Experimental report

10/03/2020

Proposal: 8-02-865 **Council:** 4/2019

Title: Structural characterization of pulmonary surfactant lipid-protein interfacial films

Research area: Biology

This proposal is a new proposal

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Samples: Model surfactant mixture (deuterated) (d-DPPC/d31-POPC/d31-POPG) -from SIGMA
NPulmonary surfactant reconstituted from the organic extract of porcine surfactant (containing all the lipids plus the hydrophobic proteins SP-B and SP-C)
Native Pulmonary surfactant (purified from porcine lungs)
Model surfactant mixture (DPPC/ POPC/POPG) -from SIGMA

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	4	3	06/09/2019	09/09/2019

Abstract:

Pulmonary Surfactant (PS) is a lipid-protein complex critical to maintaining operational breathing in mammalian lungs. It forms surface-active films at the air-liquid respiratory interface to stabilize the lungs at the end of expiration, so minimizing the work of breathing. Extensive research along the last decades has yielded a model for the mechanism by which surfactant proteins (mainly SP-B) promote the formation of complex multilayered surfactant films and how they modulate their biophysical properties for maximal stability under the demanding conditions imposed by compression-expansion breathing dynamics. However, there is still lacking a full description on the assembly of lipid-protein complexes that is crucial to fully understand the molecular mechanism by which pulmonary surfactant proteins sustain efficient respiratory mechanics and oxygen exchange in the lungs. We expect to get structural details on how surfactant proteins re-organize interfacial films, both laterally and three-dimensionally, by comparing samples obtained from natural sources with samples reconstituted upon a combination of surfactant phospholipids and surfactant proteins purified from animal sources.

Report – Structural characterization of pulmonary surfactant lipid-protein interfacial films

Introduction

The membranous surface-active components of pulmonary surfactant are essential to ensure operational breathing in mammalian lungs through reducing dramatically surface tension in the alveolar air-liquid interface (1). Lung surfactant is mainly composed of lipids (90% total weight), responsible for lung surfactant biophysical activity, but also proteins (10% total weight) that participate in the innate immune defence (SP-A and SP-D) and guarantee an efficient transfer of surface-active phospholipids from freshly secreted complexes into the alveolar air-liquid interface (SP-B and SP-C). It is noteworthy the role of SP-B prompting membrane-membrane and membrane-interface contacts by forming lipid channels able to mobilize phospholipids (2). On the other hand, SP-C is structured as a hydrophobic transmembrane α-helix that induces curvature and exclusion of unpackable lipids what is essential to enrich the interfacial monolayer of DPPC, thus reducing surface tension to values close to 1mN/m, at the end of the exhalation.

Aim

The main purpose of this research project was to perform a quantitative structural characterization of the interfacial films formed by both phospholipid models mimicking lung surfactant composition in the presence or absence of surfactant proteins B and C and a surface-active material purified from bronchoalveolar lavages of porcine lungs. Specifically, we analysed how the structure of interfacial films change along compression-expansion cycles. Altogether, our goal is to describe extensively how surfactant lipids and proteins interact each other and sustain efficient breathing dynamics by re-organizing both laterally and three-dimensionally.

Experimental design and samples analysed

Our experimental design is the following: hydrogenated or deuterated version of phospholipid mixtures mimicking lung surfactant composition (DPPC/POPC/POPG 50:25:15 w:w:w) in the presence or absence of physiological concentrations (1% weight) of surfactant proteins B and C were injected directly onto the air-liquid interface of a Langmuir trough (5µl approximately – to reach a surface pressure of 1-2mN/m). The aqueous subphase employed was a buffer containing 5mM Tris, 150mM NaCl, pH 7.4 in D_2O or Air Contrast Match Water (Table 1). Prior to beginning with the experiment, 15 minutes are necessary for chloroform evaporation. Afterwards, the barrier was compressed till reaching a surface pressure of first 10mN/m and then 35mN/m and neutron reflection was recorded at both surface pressures. Finally, 5 compression-expansion cycles were performed, and neutron reflection was recorded at 35mN/m.

Results

Data from FIGARO using phospholipids models highlight an increased lipid concentration at 35mN/m. We did not observe meaningful differences in the reflectivity profiles when using surfactant proteins B and C at their physiological concentrations. Remarkably, 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 surfactant protein B (Fig. 1). This phenomenon agrees with our hypothesis about surfactant protein B prompting connections between the adsorbed lipid monolayer and membrane reservoirs which is essential to ensure efficient lipid flow along respiratory cycles. However, we were not able to do

experiments using an organic extract of the porcine surfactant and its phospholipids fraction with or without their protein content to compare the lipid models with native samples due to lack of time.

Table 1: Sample list

Sample	Protein (weight percentage)	Buffer	Surface pressure (mN/m)
Control experiment	0	D2O	0
Control experiment	0	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	0
Control experiment	0	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	0
DPPC/POPC/POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	10
DPPC/POPC/POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35
DPPC/POPC/POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	10
DPPC/POPC/POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	10
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35 (after cycling)
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	10
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35 (after cycling)
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	10
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35 (after cycling)
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	10
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35
DPPC/POPC/POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35 (after cycling)
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	10
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35 (after cycling)
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	10
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	0	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35 (after cycling)
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	10
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35 (after cycling)
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	10
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-B	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35 (after cycling)
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	10
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35 (after cycling)
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	10
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35
d_{62}-DPPC/d_{31}POPC/d_5POPG (50:25:15 , w:w:w)	1% SP-C	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35 (after cycling)
Porcine Surfactant	Native Content (1% each protein)	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	10
Porcine Surfactant	Native Content (1% each protein)	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35
Porcine Surfactant	Native Content (1% each protein)	5 mM Tris NaCl 150 mM pH 7.4 (D2O)	35 (after cycling)
Porcine Surfactant	Native Content (1% each protein)	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	10
Porcine Surfactant	Native Content (1% each protein)	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35
Porcine Surfactant	Native Content (1% each protein)	5 mM Tris NaCl 150 mM pH 7.4 (ACMW)	35 (after cycling)

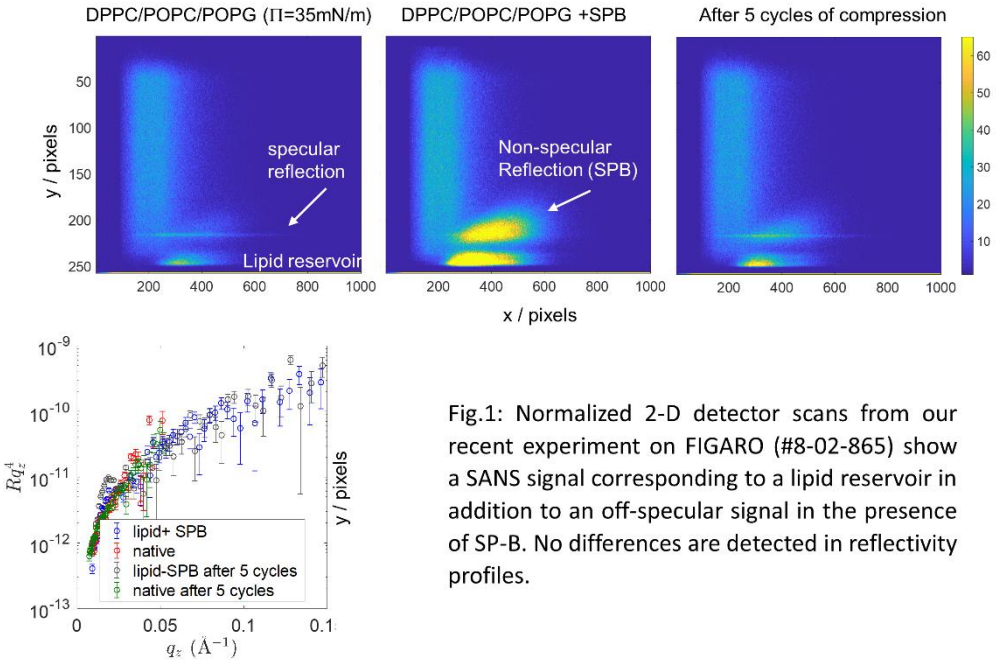

Fig.1: Normalized 2-D detector scans from our recent experiment on FIGARO (#8-02-865) show a SANS signal corresponding to a lipid reservoir in addition to an off-specular signal in the presence of SP-B. No differences are detected in reflectivity profiles.

Prospects

Our next goal is to complete the structural analyses of lung surfactant interfacial films by: 1) recording neutron reflection at pressures up to 45mN/m (where the exclusion of unsaturated phospholipids takes place and because of that the interfacial monolayer is compositionally altered), 2) employing high concentrations of surfactant proteins B and C (10% weight), 3) using materials purified from biological sources such as the organic extract of a porcine surfactant containing both lipids and proteins, its phospholipid fraction with or without their native protein content, 4) using a surface-active material isolated from human amniotic fluid since its structural and functional properties critically differ from the samples purified from lungs due to not being exposed to an air-liquid interface or subjected to respiratory mechanics.

To do so, we applied for a new beamtime (Proposal 83505) and we will do these experiments in 2-days next May.

References

(1) C. Autilio and J. Perez-Gil (2019) Understanding the principle biophysics concepts of pulmonary surfactant in health and disease. Arch Dis. Child. (doi 0.1136/archdischild-2018-315413) **(2)** B. Olmeda, B. Garcia-Alvarez, M.J. Martinez, M. Martinez-Calle, A. Cruz, J. Perez-Gil (2015) FASEB J. 29, 42364247.