Experimental report

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Proposal: 8-02-844 **Council:** 10/2018

Title: Probing the structure of natural phospholipid multilayers with Neutron Diffraction

Research area: Biology

This proposal is a continuation of 8-02-811

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Samples: Pichia pastoris Polar fraction (hydrogenous & deuterated)
Pichia pastoris PC fractions (hydrogenous & deuterated)
Ergosterol (hydrogenous & deuterated)
Silicon wafers

Instrument	Requested days	Allocated days	From	To
D16	7	7	22/07/2019	29/07/2019

Abstract:

Natural lipid bilayers extracted from P. Pastoris yeast cells are a relevant model system of complex biological membranes and a potential source of deuterated lipids for applications in neutron scattering techniques as well as NMR or IR-spectroscopy. During previous experiment on D16 we have characterized the total lipid extract (hydrogenous and deuterated, containing several phospholipid species, sterols, free fatty acids and triglycerides) and the phospholipid fraction (named Polar fraction). In particular during our last experiment we tested a lipid deposition method based on aqueous solution. This method strongly improved multilayer order and thus we are here proposing to complete our previous study by performing ND measurement with multilayers hydrated with different isotopic vapor compositions. This new data will allow us to extract the scattering length density profile from data analysis. In addition, recently we have achieved extraction and separation of hydrogenous and deuterated PC lipids, so we also propose to compare the Pol-multilayers with PC-multilayers in order to characterize the impact of the lipid headgroups on the multilayer structure.

Neutron diffraction from natural phosphatidylcholine multilayers:

impact of the preparation method on the sample structure

Introduction. In this neutron diffraction (ND) experiment, we investigated natural phospholipid multilayers prepared with hydrogenous and deuterated phosphatidylcholine (PC) lipids extracted from yeast (*Pichia pastoris*) cells, which were grown respectively in a hydrogenous and deuterated culture media. These phospholipid mixtures are characterized by having the same composition of the headgroup, but differ for the composition of the acyl chains, i.e. different length and unsaturation level. During previous ND experiments on D16 (8-02-811, INTER-442), we noticed some relevant differences in the number of lipid phases present in the characterized samples depending on the multilayer preparation method[1]. In particular, two different preparation methods were used: 1) lipid deposition form organic solvent solution; 2) lipid deposition from vesicle suspension. Both in 1) and 2) the lipid solution or suspension is deposited on the solid support by drop casting. However, in 1) the lipids are dissolved in a 1:4 chloroform: methanol mixture, while in 2) lipids are dissolved in water and subsequently sonicated to obtain a vesicle suspension. In this experiment, we explored more carefully the impact of the sample preparation method on the multilayer structure by choosing a simple, but still biologically relevant, model system, i.e. natural PC multilayers, and by systematically preparing the samples with method 1) and 2), while all the other parameters, e. g. lipid concentration, temperature, relative humidity, equilibration time, were kept fixed.

Experimental section. Lipid films corresponding to 2 mg of hydrogenous or deuterated PC lipids (hPC or dPC) were prepared by drying proper amount of lipid solution in 1:4 chloroform: methanol on the bottom of a glass vial under nitrogen. In the case of method 1 the film was re-dissolved with 200 μl of a 1:4 chloroform: methanol solution. In the case of method 2 the film was re-dissolved with 600 μl of ultrapure water. The larger solvent volume used during method 2 is due to the lower solubility of the PC lipids in water compared to the organic solvent mixture. The PC aqueous suspensions were sonicated for ~5min with a tip sonicator to obtain a homogenous sample. In this condition the PC lipids form vesicles. 200 μl of either hPC or dPC in the organic solvent solution and 600 μl of either hPC or dPC vesicle suspension were deposited on freshly cleaned quartz slides by drop casting in order to deposit on the solid support the same amount of lipids. Once the deposited samples were dried, they were subsequently stored under vacuum at 50 ^{o}C for12h (annealing). The multilayers were subsequently equilibrated at 97%RH for 24h and then placed in the humidity chambers, aligned, and equilibrated at 57% Relative Humidity (RH) for 12h. After the measurements in this condition, the samples were further equilibrated at 98% (12h) and the corresponding diffraction data were collected. The reservoir of the humidity chamber was filled with H_2O for the dPC multilayers and D_2O for the hPC multilayers. in order to guarantee the best contrast between the lipids and the hydration water. Diffraction data were collected at detector angle (γ) 12 deg by scanning the sample angle (ω) in the range -1:10 deg or -1:8 deg, with a step of 0.05 deg. Data reduction was carried out with the ILL software Lamp. The background of the data was estimated by collecting one measurement with the same scan and detector position

[1] Luchini et al., Chemistry and Physics of Lipids, 2020.

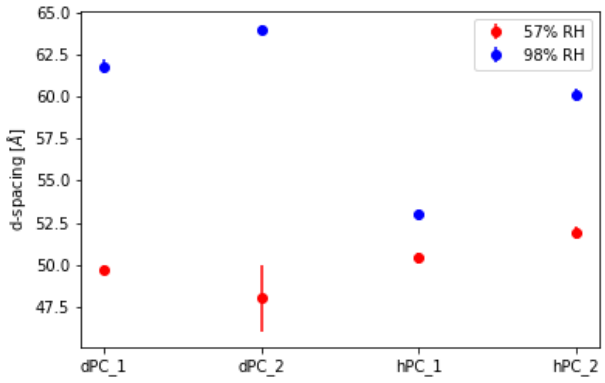

Figure 1: Calculated d-spacing values versus type and composition of lipid multilayers at 57% RH and 98% RH. hPC and dPC multilayers are labeled on the x-axis as 1 or 2 according to the preparation method used for the multilayer production.

for the empty humidity chamber. This measurement was subtracted from the ones collected for the samples. The efficiency of the detector was considered during data treatment by loading the proper calibration file in Lamp. The reduced detector images were integrated in the ω range corresponding to the observed diffraction peaks in order to obtain intensity vs 2θ plot. The positions of the Bragg peaks in the plots were determined by fitting the peaks with a Gaussian function.

Altogether, hPC and dPC lipids prepared with method 1 and 2 showed a considerable difference in the number of lipid phases present at low RH. More specifically, both hPC and dPC prepared with method 1 exhibited a single lipid phases at 57% RH, while two different lipid phases were identified in the hPC and dPC multilayers prepared with method 2. Indeed, in the organic solvent mixture the PC lipids are expected to be well-dissolved and to form a homogenous layer once deposited on the substrate. On the other hand, in the vesicle suspension the PC lipids might be already pre-organized in lamellae with different composition and structure. The co-existence of different lipid phases at 57% RH for the sample prepared with method 2 might reflect the structure of the vesicles originally present in the suspension used for the lipid deposition. At 98% RH, both method 1 and 2 produced lipid multilayers containing multiple lipid phases. Figure 4 show a comparison of the d-spacing values calculated for the investigated samples at 57% and 98% RH. As expected, all samples exhibited a larger d-spacing at 98% RH than the corresponding value at 57% RH. On average similar d-spacing values were observed for all the multilayers at 57 % RH. However, both if prepared with method 1 or 2, the dPC multilayers resulted to have a considerably larger d-spacing at 98 %RH than the corresponding hPC multilayers. In particular, the deposition from vesicle suspension resulted to produce multilayers with larger d-spacing values than the corresponding samples prepared by deposition of the lipid organic solvent solution. This was observed both in the case of dPC and hPC.

Conclusions. The multilayer preparation method resulted to have not only an impact on the number of lipid phases present in the multilayers, but also on the corresponding d-spacing. The collected results suggest that at low relative humidity (57% RH) the hPC and dPC multilayers prepared with either method 1 and method 2 exhibit a very similar structure with comparable d-spacing values. However, the multilayers prepared with method 1 were characterized by a single lipid phase, while two different lipid phases were detected in the case of the samples prepared with method 2. Interestingly both the dPC and hPC multilayers prepared with method 2 resulted to have a larger d-spacing than the corresponding samples prepared with method 1.