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Mixing of perfluorinated carboxylic acids with dipalmitoylphosphatidylcholine

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Abstract

Perfluorinated acids are emerging as an important class of persistent environmental pollutant, thus raising human health concerns. To understand the behavior of these compounds in biological systems, the mixing behavior of two perfluorinated acids, perfluorododecanoic and perfluorotetradecanoic acid, with dipalmitoylphosphatidylcholine (DPPC) was studied in monolayers at the air–water interface and in fully hydrated DPPC bilayers. The mixing behavior of both acids was indicative of an attractive interaction and partial miscibility with DPPC at the air–water interface. In the bilayer studies, the fluorinated acids cause peak broadening and elimination of the pretransition of DPPC. The onset temperature of the main phase transition remains constant in the presence of the fluorinated acids suggesting immiscibilities in the gel phase. Below X(DPPC) = 0.97 significant peak broadening of the main phase transition can be observed. These results suggest strong interaction between the respective acid and DPPC, and that both acids are able to partition into the lipid bilayer. However, their mixing behavior is far from ideal, thus suggesting the presence of domains or lipid aggregates with high acid concentrations which may (adversely) impact the function of biological mono- and bilayers.

Keywords: Perfluorinated acid; DSC; Langmuir isotherm; DPPC

1. Introduction

Fluorinated surfactants have an exceptional combination of properties, such as thermal, chemical and biological inertness, excellent spreading characteristics, and high surface activity. Because of these unique properties, perfluorinated surfactants are used for various large scale technical applications such as fire extinguishing media, electroplating bath, stain-repellent coatings on carpets, paper or fabrics, lubricants, and personal care and cleaning products [1–3]. Their extreme inertness towards chemical and biological degradation as well as their hydrophobic character contribute to their presence in the environment and, possibly, accumulation in the food chain, thus giving rise to environmental as well as human health concerns [1,4–13]. In particular, medium-chain perfluorinated acids (C10 to C14) appear to have a high potential for bioaccumulation [10,14,15].

The toxicity of perfluorinated surfactants is still poorly investigated, and it is yet unclear whether these compounds pose an risk to human health [16]. Several fluorinated surfactants such as perfluorooctadecanoic acid and perfluorooctane sulfonate (PFOS) cause developmental toxicity in laboratory animals [17,18] and are peroxisome proliferators in rodents [19]. Different mechanisms by which these fluorinated compounds may cause adverse biological effects have been proposed. Because of their hydrophobic character, one proposed mechanism is their interaction with membranes. Similar to various insecticides, such an effect can either be mediated by interaction with a membrane-bound protein or by nonspecific interactions with the lipids composing a biological membrane [20–22]. As an example, PFOS, an anionic surfactant structurally related to the perfluorinated carboxylic acids, has been reported to alter membrane fluidity and function [23,24].

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The nonspecific interaction mechanism requires the partitioning of the fluorinated acid into the lipid phase. Mixtures of fluorocarbon and hydrocarbon surfactants in the micellar state are known to exhibit highly nonideal behavior [25,26], which implies that a perfluorocarbon surfactant may also exhibit nonideal mixing in a perhydrocarbon lipid bilayer. The nonideal behavior of fluorocarbon–hydrocarbon surfactant mixtures in biomembranes has been proposed as the mechanism by which some surface active perfluorinated resins cause pulmonary toxicity after inhalation [27–30] as well as the preferred accumulation of fluorocarbon chemicals in the liver and blood, but not the adipose tissue [31,32]. Unfortunately little is known of how the hydrophobicity and lipophobicity of fluorinated surfactants determines the partitioning of these molecules into biomembranes and ultimately influences the biological function of these lipid phases.

We and others have investigated the behavior of fluorinated carboxylic acids interacting with dipalmitoylphosphatidylcholine (DPPC), an important biological phospholipid [33–35]. Similar to perhydrocarbon acids and alcohols [36], all fluorinated acids investigated to date exhibit a strong attractive interaction with DPPC and are considered to be partially miscible at the air–water interface. It is likely that strong headgroup–headgroup interactions, such as hydrogen bonding, are overwriting the nonideal alkyl fluorocarbon–hydrocarbon tail–tail interactions. Studies of the interaction of sodium perfluorooctanoate with DPPC bilayers have resulted in similar conclusions [37].

In an effort to more fully characterize the molecular details for this putative interaction, the present study investigates the mixing behavior of two environmentally relevant medium-chain perfluorinated carboxylic acids, perfluorododecanoic and perfluorotetradecanoic acid, with DPPC in monolayers at the air–water interface and fully hydrated bilayers. DPPC was chosen for this study because it is a major component of membranes, such as the outer and inner mitochondrial membranes [38,39], and pulmonary surfactant [40,41], both of which are known targets of fluorocarbon-mediated toxicity as discussed earlier.

2. Materials and methods

Perfluorododecanoic acid was purchased from Oakwood and recrystallized from toluene [42]. Perfluorotetradecanoic acid with a purity of >97% was obtained from Aldrich and used without further purification. 2-Propanol and *n*-hexanes were HPLC grade and were purchased from Fisher Scientific. Concentrated hydrochloric acid was also obtained from Fisher Scientific. Deionized water for the monolayer studies was distilled first from basic potassium permanganate followed by distillation from sulfuric acid [33,34,42,43]. Deionized water for the for the DSC experiments was obtained from a PURELAB Plus water system and had a resistance ≥ 18 MΩ.

2.1. Monolayer experiments

The monolayer experiments were described earlier using a rectangular Teflon trough (306 × 150 mm) held at 37 ± 2 °C (KSV-3000, Finland) [33,34,42–44]. The surface pressure was measured by the Wilhelmy plate method. A constant compression speed of 15 cm^2/min (10 mm/min) was used. Paper plates (15 × 58 mm) were used to avoid wetting difficulties with a platinum plate. Every surface area–surface pressure isotherm was determined on a freshly poured subphase (hydrochloric acid, pH = 1.9). The subphase was allowed to equilibrate for 10 min at 37 ± 2 °C. Surface active impurities were removed from the air–water interface with a slight vacuum after compression of the barrier. Acid, phospholipid and acid–phospholipid solutions with a concentration of 1–2 mg/ml were freshly prepared every day in *n*-hexane/2-propanol = 9:1. A known quantity (30–60 μl) of solutions was spread on the surface, and exactly 10 min at 37 ± 2 °C was allowed to elapse for solvent evaporation before the start of the compression. A constant compression speed of 15 cm^2/min (10 mm/min) was used. All experiments were repeated at least three times.

2.2. Preparation of samples for differential scanning calorimetry

Calculated amounts of the phospholipids and fluorinated acid were dissolved in chloroform–methanol (3:1, v/v) at the appropriate mole fractions [33,45]. The solvent was removed under a stream of nitrogen, and the mixtures were further dried under vacuum for at least 3 h. The samples (mixture of phospholipid and fluorinated acid or pure phospholipid) were hydrated in an excess of water (three times by weight). Samples were heated above the lipid transition temperature for 5 min and vortexed for 2 min. This process was repeated four times. Finally the samples were sonicated (ultrasonic cleaner model 450, E/MC Corporation) in a water bath above the lipid transition temperature for 30 min, followed by the heating and vortexing cycle mentioned above. Samples were stored at 4 °C for 12–16 h. Hydration of samples was always carried out a day before collecting the DSC scans.

A Thermal Analysis 2920 differential scanning instrument was used for the DSC studies. The hydrated samples were weighed into DSC aluminum pans. The DSC cell was purged with 60 ml/min and the refrigerated cooling system (RSC) with 120 ml/min dry nitrogen, respectively. Samples were cooled to 4 °C at a cooling rate of 10 °C/min and then heated from 4 to 80 °C with a heating rate of 5 °C/min. This relatively high heating rate was chosen to allow a direct comparison with previous investigations of similar systems [33,45,46]. All samples were subjected to two subsequent heating cycles. All experiments were carried out in triplicate. Onset, maximum and offset temperatures as well as peak width of the pretransition and the main phase transition

were determined for the second run using the Universal Analysis NT software [33,42,43].

3. Results and discussion

Binary mixtures of two environmentally relevant perfluorinated carboxylic acids, perfluorododecanoic and perfluorotetradecanoic acid, with DPPC were investigated at the air–water interface and in fully hydrated bilayers. We have previously reported the behavior of several fluorinated compounds at the air–water interface on a hydrochloric acid subphase (pH = 2). [33,34,42–44] This subphase was therefore chosen for the studies at the air–water interface. Due to experimental limitations, i.e. the use of aluminum pans which are not acid resistant, it was not possible to use hydrochloric acid for the DSC studies. This difference in the pH between the monolayer and the DSC studies needs to be taken into consideration when comparing the results from the two different methods. At the air–water interface the acidic subphase (pH = 2) results in a protonation of both the perfluorinated acid as well as of the phosphate residue of DPPC [47]. This is not the case for the DSC studies at pH = 7. As a result, the structures of the headgroups as well as the immediate water shell at the air–lipid interface are expected to be very different. Despite these differences in the experimental conditions and, hence, the resulting structural differences of the DPPC mono- and bilayer, these two studies still provide an insight into the question whether perfluorinated carboxylic acids can partition into lipid mono- and bilayers.

4. Monolayer studies

Two criteria for miscibility of binary mixtures of the fluorosurfactant and DPPC were applied. DPPC exhibits a phase transition from a liquid-expanded to a liquid-condensed phase at 37 °C. The concentration dependence of this phase transition was used as the first criterion for miscibility in a binary monolayer [48,49]. The concentration dependence of the average molecular area at constant surface pressure was used as the second phenomenological criterion of miscibility at the air–water interface [48]. In either ideal behavior or complete phase separation, the average area per molecule at constant surface pressure of any mixture is the sum of the areas occupied by each species at the surface

$$A(\pi) = X_{\mathrm{Acid}} A_{\mathrm{Acid}} + X_{\mathrm{DPPC}} A_{\mathrm{DPPC}} \quad [1]$$

where X is the mole fraction of each component present at the air–water interface, A is the average area per molecule of the pure component, and 'Acid' is the respective perfluorinated acid. It should be noted that mixtures will follow Eq. (1) when the components are either completely miscible or completely phase-separated. Attractive or repulsive interactions between the acid and DPPC will result in either a negative or positive deviation, respectively, from Eq. (1).

To assess the miscibility of the two binary systems using the two criteria of previously set forth, the compression isotherms of mixtures of the two perfluorinated carboxylic acids with DPPC were recorded at various compositions at 37 ± 2 °C (Fig. 1). The breakpoint of the phase transition from the liquid-expanded to the liquid-condensed state, the limiting area and, when possible, the collapse pressure for all π–A isotherms are summarized in Table 1. The A–X_{DPPC} diagrams of all carboxylic acid–DPPC mixtures are shown at surface pressures of 5, 10 and 25 mN/m in Fig. 2. These pressures were chosen because no phase transition complicating the interpretation of the A–X diagrams appears at these values.

As shown in Table 1 and Fig. 1A, a concentration-independent liquid-expanded to liquid-condensed phase

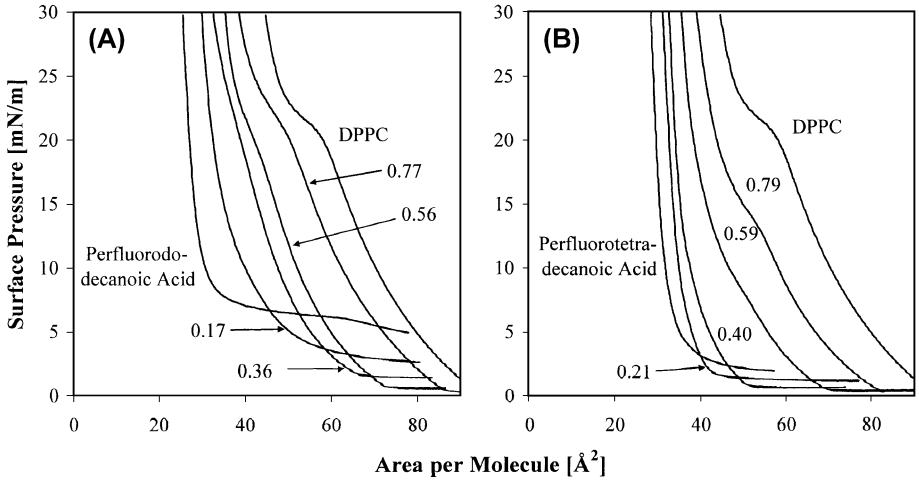

Fig. 1. Compression isotherms of mixtures of perfluorinated carboxylic acids and DPPC for different mole fractions of DPPC (37 ± 2 °C, hydrochloric acid, pH = 1.9–2.1). (A) Perfluorododecanoic acid and DPPC; (B) perfluorotetradecanoic acid and DPPC.

Table 1
Phase transition, limiting area and collapse pressure of compression isotherms of carboxylic acid–DPPC mixtures (37 ± 2 °C, hydrochloric acid, pH = 1.9–2.1)

X_{DPPC}	Surface pressure [mN/m]	Limiting area [Å^2/molecule]	Collapse pressure [mN/m]
PFDA			
0		29.0 ± 1.4	>50
0.17		33.1 ± 0.7	n.d.
0.36		36.2 ± 0.7	n.d.
0.56	18.0 ± 0.9	38.3 ± 1.4	n.d.
0.77	20.2 ± 0.3	41.3 ± 0.3	n.d.
1	20.3 ± 0.1	50.0 ± 3.0	n.d.
PFTA			
0		31.1 ± 0.5	> 50
0.21		35.2 ± 0.4	n.d.
0.40		35.4 ± 0.3	64.0 ± 0.5
0.59	7.3 ± 0.4	38.1 ± 0.9	n.d.
0.79	13.8 ± 0.7	39.6 ± 0.8	68.8 ± 0.8
1	20.3 ± 0.1	50.0 ± 3.0	n.d.

DPPC = dipalmitoylphosphatidylcholine; PFDA = perfluorododecanoic acid; PFTA = perfluorotetradecanoic acid. n.d. = not determined.

transition is observed for the perfluorododecanoic acid–DPPC system at higher concentrations of DPPC ($X_{DPPC} \geq 0.56$). Within the experimental error, the A–X_{DPPC} diagrams at surface pressures of 10 and 25 mN/m show no deviation from Eq. (1). Taking these two observations into consideration, an unambiguous interpretation of the miscibility of the two components is not possible for surface pressures of 10 mN/m and above. The A–X_{DPPC} diagram at 5 mN/m shows a significant negative deviation from Eq. (1) (Fig. 2A). This behavior suggests partial miscibility at lower surface pressures (i.e., 5 mN/m).

In the case of perfluorotetradecanoic acid and DPPC mixtures, a concentration-dependent liquid-expanded to liquid-condensed phase transition can be observed at higher concentrations of DPPC ($X_{DPPC} > 0.59$) (Fig. 1B). The monolayers of the mixtures are highly condensed for $X_{DPPC} < 0.59$. The A–X_{DPPC} diagram shows a negative deviation from Eq. (1) over the entire surface pressure range studied (Fig. 2B). The concentration-dependent phase transition, as well as the concentration dependence of the average molecular area at constant film pressure, point to partial miscibility between both compounds in the monolayer.

Similar to their behavior in the bulk phase, the mixing of fluorocarbon and hydrocarbon surfactants in micelles [25,50–53], monolayers at the air/water interface [54,55] and Langmuir–Blodgett films [56,57] often show nonideal behavior because of the low affinity of the fluorocarbon tail for the hydrocarbon tail. In general, the perfluorocarbon acid–DPPC mixtures examined in the present work do not exhibit any clear indication for the extensive phase separation reported for other fluorocarbon–hydrocarbon surfactant systems.

As seen in Fig. 2, significant negative deviations from Eq. (1) can be observed for mixtures at 5 mN/m and, less pronounced, for perfluorotetradecanoic acid–DPPC mixtures at 10 and 25 mN/m. In other studies, mixtures of DPPC with carboxylic acids [33–35,58] and long-chain alcohols [36] show a similar behavior. This negative deviation from Eq. (1) indicates the presence of strong intermolecular interactions, possibly between headgroups. However, the interaction observed in the present system appears to be stronger than those of the partially fluorinated and perhydrocarbon systems. At this time we can only speculate about the nature of these interactions, but the formation of hydrogen bonds between the carboxylic acid group and the phosphate diester function in the DPPC seems to be a plausible explanation. Similar interactions have been postulated in acid– and alcohol–phospholipid bilayers and result in the formation of complexes of 1:2 up to 1:4

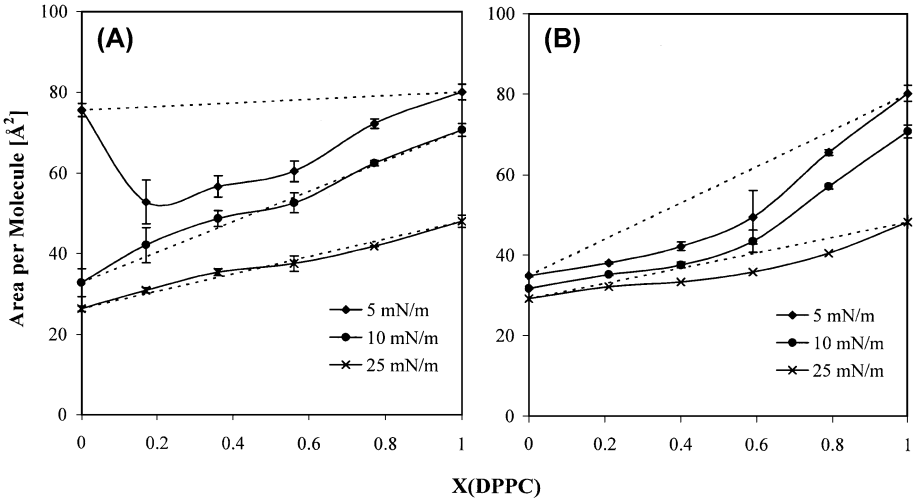

Fig. 2. A–X_{DPPC} diagrams of carboxylic acid–DPPC mixtures (37 ± 2 °C, hydrochloric acid, pH = 1.9–2.1). (A) Perfluorododecanoic acid and DPPC; (B) perfluorotetradecanoic acid and DPPC.

complexes [37,59]. Formation of a 1:1 complex has also been postulated for monolayers of hexadecanoic acid and DPPC [60].

5. Differential scanning calorimetry studies

Shown in Fig. 3 are representative DSC thermograms of mixtures of DPPC with perfluorododecanoic and perfluorotetradecanoic acid, respectively. The thermograms were used to determine the pretransition and main phase transition temperatures of the respective mixtures. The pretransition and main transition of fully hydrated DPPC occur at 36.8 ± 0.1 and 41.8 ± 0.1 °C, respectively (for literature values, see Ref. [61]). No melting transitions are observed for perfluorododecanoic or perfluorotetradecanoic acid in the same region. The addition of a small amount of either perfluorododecanoic or perfluorotetradecanoic acid results in broadening of the peak of the pretransition. The maximum of the pretransition peak appears to be constant in both mixtures while its onset is shifted to lower temperatures. This behavior is different from typical long-chain carboxylic acid–phosphatidylcholine systems where increasing the mole fraction of the acid results typically in a shift of the pretransition to higher temperatures until it merges with the main transition [33,62]. At a mole fraction below approximately $X_{DPPC} = 0.97$, no pretransition peak can be observed.

This pretransition of DPPC is the result of a shift from a tilted ($L_{\beta'}$) to a vertical ($P_{\beta'}$) configuration, and is observed for most phosphatidylcholines [61]. The tilted arrangement of the hydrophobic chains of the lipid molecules below the pretransition temperature results from packaging constrains caused by the mismatch of the cross-sectional area of the tails (40 $Å^2$ for both alkyl chains [63]) and the headgroup (46 $Å^2$ [64,65]). Alkanes, alcohols and carboxylic acids with a chain length of ≥ 12 carbon atoms as well as numerous small molecules and proteins are known to reduce or eliminated the pretransition at low concentrations [59]. These molecules are incorporated into the lipid bilayer, thus allowing the more optimal vertical alignment of the tails of the lipid molecules by reducing the head–tail mismatch. The presence of fluorinated acids results in the disappearance of the pretransition, which may suggest a change in the tilt angle of DPPC in the lipid bilayer.

In the perfluorododecanoid acid–DPPC system, $X_{DPPC} \geq 0.78$, the onset of the main transition occurs at the narrow temperature range of 40 to 42 °C. Beginning at $X_{DPPC} = 0.97$, the main transition peaks become broader with increasing amount of fluorinated acid and exhibiting a peak half-width of about 5° (Fig. 4). At $X_{DPPC} = 0.94$, at least two phase transitions can be observed (shoulder at 44.5 ± 0.2 °C), and there appears to be a minimum in the onset temperature (see Fig. 5A). Below this mole fraction two distinctive phase transitions can be observed, a relatively sharp lower temperature transition peak with almost constant onset temperature and a higher temperature transition peak which shifts to increased temperatures with acid concentration. The later phase transition also broadens significantly with increasing acid concentration. It is noteworthy that besides these two peaks, no peak (or peaks) at higher temperatures (i.e. closer to 80 °C) was observed over

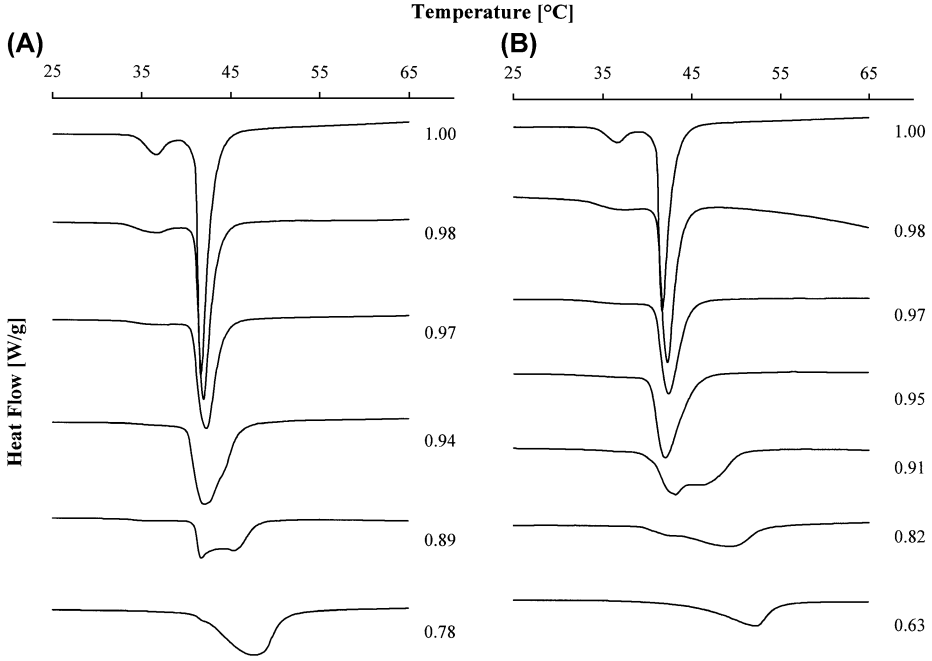

Fig. 3. Calorimetric scans for mixtures of DPPC with (A) perfluorododecanoic acid and (B) perfluorotetradecanoic acid in excess water. The mole fraction of DPPC is indicated besides each scan. The heating rate was 5°/min from 4 to 80 °C (only the part of the curve with a phase transition is shown).

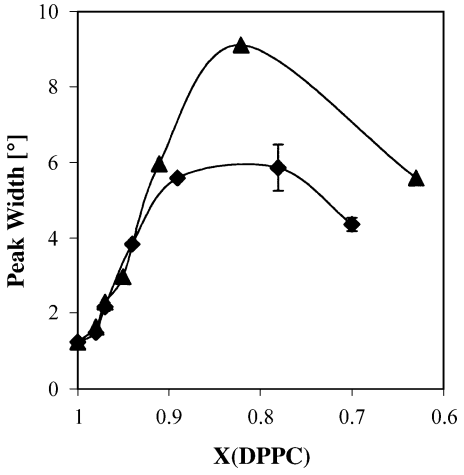

Fig. 4. Mole fraction-dependent changes in the half width of the main phase transition of mixtures of DPPC with (♦) perfluorododecanoic acid and (▲) perfluorotetradecanoic acid in excess water.

the entire concentration range studied. Such peaks would be expected if a perfluorododecanoic acid-rich lipid assembly (or assemblies) were formed due to the nonideality of the mixing of the acid and DPPC.

The DSC thermograms of the perfluorotetradecanoic acid–DPPC system show features similar to the perfluorododecanoic acid–DPPC system (Fig. 3B). The onset of the main phase transition is almost constant for X_{DPPC} in the range of 1 to 0.82. Significant peak broadening can be observed at $X_{DPPC} = 0.97$, while at least two distinct phase transitions can be observed at $X_{DPPC} = 0.91$. Here too, the low temperature phase transition remains more or less constant, but, in contrast to the perfluorododecanoic acid–DPPC system, shows some peak broadening. The peak corresponding to this phase transition disappears at X_{DPPC} below 0.82. A higher temperature transition (from $X_{DPPC} < 0.95$) shows significant peak broadening with peak widths up to 9° (Fig. 4) and shifts to higher temperatures with increasing acid concentration. No phase transition corresponding to an acid-rich phase was observed over the concentration range studied.

The main phase transition of both acid–DPPC mixtures exhibits some characteristics of longer-chain perhydrocarboxylic acid mixtures (12–18 carbon atoms) with phosphatidylcholines, but also shows some distinct differences. In general, perhydrocarbon carboxylic acids cause peak broadening of the main phase transition of fully hydrated phosphatidylcholines. In addition, the onset temperature in the perhydrocarbon systems increases because of the formation of a 2:1 compound (i.e. an azeotropic behavior of the mixture). For example, the melting point of the hexadecanoic acid–DPPC 2:1 compound is more than 20° above that of pure DPPC [62]. In both perfluorocarbon acid–DPPC mixtures studied in the present work, a significant peak broadening can be observed (Fig. 5), although the peaks are not as broad compared to typical perhydrocarbon acid–phosphatidylcholine systems. One difference is that the onset temperature in both fluorinated systems remains roughly constant until an $X_{DPPC} \sim 0.8$, which may indicate immiscibilities in the gel phase. Although some increase in transition temperature can be observed at the highest acid concentration studied ($X_{DPPC} = 0.70$ or 0.63, respectively), the temperature increase is not nearly as significant as in the perhydrocarbon systems. On the other hand, mixtures of partially fluorinated acids such as 11-(perfluorohexyl)-undecanoic acid with phosphatidylcholines show even less pronounced changes (lesser peak broadening and only a modest increase in onset temperature due to complex formation) compared to the perfluorocarbon and perhydrocarbon mixtures [33].

The similarities as well as the differences between the perhydrocarbon, partially fluorinated and perfluorocarbon systems can partly be explained by differences in the packing of the molecules in the bilayer. The drastic increase in the temperature of the phase transition in the perhydrocarbon systems has been explained by the incorporation of two alkyl chains per phosphatidylcholine molecule into the

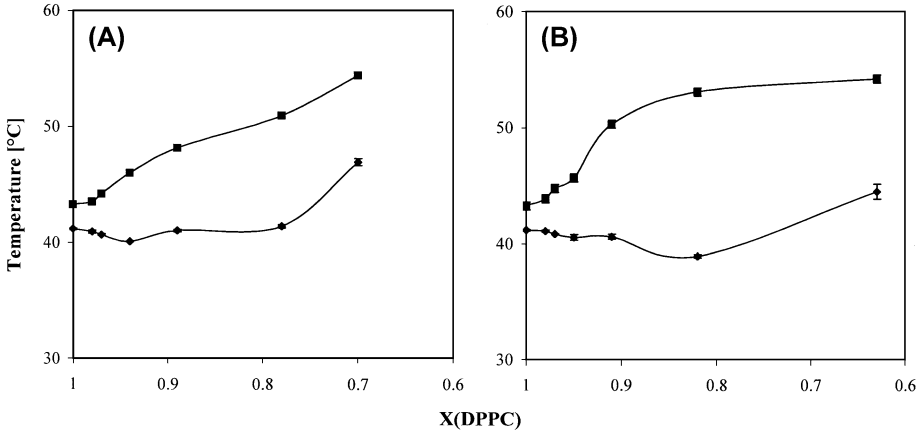

Fig. 5. Partial phase diagram of mixtures of DPPC with (A) perfluorododecanoic acid and (B) perfluorotetradecanoic acid in excess water. (♦) Onset temperature of main transition; (▲) offset temperature of main transition. The onset and offset temperatures of the pretransition are not shown.

bilayer which overcomes the head–tail mismatch and allows for an optimal packing of the alkyl tails as well as the headgroups. This increases tail–tail interactions in the bilayer and gives rise to a higher melting point of the 2:1 compound compared to the pure phosphatidylcholine. The strong attractive interactions observed in our monolayer studies, the elimination of the pretransition, as well as the absence of acid-rich phase transitions in the DSC studies suggest that both fluorinated acids have partitioned into the DPPC bilayer. However, a perfluoroalkyl chain is significantly larger compared to a hydrocarbon chain (30 vs. 20 $Å^2$, see also Table 1 for limiting molecular areas) and is liphophobic. The carboxylic headgroup of the fluorinated acids is more acidic compared to the hydrocarbon analogs which will likely disturb the network of charges and hydrogen bonds at the lipid–water interface differently than the less acidic hydrocarbon acid. This may result in unique structural changes of the DPPC headgroups at the lipid–water interface compared to the hydrocarbon systems. For example, these factors could prevent the formation of a 2:1 complex as seen with optimal packing of the hydrocarbon/fluorocarbon tails in the bilayer. Instead, the fluorocarbon acid–DPPC mixture could adopt a different, but less optimal, packaging in the bilayer to accommodate these additional factors. Thus, the phase behavior of the perfluorocarbon acid–DPPC mixtures (as well as the partially fluorinated mixtures [33]), as expected, differs significantly from perhydrocarbon acid–DPPC mixtures.

The changes in the thermodynamic properties of DPPC bilayers in the presence of small quantities of perfluorododecanoic acid or perfluorotetradecanoic acid may influence the function of biological mono- and bilayers. The fluorinated acids will be highly diluted in a given biological system. Changes in the thermodynamic properties of lipid assemblies may be, on average, too small to have any adverse effect. However, both partial phase diagrams (Fig. 5) suggest the presence of immiscibilities at physiological temperature in DPPC. Such immiscibilities could give rise to the formation of domains rich in the perfluorinated acid within a bilayer which could alter the properties of biological phospholipids mono- or bilayers and thus explain changes in properties, i.e. the membrane fluidity, of mitochondrial and cell membranes [21,23].

6. Conclusions

To further understand the behavior of perfluorinated amphiphiles, i.e. long-chain carboxylic acids, in biological systems, the mixing behavior of perfluorododecanoic and perfluorotetradecanoic acid with DPPC was studied in monolayers at the air–water interface and in fully hydrated DPPC bilayers. The mixing behavior in monolayers was assessed by analyzing the concentration dependence of (i) the average molecular area at constant film pressure (area/mole fraction or A–X diagram) and (ii) onset of the phase transition from the liquid-expanded to the liquid-condensed state of DPPC. Perfluorotetradecanoic acid showed a negative deviation from ideal behavior at surface pressures between 5 and 25 mN/m as well as a concentration dependence of the onset of the phase transition. These findings are indicative of an attractive interaction with DPPC in the mixed monolayer at the air–water interface. In the perfluorododecanoic acid–DPPC system, the onset of the phase transition showed no concentration dependence. At higher surface pressures (25 mN/m) no deviation from Eq. (1) was observed. An unambiguous interpretation of the miscibility of the two components at these high pressures is not possible. A strong negative deviation from ideal behavior was observed at lower surface pressures (5 mN/m) characteristic for strong interactions and miscibility. For the bilayer studies, DSC thermograms of fully hydrated acid–DPPC mixtures of varying mole fraction (X_{DPPC} ranging from 1 to 0.6) were recorded and the influence of the acid on the pre- and main transition of DPPC analyzed. The fluorinated acids cause peak broadening and ultimately elimination of the pretransition of DPPC. The onset temperature of the main phase transition hardly increases in the presence of the fluorinated acids, which suggests immiscibilities in the gel phase. At X_{DPPC} below 0.97, significant peak broadening with two distinct phase transitions can be observed.

The overall mixing behavior in the mono- as well as the bilayer suggests that there is a strong interaction between the acid and DPPC, and that, despite the generally highly nonideal behavior of fluorocarbon–perhydrocarbon binary systems, the perfluorinated acid is able to partition into the lipid bilayer. This observation is true regardless of the fact that the monolayer and DSC studies were preformed at different pH values. In summary, our results support previous reports [21,23] that fluorinated amphiphiles can alter the function of biological membranes at environmentally relevant concentrations, thus providing a possible mechanistic explanation for the toxicity of these compounds.

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