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Experimental Report

2014-04-03-18-20-24 Alcohol-lipid bilayer interactions.

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Introduction

The interactions of n-alkanes and n-alcohols with phospholipid bilayers are of a great interest due to several pharmacological effects they exhibit. In particular, they act as general anesthetics. The anesthetic potency could result from binding of these small molecules to a hydrophobic site in a target protein system [1], by changing the bilayer lateral pressure [2] or by influencing the phase state of lipids in membranes [3,4]. In recent papers from our group, it has been found that alcohols affect the lipid bilayer thickness in multilamellar [5] as well as in unilamellar phospholipid vesicles [6]. These results indicate that structural changes in bilayers induced by n-alcohols could be a primary cause of anesthesia. The estimation of the lipid bilayer thickness is then likely very important for the molecular interpretation of their pharmacological properties and their effects on membrane proteins. In the present study, we report on the neutron diffraction measurements of dioleoylphosphatidylcholine (DOPC) bilayer thickness changes upon the incorporation of primary aliphatic alcohols (CnOH, where n is the number of alkyl carbon atoms). We studied the effect of CnOH with n=10, 12, 14, 16 and 18 on a model membrane system prepared in a form of hydrated aligned fluid bilayers.

Experiment

DOPC was purchased from Avanti Polar Lipids (Alabaster, USA) and used as received. CnOHs with 99% purity were purchased from Sigma (St. Luis, USA). Six oriented samples were prepared on 25x65 mm^2 rectangular quartz wafers following the rock-and-roll method for creating aligned multilayers deposited from organic solvent (chloroform:methanol=3:1 by volume). The control sample comprised 20 mg of pure DOPC, while the other samples contained also an addition of CnOH at molar ratio CnOH:DOPC=0.3. The samples measured were hydrated through vapor of K_2SO_4 saturated aqueous solution (98% relative humidity) at 25°C. Three solutions were prepared with three different mixtures of D_2O/H_2O for the contrast variation (8%, 70% and 100% D_2O). This allows us to obtain the phases of diffraction peaks that are needed for the reconstruction of neutron scattering density profiles.

The data were collected at the DN-2 time-of-flight diffractometer utilizing 2D position sensitive detector. The combination of time-of-flight approach and 2D detector allows to cover a large range of scattering data even in short measurements. It is possible to follow the kinetics of various processes down to time spans of minutes. The exposure time for our data collection was set to 1 hour in accordance with the hydration kinetics of samples. Typically, we have observed changes in the diffraction peak positions within first 2-3 measurements, while no further changes confirmed the stable sample environment. Only the images obtained at the latter conditions were used for further analysis.

Results and Discussion

The typical diffraction data consisted of up to 4 Bragg diffraction peaks, suggesting a good sample quality. From the analysis of data it is clear that shorter alcohols (n=12) decrease the repeat d-spacing in DOPC multilayers, while the longer ones (n=16, 18) cause the d-spacing increase (Fig.1). Interestingly, the shortest alcohol (n=10) utilized has resulted in a slightly increased repeating distance. This behavior is unexpected, and its cause will be an object of further scrutiny. Nevertheless, we can conclude based on the preliminary results that C12OH makes the DOPC bilayer thinner, C14OH does not influence it, while longer homologues (n=16, 18) cause the increase of DOPC bilayer thickness. This is in agreement also with our previous results [5]. These different effects that shorter and longer homologues have on the bilayer thickness can, partly, explain the cut-off effect of anesthetic activity [7].

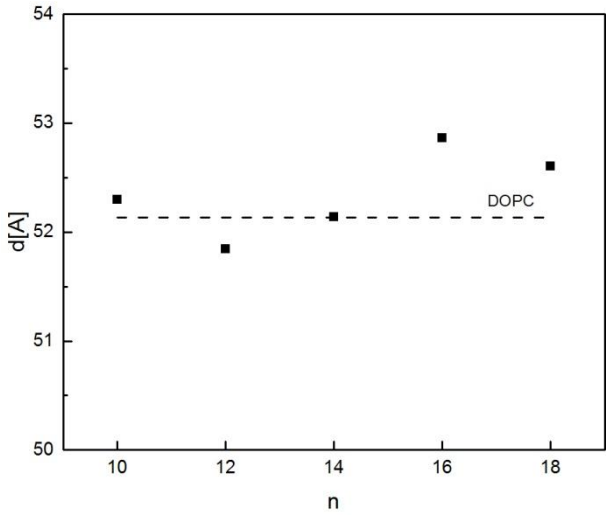

Fig. 1 Dependence of repeat period d on the length of CnOH molecules where n is the number of carbon atoms in molecule. The molar ratio of CnOH :DOPC in bilayers was 0.3.

Acknowledgments

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References

[1] Franks N.P.: Nature Reviews Neuroscience 9 (2008) 370
[2] Cantor R.S., Twyman K.S., Milutinovic P.S., Haseneder R.: Soft Matter 5 (2009) 3266
[3] Kharakoz D.P.: Biosci. Rep. 210 (2001) 801
[4] Appali R., van Rienen U., Heimburg T.: A comparison of the Hodgkin–Huxley model and the soliton theory for the action potential in nerves. Advances in Planar Lipid Bilayers and Liposomes, Volume 16, PP. 276-299, 2012, Elsevier Inc., ISSN 1554-4516
[5] Petrenko V.I., Klacsova M., Beskrovnyi A.I., Uhrikova D. and Balgavy P., Gen. Physiol. Biophys. 29 (2010) 355
[6] Klacsová M., Bulacu M., Kučerka N., Uhríková D., Teixeira J., Marrink S.J., Balgavý P.: Biochim. Biophys. Acta 1808 (2011) 2136
[7] Balgavy P., Devinsky F.: Advances Colloid Interface Sci. 66 (1996) 23