Cholesterol in unusual places

Kučerka, N.; Nieh, M. P.; Marquardt, D.; Harroun, T. A.; Wassall, S. R.; Katsaras, J.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1088/1742-6596/251/1/012038
Journal Of Physics, 251, 1, pp. 1-4, 2010

Cholesterol in unusual places

N Kučerka[1], M P Nieh[1], D Marquardt[2], T A Harroun[2], S R Wassall[3] and J Katsaras[1,2]

[1]Canadian Neutron Beam Centre, National Research Council, Chalk River, ON K0J 1J0, Canada

[2]Department of Physics, Brock University, St. Catharines, ON L2S 3A1, Canada

[3]Department of Physics, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana 46202-3273, USA

John.Katsaras@nrc.gc.ca / Norbert.Kucerka@nrc.gc.ca

Abstract. Cholesterol is an essential component of mammalian cells, and is required for building and maintaining cell membranes, regulating their fluidity, and possibly acting as an antioxidant. Cholesterol has also been implicated in cell signaling processes, where it has been suggested that it triggers the formation of lipid rafts in the plasma membrane. Aside from cholesterol's physiological roles, what is also becoming clear is its poor affinity for lipids with unsaturated fatty acids as opposed to saturated lipids, such as sphingomyelin with which it forms rafts. We previously reported the location of cholesterol in membranes with varying degrees of acyl chain unsaturation as determined by neutron diffraction studies (Harroun *et al* 2006 *Biochemistry* **45,** 1227; Harroun *et al* 2008 *Biochemistry* **47,** 7090). In bilayers composed of phosphatidylcholine (PC) molecules with a saturated acyl chain at the *sn*-1 position or a monounsaturated acyl chain at both *sn*-1 and *sn*-2 positions, cholesterol was found in its much-accepted "upright" position. However, in dipolyunsaturated 1,2-diarachidonyl phosphatidylcholine (20:4-20:4PC) membranes the molecule was found sequestered in the center of the bilayers. In further experiments, mixing 1-palmitoyl-2-oleoyl phosphatidylcholine (16:0-18:1PC) with 20:4-20:4PC resulted in cholesterol reverting to its upright orientation at approximately 40 mol% 16:0-18:1 PC. Interestingly, the same effect was achieved with only 5 mol% 1,2-dimyristoyl phosphatidylchoile (14:0-14:0PC).

1. Introduction

Polyunsaturated fatty acids (PUFAs) constitute a biologically influential group of molecules. High levels, sometimes exceeding 50 mol %, are found in the phospholipids of specialized membranes where their depletion impairs function [1]. In retinal membranes, for instance, PUFAs are particularly abundant, and nearly 30% of phosphatidylcholines (PCs) isolated from bovine rod outer segments are dipolyunsaturated [2]. Dietary consumption of PUFAs is also known to elevate the modest concentration, usually less than 10 mol %, of PUFA-containing phospholipids in the plasma membrane alleviating a number of chronic conditions [3]. While interest in the topic has centered on the omega-3 class of PUFA lipids, it has also spanned a variety of human health issues, including

PUFA-associated effects on protein signaling in inflammation and cancer [4], arteriosclerosis [5], and suppressive effects on the immune system [6].

Cholesterol is an essential component of mammalian cells and is either obtained from foods of animal origin (e.g., milk, cheese, meat, eggs, etc.) or synthesized in the endoplasmic reticulum [7]. It is required for building and maintaining cell membranes, regulates their fluidity, and may act as an antioxidant [8]. Recently, cholesterol has also been implicated in cell signaling processes, where researchers have suggested that it is involved in the formation of lipid rafts in the plasma membrane [9, 10], and has also been found to reduce the permeability of the plasma membrane to sodium and hydrogen ions [11].

The interaction of cholesterol with the fatty acids of phospholipids plays a crucial role in modulating molecular organization within membranes [7]. Its interaction with saturated fatty acid (SFA)-containing PCs is well characterized. The introduction of the rigid steroid moiety into homoacid-disaturated PC membranes disrupts the regular packing of chains in the gel or solid ordered (so) phase and restricts the reorientation of the fatty acid chains in the liquid crystalline or liquid-disordered (ld) phase [12]. The differential between the phases is smeared out until a liquid-ordered (lo) phase - characterized by rapid reorientation but high conformational order - is formed over a wide range of temperatures at concentrations >16 mol % cholesterol. Excess sterol is expelled when the content exceeds >50 mol % [13]. Within the membrane, the 3β-hydroxyl group of cholesterol locates just below the aqueous interface [14] and the steroid moiety rotates rapidly about the long molecular axis that wobbles through a narrow range of angles slightly tilted relative to the bilayer normal [15]. A similar behavior is exhibited by heteroacid-saturated-monounsaturated PC [16-18]. However, the interaction of cholesterol with polyunsaturated fatty acid (PUFA)-containing phospholipids for which the sterol has diminished affinity is much less understood and has only recently begun to receive attention.

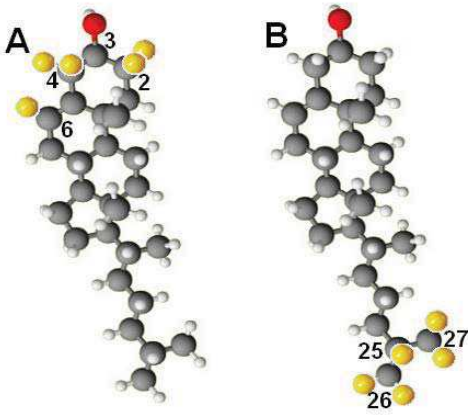

Figure 1. Deuterium label positions (yellow) of cholesterol. A) Headgroup-labeled cholesterol involving hydrogens [2,2,3,4,4,6-$^2H_6$] near the hydroxyl group on the steroid moiety. B) Acyl tail-labeled cholesterol [25,26,26,26,27,27,27-$^2H_7$]. Not all of the deuterons are visible.

2. Experimental details

Neutron diffraction data were recorded at the Canadian Neutron Beam Centre's N5 beamline, located at the National Research Universal (NRU) reactor (Chalk River, ON), using 2.37 Å wavelength λ neutrons. The appropriate wavelength neutrons were selected by the (002) reflection of a pyrolytic graphite (PG) monochromator, and a PG filter was used to eliminate higher order (i.e., $\lambda/2$, etc.) reflections. The methods of sample preparation and neutron data analysis follow exactly those described previously [19, 20]. Besides unlabeled cholesterol, experiments included 10 mol% of either "headgroup" [2,2,3,4,4,6-$^2H_6$] (Fig. 1A) or "tail" [25,26,26,26,27,27-$^2H_7$] (Fig. 1B) labeled cholesterol.

3. Results

Figure 2A shows difference (deuterated minus non deuterated cholesterol samples) scattering length density (SLD) profiles of bilayers with varying degree of acyl chain unsaturation containing 10 mol% of unlabeled or headgroup-labeled [2,2,3,4,4,6-$^2H_6$] cholesterol (Fig. 1A). For bilayers other than dipolyunsaturated 1,2-diarachidonyl phosphatidylcholine (20:4-20:4PC) the difference is dominated by a single pair of Gaussian shaped peaks symmetrically disposed on either side of the origin. On the other hand, for 20:4-20:4PC bilayers the difference SLD profile is described by a single Gaussian centered at the origin. These peaks designate where the center of mass of the six deuterated sites on the labeled sterol sits within each membrane. The solid line is a single Gaussian function fit to the difference data, which is used to determine the location and width. The appearance of a single Gaussian (20:4-20:4PC bilayers) is indicative of a single population for the label. This result clearly establishes that the cholesterol molecule has undergone a major reorientation within the dipolyunsaturated membrane, such that its hydroxyl group is now located in the middle of the bilayer instead of near the aqueous interface as is usually the case. The question that this surprising observation raises is whether the sterol lies flat between monolayers or, less likely, has become inverted in the bilayer. To answer this question we followed up with additional neutron experiments employing tail-labeled [25,26,26,26,27,27-$^2H_7$] cholesterol.

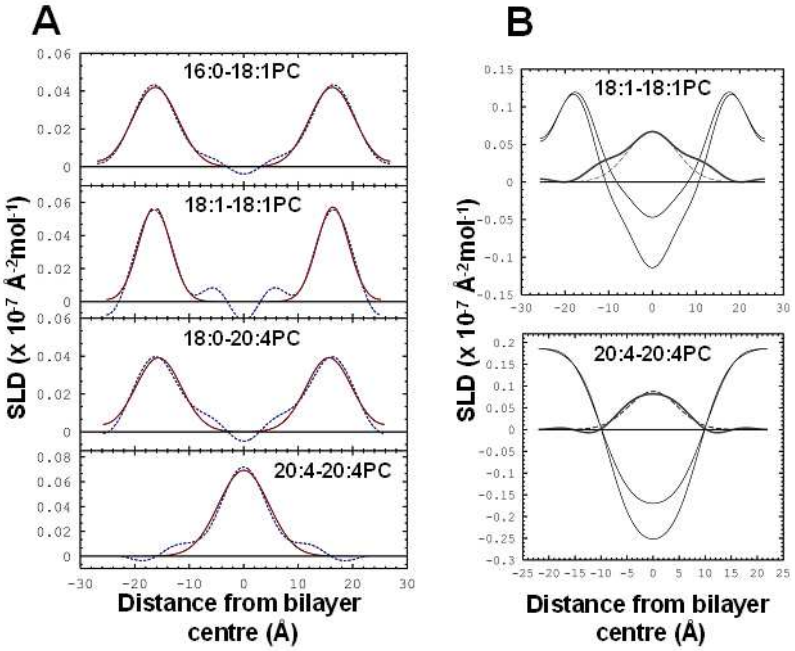

Figure 2. SLD difference profiles between labeled and unlabeled samples. A) Difference profiles for bilayers with varying levels of acyl chain unsaturation, containing 10 mol% of unlabeled or headgroup-labeled [2,2,3,4,4,6-$^2H_6$] cholesterol. B) Difference profiles for 18:1-18:1PC and 20:4-20:4PC bilayers containing 10 mol% of unlabeled or tail-labeled [25,26,26,26,27,27-$^2H_7$] cholesterol. The data were fit with single Gaussians. For details, the reader is referred to Refs. [19] and [20].

Figure 2B shows the difference SLD profiles of 1,2-dioleoyl phosphatidylcholine (18:1-18:1PC) and 20:4-20:4PC bilayers containing 10 mol % unlabeled cholesterol or [25,26,26,26,27,27,27-$^2H_7$] cholesterol. For both 18:1-18:1PC and 20:4-20:4PC bilayers there is a single peak at the center of the bilayer, indicating that cholesterol's tail is in the bilayer centre. In the case of 20:4-20:4PC, the entire sterol is now solvated at the same depth in the lipid acyl chain matrix, clear evidence that cholesterol lies flat in the midplane of 20:4-20:4PC bilayers.

3.1. Doping of 20:4-20:4PC/10 mol% cholesterol bilayers

Recently, we doped 20:4-20:4PC/10 mol% cholesterol bilayers with either 1-palmitoyl-2-oleoyl phosphatidylcholine (16:0-18:1PC) or 1,2-dimyristoyl phosphatidylcholine (14:0-14:0PC) lipids.

Preliminary data indicate that cholesterol can be induced to revert to its nominal upright orientation in 20:4-20:4PC bilayers when doped with 50 mol% 16:0-18:1PC (data not shown). However, only 5 mol% of 14:0-14:0PC is needed to accomplish the same effect (Fig. 3).

These results provide further evidence how different lipid species may affect the transmembrane, as well as the lateral distribution of cholesterol. For example, in plasma membranes, sphingolipids are primarily located in the outer monolayer [21], whereas PUFAs are preferentially incorporated into phospholipids, such as phosphatidylethanolamine, that are more abundant in the inner leaflet [22]. In light of the present results, it is conceivable that the presence of PUFA in the inner leaflet can enhance the transfer of cholesterol to the outer layer, potentially modifying raft composition and function.

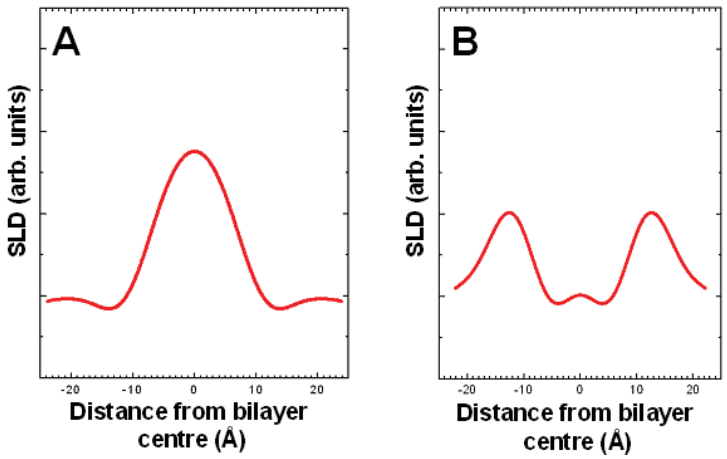

Figure 3. SLD difference profiles between headgoup-labeled and unlabeled 10 mol% cholesterol samples. A) Pure 20:4-20:4PC bilayers. B) 95% 20:4-20:4PC and 5% 14:0-14:0PC bilayers. Only 5 mol% 14:0-14:0PC is needed to flip the cholesterol in its nominal upright position.

References

[1] Salem, N Jr *et al* 1986 *Health Effects of Polyunsaturated Fatty Acids in Seafoods* Simopoulos A. P *et al* Eds (New York: Academic Press)
[2] Aveldano M I 1989 *Colloq. INSERM* **195** 87
[3] Stillwell W and Wassall S R 2003 *Chem. Phys. Lipids* **126** 1
[4] Siddiqui R A *et al* 2007 *Nutr. Clin. Pract.* **22** 74
[5] Kratz M 2005 *Handbook of Experimental Pharmacology* von Eckardstein A Ed. vol. 170 (New York: Springer)
[6] Shaikh, S R and Edidin M 2006 *Am. J. Clin. Nutr.* **84** 1277
[7] Finegold L 1993 *Cholesterol in Membrane Models* (Boca Raton FL: CRC Press)
[8] Smith L L 1991 *Free Radical Biol. Med.* **11,** 47
[9] Papanikolaou A *et al* 2005 *J. Biol. Chem.* **28** 26406
[10] Petrie R J *et al* 2000 *J. Immunol.* **165** 1220
[11] Haines T H 2001 *Prog. Lipid Res.* **40** 299
[12] Vist M R and Davis J H 1990 *Biochemistry* **29** 451
[13] Huang J *et al* 1999 *Biochim. Biophys. Acta* **1417** 89
[14] Léonard A *et al* 2001 *Langmuir* **17** 2019
[15] Marsan *et al* 1999 *Biophys. J.* **76** 351
[16] Thewalt J L and Bloom M 1992 *Biophys. J.* **63** 1176
[17] Worcester D L and Franks N P 1976 *J. Mol. Biol.* **100** 359
[18] Brzustowicz M R *et al* 1999 *FEBS Lett.* **451** 197
[19] Harroun *et al* 2006 *Biochemistry* **45** 1227
[20] Harroun *et al* 2008 *Biochemistry* **47** 7090
[21] Brown D A and London E 2000 *J. Biol. Chem.* **275** 17221
[22] Knapp H R *et al* 1994 *J. Lipid Res.* **35** 1283