Experimental report

19/02/2024

Proposal: 8-02-1004 **Council:** 4/2023

Title: COMPLETING A NEUTRON REFLECTOMETRYSTUDY OF NATIVE PULMONARY SURFACTANT INTERFACIAL FILMS AT THE HIGHESTSURFACE PRESSURES

Research area: Biology

This proposal is a continuation of 8-02-891

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Samples: Native surfactant
Amniotic fluid surfactant

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	25/09/2023	27/09/2023

Abstract:

Pulmonary surfactant is a lipid-protein complex crucial for breathing. It covers the alveolar interface in a rapid and efficient way, what is needed to prevent alveolar collapse. It is possible thanks to the accumulation of material in highly dynamic subpahse surfactant reservoirs that nurture the interface with lipids during inspiration and keep the excluded material associated to the interface during expiration. Its detailed understanding is vital for the treatment of lung pathologies and the development of new surfactant-based therapies. Usually, porcine surfactant has been used for the study of the system but recently a new surfactant isolated from human amniotic fluid has been isolated and showing the properties of a newly synthetised material. We have used neutron reflectometry to decipher the structural rearrangements occurring in this materials along respiratory mechanics still uncharacterized. 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

To keep us alive, lungs are the organs responsible for breathing mechanics, especially at the alveoli, where pulmonary surfactant is found. Pulmonary surfactant is a lipid/protein complex that covers the alveolar air-liquid interface minimizing the surface tension at the end of expiration and thus avoiding alveolar collapse [1]. Its complex structure consists on an interfacial monolayer connected to 3-D subphase reservoirs, what allows the rapid incorporation of new surfactant into the interface during inspiration and its rapid exclusion during expiration. As the detailed understanding of its dynamics is crucial for the development of surfactant based therapies, with neutron reflectometry we attempt to obtain information about the lipid/protein interactions occurring at a dynamic air-liquid interface still uncharacterized. Previous measurements on FIGARO have already provided valuable information about the structures formed along respiratory dynamics in synthetic samples. Also a couple of natural materials, one of them isolated from porcine lungs and the other one isolated from human amniotic fluid [2] have been also measured on FIGARO at low and medium surface pressures. Nevertheless, the most interesting experiments using natural materials at the highest surface pressures, where most of these structural reorganizations take place, still need to be performed.

PREVIOUS RESULTS

In previous neutron reflectometry using FIGARO, interfacial surfactant films composed of model lipids mimicking pulmonary surfactant composition (DPPC/POPC/POPG) with or without physiological and supra-physiological amounts of surfactant proteins B or C were characterized in buffer solution prepared either in D2O or Air Contrast Match Water. We studied films compressed in the Langmuir trough at surface pressures of 10 and 35 mN/m, before and after surface cycling (#8-02-865, #8- 02-891). As expected, preliminary analyses of the data confirmed the formation of reservoirs with the increase of surface pressure that is even enhanced at 10% SP-B content (Figure 1).

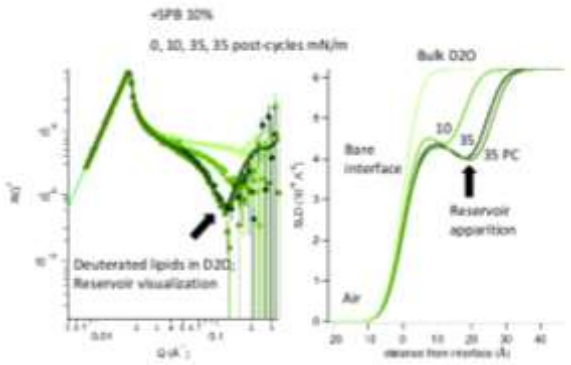

Figure 1. Reservoir appearance while increasing surface pressure in samples composed of deuterated DPPC/POPC/POPG (50:25:15 w:w:w) + 10 % SP- B in D2O. Left, reflectivity profiles. Right, SLD profiles.

AIM

The aim of this proposal was to measure native surfactant samples purified either from human amniotic fluid or porcine lungs at the highest surface pressures (50 mN/m) where the presence of the reservoir should be more obvious as most of the lipid rearrangements are taking place. By doing so, we will be able to detect lipid/protein interactions occurring throughout the remodelling of the films nowadays undescribed and complete the characterization of our systems.

EXPERIMENTAL PLAN

Pressures of 50 mN/m could not be reached due to an overflow of the trough, so measurements were performed at 45 mN/m.

1.- Native surfactant purified from porcine lungs at 45 mN/m before and after compression-expansion cycles in 2 contrasts: 100% D2O and Air Contrast Match Water.

2.- Human amniotic fluid surfactant at 45 mN/m before and after compression-expansion cycles in 2 contrasts: same measurements and contrasts as in 1.

The measurements of post-cycling condition failed due to continuous reorganizations of the interface that made impossible to stabilize the pressure at 45 mN/m, so a third contrast consisting of 50 % D2O and 50 % ACMW was used to perform the measurements at high pressures.

As some time was left after performing all these measurements, the organic extract corresponding to all the lipids and the hydrophobic proteins from surfactant (which are the components with biophysical activity) was also measured at every surface pressure tested within the whole set of experiments (10, 35, 35 post-cycling, 45 and 45 post-cycling).

RESULTS

Due to the complexity of the systems tested and the amount of data from previous proposals still in process of analysis, these experimental data are still being analysed.

REFERENCES

[1] Pérez-Gil, J. Biomed J, 2022, 45(4), 615-628.
[2] Castillo-Sánchez, J.C., Roldán, N., García-Álvarez, B., Batllori, E., Galindo, A., Cruz, A., Pérez-Gil, J. 2022, 322(2):L191-L203.