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Cholesterol-induced suppression of membrane elastic fluctuations at the atomistic level

Trivikram R. Molugu[a], Michael F. Brown[a,b,*]

[a] Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, USA
[b] Department of Physics, University of Arizona, Tucson, AZ 85721, USA

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ABSTRACT

Applications of solid-state NMR spectroscopy for investigating the influences of lipid-cholesterol interactions on membrane fluctuations are reviewed in this paper. Emphasis is placed on understanding the energy landscapes and fluctuations at an emergent atomistic level. Solid-state ^{2}H NMR spectroscopy directly measures residual quadrupolar couplings (RQCs) due to individual C–^{2}H labeled segments of the lipid molecules. Moreover, residual dipolar couplings (RDCs) of ^{13}C–^{1}H bonds are obtained in separated local-field NMR spectroscopy. The distributions of RQC or RDC values give nearly complete profiles of the order parameters as a function of acyl segment position. Measured equilibrium properties of glycerophospholipids and sphingolipids including their binary and tertiary mixtures with cholesterol show unequal mixing associated with liquid-ordered domains. The entropic loss upon addition of cholesterol to sphingolipids is less than for glycerophospholipids and may drive the formation of lipid rafts. In addition relaxation time measurements enable one to study the molecular dynamics over a wide time-scale range. For ^{2}H NMR the experimental spin-lattice (R_{1Z}) relaxation rates follow a theoretical square-law dependence on segmental order parameters (S_{CD}) due to collective slow dynamics over mesoscopic length scales. The functional dependence for the liquid-crystalline lipid membranes is indicative of viscoelastic properties as they emerge from atomistic-level interactions. A striking decrease in square-law slope upon addition of cholesterol denotes stiffening relative to the pure lipid bilayers that is diminished in the case of lanosterol. Measured equilibrium properties and relaxation rates infer opposite influences of cholesterol and detergents on collective dynamics and elasticity at an atomistic scale that potentially affects lipid raft formation in cellular membranes.

1. Introduction

Biomembranes display a tremendous complexity of lipids and proteins needed to perform the functions that cells require in the processes of life. Phospholipids are important components of these cell membranes and have a variety of roles; for example, the formation of lipid bilayers provides structural integrity, and gives an energy reservoir and source of second messenger precursors. Representative examples of glycerophospholipids and sterols found in cellular membranes are provided in Fig. 1. It can be seen that the polar head groups of the phospholipids differ in their size, capacity for hydrogen-bonding, and charge, whereas the nonpolar acyl chains vary in their length and degree and position of unsaturation. For cholesterol, the polar hydroxyl group orients the molecule at the aqueous interface, and the four fused rings together with the hydrocarbon chain constitute the nonpolar core. Most strikingly, the methyl substituents are confined to the molecularly rough β-face and the opposite α-face is smooth, in arresting contrast to its metabolic precursor lanosterol (Fig. 1). Various membrane lipids exhibit lyotropic liquid-crystalline phases under physiological conditions, involving solid-ordered (s_o), liquid-disordered (l_d), and liquid-ordered (l_o) phases. Attractive and repulsive forces for the membrane lipids entail both the polar headgroups and the non-polar moieties, and yield a substantial polymorphism with both lamellar and nonlamellar phases (Amazon and Feigenson, 2014; Brown, 1994; Feigenson, 2006, 2015; Gruner, 1989; Krepkiy et al., 2009; Phillips et al., 2009; Seddon, 1990; Seddon et al., 1997; van Meer et al., 2008; Zimmerberg and Gawrisch, 2006). Notably, the structural and dynamical properties of biomembranes are mediated by the lipid composition and interactions with the proteins, water, cholesterol,

* Corresponding author at: Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, USA.
E-mail address: mfbrown@u.arizona.edu (M.F. Brown).

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and surfactants (Brown and Chan, 2007; Coskun and Simons, 2011; Kaiser et al., 2009; Kaye et al., 2011; Kinnun et al., 2015; Leftin et al., 2014a; Mallikarjunaiah et al., 2011; Rheinstädter et al., 2004; Tyler et al., 2015). Membrane remodeling requires mesoscopic elastic deformations of the lipids (Kinnun et al., 2015) that can play a central role in biological functioning with regard to lipid-protein interactions, domain formation, and various nano- and micro-structures implicated in key cellular functions (Brown, 1997; Brown, 2012; Liang et al., 2014; Soubias et al., 2014, 2015; Teague et al., 2013).

On the other hand, it is known that many of the molecular species of lipids and proteins in membranes do not mix ideally (Ackerman and Feigenson, 2015; Amazon and Feigenson, 2014; Armstrong et al., 2012, 2013; Eriksson and Henriksson, 2007; Eriksson et al., 2006; Escribá et al., 2008; Goñi et al., 2008; Konyakhina and Feigenson, 2016). Cholesterol is one such component that may not be uniformly distributed in cellular membranes, whose distribution entails liquid-ordered raft-like domains (Ackerman and Feigenson, 2015; Amazon and Feigenson, 2014; Epand, 1998, 2006; Goñi et al., 2008; Konyakhina and Feigenson, 2016; Scheidt et al., 2013; Sodt et al., 2014). Such raft-like domains have garnered considerable attention as platforms for signaling proteins in cellular biology and pharmacology (Brown and London, 1998; Day and Kenworthy, 2015; Golebiewska and Scarlata, 2010; Klose et al., 2013; Simons and Gerl, 2010; Simons and Sampaio, 2011; Song et al., 2014; Surma et al., 2012). The concept that biomembranes are two-dimensional fluids with randomly distributed proteins (the fluid mosaic model) is challenged by the hypothesis that cellular membranes may contain such areas of lateral segregation (Bartels et al., 2008; Camley and Brown, 2010; Edidin, 2003; Feigenson, 2015; Keller and McConnell, 1999; Korade and Kenworthy, 2008; Leftin et al., 2013, 2014a; Lingwood and Simons, 2010; Meinhardt et al., 2013; Polozov and Gawrisch, 2006; Quinn, 2013; Simons and Gerl, 2010; Veatch et al., 2007; Wassall and Stillwell, 2009). For instance, raft-like domains are believed to occur in lipid systems with coexisting liquid-disordered (l_d) and liquid-ordered (l_o) phases. The l_d phase in these systems typically contains highly unsaturated lipids with a low phase transition temperature, whereas the l_o phase predominantly consists of saturated glycerophospholipids or sphingolipid components and cholesterol (Simons and Toomre, 2000). Moreover, certain proteins are endowed with the ability to interact with cholesterol via a cholesterol recognition/interaction amino acid consensus (CRAC) sequence motif (Baier et al., 2011; Fantini and Barrantes, 2013; Greenwood et al., 2008). In some cases, they include cationic clusters that allow interactions with phosphatidylinositol(4,5)bis-phosphate (PIP_2) in a cholesterol-dependent manner. Such CRAC domains are found in the *Rhodopsin* (Family A) G–protein-coupled receptors (GPCRs) (Jafurulla et al., 2011), and moreover post-translational lipid modifications (Vogel et al., 2007; Weise et al., 2013) can promote sequestration into cholesterol-rich regions or microdomains.

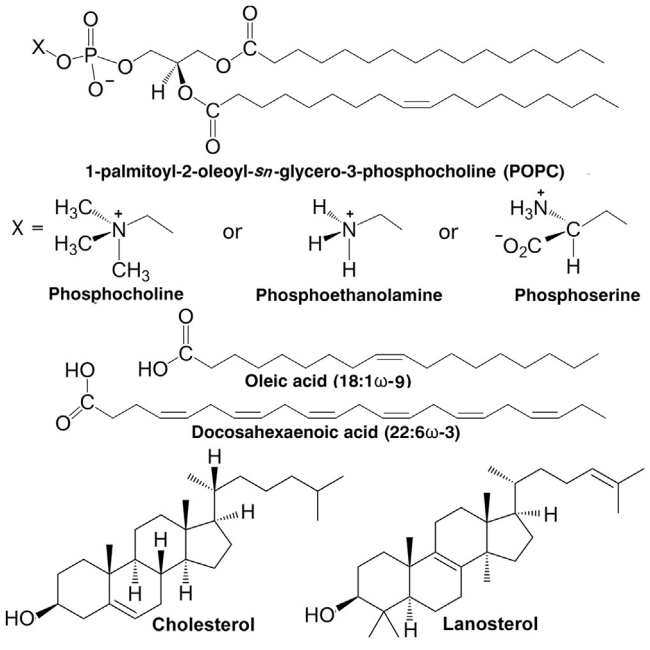

Fig. 1. Chemical structures of representative glycerophospholipids, cholesterol, and lanosterol: the polar head groups vary in size, hydrogen-bonding, and charge. Examples are shown for zwitterionic phosphocholine (PC) and phosphoethanolamine (PE) head groups, and for the anionic phosphoserine (PS) head group. Non-polar acyl chains differ in length and degree of unsaturation, as illustrated by oleic acid (18:1ω-9) and docosahexaenoic acid (22:6ω-3). Cholesterol differs in the absence of methylation at the α-face relative to its biological precursor lanosterol.

Improving our understanding of complex lipid mixtures is clearly an important target for research in pharmaceutical and physical chemistry, as well as in cellular biology. Various biophysical methods have been used to study lipid-cholesterol interactions, including electron spin resonance (ESR) (Cheng et al., 2014; Delmelle et al., 1980; Hubbell and McConnell, 1971; Lai and Freed, 2014; Manukovsky et al., 2013; Semer and Gelerinter, 1979; Stepien et al., 2015; Vitiello et al., 2015; Williams et al., 2013), Raman (Lippert and Peticolas, 1971; Mendelsohn, 1972; Tantipolphan et al., 2006), Fourier transform infrared (FT-IR) (Umemura et al., 1980), fluorescence spectroscopy (Xu and London, 2000; Yasuda et al., 2015a), atomic force microscopy (AFM), multidimensional NMR spectroscopy (Holland and Alam, 2006; Leftin et al., 2013, 2014b; Warschawski and Devaux, 2005), solid-state ^{2}H nuclear magnetic resonance (NMR) (Bartels et al., 2008; Brown, 1990; Bunge et al., 2008; Martinez et al., 2002, 2004; Matsumori et al., 2012; Stockton et al., 1976; Vogel et al., 2016; Weisz et al., 1992; Yasuda et al., 2015a), and neutron diffraction methods (Armstrong et al., 2014; Toppozini et al., 2014). However, a thorough understanding of the physical basis for these observations in relation to the intricate lipid compositions of many biological membranes to some extent remains an enigma (Feigenson, 2015; McConnell, 2005; Meinhardt et al., 2013; Sodt et al., 2014; Stanich et al., 2013). Recent developments in understanding lipid-cholesterol interactions in model membrane systems are covered in this article, including implications for cellular function as seen by solid-state nuclear magnetic resonance (NMR) spectroscopy. First, we give a brief introduction to lipid systems and solid-state NMR methods, and next we explain how solid-state NMR technology is applied for obtaining membrane structural and dynamical properties. We discuss the interactions of the phospholipids with cholesterol and lanosterol in model membranes, including the role of configurational entropy in lipid raft formation. Emphasis is placed on how the average material properties emerge from the atomistic level interactions in lipid bilayers as investigated by combining NMR spectroscopy with relaxation methods (Brown et al., 2001; Martinez et al., 2002, 2004).

2. Solid-state NMR spectroscopy of biomembranes

2.1. Deuterium solid-state NMR spectroscopy

Solid-state ^{2}H NMR spectroscopy (Brown, 1996; Kinnun et al., 2013; Leftin and Brown, 2011; Leftin et al., 2014b) of deuterated lipid molecules offers a versatile and non-invasive method for studying molecular organization within membranes. Isotopic substitution of ^{2}H for ^{1}H constitutes a minimal structural