Mode of interaction of two fluorinated-hydrogenated hybrid amphiphiles with dipalmitoylphosphatidylcholine (DPPC) at the air–water interface

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Abstract

Two-component Langmuir monolayers formed on 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl at 298.2 K were investigated for two different fluorinated-hydrogenated hybrid amphiphiles (F\(_6\)P5H\(_5\)PPhNa and F\(_8\)P5H\(_5\)PPhNa or F\(_6\) and F\(_8\), respectively) with DPPC. Surface pressure (\(\pi\)), surface potential (\(\Delta V\)) and dipole moment (\(\mu_\perp\)) as a function of molecular surface area (\(A\)) were measured by employing the Whilhelmy method and an ionizing electrode method. From the \(A\)– and \(\Delta V\)–X\(_{F6}\) (or X\(_{F8}\)) curves, partial molecular surface area (PMA) and apparent partial molecular surface potential (APSP) were determined as a function of surface mole fraction (X\(_{Fn}\)) at discrete surface pressures. Then, the behavior of occupied surface areas and surface potentials of the respective components could be made clearer. Compressibility (\(C_s\)), elasticity (\(C^{-1}\_s\)), and excess Gibbs energy (\(\Delta G_{(ex)}\)) as a function of X\(_{F6}\) (or X\(_{F8}\)) were estimated at definite pressures. These physico-chemical parameters were found to reflect the mechanical strength of monolayer films formed. The regular solution theory being applied to \(\Delta G_{(ex)}\), the activity coefficients (\(f\)) as well as the interaction parameter (\(I_p\)) between DPPC and two hybrid amphiphiles in the binary monolayers were evaluated. \(I_p\) values thus obtained indicated that F\(_8\) molecules interact more strongly with DPPC molecules than F\(_6\). Moreover, in order to better understand the morphological monolayer state, Langmuir–Blodgett (LB) films made from DPPC and fluorinated-hydrogenated hybrid amphiphiles were examined by atomic force microscopy (AFM). The miscibility of the two components in the monolayer state is evidenced by these thermodynamic quantities and AFM observations. Furthermore, AFM images demonstrated that F\(_8\) could more effectively disperse the ordered domains of DPPC than F\(_6\).

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1. Introduction

Fluorinated amphiphiles have been extensively developed through the basic material sciences and technologies for application in various aspects including biomedical one [1–9], being characterized by excellent thermal stability and chemical resistance or stability in addition to their remarkable surface activity. Since a fluorine atom has the greatest electronegativity of all elements and the denser electron cloud of fluorine atoms heads for the lower polarizability, perfluorocarbon chains are, in general, strong in intramolecular bond but very weak in intermolecular interaction [10]. Hydrocarbon chains are in contrast to the fluorocarbon ones. Therefore, fluorinated materials exhibit the limited miscibility with hydrocarbon amphiphiles [11–13] or organic solvents [14,15]. According to the first report by Mukerjee and Yang [11], a fluorocarbon surfactant (sodium perfluorocamvanoate) and a hydrocarbon surfactant (sodium dodecylsulfate)
cannot form “mixed micelles”, suggesting that two-component systems of fluorocarbon and hydrocarbon surfactants may form their own micelles or partially mixed ones in water; this is a three-dimensional (3D) event. We are, here, much interested in two-dimensional (2D) events; that is, what behavior can be seen in the case where multicomponents composed of fluorocarbon and hydrocarbon amphiphiles are spread at the air–water interface? or do they form a well miscible Langmuir film or an immiscible one?

Many studies on Langmuir films for various kinds of multicomponent monolayers made from hydrogenated surfactants spread at the air/water interface have been published [16–19]. Especially, phospholipids have attracted a great attention, because they are one of the most abundant ingredients in cell membrane [20]. Recently, we have reported the miscibility for three binary systems of dipalmitoylphosphatidylcholine (DPPC) with cholesterol, cholestanol, and stigmasterol [21]. Among the phospholipids, DPPC has been employed most for monolayer studies [22–26]. However, reports on fluorinated amphiphiles are still much fewer in number compared with conventional ones. Recently, much attention has been focused on partially fluorinated amphiphiles for pharmaceutical and medical applications such as fluorinated prodrugs for pulmonary drug delivery [27–29] because of their chemical and biological properties. In the alveolus where the gas exchange takes place, the cyclic behavior of surface tension is very important due to the extremely large surface area. Natural pulmonary surfactant consists of ∼10% proteins and 90% lipids, where DPPC is the most abundant component [30]. An important function of pulmonary surfactant is to reduce the surface tension of the air/aqueous interface in the alveolus, which is accompanied by the formation of a monolayer at the interface [31]. The monolayer is compressed and expanded successively during breathing, adjusting the surface tension dynamically [32,33].

In the present study, the so-called “hybrid” surfactants with a hydrocarbon and a fluorocarbon chain in a molecule have been synthesized in order to improve the performance of fluorinated amphiphiles [34]. Correspondingly, the authors have synthesized six novel phenyl phosphate-type hybrid surfactants; C_mF_{2m+1}C_6H_2CH(OPO_2(OCH_3)Na)C_nH_{2n+1} (F_mPH_nPPhNa; m = 4, 6, 8, n = 3, 5), expecting that fluorocarbon–hydrocarbon hybrid surfactants possessing a phenyl phosphate group may have interest aspects for physico-chemical properties such as micelle formation, surface activity, adsorptions at the liquid/solid or oil/water interfaces, etc. We have already reported interesting interfacial properties of two hybrid type amphiphiles and of binary monolayers comprised of hybrid type amphiphiles (F6PH5PPhNa and F8PH5PPhNa) and DPPC at the air/water interface in the first paper of this series study [35]. To gain a better insight into the interactions between hybrid amphiphiles (F6 and F8) and DPPC, we employed a new approach of a combination of thermodynamic analysis and morphological observation by atomic force microscopy (AFM) study [36,37] for the present binary systems. Overall, the combination of these two different approaches will provide further understanding of interactions between them in 2D phase.

2. Experimentals

2.1. Materials

Dipalmitoylphosphatidylcholine (DPPC, purity >99%) was obtained from Avanti Polar Lipids, Inc. (Birmingham, AL, U.S.A.) and used as received. Sodium phenyl 1-[(4-perfluorohexyl)-phenyl]-1-hexylphosphate (denoted as F6PH5PPhNa or F6) and sodium phenyl 1-[(4-perfluorooctyl)-phenyl]-1-hexylphosphate (denoted as F8PH5PPhNa or F8) were synthesized as reported previously [34,38]. Their chemical formulas of F6PH5PPhNa and F8PH5PPhNa are shown in Fig. 1. The other experimental materials were described in the first paper in this series work [35].

2.2. π–A and ΔV–A measurements

The surface pressure (π) was measured using an automated home-made Wilhelmy film balance. A trough made from a 48 cm × 15 cm Teflon-coated brass was used. The standard deviations for area and surface pressure measurements were ∼0.01 nm² and ∼0.1 mN m⁻¹, respectively. The monolayers were compressed at a speed of 0.13 nm² molecule⁻¹ min⁻¹, and the π–A isotherms were recorded at 298.2 ± 0.05 K. Throughout the present study, the water used was thrice-distilled (surface tension of 72.7 mN m⁻¹ at 293.2 K and electrical resistivity of 18 M Ω cm).

The surface potential (ΔV) was simultaneously monitored using an ionizing ²⁴¹Am electrode placed 1–2 mm above the interface during the π–A measurement. The standard deviation for surface potential measurements was ∼5 mV. The other conditions were the same as described in previous papers [35].
2.3. Atomic force microscopy

AFM study was performed as follows. LB-film preparations were carried out with the LB trough (KSV-minitrough, Finland) whose area is 365 mm $\times$ 75 mm. Freshly cleaved mica (Oken-shoji Co., Japan) was used as a supporting solid substrate for the film deposition. At selected surface pressures, transfer velocity of 3 mm min$^{-1}$ was used for film-forming materials on 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl. AFM images were obtained using a SPA 400 instrument (Seiko Instruments Co., Japan) at 298.2 $\pm$ 2 K in a tapping mode, which provided both a topographical image and a phase contrast one. Prior to use, the cantilevers were irradiated with ultraviolet light (8 mW/cm$^2$ at 253.7 nm) for about 20 min and then washed with distilled ethanol. The tapping mode images (512 points collected per line) were collected with scan rates of 0.5–3 Hz, using silicon tips (Olympus Co., Japan) with a nominal spring constant of 1.8 N m$^{-1}$ under the normal atmosphere. Transferred samples were checked for possible tip-induced deformation by zooming out after a region had been scanned. The domain size distribution was measured with the particle analysis function of Seiko Instruments software.

3. Results and discussion

3.1. Isotherms of surface pressure ($\pi$), surface potential ($\Delta V$), and dipole moment ($\mu_\perp$) versus molecular surface area ($A$)

In order to examine the stability of the monolayers of the respective pure systems of F6PH5PPhNa (abbreviated as F6) and F8PH5PPhNa (as F8), it has been confirmed that they are enough stable to form the insoluble monolayer at the air/water interface [35]. The part (a) in Fig. 2(A) and (B) shows the isotherms of surface pressure ($\pi$) versus mean molecular surface area ($A$) on 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl at 298.2 K. As demonstrated previously [35], DPPC forms a liquid-expanded (LE) film at the lower $\pi$ and a liquid-condensed (LC) film at the higher $\pi$, whereas hybrid surfactants form a typical disordered film. In addition, F6 and F8 have almost the same extrapolated mean molecular area ($A_0$) and the same collapse pressure. This indicates that the extension of fluorinated chain length has no influence upon the monolayer property and its two-dimensional area. The difference between F6 and F8 is seen from the surface potential value as shown in frame (b) of Fig. 2.
The surface potential (ΔV) reflects the electrostatic field gradient perpendicular to the horizontal surface and therefore varies considerably with the molecular surface orientation. In regard to the behavior of ΔV–A isotherms upon compression, the surface potential values (ΔV) of F6 and F8 were found to reach about −440 and −500 mV in closest packing state, respectively, as reported previously [35].

The vertical component of surface dipole moment (μ⊥) was calculated from the Helmholtz equation using the measured ΔV values:

$$\Delta V = \frac{\mu_\perp}{\varepsilon_0 \varepsilon A}$$

(1)

where ε0 is the permittivity of a vacuum (which is assumed to be unity) and ε the mean permittivity of the monolayer, A is the area occupied by a molecule [39]. The ΔV or μ⊥ values involve the resultant change of dipole moments carried by the polar head and hydrophobic groups, such as hydrocarbon chain, fluorocarbon chain, and phenyl group, in addition to the subphase employed. The respective changing modes of μ⊥ with compression are shown in both parts (c) of Fig. 2, indicating a big difference between DPPC and hybrid surfactants, as similar to that of ΔV–A behavior.

3.2. Ideality of mixing

3.2.1. F6 and F8/DPPC two-component systems

In Fig. 2, frames (A) and (B) exhibit the π–A, ΔV–A, and μ⊥–A isotherms for two-component monolayers of F6/DPPC and F8/DPPC systems at various mole fractions at 298.2 K on the substrate (0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl), respectively. In order to carry out the thermodynamic analysis (in the later section) from these isotherms, as was made in the previous paper [35], the above isotherms were minutely measured by changing molar fraction of F6 and F8. All the resultant isotherms of the two-component systems appeared in the order of the mole fraction between those of both pure systems. The transition from disorder to order was observable up to X_{F6} (or X_{F8}) = 0.3. The change and disappearance of the transition pressure with increasing surface pressure suggest that F6 and F8 have an ability to make DPPC miscible with themselves in the monolayers, as will be mentioned in the later section of two-dimensional phase diagram. This observation is the first evidence that both F6 and F8 are well miscible with DPPC in the monolayer state at their low mole fractions.

3.2.2. Partial molar quantities (PMA and APSP)

When π–A isotherms of a given binary system are to be analyzed, it is essential to examine whether the relation of mean molecular surface areas (A_m) with mole fraction (X) satisfies the additivity rule or not and, if not, which deviation is observed, negative or positive. All the A_m values are listed in Table 1 for the two binary systems at discrete pressures. The behavior of surface area and surface potential can be made more clearly, if their partial molar quantities are evaluated, where they have been obtained as in previous studies [40–43]. Here, A_m is assumed to satisfy the following equation:

$$A_m = X_1 A_1 + X_2 A_2$$

(2)

where X_1 and X_2 are the mole fractions of the pure components 1 and 2, respectively, and A_1 and A_2 are the partial molecular areas (PMA) in the two-component film at a definite surface pressure.

When PMA is denoted as A_1 and A_2 for components 1 and 2, respectively, A_1 and A_2 values can be determined as the respective intercepts at X_2 = 0 and 1 of a tangential line drawn at any point on the A_m–X_{F6} (or X_{F8}) curve. The relation for A_1 and A_2 are given as

$$A_1 = A_m - X_2 \left( \frac{\partial A_m}{\partial X_2} \right)_{T,\pi}$$

(3)

$$A_2 = A_m + (1 - X_2) \left( \frac{\partial A_m}{\partial X_2} \right)_{T,\pi}$$

(4)

where A_i is defined as

$$A_i = \left( \frac{\partial A_i}{\partial N_i} \right)_{T,\pi}$$

(5)

when N_1 plus N_2 molecules form a surface area A_i (≡N_1A_1 + N_2A_2), and 1 and 2 denote DPPC and each hybrid surfactant, respectively. It is noted that examination of the PMA values for respective component molecules in binary systems as a function of composition can make us see the interaction mode between the two different molecules that depends, in many cases,
on mixing ratio, i.e. which component is majority or minority [40]. The PMA value is also useful for study on the Gibbs adsorption for various binary surfactant mixtures, as has been reported in the recent literature [44].

Correspondingly, the apparent partial molecular surface potential (APSP) can be also obtained from the relationship between the average molecular surface potential and the mole fraction, which is the same as the above procedures:

\[
\Delta V_1 = \Delta V_m - X_2 \left( \frac{\partial \Delta V_m}{\partial X_2} \right)_{T, \pi} 
\]

where \(\Delta V_m\) was evaluated by dividing the measured surface potential (\(\Delta V\)) by the number of molecules in the unit area. The surface potential (\(\Delta V\)) is measured by an air electrode whose cross-sectional area is ca. 1 cm\(^2\). Therefore, we assumed its dimension to be mV cm\(^{-2}\). The average molecular surface potential in mV molecule\(^{-1}\) unit can be obtained from the \(\Delta V_m\) divided by the number of molecules per 1 cm\(^2\) calculated from the \(\pi\)–\(A\) isotherm. When APSP is denoted as \(\Delta V_1\) and \(\Delta V_2\) for components 1 and 2, they are determined by the respective intercepts at \(X_2 = 0\) and 1 of a tangential line drawn at any given points on the \(\Delta V_m-X_{F6}\) (or \(X_{F8}\)) curve.

The calculations of PMA and APSP were applied to these binary systems. The changes in PMA as a function of mole fraction are shown in Fig. 3. It is noted that if the two components are ideal mixing, PMA of each component should be parallel to the abscissa of \(X_{F6}\) (or \(X_{F8}\)) (the additivity rule). The PMA of F6 and DPPC does remain nearly constant over the whole mole fraction range at 5 mN m\(^{-1}\) (Fig. 3(A)). On the other hand, at 15 and 25 mN m\(^{-1}\), both F6 and DPPC molecules show characteristic PMA changes depending much on the composition. The positive deviations may be related to the LE/LC phase transition of DPPC molecule. Interestingly, both combinations of (A) F6/DPPC and (B) F8/DPPC show almost the same variation at each pressure, and the composition dependence in the variation may be divided into two parts; that is, \(X < 0.5\) (F6 or F8 is a minority) and \(X > 0.5\) (F6 or F8 is a majority). In the range of \(X < 0.5\), the major component DPPC slightly increase with increasing mole fraction of F6 or F8, while as the mole fraction approaches zero in DPPC major region, PMA of the minority F6 or F8 increases markedly at 15 and 25, and 35 mN m\(^{-1}\) both F6 and F8 do change its trend toward decreasing one. On the other hand, in the range of \(X > 0.5\), the major components F6 and F8 seem to keep their PMA almost constant at 15 and 25 mN m\(^{-1}\), while DPPC shows a small maximum followed by a slight decrease at 15 and 25 mN m\(^{-1}\) and an increasing trend at 35 mN m\(^{-1}\). Although the region in regard to the variation was divided at \(X = 0.5\), the distinctive boundaries may be found at \(X = 0.25–0.3\) and \(0.75–0.8\). Anyway the interaction is governed by the two kinds of components of overwhelming majority. It is suggested that the fluorocarbon chain can fully play the own lipophobicity against the hydrocarbon chains of DPPC molecules in the binary monolayer. In F8/DPPC system, the results similar to Fig. 3(A) were obtained as is seen in Fig. 3(B). Variations of PMA with surface pressure and molar fraction suggest that F8 molecules change their conformations appropriately to coexist with DPPC at the surface.

It is noted here that the previous study on the binary systems of DPPC with three kinds of sterols (cholesterol, stigmasterol, and...
cholestanol) has shown so conspicuous negative deviation from additivity rule (ideal mixing) that the value of sterols’ partial molecular area (PMA) became negative in mole fraction range below 0.3 at the surface pressure range below \( \pi = 10 \text{ mN m}^{-1} \) (below the transition pressure of DPPC), while at the higher pressure range above \( \pi = 15 \text{ mN m}^{-1} \) negligibly small deviation from the additivity rule was observed for all the three combinations of DPPC with sterols [21]. However, F6/DPPC and F8/DPPC indicated some deviations between them. This reflects a difference of the degree of molecular orientations and packing between the two components.

The APSP–\( X_{F6} \) (or \( X_{F8} \)) curves for these two systems are shown in Fig. 4. In contrast to PMA, APSP for both F6/DPPC and F8/DPPC systems does not show the difference in fluorocarbon chain length but indicates the similar behavior at each surface pressure. That is, their molecules are likely to show almost similar orientation from 5 to 35 mN m\(^{-1} \), though they are different in the length of fluorocarbon chains by two carbon atoms. It is found that APSP of F6 molecules goes down toward greater negative with approaching \( X = 0 \) in the range of \( X_{F6} < 0.5 \) and reaches their own value in the range of \( X_{F6} > 0.5 \) at 5–35 mN m\(^{-1} \). In the monolayer state, it is assumed that the extrapolated area \( (A_0) \) of F6 is much larger than that of DPPC because its hydrophilic part is extremely large. This decrease of APSP of F6 in DPPC majority region results from improving a tilt angle of fluorocarbon chain. The same story is also the case for F8. It cannot be explained yet why APSP of DPPC does not distinctively reflect the transition from LE to LC.

From examination of correlation between PMA and APSP curves at 35 mN m\(^{-1} \), it may be said that: (i) in the range of both \( X_{F6} \) and \( X_{F8} > 0.7–0.8 \) (DPPC of minority and F6 or F8 of majority) PMA of F6 or F8 as well as APSP is kept almost constant at the mole fraction of \( X_{F6} \) or \( X_{F8} \) close to 1, while those of DPPC are enhanced and (ii) over the range of \( X_{F6} \) or \( X_{F8} < 0.2–0.5 \) the major DPPC keeps PMA as well as APSP nearly constant, while the minor F6 or F8 decreases the both values as the mole fraction of F6 or F8 approaches 0. These trends are generalized to be that the minor component exhibits more remarkable changes in PMA and APSP, while the majority shows little change in both terms. This generalization may be true for all the states at differing surface pressures.

### 3.2.3. Compressibility

The compressibility \( C_s \) or elasticity \( C_s^{-1} \) may be used as a tool for assessing one of physical properties of Langmuir monolayers on the basis of \( \pi–A \) isotherms [21,45]. The characteristic value of \( \left( \frac{\partial \pi}{\partial A} \right)_T \) is related to compressibility:

\[
C_s = -(1/A)(\partial A/\partial \pi)_T
\]  

The reciprocal of compressibility, \( C_s^{-1} \), corresponds to elasticity which is more often used. Therefore, the maximum of \( -\left( \frac{\partial \pi}{\partial A} \right)_T \) value corresponds to the minimum elasticity. In this paper, the mechanical property is described in terms of elasticity \( (C_s^{-1}) \). It has been known that \( C_s^{-1} \) values distribute from 10 to 50 mN m\(^{-1} \) for disordered phases and from 100 to 250 mN m\(^{-1} \) for ordered phases [46]. In Fig. 5, \( C_s^{-1} \) values which were determined at different pressures are plotted as a function of \( X_{F6} \) (or \( X_{F8} \)) for these two-component systems. As for pure components, at high surface pressures, the \( C_s^{-1} \) value of DPPC is higher than that of F6 or F8, suggesting F6 and F8

![Fig. 4. Apparent partial molecular surface potential (APSP) of (A) F6PH5PPhNa/DPPC and (B) F8PH5PPhNa/DPPC two-component systems as a function of the composition of F6PH5PPhNa or F8PH5PPhNa at four different pressures.](image-url)
are more compressible than DPPC. In the binary systems, at 5 and 10 mN m\(^{-1}\) before their transition pressures, the \(C_s^{-1}\) values are almost parallel to the abscissa of \(X_{F6}\) (\(X_{F8}\)). However, above the transition pressures, the monolayer exists as ordered films in \(0 \leq X \leq 0.3\), and the \(C_s^{-1}\) values decrease with increasing \(X_{F6}\) (\(X_{F8}\)), although the \(C_s^{-1}\) values are almost same in \(0.3 < X \leq 1\). It is suggested that the dependence of \(C_s^{-1}\) on molar fractions in the binary monolayers was classified into two ranges \(0 \leq X \leq 0.3, 0.3 < X \leq 1\). Nevertheless, the difference between F6 and F8 is not clear, and therefore, the results are not well ascribable to differences between these hybrid amphiphiles. This obscurity between two hybrid surfactants may come from the well-known weak cohesive interaction among fluorocarbon chains. Anyway it is of interest that 10–20% addition of F6 or F8 to DPPC monolayer causes a rapid decrease in elasticity of the Langmuir film.

3.2.4. Excess Gibbs energy

As a powerful tool for evaluating the interaction among molecules and its thermodynamic stability in the monolayer consisting of two or more components, the surface excess Gibbs energy, \(\Delta G_{(ex)}\), can be used [20,47–49]. The Gibbs energy change in the mixing systems \((\Delta G_{mix})\), is considered to be a sum of ideal and excess Gibbs energy changes \(\Delta G_{mix} = \Delta G_{(id)} - \Delta G_{(ex)}\). For ideal mixing, the Gibbs energy change involves only the entropy term as \(\Delta G_{(id)} = RT(X_1 \ln X_1 + X_2 \ln X_2)\), where \(X_i\) \((i = 1, 2)\) denotes mole fraction in the mixture and \(RT\) is the product of gas constant and Kelvin temperature. Further, the excess Gibbs energy can be expressed as

\[
\Delta G_{(ex)} = \int_0^\pi [A_{12} - (X_1 A_1 + X_2 A_2)] d\pi
\]  

(8)

where \(A_{12}, A_1,\) and \(A_2\) represent the area of binary system and the areas of the respective pure systems of components 1 and 2, respectively. It is noted that \(\Delta G_{(ex)}\) for ideal mixing becomes zero because of the additivity rule: \(A_{12} = X_1 A_1 + X_2 A_2\) [50,51] (Table 2).

Fig. 6 shows the plots of \(\Delta G_{(ex)}\) against \(X_{F6}\) or \(X_{F8}\) at selected pressures (5, 15, 25, and 35 mN m\(^{-1}\)). \(\Delta G_{(ex)}\) was calculated using Eq. (8). The calculated values are listed in Table 2. From Fig. 6(A), the \(\Delta G_{(ex)}\) values as a function of pressure for F6/DPPC system were found to increase with an increase in surface pressure and show a maximum at \(X_{F6} = 0.4\). In F8/DPPC system (Fig. 6(B)), the positive value of \(\Delta G_{(ex)}\) was observed and a smaller maximum compared to that of F6/DPPC system appeared at \(X_{F8} = 0.4\) above 25 mN m\(^{-1}\), whereas no remarkable variation of excess Gibbs energy was found below 15 mN m\(^{-1}\). Fig. 6 tells us that the positive values were obtained for both binary systems at the higher pressures than 25 mN m\(^{-1}\), suggesting that the interaction between DPPC and F6 or F8 molecules is not attractive but repulsive to some extent and that from the results shown in Figs. 5 and 6 the weaker elasticity of the film seems to some extent to be in parallel with the positive \(\Delta G_{(ex)}\) values. Interestingly, the greatest values of \(\Delta G_{(ex)}\) found at mole fraction 0.4 for both binary systems make us conclude that the repulsive interaction between F8 and DPPC is less than that between F6 and DPPC; the shorter fluorocar-
Table 2
Excess Gibbs energy as a function of the composition of F6PH5PPhNa and F8PH5PPhNa at four different pressures

<table>
<thead>
<tr>
<th>Mole fraction, X</th>
<th>Excess Gibbs energy (×10³ J mol⁻¹)</th>
<th>π (mN m⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
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<tr>
<td>(a) F6/DPPC system</td>
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<tr>
<td>0.1</td>
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</tr>
<tr>
<td>0.9</td>
<td>−0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>1.0</td>
<td>0.02</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 2 shows the excess Gibbs energy as a function of the composition of F6PH5PPhNa and F8PH5PPhNa at four different pressures. The table is structured to display the mole fraction (X) and the excess Gibbs energy (×10³ J mol⁻¹) for different pressures (π (mN m⁻¹)).

A long carbon chain has resulted in the stronger repulsive force. This may be related to the more ease of the 2D motion of F6 molecules compared with F8 ones.

Assuming that the regular solution theory is applicable to the present binary systems, the real entropy term $-TΔS_{mix}^{\text{real}}$ can be regarded as equal to $-TΔS_{mix}^{\text{ideal}}$ [21,52]. When the chemical potential of $i$ ($i=1, 2$) in the monolayer is given as $μ_i = μ_i^0 + RT \ln f_i X_i$, where $μ_i^0$ is the standard chemical potential and $f_i$ is the activity coefficient, the $ΔG_{(ex)}$ can be expressed as

$$ΔG_{(ex)} = RT(X_1 \ln f_1 + X_2 \ln f_2)$$

(9)

The right side of this equation can be regarded as the same as that of Eq. (8). The activity coefficient $f_i$ is known to reflect the intermolecular interaction, and it is given as follows:

$$\ln f_1 = \frac{ω(1 - X_1)^2}{RT}, \quad \ln f_2 = \frac{ω(1 - X_2)^2}{RT}$$

(10)

where $ω$ is the exchange energy which is ascribed to cohesive forces between different molecules [21,52,53]. From Eq. (10), the following relation is obtained:

$$\ln f_1 = \frac{(1 - X_1)^2}{X_1^2} \ln f_2$$

(11)

Substituting Eq. (11) into Eq. (9), we have

$$\frac{ΔG_{(ex)}}{RT} = \left( \frac{X_2^2}{1 - X_2} + X_2 \right) \ln f_2$$

(12)

The activity coefficients $f_1$ and $f_2$ can be calculated by using the values in Table 2 [21]. In addition, the substitution of Eq. (10) into Eq. (9) allows calculation of the exchanging energy $ω$ from the following equation [21,54]:

$$ω = \frac{ΔG_{(ex)}}{X_1 X_2}$$

(13)

and the interaction parameter is defined here as

$$I_p = \frac{ω}{RT}$$

(14)

The obtained values of $I_p$ and the activity coefficients for the respective systems are listed in Tables 3 and 4. The $I_p$ values are negative or positive depending on the variation of $ΔG_{(ex)}$ values and the greater absolute values are obtained for both binary systems at the higher surface pressures at $X_{F_6} = 0.4$ (see Table 4). The greater $I_p$ values of F6 molecules than those of F8 molecules correspond to the weaker interaction with DPPC molecules as discussed above.
3.2.5. Two-dimensional phase diagrams

From the $\pi-A$ isotherms for the two-component systems of F6/DPPC and F8/DPPC, their two-dimensional phase diagrams were constructed using the data of the transition pressure ($\pi_{ct}$) and the collapse pressure ($\pi_c$) changes at various molar fractions of F6 or F8. Resultant phase diagrams at 298.2 K are shown in Fig. 7(A) and (B) for F6 and F8, respectively.

Firstly, in F6/DPPC system, the transition pressures from disorder (gaseous or liquid-expanded) to order (liquid-condensed) phase are plotted against mole fraction of F6 in Fig. 7(A), showing that over the range from $X_{F6}=0$ to 0.3, the phase transition pressure ($\pi_{ct}$) increases almost linearly with $X_{F6}$. Judging from the change of the transition pressure, two components are miscible each other at the mole fraction range, which is the first experimental evidence of the miscibility of the two components within the monolayer films as described above. This can be explained by the fact that film-forming molecules become more dense by compression, accompanying more depression in surface tension by the film-forming molecules themselves. Then, the resultant surface pressure was raised. The rise in the transition pressure with mole fraction of F6 means that the transition of DPPC takes place when the film-forming molecules become denser with the mole fraction. These phenomena resemble the elevation of boiling point in the mixed solution.

Assuming that the surface mixtures behave as a surface regular solution with a hexagonal lattice, the coexistence of phase boundary between the ordered monolayer phase and the bulk phase can be theoretically simulated by the following Joos equation, and the interaction parameter ($\xi$) can be calculated from the extent of deviation from ideal mixing [55] too:

$$\begin{align*}
1 &= x_1^4 y^4 \exp \left( \frac{\pi_{ct}^c - \pi_{ct}^s}{k_B T} \omega_1 \right) \exp \left( \xi (x_2^c)^2 \right) \\
&+ x_2^4 y^2 \exp \left( \frac{\pi_{ct}^c - \pi_{ct}^s}{k_B T} \omega_2 \right) \exp \left( \xi (x_1^c)^2 \right)
\end{align*}
$$

(15)

where $x_1^c$ and $x_2^c$ denote the mole fraction in a given binary monolayer composed of components 1 and 2, respectively, $\pi_{ct}^1$ and $\pi_{ct}^2$ the corresponding collapse pressures of components 1 and 2, and $\pi_{ct}^s$ ($m=1,2$) is the collapse pressure of the monolayer at given compositions of $x_1^c$ and $x_2^c$. In addition, $\omega_1$ and $\omega_2$ are the respective limiting molecular surface areas at the collapse points, $\gamma^1$ and $\gamma^2$ are the surface activity coefficients at the respective collapse points, $\xi$ is the interaction parameter, and $k_B T$ is the product of the Boltmann constant and the Kelvin temperature.

Calculation using Eq. (15) resulted in the solid curve along the measured points in the phase diagram, which was made coincident with the experimental values by adjusting $\xi$.

In these figures, M. indicates a two-component monolayer with a hexagonal lattice, the coexistence of phase boundary, the experimental evidence of the miscibility of the two components is also shown by filled circles, where the dotted line shows the case where the interaction parameter ($\xi$) is zero for ideal mixing.
The interaction energy was calculated to be plotted against mole fraction of F6 or F8. However, the closed packing in solid must have an interaction in 3D states involves a difficulty for interpretation. Anyway, $\xi(X)$ for a pair of F6 and DPPC molecules over the range of $X_\text{F6} = 0–0.3$. As the result, F6 and DPPC are completely miscible in the condensed state as well as in the expanded state, and this behavior is classified into the positive azeotropic type.

From the diagram, the new finding is that the monolayer state of F6/DPPC two-component system can be divided into two regions; that is, $\xi = 0.33$ over the range of $X_\text{F6} = 0–0.3$ while $\xi = 0$ over $X_\text{F6} = 0.3–1$. The negative interaction parameter means that they act mutually attractive interaction between two components in the binary monolayer that is stronger than the mean of the interactions between pure component molecules themselves. The interaction energy $-\Delta \varepsilon$ can be calculated by the following equation:

$$-\Delta \varepsilon = -\frac{\xi RT}{6}$$

The interaction energy was calculated to be $-\Delta \varepsilon = 136$ J mol$^{-1}$ for a pair of F6 and DPPC molecules over the range of $X_\text{F6} = 0–0.3$. As the result, F6 and DPPC are completely miscible in the condensed state as well as in the expanded state, and this behavior is classified into the positive azeotropic type.

Secondly, F8/DPPC system demonstrates that the monolayer behavior may also be divided into two parts at the mole fraction of $X_\text{F8} = 0.3$ similar to F6/DPPC system. Their interaction parameter and interaction energies were calculated to be $\xi = -0.60$ for the mixture over the range of $X_\text{F8} = 0–0.3$, ($\xi = 0$ over $X_\text{F8} = 0.3–1$) and $-\Delta \varepsilon = 248$ J mol$^{-1}$. Thus, these two components of both combinations are regarded as complete mixing. Here, it should be noted that according to the analysis of excess Gibbs energy the interaction parameter ($I_p$) between F6 or F8 and DPPC molecules are positive in the binary monolayer (a weakly repulsive force between different molecules) even at the higher surface pressure (see Table 3), whereas those in the bulk phase (3D phase) are negative ($0 \leq X_\text{F6}$ or $X_\text{F8} \leq 0.3$) or zero ($0.3 < X_\text{F6}$ or $X_\text{F8} < 1$). This disagreement regarding the interaction manner between the binary monolayer ($I_p$ in 2D) and solid ($\xi$ in 3D) states involves a difficulty for interpretation. Anyway, however, the closed packing in solid must have an interaction enhanced due to more close distance or due to more favorable steric conformation.

Consulting the previous report on the binary monolayers formed by three combinations of DPPC with sterols (cholesterol, stigmasterol, and cholesterol) [21], these three combinations all have demonstrated, contrary to the present combinations of DPPC with F6 and F8, negative values of $\Delta G_{\text{ex}}$ as well as negative interaction parameters ($I_p$) the extrema of which were found at the mole fraction of sterols of $X_{\text{st}} = 0.4–0.5$. In the phase diagram, on the other hand, the $\pi^\text{eq}$–$X_{\text{st}}$ relations have shown a greatly positive deviation from ideal mixing ($\xi > 0$, in particular at the range $0 < X_{\text{st}} < 0.4$), being remarkable for the present two binary systems. It may be ascribed to the big difference in molecular structure; that is, the hybrid type amphiphiles have a fluorocarbon chain and much wider extrapolated area $[A_0 (\text{nm}^2) = 0.89$ and 0.87 for F6 and F8; on the other hand, $A_0 (\text{nm}^2) = 0.38$ (cholesterol), 0.39 (stigmasterol), and 0.37 (cholestanol)]. On the other hand, the inconsistent signs of $I_p$ and $\xi$ values are found for both combination systems.

### 3.2.6. Atomic force microscopy

AFM for this study provided both topography and phase contrast images. The topography image reflects the sample topography, while the phase contrast image, which is originated from the energy loss of the oscillating AFM tip, shows the chemical structures of heterogeneous samples. Also on surfaces with local variations of mechanical properties such as biological samples, the AFM phase image provides the best contrast of fine morphological and nanostructural features [56–59]. Fig. 8 shows AFM images of pure F6 monolayer and two binary systems (DPPC/F6) transferred to mica substrates before collapse pressure at 35 mN m$^{-1}$ on 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl. We have employed AFM in the tapping mode which has various advantages for imaging soft matter and biological sample against contact mode [60].

AFM images of pure F6 at 35 mN m$^{-1}$ were homogeneous and flat, and no domains were observed in Fig. 8(A). A cross-sectional profile along the white line in the frame is shown at the bottom, which provide us the information of vertical direction. Pure DPPC monolayer provided a homogeneous AFM image as shown in the previous paper [61].

In contrast, AFM images (5 $\mu$m $\times$ 5 $\mu$m) of the monolayer containing F6 ($X_{\text{F6}} = 0.1$) at 35 mN m$^{-1}$ show two different phases as shown in Fig. 8(B). Because non-tilted DPPC molecules at the interface are about 2.5 nm in height without estimating polar head group by a computer simulation (CS ChemOffice UltraTM 5.0), bright phases represent DPPC monolayer and dark ones indicate F6 monolayer in the topography. The cross-sectional profile indicates that the average height is about 1.2 nm, which is almost equal to the difference in the predicted chain length between the DPPC monolayer (2.5 nm) and F6 (1.4 nm). The behavior of these AFM images agrees with that of FM images, where a small amount of F6 added can disperse the ordered domains of DPPC as mentioned before [25]. To understand the surface morphology of $X_{\text{F6}} = 0.1$ system in more detail, a zoomed image (2 $\mu$m $\times$ 2 $\mu$m) is presented in Fig. 8(C). DPPC bright domains coexist in the F6 domains, which indicate enhanced due to more close distance or due to more favorable steric conformation.
that F6 and DPPC do not have a strong repulsion each other in nanoscale.

AFM images at the mole fraction \(X_{F6}\) 0.5 for DPPC/F6 system are shown in Fig. 8(D) and (E), whose frame size are 5 \(\mu m\) \(\times\) 5 \(\mu m\) and 1 \(\mu m\) \(\times\) 1 \(\mu m\), in turn. In Fig. 8(D), F6 was found to form a continuous phase, where DPPC islands were embedded. This phenomenon is attributed to the exclusion of excess DPPC phase from F6 phases (dark regions). The shapes of the domains are irregular and the height differences between the two phases were about 1.0 nm. Fig. 8(E) is the zoomed image for the homogeneous F6 regions (dark region) in Fig. 8(D). Its image looks flat in Fig. 8(D) but numerous small and higher domains appears like a worm in Fig. 8(E). The dark phase includes mainly F6PH5PPhNa phase, indicating that the morphological patterns happen to a nanophase separation. The percentage of DPPC ordered domain in Fig. 8(E) becomes \(\sim 40\%\), indicating that the ratio of the two different phases shows a good agreement with that estimated by the composition

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**Fig. 8.** AFM images of the DPPC monolayer containing F6 (at \(X_{F6}\) = 0.1 and 0.5) in a tapping mode transferred onto mica at 35 mN m\(^{-1}\): topography (A) pure F6PH5PPhNa monolayer at the scan area of 5 \(\mu m\) \(\times\) 5 \(\mu m\), (B) \(X_{F6}\) = 0.1 at the scan area of 5 \(\mu m\) \(\times\) 5 \(\mu m\), (C) \(X_{F6}\) = 0.1 at the scan area of 2 \(\mu m\) \(\times\) 2 \(\mu m\), (D) \(X_{F6}\) = 0.5 at the scan area of 5 \(\mu m\) \(\times\) 5 \(\mu m\), and (E) \(X_{F6}\) = 0.5 at the scan area of 1 \(\mu m\) \(\times\) 1 \(\mu m\).
Fig. 9. AFM images of the DPPC monolayer containing F8 (at $X_{F8} = 0.1$ and 0.5) in a tapping mode transferred onto mica at 35 mN m$^{-1}$: topography (A) pure F8PH$_5$PPhNa at the scan area of 5 $\mu$m $\times$ 5 $\mu$m, (B) $X_{F8} = 0.1$ at the scan area of 5 $\mu$m $\times$ 5 $\mu$m, (C) $X_{F8} = 0.1$ at the scan area of 1 $\mu$m $\times$ 1 $\mu$m, (D) $X_{F8} = 0.5$ at the scan area of 5 $\mu$m $\times$ 5 $\mu$m, and (E) $X_{F8} = 0.5$ at the scan area of 1 $\mu$m $\times$ 1 $\mu$m.

and respective cross-sectional areas of pure components. These AFM images seem to agree with the FM images in the sense that LC domains of DPPC are dispersed by adding F6 molecules, as described in the previous paper [25].

Next, AFM images of the F8/DPPC binary system under the same condition as F6/DPPC binary system are shown in Fig. 9(A)–(E). AFM image of pure F8 in Fig. 9(A) also shows a homogeneous and flat one. On the contrary, for F6/DPPC binary system at $X_{F6} = 0.1$, the bright (higher) domains indicate the DPPC phase in Fig. 8(B) and (C), where the lower regions correspond to F8-rich phase. There are small and darker domains of F8 (less than $\sim 1 \mu$m in size) in Fig. 8(B) and the height difference between the bright (higher) domains and the dark ones is $\sim 1.0$ nm. In comparison with F6/DPPC system for $X_{F6} = 0.1$ (in Fig. 8(C)), Fig. 9(C) demonstrates that the number of DPPC domains decreases at F8 dark regions in the frame. Fig. 9(D)
of F8/DPPC system for $X_{F6} = 0.5$ shows that the lower domains (dark phase) are no longer homogeneous in image contrast to Fig. 8(D). As shown in zoomed image of Fig. 9(E), the small bright domains are approximately $\sim$1.0 nm higher than their surroundings. The bright domains occupy $\sim$20% in the frame, which is significantly lower than expected. This means that F8 molecules can more effectively disperse the ordered domains of the DPPC than F6 ones. In spite of the difference by two CF$_2$ group between two different fluorinated-hydrogenated hybrid amphiphiles (F6PH5PPhNa and F8PH5PPhNa), F8 molecule can disperse the lipid more, which may be crucial for the performance of this material during the dimensional changes of the monolayer imposed.

4. Conclusions

The monolayer properties of the two binary systems of F6PH5PPhNa/DPPC and F8PH5PPhNa/DPPC as well as the respective pure systems on 0.02 M Tris buffer solution (pH 7.4) with 0.13 M NaCl at 298.2 K were investigated as a function of mole fraction of F6 or F8. The $\pi - A$, $\Delta V - A$ isotherms of F6PH5PPhNa/DPPC and F8PH5PPhNa/DPPC mixtures showed that the two components are miscible in the monolayer state over the whole range of $X_{F6}$ (or $X_{F8}$) and of surface pressures investigated. The miscibility was evidenced by the changes of the transition pressure ($\pi^{eq}$) and the collapse pressure ($\pi^c$).

The PMA and APSP were analyzed for the present binary systems as partial molar quantities. It was found that the two hybrid surfactants have almost similar orientation at each surface pressure, though they are different in the length of fluorocarbon chains by two. Moreover, the compressibility, $(C_s^{-1})$ and the surface excess Gibbs energy, $(\Delta G_{ex})$ was used to determine the interaction among molecules in the monolayers formed. The surface excess Gibbs energy, $(\Delta G_{ex})$ has enabled us to evaluate the interaction parameter ($I_p$) and activity coefficients of the respective components ($f_1$ and $f_2$) in the binary monolayers (2D phase) at various mole fractions. It was found that a weak repulsive force acts between DPPC and the hybrid surfactants ($I_p > 0$). The two-dimensional phase diagram and the Joos equation allowed calculation of the interaction parameter ($\xi$) and interaction energy ($-\Delta \epsilon$) for F6PH5PPhNa/DPPC and F8PH5PPhNa/DPPC systems. All these systems were classified into “positive azeotropic type”. In these systems over the range of $X = 0.0$–0.3, $\pi - A$ isotherm displays a phase transition pressure ($\pi^{eq}$) that increases almost linearly with $X_{F6}$ (or $X_{F8}$). The present two binary monolayers can be divided at least into two ranges above and below mole fraction of 0.3, and it enables us to expect a developing perspective in regard to study on amphiphile mixtures.

Finally, atomic force microscopy (AFM) on their pure and two-component monolayers provided the information of a nanometer-scale. It demonstrated that F8PH5PPhNa could more effectively disperse the DPPC ordered domains than F6PH5PPhNa. In spite of the difference only by two CF$_2$ group between two different fluorinated-hydrogenated hybrid amphiphiles (F6PH5PPhNa and F8PH5PPhNa), F8 molecule can disperse the lipid more, which may be crucial for the performance of this material during the dimensional changes of the monolayer imposed.

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