Experimental report

12/04/2022

Proposal: 8-02-969 **Council:** 4/2021

Title: Interaction of a recombinant version of the SARS-Cov-2 spike protein with pulmonary surfactant interfacial monolayers

Research area: Biology

This proposal is a new proposal

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Samples: Dipalmitoylphosphatidylcholine (DPPC)
Surfactant protein B (SP-B)
Surfactant protein C (SP-C)
Native pulmonary surfactant
Organic extract from pulmonary surfactant
Recombinant SARS-CoV-2 spike protein

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	2	2	11/10/2021	13/10/2021

Abstract:

COVID-19 pandemic is having a drastic impact around the world. SARS-CoV-2 is a respiratory virus belonging to the family of coronavirus and is mainly transmitted through respiratory droplets and aerosols. In such way, this virus enters the host via respiratory airways and binds to the angiotensin-converting enzyme 2 (ACE2), protein expressed by several cells in the respiratory tract, through the so-called protein spike. This infection can progress to the clinical condition known as acute respiratory distress syndrome (ARDS). Pulmonary surfactant is a complex mixture of lipid and specific proteins that constitute the first barrier to pathogens at the level of the alveolar epithelium and it is highly affected in ARDS. In this context, we propose to perform neutron reflectometry experiments in interfacial monolayers composed of pure DPPC, as model lipid, and native surfactant complexes isolated from different biological sources, exploring the effect of the incorporation of recombinant versions of the spike protein S of SARS-CoV-2. The interaction of the virus with surfactant through this protein is likely a primary event defining the fate of viral infection into the airways.

PRELIMINARY REPORT

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INTRODUCTION

The coronavirus Sars-CoV-2 has been on everybody's lips since the end of 2019 when the current pandemic situation started. This is why investigators of respiratory pathologies have moved their focus to understand this virus infection and the consequences of COVID-19 in human health. The research of our group focuses on the characterization of the pulmonary surfactant system. Pulmonary surfactant is a lipid/protein complex located at the alveolar air-liquid interface which main function is to reduce the surface tension at the end of expiration preventing alveolar collapse [1]. This system is the first barrier that pathogens, as Sars-CoV-2, find once they have been inhaled, thus, its proper performance could impair virus infection and reduce respiratory disorders. Pulmonary surfactant is composed by lipids, mainly phospholipids, and four associated proteins. The saturated phospholipid DPPC is the major surfactant component and the one in charge of sustaining such a strong reduction of surface pressure because of its ability to pack efficiently in the interface upon compression [2]. The other lipid components are excluded from the interface and stored in reservoirs placed in the aqueous subphase. With regard to the proteins, we consider SP-B to be crucial for pulmonary surfactant's biophysical function. It is a small and highly hydrophobic protein with the ability of creating membrane contacts and transference of lipids from the subphase reservoirs to the interface and its absence turns out to be lethal [3].

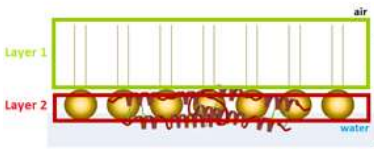
AIM

Due to the little evidence about how Sars-CoV-2 interacts with pulmonary surfactant interfacial films, we requested for a beam time to study the interaction of a recombinant version of the ectodomain of the Sars-CoV-2 spike protein (IBS, Institute de Biologie Structurale, Grenoble, France) with model lipid systems mimicking pulmonary surfactant composition. These experiments would serve as a first approach to shed light on the way in which the Sars-CoV-2 virus interacts with pulmonary surfactant once it reaches the respiratory interface.

PREVIOUS RESULTS

In previous experiments using FIGARO (#8-02-865 and #8-02-891) we demonstrated that SP-B interacts 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, as has been previously reported (*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. All these results indicate that SP-B could be connecting the interfacial monolayer with subphase

reservoirs of surfactant, as well as the different bilayers of these surfactant reservoirs between them, being essential for the dynamism of the interfacial monolayer along breathing cycles. Although the crucial action of the protein for the biophysical function of the system is unquestionable, we still need to go further in the characterization of the molecular mechanism by which the protein acts.



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

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

EXPERIMENTAL PLAN

In the present experimental plan we tried to understand how the presence of a recombinant version of the spike protein affects the structure of pulmonary surfactant model films.

We first measured the reflectivity profile of a hydrogenated or deuterated version of DPPC when it is in contact with our variant of the spike protein. A DPPC solution in chloroform/methanol 2:1 (v/v) 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 Nule Reflecting Water. Then, 10 minutes were left for chloroform evaporation. Subsequently, 30 µL of the spike protein were injected close to the interface, and 10 minutes were left for film stabilization. Before measuring, the barrier was compressed until the surface pressure of interest (10mN/m, 35mN/m or 50mN/m) and neutron reflection was then recorded. The same kind of experiments were repeated in the presence of 1 % by weight of SP-B and with or without spike protein in order to address differences between the obtained reflectivity profiles.

Finally, we moved to a more complex system and 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 of 1 % by weight of SP-B with or without the addition of the spike protein. We followed the same protocol described previously, including the additional performance of 5 compression-expansion cycles in order to emulate structural rearrangements of the interface after being subjected to *in vivo* breathing cycles.

RESULTS

Reflectivity profiles were acquired for the following samples:

Table 1. Samples measures within the proposal #8-02-969

DPPC + S

SAMPLE	H/D	CONTRAST	PRESSURES (mN/m)		
DPPC	H	D2O	10	35	50
dDPPC	D	NRW	10	35	
dDPPC	D	D20	10	35	45

DPPC + 1 % SP-B + S

SAMPLE	H/D	CONTRAST	PRESSURES (mN/m)		
DPPC + 1% SP-B	H	D2O	10	35	45
dDPPC + 1% SP-B	D	NRW	10	35	45
dDPPC + 1% SP-B	D	D20	10	35	45

LIPID MODEL + 10 % SP-B + S

SAMPLE	H/D	CONTRAST	PRESSURES (mN/m)		
DPPC POPC POPG + 10% SP-B	H	D2O	10	35	35 post-cycling
dDPPC dPOPC dPOPG + 10% SP-B	D	D2O	10	35	25 post-cycling
dDPPC dPOPC dPOPG + 10% SP-B	D	NRW	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 samples corresponding to the deuterated lipids in NRW were not measured owing to lack of time.

These experimental data are still being analysed.

FUTURE PROSPECTS

For further characterization of the interaction between our recombinant spike protein with pulmonary surfactant we would like to address the effect of the spike protein with model lipid mixtures containing SP-C, the other small hydrophobic protein essential for the biophysical function of pulmonary surfactant. We would also like to compare the neutron reflectivity profiles obtained in the presence of spike protein, with the one of a native surfactant isolated from porcine lungs, which is used nowadays in clinical surfactant preparations.

REFERENCES

[1] Lopez-Rodriguez E, Pérez-Gil J. Structure-function relationships in pulmonary surfactant membranes: From biophysics to therapy. Biochim Biophys Acta (2014) **1838:** 1568–1585.
[2] Yu SH, Possmayer F. Lipid compositional analysis of pulmonary surfactant monolayers and monolayer-associated reservoirs. J. Lipid Res. (2003) **44**: 621-629.
[3] 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**: 4236–4247.