

Synthesis and Properties of Alkyl Phosphorylcholine Amphiphiles with a Linear and an Asymmetrically Branched Alkyl Chain

Eui-Chul Kang,* Shingo Kataoka, and Kenji Kato

Tsukuba Research Laboratory, NOF Corporation, 5-10 Tokodai, Tsukuba, Ibaraki 300-2635

Received July 13, 2004; E-mail: euichul_kang@nof.co.jp

Alkyl phosphorylcholine amphiphiles bearing one linear chain and one asymmetrically branched alkyl chain were successfully synthesized using 2-chloro-2-oxo-1,3,2-dioxaphospholane in tetrahydrofuran or ethyl acetate. ^{1}H and ^{31}P NMR studies revealed that the linear alkyl phosphorylcholines (C_n-PC) provide aqueous micelles in D_2O and reverse micelles in $CDCl_3$, while the branched alkyl phosphorylcholines (ISOFOL$_n$-PC) give vesicles in D_2O. The critical micelle concentrations (CMCs) of C_n-PC were measured by fluorescence dye solubilization methods: the CMCs of C_{12}-PC, C_{14}-PC, C_{16}-PC, and C_{18}-PC were 1.6, 0.38, 0.16, and 0.11 mM, respectively, in water at 25 °C. The critical association concentrations (CACs) of ISOFOL$_{16}$-PC, ISOFOL$_{20}$-PC, and ISOFOL$_{24}$-PC were 0.068, 0.005, and 0.077 mM, respectively, in water at 25 °C. The vesicle size of ISOFOL$_n$-PC in aqueous solution was measured by the dynamic light scattering method. The mean diameter of ISOFOL$_n$-PC vesicles was approximately 30 nm and the size distribution was relatively monodisperse. The ISOFOL$_n$-PC vesicles formed were colloidally stable in water over the period of several weeks.

Low molecular weight amphiphiles such as surfactants and phospholipids have been widely used in many fields, such as cosmetics, pharmaceuticals, and paints. There are classified as anionic, cationic, nonionic, or zwitterionic surfactants according to their polar hydrophilic head groups. During the past four decades, many research groups have extensively studied the fundamental and physicochemical properties of these amphiphiles with regard to the roles of molecular structure, polar head groups and counter ions in aqueous solutions.[1–3] Of those, however, the studies of zwitterionic surfactants were relatively fewer than those of other anionic, cationic, and nonionic surfactants. The phosphatidylcholine is one of the representative zwitterionic amphiphiles, which most widely exist on cell membranes.

Various polymers having a phosphorylcholine polar group were synthesized and studied.[4–9] Several phosphorylcholine polymers, 2-(methacryloyloxy)ethyl phosphorylcholines (MPCs), have shown the enhanced biocompatibility of the substrate surfaces which are coated by them.[6–9] Several phosphorylcholine amphiphiles with a linear alkyl chain were also synthesized,[10–12] and their physicochemical and biological properties were investigated in regard to the critical micelle concentration (cmc),[13,14] the solubility in aqueous and organic media,[12,15] the interaction of phospholipase A_2,[16] and antimicrobial properties.[14] The phosphorylcholine amphiphiles bearing a single symmetrically branched alkyl chain were also studied by Overmars and his co-workers.[17] These symmetrically branched alkyl phosphorylcholine amphiphiles exhibited the formation of stable vesicles with diameters of 30–100 nm, as was confirmed by electron microscopy, fluorescence depolarization measurements, and differential scanning calorimetry (DSC). These symmetrically branched alkyl phosphorylcholines were synthesized by reaction of 2-chloro-1,3,2-dioxaphospholane and the secondary alcohol of a symmetrically branched alkyl chain.[17] However, the asymmetrically branched alkyl phosphorylcholines used in this study were synthesized by the reaction of 2-chloro-2-oxo-1,3,2-dioxaphospholane and a primary alcohol with an asymmetrically branched alkyl chain. In this paper, the new synthesis and properties of alkyl phosphorylcholines with one linear and one asymmetrically branched alkyl chain are described in detail using NMR, DSC, dynamic light scattering, fluorescence spectroscopy, and static surface tension measurements.

Experimental

Materials. 1-Dodecanol (C_{12}-OH), 1-tetradecanol (C_{14}-OH), 1-hexadecanol (C_{16}-OH), and 1-octadecanol (C_{18}-OH) were purchased from Tokyo Kasei Co., Tokyo, Japan. 2-Hexyl-1-decanol (ISOFOL$_{16}$), 2-octyl-1-dodecanol (ISOFOL$_{20}$), and 2-decyl-1-tetradecanol (ISOFOL$_{24}$) were purchased from Sasol Germany GmbH, Brunsbuettel, Germany. 2-Chloro-2-oxo-1,3,2-dioxaphospholane and L-α-dipalmitoyl phosphatidylcholine (DPPC) are our products (Nippon Oil & Fats Co., Ltd., Tokyo, Japan). Tetrahydrofuran, ethyl acetate, and acetonitrile were purchased from Kanto Chemicals Co., Tokyo, Japan, as dehydrated grade solvents. Triethylamine and diisopropylamine were purchased from Wako Pure Chemicals Co., Tokyo, Japan. Trimethylamine was purchased from Tokyo Teisan Co., Ltd., Tokyo, Japan. All other solvents and chemicals were used without further purification.

Synthesis of Linear Alkyl Phosphorylcholines (C_n-PCs). The synthetic procedure of linear alkyl phosphorylcholine was as follows, in general. A solution of 10.0 g (70.0 mmol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane in 20 mL of dry ethyl acetate was added dropwise to a mixture of 13.04 g (70.0 mmol) of 1-dodecanol and 7.08 g (70.0 mmol) of diisopropylamine in 100 mL of dry ethyl acetate at 0 °C under vigorous stirring. After the addition was completed, stirring was continued at 0 °C for 1 h and at room temperature for another 1 h under nitrogen atmosphere. The diisopropylamine hydrochloride that precipitated

Published on the web August 4, 2005; DOI 10.1246/bcsj.78.1558

was filtered off. The filtrate was evaporated under reduced pressure up to half of the content. Each concentrated solution of 1-(2-oxo-1,3,2-dioxaphospholan-2-yloxy) dodecane and 150 mL of dry acetonitrile was placed into a glass pressure bottle. After the mixture was cooled down to −20 °C, 8.3 g (0.14 mol) of anhydrous trimethylamine was added, and the reaction was carried out at 70 °C for 12 h. After 12 h, the reaction mixture was again cooled down to −20 °C to precipitate dodecyl phosphorylcholine (C_{12}-PC). The precipitates were separated by filtration, and dissolved in a small amount of ethanol. C_{12}-PC was reprecipitated by pouring the C_{12}-PC/ethanol solution into an excess amount of ethyl acetate. Subsequent drying in vacuum for 24 h at 50 °C yielded 9.1 g (37%) of a hygroscopic white solid.

^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, CH_3), 1.25 (m, 18H, $(CH_2)_9$), 1.57 (m, 2H, OCH_2CH_2), 3.41 (s, 9H, $^+N(CH_3)_3$), 3.77–3.84 (m, 4H, CH_2N^+, CH_2OP), 4.29 (br, 2H, $POCH_2$). ^{31}P NMR δ 0.61. MS (FAB) m/z: 352 (MH^+). Anal. Calcd for $C_{17}H_{39}NPO_4$.

For the synthesis and purification of tetradecyl phosphorylcholine (C_{14}-PC), hexadecyl phosphorylcholine (C_{16}-PC), and octadecyl phosphorylcholine (C_{18}-PC), tetrahydrofuran was used for ethyl acetate as the reaction solvent. C_{14}-PC was purified by ethanol/ethyl acetate. C_{16}-PC was purified by ethanol/diethyl ether. For the purification of C_{18}-PC, tetrahydrofuran was used.

^{1}H NMR ($CDCl_3$) δ for C_{14}-PC: 0.88 (t, 3H, CH_3), 1.25 (m, 22H, $(CH_2)_{11}$), 1.58 (m, 2H, OCH_2CH_2), 3.41 (s, 9H, $^+N(CH_3)_3$), 3.76–3.84 (m, 4H, CH_2N^+, CH_2OP), 4.27 (br, 2H, $POCH_2$); for C_{16}-PC: 0.88 (t, 3H, CH_3), 1.25 (m, 26H, $(CH_2)_{13}$), 1.57 (m, 2H, OCH_2CH_2), 3.40 (s, 9H, $^+N(CH_3)_3$), 3.76–3.83 (m, 4H, CH_2N^+, CH_2OP), 4.27 (br, 2H, $POCH_2$); and for C_{18}-PC: 0.88 (t, 3H, CH_3), 1.25 (m, 30H, $(CH_2)_{15}$), 1.57 (m, 2H, OCH_2CH_2), 3.40 (s, 9H, $^+N(CH_3)_3$), 3.76–3.83 (m, 4H, CH_2N^+, CH_2OP), 4.27 (br, 2H, $POCH_2$). ^{31}P NMR δ for C_{14}-PC: 0.58; for C_{16}-PC: 0.54; and for C_{18}-PC: 0.54. MS (FAB) m/z: 380 (MH^+): Anal. Calcd for $C_{19}H_{43}NPO_4$, 408 (MH^+): Anal. Calcd for $C_{21}H_{47}NPO_4$, and 436 (MH^+): Anal. Calcd for $C_{23}H_{51}NPO_4$.

Synthesis of Branched Alkyl Phosphorylcholines (ISOFOL$_n$-PCs). The synthetic procedure of branched alkyl phosphorylcholine was as follows, in general. A solution of 10.0 g (70.0 mmol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane in 30 mL of dry tetrahydrofuran was added dropwise to a mixture of 24.78 g (70.0 mmol) of 2-decyltetradecanol and 7.1 g (70.0 mmol) of triethylamine in 140 mL of dry tetrahydrofuran at 0 °C under vigorous stirring. After the addition was completed, stirring was continued at 0 °C for 1 h and at room temperature for another 1 h under nitrogen atmosphere. The triethylamine hydrochloride that precipitated was filtered off. The filtrate was evaporated under reduced pressure up to half of the content. Each concentrated solution of 1-(2-oxo-1,3,2-dioxaphospholan-2-yloxy) 2-decyltetradecane and 150 mL of dry acetonitrile was placed into a glass pressure bottle. After the mixture was cooled down to −20 °C, 8.3 g (0.14 mol) of anhydrous trimethylamine was added, and the reaction was carried out at 70 °C for 12 h. After 12 h, the reaction mixture was again cooled down to −20 °C to precipitate 2-decyltetradecyl phosphorylcholine (ISOFOL$_{24}$-PC). The precipitates were separated by filtration. ISOFOL$_{24}$-PC was purified by tetrahydrofuran and acetone. Subsequent drying in vacuum for 24 h at 50 °C yielded 15.2 g (41.8%) of a hygroscopic white solid.

^{1}H NMR (CD_3OD) δ 0.89 (t, 6H, CH_3), 1.29 (m, 40H, $(CH_2)_9$, $(CH_2)_{11}$), 1.58 (m, 1H, OCH_2CH), 3.22 (s, 9H, $^+N(CH_3)_3$), 3.62 (m, 2H, CH_2N^+), 3.77 (t, 2H, CH_2OP), 4.23 (br, 2H, $POCH_2$). ^{31}P NMR δ 1.25. MS (FAB) m/z: 520 (MH^+). Anal. Calcd for $C_{29}H_{63}NPO_4$.

For the purification of 2-hexyldecyl phosphorylcholine (ISOFOL$_{16}$-PC), diethyl ether was used, and for that of 2-octyldodecyl phosphorylcholine (ISOFOL$_{20}$-PC), tetrahydrofuran/acetonitrile was employed.

^{1}H NMR (CD_3OD) δ for ISOFOL$_{16}$-PC: 0.89 (t, 6H, CH_3), 1.37 (m, 24H, $(CH_2)_5$, $(CH_2)_7$), 1.58 (m, 1H, OCH_2CH), 3.23 (s, 9H, $^+N(CH_3)_3$), 3.62 (m, 2H, CH_2N^+), 3.77 (t, 2H, CH_2OP), 4.24 (br, 2H, $POCH_2$) and for ISOFOL$_{20}$-PC: 0.89 (t, 6 H, CH_3) 1.31 (m, 32 H, $(CH_2)_7$, $(CH_2)_9$) 1.58 (m, 1 H, OCH_2CH), 3.22 (s, 9H, $^+N(CH_3)_3$), 3.62 (m, 2H, CH_2N^+), 3.77 (t, 2H, CH_2OP), 4.24 (br, 2H, $POCH_2$). ^{31}P NMR δ for ISOFOL$_{16}$-PC: 1.24; and for ISOFOL$_{20}$-PC: 1.24. MS (FAB) m/z: 408 (MH^+): Anal. Calcd for $C_{21}H_{47}NPO_4$ (MH^+); and 464 (MH^+): Anal. Calcd for $C_{25}H_{55}NPO_4$.

IR and NMR Measurements. IR spectra with KBr were recorded with a Fourier transform infrared spectrometer (FTIR-7300, Jasco Co., Tokyo, Japan). ^{1}H NMR (270 MHz) studies were carried out in D_2O (deuterated water), CD_3OD (deuterated methanol), and $CDCl_3$ (deuterated chloroform) by using a JEOL JNM-EX270 spectrometer. The chemical shifts were recorded as parts per million (ppm) with a reference to residual solvent resonance. ^{31}P NMR (109 MHz) studies were made with the same spectrometer using phosphoric acid as the external standard. Line widths were measured as the full width at half-height maximum intensity. The concentration of NMR samples was approximately 2.0 wt % in all experiments. The ISOFOL$_n$-PC samples were prepared in deuterated water by sonication.

Vesicle Size Measurements. The ISOFOL$_n$-PC samples (20.0 mg) were suspended in water (10.0 mL) under stirring for 30 min at 50 °C. The resulting suspension was sonicated using a probe-type sonifier (Branson Sonifier 250) for 10 min at 40 W. The size of ISOFOL$_n$-PC vesicles was measured by NICOMP 380ZLS Particle Sizer with DPSS laser (wavelength, 532 nm).

Static Surface Tension Measurements. Aqueous ISOFOL$_n$-PC solutions were prepared by dissolving the ISOFOL$_n$-PC in Milli-Q water to the desired concentration. The static surface tension of aqueous ISOFOL$_n$-PC was measured with a Surface Tensiometer CBVP-A3 by the Wilhelmy plate technique (Kyowa Interface Sci. Co., Tokyo, Japan).

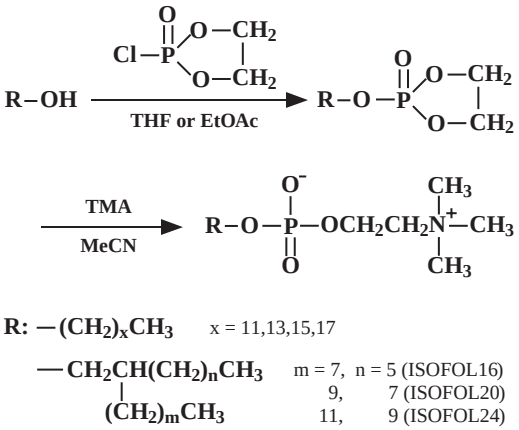
Fluorescence Measurements. Aqueous C_n-PC solutions were prepared by dissolving the C_n-PC in Milli-Q water to the desired concentration. The fluorescence dye solubilization method was employed to determine the onset of surfactant micellization (CMC). A stock solution of 1.0 mM pyrene in THF was prepared. A 10 μL aliquot of the pyrene/THF stock solution was added to 2.0 mL of C_n-PC solution, so that the final C_n-PC solution contained 0.5% v/v THF and 0.005 mM pyrene. The solution was left in the dark to equilibrate for 30 min before the fluorescence measurement. Fluorescence spectra were recorded on a Hitachi F-3010 fluorescence spectrometer. The excitation wavelength was 330 nm, and the slit widths were set at 5.0 nm (excitation) and 3.0 (emission). The I_1/I_3 ratio of the pyrene emission was taken as the ratio of the emission intensity at 373 nm to that at 384 nm. The temperature of the water-jacketed cell holder was controlled with an Omron circulating controller.

Results and Discussion

Syntheses of several alkyl phosphorylcholine amphiphiles with linear and branched alkyl chains succeeded: C_{12}-OH, C_{14}-OH, C_{16}-OH, C_{18}-OH, ISOFOL$_{16}$, ISOFOL$_{20}$, and ISOFOL$_{24}$ with 2-chloro-2-oxo-1,3,2-dioxaphospholane in tet-

rahydrofuran or ethyl acetate (Scheme 1).

The IR spectra of alkyl phosphorylcholines are shown in Fig. 1. The absorption peaks characteristic of the alkyl chains appeared at 2924 cm^{-1}, 2854 cm^{-1} (C–H stretching), and 1468 cm^{-1} (C–H bending). The absorption bands at 1247 cm^{-1} (P=O stretching), 1089 cm^{-1} (C–O–P stretching), and 970 cm^{-1} ($N^+(CH_3)_3$ stretching) are attributed to the existence of phosphorylcholine groups. The alkyl phosphorylcholines did not exhibit an absorption band at 1738 cm^{-1} (C=O stretching) in comparison with those of DPPC. All other absorptions coincided with the structure of DPPC.



Scheme 1. Synthetic route of alkyl phosphorylcholine amphiphiles.

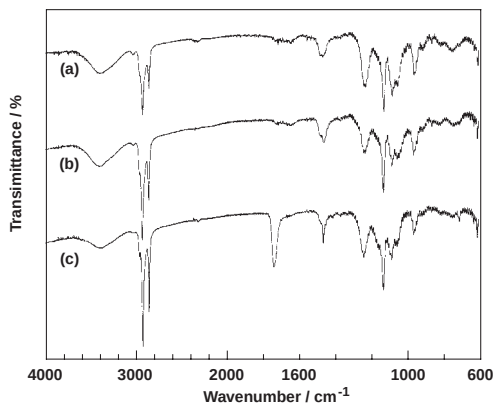


Fig. 1. IR spectra of (a) dodecyl phosphorylcholine (C_{12}-PC), (b) 2-decyltetradecyl phosphorylcholine ($ISOFOL_{24}$-PC), and (c) L-α-dipalmitoyl phosphatidylcholine (DPPC).

The ^{1}H NMR spectra and line width of the major peaks of C_{12}-PC in various solvents are shown in Fig. 2 and Table 1. In D_2O, the peaks due to the choline methyl group were relatively sharp at 3.14 ppm, whereas the peaks at 0.78 ppm (methyl) and 1.19 ppm (methylene) assigned to the dodecyl group protons were broad. This broadening suggests that the motion of the corresponding protons is restricted, providing evidence for the formation of a sort of aggregate via association of the dodecyl chains. But this aggregation disappears when C_{12}-PC is dissolved in methanol. The spectrum of C_{12}-PC in CD_3OD appeared as sharp peaks for all the proton signals. On the other hand, in $CDCl_3$, the peaks attributed to the dodecyl protons appeared as sharp peaks, while the peaks due to the trimethylammonium protons of choline group were observed as a broad peak with a downfield shift in comparison with the locations in CD_3OD. The peaks attributed to the methylene protons of choline group at 3.57 ppm in D_2O were observed as broad and partially overlapping peaks at 3.81 ppm in $CDCl_3$.

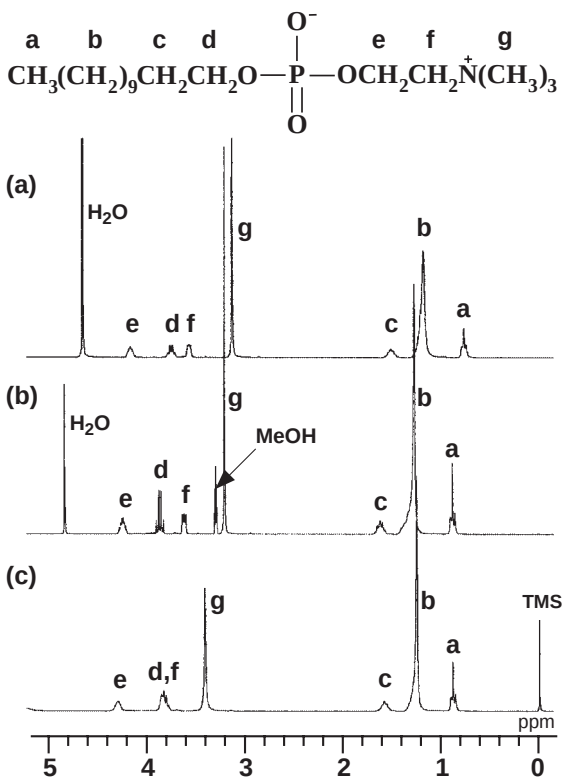


Fig. 2. ^{1}H NMR spectra of dodecyl phosphorylcholine (C_{12}-PC) in (a) D_2O, (b) CD_3OD, and (c) $CDCl_3$ at 25 °C.

Table 1. ^{1}H NMR Chemical Shifts (ppm) and Line Widths[a)] (Hz) of C_{12}-PC (with Reference to Fig. 2)

Solvent	a	b	c	d	e	f	g
D_2O	0.78	1.19 (10.8 Hz)	1.51	3.75	4.16	3.57	3.14 (2.2 Hz)
CD_3OD	0.89	1.28 (4.1 Hz)	1.61	3.86	4.24	3.62	3.21 (1.7 Hz)
$CDCl_3$	0.88	1.25 (4.3 Hz)	1.57	3.81	4.29	3.81	3.41 (4.2 Hz)

a) Line widths were measured as the full width at half-height maximum intensity.

Thus, the motion of the choline group of phosphorylcholine is highly restricted in $CDCl_3$. This indicated that the phosphorylcholine moieties are confined in a restricted environment, while the alkyl chains can rotate freely. The phosphorus peak of phosphorylcholine in C_{12}-PC appeared as a sharp peak at 0.53, 1.09, and 0.61 ppm in D_2O, CD_3OD, and $CDCl_3$, respectively (Fig. 4a). ^1H and ^{31}P NMR of other linear alkyl phosphorylcholines, C_{14}-PC, C_{16}-PC, and C_{18}-PC, also showed spectra similar to those of C_{12}-PC. Results of ^1H and ^{31}P NMR indicate that the linear alkyl phosphorylcholines would form aqueous micelle in D_2O and reverse micelle in $CDCl_3$.

Interestingly, ^1H and ^{31}P NMR spectra of the branched alkyl phosphorylcholines exhibited chemical shifts slightly different from those of the linear alkyl phosphorylcholines. The ^1H NMR spectra and line width of the major peaks of $ISOFOL_{24}$-PC in various solvents are shown in Fig. 3 and Table 2. In CD_3OD, the signals of the choline group and of the 2-decyltetradecyl group were relatively sharp. In $CDCl_3$, the 2-decyltetradecyl signals were also sharp, while the peaks of choline methyl protons were observed as broad at a downfield location. This also indicates that the phosphorylcholine moieties locate in a restricted environment, while the alkyl chains can rotate relatively freely. On the other hand, in D_2O, the trimethylammonium protons of the choline group at 3.15 ppm and the 2-decyltetradecyl protons at 0.83 ppm (methyl) and 1.23 ppm (methylene) all were broad. The peaks at 3.81 ppm assigned to the methylene protons of the 2-decyltetradecyl group in CD_3OD appeared as a broad band and overlapped with peaks observed at 3.58 ppm in D_2O. This result indicates that the phosphorylcholine moieties and the alkyl chain are both in restricted microenvironments.

Figure 4b shows the ^{31}P NMR spectra of $ISOFOL_{24}$-PC in D_2O, CD_3OD, and $CDCl_3$ at 25 °C. The phosphorus peak of the phosphorylcholine of $ISOFOL_{24}$-PC observed at 1.24 ppm in CD_3OD was sharp, similarly to the case of C_{12}-PC. However, in D_2O, the phosphorus peak of $ISOFOL_{24}$-PC appeared as a broad and symmetric peak with a peak width of 38.2 Hz at 0.54 ppm. This also indicates that the molecular motion of the phosphorylcholine moieties of $ISOFOL_{24}$-PC in D_2O is more highly restricted than that of C_{12}-PC. ^1H and

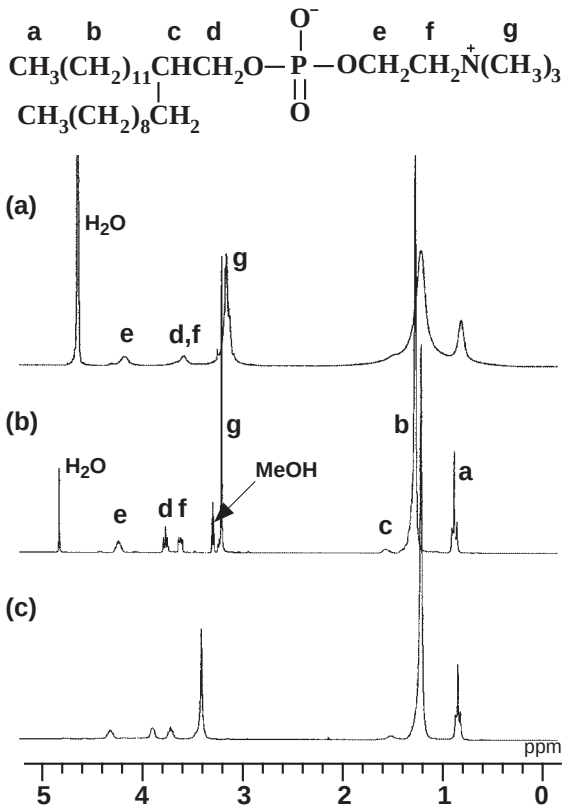


Fig. 3. ^1H NMR spectra of 2-decyltetradecyl phosphorylcholine ($ISOFOL_{24}$-PC) in (a) D_2O, (b) CD_3OD, and (c) $CDCl_3$ at 25 °C.

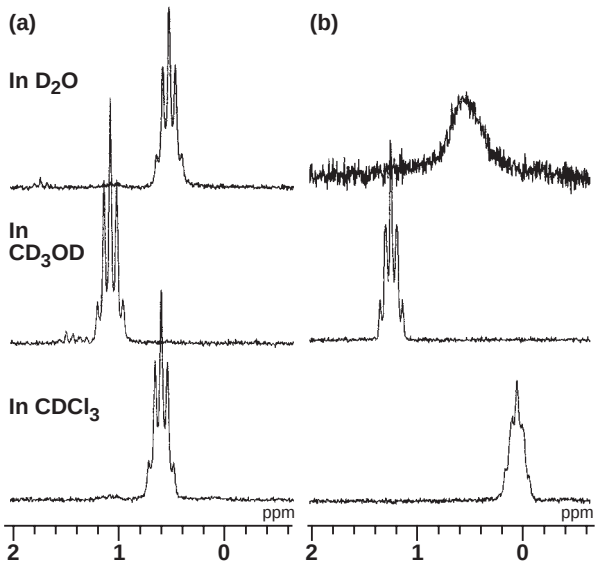


Fig. 4. ^{31}P NMR spectra of (a) dodecyl phosphorylcholine (C_{12}-PC) and (b) 2-decyltetradecyl phosphorylcholine ($ISOFOL_{24}$-PC) in D_2O, CD_3OD, and $CDCl_3$ at 25 °C.

Table 2. ^1H NMR Chemical Shifts (ppm) and Line Widths[a)] (Hz) of $ISOFOL_{24}$-PC (with Reference to Fig. 3)

Solvent	a	b	c	d	e	f	g
D_2O	0.83	1.23 (31.5 Hz)	—	3.58	4.18	3.58	3.15 (9.0 Hz)
CD_3OD	0.90	1.29 (3.9 Hz)	1.60	3.81	4.28	3.65	3.22 (1.7 Hz)
$CDCl_3$	0.85	1.22 (6.4 Hz)	1.52	3.89	4.31	3.71	3.41 (4.4 Hz)

a) Line widths were measured as the full width at half-height maximum intensity.

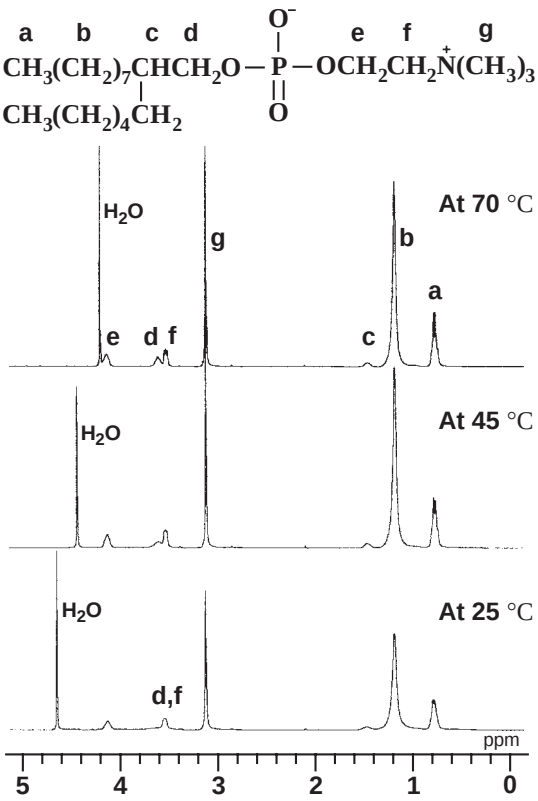


Fig. 5. ^{1}H NMR spectra of 2-hexyldecyl phosphorylcholine (ISOFOL$_{16}$-PC) in D_2O at different temperatures.

^{31}P NMR spectra of other branched alkyl phosphorylcholines, ISOFOL$_{16}$-PC (peak width of phosphorus peak, 17.2 Hz) and ISOFOL$_{20}$-PC (peak width of phosphorus peak, 28.7 Hz), were also similar to those of ISOFOL$_{24}$-PC. Generally, the phosphorus peak of the conventional egg PC liposomes exhibits a broad and asymmetric peak in D_2O.[18,19] The shape of the peak is characteristic of the lamellar structure of lipid bilayers. ^{31}P NMR spectrum of the ISOFOL$_{24}$-PC in this work was also similar to those of the egg PC liposomes.[18,19] These NMR results would suggest the formation of vesicles via self-association of asymmetrically branched alkyl chains in D_2O.

To understand the molecular motion in solution in more detail, NMR studies were carried out at different temperatures over 25–70 °C. Figures 5 and 6 show results of ^{1}H and ^{31}P NMR spectra of ISOFOL$_{16}$-PC measured at different temperatures. With increasing temperature, the methyl protons at 0.79 ppm and the methylene protons at 1.19 ppm of the 2-hexyldecyl group, and the trimethylammonium protons at 3.14 ppm of the choline group became sharper and more intense, indicating an increase in the motion of both the phosphorylcholine moieties and the 2-hexyldecyl chains. We noted a broad peak that would be attributable to the methylene protons (signal, d) of the 2-hexyldecyl and that overlapped with the methylene protons (signal, f) of the choline group at 3.53 ppm. This broad peak at 3.53 ppm at 25 °C separated into two peaks at 3.53 and 3.62 ppm above 45 °C. In ^{31}P NMR spectrum, the phosphorus peak also shifted from 0.63 ppm at 25 °C to 1.23 ppm at 70 °C and slightly sharpened (Fig. 6). These results indicate that the mobilities of the phosphorylcholine moieties and the alkyl chains both significantly increase with increasing temperature.

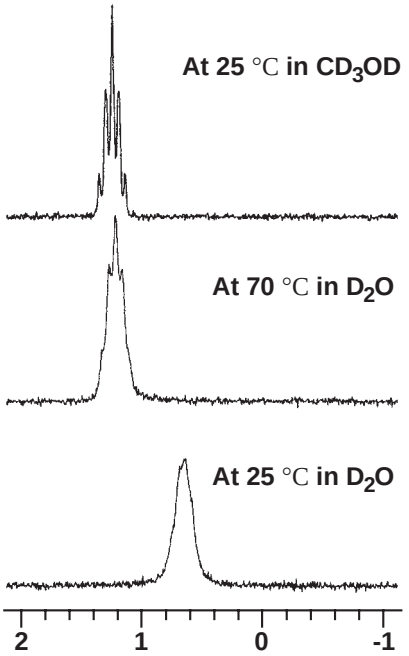


Fig. 6. ^{31}P NMR spectra of 2-hexyldecyl phosphorylcholine (ISOFOL$_{16}$-PC) under different conditions.

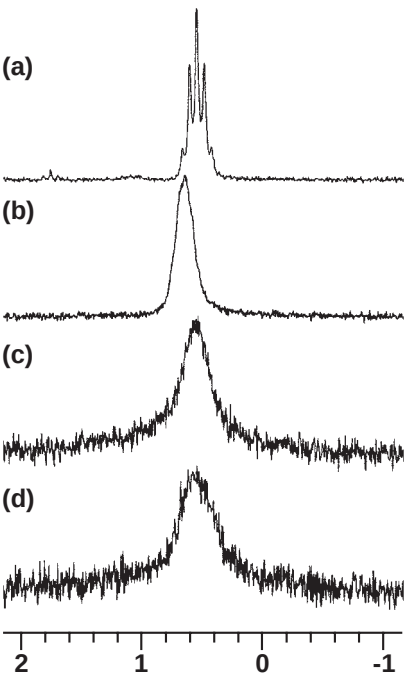


Fig. 7. ^{31}P NMR spectra of (a) C_{12}-PC, (b) ISOFOL$_{16}$-PC, (c) ISOFOL$_{20}$-PC, and (d) ISOFOL$_{24}$-PC in D_2O at 25 °C.

Figure 7 shows the ^{31}P NMR spectra of linear and branched alkyl phosphorylcholines, C_{12}-PC, ISOFOL$_{16}$-PC, ISOFOL$_{20}$-PC, and ISOFOL$_{24}$-PC in D_2O at 25 °C. The phosphorus peak of alkyl phosphorylcholines in D_2O was observed at 0.53–0.63 ppm. Though the phosphorus peaks of linear alkyl phosphorylcholines all appeared relatively sharper and symmetric, those of all branched alkyl phosphorylcholines appeared broader

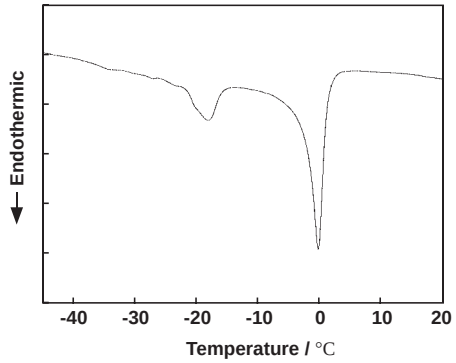


Fig. 8. DSC curve of $ISOFOL_{24}$-PC obtained on heating.

and unsymmetric. With the branched alkyl phosphorylcholines, the phosphorus peak became gradually broader with increasing alkyl chain length. These results indicate that the mobility values of the branched alkyl phosphorylcholines are significantly restricted in water compared to those of unbranched alkyl phosphorylcholines. This difference would be related with the solution structures of different aggregates such as micelle and vesicle.

Thermograms of $ISOFOL_n$-PC were recorded on a differential scanning calorimeter (DSC; Seiko DSC-210) at a heating rate of 5 °C/min (Fig. 8). The phase transition temperature between the gel and the liquid-crystal phase was determined from the peak temperature of the main endothermic transition. The phase transition temperature of $ISOFOL_{16}$-PC was not observed in the temperature range of −70 to 20 °C. The phase transition temperatures of $ISOFOL_{20}$-PC and $ISOFOL_{24}$-PC were −63 and −21 °C, respectively.

The sizes of the branched alkyl phosphorylcholine aggregates in aqueous solution were measured by the dynamic light scattering method. The mean diameters of $ISOFOL_{20}$-PC and $ISOFOL_{24}$-PC were approximately 32.1 ± 4.9 and 29.0 ± 3.8 nm, and were relatively monodisperse. The $ISOFOL_n$-PC aggregates were colloidally stable in water over several weeks. The result also suggests that the branched alkyl phosphorylcholines form aggregates larger in size than those of the unbranched alkyl phosphorylcholines.

The CMC of C_n-PC was studied by the fluorescence dye solubilization method. The fluorescence dye solubilization methods, based upon changes in fluorescence intensity of a dye upon incorporation into micelles, are among the most sensitive and convenient assays for CMC.[20,21] The solvent polarity dependence of the pyrene emission is expressed in terms of the ratio, I_1 (373 nm)/I_3 (384 nm) of the intensities of the (0,0) band (I_1) to that of the (0,2) band (I_3) of the emission. The CMC of surfactants can be obtained from measurements of the changes in I_1/I_3 ratio of the intensity of the pyrene emission as a function of surfactant concentration. The values typically range from about 1.9 in polar solvents to about 0.6 in hydrocarbons.[21,22] Figure 9 shows the changes of the I_1/I_3 ratio of pyrene emission in aqueous solution as a function of log alkyl phosphorylcholine concentration at 25 °C. The I_1/I_3 ratio of pyrene sharply decreased with an increase of alkyl phosphorylcholine concentration, and leveled off (≈1.21). This indicated that the pyrene is solubilized in the hydrophobic interior as the alkyl phosphorylcholine concentrations increase

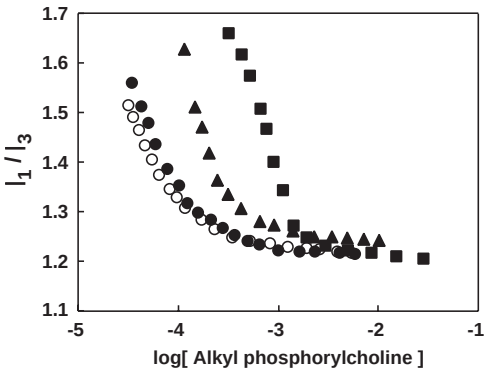


Fig. 9. Changes in I_1/I_3 ratio of pyrene emission in aqueous solution as a function of the logarithm of alkyl phosphorylcholine concentration at 25 °C: C_{12}-PC (■); C_{14}-PC (▲); C_{16}-PC (●); C_{18}-PC (○).

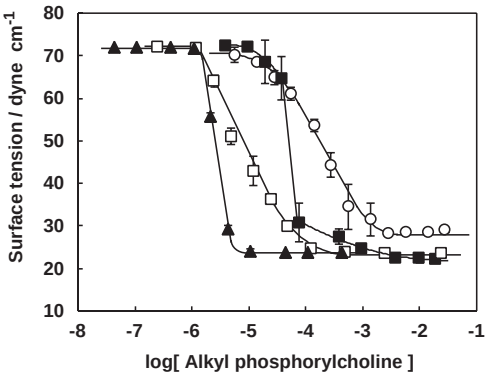


Fig. 10. Static surface tension in aqueous solution as a function of the logarithm of alkyl phosphorylcholine concentration at 25 °C: C_{12}-PC (○); $ISOFOL_{16}$-PC (□); $ISOFOL_{20}$-PC (▲); $ISOFOL_{24}$-PC (■).

above the CMC. The CMC was determined from the inflection of the I_1/I_3 values vs alkyl phosphorylcholine concentration curves. The CMC of C_n-PC decreased with an increase of the hydrophobic alkyl chain length: the CMCs of C_{12}-PC, C_{14}-PC, C_{16}-PC, and C_{18}-PC were 1.6, 0.38, 0.16, and 0.11 mM, respectively, in water at 25 °C. In the CMC of C_{12}-PC and C_{14}-PC, the CMC decreased to approximately a half when one methylene group is added in the hydrophobic alkyl chain. On the other hand, the change of CMC between C_{16}-PC and C_{18}-PC was very small. These results are agreement with the experimental values of ionic and nonionic surfactants.[23]

Figure 10 shows the surface tension curves of alkyl phosphorylcholine in aqueous solution as a function of log alkyl phosphorylcholine concentration at 25 °C. The surface tension decreased with an increase of alkyl phosphorylcholine concentration. Especially, the surface tension of $ISOFOL_{20}$-PC and $ISOFOL_{24}$-PC dramatically decreased with an increase of alkyl phosphorylcholine concentrations. The critical association concentration (CAC) of $ISOFOL_n$-PC was determined from the inflection of the surface tension values vs alkyl phosphorylcholine concentration curves. The CACs of $ISOFOL_{16}$-PC, $ISOFOL_{20}$-PC, and $ISOFOL_{24}$-PC were 0.068, 0.005, and 0.077 mM, respectively, in water at 25 °C. The association

concentrations of the branched alkyl phosphorylcholines were lower than those of the unbranched alkyl phosphorylcholines in aqueous media.

To characterize these aggregates formed in aqueous media as spherical micelle, mesophase, or vesicle, Tanford[24] and Israelachvili[25,26] proposed a concept of packing parameter. The critical packing parameter (CPP) is defined as $v/a_o \cdot l_c$, where v is the hydrocarbon chain volume, a_o is the optimal cross-sectional surface area per head group, and l_c is the critical chain length of the alkyl chains.[24] The amphiphiles form micelles when the CPP-value is below 0.5, while they form vesicles with the CPP-value is between 0.5 and 1.0. We calculated the CPP-value of alkyl phosphorylcholines with the values of 62.0–71.7 Å^2 for hydrated choline head group of phosphatidylcholine vesicle.[25,26] The calculated CPP-values are 0.29–0.34 for the linear alkyl phosphorylcholines and 0.61–0.71 for the branched alkyl phosphorylcholines; such values are consistent with the micelle-forming properties in the linear alkyl phosphorylcholines and the vesicle-forming properties in the branched alkyl phosphorylcholines, respectively. These results are consistent with the present results of NMR, DSC, dynamic light scattering, fluorescence spectroscopy, and static surface tension measurements for the alkyl phosphorylcholine amphiphiles.

References

1 K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants," Academic Press, New York (1963).

2 M. J. Schick, "Nonionic Surfactants: Physical Chemistry," Marcel Dekker, New York (1987).

3 A. K. Chattopadhyay and K. L. Mittal, "Surfactants in Solution," Marcel Dekker, New York (1996).

4 T. Doiuchi, T. Nakaya, and M. Imoto, *Makromol. Chem.*, **175**, 43 (1974).

5 T. Nakaya and Y. J. Li, *Prog. Polym. Sci.*, **24**, 143 (1999).

6 K. Ishihara, R. Aragaki, T. Ueda, A. Watanaba, and N. Nakabayashi, *J. Biomed. Mater. Res.*, **24**, 1069 (1990).

7 K. Ishihara, H. Oshida, Y. Endo, T. Ueda, A. Watanaba, and N. Nakabayashi, *J. Biomed. Mater. Res.*, **26**, 1543 (1992).

8 A. L. Lewis, P. D. Hughes, L. C. Kirkwood, S. W. Leppard, R. P. Redman, L. A. Tolhurst, and P. W. Stratford, *Biomaterials*, **21**, 1847 (2000).

9 A. L. Lewis, Z. L. Cumming, H. H. Goreich, L. C. Kirkwood, L. A. Tolhurst, and P. W. Stratford, *Biomaterials*, **22**, 99 (2001).

10 R. Hirt and R. Berchtold, *Pharm. Acta Helv.*, **33**, 349 (1958).

11 P. Chabrier, N. T. Thuong, D. L. Maitre, and M. Perat, *C. R. Acad. Sci., Ser. C*, **267**, 732 (1968).

12 M. Okazaki and I. Hara, *Yukagaku*, **30**, 553 (1981).

13 M. C. E. van Dam-Mieras, A. J. Slotboom, W. A. Pieterson, and G. H. de Haas, *Biochemistry*, **14**, 5387 (1975).

14 F. Kanetani, K. Negoro, and E. Okada, *Nippon Kagaku Kaishi*, **1984**, 1452.

15 M. Okazaki, I. Hara, Y. K. Tsutsui, and T. Fujiyama, *Bull. Chem. Soc. Jpn.*, **54**, 2399 (1981).

16 K. Teshima, K. Ikeda, and K. Yamaguchi, *J. Biochem.*, **89**, 1163 (1981).

17 F. J. J. Overmars, J. B. F. N. Engberts, and W. D. Weringa, *Recl. Trav. Chim. Pays-Bas*, **113**, 293 (1994).

18 J. Seelig, *Biochim. Biophys. Acta*, **515**, 105 (1978).

19 N. Morone, M. Yamauchi, Y. Okumura, and J. Sunamoto, *J. Bioact. Compat. Polym.*, **16**, 167 (2001).

20 E. D. Vendittis, G. Palumb, G. Parlato, and V. Bocchini, *Anal. Biochem.*, **115**, 278 (1981).

21 K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, **99**, 2039 (1977).

22 H. Ringsdorf, J. Venzmer, and F. W. Winnik, *Macromolecules*, **24**, 1678 (1991).

23 A. Kitahara and K. Aoki, "Introduction to Colloid and Surface Chemistry," 3rd ed, Hirokawa Publishing Co., Tokyo (1987), Chap. 4, p. 75.

24 C. Tanford, *J. Phys. Chem.*, **76**, 3020 (1972).

25 J. N. Israelachvili, D. J. Mitchell, and B. W. Ninham, *J. Chem. Soc., Faraday Trans. 2*, **1976**, 1525.

26 J. N. Israelachvili, S. Marcelja, and R. G. Horn, *Q. Rev. Biophys.*, **13**, 121 (1980).