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Physicochemical Studies of Bacteriorhodopsin Reconstituted in Partially Fluorinated Phosphatidylcholine Bilayers

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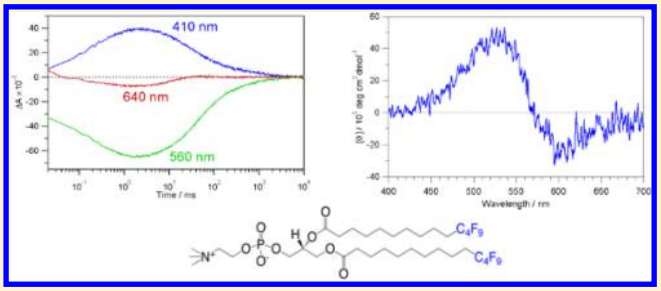
Supporting Information

ABSTRACT: A membrane protein bacteriorhodopsin (bR) that is successfully reconstituted in liposome of a novel partially fluorinated analog of dimyristoylphosphatidylcholine (DMPC) with the perfluorobutyl segments in the myristoyl groups, diF4H10-PC, has been investigated by some spectroscopic and X-ray diffraction techniques to clarify effects of substitution of nine hydrogen atoms by fluorine atoms on structural and physical properties of the membrane protein by comparison with the previous results on proteoliposome of bR and DMPC. Below the gel-to-liquid crystalline phase transition of diF4H10-PC bilayer, bR molecules adopt the two-dimensional lattice structure of trimers as the structural unit and show a photocycle very similar to that of native purple membrane like reconstituted bR in DMPC liposome in the gel phase. Even upon heating up to temperatures well above the phase transition, the nativelike functional reconstitution and higher structural stability of bR molecules in diF4H10-PC liposome are retained, which strikingly contrasts with lipid phase transition-induced disaggregation of protein molecules and light-induced denaturation in DMPC liposome. Greater membrane rigidity and low affinity between bR and fluorinated lipid molecules are proposed as a driving force for keeping nativelike properties of bR molecules in diF4H10-PC liposome even in the fluid phase.



1. INTRODUCTION

Recent genome sequence analyses for various kinds of organisms have indicated that membrane proteins, one of the most essential components of biomembranes in the cell, are encoded by approximately 25−30% of the total open reading frames (ORFs), irrespective of organisms analyzed,[1−4] and are attracting more and more attention from the viewpoints of fundamental bioscience and application to drug discovery. Membrane proteins are exposed to complex surrounding environments with two regions different in physicochemical properties, that is, amphiphilic lipid bilayer and water. For isolation, purification and further biochemical and biophysical studies of membrane proteins in aqueous media, amphiphilic molecules such as surfactants and phospholipids assume crucial roles to keep membrane proteins in the functional form. Because of serious difficulties in handling membrane proteins in water with conventional surfactants and lipids,[5] however, a limited number of experimental data of three-dimensional structure of membrane proteins are available to date, which is opposite to water-soluble proteins. To overcome the difficulties and carry out further analyses of function, structure and dynamics of membrane proteins, the design and synthesis of novel surfactants and lipids that provide membrane proteins with suitable environments have been desired for a long time.[6] One of the most promising approaches is the substitution of hydrogen atoms by fluorine atoms in amphiphilic molecules, since the introduction of fluorine atoms in the hydrophobic tail produces physical properties remarkably different from non-fluorinated molecules, for example, greater rigidity and weaker interaction with membrane proteins and/or native lipid molecules, upon formation of molecular assembly such as micelles and bilayer membrane. Fluorinated surfactants for membrane protein research have been originally developed in the 1990s by some groups.[7−11] Especially, hemifluorinated surfactants (HFS), which refer to surfactants with a fluorinated hydrocarbon chain that ends with a hydrogenated tip, originally developed by collaboration of Popot and Pucci, have been successfully utilized for membrane protein research, as HFSs

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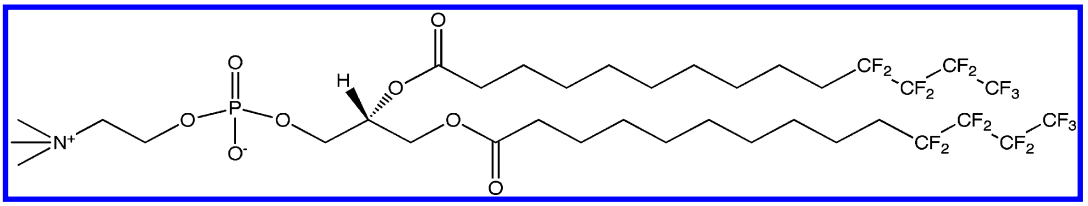

Figure 1. Chemical structure of diF4H10-DMPC, 1,2-di(11,11,12,12,13,13,14,14,14-nonafluorotetradecanoyl)-*sn*-glycero-3-phosphocholine.

can improve interaction with transmembrane surfaces of membrane proteins while preserving the overall lyophobic character of fluorinated surfactants.[6,10−13]

On the other hand, fluorinated lipids, which were originally developed as materials for highly stable liposome applicable to drug delivery system by Riess, Vierling, and co-workers, have attracted much attention mainly in the field of physical chemistry.[14−23] Although the previous physicochemical works have shown very unique physical and structural properties characteristic of partially fluorinated lipid membranes, for example, rigidifying effect because of the terminal fluorinated segments, high thermal stability, high permeability, and more tilting of the acyl chain to the bilayer normal, no attempts to utilize partially fluorinated lipids as materials for incorporating membrane proteins have been reported so far. Takagi and co-workers have recently designed and synthesized a series of double-chained phosphatidylcholines (PCs) containing perfluoroalkyl groups of different length with a C−C triple bond as promising biomaterials aiming at membrane protein researches.[24−26] Their measurements on equilibrium spreading pressure Π_e at the air−water interface on the partially fluorinated lipids and the corresponding nonfluorinated counterpart demonstrated that the substitution of at least five hydrogen atoms at the terminal hydrophobic segment by fluorine atoms is required to exhibit stabilizing effect, whereas further fluorination of PC with the perfluorobutyl group has no significant effect on the monolayer stability.[25−27] Furthermore, as the first attempt of physicochemical studies on a series of partially fluorinated saturated PCs with perfluoroalkyl groups of different length, a novel partially fluorinated PC 1,2-di-(11,11,12,12,13,13,14,14,14-nonafluorotetradecanoyl)-*sn*-glycero-3-phosphocholine (diF4H10-PC, Figure 1), an analog of a common 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) with the perfluorinated butyl segment in the myristoyl group, has been synthesized and its thermal and interfacial properties have been investigated by Sonoyama and co-workers.[28] The previous work demonstrated remarkable properties characteristic of the partially fluorinated PC that in the liquid crystalline phase, diF4H10-PC membrane has greater rigidity and larger occupied areas despite higher polarity in the hydrophilic/hydrophobic interface region and decrease in the gel-to-liquid crystalline phase transition temperature to 5.4 °C, compared to ordinary hydrocarbon analog DMPC (T_m = 23.6 °C). Furthermore, Takahashi and co-workers have recently demonstrated that by thermodynamic analyses of DSC data with the regular solution theory, a binary mixture liposome of diF4H10-PC and DMPC exhibits a remarkable phase separation to the diF4H10-PC-rich and the DMPC-rich domains.[29]

In the present study, membrane properties of the partially fluorinated PC as a material for membrane protein research have been probed by reconstituting one of the best characterized membrane protein bacteriorhodopsin (bR), a light-driven proton pump with seven transmembrane helices from *Halobacteirum salinarum*, into diF4H10-PC liposomes. Proton translocation across the membrane is accomplished through a photocycle including several spectrally distinct intermediates (J, K, L, M, N and O) after the absorption of visible light. Among the photointermediates, M is a key intermediate for the pump switch from proton release form to proton uptake form and two or three substates with almost the same absorption maximum are reported.[30] Structural and functional properties of reconstituted bR in diF4H10-PC liposome have been investigated with UV absorption, visible circular dichroisim (CD), laser flash photolysis and X-ray diffraction, and compared with the previous works on reconstituted bR in the corresponding nonfluorinated DMPC.[31,32] As a result, it has been shown that even in the liquid crystalline phase, bR molecules in diF4H10-PC liposome retain the quaternary structure like the native purple membrane (PM) and remarkably high stability in the functional state, whereas they disassemble into protomer in DMPC and consequently undergo irreversible denaturation of bR molecules.

2. MATERIALS AND METHODS

DiF4H10-PC used in this study was synthesized by the combination of the methods for partially fluorinated lipids developed by Takagi and co-workers.[24−26] HPLC purification of the synthesized diF4H10-PC was carried out on a Inertsil ODS-3 column (20 mm i.d. × 150 mm, GL Sciences Inc., Tokyo, Japan) and an ODS-80TS column (20 mm i.d. × 300 mm, TOSOH Corp., Tokyo, Japan) with methanol as the mobile phase at a flow rate of 8.0 mL/min and eluate peak was monitored with a JASCO RI-930 detector. The final product of diF4H10-PC showed a single elution peak in HPLC with RI-detector. DMPC was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All other chemicals were of best research grade and were used without further purification. PMs from *H. salinarum*, strain R1M1, were isolated and purified according to the established method by Oesterhelt and Stoeckenius.[33] The purified PMs were suspended in 100 mM phosphate buffer at pH 7.0. The protein concentration of bR in PM was determined from the absorption maximum at 568 nm recorded at room temperature using the extinction coefficient value of 62 700 M^{-1} cm^{-1}.[34]

The method for preparation of bR embedded in diF4H10-PC liposome in this study was very similar to the previous one used for reconstituting bR molecules into three kinds of nonfluorinated saturated phosphatidylcholine liposomes with different acyl-chain lengths.[31,32] The outline of the reconstitution method is as follows. The mixture of 10 μM PM and 5 mM Triton X-100 was incubated at 25 °C overnight in the dark and then solubilized bR was obtained as the supernatant of the mixture after ultracentrifugation at 100 000*g* for 1 h at 4 °C. Triton X-100 was selected as a detergent in this study because bR solubilized with Triton X-100 is highly stable in the dark up to ∼50 °C, as reported by Sasaki et al.[35] The solubilized bR in

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Triton X-100 micelle was mixed with diF4H10-PC suspension at the concentration ratio of 1:150 ([bR]/[diF4H10-PC]) and was gently stirred for 6 h, followed by gradual removal of Triton X-100 with Biobeads SM-2 (Bio-Rad Laboratories, Hercules, CA) hydrophobic and polar interaction adsorbents. The obtained suspension was purple. The final concentrations of bR and lipid in the obtained proteoliposome were approximately 8 μM and 1.5 mM, respectively. Samples for spectroscopic measurements were diluted twice. Proteoliposome of pure DMPC and bR was also prepared with the same procedure.

UV−visible spectra were recorded with a DU-7500 photodiode array spectrophotometer (Beckmann Coulter, Inc., Brea, CA). Spectroscopic measurements for bR/diF4H10-PC and bR/DMPC proteoliposome under visible light illumination were carried out using a light irradiation system that consists of a Xe lamp (average light power: 200 mW·cm^{-2}, Wacom Electric Co. Ltd., Saitama, Japan,), a Y52 color filter and a heat-cut filter for filtering out the light wavelengths shorter than 520 nm and longer than 700 nm. The temperature of the cell holder was controlled using a F25 refrigerated/heating circulator (JULABO, Seelbach, Germany) with the temperature stability of ±0.03 °C.

A J-820 spectropolarimeter equipped with a JWJTC-484 Peltier temperature controller (JASCO Co., Tokyo, Japan) was employed for obtaining visible CD spectra that provides information on the state of molecular assembly of bR in proteoliposome. The visible CD spectra in the region of 400−700 nm were measured with the 10-mm cuvette. Typically, eight scans were averaged for each spectrum.

To perform transient visible absorption experiments, a computer-controlled flash-photolysis apparatus was constructed based on the system developed previously.[36] In this system, actinic flash (532 nm, 7 ns, ∼5 mJ/pulse) was the second harmonic of the fundamental beam of a Q-switched Nd:YAG laser (Minilite II, Continuum, Santa Clara, CA). The monitoring light source was a 150-W halogen lamp (MHAB-150W-100 V, Moritex, Tokyo, Japan) and the beam was perpendicular to that of the actinic flash. A photomultiplier (R928, Hamamatsu Photonics K. K., Hamamatsu, Japan) was used to detect the light passing through the sample. To select the measuring wavelength and exclude the scattered actinic flash from the sample, we placed two monochromators (CT-10 and CT-10S, JASCO Corp., Tokyo, Japan) in the rear of the monitoring light source and in front of the photomultiplier. The output of the photomultiplier was further amplified and filtered by a homemade amplifier and then stored in a computer equipped with an A/D converter. By using the digital delay unit (MODEL DG535, Stanford Research Systems, Inc., Sunnyvale, CA), the laser pulse timing was adjusted to come after a few tens of millisecond delay from the start of the data acquisition. The data before the laser pulse were adopted as a baseline for the calculation of the following absorption changes.

X-ray diffraction measurements with synchrotron radiation source were performed at the beamline 6A of the Photon Factory (Tsukuba, Japan). Although all data presented in this paper were obtained at the beamline 6A, measurements were also carried out at the beamline 9C of the Photon Factory to test reproducibility. The details of the beamlines 6A[37] and 9C[38] have been reported elsewhere. The wavelength of X-ray beam was 0.15 nm and the detector-to-sample distance was about 500 mm. X-ray diffraction patterns were recorded using an X-ray photon counting two-dimensional pixel array detector PILATUS100K (DECTRIS, Switzerland).[39] Typical exposure time was 60 s. The two-dimensional data of PILATUS100K detector were transformed into one-dimensional data using FIT2D software.[40] The scattering angle was calibrated using diffraction patterns of silver behenate. In this report, we used the reciprocal spacing (S), $S = 1/d = (2/\lambda)\sin\theta$ (where d is the lattice spacing, 2θ is the scattering angle, and λ is the wavelength of X-ray) for horizontal axis to display one-dimensional data. The sample was mounted on a DSC apparatus for an optical microscope (FP-84, Mettler-Toledo International Inc., Columbus, OH), which was used as a temperature controller.[41] For X-ray diffraction studies, the bR/diF4H10-PC-membrane samples were pelleted by centrifugation at 18 000g for 30 min at 4 °C with a temperature-controlled centrifuge (MX-150, Tomy Ltd., Tokyo, Japan).

The degree of irreversible light-induced denaturation of bR was investigated as follows to compare structural stability of bR in diF4H10-PC and DMPC proteoliposome in the functional state. After continuous illumination of reconstituted bR with visible light at 30 °C for 1, 2, 4, 8, or 16 h, each sample was quickly cooled down and incubated in the dark at 4 °C overnight. Absorption spectra of the incubated samples were recorded in the dark the next day. The degree of light-induced denaturation was estimated by dividing the absorbance at the wavelength of the absorption maximum after the incubation by that obtained before light illumination.

3. RESULTS

The purple suspension obtained by the above-mentioned reconstitution experiments of bR into diF4H10-PC was ultracentrifuged at 100 000g for 1 h, which resulted in the complete separation of homogeneous purple precipitate and transparent supernatant, as shown in Figure 2. This indicates that bR molecules are completely embedded in diF4H10-PC liposome, that is, the successful formation of proteoliposome composed of bR and diF4H10-PC. UV−visible spectra of the

Figure 2. Photographs of purple suspension prepared by reconstitution experiments shown in Materials and Methods (A) before and (B) after ultracentrifuge.

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obtained bR/diF4H10-PC proteoliposome suspension in the dark and under visible light irradiation at 2 °C are shown in Figure 3. The wavelength of absorption maximum λ_{max} is

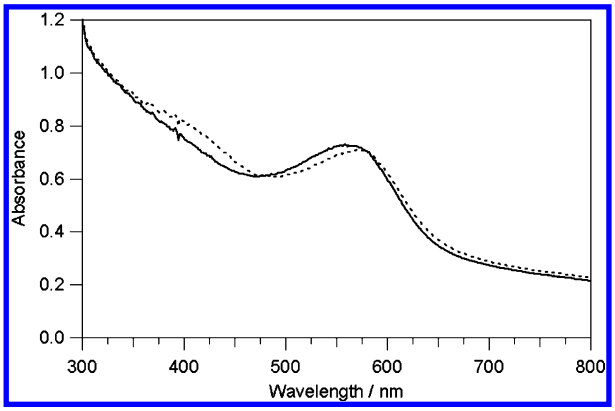

Figure 3. UV−visible spectra of bR reconstituted in diF4H10-PC liposome in the dark (solid line) and under visible light illumination (broken line) at 2 °C.

observed at 570 nm in the dark and shifts to 580 nm with the appearance of another peak at 410 nm upon light illumination. The band at 410 nm is attributable to the M intermediate, as it disappeared with the concomitant increase in absorbance at 570 nm after stopping illumination. The large redshift of λ_{max} by irradiation of visible light is an indication that bR molecules in diF4H10-PC liposome retain the photoconversion ability from the dark-adapted to the light-adapted states as in native PM. It is to be noted that in the both dark-adapted and the light-adapted states, λ_{max} of bR in diF4H10-PC showed the redshift of ∼8 nm against bR in native PM, whereas the blueshift of ∼3 nm was observed for bR in DMPC liposome (Supporting Information Figure S1). The difference in λ_{max} between bR molecules in diF4H10-PC and in DMPC liposomes is as much as ∼11 nm. As many experimental and theoretical works on spectral tuning in visible absorption of bR demonstrated that λ_{max} of bR is strongly dependent on structure around retinal pocket,[42] the remarkable peak shift in bR/diF4H10-PC proteoliposome is attributable to some structural differences near retinal pocket. Because the value of λ_{max} reflects electrostatic environments around retinal pocket, a most plausible structural properties responsible for the difference in λ_{max} is local structural arrangements of the chromophore and some amino acid residues in the vicinity, for example, relative distances between retinal and amino acid residues. The perturbation to the local structural arrangement around retinal pocket by reconstituting into artificial lipid membrane would be different between bR/diF4H10-PC and bR/DMPC, although details are not clear at present.

Visible CD spectra of bR/diF4H10-PC proteoliposome were measured to examine quaternary structure of bR molecules in diF4H10-PC liposome. The representative spectra at 2 °C (the gel phase) and 30 °C (the liquid crystalline phase) are shown in Figure 4. The CD spectrum at 2 °C shows a characteristic exciton features with a positive peak and a negative peak that is very similar to that of native PM, indicating that in the gel state, bR molecules in diF4H10-PC liposome adopt trimeric structure as PM[43,44] and bR/DMPC in the gel state.[31,32,45,46] Even on temperature elevation to 30 °C, no significant spectral changes in the visible region were observed, as shown in Figure 4. Because the wide-angle X-ray diffraction (WAXD) profiles of

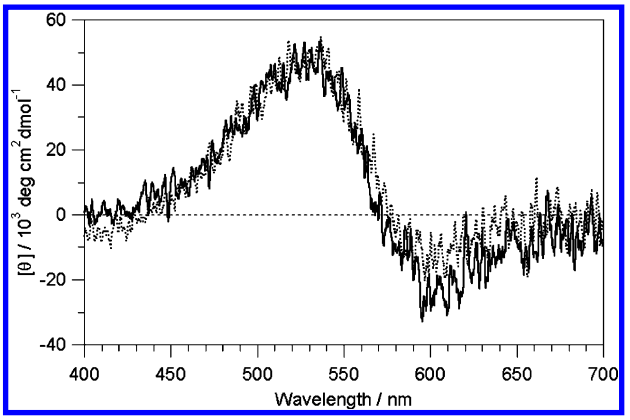

Figure 4. Visible CD spectra of bR reconstituted in diF4H10-PC liposome at 2 °C (solid line) and 30 °C (broken line).

bR/diF4H10-PC proteoliposome at 10 °C clearly showed the chain melting of diF4H10-PC, these CD results demonstrates that trimeric structure of bR molecules is retained in diF4H10-PC liposome even in the liquid crystalline phase. This strikingly contrasts with disassembly of bR trimres into protomers through the gel-to-liquid crystalline phase transition of PC lipid bilayer when reconstituted into liposome of three kinds of ordinary saturated PCs with different acyl length including DMPC.[31,32,45,46]

For further analysis on higher-order structure of bR/diF4H10-PC proteoliposome, X-ray diffraction measurements were performed. As shown in the diffraction pattern at 2 °C (Figure 5A), strong peaks are observed at 0.321 and 0.372

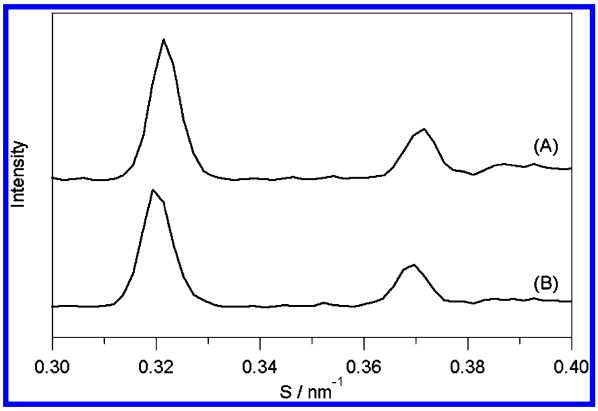

Figure 5. WAXD patterns of bR reconstituted in diF4H10-PC liposome at (A) 2 and (B) 30 °C.

nm^{-1}. The positions of the two diffraction peaks are very close to the ones at 0.319 and 0.370 nm^{-1} for native purple membrane, which are attributable to (1,1) and (2,0) reflections, respectively,[47,48] indicating that in the gel phase, bR/diF4H10-PC proteoliposome adopts the nativelike two-dimensional crystalline structure. The lattice constant of 6.21 nm obtained for bR/diF4H10-PC proteoliposome was very similar to the value of 6.27 nm for native PM.[47] The formation of nativelike lattice structure was also reported for bR/DMPC proteoliposome in the gel state, although the nativelike higher-order structure is lost in the liquid crystalline phase based on the XRD results.[45] The diffraction pattern shows no significant changes even upon heating bR/diF4H10-PC proteoliposome to 30 °C in the liquid crystalline phase (Figure 5B), demonstrating that even after the thermotropic lipid phase transition to the

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liquid crystalline phase, bR/diF4H10-PC complex retains two-dimensional crystalline structure. This is very consistent with the visible CD results showing no significant changes of trimeric structure above the phase transition temperature.

Laser flash photolysis experiments were carried out for examining structural properties of some photointermediates of bR in diF4H10-PC liposome. Representative profiles at 2 °C for the transient absorption changes at 410, 640, and 560 nm, which are assigned to M, O, and ground states, respectively, are shown in Figure 6. These profiles are very close to those for

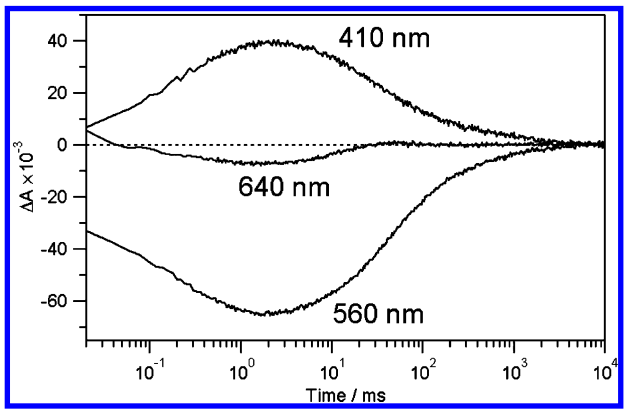

Figure 6. Flash-induced absorbance changes at 410, 560, and 640 nm for bR reconstituted in diF4H10-PC liposome at 2 °C.

native PM, although the photocycle is elongated several fold, showing that bR/diF4H10-PC proteoliposome has proton pumping activity like native PM. It is likely that due to weaker protein−protein interaction in the artificial membrane, magnitude and rate of structural changes in reconstituted bR are different from those of native bR, which would result in elongated photocycle for bR/diF4H10-PC. Even at temperatures above the gel-to-liquid crystalline phase transition, similar flash-induced absorbance changes at 410, 560, and 640 nm were observed. These results represent that both in the gel and the liquid crystalline phase, bR molecules reconstituted in diF4H10-PC liposome have nativelike photocycle, which are consistent with the results of UV−vis, visible CD, and X-ray diffraction measurements. Furthermore, even after the lipid phase transition, λ_{max} of the fast and the late M photointermediate was almost constant at 410 nm like native PM. This is in a stark contrast with the phase transition-induced significant blue shift of the late M photointermediate of bR/DMPC proteoliposome.[31] It should be stressed that substitution of nine hydrogen atoms to fluorine atoms in the terminal butyl group in the hydrophobic chains of DMPC dramatically changed undesirable nonnative structure of bR[31,32] to nativelike active one in the fluid proteoliposome.

As mentioned above, Yokoyama et al. reported[32] that in the liquid crystalline phase, bR molecules reconstituted into DMPC disassemble to protomers and undergo irreversible denaturation induced by continuous irradiation of visible light that originally trigger the photocycle of bR. To compare structural stability of reconstituted bR in the functional state between diF4H10-PC and DMPC, denaturation experiments under light illumination were performed at 30 °C. Irreversible denatured component was estimated from absorbance changes at 560 nm before and after light irradiation for 1−16 h. As shown in Figure 7, the irreversible denatured component for bR/DMPC gradually increases with illumination time and reached to

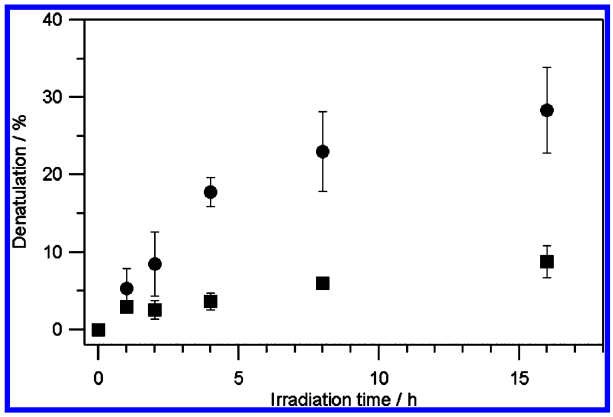

Figure 7. Light-induced irreversible denaturation component of bR/diF4H10-PC (squares) and bR/DMPC (circles) as function of light irradiation time.

∼30% at 16 h, while that for bR/diF4H10-PC remarkably reduces to ∼8% even after 16 h light illumination. These denaturation experiments indicate that bR molecules in diF4H10-PC liposome are much more stable those in DMPC liposome. It is plausible that the higher stability of bR/diF4H10-PC in the liquid crystalline phase is due to nativelike higher-order structure of bR molecules, that is, the two-dimensional crystal structure composed of trimers of the molecule and lipids as the structural unit, although the higher stability of bR/diF4H10-PC does not surpass native PM that is extremely stable up to ∼60 °C under light irradiation.[49−52]

4. DISCUSSION

We have succeeded in functional reconstitution of a membrane protein bR into liposome of the novel partially fluorinated PC, diF4H10-PC. By comparison of the present results with the previous works on reconstitution of bR molecules into artificial lipid membrane, it is obvious that the most significant feature for the obtained bR/diF4H10-PC proteoliposome is the preservation of bR molecular assembly even after the lipid phase transition. Some groups reported that bR molecules in the PM undergo irreversible light-induced denaturation by the loss of the two-dimensional crystalline structure by heating, alkalization or solubilization.[35,49,50,53−57] The structural order of bR molecular assembly is crucial for light-driven proton pumping activity. Therefore, it should be stressed that substitution of nine hydrogen atoms with fluorine atoms in the hydrophobic chains of DMPC leads to formation of the two-dimensional lattice of bR trimers in the liquid crystalline phase without irreversible light-induced denaturation.

What is responsible for retaining the two-dimensional lattice structure of bR trimers in diF4H10-PC liposome in the liquid crystalline phase? The possible candidates for the driving force are membrane properties induced by fluorocarbon segments in the core region or segregation of protein molecules from lipid molecules because of the low affinity between bR and diF4H10-PC. Our recent surface pressure-molecular area (Π-A) isotherm curve measurements in the liquid crystalline phase indicated larger values of the collapse pressure Π_c for the diF4H10-PC monolayer at the air/water interface than DMPC,[28] suggesting greater rigidity of diF4H10-PC bilayer. Santaella and Vierling reported a similar rigidifying effect for an acyl chain ended by a fluorinated tail in the fluorinated PC membrane in the fluid phase.[18] They found that membrane fluidity was almost unaffected by the fluorocarbon or hydrocarbon nature of the

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bilayer in the gel phase, while fluidity decreased substantially in the liquid crystalline phase when fluorinated chains are present, and the order in the fluorinated PC membrane is higher than in fluid conventional PC membranes. Molecular dynamics simulation studies by Shinoda et al. also demonstrated largely restricted wobbling and lateral diffusional motions in fluorinated PC bilayer.[58] These results suggest that even after the main transition from the gel to the fluid phase, fluorinated lipid bilayer rather retain physical properties characteristic of the gel phase. This picture would be essentially consistent with membrane structural properties of a DPPC analog (F6-DPPC) with 6 terminal perfluorinated methylene units reported by Schuy et al.[23] Their FT-IR spectroscopy, ellipsometry, and AFM study showed that during the gel-to-liquid crystalline phase transition, both the relative bilayer thickness and the relative area changes are substantially smaller for F6-DPPC than for DPPC. It is plausible that diF4H10-PC bilayer studied in the present study has also similar physical and structural properties characteristic of the fluorinated PCs reported in the previous works. Then, bR molecules reconstituted in diF4H10-PC liposome are thought to be in lipid bilayer where fluidic motions are largely restricted even in the liquid crystalline phase, which would resulted in the preservation of the two-dimensional crystalline structure like in the gel phase.

Another candidate, that is, segregation of bR molecules from diF4H10-PC lipid molecules, is related to the mixing or demixing behavior of protein and lipid molecules. It is well-known that amino acid residues in the transmembrane region have a side-chain of hydrocarbon segment with hydrophobic nature. Because of this nature, membrane proteins can be embedded into lipid bilayer in biomembrane. In other words, the hydrophobic nature greatly contributes to structure formation and functional expression of membrane proteins. Then, what would happen to bR in PC liposome, when a fluorocarbon segment is introduced into the terminal region of hydrocarbon tails of lipid molecules like diF4H10-PC? The tendency that fluorocarbons do not easily mix with hydrocarbons[59,60] is generally thought to be also applicable for assembly of amphiphilic molecules with fluorocarbon moiety. Actually, fluorinated surfactants often tend to be segregated from nonfluorinated ones in micelles.[61,62] Furthermore, previous works on binary bilayer of fluorinated and nonfluorinated phospholipid mixtures have qualitatively shown the tendency of demixing between the two kinds of phospholipids.[22,63−66] Our recent calorimetric analysis of immiscible binary PC bilayer composed of diF4H10-PC and DMPC has strongly suggested that the mutual phobic interaction between hydrocarbon and fluorocarbon segments is one of the dominant factors of the highly immiscible diF4H10-PC/DMPC mixture.[29] Taking into account the tendency of demixing of fluorocarbon with hydrocarbon moieties in molecular assemblies of ammphiphile, it is highly probable that bR molecules with many hydrocarbon side-chains in the transmembrane region are highly immiscible with diF4H10-PC with perfluorocarbon chains, which would result in bR molecular assembly of the two-dimensional crystalline structure. This idea may be supported by our preliminary work on reconstitution of bR molecules into immiscible binary liposome of diF4H10-PC and DMPC. When bR molecules are reconstituted in the binary liposome, they are not equally distributed into the diF4H10-PC-rich and the DMPC-rich domains, but selectively into the latter, indicating that bR molecules show much higher affinity with nonfluorinated lipid DMPC than partially fluorinated lipid diF4H10-PC. Therefore, the proposed idea of promoting molecular assembly of bR by segregation from diF4H10-PC molecules because of low affinity between the protein and fluorinated lipid is a likely scenario.

5. SUMMARY

A novel partially fluorinated analog of a common phosphatidylcholine DMPC, diF4H10-PC, that shows very unique membrane properties, for example, greater rigidity and lower phase-transition temperature, compared to nonfluorinated DMPC, was used as a material for incorporating a photoreceptor membrane protein bR. The reconstituted bR molecules are arranged in a two-dimensional hexagonal lattice of protein trimers in the diF4H10-PC lipid bilayer and have cyclic photoreaction like native proteins in the purple membrane. The noteworthy properties of the proteoliposome of bR/diF4H10-PC are that even in the liquid crystalline phase, it retains the nativelike structure, function and higher stability against visible light, which are in stark contrast with reconstituted bR molecules in DMPC liposome showing that phase transition-induced disassembly of protein molecules and remarkable irreversible denaturation by visible light irradiation. The structurally and functionally nativelike and highly stable reconstitution of the membrane proteins into the novel partially fluorinated phosphatidylcholine liposome not only in the gel phase but also in the liquid crystalline phase strongly suggest that it is very powerful for studying membrane proteins.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1 showing UV−visible spectra of bR reconstituted in DMPC liposome in the dark and under visible light illumination at 2 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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