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Membrane properties of cholesterol analogs with an unbranched aliphatic side chain

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ABSTRACT

The interactions between cholesterol and other membrane molecules determine important membrane properties. It was shown that even small changes in the molecular structure of cholesterol have a crucial influence on these interactions. We recently reported that in addition to alterations in the tetracyclic ring structure, the *iso*-branched side chain of cholesterol also has a significant impact on membrane properties (Scheidt et al., 2013). Here we used synthetic cholesterol analogs to investigate the influence of an unbranched aliphatic side chain of different length. The ^{2}H NMR order parameter of the phospholipid chains and therefore the molecular packing of the phospholipid molecules shows a significant dependence on the sterol's alkyl side chain length, while, membrane permeation studied by a dithionite ion permeation assay and lateral diffusion measured by ^{1}H MAS pulsed field gradient NMR are less influenced. To achieve the same molecular packing effect similar to that of an *iso*-branched aliphatic side chain, a longer unbranched side chain (*n*-dodecyl instead of *n*-octyl) at C17 of cholesterol is required. Obviously, sterols having a branched *iso*-alkyl chain with two terminal methyl groups exhibit altered cholesterol–phospholipid interactions compared to analogous molecules with a straight unbranched chain.

1. Introduction

The properties and therefore the functions of cellular membranes are determined by a multitude of physical interactions between many different molecules, which form nature's most important interface. Especially, the interactions between cholesterol and phospholipids and also membrane proteins have been the objective of an enormous number of studies, since cholesterol plays a major role in modifying membrane properties. In fungi and plants, other sterols such as ergosterol, campesterol, sitosterol, and stigmasterol fulfil the role of cholesterol. Special attention has been paid to the influence of various sterols on domain formation and the raft hypothesis (Xu et al., 2001; Leslie, 2011; Simons and Ikonen, 1997; Marsh, 2009). Specific interactions between cholesterol and phospholipid molecules, in particular with their saturated acyl chains, induce tight molecular packing by ordering the acyl chain of the phospholipids (Oldfield et al., 1978). The increased packing density changes essential membrane properties such as membrane permeability (Demel et al., 1972; Huster et al., 1997) and lateral lipid diffusion (Filippov et al., 2003; Scheidt et al., 2005). The membrane interactions of cholesterol are mostly described as attractive van der Waals forces between the saturated acyl chain of phospholipids and the ring system of cholesterol (Wang et al., 2004; Davies et al., 1990). Moreover, hydrogen bonds between the 3β-hydroxyl group of cholesterol and the neighboring phospholipids play an important role (Soubias et al., 2004). Several studies have shown that even small changes in the cholesterol ring system disturb these interactions and change the membrane properties of cholesterol, sometimes dramatically (Demel et al., 1972; Xu and London, 2000; Wang et al., 2004; Scheidt et al., 2003; Huster et al., 2005; Endress et al., 2002; Milles et al., 2013; Shrivastava et al., 2008; Benesch and McElhaney, 2014).

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; NBD-PE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl); PFG MAS NMR, pulsed field gradient magic angle spinning nuclear magnetic resonance; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

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Recently, we demonstrated that not only the tetracyclic ring system of cholesterol, but surprisingly, also the length of the *iso*-branched aliphatic side chain of cholesterol significantly affects membrane properties (Scheidt et al., 2013). This study indicated that the *iso*-branched side chain of cholesterol is responsible for 40–60% of the ordering effect of cholesterol. Therefore, the interactions between the alkyl side chain of cholesterol with the neighboring molecules should be considered in order to fully understand the impact of cholesterol on various properties of lipid membranes.

Here, we address the specificity of the *iso*-branched side chain of cholesterol. To this end, we investigated the influence of an unbranched aliphatic side chain on the membrane properties of cholesterol by using synthetic sterols bearing an unbranched side chain with 3–14 carbons attached to C17 of cholesterol (see Fig. 1). In particular, we investigated the influence of this cholesterol modification on the molecular packing of the membrane lipids, their lateral diffusion, and the membrane permeability of bilayers composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and the respective cholesterol analog. While membrane permeation and lateral diffusion are influenced only to a small extent by the sterol's alkyl side chain length, a significant dependence on the molecular packing of the phospholipid molecules was observed.

2. Materials and methods

2.1. Materials

The phospholipids and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL). Androst-5-en-3-β-ol (androstenol) was from Sigma–Aldrich (Steinheim, Germany). These chemicals were used without further purification. All other chemicals were from Sigma–Aldrich. The structures of the cholesterol analogs comprising varying lengths of the unbranched side chain are shown in Fig. 1; they were synthesized by modifications of previous procedures (Vilchèze et al., 1996; Chia et al., 1993; Baek and Bittman, 2013).

2.2. Preparation of NMR samples

For the NMR measurements, chloroform solutions of phospholipids and sterol analogs were mixed in a glass tube. After the solvent was evaporated, the samples were dissolved in cyclohexane and lyophilized overnight at a pressure of 0.2 mbar. The resulting fluffy powder was hydrated to 40 wt% with deuterium-depleted H_2O for ^{2}H NMR or D_2O for ^{1}H PFG MAS NMR. After homogenization by ten freeze-thaw cycles and centrifugation steps, the samples were transferred to 5 mm glass vials for static ^{2}H NMR or 4 mm MAS rotors for ^{1}H PFG MAS NMR experiments.

2.3. ^{2}NMR experiments

The ^{2}H NMR experiments were performed on a Bruker Avance 750 MHz NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) at a resonance frequency of 115.0 MHz for ^{2}H using a solids probe with a 5 mm solenoid coil. ^{2}H NMR spectra were acquired using a quadrupolar echo pulse sequence with a relaxation delay of 1 s. The two $\pi/2$ pulses with a typical length of around 3 μs were separated by a 60 μs delay. The spectral width was 500 kHz. ^{2}H NMR spectra were dePaked and smoothed order parameters were determined as described previously (Huster et al., 1998). From these order parameters the lipid chain extent as a measure of the lipid chain length was calculate according to the mean torque model (Petrache et al., 1999; Petrache et al., 2000).

2.4. ^{1}PFG MAS NMR measurements

^{1}H PFG MAS NMR experiments were carried out on a Bruker DRX 600 NMR spectrometer equipped with a 4 mm HR MAS probe with a gradient coil along the magic angle. Experiments were conducted at a spinning frequency of 6 kHz at 318 K. A stimulated echo sequence (Cotts et al., 1989) with sine-shaped bipolar gradient pulses with a length of 6 ms, an eddy current delay of 5 ms was used and typical ^{1}H $\pi/2$ pulse lengths were 11–13 μs. The relaxation delay was set to 5 s. The gradient strength, which was calibrated using a sample of pure water and the known diffusion coefficient of water, was varied in seven increments between 0.03 and 0.45 T/m. The diffusion time was varied in five increments between 25 and 300 ms. Apparent diffusion coefficients D_{app} were determined from a plot of peak intensity vs. gradient strength according to (Gaede and Gawrisch, 2003); furthermore, the diffusion coefficients were corrected for the radius of curvature of the vesicles using diffusion time dependent measurements (Gaede and Gawrisch, 2003; Scheidt et al., 2005).

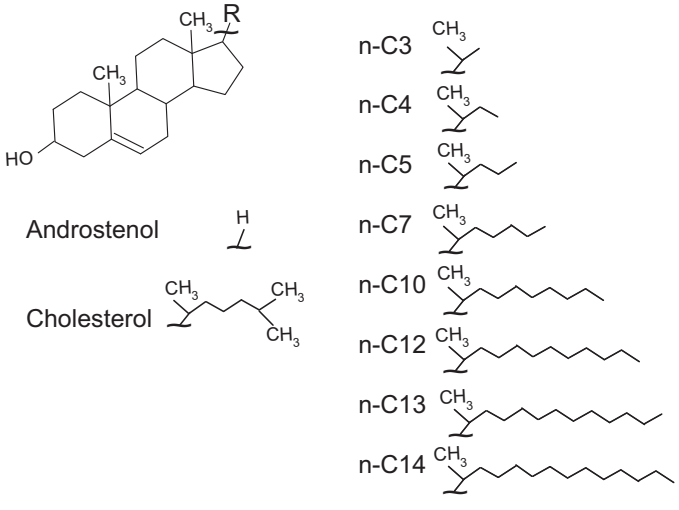

Fig. 1. Chemical structures of the sterol analogs used in this study.