Experimental report

19/02/2024

Proposal: 8-02-990 **Council:** 10/2022

Title: Completing the structural characterization of pulmonary surfactant model films by neutron reflectometry

Research area: Biology

This proposal is a continuation of 8-02-891

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Samples: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)
Surfactant protein B (SP-B)
Surfactant protein C (SP-C)
Organic extract from pulmonary surfactant
2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC)
2-Oleoyl-1-palmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (POPG)

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	3	3	19/06/2023	22/06/2023

Abstract:

Pulmonary surfactant is a lipid/protein complex that coats the alveolar air-liquid interface reducing the surface tension at the end of expiration. Its correct functioning is crucial for the development of breathing cycles, being otherwise alveoli at risk of collapse. As it needs to cover the interface rapidly, there are highly dynamic subpahse surfactant reservoirs that nurture the interface with lipids during inspiration and keep the excluded material associated to the interface during expiration. These lipid trafficking is possible thanks to the action of both hydrophobic proteins of the system, surfactant proteins B and C. However, their mechanism of action is still far from completely understood. This is why we used neutron reflectometry to decipher protein/lipid interactions occurring along respiratory mechanics still uncharacterized. Previous measurements on FIGARO have already provided valuable information about 3-D structures formed along respiratory dynamics. Nevertheless, the most interesting experiments at the highest surface pressures (= lowest surface tensions) where most of these structural reorganizations take place still need to be performed.

FINAL REPORT

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INTRODUCTION

Pulmonary surfactant is a lipid/protein complex located lining the alveolar air-liquid interface that minimizes the surface tension of the respiratory interface allowing breathing dynamics and preventing alveolar collapse [1]. To do so, it needs to cover rapidly and efficiently the whole respiratory surface forming a lipid-based monolayer that keeps associated to a subphase reservoir that provides the interface with lipids when needed (during inspiration). This is possible thanks to the action of both hydrophobic proteins of the system: surfactant proteins B (SP-B) and C (SP-C). SP-B is thought to oligomerize creating a ring with a central cavity that allows a rapid lipid flow in and out of the interface [2]. SP-C creates membrane curvature making possible the exclusion of unsaturated phospholipids out of the interface to reach the minimum surface tension needed to stabilise the alveoli at the end of expiration [3]. However, the molecular interactions that take place are not completely characterized. To be able to study this complex films not only 2-dimensionally but also characterizing the 3-D structures formed along film compression, we performed neutron reflectometry measurements of adsorbed model surfactant films in an air-liquid interface subjected to compression-expansion cycles, obtaining information about lipid/protein interactions occurring during breathing dynamics previously uncharacterized.

PREVIOUS RESULTS

In previous neutron reflectometry experiments of interfacial surfactant films using FIGARO we characterized interfacial films composed of model lipids mimicking pulmonary surfactant composition (DPPC/POPC/POPG) with or without physiological amounts of surfactant proteins B or C (1% by mass) in buffer solution containing Tris 5mM, NaCl 150mM, pH 7.4 either in D2O or Air Contrast Match Water and at surface pressures of 10 and 35 mN/m before and after surface cycling (Figaro experiment: #8-02- 865). Data obtained from lipids models pointed out an increased lipid concentration at 35mN/m and, although no significant differences in the reflectivity profiles were detected, we recorded a small-angle-neutron scattering (SANS) signal corresponding to a lipid reservoir in bulk, as well as an increase in the off-specular signal when using samples containing SP-B. These data are agreement with our hypothesis that SP-B could be connecting the interfacial monolayer with subphase reservoirs, being essential for the rapid reorganization of the monolayer along breathing cycles. Taking these preliminary data into consideration, we performed experiments for lipid mixtures containing higher protein amounts (10% by mass) to determine in more detail how lipids and proteins interact at higher surface pressures (50 mN/m) since the remodelling of the films occur at pressures around 40mN/m (#8-02-891). We measured the same samples as before (hydrogenated or deuterated versions of phospholipid mixtures mimicking lung surfactant composition: DPPC/POPC/POPG 50:25:15 w:w:w) in the presence or absence of 10% weight of SP-B or SP-C. Preliminary analysis of the data confirm the formation of reservoirs that is even

enhanced at 10% SP-B content. However, due to lack of time, there were some crucial experiments/contrasts not performed for a full structural analysis of the data.

AIM

The main purpose of this research project was to thoroughly characterize how surfactant lipids and proteins interact with each other both laterally and three-dimensionally to sustain efficient breathing dynamics. Having already characterized a phospholipid model mimicking pulmonary surfactant composition in the presence or absence of surfactant proteins SP-B or SP-C at their physiological concentration (proposal #8-02-865) we increased the concentration of SP-B and SP-C and the pressure at which we recorded neutron reflection to try to reach the exclusion zone of unsaturated lipids and proteins where a compositional alteration of the interfacial monolayer takes place. However, we did not have time to record all samples and all pressures (proposal #8-02-891), as well as samples corresponding to the organic extract of native surfactant (containing all surfactant lipids, SP-B and SP-C) to compare their behaviour with the one for the synthetic lipid mixtures.

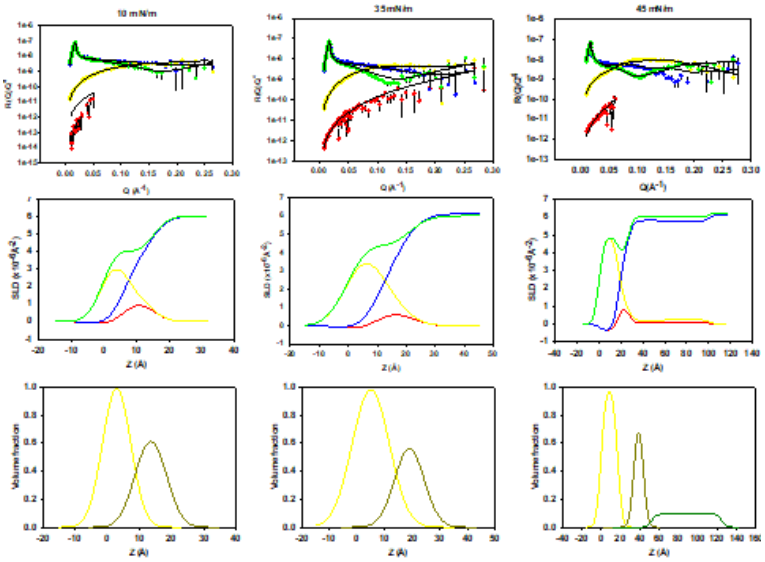
EXPERIMENTAL PLAN

Model surfactant system (hydrogenated and deuterated DPPC/POPC/POPG) in absence or presence of SP-B or SP-C (10%, weight) was measured at all pressures left in 2 contrasts: 100% D2O and Air Contrast Match Water to complete the tables above. As an overflow of the trough occurred at 50 mN/m, all measurements were repeated at 45 mN/m.

RESULTS

The results of these proposals (#8-02-865, 8-02-891, 8-02-990) are going to be published shortly. No differences were seen in samples before and after cycling, so only samples before cycling are represented:

a)



b)

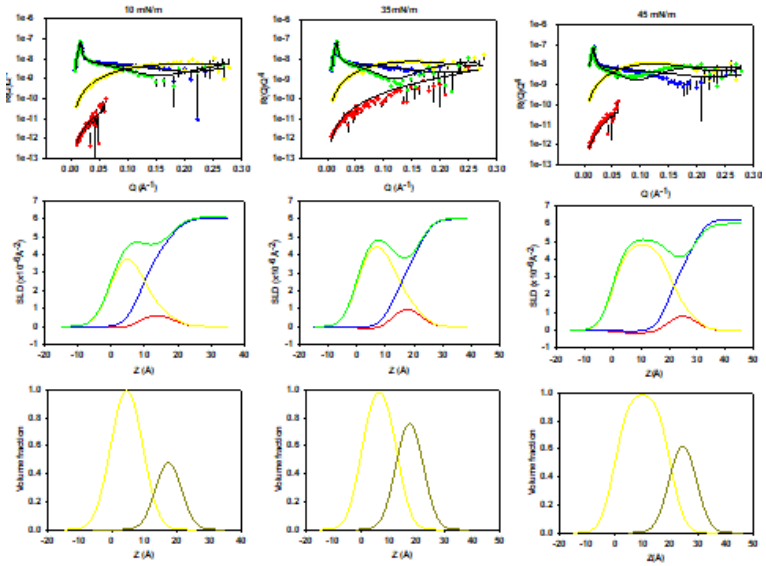


Figure 1. Reflectivity (top), SLD profiles (middle) and volume fractions (bottom) of A) DPPC/POPC/POPG (50:25:15 w:w:w) + 10 % SP-B and B) + 10 % SP-C. Four contrasts are fitted altogether to enhance the quality of the analysis: hydrogenous lipids in D_2O in yellow, hydrogenous lipids in ACMW in red, deuterated lipids in D_2O in green and deuterated lipids in ACMW in blue. Continuous lines in reflectivity profiles show the fit of the chosen model to the experimental data. For volume fractions, light yellow shows the first layer, dark yellow the second one and when there is a third layer, it is represented in dark green.

Table 1. Structural parameters obtained from the fit of all samples tested.

		SP-B			SP-C		
Layer	**Parameter**	**10 mN/m**	**35 mN/m**	**45 mN/m**	**10 mN/m**	**35 mN/m**	**45 mN/m**
Tails	Thickness (Å)	5.9±0.1	11±0.2	17.64±0.2	8.58±0.2	12.93±0.2	19.99±0.2
Heads	Hydration (0:1)	0.39±0.05	0.43±0.05	0.4±0.06	0.52±0.06	0.27±0.05	0.379±0.05
Reservoir	Thickness (Å)	-	-	73.38±0.2	-	-	-
	Hydration (0:1)	-	-	0.92±0.06	-	-	-
	σ (Å)	4±1E-10	6±1E-4	4±1E-10	4±1E-10	4±1E-10	4±1E-10

REFERENCES

(1) Pérez-Gil J. Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions. Biochim Biophys Acta (2008) 1778:1676–1695.

(2) Olmeda B, García-Álvarez B, Gómez MJ, Martínez-Calle M, Cruz A, Pérez-Gil J. A model for the structure and mechanism of action of pulmonary surfactant protein B. FASEB J (2015) 29(10): 4236-4247.

(3) Parra E, Moleiro LH, López-Montero I, Cruz A, Monroy F, Pérez-Gil J. A combined action of pulmonary surfactant proteins SP-B and SP-C modulates permeability and dynamics of phospholipid membranes. Biochem J. (2011) 438: 555–564.