Experimental report

18/02/2022

Proposal: 8-02-891 **Council:** 10/2019

Title: Going deeper in the structural characterization of pulmonary surfactant films at the air-liquid interface

Research area: Biology

This proposal is a new proposal

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Samples: POPC, CAS Number 26853-31-6
16:0-d31-18:1 PC, CAS Number 179093-76-6
Purified surfactant protein B (SP-B)
Purified surfactant protein C (SP-C)
DPPC, CAS Number 63-89-8
POPG, CAS Number 268550-95-4
16:0 PC-d62, CAS Number 25582-63-2
16:0-18:1 D5 PG, CAS Number 1246298-34-9
Porcine lung surfactant
Human amniotic fluid lung surfactant (AFS)

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	4	2	17/02/2021	19/02/2021

Abstract:

Covering the alveolar air-liquid interface, pulmonary surfactant is essential to maintain operational breathing by reducing surface tension. Pulmonary surfactant forms a highly dynamic multilamellar membrane film in the alveolar air-liquid interface. Triggered by respiratory mechanics, surfactant membranes undergo remarkable structural changes that are not well characterised yet. That is the reason why neutron reflectometry of adsorbed surfactant films in an air-liquid interface and subjected to compression-expansion cycles appears to be a promising technique to address such quantitative structural characterization. Here, we aim to understand in detail how surfactant lipids and proteins interact in such dynamic surface-active material. For this purpose, we will perform experiments on FIGARO using, not only model lipids in combination with surfactant proteins B and C, but also surfactant from biological sources that keep the native lipid-protein interactions. In such a way, we expect to be in position to attribute a structural role in surfactant homeostasis to both lipids and proteins.

FINAL REPORT

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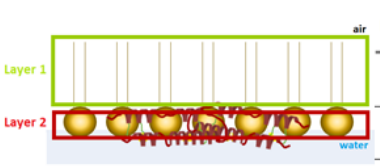
INTRODUCTION

Pulmonary surfactant is a lipoprotein complex that covers rapidly and efficiently the whole mammalian respiratory surface forming an interfacial monolayer. Its function is to maintain surface tension near equilibrium at the end of inspiration and to allow reaching minimal surface tensions during expiration to stabilize the alveoli and prevent alveolar collapse (1). The formation of this interfacial monolayer in an efficient way is of utmost importance in order to guarantee the correct development of the process of breathing. The rapid adsorption of surfactant into the interface is facilitated by the previous accumulation of pulmonary surfactant in reservoirs connected to it. Pulmonary surfactant is an extremely complex mixture of components being 90% in mass lipids, mainly phospholipids, and 10% proteins, which are essential for surfactant functionality. Especially, the hydrophobic proteins SP-B and SP-C are small proteins that strongly interact with phospholipids, playing critical roles in formation and stabilization of pulmonary surfactant films and their associated reservoirs. SP-B has been described to promote the exchange of lipids between bilayers and could help to connect the interface with its associated reservoirs (2). SP-C would insert in the monolayer creating negative curvature to facilitate the exclusion of unsaturated lipids to reach the minimum surface tension needed to stabilise the alveoli at the end of expiration (3).

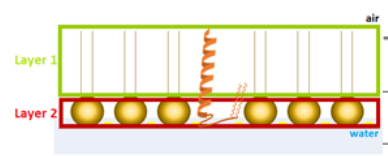
PREVIOUS RESULTS

In previous experiments using FIGARO we demonstrated that adding SP-B to its physiological concentration (1 %) to a mixture of phospholipids mimicking pulmonary surfactant composition, it interacts only with lipid polar headgroups of surfactant phospholipids in a peripheral disposition causing their dehydration at 10 mN/m and their rehydration at 35 mN/m, meaning that the protein could have been excluded from the interface at high pressures, what has been previously reported. In the presence of 1 % SP-C, data suggested that the protein would interact with both phospholipid polar headgroups through its N-terminal region and with the acyl chains through its hydrophobic α-helix, which is in accordance to previous knowledge about the behaviour of SP-C in lipid monolayers. We were not able to observe the exclusion of the α-helix from the interface during compression (Fig. 1).

We also 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. Altogether with the reflectivity results, SP-B could be connecting the interfacial monolayer with surfactant reservoirs, as well as different bilayers of surfactant reservoirs between them, being essential for the efficient restructuration of the monolayer along breathing cycles.



Layer	Parameter	10 mN/m	35 mN/m pre-cycling	35 mN/m post-cycling
1	t (Å)	6.57±0.1	11.72±0.2	11.28±0.2
	f_w (%)	0	0	0
2	t (Å)	8.5	8.5	8.5
	f_w (0:1)	7.4E-09	0.231±0.05	0.082±0.06
	χ^2	24.27	33.79	38.03



Layer	Parameter	10 mN/m	35 mN/m pre-cycling	35 mN/m post-cycling
1	t (Å)	9.16±0.2	12.91±0.2	12.86±0.2
	f_w (%)	0	0	0
2	t (Å)	8.5	8.5	8.5
	f_w (0:1)	0.510±0.06	0.344±0.05	0.349±0.05
	χ^2	25.63	35.48	34.42

Figure 1. Reflectivity parameters of phospholipid tails (layer 1) and heads (layer 2) in the presence of SP-B (top) or SP-C (bottom). Parameters: *t* (thickness, Å) and f_w (hydration).

AIM

Based on previous FIGARO experiments, we tried to go deeper into the assessment of the structural rearrangements of pulmonary surfactant interfacial monolayers. We employed the same samples as before (a phospholipid model mimicking pulmonary surfactant composition in the presence or absence of surfactant proteins SP-B and SP-C) but we increased 1) the concentration of SP-B and SP-C, and 2) 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. These experiments allowed us to characterize the interactions between surfactant lipids and proteins that take place at the alveolar air-liquid interface when breathing dynamics are taking place and to address the reorganization of surfactant complexes during this process.

EXPERIMENTAL PLAN

We measured samples containing 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 and SP-C. To do so, we maintained the same protocol that in our previous experiments (proposal #8-02-865): enough volume of the samples was injected directly onto the air-liquid interface of a Langmuir trough to reach a surface pressure of 1-2mN/m. The aqueous subphase employed was a buffer containing Tris 5mM, NaCl 150mM, pH 7.4 either in D_2O or Air Contrast Match Water. Then, 10 minutes were left for chloroform evaporation. Before measuring, the barrier was compressed until the surface pressure of interest (10mN/m, 35mN/m or 50 mN/m) and neutron reflection was recorded. Subsequently, 5 compression-expansion cycles were performed, and neutron reflection was recorded again at 45mN/m.

RESULTS

During this beam time, reflectivity profiles from these samples were obtained:

Table 1. Samples measured within the proposal #8-02-891.

LIPID MODEL + 10% SP-B

SAMPLE	CONTRAST	PRESSURES (mN/m)				
DPPC/POPC/POPG + 10% SP-B	D2O	10	35	35 post-cycling		
DPPC/POPC/POPG + 10% SP-B	ACMW		35		50	50 post-cycling
dDPPC/dPOPC/dPOPG + 10% SP-B	D2O	10	35	35 post-cycling		
dDPPC/dPOPC/dPOPG + 10% SP-B	ACMW		35	35 post-cycling	50	50 post-cycling

LIPID MODEL + 10% SP-C

SAMPLE	CONTRAST	PRESSURES (mN/m)				
DPPC/POPC/POPG + 10% SP-C	D2O	10	35	35 post-cycling		
DPPC/POPC/POPG + 10% SP-C	ACMW		35		50	50 post-cycling
dDPPC/dPOPC/dPOPG +10% SP-C	D2O	10	35	35 post-cycling		

As it can be observed in the tables above, some pressures are missing, especially the higher ones, due to an overflow in the trough. The sample corresponding to the deuterated lipid mix + 10 % SP-C in ACMW was not measured owing to lack of time.

These experimental data are still being analysed.

FUTURE PROSPECTS

To complete this study we would like to compare the neutron reflectivity profiles obtained until now with those of native materials purified from biological sources: (i) a porcine surfactant used nowadays in clinical surfactant preparations, and (ii) a newly characterized material isolated from human amniotic fluid, which conserves the properties of a freshly secreted material that still has not been subjected to the dynamic process of breathing.

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.