Experimental report

17/09/2018

Proposal: 8-02-811 **Council:** 4/2017

Title: Neutron diffraction study of natural lipid-sterol membranes

Research area: Biology

This proposal is a new proposal

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Samples: Cholesterol
ergosterol
silicon wafers
Polar fraction of P. pastoris extracts (deuterated)
Polar fraction of P. pastoris extracts (hydrogenous)

Instrument	Requested days	Allocated days	From	To
D16	7	7	21/06/2018	28/06/2018

Abstract:

We have recently carried out a detailed characterization of the lipid composition of native fungal membrane extracts from Pichia Pastoris [1]. We subsequently characterized the structure of membranes composed of extracts with or without ergosterol and the structural consequences induced by the antifungal agent AmB using Neutron Reflectometry measurements [2]. Notably, the structure of yeast phospholipid membranes differs considerably from those found in typical model lipid bilayers composed of synthetic lipids and their structure depends on the degree of lipid polyunsaturation as well as the presence of the native ergosterol (10-15 mol% at natural abundance). We propose to complement this study by means of Neutron Diffraction measurements as it can shed light on the organization of the lipids in single-phase or multiple-phases system as function of the external humidity. The final goal of this study, on natural lipids multilayers in which the content of cholesterol/ergosterol will be systematically varied, is the determination of the phase diagram of the mixture of natural phospholipids and ergosterol/cholesterol.

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Introduction

Neutron Diffraction is a unique tool to investigate in details the structure of lipid membranes. In continuation with our previous works [1-3], we are interested in understanding the structure of membranes prepared with lipids extracted by *Pichia Pastoris* yeast cells grown in H_2O or D_2O media and how this is affected by the ergosterol content. The main goals are characterizing the structure of complex lipid membranes (including several lipid species), thus representative of natural cell membrane, and at the same time evaluate the possibility to exploit *Pichia Pastoris* yeast cells grown in D_2O media for the production of deuterated lipids. This second goal is particularly relevant as deuterated lipids are a valuable product for several techniques, such as neutron scattering NMR or IR spectroscopy.

Experimental section

Deuterated natural phospholipids (dPol) and hydrogenous and deuterated ergosterol (named hereafter hErg and dErg) were dissolved in H_2O to prepare lipid solutions with concentration 20 mg/ml. Samples were prepared with increasing ergosterol content in the range 0-30% mol/mol. Silicon wafers were cleaned by sequential sonication in Chloroform, Aceton, Ethanol followed by Plasma Cleaner treatment. 200 μl of each the natural lipid solutions were spread on the cleaned silicon wafers and dried. The wafers were subsequently stored under vacuum at 50 ^oC for at least 6h. The samples were then placed in the humidity chambers and measured at 60%, 80% and 100% Relative Humidity (RH).

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 0.01 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 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.

Results and Discussions

Figure 1a shows the data collected for the dPol multilayer with 30% hErg as representative of the data collected during the experiment. In all the explored humidity conditions the lipid multilayers were characterized by the coexistence of two phases named *a* and *b*. This observation is in agreement with the results reported elsewhere [3] and was observed for all samples prepared with dPol. Together with the two lipid phases, the collected data at high ergosterol content are characterized by the presence of the typical peaks of the ergosterol crystallizing outside the membranes. The comparison of the data collected for the multilayers with increasing ergosterol concentration highlighted that the mixing efficiency strongly decreased with the ergosterol concentration (particularly, in the concentration range 20%-30%). Although we have not attempted here to precisely determine the ergosterol solubility limit, it was already reported that ergosterol solubility is particularly low in unsaturated phospholipid multilayers [4] and dPol is a mixture of phospholipids with acyl chains exhibiting different level of unsaturation.

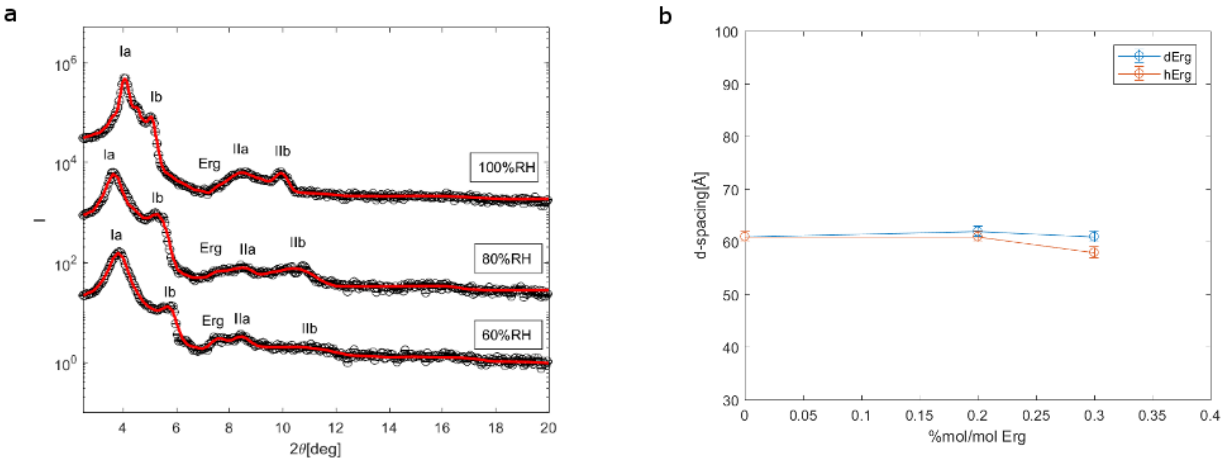

Figure 1-Experimental data collected on dPolhErg with hErg concentration 30%mol/mol in H_2O-contrast (panel a). The experimental data are reported together with the fitting curve used to identify the peak positions. Data intensity was scaled in order to allow a better visualization of the data collected at 80% and 100% RH. The d-spacing calculated for the characterized multilayers at 100% RH is plotted versus ergosterol % mol/mol (panel b).

Besides the observation that ergosterol can be efficiently mix with dPol only at concentration ≤ 10% mol/mol it is interesting to evaluate how the presence of ergosterol can affect the membrane d-spacing. By comparing the d-spacing calculated for the multilayers with increasing ergosterol concentration it is possible to observe for both dErg and hErg a gradual thinning of the membrane d-spacing (Figure1b). Interestingly this d-spacing was calculated for phase *a*. On the other hand, the d-spacing calculated for phase *b* resulted to be less sensitive to the ergosterol concentration. In contrast to the initial idea that ergosterol plays in yeast or fungi membrane the same role that cholesterol plays in mammalian plasma membrane, the here reported data sustains the hypothesis of a different biological role of ergosterol compared to cholesterol [3].

Conclusion

The experiment performed on D16 allowed to collect, for the first time, structural information on deuterated natural phospholipid multilayers with increasing ergosterol concentration. Our optimized sample preparation protocol allowed us to improve the data quality compared to previous experiments and will allow us in future experiments to exploit the contrast variation approach to extrapolate scattering length density profiles from the diffraction patterns. Indeed, in the case of hPoldErg system (data not shown) three peaks corresponding to three diffraction orders were observed. Although most of the data sets showed very interesting results, we faced some technical difficulties for some of the other measurements. In particular, misfunctioning of the humidity regulation system compromised the dataset collected for dPolhErg 10% mol/mol.

References

[1] A. de Ghellinck et al, PLoS One, 9 (2014) e92999. [2] Y. Gerelli et al, Acta Crystallogr D Biol Crystallogr, 70 (2014) 3167-3176. [3]A. Luchini et al, Colloids and Surfaces B, 168 (2018) 126-133. [4] H. Huang et al, Biophysical Journal, 110 (2016) 2026-2033.