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Effect of alkan-1-ols on the structure of dopc model membrane

Original Paper

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Abstract The effect of general anaesthetics alkan-1-ols (CnOH, where n = 10, 12, 14, 16 and 18 is the number of carbon atoms in the molecule) on the structure of dioleoylphosphatidylcholine (DOPC) model membrane was studied by small-angle neutron scattering (SANS) and small-angle neutron diffraction (SAND). Fluid bilayers were prepared at CnOH:DOPC = 0.3 molar ratio. The results of both the experiments show that bilayer thickness – a thickness parameter d_g in the case of SANS and lamellar repeat distance D in the case of SAND – increases with increasing n. A coexistence of two lamellar phases with different D was detected by measuring the C18OH+DOPC oriented sample.

Keywords *Model membrane – general anaesthetics – alkan-1-ols – small-angle neutron scattering – small-angle neutron diffraction – structure*

INTRODUCTION

Long-chain primary alcohols (alkan-1-ol or CnOH, where n is the number of carbons in aliphatic chain) display several biological activities (Fujita et al., 2008; Kubo et al., 1995; Pringle et al., 1981). The most widely known are their general anaesthetic properties (Pringle et al., 1981), which are believed to result from their interactions with biological membranes. Whilst the origin of the anaesthetic effect is discussed in terms of both their specific interactions with membrane proteins or via structural changes in lipid bilayers, neither mechanism is described adequately. Because of their amphiphilic structure, CnOHs penetrate into lipid bilayers of biomembranes and change their structural and dynamic properties. Some of these changes may be related to biological effects of CnOHs. Their anaesthetic potency increases with the increase in n up to n = 11 and then decreases. The compounds with n > 13 are non-anaesthetic (Pringle et al., 1981). Such dependence on the chain length is typical for different types of biological activities of homologous series of amphiphilic molecules with long-chain substituents and is called the cut-off effect. The lipid theory of cut-off effect supposes that amphiphiles influence physical properties of lipid bilayers, for example, the bilayer thickness, and thereby induce conformational changes in transmembrane proteins, resulting in the blockage of ion channels (Balgavý & Devínsky, 1996). In this contribution, we study the effects of CnOH (n = 8–18) on a model membrane, the dioleoylphosphatidylcholine (DOPC) bilayers, which resemble the lipid part of biological membrane. Preliminary results obtained from experiments performed by small-angle neutron diffraction (SAND) and small-angle neutron scattering (SANS) are presented.

MATERIALS AND METHOD

DOPC was purchased from Avanti Polar Lipids (Alabaster, USA), saturated and unbranched CnOHs (n = 10, 12, 14, 16, 18) were from Sigma-Aldrich (St. Louis, USA) and heavy water (99.98% D_2O) was from Izotop (Moscow, Russia) and Chemotrade (Leipzig, Germany). Spectrosil 2000 quartz plates (75 mm × 25 mm) were from Saint-Gobain Quartz (Saint-Gobain, France).
Oriented samples were prepared for SAND measurements. Calculated amounts of DOPC and CnOH were co-dissolved in chloroform–methanol mixture (3:1 v/v) in glass vials to

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achieve CnOH:DOPC molar ratio of 0.3. Approximately 20 mg of DOPC or DOPC + CnOH in solution were spread onto a 75 cm × 25 cm quartz glass and rocked during evaporation of organic solvent (Tristram-Nagle, 2007). The remaining traces of solvent were evaporated by vacuum pump, which was done at reduced temperature (–10 °C) to avoid the loss of volatile CnOHs. Before each measurement, samples were equilibrated for 24 h at 98% relative humidity (RH) and temperature of 25 °C. The samples were hydrated from a vapour phase over saturated K_2SO_4 solution at three different D_2O/H_2O contrasts (8%, 20% and 50% of D_2O) to determine the phases of structure factors for the Fourier transformation. Measurements were performed using the neutron Membrane Diffractometer V1 equipped by a ^{3}He position-sensitive detector at the BER II reactor of the Helmholtz-Zentrum Berlin für Materialien und Energie. Neutron wavelength was selected at λ = 4.5707 Å.

Dispersions of unilamellar liposomes were prepared for SANS measurements. Calculated amounts of DOPC and CnOH were co-dissolved in chloroform–methanol mixture (3:1 v/v) in glass vials to achieve CnOH:DOPC molar ratio of 0.3. Lipid was dried by a stream of gaseous nitrogen. The rest of the solvent was removed by vacuum pump. Lipid film was hydrated by 100% D_2O so that the weight percentage of DOPC + CnOH in D_2O was less than 2%. The dispersion of multilamellar liposomes arising in the process of agitation was then extruded through a polycarbonate membrane filter with 50-nm diameter pores. The extrusion technique is suitable to produce unilamellar liposomes with a reasonably homogeneous diameter distribution (polydispersity of about 30%) with a mean diameter approaching the polycarbonate membrane pore diameter (Kucerka et al., 2007). The samples of unilamellar liposomes dispersion were poured into 1-mm thick quartz cells and measured at 25.0 ± 0.1 °C. The SANS measurements were performed at the time-of-flight spectrometer YuMO with two-detector system at IBR-2 fast pulse reactor of Frank Laboratory of Neutron Physics in Joint Institute for Nuclear Research in Dubna, Russia (Kuklin et al., 2012). The scattering curves were corrected for background (Soloviev et al., 2003).

RESULTS AND DISCUSSION

We study the interaction of homologous series of alkan-1-ols with a model membrane represented by DOPC bilayers. Fully hydrated DOPC lipids form a fluid lamellar phase at temperatures above –17 °C (Lewis et al., 1988). Because of their amphiphilic structure, alcohols (CnOH, n = 8–18) are intercalated in DOPC bilayer with their hydroxyl groups in the head-group region of DOPC and the hydrophobic chains parallel to the acyl chains of DOPC. It was found that DOPC + CnOHs (n = 8–18) also form homogeneous fluid bilayers without phase separation at least to CnOH:DOPC molar ratio of 0.4 (Kotalová et al., 2008).

In oriented samples, bilayers of CnOH + DOPC are aligned parallel to the flat surface of the quartz plate. Individual lipid bilayers are separated by layers of water. The number of water molecules per one DOPC molecule is only approaching the condition of full hydration because samples were hydrated in the surroundings with 98% RH. Such partial dehydration was, however, shown not to affect, for the most part, the bilayer structural parameters (Kucerka et al., 2009). A regular arrangement of lipid bilayers separated by interlamellar water layers causes a diffraction of neutron beam applied at small scattering angle θ (angle between the incident neutron beam and the planar surface of the bilayer). Typical diffractogram contained five to seven diffraction peaks. As an example, diffractogram obtained by C10OH + DOPC oriented sample is shown in Fig. 1.

Diffraction peaks were fitted to Gaussians sitting atop of linear function describing the background. The equal distance between maxima of individual diffraction peaks confirms the lamellar arrangement. The position of peaks is given by the Bragg law,

$$h\lambda = 2D \sin(\theta), \quad (1)$$

where h is the order of diffraction peak, λ is the wavelength of neutrons and D is the minimum distance between periodically repeated structures. The repeat distance D is the sum of the thickness of the lipid bilayer D_B and the thickness of the water layer D_W ($D = D_B + D_W$). The dependence of the repeat distance D on the chain length of CnOH is shown in Fig. 2. It is clearly seen that shorter alcohols (n = 10–14) cause a decrease in D in DOPC multilayers where C10OH is the most effective. Longer alcohol, C16OH, increases the repeat distance. Two lamellar phases with different repeat spacing were detected in the sample containing C18OH (see inset to Fig. 1). C18OH and DOPC probably do not mix ideally at the molar ratio of 0.3 and level of hydration used, and two phases with different C18OH:DOPC molar ratio are created. Similar diffractograms were obtained after repeated preparation of C18OH + DOPC oriented sample.

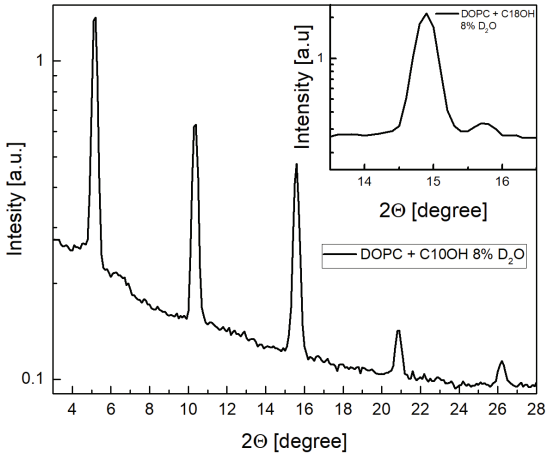

Figure1. Diffractogram of oriented C10OH + DOPC multilayers hydrated at 98% RH and D2O/H2O contrast of 8%. Inset – the third peak in the diffractogram of DOPC + C18OH multilayers documenting phase superposition.

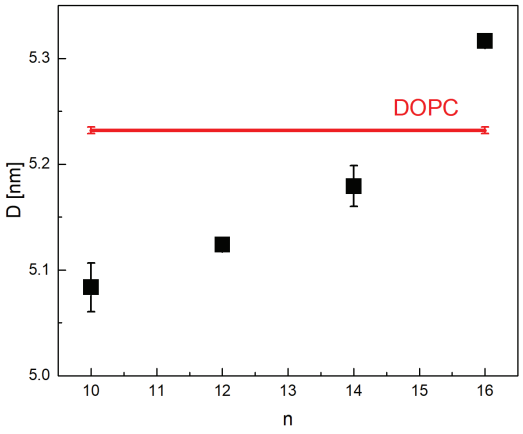

Figure2. Dependence of the repeat distance D on CnOH alkyl length n. Horizontal line – DOPC as a reference.

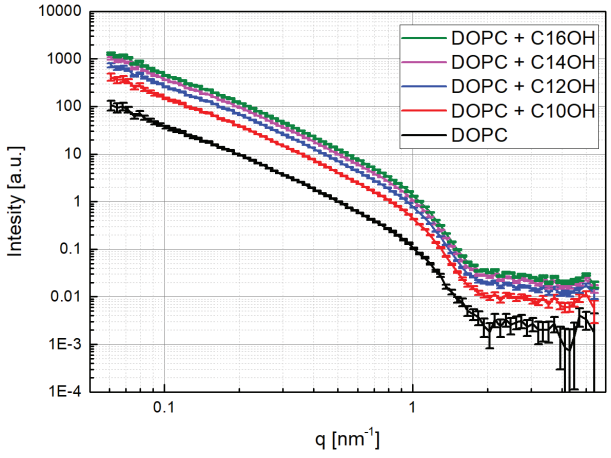

Figure3. Experimental SANS data obtained from unilamellar liposomes of DOPC + CnOH dispersed in D_2O. Scattering curves are shifted vertically for clarity of presentation. From bottom to top, they correspond to DOPC bilayers and DOPC containing C10OH, C12OH, C14OH and C16OH, respectively.

Results obtained with C18OH + DOPC, though a relative amount of the secondary phase can be estimated to be less than 5%, are, therefore, excluded from further data analysis.

A similar study was performed with non-oriented samples at full hydration (Petrenko et al., 2010). The increase in D with increasing n in CnOH:DOPC bilayer was also observed. Because of full hydration, the values of D were higher (6.03 nm for pure DOPC) and characteristic of the higher experimental error. Figure 2 shows that CnOH (n = 10–16) are able to influence the repeat distance D of DOPC-stacked bilayers at CnOH:DOPC molar ratio of 0.3. On the presented level of evaluation, it is not possible to distinguish whether D_B or D_W or both of them are influenced by CnOH. SAND patterns measured at various D_2O/H_2O contrasts also include information regarding the internal structure of the lipid bilayer. After the correction of Bragg peak intensities for incident flux, sample absorption and Lorentz correction, form factor phases can be determined through the isotopic replacement of H_2O for D_2O. Scattering length density profile of the bilayer can then be acquired using scattering form factors through their Fourier transformation. This more advanced evaluation described, for example, in Kucerka et al., 2009 will be presented in future.

Experimental SANS data are dependencies of scattered intensity I on the scattering vector modulus $q = 4\pi\sin(\theta)/\lambda$. Scattering curves for DOPC and CnOH + DOPC are shown in Fig. 3. It was experimentally shown (see, e.g. Kučerka et al., 2003, for citations) that the interparticle interaction between unilamellar liposomes is negligible at the lipid concentrations and liposome sizes used. When the bilayer thickness is small compared to liposome radii, neutron scattering on liposomes can be approximated by the scattering on randomly oriented planar sheets having the same thickness d_g. The Kratky–Porod approximation can be used in some range of q:

$$I(q)=Aq^{-2}\exp(-q^2R_g^2), \quad (2)$$

where A is a scaling constant and R_g is the radius of gyration. The thickness of two-dimensional planar sheet d_g ($d_g^2=12R_g^2$) can be determined using the Kratky–Porod plot ($Ln(Iq^2)=f(q^2)$). We found that d_g = 3.93 ± 0.08 nm for DOPC without CnOH similar to 3.78 ± 0.02 nm (Kučerka et al., 2003), 3.90 ± 0.08 nm (Uhríková et al., 2000), 3.92 ± 0.06 nm (Uhríková et al., 2003) and 3.91 ± 0.02 nm (Uhríková et al., 2001).

It was shown that bilayer thickness parameter d_g is linearly correlated with the transbilayer phosphate–phosphate distance in unilamellar diacylphosphatidylcholine liposomes (Balgavý et al., 2001). This indicates that changes in thickness parameter d_g can reflect rather well changes in the bilayer thickness. Obviously, the values of d_g are smaller than the steric thickness (e.g. 4.97 ± 0.07 nm for pure DOPC; Kučerka et al., 2007) obtained when taking into account the internal structure of the bilayer with some water molecules penetrating into the polar region of the bilayer. Figure 4 shows that the bilayer thickness parameter d_g increases with increasing n reaching the d_g value of pure DOPC bilayer at C16OH. However, the decrease in d_g caused by the shortest alcohol C10OH is only mild, around 0.1 nm. Unfortunately, the experimental error of d_g is quite large. We suppose that more precise values of the bilayer thickness will be obtained by fitting of the scattering curve in a broader range of q in advanced evaluation using a more realistic model of SANS on unilamellar vesicles as shown in our previous papers (e.g. Gallová et al., 2011).

Similar effect of CnOH on the lipid bilayer thickness as shown in Fig. 4 was observed in Klacsová et al. (2011), wherein the steric thickness of the mixed DOPC–DOPS (dioleoylphosphatidylserine) bilayer (DOPC:DOPS molar ratio of ~25) was studied by SANS. The decrease in the bilayer thickness caused by short alcohols was explained by the mismatch between the chain length of CnOH and lipid– the insertion of shorter alcohol molecule into a lipid bilayer creates a free space under its terminal methyl group, which is filled-in

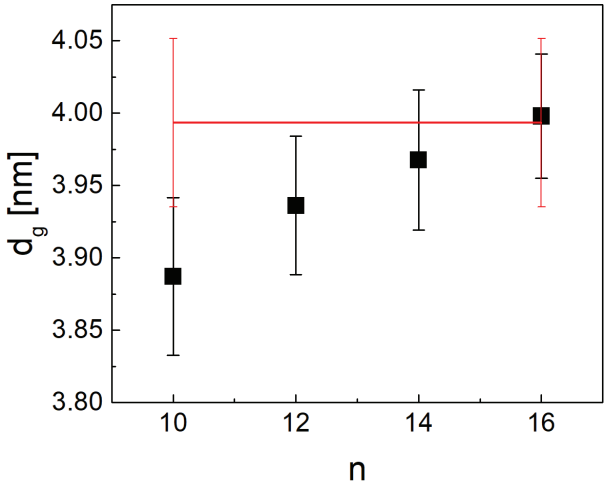

Figure4. Dependence of the bilayer thickness parameter dg on CnOH alkyl length n. Horizontal line – DOPC as a reference.

with neighbouring lipid acyl chains. This causes a decrease in the bilayer thickness. Such defects become smaller when longer-chain alcohol is intercalated in the same bilayer.

In conclusion, the preliminary results of SAND experiments on aligned planar bilayers with limited hydration show that shorter alcohols (CnOH, n = 10–14) decrease the repeat distance, whilst C16OH causes its increase. It was determined using SANS that the parameter of the bilayer thickness d_g increases with increasing n reaching the d_g value of pure DOPC bilayer at C16OH.

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