Experimental report

14/01/2020

Proposal: 8-02-847 **Council:** 10/2018

Title: Phospholipid bilayer of pulmonary surfactant: the effect of SP-B protein and Polymyxin B

Research area: Other...

This proposal is a new proposal

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Samples: Cur+PxB
DPPC/POPC/PLPC/DOPG+SP-B protein
DPPC/POPC/PLPC/DOPG+PxB
porcine surfactant Cur
DPPC/POPC/PLPC/DOPG
DPPC/POPC/PLPC/DOPG+SP-B+PxB

Instrument	Requested days	Allocated days	From	To
D16	6	6	19/06/2019	20/06/2019
			24/06/2019	04/07/2019

Abstract:

Pulmonary surfactant is a unique mixture of phospholipids and surfactant specific proteins (< 10 %) which decreases surface tension at the alveolar air-liquid interphase. The hydrophobic protein SP-B acts as a promoter for approaching and binding of the two membrane surfaces together and stimulates formation of lamellar structures in water hypophase that are essential to facilitate the formation of surface active films. Polymyxin B (PxB), peptid based antibiotic, was found to mimic functional properties of SP-B. Oriented bilayers mimic well the biological system of interest. We propose neutron diffraction experiment to determine structural parameters of the surfactant organized in bilayers and to compare the effect of SP-B and PxB. Aligned bilayers will be prepared either from therapeutically used porcine lung surfactant (Cur) or synthetic surfactant (PPS) composed of DPPC/POPC/PLPC/DOPG. Biological experiments confirmed PPS mixture as promising substitution of an expensive porcine surfactant Cur. The aim of the experiment is to answer questions: Is there a structural similarity in Cur and PPS+SP-B? How is revealed the fusogenic effect of SP-2 and PxB? Can PxB substitute SP-B?

Experimental Report

Experiment title: Phospholipid bilayer of pulmonary surfactant: the effect of SP-B protein and Polymyxin B

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Abstract:
Pulmonary surfactant (PS) is a unique mixture of phospholipids and surfactant specific proteins which decreases surface tension at the alveolar air-liquid interphase. The hydrophobic protein SP-B stimulates formation of lamellar structures in water hypo-phase that are essential to facilitate the formation of surface active film. Polymyxin B (PxB), peptid based antibiotic, was found to mimic functional properties of SP-B. We performed neutron diffraction experiment on oriented lipid bilayers mimicking PS to determine structural parameters and to examine the effect of PxB. Bilayers prepared from therapeutically used porcine lung surfactant (PSUR), a mixture of various lipids, have shown 2-3 diffraction maxima. PxB induces structural changes in aligned bilayers composed of DPPC/POPC/PLPC/DOPG, model system of PS.

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 and, due to its ability to generate contacts between the lipid bilayers (Fig. 1A) [1]. These lamellar structures are likely equivalent to the disk-like structures and to the "nanosilos" or protrusions that are associated with the air-liquid interface [1]. SP-B is difficult to synthesize; that prevents characterization of its 3D structure [2]. 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 [3]. It was found that PxB improves physiological properties of therapeutically used formulation, surfactant Curosurf (PSUR) [4].

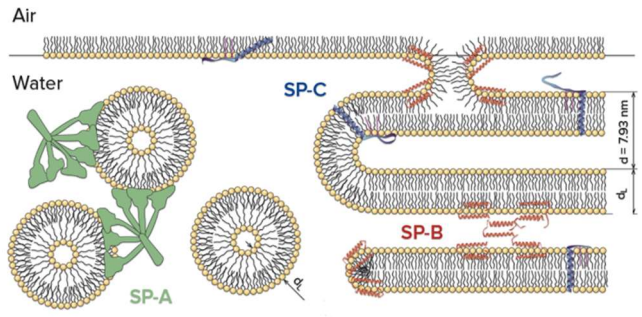

Fig. 1. Sketch of pulmonary surfactant structure. The repeat distance d=79.3 Å determined by SAXS on fully hydrated bilayers of PSUR [5].

Curosurf (PSUR) is clinically used preparation obtained from porcine lungs. It is composed of at least 50 different phospholipids and a small amount of SP-B (~ 2 wt %) [1]. Therefore, a great effort is put into a development of synthetic surfactants for the treatment of lung pulmonary diseases. The composition of the mixture of dipalmitoylphosphatidylcholine (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% (PPS) used in our experiments is based on its recent tests on animal model [6].

Experiment:
Phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Approximately 12 mg of PPS 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. PPS was incubated with PxB prior the dispersion deposition. The dispersions were deposited on leveled silicon wafers that were heated to 50^{o}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 curve 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 4.493Å wavelength were selected by the (002) reflection of a pyrolytic graphite (PG) monochromator. Incoming beam was formed by the set of slits (S1=150x6 mm^2 and S2=25x6 mm^2) and sample-to-detector distance was 0.95 m. All samples were measured at two detector positions with 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 ^{o}C to secure liquid-crystalline state of the lipid.

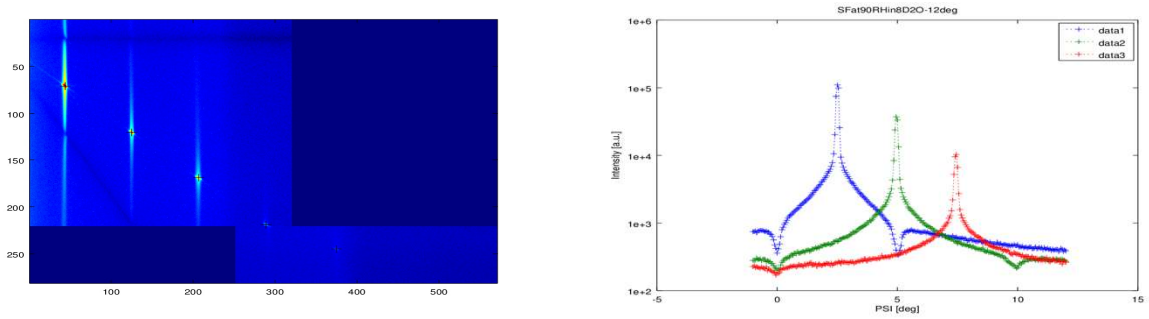

Fig. 2. Diffraction patterns of PPS and the rocking curves at the three most pronounced diffraction orders.

Results and discussion:

Fig. 3A shows examples of constructed diffraction curves of samples hydrated at 90 % RH of 8 % D_2O. We detected significant difference in the repeat distances of PS prepared from synthetic lipids, PPS, *d*=51.4±0.3 Å and clinically used PSUR, *d*=70.4±1.5Å. The obtained *d* of PSUR corresponds well with the value *d* ~ 79.3 Å found by SAXS for fully hydrated multilamellar vesicles [5]. Rather high uncertainty of the repeat distance of PSUR, and only two diffraction peaks indicate lower quality of lipid bilayers alignment that results from the PSUR composition. On the other hand, all samples of PPS, PPS + PxB have shown SAND patterns with up to 4-5 peaks. Samples were hydrated at 5-6 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 PPS and the surfactant incubated with PxB (PxB+Model PS). Note, the repeat distance *d* of PPS shows quasi-parabolic dependence on RH. The value, *d*=52.3±0.3 Å at 80 % RH, we attribute to the gel state of the lipid. PxB decreases the temperature of the gel to liquid-crystalline phase transition. Globally, the repeat distances linearly increase with RH% what can be attributed to swelling of lamellae due to the increase of the water layer localized between adjacent lamellae. However, PxB affects the periodicity in non-linear way. Fig. 4A shows the effect of PxB on the repeat distance at selected humidity. *d* of PxB+PPS slightly decreases and consecutively increases with the concentration of PxB in comparison to the repeat distance of PPS (dashed line). PPS is composed of 10 wt% of negatively charged POPG. Thus the observed non-linear course of *d* with increasing cationic PxB can reflect electrostatic interactions between individual compounds, however we can not exclude the effect of PxB on the thickness of the lipid bilayer.

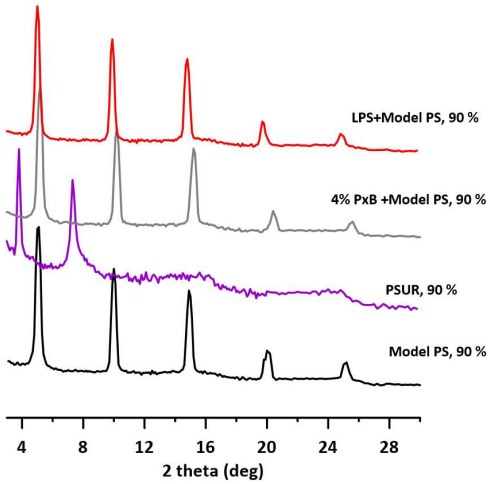

Fig. 3A Diffraction curves of selected samples at 90 % RH. Intensities are plotted in logarithmic scale.

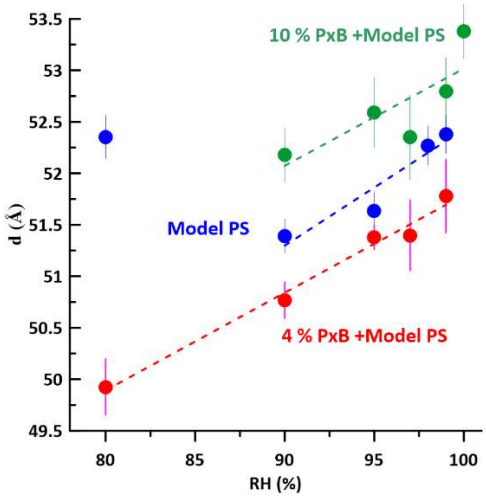

Fig.3B Repeat distances as a function of RH % for model system (PPS) and PxB+PPS.

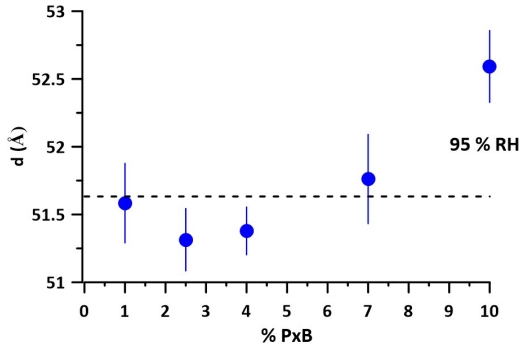

Fig.4A The effect of PxB on the repeat distance of PPS (dashed line) at 95 % RH.

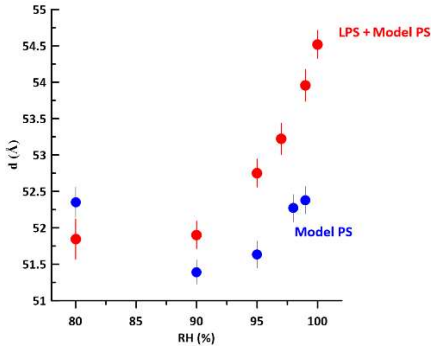

Fig. 4B The repeat distance of PPS and PPS+10% of LPS as a function of hydration.

The reconstruction of neutron scattering density profiles will help to resolve these two effects. Fig. 4B shows the increase of the repeat distance *d* with RH% for LPS+PPS resulting from sweeling of lamellae due to electrostatic repulsion between negatively charged LPS+PPS bilayers.

Part of obtained results was presented at BILL 2019 at ILL, Grenoble (December 11-13, 2019) under "Structural changes of pulmonary surfactant induced by bacterial lipopolysaccharide and by Polymyxin B" (Uhríková et al.; oral).

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