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Molecular dynamic simulation study of cholesterol and conjugated double bonds in lipid bilayers

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ABSTRACT

Conjugated linoleic acids (CLA) are found naturally in dairy products. Two isomers of CLA, that differ only in the location of *cis* and *trans* double bonds, are found to have distinct and different biological effects. The *cis* 9 *trans* 11 (C9T11) isomer is believed to have anti-carcinogenic effects, while the *trans* 10 *cis* 12 (T10C12) isomer is believed to be associated with anti-obesity effects. In this paper we extend earlier molecular dynamics (MD) simulations of pure CLA–phosphatidylcholine bilayers to investigate the comparative effects of cholesterol on bilayers composed of the two respective isomers. Simulations of phosphatidylcholine lipid bilayers in which the sn-2 chains contained one of the two isomers of CLA were performed in which, for each isomer, the simulated bilayers contained 10% and 30% cholesterol (Chol). From MD trajectories we calculate and compare structural properties of the bilayers, including areas per molecule, thickness of bilayers, tilt angle of cholesterols, order parameter profiles, and one and two-dimensional radial distribution function (RDF), as functions of Chol concentration. While the structural effect of cholesterol is approximately the same for both isomers, we find differences at an atomistic level in order parameter profiles and in two-dimensional radial distribution functions.

1. Introduction

Isomers of linoleic acid that contain conjugated double bonds (conjugated linoleic acids, CLA) are found in dairy products and meats, and are associated with anti-carcinogenic effects, reduction in adiposity, and improvement of immune function (Pariza, 2004; Wahle et al., 2004; Belury, 2002) in experimental animals. Sixteen isomers of CLA have been identified in natural products, but two, *cis* 9 *trans* 11 (80%) and *trans* 10 *cis* 12 isomers (10%) dominate in dairy products. The two isomers differ significantly from each other in their biological effects (Churruca et al., 2009; Wahle et al., 2004; House et al., 2005; Taylor and Zahradka, 2004), suggesting that the location of the *cis* and *trans* double bonds in the acyl chain plays a critical role in the biological function. The mechanism of action, and the molecular basis for the divergent effects of CLA isomers remain unclear, despite these and other studies in experimental animals and cultured cells. Although it is possible that at least some of the effects of CLA are through modification of membrane structure and function (Stulnig et al., 2001; Liu et al., 1994; Li et al., 2005; Ma et al., 2004) our earlier MD study of pure bilayers composed of the two isomers revealed very little difference in structural and dynamical properties (Zhao et al., 2011). Since Cholesterol (Chol) comprises approximately 25–40 mol% of the lipid portion of the mammalian plasma membranes, and is thought to modulate the physico-chemical properties required for cell viability and proliferation, it is natural to next consider the interactions of the two isomers with Chol. Numerous experimental studies have demonstrated that Chol also reduces the passive permeability of membranes, increases membrane mechanical strength, and modulates membrane enzymes (Yeagle, 1993). Because of the unique distribution of double bonds in the acyl chain, the CLA-containing phospholipids may interact with cholesterol differently from other phospholipids, resulting in the alteration of the overall function of the membrane-associated receptors, enzymes, and channels. To test this hypothesis, we conducted a molecular dynamics (MD) simulation study of bilayers of phosphatidylcholine (PC), which contained a palmitic acid at sn-1 position, and *cis* 9 *trans* 11 CLA, or *trans* 10 *cis* 12 CLA, at the sn-2 position (hereafter referred to as CLA–PC bilayers). We incorporated 10 and 30 mol% of cholesterol into the PC bilayer and studied the atomic level interactions between PC and cholesterol in the bilayer.

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Molecular dynamics simulations of phospholipid bilayers are widely used to study the physical properties of specific bilayers with different lipid compositions, cholesterol, or embedded peptide/proteins and achieve good agreement with known experimental properties. Simulations can add atomic level insight into microscopic interactions between lipids, cholesterol, and water (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). Although the effect of isolated double bonds has been studied by MD simulation (Bachar et al., 2004; Martinez-Seara et al., 2008a,b, 2008c), no studies have been conducted on the effect of conjugated double bonds on phospholipid bilayers containing cholesterol. In this paper, we focus on linoleic acids *cis* 9 *trans* 11 and *trans* 10 *cis* 12 as these two CLA isomers bear important and interesting physiologically functions. We extend our earlier work (Zhao et al., 2011) to study the potential interactions of cholesterol with conjugated double bonds brought to polyunsaturated lipid bilayer. We study the effect of cholesterol on the structure of CLA–PC bilayers of both isomers at different cholesterol concentrations, and we examine CLA–cholesterol interactions in atomistic detail.

2. MD method

This paper describes MD simulations that follow on earlier simulations of pure CLA–PC bilayers of both *cis* 9 *trans* 11 and *trans* 10 *cis* 12 isomers (Zhao et al., 2011). Here we summarize simulation methodology and force fields that are described in more detail in the earlier paper. The force-field parameters used for this work were taken from the newly developed GROMOS 43A1-S3 parameter set (Chiu et al., 2009). For the CLA simulations, we added additional dihedral parameters for dihedrals that contained one or both of the CLA conjugated double bonds to the 43A1-S3 force field. The calculations of these parameters was done following the procedure used for the 43A1-S3 force field (Chiu et al., 2009). In our earlier paper (Zhao et al., 2011) we provided a detailed description of the procedure. For bonding parameters not part of the –CH=CH–CH=CH– dihedral we used existing parameters in the 43A1-S3 force field, including parameters for the dihedrals that are next to, but do not include, double bond carbons. In supplemental material we provide the energy profiles for double bonds that verify that we have included skew states as emphasized by the work of Bachar et al. (2004). All force field parameters used in this paper can be uploaded from URLs given in supplemental information.

Fig. 1 shows the structures and numbering schemes for each of the CLA–PC isomers and for cholesterol. The total number of lipids in each bilayer was 200, or 100 per leaflet. The simulations were constructed as described earlier (Zhao et al., 2011). After building CLA–PC lipids for both C9T11 and T10C12 from a POPC molecular structure, bilayers were constructed by placement of 100 CLA–PC molecules per leaflet. Then, randomly selected CLA–PC molecules were replaced with Chol molecules for each simulated Chol concentration. The bilayers were hydrated with 6800 SPC-E water molecules, for a hydration level of 34 water molecules per lipid. A 200 ps MD simulation was performed on each system at 500 K. This was done to ensure proper disordering of the hydrocarbon chains. Temperatures were then brought down to the target temperature of 298 K in steps of 50 K. At each temperature step a small 200 ps MD simulation was performed on each system. Next, the systems were simulated for 180 ns of MD with regeneration of velocities from a Maxwellian distribution at 298 K after every 200 ps. Then 100 ns continuous MD trajectories were generated and used for analysis. To verify that simulation results were not dependent on initial conditions, we built two additional systems with different initial bilayer configurations, including one larger system (400 lipids), and

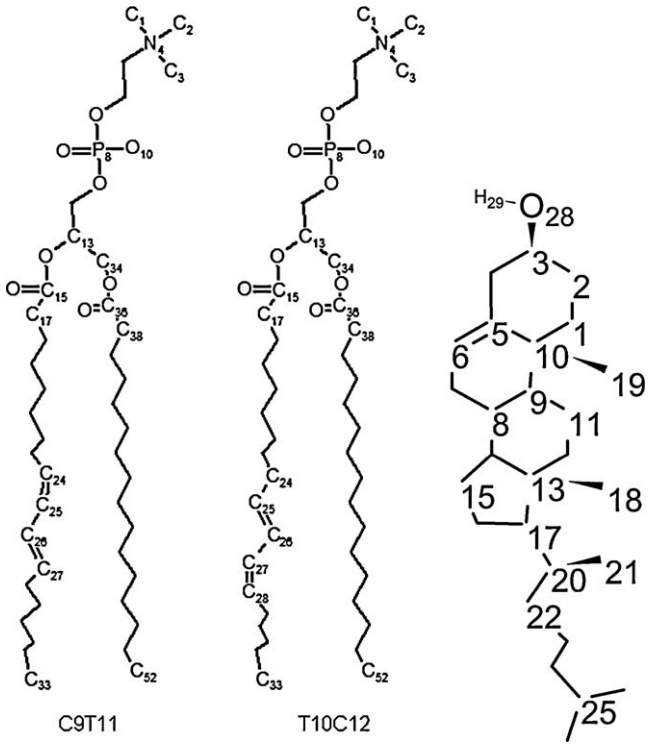

Fig. 1. Structures of CLA–PCs and Chol molecules, with the numbering scheme used in the simulations.

we used a longer annealing time. All simulations were performed using the GROMACS 4.05 package (Hess et al., 2008). The LINCS algorithm was used to constrain all the bonds in the system (Hess et al., 1997). The integration time step was 2 fs. Periodic boundary conditions were applied in all three dimensions and long range electrostatics were calculated using the PME algorithm (Essmann et al., 1995) with a real space cutoff of 10 Å. A cutoff of 16 Å was employed for van der Waals interactions, as is required for the 43A1-S3 force field. All simulations were performed at 298 K using the Nose–Hoover temperature coupling scheme. The system was simulated in an NPT ensemble using the Parrinello–Rahman pressure coupling scheme at a constant pressure of 1 bar. The systems were energy minimized to remove bad contacts. Calculated properties from those runs are quantitatively identical to results presented here. Throughout the simulations we monitored the dimensions of the simulation cells. The area per lipid in all simulations was stable, with fluctuations of ± 2.5 Å^2/mol, as shown in Fig. 2.

3. MD results and discussion

3.1. Structural properties

Table 1 lists the values of the simulated areas per molecule for both isomers at 0% (from Zhao et al., 2011), 10% and 30% Chol. The molecular areas were calculated by dividing the simulation box area by the number of lipids per leaflet. Corrections for undulation fluctuations (Braun et al., 2011) were not made as the system sizes are sufficiently small that these terms should be small, and in any case should be the same for all systems we simulated. For binary CLA–Chol simulations, one could alternatively calculate partial molecular areas for CLA and Chol separately using the method described by Edholm and Nagle (2005) but this requires at least 3–4 Chol concentrations, and the partial areas areas not measured experimentally. Thus Fig. 2 shows time traces, over the duration of the production runs, of the area per molecule calculated from the