Comparison of two different partially fluorinated phosphatidylcholines with the perfluorobutyl group on thermotropic properties of the bilayer membrane and reconstituted bacteriorhodopsin

Tamami Yanagi¹ · Toshiyuki Takagi² · Hiroshi Takahashi³ · Takashi Kikukawa^{4,5} · Hideki Amii¹ · Masashi Sonoyama^{1,6,7}

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Introduction

Fluorinated surfactants (Shepherd and Holzenburg 1995; Popot 2010; Frotscher et al. 2015) and phospholipids (Santaella et al. 1991; Yoder et al. 2007; Takai et al. 2008; Gagnon et al. 2018) are now attracting more and more attention in biophysical fields because the incorporation of fluorine atoms provides distinctive physical properties to amphiphilic molecules, which would open up a new way of structural and functional characterization of membrane proteins (MPs). In particular, a novel partially fluorinated analog of dimyristoylphosphatidylcholine (DMPC), F4-DMPC (Fig. 1, $m = 9$; diF4H10-PC was used as the former abbreviation in the previous papers) (Yoshino et al. 2012; Takahashi et al. 2013; Okamoto et al. 2016), is of great promise for biophysical studies of MPs. Bacteriorhodopsin (bR) molecules in the F4-

DMPC bilayer membrane form the native purple membrane (PM)-like two-dimensional crystals of trimers as the structural unit in the liquid-crystalline phase as well as in the gel phase (Yoshino et al. 2013; Takahashi et al. 2019), which shows a striking contrast with phase transition-induced disassembly of bR trimers to monomers upon reconstitution into vesicles of the corresponding hydrogenated DMPC (Cherry et al. 1978; Heyn et al. 1981; Sonoyama et al. 2009; Yokoyama et al. 2010). As a further attempt to extend the novel partially fluorinated phosphatidylcholine (PF-PC) F4-DMPC to phospholipids with longer acyl chains, we have recently developed a novel analog of dipalmitoylphosphatidylcholine (DPPC) with the perfluorobutyl group like F4-DMPC, F4-DPPC (Fig. 1, $m = 11$). In this short report, comparison of F4-DPPC with F4-DMPC on structural and physical properties of the phospholipid bilayer membrane and reconstituted bR is discussed.

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✉ Toshiyuki Takagi
t.takagi@aist.go.jp

✉ Hiroshi Takahashi
hirotakahashi@gunma-u.ac.jp

✉ Masashi Sonoyama
sonoyama@gunma-u.ac.jp

¹ Division of Molecular Science, Faculty of Science and Technology, Gunma University, Kiryu, Gunma 376-8515, Japan

² Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8565, Japan

³ Division of Pure and Applied Science, Graduate School of Science and Technology, Gunma University, Maebashi, Gunma 371-8510, Japan

⁴ Faculty of Advanced Life Science, Hokkaido University, Sapporo 060-0810, Japan

⁵ Global Station for Soft Matter, GI-CoRE, Hokkaido University, Sapporo 060-0810, Japan

⁶ Gunma University Center for Food Science and Wellness (GUCFW), Gunma University, Maebashi, Gunma 371-8510, Japan

⁷ Gunma University Initiative for Advanced Research (GIAR), Gunma University, Maebashi 371-8511, Japan

Thermotropic properties of the F4-DPPC bilayer membrane

Based on an endothermic peak at 24.1 °C (Fig. 2a) and temperature-dependent wide-angle X-ray diffraction pattern changes (Yanagi et al., unpublished results) of the F4-DPPC membrane, the drastic decrease of ~20 °C in the gel-to-liquid crystalline phase transition temperature (T_m) against DPPC was induced for hydrated partially fluorinated DPPC by the perfluorobutylation of the palmitoyl chain, which is very similar to the previous work on F4-DMPC, as shown in Fig. 2a (Yoshino et al. 2012). It is reasonable to mention that the partial fluorination-induced drop of ~20 °C in T_m observed for F4-DPPC and F4-DMPC is due to the mismatch between the methylene and the perfluorobutyl moieties in the occupied areas in the lateral dimension of the membrane (Krafft and Riess 2009). The phase transition temperature of 24.1 °C for F4-DPPC provides a very significant basis to an application for biophysical studies of MPs in that under physiological conditions, the F4-DPPC bilayer membrane shows the liquid crystalline phase, i.e., fluidic properties. It is of great significance that the remarkable decrease of ~20 °C in T_m is induced not by chemically unstable unsaturated C=C bond but by the perfluorobutyl group including the most chemically stable C-F bond. Thermodynamic parameters characterizing the phase transition of the F4-DPPC bilayer membrane are $34.8 \pm 7.2 \text{ kJ mol}^{-1}$ for ΔH and $117 \pm 26 \text{ J mol}^{-1} \text{ K}^{-1}$ for ΔS , which are larger than its corresponding hydrogenated DPPC ($\Delta H = 27.3 \pm 1.5 \text{ kJ mol}^{-1}$ and $\Delta S = 86.8 \pm 5.5 \text{ J mol}^{-1} \text{ K}^{-1}$). The remarkable increase in the thermodynamic parameters of F4-DPPC against DPPC is very similar to the results of F4-DMPC against DMPC (Yoshino et al. 2012). The dominant factors for the differences in the thermotropic transition between the PF-PCs and their corresponding hydrogenated PCs will be discussed based on experimental results by X-ray diffraction (Yanagi et al., unpublished results).

Molecular assembly state of bR in the F4-DPPC bilayer membrane

Two-dimensional crystals of bR trimers (Henderson 1975), which are essential for efficient and stable light-driven proton pumping, are formed like the native PM even in the artificial membrane of PCs with saturated acyl chains (e.g., DMPC and DPPC) in the gel state (Cherry et al. 1978; Heyn et al. 1981;

Fig. 1 Chemical structure of F4-DMPC ($m = 9$) and F4-DPPC ($m = 11$)

Fig. 2 a DSC thermograms for bilayer vesicles reconstituted with and without bR. The scalar bar corresponds to 200 μW. b Temperature dependence of minimum molar ellipticity around 600 nm for bR reconstituted into vesicles

Sonoyama et al. 2009; Yokoyama et al. 2010). Upon thermotropic transition to the liquid crystalline phase, however, reconstituted bR molecules undergo disassembly into monomers at a high lipid-to-protein ratio ($L/P > \sim 100$) (Cherry et al. 1978; Heyn et al. 1981; Sonoyama et al. 2009; Yokoyama et al. 2010). This quaternary structural transition of bR is readily monitored by molar ellipticities of the negative minimum around 600 nm ($[\theta]_{600 \text{ nm}}$) in visible circular dichroism spectra (Sonoyama et al. 2009; Yokoyama et al. 2010), as shown in Fig. 2b. The midpoints of the structural transition estimated by temperature-dependent changes of $[\theta]_{600 \text{ nm}}$ are ~18 °C for bR/DMPC and ~36 °C for bR/DPPC, which are in good agreement with T_m obtained by DSC measurements (Fig. 2a). Of note is that compared with pure PC membranes, the endothermic peaks of the reconstituted membrane of DMPC and DPPC showed a shift toward lower temperatures and significant broadening with a gradual rise in the low-temperature side (Yokoyama et al. 2010). These experimental results indicate that bR molecules are highly dispersed in the fluidic DMPC and DPPC bilayer membrane due to high miscibility between DMPC or DPPC and bR. As reported in the previous paper (Takahashi et al. 2019), on the other hand, thermotropic behaviors of bR molecules in the F4-DMPC (bR/F4-DMPC) membrane are substantially different from

those in DMPC and DPPC: two-dimensional crystalline arrays of bR trimers are retained even above $\sim 40^\circ\text{C}$ (Fig. 2b), $\sim 35^\circ\text{C}$ higher than T_m (3.5°C) of bR/F4-DMPC revealed by a calorimetric measurement (Fig. 2a). It should be pointed out that in the bR/F4-DMPC system, a second endothermic peak at 5.8°C also appears, which indicates coexistence of two regions composed of almost pure F4-DMPC and F4-DMPC interacting with bR molecules (Takahashi et al. 2019). The heterogeneity of bR/F4-DMPC strongly suggests that F4-DMPC tends to be less miscible with bR than DMPC and DPPC, which is the most likely predominant driving force for retaining two-dimensional crystals of bR trimers even in the fluid phase.

Upon reconstitution into F4-DPPC, on the other hand, bR trimeric molecules disassemble to monomer in a thermotropic transition-dependent manner: the midpoint temperature (approximately 22°C) of quaternary structural changes estimated by $[\theta]_{600\text{ nm}}$ (Fig. 2b) is in good agreement with T_m (22.0°C) of the bR/F4-DPPC bilayer revealed by a DSC measurement. In addition to the lipid bilayer phase transition-induced monomerization, a single broad endothermic peak is observed with a gradual rise in the lower-temperature side, which is also markedly different from bR/F4-DMPC and is instead very similar to bR/DMPC and bR/DPPC. These experimental results suggest that in contrast to the immiscible bR/F4-DMPC binary system even in the liquid crystalline phase, bR molecules exhibit an affinity with F4-DPPC, which resulted in the well-mixed bR/F4-DPPC binary membrane in the fluid phase. The reason is probably that hydrocarbon segments in the acyl chains of F4-DPPC are longer than those of F4-DMPC. The hydrocarbon moieties have an affinity to bR molecules by contrast to the fluorocarbon ones.

Concluding remarks

Thermotropic behaviors of the phospholipid bilayer membrane and reconstituted bR were compared between two kinds of PF-PCs bearing a perfluorobutyl group in common in the terminal region of an acyl chain with different lengths. Thermotropic properties of the F4-DPPC and F4-DMPC membranes are very similar to each other, whereas thermally induced quaternary structural changes of bR reconstituted in the F4-DPPC and F4-DMPC membranes occurred in a starkly different manner. It is of great significance that even upon introduction of the same perfluoroalkyl group into the terminal region of acyl chains, the complex system of MPs and PF-PCs with different acyl chain lengths exhibited different properties, which show the high potential of PF-PCs for biophysical studies of MPs in various circumstances. The artificial bilayer membrane of F4-DPPC, which is miscible with MPs and fluidic under physiological conditions, would become a

useful platform for functional and structural characterization of MPs *in vitro*.

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Compliance with ethical standards

Conflict of interest Tamami Yanagi declares that she has no conflict of interest. Toshiyuki Takagi declares that he has no conflict of interest. Hiroshi Takahashi declares that he has no conflict of interest. Takashi Kikukawa declares that he has no conflict of interest. Hideki Amii declares that he has no conflict of interest. Masashi Sonoyama declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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