Effective additives to pulmonary surfactant (PS) preparations for therapy of respiratory distress syndrome (RDS) are being intensively sought. We report here the investigation of the effects of partially fluorinated amphiphiles (PFA) on the surface behavior of a model PS formulation. When small amounts of a partially fluorinated alcohol C_{8}F_{17}C_{n}H_{2m}OH (F8HmOH, m = 5 and 11) are added to the PS model preparation (a dipalmitoylphosphatidylcholine (DPPC)/Hel13–5 peptide mixture) considered here, the effectiveness of the latter in vitro pulmonary functions is enhanced. The mechanism for the improved efficacy depends on the hydrophobic chain length of the added PFA molecules. The shorter PFA, F8H5OH, when incorporated in the monolayer of the PS model preparation, promotes a disordered liquid-expanded (LE) phase upon lateral compression (fluidization). In contrast, the addition of the longer PFA, F8H11OH, reduces the disordered LE/ordered liquid-condensed (LC) phase transition pressure and promotes the growth of ordered domains (solidification). Furthermore, compression—expansion cycles suggest that F8H5OH, when incorporated in the PS model preparation, undergoes an irreversible elimination into the subphase, whereas F8H11OH enhances the squeeze-out phenomenon of the SP-B mimicking peptide, which is important in pulmonary functions and is related to the formation of a solid-like monolayer at the surface and of a surface reservoir just below the surface. F8H11OH particularly reinforces the effectiveness of DPPC in terms of minimum reachable surface tension, and of preservation of the integrated hysteresis area between compression and expansion isotherms, the two latter parameters being generally accepted indices for assessing PS efficacy. We suggest that PFA amphiphiles may be useful potential additives for synthetic PS preparations destined for treatment of RDS in premature infants and in adults.

1. Introduction

Alveolar surfaces of mammalian lungs are covered with a surface-active material called pulmonary surfactant (PS). Specific roles of PS are roughly classified into two important categories: biophysical activity across the air—alveolar fluid interface and immunological barrier against pathogens. These functions have been summarized in review articles.1–7 The interfacial and biophysical PS functions include prevention of alveolar collapses at the end of expiration and minimization of the work of breathing by reducing surface tension at the alveolar surface during a respiratory inspiration.8 These functions are achieved by lipid monolayers primarily dipalmitoylphosphatidylcholine (DPPC) and bilayer or multilayer structures (surface-associated reservoirs) that are closely attached to the monolayers. PS deficiency causes neonatal respiratory distress syndrome (NRDS) in premature infants, whereas lung injury often ends in acute RDS (ARDS) in children and adults. Surfactant preparations based on bovine or porcine lung extracts are commonly administered to NRDS patients for therapy.7,8 Although these preparations are effective, they carry a risk of infection and involve costly purification procedures in order to achieve batch-to-batch reproducibility. Therefore, preparations made of synthetic components and proteins or peptides are desirable for clinical surfactant replacement therapy for NRDS and ARDS.9–13 Several additional to PS formulations have been considered for restoration of pulmonary functions that have been inactivated by serum proteins such as albumin. Polymyxin B/PS mixtures have increased resistance to inactivation by meconium.14,15 Furthermore, several hydrophilic polymers such as dextran, polyethylene glycol, and hyaluronan enhance the ability of clinical PS preparations to resist inactivation by serum proteins.16–22 However, these polymers (especially the nonionic polymers) need to be...
formulated at relatively high concentrations, which results in viscous solutions that hinder instillation to the patients. Recently, chitosan has been investigated as a PS additive. The resistance of chitosan/PS mixture to albumin-induced inactivation was found to be more effective than that induced by the above-mentioned polymers.

PS films show specific changes in fluidity and rigidity during compression and expansion of alveolar surfaces. Film rigidity is ensured by dipalmitoylphosphatidylcholine (DPPC), which is a major component of native PS extracts. However, DPPC alone cannot fulfill PS functions due to slow adsorption and poor respreading on the surface. Partially fluorinated amphiphile (PFA) on the surface unique features including high gas-dissolving capacity, low surface tension, and high fluidity and have triggered numerous studies for applications in diagnosis and therapy. Although highly fluorinated compounds tend to accumulate in the gaseous PFOB promotes the respreading of a DPPC monolayer of the semicrystalline (liquid-condensed) DPPC domains and found that the fluorocarbon gases effectively inhibit the formation of guaranteed reagent grade for the preparation of the subphase Tris(hydroxymethyl) aminomethane (Tris) and acetic acid (HAc) of 12634 and the electrical resistivity = 18 MΩ.

In this paper we suggest, as a new approach to PS substitute additives, the use of a hybrid-type PS formulation that incoro-pTera molecules to improve the efficiency of the PS preparation for treatment of NRDS. The primary goal of the study is to evaluate the effect of partially fluorinated alcohols on model PS preparations consisting of DPPC with and without a PS mimicking peptide (Hel 13–59) using the monolayer technique. Since PS forms monolayer films at the alveolar surface, it make sense to study their surface properties using a Langmuir monolayer model. (perfluorooclyl)pentanol (C₈F₁₇H₅OH, F₈H₅OH) and (perfluorooclyl)undecanol (C₁₁F₂₃H₈OH, F₈H₁₁OH) which both have a C₈F₁₇ chain, were selected as the PS molecules, because their molecular structure is relatively simple and because they have thoroughly been investigated previously. In order to understand the basic interfacial behavior of the PS preparations used here, the surface pressure–molecular area (π–a) and surface potential–area (Δπ–a) was systematically measured under physiological and ion strength conditions. Phase and morphology variations of the monolayer were visualized using fluorescence microscopy (FM) and atomic force microscopy (AFM). Furthermore, we investigated hysteresis of the π–a and Δπ–a isotherms, where the monolayer was alternatively compressed and expanded repeatedly to mimic the biophysical situation found at the alveolar surface during breathing, although the dynamics are somewhat different.

2. Materials and Methods

2.1. Materials. The Hel 13–5 (NH₂–KLLKLLLKLWLKL-LKLLLL-COOH, see Figure S1(A) in the Supporting Information) peptide was synthesized by the Fmoc (9-fluorenylmethoxycarbonyl) technique and purified with reverse-phase HPLC as described in the literature. More detailed procedures for the synthesis, purification, and analysis of Hel 13–5 were reported previously. (Perfluorooclyl)pentanol (F₈H₅OH) and (perfluorooclyl)undecanol (F₈H₁₁OH) were synthesized as reported previously (see Figure S1(B)). 1-α-Dipalmitoylphosphatidylcholine (DPPC; purity > 93%) and the fluorescent probe, 1-palmitoyl-2-[6-(7-nitro-2,1,3-benzoxadiazol-4-yl)amino][hexa-4-3-phospho- choline (NB-D-PC), were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL) and were used without further purification. n-Hexane (99.5%) and ethanol (99.5%) was used as spreading solvents and were purchased from Cica-Merck (Uvasol, Tokyo, Japan) and nacalai tesque (Koto, Japan), respectively. Tris(hydroxymethyl) aminomethane (Tris) and acetic acid (HAc) of guaranteed reagent grade for the preparation of the subphase were obtained from nacalai tesque. Sodium chloride (nacalai tesque) was roasted at 1023 K for 24 h to remove all surface-active organic impurities. The substrate solution was prepared using distilled water (the surface tension = 71.96 mN m⁻¹ at 298.2 K and the electrical resistivity = 18 MΩ.


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2 Materials and Methods

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an automated homemade Wilhelmy balance. The surface pressure balance (Mettler Toledo, AG-64) had a resolution of 0.01 mN m\(^{-1}\). The pressure-measuring system was equipped with a filter paper (Whatman 541, porosity = 4 cm). The trough was made from Teflon-coated brass (area = 750 cm\(^2\)), and Teflon barriers (both hydrophobic and lipophobic) were used. The compression of a typical phospholipid such as DPPC can reach a surface pressure close to 70 mN m\(^{-1}\) without leakage of the material by using hydrophobic barriers. The collapse value of DPPC monolayers is a controversial subject discussed in the literature.\(^{46-50}\) Namely, an entirely Teflon-made system can prevent adsorption of material onto the barriers, and allow accurate quantitative analyses of phospholipid monolayers up to collapse. In this study, the first kink point on the \(\pi-A\) isotherms at higher surface pressures (>50 mN m\(^{-1}\)) is defined as a monolayer collapse.\(^{45-50}\) The surface potential measurement supports this behavior. The \(\pi-A\) isotherms were recorded at 298.2 ± 0.1 K. Stock solutions of DPPC (1.0 mM), FSH(1OH (1.0 mM), and Hel 13–5 (0.1 mM) were prepared in \(n\)-hexane/ethanol (9/1, v/v) and (4.5/5.5, v/v for Hel 13–5). The spreading solvents were allowed to evaporate for 15 min prior to compression. The monolayer was compressed at a speed of <0.11 nm\(^2\) molecule\(^{-1}\) min\(^{-1}\). Cyclic compression–expansion isotherms (hysteresis curves) were drawn by recording five compression and expansion cycles at a rate of ∼0.24 nm\(^2\) molecule\(^{-1}\) min\(^{-1}\) (or ∼90 cm\(^2\) min\(^{-1}\)). The standard deviations (SD) for molecular surface area and surface pressure were ∼0.01 nm\(^2\) and ∼0.1 mN m\(^{-1}\), respectively. The pH of the subphase was controlled with 0.02 M Tris buffer and 0.13 M NaCl and was adjusted to 7.4 with an adequate amount of HAc.

2.2.2. Surface Potential–Area Isotherms. The surface potential (\(\Delta\phi\)) was recorded simultaneously with surface pressure using Keithley 614 and 6517 electrometer, where the monolayer was compressed and expanded at the air/water interface. It was monitored by using an ionizing 241Am electrode located at 1–2 mm above the interface, while a reference electrode was dipped in the subphase. The SD for the surface potential was 5 mV.\(^{51,52}\)

2.2.3. Fluorescence Microscopy (FM). The film balance system (KSV Minitrough) was mounted onto the stage of an Olympus microscope BX51WI (Tokyo, Japan) equipped with a 100 W mercury lamp (USHI-1030 L), an objective lens (SLMPlan5×, working distance = 15 mm), and a 3CCD camera with a camera control unit (IKTUS1CU, Toshiba, Japan). A spreading solution of the sample investigated was prepared and doped with 1 mol % of the fluorescence probe (NBD-PC). Image processing and analysis were carried out using the software, Adobe Photoshop Elements ver. 7.0 (Adobe Systems Incorporated, CA). The total amount of ordered domains (dark contrast regions) was evaluated and expressed as a percentage per frame by dividing the frame into dark and bright regions. For the percentage, the resolution was 0.1% and the maximum SD in this study was 8.9%. More details concerning the FM measurements were provided in a previous paper.\(^{9}\)

2.2.4. Atomic Force Microscopy (AFM). Langmuir–Blodgett (LB) film preparations were carried out with the KSV Minitrough. Freshly cleaved mica (Okenshoji Co., Tokyo, Japan) was used as a supporting solid substrate for film deposition (a vertical dipping method). At selected surface pressures, a transfer velocity of 5 mm min\(^{-1}\) was used for the film-forming materials on a 0.02 M Tris buffer and 0.13 M NaCl (pH 7.4) at 298.2 K. The transfer was achieved so that the hydrophilic part of the monolayer was juxtaposed to the mica while the hydrophobic part was exposed to air. LB films with deposition rate of ∼1 were used in the experiments. The AFM experiments were performed in the air. The LB technique includes the risk that the original monolayer structures may be modified by the electric charge of the samples and the draining force during the deposition procedure. However, AFM images of deposited LB films do provide useful information for understanding pulmonary functions. AFM images were obtained using an SPA 400 instrument (Seiko Instruments Co., Chiba, Japan) at room temperature in the tapping mode, which provided both topographical and phase-contrast images. Other details about AFM measurements were reported previously.\(^{9}\)
3. Results

3.1. Effects of Added F8HmOH on DPPC Monolayers.

The $\pi$-$A$ and $\Delta V$-$A$ isotherms of DPPC monolayers containing 0 to 20 wt % of F8H5OH and F8H11OH are shown in Figure 1, panels A and B, respectively. In the DPPC/F8H5OH system (Figure 1A), the $\pi$-$A$ isotherm of DPPC monolayers is seen to shift toward larger molecular area as the percentage of added F8H5OH increases. In the conditions used, DPPC monolayers undergo a first-order transition (at $\sim$11 mN m$^{-1}$) from a liquid-expanded (LE) to a liquid-condensed (LC) phase upon compression. Further compression leads to monolayer collapse at $\sim$55 mN m$^{-1}$. These phase transitions and properties of DPPC monolayers including their $\Delta V$ behavior have been steadily discussed in the previous papers.34,39 The LE/LC transition pressure increases from $\sim$11 to $\sim$14 mN m$^{-1}$ upon addition of 0 to 20 wt % of F8H5OH (Figure 1A), which means that the DPPC monolayers undergo a fluidization process in the presence of this compound.34 Also, a slight increase of the monolayer’s collapse pressure by $\sim$3 mN m$^{-1}$ is observed (see inset in Figure 1A). This rise provides evidence that DPPC and F8H5OH are miscible in the monolayer state.34 The general shape of the $\Delta V$-$A$ isotherms does not change upon addition of F8H5OH. However, the maximum $\Delta V$ value in the close-packed state decreases from $\sim$550 (curve 1) to $\sim$370 mV (curve 5). This negative variation arises from the contribution of the fluorocarbon moiety of F8H5OH, since negative $\Delta V$ values have previously been reported for pure F8H5OH monolayers.34,41

Contrary to the DPPC/F8H5OH system, the DPPC/F8H11OH system (Figure 1B) exhibits a decrease of the LE/LC transition pressure from $\sim$11 to $\sim$5 mN m$^{-1}$ as the percentage of F8H11OH increases. This reduction means that F8H11OH addition causes the DPPC monolayers to become more rigid or ordered. In line with this observation, the collapse pressure increases significantly from 55 up to $\sim$65 mN m$^{-1}$ (see inset in Figure 1B). The $\Delta V$ values near the monolayer collapse decrease substantially from $\sim$550 to $\sim$240 mV (curve 5). This larger $\Delta V$ reduction in the DPPC/F8H11OH system as compared to DPPC/F8H5OH system reflects the longer carbon chain of F8H11OH (Figure S2 in the Supporting Information).

Figure 2 shows fluorescence micrographs of DPPC monolayers with 0, 5, and 10 wt % of F8HmOH added in the LE/LC coexistence region. Fluorescent probes (here, NBD-PC) selectively dissolve into disordered phases in monolayers. Therefore, bright and dark regions in the FM images correspond to disordered (or LE) and...
ordered (or LC) phases, respectively. Below the transition pressure of $\sim 11$ mN m$^{-1}$ and in the absence of $F8HmOH$, the FM images of DPPC monolayers are homogeneously bright. When this pressure is reached, nucleation of LC domains starts and further monolayer compression leads to growth of LC domains as seen in Figure 2a. The percentage of surface occupied by ordered domains in each FM image increases with increasing surface pressure and reaches $\sim 95\%$ beyond 20 mN m$^{-1}$ (Figure S3 in the Supporting Information). The ordered domains with counterclockwise arms are characteristic of DPPC ($L_{type}$) monolayers. Upon addition of 5 wt % $F8H5OH$ to DPPC (Figure 2b), the arms of the domains become slimmer and start branching out. Further addition of $F8H5OH$ (Figure 2c) increases the morphological variations. The shape of the ordered domain is commonly controlled by line tension of phase boundaries and long-range dipole interactions. That is, the FM phase behavior in the DPPC/$F8H5OH$ systems is dominated by the long-range dipole interactions induced by the addition of $F8H5OH$. This is readily understandable from the fact that monolayers of $F8H5OH$ alone display an opposite variation of $\Delta V$ value as compared to DPPC monolayers. As a result, the dipole interactions induce fluidization of the DPPC monolayer. Contrary to the DPPC/$F8H5OH$ case, addition of 5 wt % $F8H11OH$ causes an increase of the width of the arms of the ordered domains (Figure 2d). For the DPPC monolayers with 10 wt % $F8H11OH$ (Figure 2e), the arms can no longer be distinguished and the ordered domains are essentially round (at 13 and 15 mN m$^{-1}$) or elongated (at 20 mN m$^{-1}$) in shape. This domain variation means that addition of $F8H11OH$ considerably changes the line tension of the phase boundary between coexisting two-dimensional phases in monolayers and the long-range dipole interaction to the domain shape. In other words, a dominant factor of the domain shape is expected to shift from the positive line tension ($\pi \geq 15$ mN m$^{-1}$) to the dipole interaction ($\pi \geq 20$ mN m$^{-1}$). Considering that the ordered domains are larger in the DPPC/$F8H11OH$ system as compared to the DPPC/$F8H5OH$ system, it appears that $F8H11OH$ preferably interacts with the LC phase of DPPC monolayers, which results in an improvement of monolayer order and rigidity.

3.2. Effects of $F8HmOH$ Addition on a Pulmonary Surfactant Model. 3.2.1. Compression Isotherms. The $\pi-A$ and $\Delta V-A$ isotherms of the binary DPPC/Hel 13–5 monolayer (X$_{Hel13–5} = 0.1$), which is a simple model mixture of synthetic pulmonary surfactants, are shown in Figure 3 (curve 1). This monolayer has its disordered/ordered phase transition at $\sim 14$ mN m$^{-1}$, and the plateau at $\sim 42$ mN m$^{-1}$ (indicated by arrows), when Hel 13–5 begins to be squeezed out of DPPC monolayers. Indeed, the squeezed-out behavior of Hel 13–5 corresponds to the vertical phase separations found for other phospholipid containing systems. Such squeezing-out behavior also takes place for native pulmonary surfactants and has been discussed extensively. Upon addition of $F8H11OH$ (Figure 3A), the $\pi-A$ isotherm shifts very slightly toward larger molecular areas with increasing amount of added $F8H5OH$ below the plateau pressure. Although the plateau pressure remains almost constant regardless of the amount of $F8H5OH$ added, the plateau region becomes more pronounced. It is suggested that $F8H5OH$ enhances the squeezed-out of Hel 13–5 upon compression. This view is also supported by the fact that the $\pi-A$ isotherms move to smaller molecular areas upon $F8H5OH$ addition when pressure becomes larger than 42 mN m$^{-1}$ (see inset in Figure 3A). The maximum $\Delta V$ values measured for mixtures containing 3 to 10 wt % $F8HmOH$ are characteristic of DPPC ($L_{type}$) monolayers.

Figure 3. $\pi-A$ and $\Delta V-A$ isotherms of monolayers containing the model pulmonary surfactant preparation (the binary DPPC/Hel 13–5 mixture with the fixed ratio of X$_{Hel13-5} = 0.1$) plus 0 to 20 wt % of $F8H5OH$ (A) and 0 to 20 wt % of $F8H11OH$ (B) on a 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl at 298.2 K. (Inset) Enlarged $\pi-A$ isotherms at high surface pressures in the vicinity of the monolayer’s collapse.

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of F8H5OH in the close-packed states are almost the same as that for the DPPC/Hel 13–5 mixture (curve 1), which indicates the contribution of the F8H5OH addition to the ΔV value is small. Interestingly, it is found that F8H5OH is also squeezed out of the monolayer along with Hel 13–5. However, upon further addition of F8H5OH (curve 5), the surplus amount of F8H5OH at the interface achieves a substantial negative contributions to the ΔV value.

In the case of F8H11OH (Figure 3B), the π–A isotherms exhibit a different, more complex behavior. A reduction in the disordered/ordered transition pressure by F8H11OH is seen at lower surface pressures. The π–A isotherms clearly move to larger molecular areas in the vicinity of plateau pressures. In addition, the plateau region becomes wider as the amount of added F8H11OH increases. The plateau region in the F8H11OH-containing systems is larger compared with that of the F8H5OH-containing systems (Figure 3A). The decrease in ΔV values in the close-packed state with increasing F8H11OH amount is more pronounced than for the F8H5OH systems. This negative variation indicates that F8H11OH molecules remain in the monolayer above the plateau regions. Therefore, it is suggested that F8H11OH molecules improve the packing of DPPC monolayers by their presence at the interface and thus promote the squeeze-out motion of Hel 13–5 at the higher surface pressures.

3.2.2. Fluorescence Micrographs. FM images of the DPPC/Hel 13–5 monolayers (XHel13–5 = 0.1) with and without F8H5OH are shown in Figure 4. In Figure 4a, the ordered (dark) domains correspond to the LC phase of DPPC monolayers, since Hel 13–5 itself is known to form a homogeneous disordered (bright) phase. Although the LC domains grow in ratio upon compression (see Figure S3), the domain boundaries become diffuse. This may be attributed to an increase in surface concentration of Hel 13–5, which is relatively huge molecule as compared to DPPC. In the case of the 5 wt % F8H5OH addition (Figure 4b), the number and size of the ordered domains diminish as a result of the fluidization effect of F8H5OH. However, the further addition of F8H5OH conversely brings about a growth in size of the ordered domains as seen in Figure 4c. The morphology of the domains resembles that seen for pure DPPC monolayers (Figure 2a). Considering the phase variation observed in Figure 2a–c, this implies that F8H5OH hinders the interaction between DPPC and Hel 13–5, or that F8H5OH interacts preferably with Hel 13–5 in the 10 wt % addition system. On the other hand, in the DPPC/Hel 13–5/F8H11OH system (Figure 4d,e), the phase behavior and its variation are similar to those observed in the absence of Hel 13–5 (Figure 2d,e). As opposed to F8H5OH, F8H11OH interacts favorably with the LC domains of the DPPC monolayers, in spite of the presence of Hel 13–5.
3.2.3. AFM Topographic Images. AFM observations of Langmuir–Blodgett (LB) films transferred onto mica were performed to elucidate the PFA addition effect at the nanometer level. The AFM topographies of the ordered domains (dark areas in the in situ FM observations) showed almost homogeneous images (data not shown). Therefore, our attention was focused on the disordered phase observed by FM. The AFM topographic images collected for the PS model of DPPC/Hel 13–5 (X = 0.1) containing 0–10 wt % F8HmOH are shown in Figures 5 and 6. For the DPPC/Hel 13–5 mixture alone (Figure 5A), the dark (lower in height) and bright (higher in height) domains observed at 35 mN m\(^{-1}\) and bright (higher in height) domains observed at 35 mN m\(^{-1}\) are composed of Hel 13–5 and of DPPC, respectively.\(^{39}\) Further compression of the monolayers at 45 mN m\(^{-1}\) (the plateau pressure of ~32 mN m\(^{-1}\) in pure Hel 13–5) causes the emergence of protruding masses (indicated by arrows) that correspond to the squeezed-out Hel 13–5. Similar protruding masses were also seen in monolayers of DOPG and a peptide dimer mimicking SP-B and called “nanosilos”.\(^{62}\) The protruding masses grow in size and number at 55 mN m\(^{-1}\), and the height difference (~2.8 nm) between the masses and the surrounding monolayers corresponds to 2–3 molecular layers of Hel 13–5 peptides.\(^{39}\) The formation of such particles is quite an important process in pulmonary function.\(^{9,59}\)

When F8H5OH is added (Figure 5B,C), the monolayer topography observed at 35 and 45 mN m\(^{-1}\) show little difference with the AFM images of Figure 5A. However, at 55 mN m\(^{-1}\) and for a 10 wt % addition, the images show that the size of the protruding masses increases in diameter from 23 ± 13 nm (Figure 5A) to 52 ± 13 nm (Figure 5C). The F8H5OH addition also enhances their height, which then reaches ~6 nm (Figure 5D). According to the \(\Delta V_A\) isotherms (Figure 3A), the protruding masses are thought to comprise F8H5OH molecules excluded from the surface monolayers along with Hel 13–5.

For the F8H11OH addition system (Figure 6), some changes of the monolayer’s morphology are observed as compared to the images collected for the DPPC/Hel 13–5 mixture (Figure 5A). The Hel 13–5 network (dark) is preserved and continues to be interlinked even at 55 mN m\(^{-1}\), which indicates that F8H11OH enhances phase separation between DPPC and Hel 13–5 in the...
monolayers by forming a closer-packed monolayer with DPPC. Huge protruding masses with a diameter of 137 ± 21 nm and a height that reaches ~13 nm are observed at 55 mN m⁻¹ (Figure 6B). These protruding masses differ from those observed for the F8H5OH systems in number, diameter, and height (Figure 6C). The formation of large protruding masses may be attributed to the stability of the rigid mixed DPPC/F8H11OH monolayer at the interface, which induces the elimination of Hel 13–5 from the monolayer. No surface micelles, such as those reported for (F-alkyl)alkyldiblocks,⁹ were observed.

3.2.4. Repeated Compression–Expansion Isotherms. Repeated cycling π→A and ΔV→A isotherms of the preparations investigated were measured systematically. The monolayers were compressed up to their collapse pressure and then expanded to the initial molecular areas. This process was repeated five times to ascertain the repeatability of the respreading process. Typical compression–expansion isotherms are shown in Figure 7. The formation of large protruding masses may be attributed to the stability of the rigid mixed DPPC/F8H11OH monolayer at the interface, which induces the elimination of Hel 13–5 from the monolayer. No surface micelles, such as those reported for (F-alkyl)alkyldiblocks,⁹ were observed.

Figure 6. Typical AFM topographic images of the DPPC/Hel 13–5 preparation with 5 wt % of F8H11OH added (A) and with 10 wt % of F8H11OH added (B) compressed at 35, 45, and 55 mN m⁻¹ on a 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl at 298 K and transferred on mica. The scanned area is 1 × 1 μm and the scale bar in the lower-left corner represents 300 nm. The cross-sectional profiles along the white scanning line a and b are given in panel C. The height difference between the arrowheads is indicated in the cross-sectional profiles. The scanned area is 1 × 1 μm and the scale bar in the lower-left corner represents 300 nm. The cross-sectional profiles along the white scanning line a and b are given in panel C. The height difference between the arrowheads is indicated in the cross-sectional profiles.

4. Discussion

To our knowledge, this is the first investigation of a phospholipid/protein hybrid-type lung surfactant formulation incorporating fluorinated molecules intended for the development of new synthetic PS preparations. Besides the unique features mentioned in the Introduction, it should be noted that the PFA molecules used are not macromolecules, contrary to Hel 13–5, and that there is no need to worry about thermal stability and antigen recognition related to biophylaxis. In particular, the relative thermal stability of PFA molecules is an advantage in terms of long-time storage and large-volume production. When a small amount of the PFA molecules used here is added to the model PS preparation, the squeezing-out of Hel 13–5 during compression is enhanced. This squeezing-out phenomenon observed on the π→A isotherms at ~42 mN m⁻¹ is closely related with the reduction in work of breathing at the alveolar surface and thus is an important index to

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Figure 7. Cyclic compression and expansion (or hysteresis curves) for surface pressures and surface potentials of monolayers containing the DPPC/Hel 13–5 preparation (A), the preparation with 10 wt % of F8H5OH added (B), and with 10 wt % of F8H11OH added (C). The subphase was a 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl. The temperature was 298.2 K. The compression—expansion cycle was performed five times at the compression rate of ~0.25 nm² molecule⁻¹ min⁻¹.

assess the efficacy of artificial PS preparations. From the point of view of enhancing product effectiveness for NRDS patients, positive in vitro effects of PS additives include an increase of the maximum molecular collapse pressure, an increase of the integrated hysteresis area for the π–A isotherms, and an improved respreading ability at low additive concentrations.26,64 The respreading effect is complemented for NRDS treatment by the fact that instillation of PS preparations reactivates alveolar type II cells so that native PS secretion is resumed. Our study shows that incorporation of small amounts of both F8H5OH additives has beneficial effects on the preparation.

The administration of PS formulations is a common treatment for NRDS infants, and many researchers investigate new synthetic preparations.9–13 In particular, use of air saturated with perfluorocarbon gases has recently been considered for RDS treatment.35–38,65 This followed the observation that FC gases can prevent the formation of an LC phase in Langmuir monolayers of DPPC and can promote dissolution of existing LC domain.35–38 A predominant symptom in RDS patients is dyspnea arising from dysfunction and inactivation of PS. Partial ventilation with air and fluorocarbon gases has been considered useful as RDS treatment.56,67 However, this application is still under investigation due to the difficulty of controlling the partial pressure of the gases, which strongly depends on the state of the lungs. In the present study, F8H5OH molecules (m = 5 and 11) are incorporated in the PS preparation as potential additives. Both F8H5OH monolayer molecules investigated improve the squeezing-out behavior of Hel 13–5 during compression but appear to involve different mechanisms; F8H5OH undergoes an irreversible ejection from the surface monolayers along with Hel 13–5, whereas F8H11OH promotes Hel 13–5 squeeze-out by remaining present within the DPPC monolayer at the surface even at high surface pressures. It is noticeable that the squeeze-out enhancing mechanism drastically depends on the total length of the hydrophobic chain of F8H5OH, although both compounds have the same perfluoroocetyl moiety. Because it stays in the monolayer, F8H11OH is deemed to be more useful in terms of the improved in vitro efficacy. Another additive to PS preparations, namely hexadecanol (HD), which is present in the commercially available formulation, Exosurf, has been investigated. A small amount of HD induces a decrease of the LE/LC transition pressure of DPPC monolayers68 and in the collapse pressure of Infasurf,69 which is also one of the commercial formulations. In terms of reduction of the transition pressure, HD exercises an effect similar to that of F8H11OH rather than of F8H5OH. However, the morphological phase behavior induced by F8H5OH addition in this study is significantly different from that observed with molecules with hydrocarbon chains.

A quantitative assessment of the variation of the integrated hysteresis areas during successive compression/expansion π–A cycles, which are related to the pressure—volume (P–V) curves measured in the lung,30–32 is indispensable to confirm such usefulness. In the case of F8H5OH incorporation (Figure 8A), the integrated hysteresis area decreases with increasing compression/expansion cycle number and eventually converges to almost the same value as in the absence of additive. This result also indicates the irreversible elimination of F8H5OH from the surface monolayer. On the other hand, the integrated hysteresis area decreases...
be more complex than those observed with fluorocarbon gases and particularly, van der Waals, electrostatic, and dipole actions) between molecules of their hydrophobic chains in the air. Because fluorocarbon gases should interact only with the hydrophobic chains of the monolayers, whereas F8H11OH can interact both with their OH and PS components may concern their physical states (solid or gas) at room temperatures and the presence or absence of hydrophilic headgroups in their structures. Priori, fluorocarbon gases should interact only with the hydrophobic chains of the monolayers, whereas F8HmOH can interact both with their polar head, which can be anchored at the water surface and with their hydrophobic chains in the air. Because F8HmOH compounds are essentially restricted to the surface, mutual interactions (in particular, van der Waals, electrostatic, and dipole–dipole interactions) between molecules of F8HmOH and PS components may be more complex than those observed with fluorocarbon gases and them. The results obtained here suggest that fluorinated amphiphiles may be useful potential additive to PS formulations. Furthermore, considering the application of gaseous fluorocarbons to alumin-covered surfaces,38 the concept described here could possibly be extended to the use of such formulations in ARDS patients. However, while a substantial amount of data is available on the toxicity and pharmacodynamics of fluorocarbons and fluorocarbon/hydrocarbon diblocks and generally indicate considerable biological inertness,73–75 the toxicity, pharmacodynamics and biodegradability of the fluorinated amphiphiles with polar headgroups are still poorly documented in the literature, which could hinder the development of their possible applications.76,77 Therefore, the effect of highly fluorinated compounds on PS formulations warrants further research, in particular in terms of adsorption kinetics and biocompatibility, as well as of practical aspects, including feasibility, and acceptability.

In conclusion, the present in vitro surface chemistry study provides some fundamental information that supports the potential of fluorinated amphiphiles for NRDS surfactant replacement therapy. The FM images illustrate the morphological variations (shape and size) that occur in ordered domains of the model PS formulation upon addition of F8HmOH (m = 5 and 11). They suggest a fluidizing (for m = 5) or a condensing (for m = 11) effect on the LC domains that depends on hydrophobic chain length. The topographic AFM image reveal that the formation of protruding masses (or surface-associated reservoirs) is improved in size and number by F8HmOH addition above the squeeze-out pressure of Hel 13–5. Finally, hysteresis measurements provide definite evidence that (i) F8H5OH undergoes an irreversible exclusion from the surface monolayer, (ii) F8H11OH enhances the squeeze-out behavior of Hel 13–5 by remaining at the surface to form a solid-like monolayer with DPPC at high surface pressures, and (iii) the 5–10 wt % F8H11OH addition enhances the effectiveness of the PS model preparation in terms of both integrated hysteresis area preservation and of maximum reachable surface pressure. These results suggest that a hybrid PS formulation incorporating fluorinated amphiphiles may provide a useful basis for the design of novel synthetic PS preparations and for effective formulations for NRDS therapy.

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Supporting Information Available: Chemical structures of Hel 13–5 and of the fluorinated alcohols; the π–A and ΔV′–A isotherms of F8H11OH monolayers at different temperatures; the ratio of ordered domain areas in fluorescence micrographs; the hysteresis curves for the DPPC/F8H5OH and DPPC/F8H11OH systems. This material is available free of charge via the Internet at http://pubs.acs.org.

Figure 8. Variation of the integrated hysteresis area between compression and expansion isotherms as a function of the number of compression/expansion cycles; the DPPC/Hel 13–5 preparation incorporating 0–20 wt % of F8H5OH (A) or 0–20 wt % of F8H11OH (B).