Access Highlights - KLACSOVÁ Mária

Interaction of 1-alkanols with lipid bilayers from monounsaturated phosphatidylcholines

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The experiment

Thanks to their general anesthetic potency, alkanols are widely used in studies concerning the mechanism of anesthesia. The general anesthetic potency of primary aliphatic alkanols CnOH increases up to C11OH and than decreases, compounds longer than C13OH are non-anesthetic, i.e. the homologous CnOH series display a “cut-off” in the anesthetic potency. This “cut-off” effect can be caused directly or indirectly by dissolving of alkanol molecules in lipid bilayers of biomembranes.

We have chosen to study the effect of long aliphatic alkanols (C8OH – C18OH) on structural parameters of fluid bilayers in unilamellar dioleoylphosphatidylcholine: dioleoylphosphatidylserine (DOPC:DOPS = 96:4 wt%) vesicles (ULVs) at different 2H2O/H2O outer contrasts. We prepare ULVs by extrusion. DOPS in DOPC bilayers charges the bilayer surface negatively and thus prevents oligolamellar vesicle formation during extrusion and vesicle aggregation after extrusion. Small-angle neutron scattering (SANS) measurements were performed at the PAXE spectrometer at LLB Saclay. The scattering data were acquired at sample-detector distances 1.3 m and 5.05 m, the vawelength of neutrons was λ=0.6 nm. The experimental normalized SANS intensity I(q) as a function of the scattering vector modulus q was evaluated using a strip model of lipid bilayer. The model assumes that extruded ULVs are polydisperse hollow spheres with the single bilayer separating the inside and outside aqueous compartments, and that the bilayer can be divided into three strips corresponding to two polar headgroup regions (one on each side of the bilayer) and the bilayer center spanning hydrocarbon region. The polydispersity of ULVs is described by the Schulz distribution function of vesicle radii.

For evaluation of SANS data obtained at the single contrast using the described model, the value of polar region thickness DH must be constrained. Contrast variation experiments were therefore aimed to find whether DH changes in presence of CnOHs. We found that DH of the PCPS bilayer slightly decreases after incorporation of CnOH molecules and that this decrease is a function of CnOH chain length, i.e. with increasing n the thickness DH decreases. By subsequent fitting of experimental data from samples prepared at a single contrast (in heavy water) we found that the bilayer thickness D decreases with increasing CnOH:PCPS molar ratio for all alkanols studied. At the constant (0.4) molar ratio, the bilayer thickness increases with increasing CnOH chain length, reaching for C16OH and

C18OH the D value of the pure PCPS lipid system, apparently due to CnOH and lipid chain length mismatch. The lateral area of the unit cell AUC consisting of a phospholipid molecule and a particular fraction of the alkanol at the bilayer – aqueous phase interface was found to increase with increasing CnOH:PCPS molar ratio. Further, AUC rises significantly at a constant (0.4) molar ratio, in comparison with pure PCPS bilayers, already in the presence of the shortest alcohol studied; for longer alcohols further increase of AUC was observed. Hereby, the partial surface area ACnOH of alkanol molecules was found to linearly increase with the alkyl chain length. The values ACnOH<20 Å2 obtained for shorter – anesthetically active – alkanols are surprisingly low, as the mean surface area 18.8 Å2 is typical for crystalline states of alkanes and ~20 Å2 for solid rotator phase of alkanes. The experimental trends of D and AUC observed were reproduced with coarse-grained molecular dynamics simulations of DOPC bilayers with CnOH molecules inserted. We suppose that the anomaly in ACnOH is caused by the lipid headgroup which interfacial area is larger or equal comparing to the sum of hydrocarbon chains cross section areas, so that a small OH group is located underneath at the lipid glycerol fragment, in analogy to the “umbrella” model suggested for cholesterol location in bilayers. To verify this hypothesis additional SANS experiments with varying lipid headgroup size will be performed.

Support
This research project has been supported by the VEGA 1/0295/08 grant and by the European Commission under the 7th Framework Programme through the 'Research Infrastructures' action of the 'Capacities' Programme, Contract No: CP-CSA_INFRA-2008-1.1.1 Number 226507-NMI3. The NMI3 suport was get via Research Proposal at LLB (http://www-llb.cea.fr/fr-en/proposal.php).

The Facility
Experiment at small-angle neutron scattering spectrometer PAXE, LLB, France

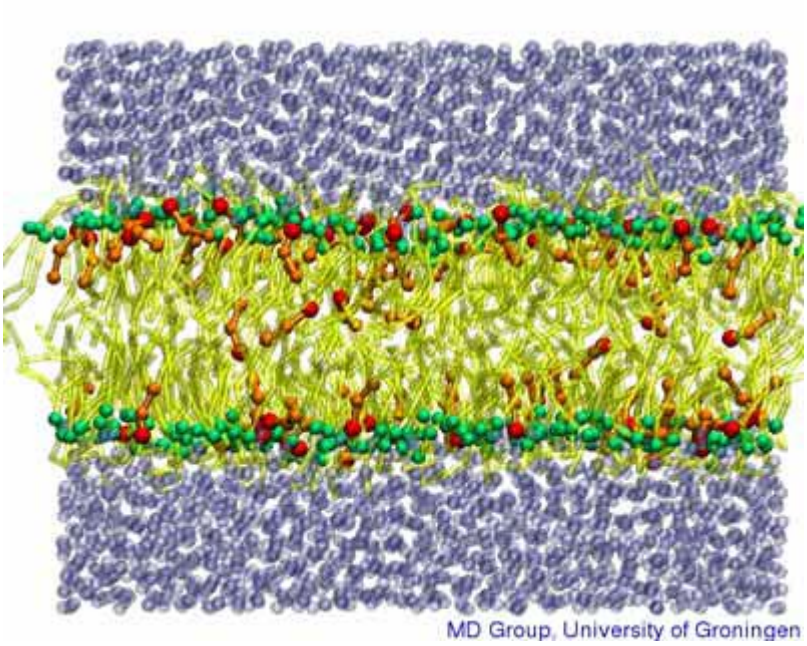
Publication: M. Klacsová, M. Bulacu, N. Kučerka, D. Uhríková, J. Teixeira, S.-J. Marrink, P. Balgavý: Effects of aliphatic alcohols on the fluid bilayer in unilamellar DOPC+DOPS vesicles: SANS and MD study, Biochim. Biophys. Acta, in preparation

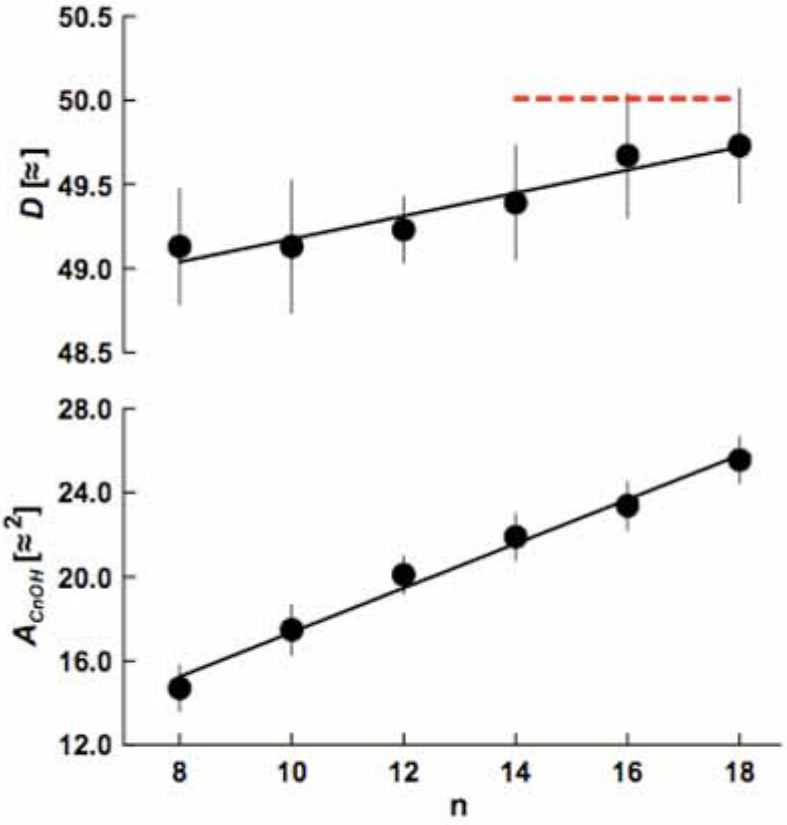
The team
The effect of amphiphilic molecules (local and general anesthetics, β-blockers, sterols and different kinds of biologically active surfactants) on structure and physico-chemical properties of lipid bilayers has been the aim of study in Biophysical laboratory at Faculty of Pharmacy, Comenius University for many years. The experimental techniques involved span from classical fluorescence and microcalorimetry measurements, across NMR and ESR spectroscopy, up to neutron and X-ray scattering.

I have joined the scientific group of Biophysical laboratory six years ago as a master student under supervision of Ass.Prof. Daniela Uhríková. It was my first experience with experiments on large facility – synchrotron – at HASYLAB DESY, Hamburg. After finishing master degree I have continued on PhD studies, where my supervisor is Prof. Pavol Balgavý. The scope of my thesis is the study of influence of long aliphatic 1 alkanols on structural parameters of phospholipid bilayers using different techniques, including small and wide angle X-ray diffraction and small angle neutron scattering. I have performed several SANS experiments at LLB Saclay and futher experiments at HASYLAB

in cooperation with Ass.Prof. Uhríková and our local contact at LLB Dr. José Teixeira. The evaluation of experimental data, especially of SANS experiments, was performed in collaboration with Dr. Norbert Kučerka, former student of Prof. Balgavý, who is also the creator of advanced models used for evaluation.

The evaluation of experimental data gave, however, suprising results, as discussed within the abstract. We have therefore decided to ask the group of Prof. Sievert-Jan Marrink in Groningen, Netherlands for help with interpretation of our results using molecular dynamic simulations. The simulations were done by his post-doc student Dr. Monica Bulacu, with whom I shared also the presentation at NMI3 General assembly. Based on the results of MD simulation we can conclude, that the combination of scattering techniques and simulations is a usefuls tool for better interpretation of experimental data.

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Maria Klacsová & Monica Bulacu at NMI3 General assembly in Barcelona, May 2010