Experimental Report

28/04/2014

Proposal:	**9-13-498**	**Council:**	10/2012

Title: Structure of floating DPPC bilayerin presence of long-chain alcohols

This proposal is resubmission of: 9-13-449

Research Area: Soft condensed matter

Main proposer: **BALGAVY Pavol**

Experimental Team: BALGAVY Pavol
BELICKA Michal

Local Contact: FRAGNETO Giovanna

Samples: octanol
decanol
dodecanol
tetradecanol
hexadecanol
dipalmitoylphosphatidylcholine
hexanol
heavy water 99.99%

Instrument	Req. Days	All. Days	From	To
D17	6	3	11/03/2013	14/03/2013

Abstract:

Primary aliphatic alcohols (CnOH, n is the number of alkyl carbons) are general anesthetics, they also display a wide range of toxic effects. In pharmacy, they are used as penetration enhancers in transdermal drug delivery. Their potency in most biological activities depends quasi-parabolically on alkyl length with a maximum at n~10-12. CnOHs partition in the lipid bilayer of biomembranes, changing its physical properties anisotropically depending on the concentration and chain length n. This leads to conformational changes in membrane proteins coupled to structural and dynamical changes of bilayer resulting in biological effects. The neutron reflectometry experiments proposed will determine how CnOH alcohols affect the bilayer structure. As a model membrane system, bilayers prepared from dipalmitoylphosphatidylcholine (DPPC) and freely floating above another bilayer adsorbed on a smooth solid substrate will be used. Final aims of reflectometry experiments will be determination of CnOH effects on the floating bilayer thicknessthickness and roughness, on the lipid and alcohol interface area and on the hydration of bilayer polar region.

Title: Structure of floating DPPC bilayer in presence of long-chain alcohols.

Experiment: 9-13-498 Dates: 11/03/2013 – 14/03/2013 Instrument: D17

Team: M. Belička, G. Fragneto, N. Kučerka, P. Westh, P. Balgavý

Local contact: G. Fragneto

Abstract:

Primary aliphatic alcohols (CnOH, n is the number of alkyl carbons) are general anesthetics, they also display a wide range of toxic effects. In pharmacy they are used as penetration enhancers in transdermal drug delivery. Their potency in most biological activities depends quasi-parabolically on alkyl length with a maximum at n ~ 10 – 12. C*n*OHs partition in the lipid bilayer of biomembranes, changing its physical properties anisotropically depending on the concentration and chain length n. This leads to conformational changes in membrane proteins coupled to structural and dynamical changes of bilayer structure. As a model membrane system, bilayers prepared from dipalmitoylphosphatidylcholine (DPPC) and freely floating above another bilayer adsorbed on a smooth solid substrate will be used. Final aims of reflectometry experiments will be determination of CnOH effects on the floating bilayer thickness and roughness, on the DPPC and CnOH interface area on the hydration of bilayer polar regions.

Introduction

The cut-off effect observed in anesthetic potency of alcohols, but also other biologically active substances, does not correspond with behavior of their partition coefficient (Franks and Lieb, 1986), therefore it is not caused by any anomaly in partition equilibria. In previous experiments (Klacsová et al., 2011) it was found using the technique of small-angle neutron scattering that alcohols with different alkyl chain length influence the structure parameters – the lateral area per a lipid molecule and the bilayer thickness – in a different way. There are two principle ways how general anesthetics interact with biological systems, through direct contact with membrane proteins or through the change of physical and mechanical properties of lipid bilayers, which can influence the lipid membrane proteins as well. Another differences between the impact of shorter and longer alcohols intercalation on lipid bilayers were found in densitometry study of Aagaard et al. (Aagaard et al., 2006) where shorter alcohols tend to increase their volumes by the intercalation into lipid bilayers from aqueous environment, whereas alcohols with medium or longer alkyl chain lengths tend to decrease their molecular volume. The mentioned results led us to conclusion that the mentioned results are the consequences of different kind of interactions shorter and longer are exposed to. And thus, the cut-off effect might be correlated with the position of intercalated molecules inside the membrane.

Preliminary Report of Specular Neutron Reflectometry Results

SNR measurements were performed on the high flux D17 reflectometer with horizontal scattering geometry in time-of-flight mode using an interval of neutron wavelengths between 2 and 18 Å with incident angles 0.8° and 3.2°, what covered q range from 0.005 to 0.2 Å. In contrast to the previous measurements mainly the supported bilayers consisting of mixtures DMPC+C*n*OH (prepared by depositions in Langmuir trough and by vesicle fusion) were measured. Only one sample formed by a floating bilayer system of DPPC+C16OH bilayer over a supported diC22:0PC bilayer was measured. The reason was the last mentioned sample was destroyed during the previous measurements. The samples were measured in three different SLDs of water solvent at 25 °C and 55 °C. The direct experimental data obtained from the reflectometer were treated using LAMP software package, through which they were normalized and the corresponding resolution was determined.

The measurements of the samples prepared by vesicle fusion were found to be failed as there was a problem with a water pump. The rest of the samples was measured successfully. Currently only the scattering curves of a pure DPPC floating bilayer system were completely evaluated. In the case of a measurement at 25 °C we were able to fit only the overall thickness of the floating bilayer, but we did not succeeded with the internal structure determination. As the bilayer hydrocarbon core thickness $2D_C$ = 35.9 ± 0.1 Å was found to be higher than the results of other groups $2D_C$ = 34 Å (Charitat et al., 1999) or $2D_C$ = 32.0 ± 2 Å (Fragneto et al., 2003), this might be a direct consequence of a higher disorder inside the floating bilayer in the gel phase after it deposition.

On the other side, the reflectivity curves corresponding to the floating DPPC bilayer at 55 °C were evaluated completely. Even despite the complexity of a system. The supported diC22:0PC bilayer structure was the same as mentioned in the previous report. Only its coverage/hydration was changed. The thickness of a water layer between the supported bilayer and the floating bilayer was decreased by ca 2 Å during the phase transition of the floating DPPC bilayer. That is in a very good agreement with the previously published results of Fragneto et al., (2012). The obtained fits and scattering length density profiles are depicted on Figures below.

References

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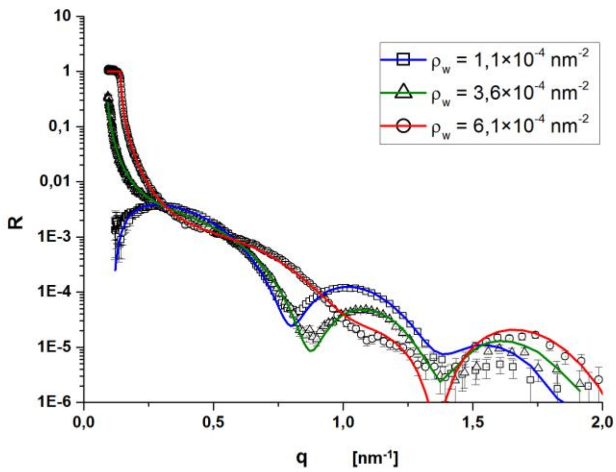

Fig. 1: The normalized reflectivity curves of a floating d62-DPPC bilayer over a diC22:0PC bilayer at 55 °C in three different contrasts. The lines represent the best simultaneous fit of the bilayer model to the data.

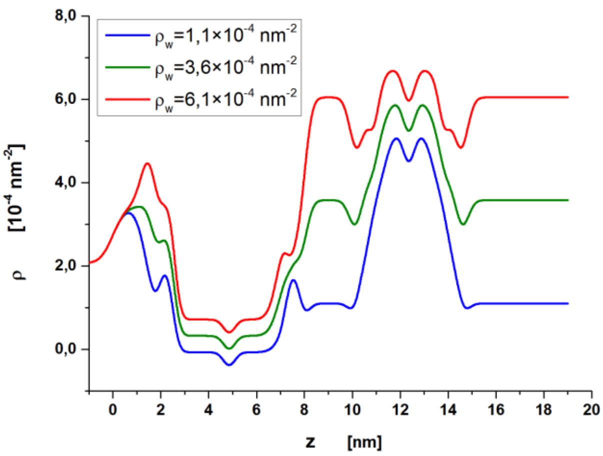

Fig. 2: The scattering length density profiles of the system of a floating d62-DPPC bilayer over a diC22:0PC bilayer at 55 °C in three different contrasts. The profiles correspond to the fitting lines in Fig. 1.