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Experimental Report

Location of the general anesthetics in model membranes

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Introduction

Alkanes and primary aliphatic alcohols have several pharmacological effects, which are believed to result from their interactions with constituents of biological membranes. In particular, they are able to act as general anesthetics. While the origin of the anesthetic effect is discussed both in terms of their specific interactions with membrane proteins, and the effects via structural changes of lipid bilayers, neither mechanism is described adequately. In addition, it has been known for many years that their anesthetic potency displays a sudden drop, called the cut-off effect[1]. For instance, the changes in bilayer thickness appear to be the primary mechanism when it comes to the modulation of the function of Ca^{2+} transporting ATPase. Its enzymatic activity achieves maximum in the mid-length-chain membranes, while it falls four-fold in membranes composed of shorter or longer chain lipids[2]. This effect was corroborated also by the bilayer thickening achieved as a response to the addition of cholesterol[3].

Similar model was proposed in the case of anesthetic effect of n-alkanes. Interestingly, the detailed investigation of the system with n-decane suggested two regimes for its interaction with lipid bilayers[4]. The hydrophobicity of n-decane molecule dictates its partition into the hydrophobic region, first in the orientation perpendicular to lipid acyl chains. The increasing concentration of n-decane however results into nonlinear increase of bilayer thickness, accompanied also by the increase of area per lipid. The most likely explanation suggests that n-decane is locating itself parallel in-between the lipid chains after filling the space available in the bilayer center[4]. Nevertheless, and despite the importance of understanding the mechanism of anesthesia, the model has not been confirmed by direct measurements.

Experiment

DOPC was purchased from Avanti Polar Lipids (Alabaster, USA) and used as received. Both labelled (i.e., deuterated n-decane) and non-labelled versions were purchased from Sigma (St. Luis, USA). Four oriented samples were prepared on 25x65 mm^2 rectangular single crystal silicon wafers following the rock-and-roll method for creating aligned multilayers deposited from organic solvent (chloroform:methanol=3:1 by volume)[5]. The samples with two different concentrations of n-decane were selected from the two different regimes of interaction model (mol:mol, 0.3:1 for the location in bilayer center, and 1:1 for the location also parallel to lipid chains), while each sample was prepared in both labelled (i.e., deuterated n-decane) and non-labelled versions. The control sample was made of 20 mg of pure DOPC.

The samples measured were hydrated through vapor of K_2SO_4 saturated aqueous solution (98% relative humidity[6]) at 25 °C. Each of the five samples were measured in four different contrast conditions (i.e., 100%, 70%, 40%, and 8% D_2O) to resolve the scattering phase problem, and to provide the water penetration profile[7]. The data were collected at the DN-2 time-of-flight diffractometer with 2D position sensitive detector.

Results and discussions

Most of the samples exhibit 3 diffraction peaks only (see Fig.1), while a minimum of 4 peaks is required for correct Fourier transform analysis [8]. Unfortunately, it is not possible to obtain the exact position of n-decane as mentioned in the introduction from measured data. The present data allow us to evaluate bilayer thickness only, which we have however obtained from different experiments already.

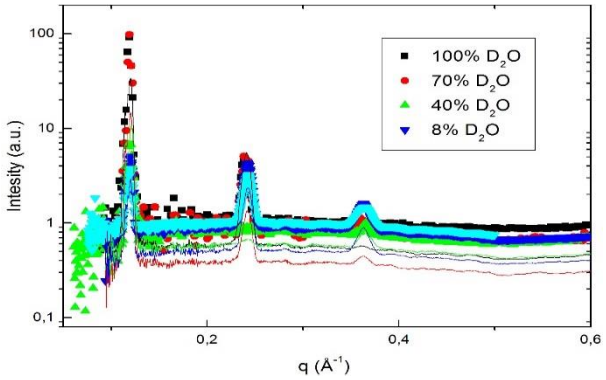

Fig. 1 Diffraction spectra from the sample made of n-decane:DOPC at 0.3:1 molar ratio and measured at various D_2O/H_2O contrasts (shifted vertically for the clarity of presentation).

Despite the unsuccessful measurements, we suggest to continue our studies by focusing on the improvement of oriented sample preparation method. The future experiments utilizing highly aligned stacked bilayers will then allow us to answer the questions outlined.

References

[1] P. Balgavý, F. Devínsky, Cut-off effects in biological activities of surfactants, Adv. Colloid Interface Sci. 66 (1996) 23–63.
[2] J. Karlovská, D. Uhríková, N. Kučerka, J. Teixeira, F. Devínsky, I. Lacko, et al., Influence of N-dodecyl-N,N-dimethylamine N-oxide on the activity of sarcoplasmic reticulum Ca^{2+}-transporting ATPase reconstituted into diacylphosphatidylcholine vesicles: Effects of bilayer physical parameters, Biophys. Chem. 119 (2006) 69–77.
[3] F. Cornelius*, Modulation of Na,K-ATPase and Na-ATPase Activity by Phospholipids and Cholesterol. I. Steady-State Kinetics†, (2001).
[4] N. Kučerka, D. Uhríková, J. Karlovská, P. Balgavý, Štúdium interakcie n-dekánu s fosfatidylcholínovými dvojvrstvami pomocou difrakcie röntgenového žiarenia, Farm. Obz. 71 (2002) 182–187.
[5] Tristram-Nagle, S. Preparation of oriented, fully hydrated lipid samples for structure determination using X-ray scattering. *Methods Mol.Biol.* **2007,** *400* 63-75.
[6] Greenspan, L. Humidity Fixed Points of Binary Saturated Aqueous Solutions . *JOURNAL OF RESEARCH of the National Bureau of Standards - A.Phys ics and Chemistry* **1977,** *81A*(1), 89-96.
[7] Kučerka, N.; Nieh, M. P.; Pencer, J.; Sachs, J. N.; Katsaras, J. What determines the thickness of a biological membrane. *Gen.Physiol Biophys.* **2009,** *28*(2), 117-125.
[8] J.F. Nagle, R. Zhang, S. Tristram-Nagle, W. Sun, H.I. Petrache, R.M. Suter, X-ray structure determination of fully hydrated L alpha phase dipalmitoylphosphatidylcholine bilayers., Biophys. J. 70 (1996) 1419–31.