Hydrogen-bonding patterns of cholesterol in lipid membranes

Jérôme Hénin, Christophe Chipot *

Equipe de dynamique des assemblages membranaires, UMR CNRS/UHP 7565, Université Henri Poincaré, BP 239, 54506 Vandœuvre-lès-Nancy Cedex, France

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Abstract

Correlation between the rotation of the cholesterol hydroxyl group and the formation of hydrogen bonds with its lipid environment is examined through molecular dynamics (MD) simulations and compared with recently reported NMR experiments. All atom MD simulations of a fully hydrated 1:2 cholesterol–dimyristoylphosphatidylcholine bilayer have been performed. Precise reproduction of the cholesterol cell parameters via simulation of its P1-group crystal validates the force field utilized. The lipid–cholesterol hydrogen-bonding pattern reflects the coexistence of alternative dimer motifs with comparable conformer populations, in line with the estimated free energy differences for the rotamers of the cholesterol C=O bond.

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1. Introduction

Before a full understanding of the behavior of complex molecular assemblies like biological membranes can be reached, an accurate description of the role played by the elementary bricks that form the lipidic environment is highly desirable. Among these elementary bricks, cholesterol is the dominant sterol component in mammal cell membranes [1,2], regulating the fluidity of the latter [3]. It is known to broaden the main transition between the L_β^0, gel, and the L_α, liquid crystal, phases of lipid bilayers [4], thereby increasing the order in the high-temperature liquid crystalline phase [5]. Through modulation of its fluidity, cholesterol also modifies the mechanical and transport properties of the cell membrane [6]. Although many molecular dynamics (MD) investigations have been performed on model systems in recent years [7], quantitative agreement with experiment for several relevant physical observables has seldom been achieved [8]. This may be easily understood, given the inherent difficulties raised by such studies, arising principally from the significant time scales involved in the slow relaxation of collective degrees of freedom, that justify the requirement of long, multi-nanosecond MD simulations to obtain fully equilibrated systems [9].

Understanding the formation of lipid–cholesterol complexes in mixed lipid bilayers constitutes the main thrust of the present contribution. After probing the potential energy function of cholesterol through the reproduction of the cell parameters of its P1 crystal, all atom molecular dynamics simulations of a fully hydrated dimyristoylphosphatidylcholine (DMPC) bilayer with a 33% molar cholesterol concentration were carried out, from which selected structural properties of the supramolecular assembly were derived. The reasonable agreement with the experimentally determined observables—viz. neutron diffraction profiles, order parameters of the lipid alkyl chains and molecular order parameter of the cholesterol—provides a convenient framework for investigating at the atomic level whether and how rotation of the cholesterol hydroxyl group and its specific association with lipids are intertwined.

2. Methods and computational details

The molecular assembly examined herein consisted of a fully hydrated DMPC bilayer formed by 128 lipid units, in
equilibrium with 3240 water molecules, and interacting with 64 cholesterol molecules, which corresponds to a cholesterol molar concentration of 33% in the lipid phase.

All MD simulations were carried out using the NAMD program [10] with the all atom CHARMM27 force field [11,12]. The net atomic charges borne by cholesterol were determined with the OPEP program [13] from the molecular electrostatic potential computed at the RHF/6-31 G(d,p) level of theory and polarized by means of a self-consistent reaction field (SCRF) description of the environment [14] (see Fig. 1). The optimized geometry was obtained at the same level of theory. Consistency of the parameters adopted for the description of cholesterol was probed through the simulation of its P1 crystal, which consisted here in a replication of the unit cell containing eight molecules in the \( x \) and \( z \) directions of Cartesian space, thereby forming an ensemble of 72 cholesterol molecules. Average of the cell parameters over the last 500 ps of a 1 ns run did not reveal any spurious drift in the crystal structure—viz. \( a = 14.30 \text{ Å}, \ b = 34.11 \text{ Å}, \ c = 10.58 \text{ Å}, \ \alpha = 94.7^\circ, \ \beta = 90.7^\circ \) and \( \gamma = 96.2^\circ \), in good agreement with the available experimental values [15] of \( a = 14.172 \text{ Å}, \ b = 34.209 \text{ Å}, \ c = 10.481 \text{ Å}, \ \alpha = 94.64^\circ, \ \beta = 90.67^\circ \) and \( \gamma = 96.32^\circ \), as well as previous theoretical estimates [16].

The results presented here were obtained from a 19 ns production trajectory. During all simulations, the system was maintained at a constant temperature of 313 K, using Langevin dynamics, and at a constant normal pressure of 1 bar and constant surface tension of 42 dyn/cm, by means of the Langevin piston algorithm applied to a fully flexible periodic box. The equations of motion were integrated with a time step of 2 fs.

MD simulations at zero surface tension of pure DMPC bilayers described by the CHARMM force field [11,12] cannot provide a realistic reproduction of its structural features [17]. Equilibrated lipid bilayers tend to exhibit smaller surface areas per lipid and higher alkyl chain ordering than expected. The choice of 42 dyn/cm was found to yield a pure DMPC bilayer, the structural parameters of which agree reasonably well with the available experimental data. For consistency reason and owing to the paucity of reliable experimental data on lipid–cholesterol mixtures [18], the simulations reported here were performed following the same strategy.

The initial system was obtained by replicating 32 times a unit cell containing two DMPC units plus one cholesterol molecule, and duplicating the resulting monolayer. The crystal-like bilayer was hydrated and underwent energy minimization, prior to MD simulation for an overall duration of 30 ns, without application of a surface tension in the first 15 ns. During this equilibration stage, two short simulated annealing steps were performed, and significant in-plane rearrangement was observed. The key structural features of the system were found to be stable over several ns, and in overall agreement with the available experimental values. Orientational order in lipid bilayers can be measured through nuclear magnetic resonance (NMR) experiments, by means of the order parameter of deuterated alkyl chains:

\[
\begin{align*}
0.4639 & +0.0619 \\
0.0848 & +0.0247 \\
-0.1343 \\
-0.0998 & +0.0168 \\
-0.3359 & +0.2406 \\
-0.2745 & +0.1975 \\
-0.4985 & +0.2338 \\
-0.314 & +0.1187 \\
-0.1061 & +0.0530 \\
+0.1415 & +0.0005 \\
+0.0226 & +0.0113 \\
+0.0010 & +0.0005 \\
+0.0264 & +0.0132 \\
+0.1256 & +0.0299 \\
-0.1465 & +0.0299 \\
\end{align*}
\]

Fig. 1. Left: Molecular diagram of cholesterol featuring the net atomic point charges derived from the quantum mechanical molecular electrostatic potential. Right: Snapshot of the initial mixed lipid bilayer structure. DMPC units are rendered as sticks, and cholesterol as a yellow space-filling representation. For clarity, only one leaflet is shown. Molecular graphics rendered using VMD [35] (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.).
where \( \theta \) is the angle between a C—D chemical bond and the axis of the static magnetic field—viz. typically, the normal to the bilayer [19]. This quantity is averaged over all molecules present in the sample and may be derived from MD trajectories, thereby making direct comparison between theory and experiment possible.

The order parameter, \( S_{\text{mol}} \), associated to the orientation of the rigid polycyclic part of cholesterol may also be estimated from NMR experiments, using selectively deuterated molecules [20]. In order to obtain this parameter from MD trajectories, orientation of the principal inertial axes of the rigid part of cholesterol with respect to the normal was evaluated.

Neutron diffraction profiles were computed, considering that each atom contributes a peaked term to the scattering amplitude density, the integral of which is equal to its neutron scattering length. The experimental profiles were reconstructed from previously published [21] structure factors, \( F(k) \), in the form of a truncated Fourier series:

\[
\rho(z) = \frac{2}{d} \sum_{k} F(k) \cos \left( \frac{2\pi k z}{d} \right)
\]

where \( z \) is the position along the normal to the bilayer, \( d \) is the repeat distance of the lamellar structure, and the values of \( 2\pi k/d \) are the wave vectors for which structure factors are available.

Hydrogen bonds were detected using geometrical criteria. A bond is considered to be formed between donor group \( d-H \) and acceptor atom \( a \) if the \( d-a \) distance is less than 3.3 Å, and the angle formed by the \( d-H \) bond and the \( d-a \) direction at most 30°.

3. Results and discussion

3.1. Reproduction of structural properties

The order parameters of the \( sn-2 \) alkyl chain of DMPC, determined from MD simulations and NMR measurements [22,23] are depicted in Fig. 2. Noteworthily, theory and experiment agree particularly well, which is suggestive that both the potential energy function of cholesterol and the simulation strategy adopted here are adequate. Not too surprisingly, addition of cholesterol into the bilayer leads to higher ordering of the lipid chains [4]. Compared to the reference experimental data for a pure hydrated DMPC bilayer [24], the plateau corresponding to positions 4–6 in the \( sn-2 \) alkyl chain is raised by ca. 0.25. At the atomic level, this result corresponds to a pronounced propensity towards the all-trans conformation of the chains. MD simulations also give access to the order parameter characterizing the orientation of cholesterol. From the present simulations, \( S_{\text{mol}} \) is found to be equal to 0.94 ± 0.01, identical to the value derived from deuterium NMR spectra by Marsan et al. [20].
3.2. Complexation of DMPC by cholesterol

Analysis of the lipid–cholesterol complexes formed in the course of the MD simulation reveals different hydrogen-bonding possibilities [29], wherein the sterol and the phospholipid may act both as a donor and an acceptor. Table 1 summarizes the gross populations of hydrogen bonds involving, on the one hand, cholesterol, and, on the other hand, water and the phosphate, the ester and the choline moieties of DMPC. Comparison with the experimentally determined populations [30] indicates a good accord for the phosphate group and the donor water molecules. The same cannot be said, however, for the ester moiety, albeit the width of the corresponding experimental confidence interval suggests that the theoretical estimate falls within the error bar of the experimental one. The main discrepancy between theory and experiment lies in the population of hydrogen bonds created by acceptor water molecules, which seems to be exaggerated in the present MD simulations. A plausible, yet limited reason for this discrepancy lies in the existence of choline-cholesterol bonds, that were not taken into account in the calculations of Soubias et al. [30]. Definition of hydrogen-bonding is subject to tolerances in the geometrical criteria utilized. In the present work, a rather stringent definition has been adopted, likely to modulate the hydrogen bond populations, albeit not revert the observed trends. Interestingly enough, Soubias et al. acknowledge that hydrogen bond populations involving ester or water acceptor groups are poorly defined, whereas the sum of these populations is determined with a far greater accuracy—viz. 43 ± 14%. The corresponding computational estimate of 56 ± 4% falls within the statistical confidence interval. Association through pseudo-hydrogen bonds [31] involving a carbon atom of the glycerol moiety is occasionally detected, with a probability of about 0.2%. In sharp contrast, such a non-conventional hydrogen-bonding pattern is far more frequently observed in the case of the choline group.

The various possible complexation stoichiometries of cholesterol and DMPC or water are listed in Table 2, together with their respective population. DMPC–cholesterol complexes appear to be mostly 1:1, less than 10% of them involving two DMPC molecules (see Fig. 3). It should be noted that such complexes require one of the two DMPC units to act as a hydrogen acceptor and the other one as a donor through its choline group. As has just been mentioned, the latter occurs marginally, and about one third of the hydrogen bonds involving choline participate in 2:1 DMPC–cholesterol complexes. In contrast, complexes with several water molecules are frequent, supporting the view of a largely hydrated hydroxyl moiety of cholesterol. A somewhat different picture was painted by Pandit et al. [29], who described dipalmitoylphosphatidylcholine (DPPC) and dilaurylphosphatidylcholine (DLPC) bilayers with a 40% molar content of cholesterol. These authors report DPPC–cholesterol complexes with a 2:1 stoichiometry to constitute the most probable form, whereas 1:1 complexes have the highest population in the case of DLPC.

Formation of alternate hydrogen bonded complexes of cholesterol, water and lipid molecules occurs through the rotation of the C–O covalent bond of the cholesterol hydroxyl functional group. The free energy profile and probability distribution for this torsion are depicted in Fig. 4 and the corresponding percentile populations are gathered in Table 3. From the onset, it can be seen that the gauche+ and gauche− states are marginally more stable than the anti conformation—viz. within 0.5 kcal/mol, albeit the barriers between these states are appreciable, yet not unsurmountable at 313 K, ranging from ca. 1.0 to 1.5 kcal/mol.

The computed gross conformer populations and those determined by Soubias et al. [30] appear to be at variance, the latter favoring the anti rotamer. Rationalizing differences in conformational preferences is a difficult task. In particular, conformational equilibria are anticipated to be influenced substantially by the choice of the force field—whether specific, newly developed potential energy functions for sterols [32] are able to address this subtle issue deserves additional investigation. It may be desirable to relate the present conformational equilibrium to preferential hydrogen-bonding with the surrounding lipid and water molecules.

The populations of the cholesterol rotamers involved in such hydrogen bonds are gathered in Table 3. They illuminate the specificity of cholesterol towards gauche+ conformations, with a marked propensity for the formation of hydrogen bonds with the phosphate moiety of DMPC. This result is in line with the measured lifetime

Table 1

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>Theory</th>
<th>Experiment [30]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor cholesterol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ester</td>
<td>7 ± 2</td>
<td>26 ± 19</td>
</tr>
<tr>
<td>Phosphate</td>
<td>28 ± 3</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Acceptor water</td>
<td>49 ± 2</td>
<td>17 ± 10</td>
</tr>
<tr>
<td>Ester + acceptor water</td>
<td>56 ± 4</td>
<td>43 ± 14</td>
</tr>
</tbody>
</table>

| **Acceptor cholesterol**    |        |                 |
| Choline                     | 9 ± 1  | N/A             |
| Donor water                 | 74 ± 2 | 76 ± 14         |

Table 2

<table>
<thead>
<tr>
<th>Participating molecules</th>
<th>Unbound</th>
<th>1:1</th>
<th>2:1</th>
<th>3:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC–cholesterol</td>
<td>59 ± 2</td>
<td>37 ± 3</td>
<td>3 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>Water–cholesterol</td>
<td>17 ± 1</td>
<td>48 ± 2</td>
<td>31 ± 2</td>
<td>4 ± 0.6</td>
</tr>
</tbody>
</table>
Fig. 3. Complexation of DMPC with cholesterol in a 1:1 stoichiometry through hydrogen-bonding of the hydroxyl moiety of cholesterol with the phosphate moiety of DMPC (a) and with its ester functional group (b), or through pseudo-hydrogen bonding, viz. C-H...O, with the choline group (c). In a 2:1 stoichiometry, cholesterol acts both as hydrogen bond donor and acceptor (d). Molecular graphics rendered using VMD [35].

Fig. 4. Free energy difference for the rotation of the hydroxyl group of cholesterol about its C=O covalent bond. Inset: Probability distribution for the C<sub>2</sub>–C<sub>3</sub>–O–H torsional angle.

Table 3
Computationally determined percentile populations of cholesterol rotamers involved in hydrogen bonds with surrounding DMPC and water molecules, as a function of the donor and the acceptor

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>Gauche+</th>
<th>Anti</th>
<th>Gauche−</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ester</td>
<td>42 ± 9</td>
<td>30 ± 9</td>
<td>28 ± 15</td>
</tr>
<tr>
<td>Phosphate</td>
<td>51 ± 5</td>
<td>22 ± 4</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Acceptor water</td>
<td>45 ± 4</td>
<td>23 ± 3</td>
<td>31 ± 3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>47 ± 3</td>
<td>23 ± 3</td>
<td>30 ± 3</td>
</tr>
<tr>
<td><strong>Total (experiment) [30]</strong></td>
<td>31 ± 4</td>
<td>41 ± 9</td>
<td>28 ± 12</td>
</tr>
</tbody>
</table>
of lipid–cholesterol hydrogen bonds—viz. phosphate > acceptor water > ester > donor water. Whereas donor water molecules bound to the hydroxyl group of cholesterol exchange rapidly, viz. typically within 15 ps, the life span of lipid–cholesterol association through the phosphate moiety of the latter may exceed the 50 ps time scale. Significantly longer lifetimes have been reported previously for lipid–cholesterol hydrogen bonds [8]. Discrepancies with respect to the present values may be ascribed to the very strict definition used here, as well as the intermittent breaking and reforming of individual bonds on short time scales, which was taken into account here. In contrast, lipid–cholesterol complexes, as a whole, persist over much longer time scales, presumably dictated by the slow lateral diffusion within the lipid bilayer, rather than intrinsic hydrogen-bonding properties.

4. Conclusion

To gain novel, atomic-detail insight into the role played by cholesterol in lipid membranes, in particular how it modulates their physical and chemical properties [2,3] and promotes the formation of so-called raft domains [33,34], precise understanding of the underlying lipid–cholesterol interactions is of paramount importance. In the present contribution, the interaction of a DMPC bilayer with cholesterol at high molar concentration has been examined by means of multi-nanosecond MD simulations. The brute structural properties of the molecular assembly compare reasonably well with the available experimental data [20–23] and coincide with previous theoretical studies [7–9,29].

So far, little information has been accrued on the specificity of lipid–cholesterol interactions, and it has been hitherto inferred indirectly from NMR experiments [30]. Thorough analysis of the rotamers of cholesterol about the C—O covalent bond of its hydroxyl moiety, on the one hand, and the hydrogen bond populations involving cholesterol, both as an acceptor and a donor, and surrounding water and lipid molecules, on the other hand, sheds new light on their interplay. Rotation of the hydroxyl group, although related to the possibilities of hydrogen-bonding with neighboring water and DMPC molecules, does not exhibit a strong dependence on the type of bonds formed. The overall preference towards the gauche+ conformer is at variance with the conclusions drawn from solid-state NMR measurements [30]. It should, however, be remembered that the conformer populations predicted by Soubias et al. are an indirect interpretation of experimental results based on theoretical calculations requiring subtle underlying assumptions. Beyond the experimental uncertainty, the apparent discrepancy between theory and experiment illustrates the tremendous difficulties faced by current, state-of-the-art atomistic simulations and macromolecular force fields to reproduce accurately subtle structural features of mixed model membrane systems.

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