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Molecular origins of bending rigidity in lipids with isolated and conjugated double bonds: The effect of cholesterol

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ABSTRACT

We examine the effects of cholesterol (Chol) on the mechanical properties of membranes consisting of 16:0/18:1 POPC lipid and of lipids with conjugated linoleic acid (CLA), *cis-9/trans-11* CLA (C9T11) and *trans-10/cis-12* CLA (T10C12). Atomistic molecular dynamics (MD) simulations of POPC–Chol and CLA-Chol mixtures at various Chol concentrations are employed within a recently developed and validated computational methodology (Khelashvili et al., 2013) that calculates from MD trajectories the bending rigidity (K_C) for these systems. We have found that the addition of 30% Chol stiffens POPC lipid membranes much more significantly (2.3-fold) than it does C9T11 (1.5-fold) or T10C12 (1.75-fold) lipid bilayers. Extensive comparative structural analysis of the simulated mixtures supports a molecular mechanism for the differential effects of cholesterol, whereby the sterol molecules tilt more significantly in CLA membranes where they also insert deeper inside the hydrocarbon core. The observed distinct arrangement of Chol molecules in CLA and POPC bilayers, in turn, is dictated by the interplay between the specific location of the *trans* double bond in the two CLA lipid isomers and the preferential interaction of the rigid Chol ring with the saturated segments of the lipid tails. The simulations and analysis described in this paper provide novel insights into the specific modes of molecular interaction in bilayers composed of mixtures of Chol and unsaturated lipids that drive emergent macroscopic properties, such as the membrane's bending modulus.

1. Introduction

A wealth of experimental (see Pan et al., 2009, 2008; Mouritsen and Zuckermann, 2004; Veatch, 2007; Marsh, 2009; Elson et al., 2010; Korade and Kenworthy, 2008; Gracia et al., 2010 and citations therein) and computational studies (see Chiu et al., 2001; Tu et al., 1998; Smondyrev and Berkowitz, 1999; Pasenkiewicz-Gierula et al., 2000; Scott, 2002; Pandit et al., 2004, 2007; Bewrkowitz, 2009; Róg et al., 2009; Pandit and Scott, 2009; Khelashvili et al., 2010a; Khelashvili and Harries, 2013a to cite a few) reported over the past decade have established that cholesterol (Chol), a key component of mammalian cell plasma membrane, interacts more strongly with saturated chain phospholipids (PCs), compared to lipids with double bonds in their chains. This selective behavior allows Chol to differentially regulate structural, thermodynamic, and mechanical properties of saturated and unsaturated lipid membranes. Especially intriguing is the differential effect of Chol on the mechanical properties of bilayers composed of lipids of different levels of hydrocarbon chain saturation (Pan et al., 2009, 2008; Gracia et al., 2010). Experimental and modeling evidences suggest that the addition of up to 40% Chol does not change the bending rigidity, K_C, of membranes consisting of mono-unsaturated dioleoylphosphocholine (DOPC) lipids. But similar amounts of Chol in lipid bilayers containing saturated lipids, such as dimyristoylphosphatidylcholine (DMPC) or 18:0/18:1 sphingomyelin (SM), raises K_C by as much as 5-fold (Pan et al., 2009, 2008; Gracia et al., 2010). To uncover molecular origins of these experimental findings, we recently developed a computational approach (Khelashvili et al., 2013) that utilizes molecular dynamics (MD) trajectories to calculate the fluctuations in tilt of all the membrane components, and of the splay of all possible pairs of molecules in the membrane. Our earlier work suggests that tilt and splay deformations are important contributors to the overall elasticity of the mixed lipid membranes, and that they can account for the experimentally established differences between the liquid ordered SM/Chol bilayers and fluid DOPC/Chol bilayers (Khelashvili and Harries, 2013a).

Experiments and simulations have shown that the addition of cholesterol to membranes composed of mixed (hybrid)

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phospholipids containing one saturated and one monounsaturated hydrocarbon chain (i.e. POPC, 16:0–18:1 phosphoryl-oleyol-phosphatidylcholine), leads to mechanical properties that are intermediate to those measured in the liquid ordered and fluid lipid membranes (Arriaga et al., 2009; Pandit et al., 2008). On the molecular level, the mechanistic origins of these effects can be understood in the context of the two faces of the Chol tetracyclic ring – the rough face where methyl groups protrude, and the opposite, smooth face of the ring (Pandit et al., 2004). Molecular simulations have shown that the saturated tails of PC lipids predominantly associate with the smooth face, leaving the rough face exposed to a greater extent to the unsaturated chains (Pandit et al., 2004; Martinez-Seara et al., 2008). MD computations also revealed that the effect of Chol on the properties of lipid bilayers is determined not only by the simple presence of the unsaturation in the lipid tails, but also by the location of the double bond (Martinez-Seara et al., 2008) along the chain. By systematically varying the position of the double bond along the acyl chains in MD simulations, Martinez-Seara et al. (2008) found that, when the double bond was not in contact with the cholesterol ring, the membrane properties were closest to those of the saturated bilayer. However, any contact of the double bond in the lipid tails with the rough side of Chol ring produced increased membrane disordering.

The effects of Chol on lipids possessing conjugated double bonds are even more complex and have generally been less extensively examined. Interesting lipid species in this context are isomers of linoleic acid (conjugated linoleic acids, CLA). CLA lipids are found in dairy products and meats, and are associated with numerous physiological effects in laboratory animals (Pariza, 2004; Wahle et al., 2004; Beruly, 2002). While at least 16 isomers of CLA are found in various food products, two isomers, with *cis* 9 *trans* 11 (80%) (C9T11) and *trans* 10 *cis* 12 (10%) (T10C12) conjugated double bonds (Fig. 1) are predominant in dairy products. The two isomers differ significantly from each other in their biological effects (Wahle et al., 2004; Churruca et al., 2009; House et al., 2005; Taylor and Zahradka, 2004), suggesting that the location of the *cis* and *trans* double bonds in the acyl chain is critical in physiological contexts. The mechanism of action and the molecular basis for the divergent effects of CLA isomers are not well understood. In earlier computational work (Zhao et al., 2011a,b), we used atomistic MD simulations to address the hypothesis that the differential effect of the two CLA isomers is due to differential intermolecular interactions within the lipid membranes both with and without cholesterol. Surprisingly, despite of the unique distribution of double bonds in the acyl chain of C9T11 and T10C12 isomers, we found only subtle differences in the structural properties of these CLA-containing membranes, both in the presence and in the absence of Chol, with differences arising primarily in the localized radial distributions of CLA atoms around Chol molecules (Zhao et al., 2011).

In the present work, we have revisited the MD simulation trajectories of Zhao et al. (2011a,b), for C9T11/Chol and T10C12/Chol binary mixtures. The purpose of the current study was to calculate mechanical properties of these bilayers. Specifically, we applied our new methodology (Khelashvili et al., 2013), to compare the bending rigidity, K_C, for the C9T11/Chol and T10C12/Chol systems. Furthermore, since C9T11 lipid structurally differs from POPC only in its additional *trans* double bond (Fig. 1), we also generated and analyzed MD trajectories for POPC/Chol mixtures to compare structural and mechanical properties of POPC/Chol and CLA/Chol bilayers. The results suggest that while small amounts (up to 10%) of Chol nearly uniformly affect the bending modulus of CLA and POPC lipid membranes, in the presence of a high concentration of Chol (30%) these bilayers stiffen differently. The largest increase in K_C was observed in POPC membranes, and the smallest increase – in C9T11 bilayers. In this paper we describe these findings, and interpret them in the context of differential intermolecular interactions observed in the simulations. Our results provide novel mechanistic insights regarding the distinct effects of cholesterol on lipids with isolated and conjugated double bonds.

2. Methods

2.1. Molecular dynamics simulations

The atomistic MD trajectories of C9T11/Chol and T10C12/Chol systems analyzed in this work are described in our earlier papers (Zhao et al., 2011a,b). New MD simulations were performed for hydrated POPC/Chol mixtures at 0%, 10% and 30% Chol concentrations and at a temperature of 298 K (corresponding to the temperature at which CLA/Chol mixtures were simulated in the earlier studies (Zhao et al., 2011a,b)). For the pure POPC bilayer, an equilibrated 50 ns trajectory was generated by extending previous 70 ns simulations of POPC membranes containing 128 lipids and 3552 water molecules (Pandit et al., 2007). For cholesterol-containing POPC mixtures, we started with previously equilibrated 200-lipid (including Chol) size C9T11/Chol bilayers at 10% and 30% sterol concentrations (Zhao et al., 2011b). The C9T11 lipids were transformed into POPC and 200 ps MD simulations were performed on each system at 500 K to ensure sufficient disordering of the hydrocarbon chains. The temperature was then lowered to the target 298 K in steps of 50 K. At each temperature step, 200 ps MD simulations were performed on each system. After this equilibration phase, long (140 ns) trajectories were accumulated from which the last 70 ns segment was used for the analysis.

For all the simulations we used the GROMACS simulation suite (Hess et al., 2008) with the LINCS algorithm (Hess et al., 1997) to constrain all bond lengths allowing for 2 fs time steps. Periodic boundary conditions were applied in all three dimensions, and long-range electrostatics were calculated using the Particle-Mesh-Ewald algorithm (Essmann et al., 1995). A cutoff of 16 Å was employed for van der Waals interactions. The systems were simulated in an NPT ensemble. A constant pressure of 1 atm was maintained using the Parrinello–Rahman semi-isotropic pressure coupling scheme (Nose and Klein, 1983; Parrinello and Rahman, 1981). The temperature was controlled by the Noose–Hoover temperature coupling method (Evans and Holihan, 1985). The recently improved united atom GROMOS96 43A1-S3 force-field was utilized throughout (Chiu et al., 2009). This parameter set, described in detail in Chiu et al. (2009), has been already successfully tested for various lipid membrane systems, including POPC/Chol mixtures (Pandit et al., 2007), and is publicly available from http://www.nanoconductor.org.

2.2. Trajectory analysis

2.2.1. Methodology to extract bending rigidities from atomistic simulations

Bending rigidities (K_C) of the simulated bilayers were calculated using a recently-developed analysis tool (Khelashvili and Harries, 2013a; Khelashvili et al., 2013). Briefly, the method uses equilibrated MD trajectories (where the model membrane patch satisfies its experimentally validated structural properties) as input, and from these calculates fluctuations in splay of all possible pairs of molecules in the lipid membrane (i.e. lipid/lipid, Chol/Chol, lipid/Chol). Corresponding splay angle probability distributions ($P(\alpha)$, α being the splay angle) are then constructed. These probability distributions, in turn, allow us to derive the splay modulus, χ_{12}, for each molecular pair by performing a quadratic fit in the interval of small α angles to the function $\mathrm{PMF}(\alpha) = -k_BT \ln[P(\alpha)/\sin\alpha]$, which describes the two-molecule potential of mean force (PMF) for splay (Khelashvili et al., 2013). By weighting each splay