Experimental report

18/10/2017

Proposal: 8-02-777 **Council:** 4/2016

Title: Protein and DPPC competitive adsorption can be regulated by fluorocarbon gases and sinusoidal oscillations close to respiration frequencies

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Bovine serum albumin (BSA),
Fluorocarbons (perfluorohexane and perfluorooctylbromide)
Dipalmitoylphosphatidylcholine (DPPC)

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	4	6	07/11/2016	10/11/2016
			28/02/2017	03/03/2017

Abstract:

It is appreciated that proteins such as albumin and fibrinogen interfere with the adsorption of components of lung surfactant to the surface of the alveoli, thus disrupting normal pulmonary function, which can lead to serious consequences. Recently we have shown that direct interactions of films with biocompatible fluorocarbon gases under sinusoidal oscillations of the surface area of films appear to inhibit these effects as the surface pressure then converges on values closer to that of lipid than protein. A direct quantification of these effects is however missing to date. Here we propose to exploit new methodology developed recently on the high flux FIGARO reflectometer at the ILL to resolve the composition at the air/water interface of mixed protein/lipid systems in situ while they are subjected both to fluorocarbon gases and to sinusoidal oscillations. The strong scattering from the fluorocarbons and deuterated lipid will be exploited. This work will give important new insight into the fundamental mechanisms by which proteins inhibit lung function, and pave the way for studies on more complex systems involving lung surfactant targeted therapies in the future.

Final Report on FIGARO experiment #8-02-777

Protein and DPPC competitive adsorption can be regulated by fluorocarbon gases and sinusoidal oscillations close to respiration frequencies

INTRODUCTION

Serum proteins interfere with the adsorption of components of lung surfactant to the surface of alveoli, thus disrupting normal pulmonary function and leading to serious health consequences. We hypothesized that the interaction between phospholipids (the main components of the lung surfactant) and fluorocarbons may favor enhanced adsorption of phospholipids, thereby counteracting the proteins' inhibition of the lung surfactant. Recently, we have investigated the influence of prolonged sinusoidal oscillations on the competitive adsorption of dipalmitoylphosphatidylcholine (DPPC) vesicles and bovine serum albumin (BSA) at a perfluorohexane (*F*-hexane)-saturated air/water interface (Nguyen,…, Krafft, *Soft Matter* **2013**, *9*, 9972). In a first study, we found that the oscillations can fully *reverse* the usual outcome of the competitive adsorption. Thus, after ~5 h, albumin started being progressively expelled from the interface and replaced by DPPC. The oscillations strongly accelerated the replacement of albumin by DPPC, which was then obtained in ~10 h as compared to ~22 h in air (Fig. 1). In another study, we demonstrated that the introduction of *F*-hexane in the air phase strongly accelerated the displacement of albumin and its replacement by DPPC (Fig. 1, 2) (Nguyen,…, Krafft, *Chem. Commun.* **2014**, *50*, 11576).

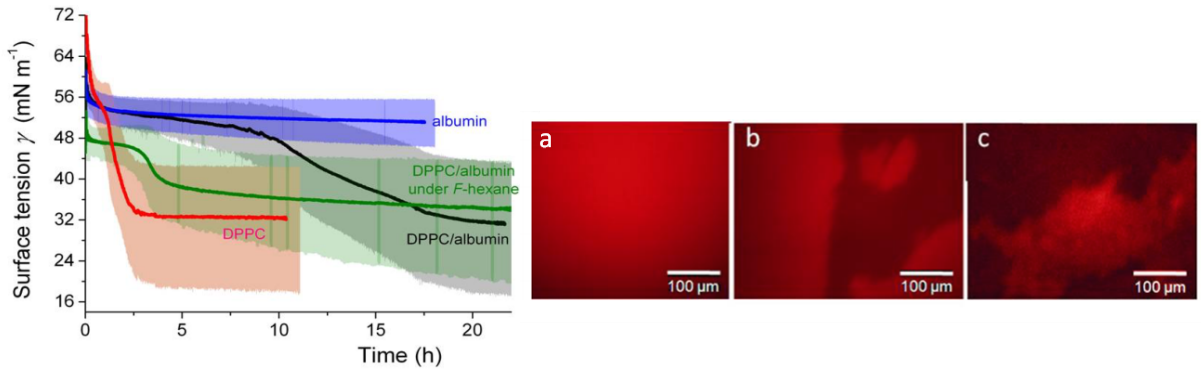

Fig 1 (left). Kinetics of adsorption (37°C) at the surface of an air bubble having a shell of DPPC (10^{-3} mol L^{-1}, red), albumin ($7.5\ 10^{-7}$ mol L^{-1}, blue), or a DPPC/albumin ($1\ 10^{-3}$:$7.5\ 10^{-7}$ mol L^{-1}, black) combination. Also shown is the absorption kinetics of the DPPC/albumin combination at the *F*-hexane-saturated air/water interface (green). The bubbles were submitted to oscillations (*T* 10 s, Δ*A* 15%) throughout the experiments. The lightly colored areas represent the fluctuations in surface tension associated with the oscillations. **Fig 2 (right).** Fluorescence micrographs of the sequential adsorption of albumin and DPPC at the air/buffer interface of a Langmuir trough in the presence or absence of *F*-hexane. At $t = 0$, albumin (+ 2 mol% of albumin-Texas-Red) is injected in the sub-phase. DPPC is injected 1.5 h after albumin. It takes ~1.5 h (in the presence of *F*-hexane) and ~6 h (in its absence) to replace albumin from ~60% of the interface.

AIMS

The project extension to neutron reflectometry aimed at quantifying the two components, dipalmitoylphosphatidylcholine DPPC and bovine serum albumin BSA, in mixed monolayers formed at air-water interface, in the presence or absence of *F*-hexane using the high flux FIGARO reflectometer at the ILL. Measurements involving normal and deuterated lipids were performed to determine the composition of the mixed monolayers using the low-Q compositional analysis method (Campbell, *Soft Matter*, **2015**, *9*, 5304). This work was designed to provide essential new insight into the mechanisms by which proteins perturb the integrity of phospholipid monolayers, and thus lung function, and pave the way for the design of systems involving lung surfactant targeted therapies based on fluorocarbons (Krafft, *Soft Matter*, **2015,** *11*, 5982).

Final Report on FIGARO experiment #8-02-777

FIGARO: EXPERIMENTAL PLAN

The 3-day experiment using the FIGARO reflectometer took place in November 2016. Due to a problem with the detector that badly affected the results, we were granted another 3-day experiment in February 2017. Our initial plan was to study the competitive and sequential adsorption of DPPC vesicles and BSA in the presence or absence of the fluorocarbon gas. The concentration of DPPC was to be quantified using the isotopic contrast difference between h-DPPC and d-DPPC. Then, upon flushing the fluorocarbon gas out, the partitioning between the BSA and DPPC left trapped at the interface would be determined. A Langmuir trough with oscillating barriers (KSV-NIMA) was installed in a sealed box for the first time. Oscillations were indeed an indispensible factor for DPPC vesicles to adsorb at the air-water interface. A first technical problem was encountered, namely that it proved impossible to saturate the box in which the trough was enclosed with *F*-hexane. A second problem was that, quite unexpectedly, the adsorption of h-DPPC and d-DPPC vesicles at the interface under oscillation occurs at significantly different rates, thus invalidating the treatment of the data. In our second experiment, we succeeded in circumventing both issues, by conducting the experiments on Langmuir monolayers of DPPC spread at the interface after having injected the protein in the Hepes buffer sub-phase (pH 7) prepared with a mixture of 8.1% by volume D_2O in H_2O (ACMW) in the FIGARO adsorption troughs (no barriers). Two concentrations of DPPC were deposited in order to obtain either a liquid expanded (LE, 8 mN m^{-1}) or a liquid condensed (LC, 30 mN m^{-1}) phase.

FIGARO EXPERIMENT: RESULTS

For DPPC monolayers in the LE state (8 mN m^{-1}), in the presence of BSA the surface excess of DPPC decreased both in air (from 1 to 0.6 mg m^{-2}) and in *F*-hexane-saturated air (from 1.2 to 0.5 mg m^{-2}) (Fig. 3). In the absence of BSA, DPPC surface excess remained constant (~1.5 mg m^{-2}, not shown) in air and in *F*-hexane, which indicated that BSA was responsible for this loss of lipid. When *F*-hexane was flushed out and replaced by air, the DPPC surface excess unexpectedly increased back to its initial value (1.5 mg m^{-2}). The surface excess of BSA strongly increased over time in *F*-hexane-saturated air (from 0.2 to 1.5 mg m^{-2}) while it remained unchanged in air (0.2 mg m^{-2}). When *F*-hexane was removed, the surface excess of BSA immediately decreased to 0.4 mg m^{-2}, a value still higher than that in air. A working hypothesis is that the adsorption of *F*-hexane at the interface results in a compression of the molecules at the interface, inducing a change in BSA conformation. The effect of the removal of *F*-hexane would correspond to an expansion of the monolayer, leading to the re-expansion of DPPC at the interface and concomitant desorption of BSA.

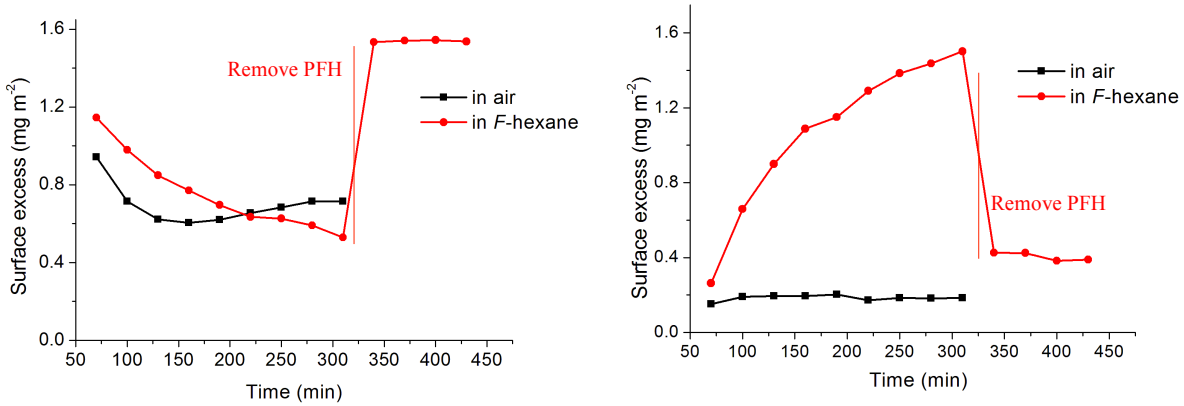

Fig. 3. Surface excesses of DPPC (left) and BSA (right) measured over time by neutron reflectometry for a DPPC monolayer (LE state) in contact with BSA in the sub-phase and in the presence or absence of *F*-hexane.

In the case of a DPPC monolayer in the LC state (30 mN m^{-1}) (Fig. 4), the DPPC surface excess remained almost unchanged in air, as well as in *F*-hexane-saturated air, and after *F*-hexane release. The BSA surface excess remained small (0.1 mg m^{-2}) in air, while it abruptly increased to 1.5 mg m^{-2} in *F*-hexane-saturated air, and then progressively decreased to 1 mg m^{-2} within 5 h. After removal of *F*-hexane, it immediately fell to 0.1 mg m^{-2}. These results show that DPPC monolayers in the LC state are more resistant against desorption by BSA than in their LE state.

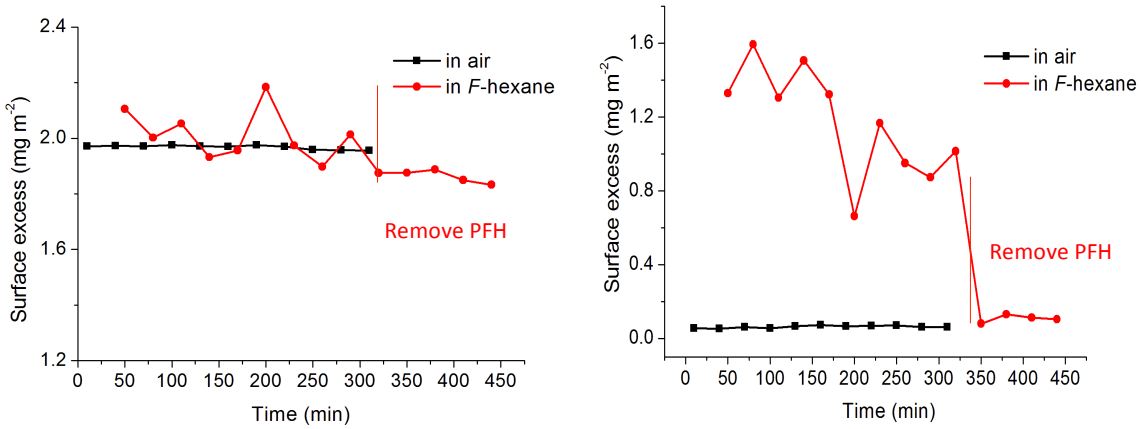

Fig. 4. Surface excesses of DPPC (left) and BSA (right) measured upon time by neutron reflectometry for a DPPC monolayer (LC state) in contact with BSA in the sub-phase and in the presence or absence of *F*-hexane in the air phase.

SUBSEQUENT LABORATORY EXPERIMENT

Since BSA adsorbs more rapidly than DPPC vesicles at the air-water interface, we chose to spread the DPPC monolayer over an aqueous sub-phase that already contained the protein. In order to investigate whether the order of injection of DPPC and BSA affects the results, we have achieved an ellipsometry study (in the Partnership for Soft Condensed Matter) in the presence and absence of *F*-hexane. We found that at equilibrium (after 2.5-3 h) the BSA surface excess reached the same level whether or not the DPPC monolayer (LC state) was spread first.

CONCLUSIONS

An instrument problem led to a follow-up experiment, and two unanticipated experimental difficulties led to refinement of our initial experimental plan, resulting in a very successful outcome. According to our modified plan, we achieved quantification of DPPC and BSA in mixed interfacial monolayers in the presence or absence of *F*-hexane. DPPC was deposited as a Langmuir monolayer on a BSA-containing sub-phase. When the DPPC monolayer was in the LE state, BSA adsorbed in the DPPC monolayer, partially desorbing DPPC molecules from the interface in both air and *F*-hexane-saturated air. Remarkably, in the latter case we found that, when *F*-hexane is removed, the surface excess of DPPC returned to its initial value, indicating that the DPPC molecules remain in a sub-surface layer and are able to respread at the interface. *F*-hexane recruits BSA molecules at the interface, but the protein is released when *F*-hexane is removed. When in the LC state, the compact phospholipid monolayer is not desorbed by the protein. Adsorption of BSA is increased, but the protein is desorbed when *F*-hexane is removed.

The co-adsorption of a phospholipid and albumin in interfacial monolayers in the presence of *F*-hexane has been quantified for the first time thanks to FIGARO. The results will be soon published. This study also sheds light on the mechanisms of adsorption/desorption of a phospholipid/protein mixture at the surface of water, and on how they are affected by a fluorocarbon gas. In order to pursue our investigations on the competitive adsorption of DPPC vesicles and BSA, we will need to design a Langmuir trough with oscillating barriers in a box that is small enough to allow efficient saturation by the fluorocarbon gas. These experiments should contribute to better understand how the fluorocarbon gas counteracts the inhibiting effect of albumin on respiration, thus ascertaining the role of fluorocarbons in lung therapy.