Rather, we show only the temporal development of the area per lipid in Fig. 1. The results of the earlier simulations (Falck et al. 2004b) carried out using PME are also shown in the figure in compressed form from $t = -100$ to 0 ns. From Fig. 1, it is then immediately obvious that changing the electrostatics treatment from PME to the reaction field at $t = 0$ ns has no relevant effect on the systems, and that the systems are in equilibrium.

Results

Pressure profiles

The computed lateral pressure profiles are summarised in Fig. 2. We first discuss a few general features.

First, for low cholesterol concentration, the pressure does not decrease to zero at the edges of the simulation

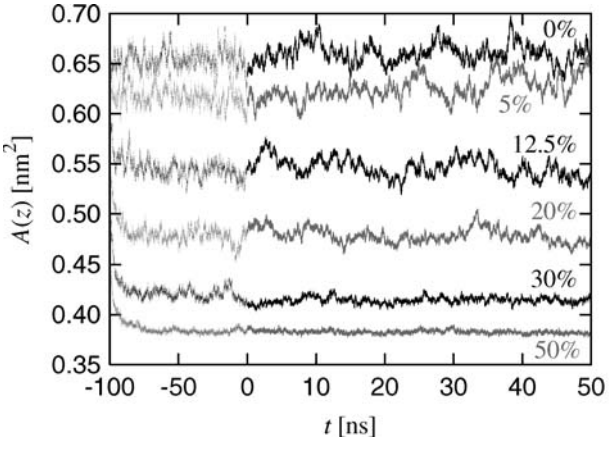

Fig. 1 Temporal development of the area per lipid for the different cholesterol concentrations from $t = 0$ to 50 ns. The results computed using PME are shown in condensed form from $t = -100$ to 0 ns

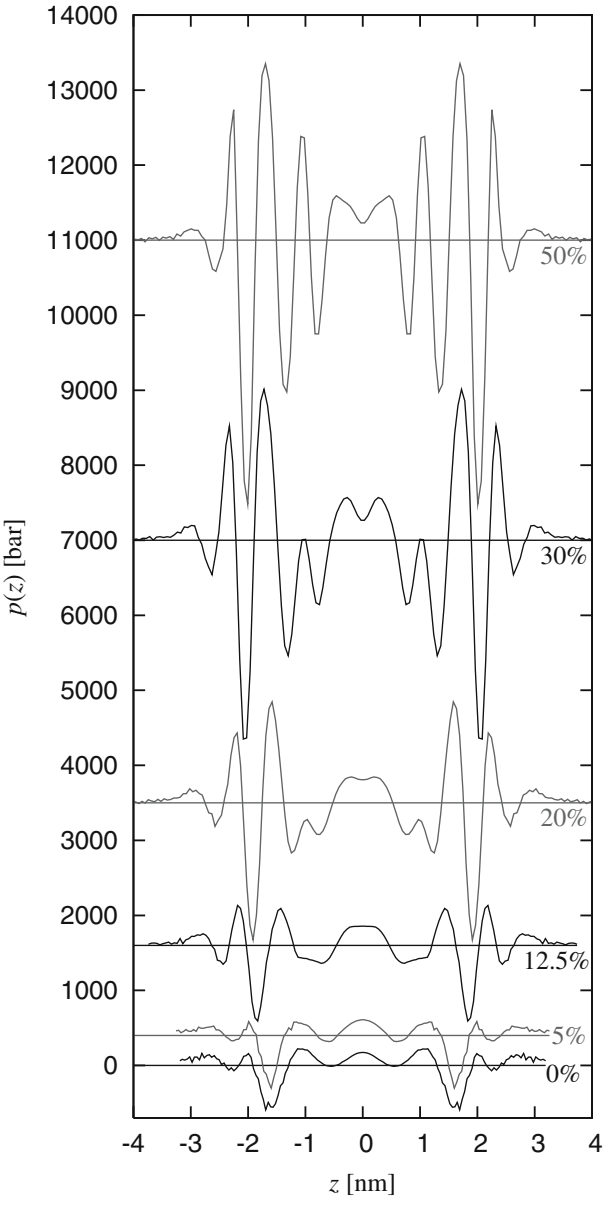

Fig. 2 Lateral pressure profiles through the bilayer for different cholesterol concentrations. The different curves have been shifted for clarity. The *labels on the graphs* give the cholesterol concentration

box. This is an indication that the bilayer is not fully hydrated, and it was estimated that an additional four to five water molecules per lipid would be needed for complete hydration (Lindahl and Edholm 2000). This number is in agreement with our results. Increasing cholesterol concentration means a decrease of the number of lipid molecules, hence an increase in the number of water molecules per lipid. At 12.5% cholesterol, the number of water molecules per lipid has increased by four. For this and higher cholesterol concentrations the pressure in the bulk water phase indeed becomes zero, as can be seen from Fig. 2.

Second, the lateral pressure profile is not flat but some parts of the bilayer would like to expand, at the same time that other parts would like to contract. While the net pressure, averaged over the entire bilayer, is small, the local pressure can be much higher than typical macroscopic pressures. A simple estimate shows that the local lateral pressure in the bilayer core can be well over 300 bar and can reach values exceeding 1,000 bar close to the interface (Gullingsrud and Schulten 2004). Our results for pure DPPC agree both qualitatively and quantitatively with earlier results (Lindahl and Edholm 2000) with small differences in the peak positions close to the interface due to the different treatment of electrostatics in that study.

Third, the magnitude of the local lateral pressure becomes higher as the cholesterol concentration increases. While for small cholesterol concentrations the local pressure is of the order of a few hundred bars, it increases to thousands of bars for the highest cholesterol concentrations. There is no straightforward explanation for this, but this phenomenon is very likely related to higher bilayer rigidity at high cholesterol concentration since pressure gradients are ultimately related to the elastic modulus.

Finally, the pressure profiles possess additional structure in the presence of cholesterol. Without cholesterol, the lateral pressure in the lipid tail region of the bilayer is strictly nonnegative. Already for 5% cholesterol, a small region of negative lateral pressure is seen in the figure. For cholesterol concentrations of 20% and higher, an additional structure is seen. This reflects that cholesterol is not some generic structureless object but rather possesses an internal structure. One could call this the *specific* effect of cholesterol, compared to the *unspecific* effects that are also observed.

Partial pressures

The pressure tensor (1) arises from inter-molecular and intramolecular pairwise forces [cf. Σ from (3)] as well as from the kinetic motion of the atoms [cf. E from (2)]. There are three components in the bilayer system (DPPC, cholesterol and water), which give six possible combinations for the pairwise forces, and Σ can thus be split into six contributions (DPPC–DPPC, DPPC–cholesterol, and so on), according to which kind of molecules the two atoms i and j causing the force $\boldsymbol{F}_{ij}$ belong to.

Similarly, the kinetic energy tensor E can be split into three contributions. Thus, we divide the lateral pressure profile $p(z)$ into nine contributions—six from Σ and three from E. The partial pressure profiles in Fig. 3 show the different contributions marked by different shades. The size of the shaded area directly gives the pressure due to that contribution. The arrangement of the areas, i.e. whether a given area is close to the z-axis or further away, has no physical meaning. The kinetic contribution from the DPPC molecules is marked with the same shade of grey but with dots.

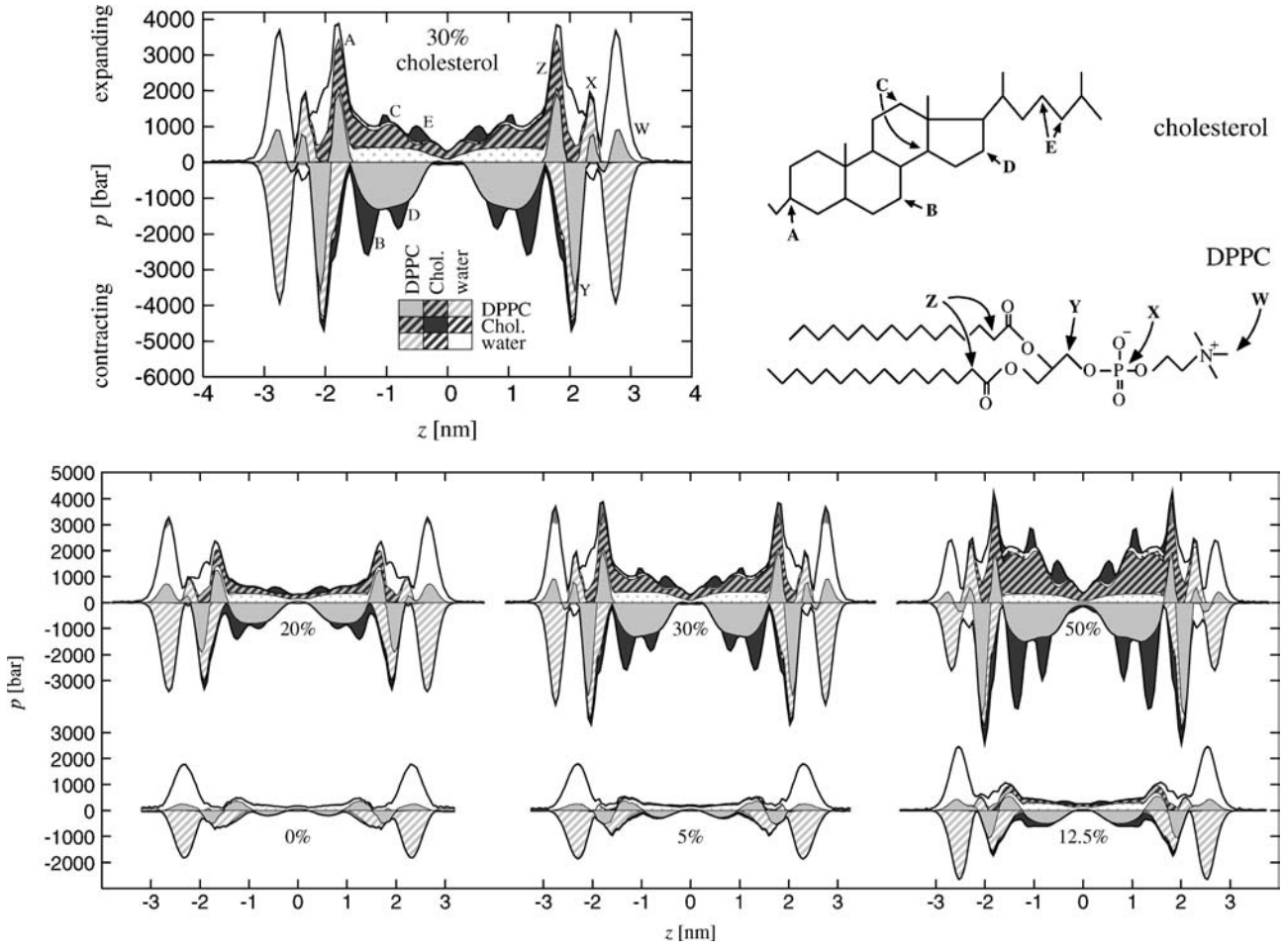

Fig. 3 Partial pressure profile through the bilayers. Expanding ($p > 0$) and contracting ($p < 0$) contributions are shown separately. *Light grey* stands for DPPC, *dark grey* for cholesterol and white for water. The pressure due to the interaction between two different components is shown with *stripes* while kinetic contributions are shown with *dots*. The label on the curves gives the cholesterol concentration (*bottom*). On the *top*, an enlarged version of the profile for 30% cholesterol is shown where important peaks are labelled according to which position in a DPPC and/or cholesterol molecule they relate to. Schematic drawings of these molecules are included on the right. The peaks for other cholesterol concentrations could not be labelled for space reasons but they are easily mapped to the *top figure*

There exists a fundamental difference between the tensor Σ, in that it depends only on positions and forces and thus describes only the static properties of the system, and E, which depends only on the velocities and thus is a purely dynamic quantity. Each degree of freedom contains an amount $k_B T$ of energy, and the kinetic energy per particle computed from (2) can thus be larger than $k_B T$. The increase in kinetic pressure E compared to an ideal gas at the same density is due to the kinetic energy stored in harmonic bonds, angular potentials and other interactions. As the bonds in the acyl chains are preferably aligned normal to the x–y plane, isotropy is broken, and E becomes anisotropic. E thus measures (in addition to the particle density) how the lipids are oriented, and in this it complements the traditional deuterium or NMR order parameter $|S_{CD}|$ that is used to quantify orientational orientation along a chain (Tieleman et al. 1997).

Each contribution can be either expanding (positive pressure) or contracting (negative pressure). The sum of all expanding contributions is shown as a black line in the figure. The same applies to the sum of all contracting contributions. The difference between these two values gives the pressure profile that was shown in Fig. 2. It should be noted that this difference is significantly smaller in magnitude than each of the two original terms.

As more cholesterol is added, the positions of the pressure profile peaks shift. Simultaneous analysis of both the pressure profile and the atom density profiles as a function of cholesterol concentration allows us to find correlations between pressure and atom positions. The result of this analysis is included in Fig. 3. Labels "A"–"E" refer to the peak positions caused by cholesterol, and labels "W"-"Z" to DPPC. Let us now take a closer look at the different regions in the bilayer.

Interface region

There is hardly any contact between the water and cholesterol molecules, so one might be tempted to assume that the interface region (also referred to as head group region) of the bilayer should be hardly influenced by cholesterol at all. The opposite is the case, as is seen from Fig. 3. Cholesterol reduces the area per lipid of the bilayer, and that strongly influences also the interface region, but the effects there are mainly generic and unspecific—any substance that would reduce the area per lipid would have a similar effect.

The outermost part of the bilayer is formed by the choline group of the DPPC molecules (labelled as "W"), and the total pressure there is negative. The attraction is dominated by the solvation energy between water and the polar lipid headgroups. Most of the contracting pressure is compensated by the positive pressure among the water molecules. Entropy plays an important role in the latter as water molecules become ordered in the electrostatic field from the zwitterionic headgroups (Lindahl and Edholm 2000). The behaviour of this region of the pressure profile depends only weakly on cholesterol concentration as the hydration energy depends only weakly on the area per lipid (Marsh 2002). Note that both the DPPC bilayer (grey) and the water phase (white) would like to expand, and it is only the interaction between these two (alternating grey and white stripes), commonly called simply "hydrophobicity", that is trying to reduce the area of the interface.

A bit further down in the bilayer, the phosphate groups of the DPPC molecules are located. The mutual arrangement between the choline and phosphate groups depends heavily on the area per lipid (Gurtovenko et al. 2004; Falck et al. 2004b). For large area per lipid, and thus for low cholesterol concentration, both groups are, on average, located in the same plane. In this case, the effects of the phosphate and the choline group cannot be separated in the pressure profile. At sufficiently large cholesterol concentration, the choline group is tilted up, and an additional peak ("X") thus appears. The pressure is positive as the water molecules are oriented according to the charge of the choline group and thus have an unfavourable interaction with the oppositely charged phosphate group.

The inner boundary of the interface region is marked by the *sn*-3 carbon (labelled as "Y"). This is the furthest extension to where there is noteworthy penetration of water into the bilayer. Furthermore, this is the outermost position of cholesterol, occupied by its hydroxyl group ("A"). Only inside this peak, cholesterol has, in addition to its change of the area per lipid, also direct effects.

Acyl chain region

The acyl chain region is of special importance to the study of cholesterol-containing bilayer as here are located not only the acyl chains of the DPPC (hence the name) but also the rigid four-ring structure of the cholesterol body. In the presence of cholesterol, a characteristic structure in the pressure profile develops, to the point that one might speculate on whether this is sufficient to explain specific effects, such as observed for the nicotinic acetylcholine receptor (Rankin et al. 1997).

Upon increase of cholesterol concentration, an ordering of the DPPC acyl chains takes place. This ordering increases the attraction among the DPPC tails, thereby increasing the compressing pressure component, while at the same time introducing orientational correlations into the motion of the atoms of the tails, thereby increasing the expanding kinetic pressure. The kinetic pressure per DPPC molecule increases monotonously by almost a factor of 4 from 0 to 50% cholesterol, and per cholesterol molecule it increases by a factor of 3. Cholesterol thus induces significant correlations into the motion of the atoms, and the ordering induced by cholesterol is reflected much stronger in the correlated motion of the atoms than it is in the order parameter $|S_{CD}|$.

The rigid four-ring structure of cholesterol offers a much more interesting chemical structure than the basically linear chains of the DPPC molecules. This shows also in the pressure profile that exhibits several well-pronounced peaks, making the pressure profiles of cholesterol-containing membranes very different from previously reported pressure profiles (Lindahl and Edholm 2000; Gullingsrud and Schulten 2004). There are several atoms in the cholesterol molecules that are correlated with peaks in the pressure profile, see the labels in Fig. 3. Steric arguments can explain only some of the correlations. This should come as no surprise since the pressure $p(z)$ at a given depth z in the bilayer cannot be computed solely from atomic information from that depth—the stresses in different parts of the membrane are coupled due to the finite elastic modulus. The picture becomes even more difficult if one accounts also for chemistry, i.e. for favourable and unfavourable interactions between certain pairs of atoms.

The orientational correlations in the kinetic pressure, related to entropy and giving rise to kinetic pressure, complicate matters even more. Thus, trying to explain the origin of every peak in the lateral partial pressure profile, especially since at the moment there is little possibility to prove or disprove any suggestion, is of little importance. What is definite, however, is that all the features of the lateral pressure profile are in some way related to the chemistry and structure of cholesterol.

Cholesterol is by far the most prominent steroid in any Eukaryote but it is only one member of a large group of chemically related substances that are varying only by seemingly minor changes in their structure, such as a single additional double bound or a different placement of the two carbon atoms that project out from the rigid cholesterol body. Still, their properties in the membrane are distinguishably different (Endress et al. 2002; Scheidt et al. 2003), and only in special cases one type of steroid can replace another in biological systems. Our results suggest that cholesterol relatives, such as lanosterol or

ergosterol, would have a pressure profile that is different from the pressure profile of cholesterol systems. Unfortunately, no such simulations, let alone experimental measurements, are available at the moment.

Centre of the bilayer

The centre of the bilayer is formed by the ends of the acyl chains of the DPPC and cholesterol molecules. Both mass and electron density have a minimum at $z = 0$ known as the methyl trough. For low cholesterol concentration, there is a single peak in the pressure profile at $z = 0$ that has been suggested to be related to interdigitation (van den Brink-van der Laan et al. 2004). Understanding the centre of the bilayer is difficult as the peak in the pressure profile is found only in atomistic MD simulations (Lindahl and Edholm 2000; Gullingsrud and Schulten 2004) whereas analytical theories and coarse-grained models fail to reproduce it (van den Brink-van der Laan et al. 2004). Experimentally it was found that cholesterol reduces interdigitation but it should be noted that even pure DPPC bilayers are only moderately interdigitated (Siminovitch et al. 1987). This is in agreement with our results in Fig. 4.

For increasing cholesterol concentration, the single peak in the pressure profile at $z = 0$ splits into two peaks as the pressure close to $z = 0$ increases faster than precisely at $z = 0$. Simultaneously, in addition to a reduction in interdigitation, the end point of the acyl chains becomes more pronounced in the mass density profile. At the highest cholesterol concentrations, even a small peak in the mass density profiles shown in Fig. 4 develops near the end of the chains.

The relation between interdigitation and the pressure profile in the centre thus is not obvious—our results are more consistent with the absence of such a relation. From Fig. 3, it is observed that the peak in the pressure profile around the bilayer centre does not originate in an increase of the expanding pressure components but rather from a sharp decrease of the condensing pressure. A possible explanation is that, as they are less ordered than the other atoms, the atoms at the end of the acyl chains cannot make favourable contact with neighbouring chains, thereby losing van der Waals attraction. The shape of the pressure profile near the centre of the bilayer would thus be dominated not by the mass density profile itself but by the spatial distribution of the end points of the chains. For cholesterol, this gives a peak at $z = 0$ for small cholesterol concentration that splits into two peaks as interdigitation is reduced, in agreement with the computed pressure profiles.

Relation to membrane penetration

The lateral pressure profile is related to the ability of small molecules to penetrate into the bilayer. Negative pressure means that the system would like to contract. Regions with negative lateral pressure thus mark regions where it would be energetically favourable for additional particles or molecules to be inserted. Ultimately, the partitioning of a particular solute into the bilayer is determined by the free-energy profile (Marrink and Berendsen 1996).

The lateral pressure profile gives the volume contribution $p\ \mathrm{d}V$ to the free-energy profile, and for nonpolar

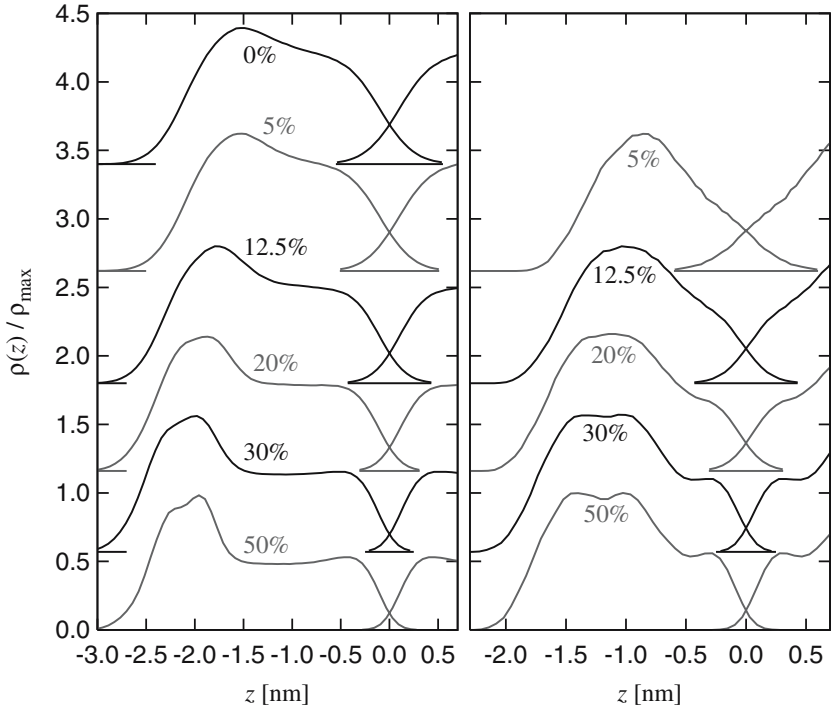

Fig. 4 Mass density profiles with the contributions from both leaflets plotted separately (*left* for DPPC, *right* for cholesterol). The curves have been scaled by their maximum and shifted vertically. For symmetry reasons, only a small part of the right leaflet is shown. The numbers on the curves give the cholesterol concentration. The amount of interdigitation is significantly reduced if cholesterol is present

or moderately polar solutes, the volume contribution is a good approximation to the full free-energy profile. The relation between the pressure profile and the volume contribution is exact for infinitesimally small solutes as their introduction does not change the pressure profile. For large solutes, such as proteins, the change of pressure profile induced by the solute can become relevant, however, and the volume contribution to the free-energy profile cannot be computed quantitatively from knowledge of the unperturbed pressure profile alone.

On a first view, the effect of the pressure profile seems to be related to free-volume theories, which state that the amount of unoccupied space inside the bilayer determines its penetrability. In the context of pressure profiles, "occupied volume" is equivalent to large steric repulsive forces, resulting in a large expanding static pressure. From the discussion of the partial pressure profiles above it should be obvious that free-volume theories face an immense problem as much of the pressure inside the bilayer is either attractive or of kinetic origin.

One of the many reasons why cholesterol-containing membranes are interesting systems to study is that cholesterol affects the membrane spatially inhomogeneously (Lee and Petersen 2004). For example, the addition of cholesterol increases the penetration of water into most regions of the bilayer, while it reduces the water concentration in the very centre of the bilayer (Marsh 2002).

These observations are easily explained within the context of lateral pressure profiles but not within free-volume theories. In the pure DPPC bilayer, the pressure in the acyl chain region is small but positive. As the cholesterol concentration is increased from 0 to 30%, the pressure in most of the acyl chain region becomes negative (allowing for easier penetration) while in the centre of the bilayer it becomes more positive (thus expelling particles there). Also at 50% cholesterol, there are ample regions with negative pressure even though they become disconnected by high peaks of positive pressure.

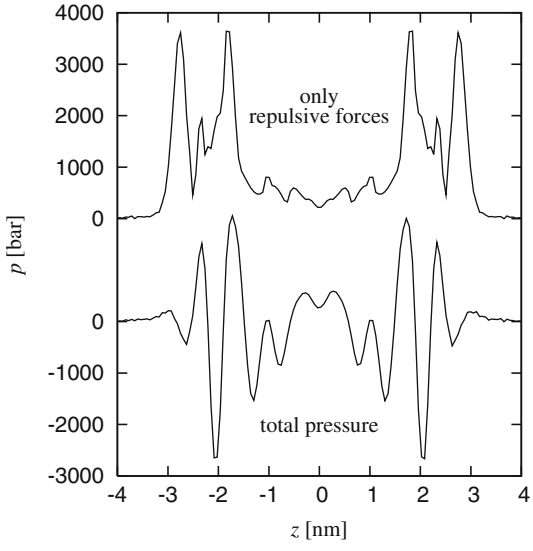

Fig. 5 Pressure profiles for 30% cholesterol. The *lower curve* gives the correct pressure profile whereas in the *upper curve* only static expanding contributions to the pressure have been included

To make contact with free-volume theories it is instructive to "compute" from Fig. 3 a "fake" pressure profile that only includes static expanding pressure contributions, shown in Fig. 5. The "correct" lateral pressure profile changes strongly as cholesterol is added, with several new peaks appearing in the pressure profile, and the magnitude of the pressure changing by about one order of magnitude. In contrast, the free-volume profile lacks the additional structure induced by cholesterol and only changes monotonously as the cholesterol concentration is changed, and the changes are rather moderate (at least compared to the lateral pressure profile) (Falck et al. 2004a, b)—both observations apply also to the "fake" pressure profile.

The free-volume fraction is highest in the centre of the bilayer, which is simply a reflection of the bilayer trough with reduced atom density (Falck et al. 2004a), and molecules such as water should thus preferably be located there. From this contradiction, it was thus realised already early that it is not the average amount of free volume that determines the penetration but rather rare fluctuations (Marrink and Berendsen 1994).

Free-volume theory can be applied to compare penetration of solutes of different sizes through the same bilayer, as penetration then is rate-limited by a single point in the membrane, usually located at the bilayer interface (Killian and van Meer 2001). Already when comparing the same bilayer at different temperatures, such a one-parameter approach may break down (Sutter et al. 2004). The lateral pressure profile, on the other hand, allows to compare also very different systems, such as bilayers with a different cholesterol concentration, as the description of the bilayer is in full detail. (The description of the solute is still reduced to solely its volume, and the most straightforward improvement to this simplification are free-energy profiles.)

Discussion

In this paper we have studied lateral pressure profiles in cholesterol–DPPC bilayers at varying composition ratios. We found that the pressure profiles become more structured and complicated as the cholesterol fraction is increased (cf. Fig. 2). This is related to the structural changes in the bilayer upon addition of cholesterol. These changes can have biological consequences: proteins or other molecules are able to anchor at a given depth inside a cell membrane guided by the pressure profile (van den Brink-van der Laan et al. 2004).

Biological interactions can be divided into specific and unspecific. The general view in the literature seems to be that changes via the lateral pressure profile are rather unspecific, i.e. proteins or other molecules in a cell

membrane are affected by the lateral pressure profile but it is of secondary importance of how a change in the pressure profile was induced. For a specific interaction, a direct contact between cholesterol and, e.g., a membrane channel would be needed.

In view of the lateral pressure profiles presented in this paper one could speculate whether a specific interaction via the pressure profile is possible. The profile contains a wealth of additional structure, very different from what could be achieved by simply compressing the bilayer. This structure in the lateral pressure profile might be specific enough to allow molecules such as the nicotinic acetylcholine receptor (Rankin et al. 1997) to sense the presence or absence of cholesterol solely via the lateral pressure profile.

This might be an additional reason for the abundance of cholesterol in eukaryotic cell membranes, and why there is so much cholesterol but so few other chemically closely related steroids—in contrast to the multitude of different lipids found in cell membranes. From that point of view, it would be interesting to see the difference between the different sterols on pressure profiles, e.g. how the pressure profile would change if cholesterol was replaced by lanosterol.

The results presented in this paper also demonstrate that the effects of cholesterol cannot be captured by simple models. Still, generic models have their advantages, and the importance of lateral pressure profiles would not have been accepted without the pioneering analytical work by Cantor in the late 1990s (Cantor 1997a, b, 1999a.b). His most important contribution were not so much the results themselves—much of it was already known—but to cast them into a single consistent form that, e.g., allowed to relate pressure profiles to ''nonbilayer lipids'' or spontaneous membrane curvature.

A characteristic of such analytical theories is that they are very successful in describing plain acyl chains but already their treatment of the headgroups is rather crude (Ben-Shaul 1995; van den Brink-van der Laan et al. 2004). Cholesterol is well beyond the reach of such models. In the headgroup region, the problem is the description of the mutual headgroup interactions and, even though this effect is generic, their change upon decrease in the area per lipid induced by cholesterol. In the acyl chain region, the problem is the nongeneric nature of the rigid cholesterol body.

This and other atomistic studies show the power of computer simulations to resolve the details of molecular systems and to provide further insight to complex multicomponent systems. Pressure profiles are an excellent example for this power due to the severe limitations encountered in the experiment, coarse-grained simulations and analytical theories.

Acknowledgements I would like to acknowledge valuable discussions with Mikko Karttunen and the support from the European Union (MRTN-CT-2004-512331).

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