Experimental report

03/04/2022

Proposal: 8-02-927 **Council:** 4/2020

Title: Phospholipid bilayer of pulmonary surfactant: the effect of lipopolysaccharide and Polymyxin B

Research area: Other...

This proposal is a new proposal

Main proposer: Daniela UHRIKOVA

Experimental team: Daniela UHRIKOVA
Norbert KUCERKA

Local contacts: Bruno DEME

Samples: DPPC/POPC/PLPC/POPG
DPPC/POPC/PLPC/POPG + SP-B
DPPC/POPC/PLPC/POPG +LPS (10 wt%)
DPPC/POPC/PLPC/POPG +LPS + PxB (1; 2.5; 7 and 10 wt %)
DPPC/POPC/PLPC/POPG +LPS + SP-B (0.1, 0.3, 1 wt%)

Instrument	Requested days	Allocated days	From	To
D16	6	5	17/06/2021	22/06/2021

Abstract:

After inhalation, bacterial lipopolysaccharide (LPS) molecules interfere with a pulmonary surfactant (PS), a unique mixture of phospholipids and specific proteins (< 10 %) that decreases surface tension at the air-liquid interphase of lungs alveoli. LPS incorporated into clinically used exogenous porcine surfactant prevents the PS to reach the necessary low tension during area compression and disturbs its lamellar structures in water hypophase by swelling as resulted from our SAXS experiments. Polymyxin B (PxB), peptid based antibiotic that mimics functional properties of pulmonary specific protein SP-B, acts as an inhibitor of these structural changes. Oriented lipid bilayers hydrated from vapour mimic well the biological system of interest. We propose neutron diffraction experiment at D16 beamline to determine structural parameters of synthetic surfactant (PPS) composed of DPPC/POPC/PLPC/POPG and LPS, interacting with PxB and SP-B protein. The aim of the experiment is to specify: a) the effect of bacterial LPS on the lipid bilayer thickness of PPS and its hydration; b) the effect of PxB and SP-2 protein on PPS under pathological conditions induced by LPS.

Experimental Report

Experiment title: Phospholipid bilayer of pulmonary surfactant: the effect of lipopolysaccharide and Polymyxin B

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Abstract:
After inhalation, bacterial lipopolysaccharide (LPS) molecules interfere with a pulmonary surfactant (PS), a unique mixture of phospholipids and specific proteins (< 10 %) that decreases surface tension at the air-liquid interphase of lungs alveoli. LPS incorporated into clinically used exogenous porcine surfactant prevents the PS to reach the necessary low tension during area compression and disturbs its lamellar structures in water hypophase by swelling as resulted from our SAXS experiments. Polymyxin B (PxB), a peptide antibiotic that mimics functional properties of pulmonary specific protein SP-B, acts as an inhibitor of these structural changes [1]. Oriented lipid bilayers hydrated from vapour mimic well the biological system of interest. We performed a neutron diffraction experiment at D16 beamline to determine structural parameters of synthetic surfactant (MPS) composed of DPPC/POPC/PLPC/POPG and LPS, interacting with PxB and SP-B protein. The aim of the experiment was to specify: a) the effect of bacterial LPS on the lipid bilayer thickness of model PS and its hydration; b) the effect of PxB and SP-2 protein on model PS under pathological conditions induced by LPS.

Background:
Pulmonary surfactant (PS) is composed of ~ 90% lipids and 8-10% specific surfactant associated proteins. The hydrophobic protein SP-B is essential to facilitate the formation and proper performance of surface active films at the air-liquid interface. On the other hand, SP-B generates a multilamellar organization of phospholipid bilayers due to its fusogenic and lytic properties (Fig. 1) [2]. SP-B is difficult to synthesize; that prevents characterization of its 3D structure. It is thus important that the functional properties of SP-B can be mimicked by polymyxin B (PxB), an acyclic amphipathic decapeptide with five positively charged side chains and an acyl chain at the N terminus (Fig. 1) [3]. It was found that PxB improves physiological properties of therapeutically used formulation, exogenous surfactant Curosurf [4]. After inhalation, bacterial LPS molecules (Fig. 1) interfere with pulmonary surfactant.

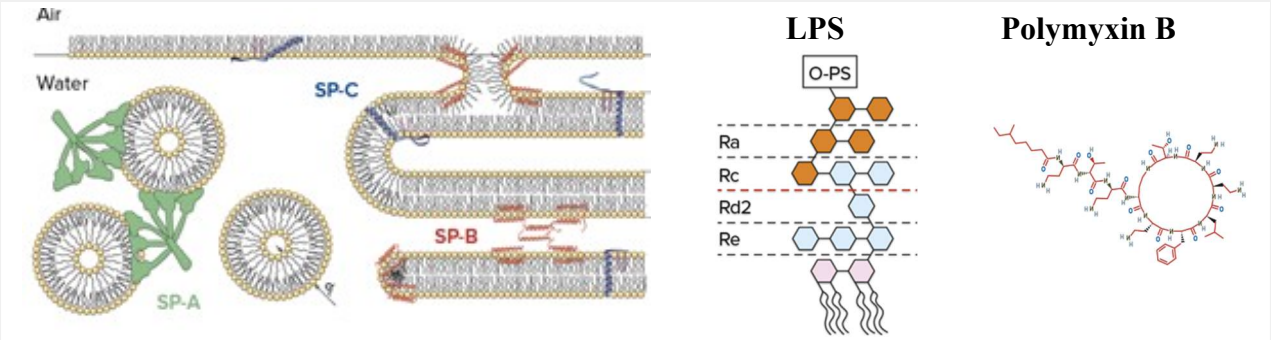

Fig. 1. Sketch of pulmonary surfactant structure, bacterial lipopolysaccharide molecule (LPS) and Polymyxin B.

Curosurf is obtained from porcine lungs. It is composed of at least 50 different phospholipids and a small amount of SP-B (~ 2 wt %) [2]. Due to economic and ecological reasons, a great effort is put into the development of synthetic surfactants for the treatment of lung pulmonary diseases. The composition of the mixture of dipalmitoyl-phosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), 1-palmitoyl-2-linoleoyl-phosphatidylcholine (PLPC) and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG) at ratio DPPC/POPC/PLPC/POPG= 50:24:16:10 wt% (MPS) used in our experiments is based on its tests on animal model [5]. We focus our attention on lamellar structures present in the hypophase (Fig. 1) that are essential for proper function of PS.

Experiment:
Phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Mixtures of MPS and LPS were prepared following [6]. Approximately 12 mg of lipid mixture (thin film comprising of 2,000-3,000 bilayers having a total thickness ~15 μm when spread onto a 25 x 50 mm^2 silicon wafer) was hydrated by deionized water and mixed thoroughly when following several freeze-thaw cycles and vortexing vigorously. MPS+LPS was incubated with PxB or SP-B prior to the dispersion deposition. The dispersions were deposited on leveled silicon wafers that were heated to 50°C, and excess water left to evaporate. The care was taken to form lipid multilayers in the fluid phase, and to anneal the samples for several hours upon rehydration. Samples were hydrated in vapour of 8% D_2O (based on [7]) using an air-tight hydration chamber provided by ILL. High quality oriented stacks were confirmed by observing up to 5 orders of diffraction peaks; an example of rocking curves is shown in Fig. 2.

Small-Angle Neutron Diffraction
Neutron diffraction data were collected at the Institut Laue-Langevin (ILL) in Grenoble, France on D16 small momentum transfer diffractometer with variable vertical focusing. Neutrons of 4.466 Å wavelength were selected by the (002) reflection of a pyrolytic graphite (PG) monochromator. Incoming beam was formed by the set of slits (S1=140x6 mm^2 and S2=30x5 mm^2) and sample-to-detector distance was 0.95 m. All samples were measured at two detector positions with a 3He position sensitive detector. Γ_1=12° was utilized for the detection of up to 4th order diffraction peak, and Γ_2=27° for the detection of higher order peaks. The data of area detector were visualized and reduced by an in-house written routine and the Lamp software provided by ILL [8]. Temperature was kept to 45 °C to secure liquid-crystalline state of the lipid.

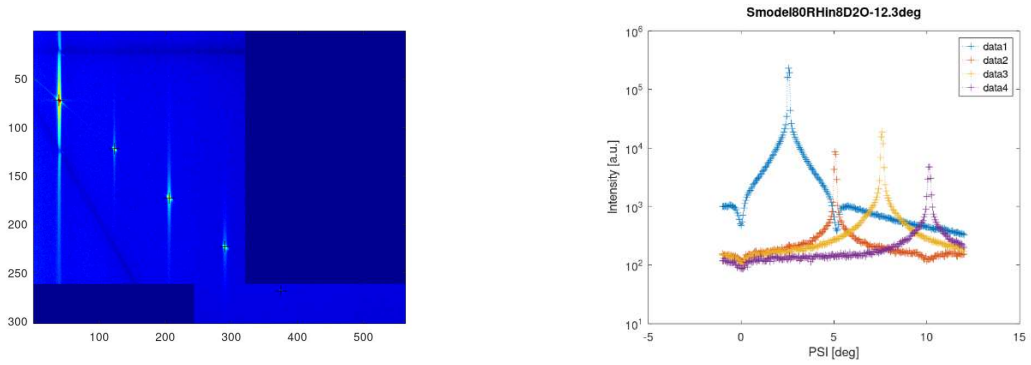

Fig. 2. Diffraction patterns of MPS and the rocking curve.

Results and discussion:
Fig. 3A shows examples of diffraction curves of samples hydrated at 95 % RH of 8 % D_2O. All samples have shown SAND patterns with up to 4-5 peaks. Samples were hydrated at 4 different RH in vapor of 8% D_2O. The obtained data thus provide both scattering amplitudes and their phases allowing Fourier transform analysis [7] and construction of neutron scattering density profiles (the analysis is in progress).

Fig. 3B shows the extracted repeat distances as a function of hydration (% RH) for MPS system and surfactant infected by LPS. The repeat distance d increases non-linearly with both, increasing hydration characterized by RH (%), and with the content of LPS. Fig. 4A summarizes and illustrates the LPS effect at two hydrations. Due to hydrophobic interactions between acyl chains of lipid A and hydrophobic core of PS, LPS intercalates into PS bilayer. $d=d_w+d_L$, and both parts the lipid bilayer thickness (d_L) and the thickness of the water layer (d_w) can be affected. The reconstruction of neutron scattering density profiles will help to resolve these two effects. While the profile corresponding to the 100% D_2O contrast condition provides the best estimate of the bilayer's steric thickness, lower contrast profiles reveal details of the lipid headgroup region. This is best exemplified by the 8% D_2O data, where the net NSLD of water is zero, essentially yielding the lipid only NSLD profile of the bilayer [7].

Model PS system (MPS) is composed of 10 wt% of negatively charged POPG, mimicking properties of natural PS. PxB is a cationic antibiotic interacting electrostatically with PS. Our previous studies have revealed that PxB affects the repeat distance of model PS system in a quasi-parabolic way, showing the most efficient charge shielding at ~ 4 wt% of PxB [9,10]. Fig. 4B shows the effect of PxB on model PS "infected" by LPS. The surfactant infected by 5 wt% of LPS shows the increase in d > 1 Å at each studied hydration (80 – 99 RH%). 4 wt% of PxB restores the original structure, repeat distances do not differ within the experimental error at

hydrations 80≤RH%≤95. Even if PxB does not reduce *d* back to original value observed for the surfactant without any additive at 99% RH, its "healing effect" is evident. The reconstruction of neutron scattering density profiles will help to resolve (and quantify) the disturbing effect of additives on the thickness of PS bilayer (d_L), and on the thickness of water layer (d_W) where electrostatic interactions at lipid/water interface are dominant.

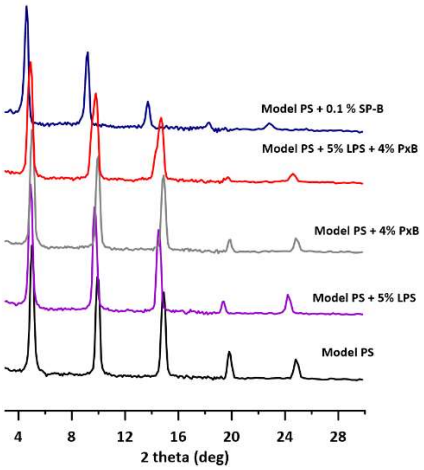

Fig. 3A Diffraction patterns of selected samples at 95 % RH. Intensities are plotted in logarithmic scale.

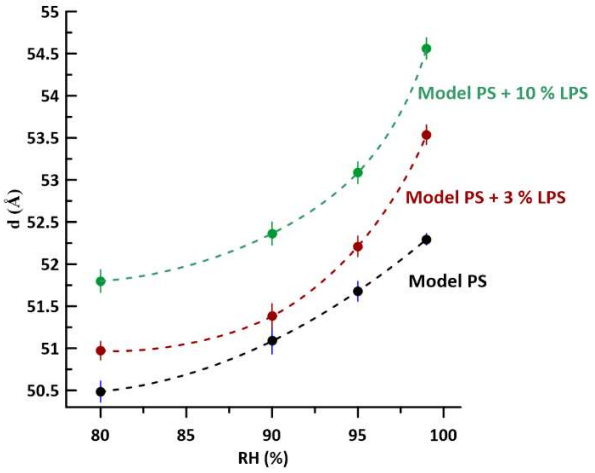

Fig. 3B Repeat distances as a function of RH % for model system (MPS) and MPS+LPS.

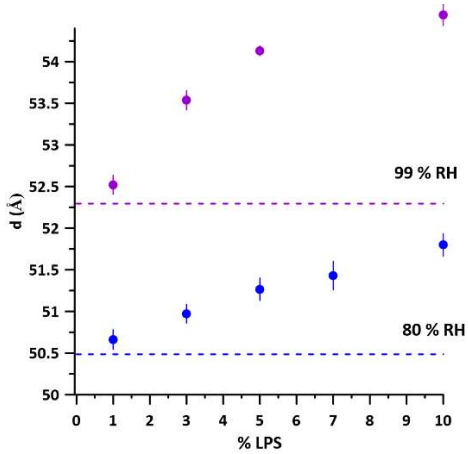

Fig. 4A The effect of LPS on the repeat distance of MPS (dashed lines) at 80 and 99 % RH, respectively.

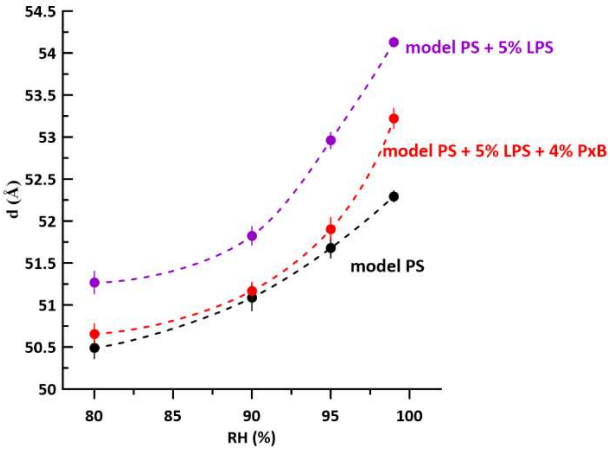

Fig. 4B The repeat distance of MPS, MPS+5% LPS and MPS+5%LPS+4%PxB as a function of hydration.

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