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Effect of the fluorination degree of hydrophobic chains on the monolayer behavior of unsaturated diacylphosphatidylcholines bearing partially fluorinated 9-octadecynoyl (stearoloyl) groups at the air–water interface

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ABSTRACT

The effect of the fluorination degree of hydrophobic chains on the monolayer behavior of unsaturated diacylphosphatidylcholines (PCs) was examined by employing a series of PCs bearing partially fluorinated 9-octadecynoyl (stearoloyl) groups (DF_nStPCs, n: the number of fluorinated carbon atoms in a stearoloyl group; $n = 1, 2, 4, 8$), including their hydrophobic parts – partially fluorinated stearolic acids (F_nStAs) – at the air–water interface. π–A isotherm measurements and Brewster angle microscope observations revealed: (i) all DF_nStPCs including F_nStAs form monolayers of liquid character at 25 °C; (ii) they form more expanded monolayers than their non-fluorinated counterparts, distearoloyl-PC (DStPC) and stearolic acid, while the monolayer stability increases with n; (iii) compared with DStPC and DF_8StPC, DF_nStPCs ($n = 1, 2, 4$) in the low-π region tend to show a weakening in their self-aggregation property and an increase in the work required for monolayer compression; (iv) although DF_8StPC forms the most expanded monolayer, the behavior of DF_8StPC resembles that of DStPC rather than that of DF_nStPCs ($n = 1, 2, 4$). The monolayer behavior of DF_nStPCs ($n = 1, 2, 4$) is explained by postulating a flatly-lying conformation of hydrophobic chains, in which three polar parts (ester group, triple bond, CF_2—CH_2 linkage) in chains are immersed in the subphase at large areas. DStPC and DF_8StPC lacking a CF_2—CH_2 linkage, however, do not likely adopt such a conformation.

1. Introduction

Natural lipid membranes have been widely utilized as reconstitution matrices for membrane proteins [1,2]. However, these membranes have some performance limitations, such as low mechanical and low chemical stability, which often hinder the use of lipid membranes in technical applications. Therefore, several methods for improving membrane stability have been proposed, e.g., the incorporation of stabilizing components such as cholesterol, polysaccharides or semifluorinated alkanes into membranes, polymerization among polymerizable lipids and membrane immobilization on solid substrates [2–4]. We focus on the use of fluorinated lipids as one of the ways to improve membrane stability because fluorinated carbon chains exhibit unique features, such as thermal and chemical stability, remarkable hydrophobicity and lipophobicity, as well as biological inertness [4–10]. From the viewpoint of protein handling, some fluorinated lipid monolayer membranes have received attention as matrices for 2D protein crystallization at the air–water interface [9,11]. Most of membrane-forming fluorinated lipids so far proposed [3,5,7–14], however, appear to be insufficient for yielding membranes possessing the required fluidity as reconstitution matrices for membrane proteins because these lipids bear saturated chains and tend to form rigid structures at physiological temperatures.

Aiming at eventually designing fluorinated phospholipids suitable for membrane protein reconstitution, we firstly considered partially fluorinated C_{18} fatty acids as building blocks for fluorinated phospholipids. Consequently, we observed that the fatty acids bearing perfluorooctyl (F_8) group as the terminal segment form monolayers with much higher equilibrium spreading pressures, π_{es}s, compared with their non-fluorinated counterparts, irrespective of the chain structure [15]. Originally, π_{es} was defined

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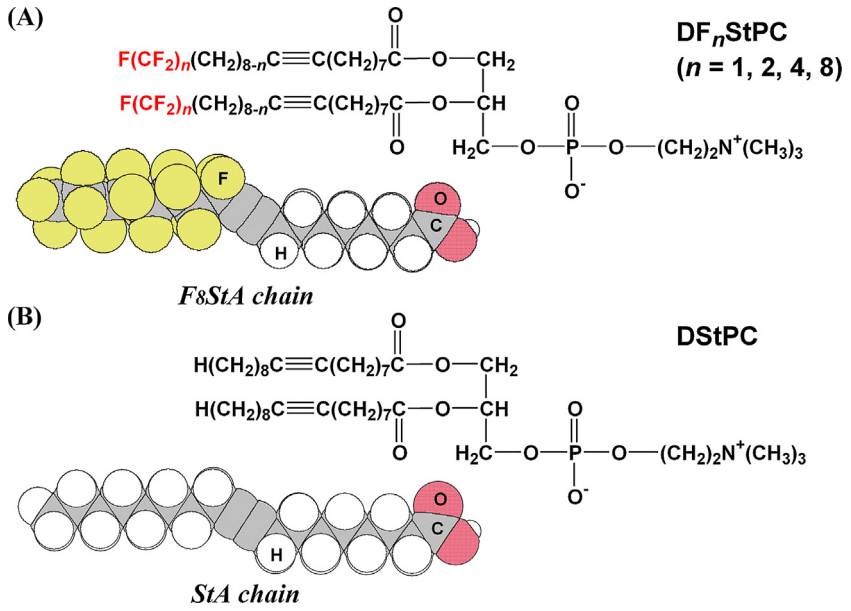

Fig. 1. Chemical structures of (A) DF$_n$StPC (n = 1, 2, 4, 8) and (B) DStPC, with space filling models of each hydrophobic chain represented by the corresponding StA analog. For DF$_n$StPCs, only F$_8$StA with a fluorinated segment adopting a 15/7 helical conformation [4,49] is illustrated.

as the surface pressure, π, of the 2D (monolayer) phase in equilibrium with the 3D (bulk) phase on the surface, and π_{es} can be taken as a measure of monolayer stability [16,17]. Therefore, the introduction of fluorinated chains into C_{18} fatty acid molecules was considered effective in improving monolayer stability against lateral compression [15]. We also observed that progressive fluorinations of the terminal segment of unsaturated fatty acids, such as 9-octadecynoic acid (stearolic acid, StA) [18] and 9-octadecenoic acid (oleic acid, OA) [19], gradually increase monolayer stability. These findings provided information on the extent to which the fluorination degree of hydrophobic chains is required to improve membrane stability. Next, by considering compatibility with membrane proteins as well as membrane fluidity, we designed a series of unsaturated diacylphosphatidylcholines (PCs) bearing partially fluorinated stearoloyl groups (DF$_n$StPCs, n: the number of fluorinated carbon atoms in a stearoloyl group; n = 1, 2, 4, 8) as analogs of distearoloylphosphatidylcholine (DStPC) and estimated their monolayer stability by π_{es} measurements [20]. It is noted that neither natural phospholipids nor glycolipids bearing stearoloyl groups are known at all, although the class of lipids containing C≡C triple bonds rarely occurs in natural plant oils; particularly, StA is one of the best known fatty acids in this class and is found in the seeds of *Santalaceae* species [21]. To date, only a few reports on the synthesis and properties of DStPC have been presented [20,22–24]. DStPC forms liquid-crystalline membranes above 0 °C and, hence, is expected as a reconstitution matrix for membrane proteins [23]. The advantage of DStPC analogs over dioleoyl-PC ones is that the former can be synthesized more readily than the latter because the isolation of the *cis–trans* isomers with respect to hydrophobic chains is unnecessary for DStPC analogs.

This work was aimed at examining the monolayer properties of DF$_n$StPCs including their hydrophobic part acids (F$_n$StAs) at the air–water interface in further detail. Besides monolayer stability, we examined the effects of the fluorination degree of chains on monolayer compressibility, self-aggregation property of lipid in monolayer, and energy required for monolayer compression. These results complemented our previous studies and newly indicated that short fluorinated chains could markedly affect conformations of lipid molecules in monolayer due to chain–water interactions.

2. Materials and methods

2.1. Materials

F$_n$StAs (n = 1, 2, 4, 8) and StA were prepared with purities of >97% as previously described [15,18]. DF$_n$StPCs (n = 1, 2, 4, 8, Fig. 1) and DStPC (Fig. 1) were prepared with purities of >98% as previously described [20]. Distearoyl-PC (DSPC, >99% pure) and dielaidoyl-PC (DEPC, >98% pure) were purchased from Sigma and used as received. Chloroform and methanol used for monolayer experiments were of spectroscopic grade from Dojindo (Kumamoto, Japan). Water was purified as previously described [25], and its resistivity was >18 MΩ cm.

2.2. Monolayer experiments

π–A isotherms were measured on a KSV (Helsinki, Finland) minitrough 2 system at 25.0 ± 0.1 °C. The constitution of this apparatus is detailed elsewhere [19]. For fatty acids, aqueous hydrochloric acid (pH 2.0) was used as the subphase to minimize the dissolution of lipids into the subphase due to their dissociation. For PCs, unbuffered water (pH ≈ 6) was used. The spreading solvents were chloroform for fatty acids and a chloroform/methanol (9:1, v/v) mixture for PCs. Fatty acid concentrations were determined gravimetrically, whereas PC concentrations were determined by the phosphorus assay [26]. Lipid concentrations were 0.5–3 mmol/L. After aspirating the subphase surface to remove impurities, monolayers were formed by spreading 10–25 μL of lipid solution on the subphase surface. The monolayers were left for ≥5 min before compression. The compression rates were 0.06–0.1 and 0.1–0.15 nm^2/molecule min for fatty acids and PCs, respectively.

Brewster angle microscope (BAM) observations of monolayers were performed with a KSV NIMA (Espoo, Finland) MicroBAM equipped with a 30-mW diode laser emitting p-polarized light at a wavelength of 659 nm, an analyzer at a fixed angle and a CCD camera (640 × 480 pixels). The incident angle was fixed to 53°, and the nominal horizontal resolution was 12 μm. The MicroBAM was coupled to the minitrough.