



Cholesterol's location in lipid bilayers



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ABSTRACT

It is well known that cholesterol modifies the physical properties of lipid bilayers. For example, the much studied liquid-ordered L_o phase contains rapidly diffusing lipids with their acyl chains in the all *trans* configuration, similar to gel phase bilayers. Moreover, the L_o phase is commonly associated with cholesterol-enriched lipid rafts, which are thought to serve as platforms for signaling proteins in the plasma membrane. Cholesterol's location in lipid bilayers has been studied extensively, and it has been shown – at least in some bilayers – to align differently from its canonical upright orientation, where its hydroxyl group is in the vicinity of the lipid–water interface. In this article we review recent works describing cholesterol's location in different model membrane systems with emphasis on results obtained from scattering, spectroscopic and molecular dynamics studies.

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1. Introduction

Few biomolecules have been scrutinized as much as cholesterol. Numerous works have been written about its life-cycle in the human body, its association with other biomolecules, and its role in human health (Myant, 1981). Moreover, many interesting physical properties have been attributed to it. Although a focused review, as this, cannot do proper justice to cholesterol's biological importance, we will focus on recent results specifying cholesterol's location in different model membranes, with special emphasis on data derived from scattering, spectroscopic and molecular dynamics studies.

Cholesterol is found in all animal cell membranes and is needed for their proper function, including membrane permeability and fluidity (Smith, 1991; Parasassi et al., 1995). It is also thought to act as an antioxidant, and has been implicated in cell signaling processes associated with functional domains in the plasma membrane (Petrie et al., 2000; Papanikolaou et al., 2005). As much as 90% of total cholesterol is found in the plasma membrane (PM) (Lange and Ramos, 1983), and accounts for up to 45 mol% of the membrane's total lipid content (Yeagle, 1993) – it should be pointed out, however, that organelle membranes are almost devoid of it (Mouritsen, 2005). Since its discovery in 1769 by de la Salle (Stillwell, 2013), cholesterol has become one of the most studied biomolecules (Brown and Goldstein, 1992). However, despite the wealth of research data, many questions remain about cholesterol's role in membranes.

Cholesterol is made up of a hydrocarbon tail, a fused planar 4-ring assembly – common to steroid hormones (i.e., testosterone and estrogen) – and a hydroxyl headgroup that helps it orient at the membrane–water interface. Although a planar molecule,

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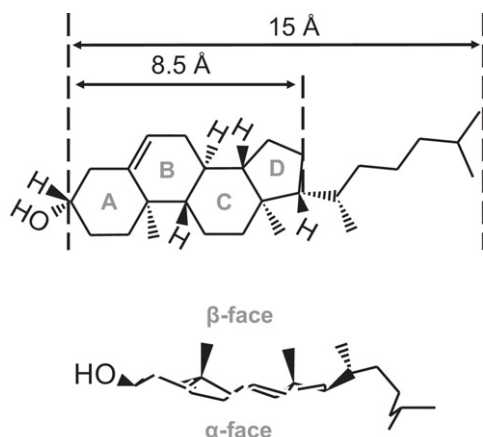


Fig. 1. (a) The structure of cholesterol showing its steroid skeleton of four fused rings, and its fatty acid tail. Molecular dimensions were taken from Marquardt et al. (2015d), Kučerka et al. (2008). (b) Schematic showing differences between the molecule's α - and β -faces. Hydrogen atoms are not shown for better viewing.

cholesterol has methyl groups on its two quaternary centres, making up its so-called “rough” or β face, while their absence on the molecule's other side account for its “smooth” or α face (Fig. 1). Although there are different cholesterol isoforms (i.e., 256), only one is naturally occurring (i.e., SRSSRRR-cholesterol) (Westover et al., 2003; Kristiana et al., 2012).

In water cholesterol aggregates, forming micelles at concentrations between 20 and 40 nM (Haberlan and Reynolds, 1973; Gilbert et al., 1975). However, due to its hydrophobic nature it has a solubility of only 1.8 mg/L (Haberlan and Reynolds, 1973). Cholesterol excluded from the membrane adopts a crystalline monohydrate form (Brzustowicz et al., 2002b). In this review we will report on cholesterol's location and influence on different lipid bilayers as studied by neutron scattering and nuclear magnetic resonance (NMR). Understanding its influence on model membranes may eventually help us to resolve some of the open questions associated with this important biomolecule.

2. Cholesterol in different bilayer systems

Cholesterol alters lipid membranes in complex ways, and over the years a number of experimental techniques have been used to construct phase diagrams of cholesterol-containing membranes. In this section we will review some of the experimental techniques used to determine the location of cholesterol in model membranes.

Determining cholesterol's location in model membranes has made use of a number of different sample preparations. For example, commonly used and easy to prepare multilamellar vesicles (MLVs) have the advantage of being easily hydrated under different buffer conditions. Unilamellar vesicles (ULVs) are in many ways similar to MLVs, but with the addition that they have to be extruded or sonicated from a parent MLV solution. An advantage of ULVs is that when extruded, their size is well defined, allowing for studies probing the effects of curvature on membrane organization. Model membranes can also be aligned on rigid substrates (e.g., using single crystal silicon or glass), forming smectic layers. Breaking the symmetry allows the experiment to probe orientation with respect to the bilayer normal, but also allows for a better signal-to-noise ratio.

2.1. Scattering

Elastic neutron and X-ray scattering have been used to determine the location of cholesterol in model membrane systems (Worcester and Franks, 1976; Franks and Lieb, 1979). A thorough list of literature determining the location cholesterol via scattering

can be found in (Marquardt and Harroun, 2014). Neutron scattering is a particularly powerful method for examining materials inherently rich in hydrogen, due to the neutron's ability to distinguish between hydrogen (^1H) and deuterium (^2H) atoms – e.g., 63 mol% of cholesterol is hydrogen. Individual or groups of hydrogen atoms within a molecule can selectively be replaced with deuterium, and because of the large difference in neutron scattering power between ^2H and ^1H , this substitution provides the necessary contrast needed to locate groups of interest. For example, the difference in measured scattering between ^2H -cholesterol and ^1H -cholesterol identifies the location and distribution of the ^2H label within the membrane (Harroun et al., 2006, 2008; Kučerka et al., 2010).

X-ray scattering is in many ways a complementary technique to neutron scattering. Compared to neutrons, X-ray sources offer far more intense beams, greater instrumental resolution, and wavelength spreads that are approximately 2 orders of magnitude finer (Marquardt et al., 2015b). These features allow for better data quality through significantly sharper diffraction peaks and a better signal-to-noise, especially at higher scattering angles, which translates in to higher resolution scattering density profiles of the membrane. For a detailed review of scattering on membranes see (Marquardt et al., 2015b).

2.2. Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectroscopy is a technique that can be used in a number of different ways, allowing for studies of cholesterol in model membranes under biologically relevant conditions. Like neutron scattering, isotopic enrichment is used to “highlight” a specific functional moiety in a biomolecule. For example, analysis of solid state ^2H NMR spectra of deuterated lipid analogs have yielded the fluctuation and orientation of acyl chain $\text{C}-^2\text{H}$ bonds with respect to the bilayer normal (Seelig, 1977; Davis, 1983). How cholesterol orients in different membranes has also been mapped with phospholipid analogs having selectively deuterated and perdeuterated acyl chains (Vist and Davis, 1990; Yasuda et al., 2014; Mihailescu et al., 2011), while cholesterol tilt in a membrane has been found to be responsive to lipid environments with deuterated analogs of cholesterol (Brzustowicz and Wassall, 1999; Shaikh et al., 2006).

The order parameter is the quantity most often measured in ^2H NMR studies. It is defined according to

$$S_{\text{CD}} = \frac{1}{2}(3\cos^2\beta - 1) \quad (1)$$

where β is the angle for a $\text{C}-^2\text{H}$ bond relative to the bilayer normal, which constitutes the axis of motional averaging for the reorientation of lipid molecules, and the angular brackets designate a time average (Seelig, 1977). The value of the order parameter describes the degree of anisotropy of molecular motion at the site of isotopic substitution. Values typically fall in the range $0 \leq S_{\text{CD}} \leq \frac{1}{2}$ for methylene segments labeled on a phospholipid chain, the lower limit corresponding to isotropic motion and the upper limit corresponding to fast axial rotation in the all-trans configuration. However, this interpretation must be modified in cases when the most probable orientation for a $\text{C}-^2\text{H}$ bond is not perpendicular to the bilayer normal. This is due to conformational constraints such as on labeled sites in the vicinity of a double bond in an unsaturated phospholipid chain (Seelig and Waespe-Sarcevic, 1978) or in the rigid steroid moiety of cholesterol (Marsan et al., 1999).

2.3. Molecular dynamics simulations

The molecular origin of how cholesterol affects lipids has been extensively probed by molecular dynamics (MD) simulations, both from a structural and thermodynamic perspective (Chiu et al.,

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