Experimental report

13/02/2019

Proposal: **INTER-442** **Council:** 10/2018

Title: Neutron diffraction study of natural lipid-sterol membranes

Research area:

This proposal is a new proposal

Main proposer: **Alessandra LUCHINI**

Experimental team: Alessandra LUCHINI
Robin DELHOM

Local contacts: Viviana CRISTIGLIO

Samples: Cholesterol
Polar fraction of P. pastoris extracts

Instrument	Requested days	Allocated days	From	To
D16	3	3	05/10/2018	08/10/2018

Abstract:

Neutron Diffraction study on lipid-sterol membranes

Introduction

We are interested in 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. Neutron Diffraction (ND) can provide unique information on the structural organization of lipid membranes. In continuation with our previous work [1-3], we have performed ND measurements to investigate the impact of ergosterol on the multilayers prepared with deuterated phospholipids extracted from *P. Pastoris*. During a previous experiment (exp. Report), we had technical problems in properly controlling the sample humidity in some of the measurements. During this additional experiment, we were able to repeat the missing measurements and complete our characterization on phospholipid multilayer with hydrogenous and deuterated ergosterol.

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 Discussion

Figure 1 shows the diffraction data collected for the pure dPol multilayer. At 57% RH the diffraction pattern was characterized by 4 diffraction peaks, which were interpreted as belonging to two different lipid phases (a and b) in agreement with the data reported elsewhere [3]. The characteristic d-spacing (d) was calculated as $d_a = (58.5 \pm 0.1)$ Å $d_b = (51.4 \pm 0.8)$ Å. By increasing the relative humidity to 98%, a structural rearrangement occurred and a single lipid phase was observed. From the position of the Bragg peaks associated to each of the identified lipid phases, the characteristic d-spacing (d) was calculated as $d = (62.1 \pm 0.6)$ Å. As expected, by increasing RH, an increment in the d-spacing was observed. The presence of hErg or dErg 10% mol/mol in mixture with dPol did not dramatically affect the multilayer structure. As for the dPol multilayer, two lipid phases where observed at low RH, which rearranged in a single phase at 98% RH.

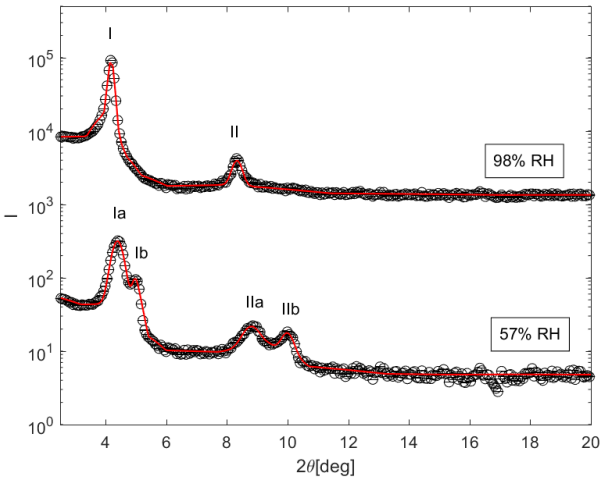

Figure 1: Intensity vs 2θ plot for the dPol multilayer at 57% and 98%RH as reported in the graph. Data at 98% RH were multiplied by a scale factor to allow a better comparison with the data collected at 57% RH.

Also, the calculated d-spacing did not show a substantial variation compared to the dPol multilayer both at 57%RH (d_a = (55.6 ± 0.6) Å, d_b = (51.4 ± 0.8) Å for dPol/hErg; d_a = (57.2 ± 0.9) Å, d_b = (55 ± 1) Å for dPol/dErg) and 98% RH (d = (62.1 ± 0.8) Å dPol/hErg; d = (62.4 ± 0.2) Å for dPol/dErg). Interestingly, very similar results were obtained both for multilayer prepared with dErg and hErg.

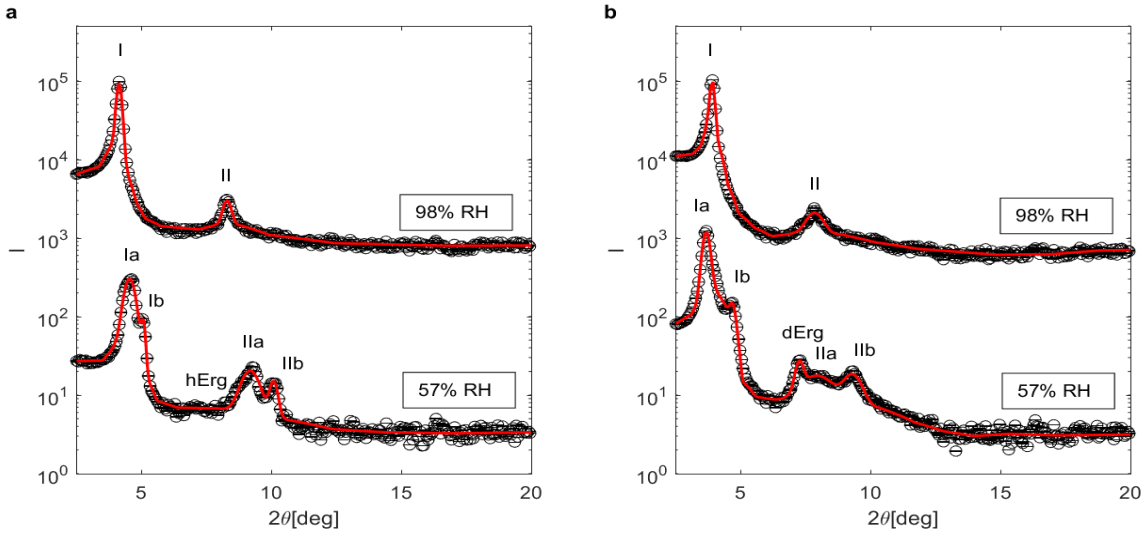

Figure 2: Intensity vs 2q plot for the dPol/hErg (a) and dPol/dErg (b) multilayer at 57% and 98%RH as reported in the graph. Data at 98% RH were multiplied by a scale factor to allow a better comparison with the data collected at 57% RH.

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.