Doping of Polyunsaturated Fatty Acid Bilayers with Monounsaturated and Disaturated Lipids Results in the Reorientation of Cholesterol

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Cholesterol is an essential component of mammalian cells. It can either be obtained from foods of animal origin (e.g., milk, cheese, meat, eggs, etc.), or synthesized in the endoplasmic reticulum [1]. It is required to build and maintain cell membranes, regulates their fluidity and may act as an antioxidant. Recently, cholesterol has also been implicated in cell signaling processes, where it has been suggested that it forms lipid rafts in the plasma membrane [2]. In biological membranes, the lateral sequestration of lipids with polyunsaturated fatty acid (PUFA) chains into membrane domains depleted of cholesterol has been hypothesized to have an important role in neurological function and in alleviating a number of health-related problems [3]. There seems to be a strong aversion of the disordered polyunsaturated chains to the planar, rigid surface of the cholesterol's steroid moiety, which is thought to be the major driving force for this kind of domain formation. Neutron studies of selectively deuterium labeled cholesterol incorporated into diC20:4PC (1,2-di-arachidonoyl-phosphatidylcholine) bilayers, revealed that the cholesterol's hydroxyl group resides at the bilayer center [4]. This result was interpreted in terms of cholesterol preferentially sequestering inside the membrane and in contrast to its usual position, where the hydroxyl group locates near the aqueous interface. Further neutron scattering experiments using tail deuterium labeled cholesterol confirmed that, in fact, the molecule is laying flat in the bilayer and not inverted with its tail near the aqueous interface [5].

Recent simulations [3] using MARTINI coarse grained modeling have shown that the angle of the cholesterol with respect to the bilayer normal varies with the number of unsaturated bonds in the lipid fatty acid chains; i.e. unsaturation increases the tilt angle. It was also shown that the frequency of cholesterol's flip-flop between bilayer leaflets dramatically increases with the number of unsaturated bonds. Therefore the ability of cholesterol to flip-flop more rapidly in the presence of PUFA lipids is enticing as a cell-signaling response mechanism to changing conditions of membrane fluidity or stress. It is not just a matter of lateral domain formation, as is so much the focus of lipid rafts research, but the wholesale movement of cholesterol across the bilayer that may regulate membrane fluidity, which in turn regulates membrane protein function. It thus follows that in a mixed bilayer of saturated and polyunsaturated lipids, cholesterol can be made to flip between upright and flat orientations.

We have carried out neutron diffraction experiments to test the hypothesis that cholesterol flips into its nominal upright position at some critical concentration of the monounsaturated 1-palmitoyl-2-oleoyl-phosphatidylcholine (C16:0-18:1PC, POPC) or disaturated 1,2-di-mirystoyl-phosphatidylcholine (diC14:0PC, DMPC) lipid in polyunsaturated bilayers prepared of diC20:4PC. Neutron diffraction data were collected at the Canadian Neutron Beam Centre's D3 beamline, located at the National Research Universal (NRU) reactor (Chalk River, ON), using 2.37 Å wavelength neutrons. The appropriate wavelength neutrons were selected by the (002) reflection of a pyrolytic graphite monochromator. Samples consisted of oriented multibilayers prepared using appropriate amounts of PUFA and POPC, or DMPC, and 10 mol% cholesterol. Besides unlabeled cholesterol, samples were prepared with 10 mol% of headgroup labeled cholesterol (2,2,3,4,4,6-D6) obtained from C/D/N Isotopes (Pointe-Claire, QB).

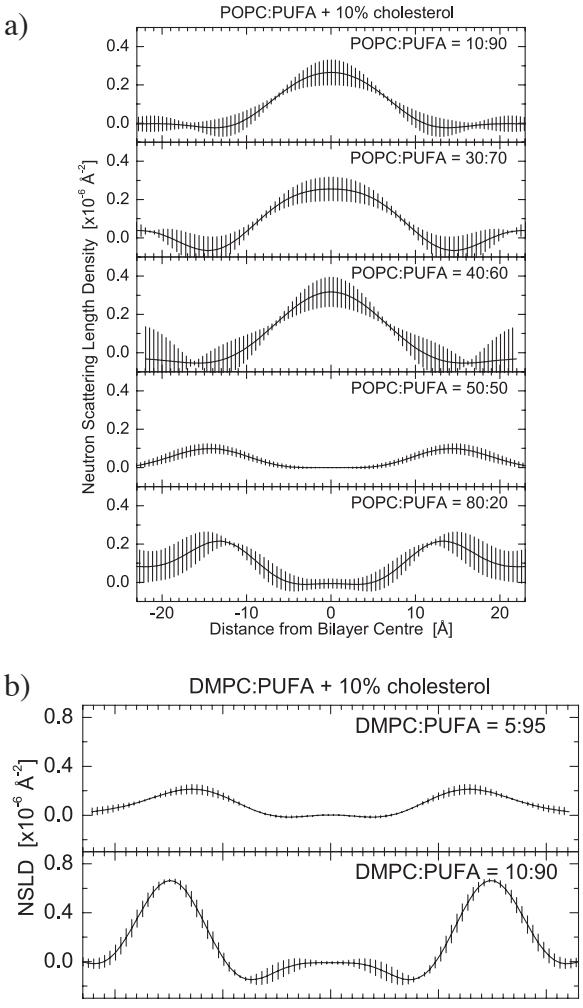

Fig 1. *NSLD difference profiles (deuterated minus non-deuterated cholesterol samples) showing the distribution of the cholesterol headgroup label. (a) PUFA bilayers doped with various amounts of POPC; (b) similar data for DMPC doped samples.*

Figure 1 shows difference neutron scattering length density (NSLD) profiles of PUFA bilayers with various doping of POPC or DMPC. Both parts confirm that cholesterol flips into its commonly assumed upright position at some concentration of dopant lipid. Figure 1a suggests the critical concentration of POPC to be between 40 and 50 mol%, while in the case of DMPC (Figure 1b), that amount is less than 5 mol%. This result clearly emphasizes cholesterol's affinity for saturated fatty acid chains.

The importance of these data may be rationalized with what we presently know of biological systems. For example, in plasma membranes sphingolipids are primarily located in the outer monolayer [6], whereas unsaturated phospholipids are more abundant in the inner leaflet [7]. Thus, the presence of PUFA in the inner leaflet may enhance the transfer of cholesterol to the outer layer, potentially modifying raft composition and membrane function.

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