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Saturation with cholesterol increases vertical order and smoothes the surface of the phosphatidylcholine bilayer: A molecular simulation study

Elżbieta Plesnar [a], Witold K. Subczynski [b], Marta Pasenkiewicz-Gierula [a,*]

[a] Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
[b] Department of Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

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ABSTRACT

Molecular dynamics (MD) simulations of a mono-*cis*-unsaturated 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) bilayer and a POPC bilayer containing 50 mol% cholesterol (POPC–Chol50) were carried out for 200 ns to compare the spatial organizations of the pure POPC bilayer and the POPC bilayer saturated with Chol. The results presented here indicate that saturation with Chol significantly narrows the distribution of vertical positions of the center-of-mass of POPC molecules and POPC atoms in the bilayer. In the POPC–Chol50 bilayer, the same moieties of the lipid molecules are better aligned at a given bilayer depth, forming the following clearly separated membrane regions: the polar headgroup, the rigid core consisting of steroid rings and upper fragments of the acyl chains, and the fluid hydrocarbon core consisting of Chol chains and the lower fragments of POPC chains. The membrane surface of the POPC–Chol50 bilayer is smooth. The results have biological significance because the POPC–Chol50 bilayer models the bulk phospholipid portion of the fiber-cell membrane in the eye lens. It is hypothesized that in the eye lens cholesterol-induced smoothing of the membrane surface decreases light-scattering and helps to maintain lens transparency.

1. Introduction

Fiber-cell membranes of the human eye lens are overloaded with cholesterol (Chol) [1,2]. Generally, when Chol exceeds the phospholipid-Chol miscibility threshold in the membrane, immiscible cholesterol bilayer domains (CBDs) within phospholipid-cholesterol domain saturated with Chol (PCD), start to form [3–5]. It is hypothesized that a high amount of Chol and the presence of CBDs help to maintain lens transparency [3,6,7] and, therefore, possibly protect against cataract formation [3,8]. However, their particular role in fiber-cell membranes of the human eye lens has not been determined.

Better understanding of the physiological role of membrane saturation with Chol requires improved molecular-level knowledge of the interactions between Chol and membrane lipids. We have investigated experimentally, using EPR spin-labeling methods, the properties of the phospholipid-cholesterol domain saturated with cholesterol, PCD, in lens lipid membranes from different animals (cow and pig [9–11]), from animals at different ages (six-month- and two-year-old cows [10–12]), and from different eye regions (the cortex and nucleus of two-year-old cows [12]). The phospholipid composition of the fiber-cell membrane is significantly different for different species [13–15], ages [15], and regions of the lens [16]. The phospholipids differ both in the chemical structure of their headgroups and the degree of saturation of their acyl chains. Nevertheless, depth dependences (profiles across the bilayer) of quantities characterizing the bulk membrane, including order parameter, hydrophobicity, and oxygen transport parameter (oxygen diffusion–concentration product), are nearly identical in all of the investigated membranes. Therefore, we concluded that a saturating content of Chol in the fiber-cell membrane keeps the bulk physical properties of the PCD the same independently of the phospholipid composition. The profiles mentioned above are very similar when the CBD is not yet formed in the membrane and when it is already formed (*i.e.*, in the PCD surrounding the CBD [9,12]), the latter of which is the case of membranes in aged eyes. This allowed us to conclude further that the CBD plays some role specific to the fiber-cell plasma membrane. The CBD provides a buffering capacity for Chol concentration in the surrounding phospholipid bilayer, keeping it at a constant saturating level to ensure certain physical properties of the membrane. These results are especially significant for human lenses. Among mammalian lenses, human lenses have the longest lifespan and changes in their phospholipid composition due to age are the most pronounced [17].

Abbreviations: CBD, cholesterol bilayer domain; PCD, phospholipid–cholesterol domain; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine; Chol, cholesterol; MD, molecular dynamics; CM, center-of-mass; RP, roughness parameter

* Corresponding author at: Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Krakow, Poland. Tel.: +48 12 664 6518; fax: +48 12 664 6902.
E-mail addresses: elzbieta.pieniazek@uj.edu.pl (E. Plesnar), subczyn@mcw.edu (W.K. Subczynski), marta.pasenkiewicz-gierula@uj.edu.pl (M. Pasenkiewicz-Gierula).

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To achieve a better understanding of the properties of the lens lipid membrane, we also carried out EPR spin-labeling studies of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) bilayers in the cholesterol-dependent liquid-ordered phase. A POPC–Chol bilayer (like other phosphatidylcholine [PC] bilayers) is in the liquid-ordered phase when the Chol content is between 30 and 50 mol%. Below this range (but above ~7 mol%), the liquid-disordered and liquid-ordered phases coexist, and above this range, a pure Chol bilayer domain (CBD) starts to form in the bilayer. Thus, 50 mol% is an approximate saturating Chol content for the POPC bilayer. When the Chol content reaches saturation, the most drastic changes that occur in the bilayer are observed in the profiles of hydrophobicity and the oxygen transport parameter [18,19]. In liquid-ordered PC bilayers of the lowest Chol content of ~30 mol%, as well as in bilayers without Chol, these profiles are bell-shaped [20,21]. At Chol saturation, the shape of these profiles becomes rectangular and is practically the same as for lens lipid membranes [22,23]. The discontinuities in these profiles (an abrupt increase) occur near the vertical positions of the ninth (C9) and tenth (C10) carbon atoms in the acyl chains (*i.e.*, approximately at the depth at which the rigid tetracyclic Chol structure is immersed in the bilayer). Hydrophobicity profiles were obtained experimentally for frozen membranes where lipid motions were halted [10]. Alternatively, profiles of the oxygen transport parameter were obtained at physiological temperatures for liquid-crystalline membranes [10,11]. In the bilayer saturated with Chol, the oxygen transport parameter from the membrane surface to the approximate depth of C9 was as low as in a gel-phase membrane, and at locations deeper than C9, it was as high as in the fluid-phase membrane. Thus, the abrupt increase in the parameter value in both bilayer leaflets occurs within the distance of one carbon–carbon bond (*i.e.*, 1.3–1.5 Å). This is difficult to explain unless one assumes that at a saturating Chol content the vertical fluctuations of lipid atoms are smaller than in membranes below a saturating Chol content. As a result, the vertical alignment of all corresponding lipid groups is high, and all Chol rings are immersed to the same membrane depth, which is close to the position of C9 in PC acyl chains. To determine the shapes of the profiles, very small probes (*i.e.*, molecular oxygen and the nitroxide moiety) are used [18]. Thus, the best way to confirm the effect of Chol on vertical alignment in the membrane is to use molecular dynamics (MD) simulation with atomic resolution. Atomistic MD simulations have proven to be extremely useful in membrane research (see [24] and references therein). They provide direct information about atomic level mechanisms not accessible by any of the current experimental techniques. Additionally, MD simulated systems are not perturbed by any probe as is often the case in experimental studies.

In the present research, we used an atomistic MD simulation method to characterize depth-dependent dynamic structures of a liquid-ordered POPC bilayer containing 50 mol% Chol and a liquid-disordered POPC bilayer without Chol. Comparison of these bilayers indicates that saturation with Chol significantly narrows the distribution of vertical positions of each lipid atom at all bilayer depths. As a result, the phospholipid bilayer surface becomes smoother, which is in accord with our hypothesis based on the experimental results discussed above.

We believe that these data contribute to a better understanding of the role of Chol in maintaining eye-lens transparency. We hypothesize that cholesterol-induced smoothing of the membrane surface should decrease light-scattering and help to maintain lens transparency.

2. Methods

2.1. System description and parameters

Atomic-scale MD simulations of two membrane systems (each composed of 200 lipid molecules) were performed. One system comprised only POPC (Fig. 1a) (POPC bilayer), and the other POPC and Chol (Fig. 1b) molecules (1:1 ratio) (POPC–Chol50 bilayer).

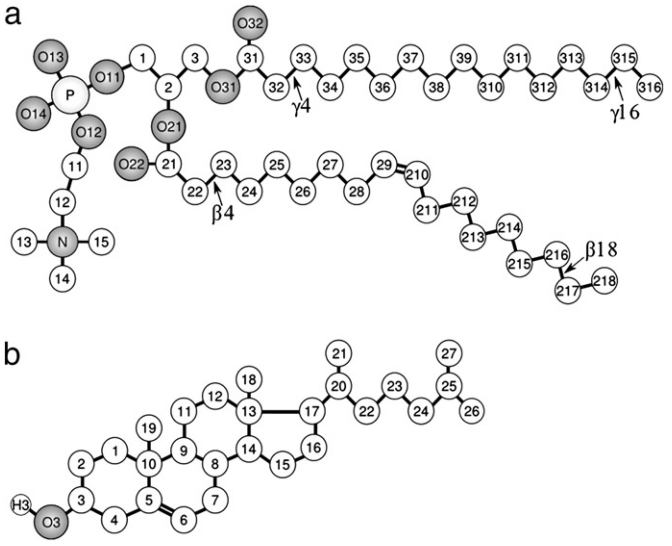

Fig. 1. Molecular structures with numbering of atoms of POPC (a) (according to Sundaralingam's nomenclature [61]) ("2" signifies oleoyl and "3" the palmitoyl chain) and Chol (b) molecules (the hydrogen atoms as well as the chemical symbol for carbon atoms, C, are omitted). Torsion angles at the beginning and end of each acyl chain are indicated.

Each of the bilayers was hydrated with 6000 water molecules (30 H_2O/lipid). Bilayers were constructed in two steps. First, 100 vertically oriented (along the *z*-axis) molecules (either POPC or POPC and Chol) were placed regularly in alternating POPC and Chol stripes in the *x*, *y* plane to form one layer (Fig. S1, Supplementary Material). Then, the second layer was obtained from the first by a 180° rotation and shifting to reduce the free volume between the layers. Each bilayer was simulated for over 200 ns using the GROMACSv4.0 software package [25]. The initial structure of Chol was the crystal structure of cholesterol molecule A, as determined by Shieh et al. [26], and that of POPC was taken from our previous bilayer system [27]. Hydrogen atoms were added to both structures.

For POPC and Chol molecules, all-atom optimized potentials for liquid simulations (OPLS-AA) [28] were used. For water, the transferable intermolecular potential three-point model (TIP3P) was used [29]. The linear constraint solver (LINCS) algorithm [30] was used to preserve the length of any covalent bond with a hydrogen atom, and the time step was set to 2 fs. The van der Waals interactions were cut off at 1.0 nm. Long-range electrostatic interactions were evaluated using the particle-mesh Ewald summation method with a β-spline interpolation order of 5 and direct sum tolerance of 10^{-5} [31]. For the real space, a cut-off of 1.0 nm, three-dimensional periodic boundary conditions, and the usual minimum image convention were used [31].

MD simulations were carried out in the *NPT* ensemble (the number of particles, pressure, and temperature were constant) at a pressure of 1 atm and temperature of 310 K, which is above the main-phase transition temperature for a pure POPC bilayer of −5 °C [32]. The temperatures of the solute and solvent were controlled independently by the Nose–Hoover method [33], with the relaxation time set at 0.6 ps. Pressure was controlled anisotropically by the Parrinello–Rahman method [34], with the relaxation time set at 1.0 ps. The list of nonbonded pairs was updated every five steps.

3. Results

3.1. Characterization of the membranes

3.1.1. Equilibration

In the molecular modeling study of a lipid bilayer, the convergence of the surface area of the bilayer is an adequate first indicator of the