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Ceramide–lipid interactions studied by MD simulations and solid-state NMR

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ABSTRACT

Ceramides play a key modulatory role in many cellular processes, which results from their effect on the structure and dynamics of biological membranes. In this study, we investigate the influence of C16-ceramide (C16) on the biophysical properties of DMPC lipid bilayers using solid-state NMR and atomistic molecular dynamics (MD) simulations. MD simulations and NMR measurements were carried out for a pure DMPC bilayer and for a 20% DMPC–C16 mixture. Calculated key structural properties, namely area per lipid, chain order parameters, and mass density profiles, indicate that C16 has an ordering effect on the DMPC bilayer. Furthermore, the simulations predict that specific hydrogen-bonds form between DMPC and C16 molecules. Multi-nuclear solid-state NMR was used to verify these theoretical predictions. Chain order parameters extracted from $^{13}C-{}^{1}H$ dipole couplings were measured for both lipid and ceramide and follow the trend suggested by the MD simulations. Furthermore, ^{1}H-MAS NMR experiments showed a direct contact between ceramide and lipids.

1. Introduction

Ceramides are important bioactive sphingolipids that act as second messengers in cell signal transduction [1,2]. Hence, they play important roles in facilitating and modulating many cell processes including apoptosis, differentiation, and senescence [3–6]. The mechanism that underlies the physiological role of ceramides has not been completely clarified yet, and thus remains a subject of active research. Recent studies have indicated that this modulatory role is related to the effect that ceramides have on cellular membranes, especially in regard to lipid rafts [7]. Rafts are rigidified membrane domains, formed by clustering of sphingomyelin with cholesterol. In these rafts, cholesterol fills the hydrophobic space inside the sphingomyelin bilayer, resulting in a more compact membrane [8]. Several studies have indicated that in the course of signal-transduction processes rafts become larger and enriched in ceramide, as a result of the hydrolysis of sphingomyelin, catalyzed by the enzyme sphingomyelinase (SMase) [9]. These domains are thought to diffuse dynamically along the membrane, and form larger macro-domains called ceramide-enriched platforms. It has been proposed that ceramide macro-domains are important to help receptors to transmit signals across the membrane [10–13].

Ceramides have a fatty-acid chain that is connected to a sphingosine base by an amide bond. The fatty-acid chain lengths found in nature vary from 2 to 28 carbon atoms, in both saturated and unsaturated forms. Short-chain ceramides contain acyl chains with less than 8–10 carbon atoms, while long-chain ceramides may consist of more than 10–12 carbons [14,15]. Free ceramides occur mainly in the stratum corneum [16], which is the outermost layer of the skin. They are also found in membranes in small amounts, as metabolites or precursors of other compounds [1,2]. Several published studies have investigated how ceramides behave and organize themselves in membranes [14,17–26]. Lateral phase separation into a ceramide-dominated gel and a phospholipid-dominated liquid crystalline phase have been observed in some cases [17,18,26]. Studies on cell cultures have also indicated that ceramides located in cell membranes can form domains [19].

Several methods have been applied to understand the influence of ceramides on the physical properties of lipid bilayers. Studies based on the fluorescence anisotropy have indicated that phospholipids tend to be more ordered in the presence of ceramides [20,23,25]; similar observations have been made using NMR, specifically for ceramides with 16-carbon chains, or C16-ceramide, in DPPC and POPC bilayers [21,22,27].

The latter studies indicated that C16 induces a phase separation only in the gel phase; above the lipid main phase transition, C16 diffuses into the phospholipid bilayer and promotes the ordering of the lipid chains. This ordering effect has also been observed in molecular dynamics (MD) simulations of POPC-C16 mixtures [24]. Studies based on both NMR and MD simulations have therefore shown that C16 influences the physical properties of several phospholipid membranes. However, not much is known about the direct interactions formed between lipids and C16.

Here, we characterize the effect of C16-ceramide on DMPC lipid bilayers. DMPC is a saturated phospholipid with 14 carbon atoms in each fatty-acid chain (Fig. 1a). This makes DMPC a good match for C16

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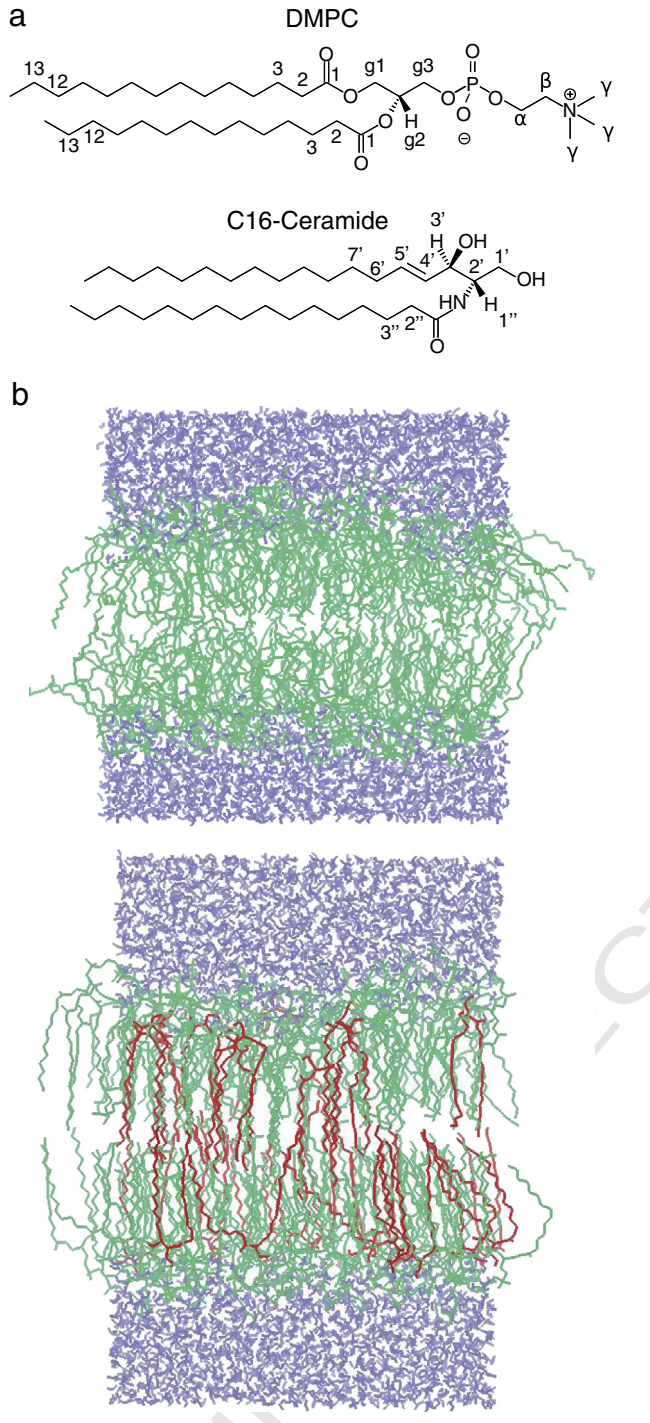

Fig. 1. (a) Chemical structures of DMPC and C16-ceramide. (b) Snapshots of the MD simulations of pure DMPC (top) and mixed DMPC/C16 (20%) bilayers (bottom), at 100 ns. The DMPC bilayer contained 128 lipid molecules (green) and 3655 water molecules (blue). The mixed DMPC/C16 bilayer contained 102 lipid molecules, 26 C16 molecules (red) and 3655 water molecules. C16 is fully incorporated into the DMPC bilayer. The temperature of the simulations was 310 K.

(Fig. 1a). DMPC is also often used as a model lipid in solid-state NMR studies, and has been extensively characterized. Therefore this lipid type is an ideal choice for ceramide–lipid interaction studies. Furthermore, C16 is known to be one of the main components of the natural ceramide pool [2]. Our methodological approach is based on atomistic MD simulations, by which we gain specific insights that are verified and expanded by solid-state NMR experiments. In the following, we show that C16 causes a significant ordering effect on DMPC membranes, which is caused by the formation of H-bonded C16–DMPC complexes dissolved within fluid lipid bilayers. Experimental evidence derives from ^{1}H-MAS-NMR, and ^{1}H-NOESY-MAS measurements, as well as from the determination of dipolar C – H order parameters.

2. Materials and methods

2.1. Sample preparation

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-d54-*sn*-glycero-3-phosphocholine-1,1,2,2-d4-N,N,N-trimethyl-d9 (DMPC-d_{67}) and N-palmitoyl-D-*erythro*-sphingosine (C16-ceramide; C16) were obtained from Avanti Polar Lipids and used without further purification. Liposome samples were prepared following standard procedures [28]. The appropriate amounts of DMPC and C16 (usually 20:4 mg/mg) were co-dissolved in chloroform (5 mL) and shaken until a clear solution was obtained. The bulk solvent was evaporated under a stream of nitrogen gas and subsequently dried overnight in a rotary evaporator under high vacuum, resulting in a dry lipid-substrate film. Samples were rehydrated slowly inside a round-bottom flask by adding 1 mL of water under slow rotation until a multi-lamellar dispersion was formed. Hydrated samples were transferred to an Eppendorf tube and pelleted by centrifugation for 1 h at 16,060 g. Bulk water was removed and samples were dehydrated by lyophilisation followed by another rehydration step through direct addition of 10 μL of water. In our experience, this additional step, which hydrates the bilayer sufficiently but avoids bulk water, is required to obtain the best-resolved MAS-NMR spectra of lipid vesicles. Four freeze–thaw cycles were performed between temperatures that were below and above the transition temperature of the lipids, to obtain more homogeneous liposomes. Finally, samples were transferred to a Bruker 3.2 mm MAS-NMR rotor.

2.2. MD simulations

A pure DMPC bilayer and a DMPC bilayer containing 20% C16 were simulated for a total of 100 ns each. The pure DMPC bilayer consisted of 128 lipids and 3655 water molecules. Initial coordinates were kindly provided by Tieleman et al. (http://wcm.ucalgary.ca/tieleman/downloads). To model the mixed bilayer, 26 DMPC molecules were randomly chosen after a 10 ns equilibration of the pure bilayer, and replaced by ceramide molecules. The initial position of the ceramide molecules in the bilayer was such that their centers of mass coincided with those of the replaced DMPC lipids. Energy minimizations were used to remove steric clashes prior to the molecular dynamics simulation.

The force-field parameters used for DMPC are those developed by Berger et al. [29]. For the ceramide acyl chains, we adapted the bonded and non-bonded interaction parameters used for DMPC, and for the ceramide head group we adapted parameters for equivalent chemical groups from the GROMOS87 protein force-field (Fig. S1) [30]. The SPC water model was used. All simulations were performed using GROMACS 4.0.3 [30] using LINCS to constraint bond-lengths involving hydrogen atoms.

Periodic boundary conditions were applied in three dimensions. Short-range Coulomb and van-der-Waals interactions were cut-off at 11 Å. The Particle-Mesh-Ewald algorithm was used to calculate long-range electrostatic interactions (no dispersion correction was used). Neighbor lists were updated every 5 integration steps using the grid method. Both simulations were carried out at a temperature of 310 K (i.e. above the DMPC gel-to-liquid crystalline transition temperature of ~297 K) and 1 bar of pressure, using the Nosé–Hoover thermostat and a semi-isotropic Parrinello–Rahman barostat. The time constants of the thermostat and barostat were 0.1 and 1 ps, respectively.

From the MD trajectories it is possible to quantify several observables that characterize the structure and dynamics of the membrane. Bilayer widths and mass-density profiles, carbon–hydrogen (C – H) order parameters, and hydrogen-bonding patterns between ceramide

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