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Cholesterol effects on the phospholipid condensation and packing in the bilayer: a molecular simulation study

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Abstract A 15-ns molecular dynamics simulation of the fully hydrated liquid–crystalline dimyristoylphosphatidylcholine–cholesterol (DMPC–Chol) bilayer containing ~22 mol% Chol was carried out. The generated trajectory was analysed to investigate the mechanism of the Chol condensing effect on DMPC hydrocarbon chains and the influence of Chol on the chain packing in the membrane. Chol was found to induce stronger van der Waals interactions among the chains, whereas its interactions with the chains were weak. In the DMPC–Chol bilayer, as in the DMPC bilayer, DMPC chains were regularly packed around a chosen chain but around a Chol molecule they were not. DMPC γ chains made closer contacts with Chol than the β chains. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Phosphatidylcholine; Molecular dynamics; Chain order; Van der Waals interaction; Atom density profile

1. Introduction

Cholesterol (Chol) is an important constituent of eukaryotic cell membranes where it amounts up to 50 mol% of the membrane lipids [1]. The biological roles of Chol involve the maintenance of proper fluidity [2], the reduction of passive permeability [3], and the increase of the mechanical strength [4] of the membrane. Because of these important roles of Chol, phospholipid–Chol interactions in the membrane have been studied extensively [5], revealing that Chol increases both the order of the hydrocarbon chains (an ordering effect) [6,7] and the surface density of the membrane (condensing effect) [8,9]. Ordering and condensing effects of Chol have been observed both in model and in biological membranes [10]. Our previous molecular dynamics (MD) simulation studies of the dimyristoylphosphatidylcholine–cholesterol (DMPC–Chol) bilayer concerned the effect of Chol on the organisation of the membrane–water interface [11] and the ordering of DMPC alkyl chains [12]. The present paper is concerned with the study of mechanisms for the Chol condensing effect at the atomic level.

The Chol condensing effect is responsible for a decreased membrane permeability for small molecules [13] and for an enhanced mechanical strength of the membrane [14,15]. The basic mechanism behind this effect is an increased van der Waals (vdW) interaction, although there is still controversy as to the main cause of the increase. It may either come from sole interactions among methyl and methylene segments of phosphatidylcholine (PC) alkyl chains [16] or, perhaps a more common view, from interactions between PC chains and the steroid ring of Chol [17,18]. There is, unfortunately, no easy experimental procedure to distinguish between these two hypotheses. Our results, reported here, support the first, less common view.

2. Materials and methods

2.1. Simulation system

The DMPC–Chol bilayer membrane used in this study consisted of 56 DMPC, 16 Chol (~22 mol% Chol) and 1622 water molecules. Details concerning the membrane equilibration and validation are described elsewhere [11,12]. The bilayer was simulated for 15 ns using AMBER 4.0 [19]. The last 8-ns fragment of the generated trajectory was used for analyses. A pure DMPC bilayer consisting of 72 DMPC and 1622 water molecules was used as a reference system [20,21].

2.2. Simulation parameters

Optimised potentials for liquid simulations (OPLS) parameters [22] were used for both DMPC and Chol. The procedure for supplementing the original OPLS base with the missing parameters is described in [21] for DMPC and in [11] for Chol. Atomic charges for DMPC and Chol are given in these papers. The united-atom approximation was applied to the CH, CH_2, and CH_3 groups of DMPC and Chol. The hydroxyl group of Chol was treated with full atomic detail. For water, TIP3P parameters were used [23].

2.3. Simulation conditions

Three-dimensional periodic boundary conditions with the usual minimum image convention were used. The SHAKE algorithm [24] was used to preserve the bond lengths of the water molecules and the hydroxyl group of Chol, and the time step was set at 2 fs. For non-bonded interactions a residue-based cutoff was used with a cutoff distance of 12 Å. The list of non-bonded pairs was updated every 25 steps.

Simulation was carried out at a constant temperature of 310 K (=37°C, which is above the main phase transition temperature of 23°C for a pure DMPC bilayer) and at a constant pressure of 1 atm. Temperatures of the solute and solvent were controlled independently. Both the temperature and pressure of the system were controlled by the Berendsen method [25]. The relaxation times for temperatures and pressure were set at 0.4 and 0.6 ps, respectively. Applied pressure was controlled anisotropically, where each direction was treated independently and the trace of the pressure tensor was kept constant at 1 atm.

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Abbreviations: PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; Chol, cholesterol; RDF, radial distribution function; vdW, van der Waals; MD, molecular dynamics; OPLS, optimised potentials for liquid simulations

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3. Results

3.1. Spatial properties of the membrane

Profiles of the atom density for pure DMPC and mixed DMPC–Chol bilayers along the normal to the membrane surface are shown in Fig. 1. This figure indicates that the mixed bilayer is thicker than the pure one. The increase in the membrane thickness can be estimated from the distances between average positions of P (N) atoms in opposite leaflets of the two bilayers. The P–P (N–N) distance is 32.9 ± 0.1 Å (36.7 ± 0.1 Å) in DMPC and 35.1 ± 0.1 Å (39.0 ± 0.1 Å) in DMPC–Chol bilayers (errors are S.E.M.; the number of determinations was 35 and 20 for DMPC–Chol and DMPC bilayers, respectively). Concomitant with an increase in the bilayer thickness, Chol causes a decrease in the cross-sectional area/DMPC from 60 ± 1 Å^2 in the pure DMPC bilayer [20,21] to 53 ± 1 Å^2 in the DMPC–Chol bilayer [12]. In effect, the surface density of the membrane, which is the total mass of all membrane lipids divided by the surface area of the membrane, increases by 10%, from 1.87×10^{-7} g/cm^2 in the DMPC bilayer to 2.04×10^{-7} g/cm^2 in the DMPC–Chol bilayer. At the same time, the density of the whole system (lipids and water), which is the total mass of all membrane molecules divided by the simulation box volume, increases by much less than 1%, from 1.135 ± 0.0003 g/cm^3 in the DMPC bilayer to 1.138 ± 0.0003 g/cm^3 in the DMPC–Chol bilayer.

3.2. Atom packing in the hydrophobic core

Radial distribution functions (RDFs) of the carbon atoms in the hydrophobic core of the bilayer relative to a DMPC alkyl chain carbon atom, for both DMPC and DMPC–Chol bilayers, are compared in Fig. 2a. The pairs of atoms linked by the bonding interactions (bond, angle, and torsion) were omitted when calculating RDFs. Both RDFs have two well resolved maxima, the first at a distance of 6 Å and the second at 9 Å, and a minimum at 7 Å. The distance of 6 Å is approximately equal to the sum of vdW radii of two C2 carbon atoms in OPLS parametrisation. The RDF for the DMPC–Chol bilayer has higher values than for the DMPC bilayer by about 5%, indicating a higher density (that is a tighter packing of atoms) of the hydrophobic core of this bilayer.

The RDFs for the DMPC–Chol bilayer shown in Fig. 2a can be decomposed into two components, shown in Fig. 2b.

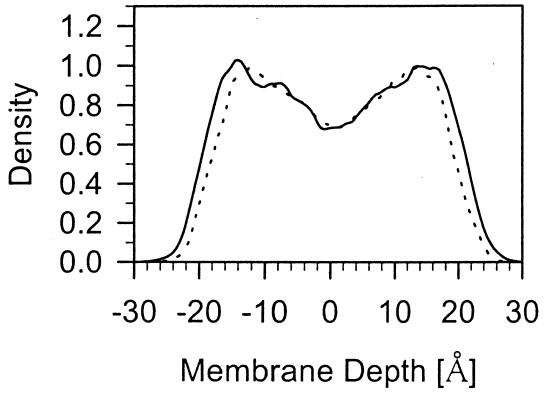

Fig. 1. Profiles of the atom density along the normal to the membrane surface, in DMPC (dotted line) and DMPC–Chol (solid line) bilayers.

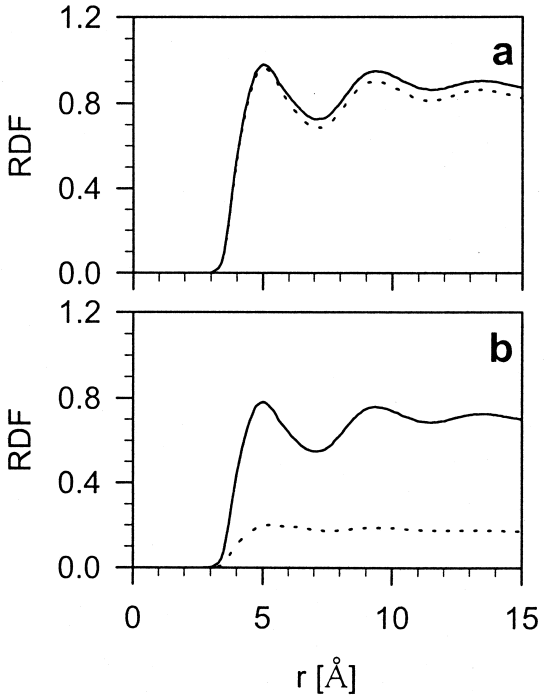

Fig. 2. Three-dimensional RDFs of (a) the carbon atoms in the hydrophobic core of the bilayer relative to a DMPC alkyl chain carbon atom, in DMPC (dotted line) and DMPC–Chol (solid line) bilayers, (b) the DMPC alkyl chain carbon atoms relative to each other (solid line) and relative to a steroid ring carbon atom (dotted line), in the DMPC–Chol bilayer. In the calculations, pairs of atoms linked by the bonding interactions (bond, angle, and torsion) were omitted.

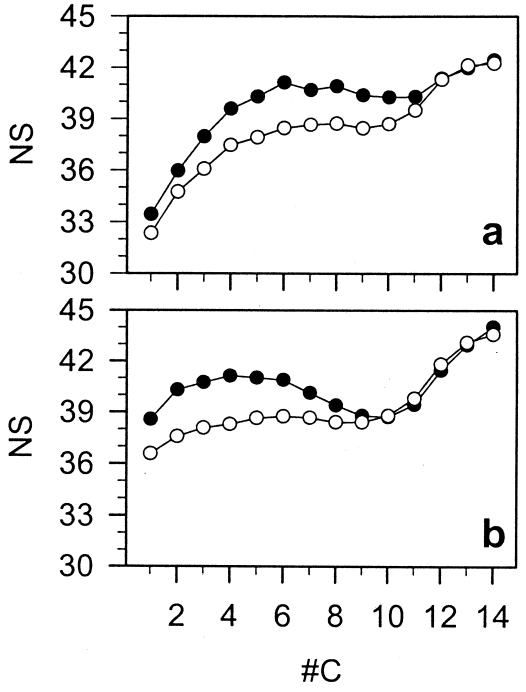

Fig. 3. Profiles of the number of neighbours (NS) (cf. text) along (a) the β chain, and (b) the γ chain, in DMPC (open symbols) and DMPC–Chol (solid symbols) bilayers.

One component is the RDF calculated solely for carbon atoms of the DMPC alkyl chains, and the other component is the RDF of the Chol ring carbon atoms relative to a carbon atom of a DMPC alkyl chain. The two maxima and the minimum at the distance of 7 Å are present only in the first curve. The shape of the second curve indicates that the steroid ring of Chol has a negligible ordering effect on the DMPC alkyl chain atoms. One can conclude that vdW interactions between steroid rings and hydrocarbon chains are not strong.

A neighbour is a carbon atom of a DMPC alkyl chain or a steroid ring, which is located not further than 7 Å (the position of the first minimum in the RDF) away from an arbitrarily chosen carbon atom of a DMPC alkyl chain. The average number of neighbours is 38.82 ± 0.05 in the DMPC bilayer and 40.15 ± 0.05 in the DMPC–Chol bilayer. Profiles of the number of neighbours along the β and γ chains in DMPC and DMPC–Chol bilayers are shown in Fig. 3. For carbon atoms 1–11 of the β chain and 1–8 of the γ chain, numbers of neighbours in the DMPC–Chol bilayer are larger than in the DMPC bilayer. These atoms penetrate to the same depth of the bilayer as the Chol ring atoms. For the remaining alkyl chain atoms, that is those which are below the Chol rings, the profiles obtained for the two bilayers do not differ from each other.

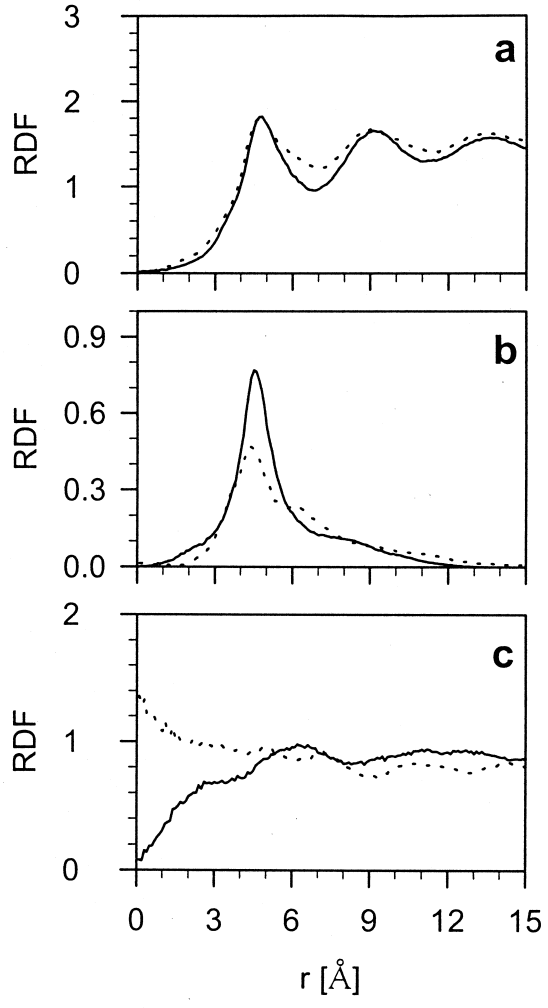

Fig. 4. Two-dimensional RDFs calculated in the *x,y* plane for the centres of masses of the DMPC alkyl chains, in DMPC (dotted line) and DMPC–Chol (solid line) bilayers. a: Alkyl chains belonging to different DMPC molecules. b: Alkyl chains belonging to the same DMPC molecule. c: Two-dimensional RDFs calculated in the *x,y* plane for centres of masses of β (solid line) and γ (dotted line) chains relative to the centre of mass of a Chol molecule, in the DMPC–Chol bilayer.

3.3. Chain packing in the hydrophobic core

Two-dimensional RDFs calculated for the centres of masses of the DMPC alkyl chains in the *x,y* plane of DMPC and DMPC–Chol bilayers are shown in Fig. 4a. The RDFs have two maxima, the first at 4.5 Å and the second at 9 Å, and a minimum at 7 Å. Both the maxima and the minimum are better resolved for the DMPC–Chol bilayer due to higher ordering of the chains. The number of chains within the distance of 7 Å from a chosen chain is equal to 3.4 in the DMPC bilayer. Within the same distance in the DMPC–Chol bilayer, there are 3.4 alkyl chains and 1.5 Chol molecules. Similar RDFs to those discussed above were obtained for a DMPC membrane parametrised in the all-atom approximation [26].

Two-dimensional RDFs calculated for centres of masses of alkyl chains belonging to the same DMPC molecule (intramolecular RDF) in DMPC and DMPC–Chol bilayers are shown in Fig. 4b. Each of these curves has only a single maximum at 4.5 Å. The average intramolecular distance between chains is 6.0 ± 0.1 Å in the DMPC–Chol bilayer and 7.1 ± 0.1 Å in the DMPC bilayer, and is smaller by more than 1 Å.

Two-dimensional RDFs calculated for centres of masses of β and γ chains relative to the centre of mass of a Chol molecule are shown in Fig. 4c. Neither of the curves has a maximum but their shapes indicate that the geometrical arrangement of β chains relative to Chol is different from that of γ chains. Using the same definition of neighbours as above, the average number of alkyl chains surrounding a Chol molecule is 5.2, of which 2.4 are β chains and 2.8 are γ chains.

4. Discussion

In accordance with experimental results, intercalation of Chol into the bilayer built of DMPC molecules increased both the membrane surface density [7,8] and the density of the membrane hydrophobic core [27]. As was shown in our previous paper [12], Chol promotes alignment of DMPC alkyl chains. This enables better packing of the chains and enhances vdW interactions among chain atoms, as average distances between them get smaller. In effect, the surface area in the DMPC–Chol bilayer decreases and the surface density increases. Although concomitant with a decreasing surface area (better packing) the bilayer thickness increases (better ordering), the density of the membrane hydrophobic core increases, due to a feedback process between chain packing and enhancement of vdW interactions.

One of the two key results of the work reported in this paper is that the increase of the vdW interactions, responsible for the Chol condensing effect, is limited to methylene and methyl groups of DMPC alkyl chains as, according to Fig. 2b, vdW interactions between alkyl chains and steroid rings are weak. Increased vdW interactions are observed for the groups both of the same DMPC molecule and of neighbour-

ing DMPCs. These results give strong support to the hypothesis postulated by Hyslop et al. [16] that Chol induces an increase in both intra- and intermolecular vdW interactions of the DMPC alkyl chains, while its vdW interactions with the chains are less favourable. The hypothesis was based on the estimated values of molar attraction of hydrophobic groups of the sterol molecule which were lower than those of methylene segments of the alkyl chain. Our MD simulation shows directly this postulated increase in the vdW interactions.

The other key result of this work concerns the effect of Chol on alkyl chain packing. The effect is confined to methylene segments, which penetrate to the same depths of the bilayer hydrophobic core as the Chol rings. The remaining chain segments (those below the steroid rings) show a packing of the chains that is similar in both DMPC and DMPC–Chol bilayers (cf. Fig. 3). A similar conclusion could be drawn from density profiles along the membrane normal obtained experimentally, which showed that for the central region of the bilayer, density was similar in pure PC and mixed PC–Chol bilayers [27]. Also, the diffusion of small molecules such as oxygen in the central region of the bilayer was not modified by the presence of Chol in the membrane [28].

The presence of two maxima in RDFs in Fig. 4a indicates that in the plane of both DMPC and DMPC–Chol bilayers, the DMPC alkyl chains pack regularly around a chosen chain. In the DMPC–Chol bilayer, the maxima are better resolved due to a better ordering of the chains [12]. The lack of any features in RDFs in Fig. 4c implies a lack of regular arrangement of DMPC chains around a Chol molecule. The difference between the two curves in this figure suggests further that DMPC γ chains make closer contact with Chol than the β chains. This result is in general agreement with results of calorimetric measurements which showed that interaction between Chol and an alkyl chain in the β position differs from that in the γ position [29].

The results presented in this paper elucidate the mechanisms of the Chol condensing effect at the atomic level and show that Chol ordering and condensing effects are interdependent. This and previous MD simulation studies indicate that Chol modifies the dynamic structure of the lipid bilayer by promoting an orientation of the PC chains that is more parallel to the membrane normal as well as a higher packing of the chains. The effect is that the bilayer containing Chol is less permeable and shows greater mechanical strength than a pure PC bilayer, thus provides a greater control of the integrity of the cell.

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