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Amphiphilic Poly(acrylamide)s Having Saturated and Unsaturated Dialkyl Chains and Phosphatidylcholine Groups in the Side Chains

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ABSTRACT: A novel phosphatidylcholine analogous vinyl monomer 1-(acrylamidomethyl)-2'-(trimethyl ammonio)ethyl phosphate (AMP) was synthesized and characterized. A series of amphiphilic poly(acrylamide)s were obtained from AMP, and some dialkylacrylamide monomers, by radical copolymerization in the presence of α, α' -azobis(isobutyronitrile) (AIBN). The polyelectrolyte behavior of these copolymers was investigated by their viscosity measurements. Moreover, the structures in condensed phases and thermal properties of these copolymers were investigated by X-ray analysis, DSC measurement, and POM observation. In addition, the phase transitions of monolayers obtained from these amphiphilic copolymers were observed by changing the temperature of the subphase, length of the alkyl chains, and observation times (t_{ob}). The LB films were prepared by using the vertical dipping method, and their surface contact angles with pure water were also measured.

Introduction

Phospholipids are basic constituents of biomembranes and are especially abundant in the brain and myelin sheaths of the nerves. All phospholipids are composed of one strongly hydrophobic portion, the fatty acids and the sphingosine chain, and one highly hydrophilic portion, which in many cases is zwitterionic. This accounts for their tendency to form bilayers in aqueous media and for their excellent emulsifying properties. Since energy, material, and information can be exchanged selectively and communicated highly efficiently through the biomembrane, it is highly important to synthesize phospholipid analogues and to study their molecular arrangements and phase behaviors in the solid state.

On the other hand, the Langmuir–Blodgett (LB) technique is an excellent method for preparing an ultrathin film that has controlled thickness and orientation of molecules. There is a growing interest in using preformed polymers for the creation of LB films because they are expected to combine increased thermal and mechanical stability with a low defect density¹ compared with multilayers obtained by polymerization of monomeric amphiphiles after deposition.^{2,3} In favorable cases, the ordered monolayers of amphiphiles at an air–water interface can be transferred onto solid supports to give LB films, and the characterizations of original monolayers strongly affect the regularity, mechanical stability, and other properties of LB multilayers. From these points of view, it seems very interesting to investigate the preparations and properties of monolayers and LB films of polymeric phospholipid analogues and further to fabricate some phospholipid analogous membranes artificially.

Many works on the study of syntheses and properties for polymeric phospholipid analogues have been re-

ported from almost 20 years ago.^{4–12} More recently, a series of amphiphilic phospholipid analogous polymers were produced from some hydrophobic acrylamide monomers having two long alkyl chains¹³ and a hydrophilic vinyl monomer containing a phosphatidylcholine-like moiety by radical copolymerizations. On the studies of monolayers, those polymers were found to display only the solid analogous phases during the condensed processes. To obtain some phospholipid analogous polymers having a better balance of hydrophilicity and hydrophobicity, we now prepare a series of new amphiphilic copolymers from the same hydrophobic acrylamide monomers reported earlier¹³ and a new hydrophilic acrylamide monomer bearing a phosphatidylcholine moiety, which occupies a smaller space but appears to have a stronger hydrophilicity than the hydrophilic monomer mentioned above.¹³ In this paper, we will report the syntheses, structures in condensed states, and thermal properties of these new copolymers; especially, we will investigate the phase transitions of the surface pressure–area isotherms of their monolayers. Furthermore, the preparation of stable LB multilayers for these new polymers will be also described below.

Experimental Section

Characterization. Proton (¹H, 400 MHz) and carbon (¹³C, 100.40 MHz) NMR spectra were recorded at 25 °C on a JEOL α -400 from CDCl₃ and CD₃OD solutions. Proton chemical shifts, reported in parts per million, were referenced to tetramethylsilane directly as an internal standard. Multiplicities of resonance peaks are indicated as singlet s and multiplet m. Infrared (IR) spectra (KBr disks) were obtained at room temperature by using a JASCO A-202 spectrometer and reported in the range of 4000–400 cm⁻¹. In the IR data presentation, m, s, and vs indicate the extent of absorption as medium, strong, and very strong. The solution viscosity was measured by using a Ubbelohde dilution capillary viscometer placed in a temperature-controlled water bath at 25 ± 0.1 °C. The melting point measurements and polarizing observations were carried out by using a micro melting point apparatus (Yanaco MP-J3). For an X-ray diffraction measurement, the specimen was completely sealed with mica in the sample

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holder. The specimen was stable during X-ray diffraction measurement, as judged from the reproducibility of the diffraction pattern. The X-ray diagram was photographed with nickel-filtered Cu K α radiation (37.5 kV, 20 mA), using a flat-plate camera of 45.4 mm passage at room temperature. Thermal analysis was performed by means of differential scanning calorimetry (DSC) on a Rigaku Thermoflex apparatus. The sample quantity was 10 mg with a 5 °C/min rate of heating from 0 to 250 °C. Polarizing optical microscopy (POM) of a thin sample, which was sandwiched between two glass slides (spacing: 12 μ m), was performed using a Nikon microscope. π -A isotherms and LB films were measured and prepared by a film-balance measuring apparatus (Lauda). Contact angles between LB multilayers and distilled water were measured by using a contact-angle meter model CA-A (Kyowa Inter Face Science Co., Ltd. Japan). The water used to prepare monolayers and LB films and to measure contact angles was purified by distillation and passed through a GS-200 deionized system (Advantec Toyo Co., Ltd. Japan).

Materials. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride. Acetonitrile and benzene were dried by distillation from phosphorus pentoxide. Anhydrous methanol was obtained by distillation in the presence of magnesium and a little iodine. Acetone was distilled from anhydrous potassium carbonate. The other solvents and chemicals which are commercially available were guaranteed (G.R.) or extra pure (E.P.) reagent grade and were used without further purification. All reagents were purchased from Nacalai Chemical Co. unless otherwise noted.

2-Chloro-2-oxo-1,3,2-dioxaphospholane, bp 103.0–105.0 °C (1.0 mbar), was prepared according to the methods of Lucas et al.¹⁴ and Edmundson.¹⁵ Trimethylamine was prepared by the reaction of trimethylamine hydrochloride with 40% sodium hydroxide.¹⁶ The syntheses and characterizations of the hydrophobic acrylamide monomers containing two long alkyl chains (R_1R_2 AAm) have been described in detail previously.¹³

2-(Acrylamidomethoxy)-2-oxo-1,3,2-dioxaphospholane (1). Into 30 mL of dry THF was placed 2.50 g (24.7 mmol) of 1-(hydroxymethyl)acrylamide, and then the mixture was filtered by using a usual filtrate to give a clear solution, which 1.82 g (18.0 mmol) of 1-(hydroxymethyl)acrylamide dissolved thoroughly in the THF. Then, this solution was transferred into a dry 100 mL four-necked round-bottom flask equipped with a mechanical stirrer, a calcium chloride drying tube, and a dropping funnel. Into the same flask was also placed 2.00 g (19.8 mmol) of triethylamine. When the mixture was cooled to –20 °C in a dry ice/methanol bath, 2.57 g (18.0 mol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane was added slowly by dropping into the stirred solution over a period of 1 h, by which procedure the homogeneous solution separated into two layers. After the completion of addition, the reaction mixture was stirred at 0 °C for a further 1 h. Then the THF in the upper layer was removed, and 100 mL of acetonitrile was added into the flask which contained the purposed compound and triethylamine hydrochloride as a white viscous liquid in the lower layer. This solution was kept at –50 °C in a dry ice/methanol bath to precipitate triethylamine hydrochloride as a white solid. The precipitate was filtered off with a glass filter and washed with 20 mL of cooled acetonitrile. The filtrate was evaporated under vacuum in a stream of nitrogen for 1 h to give compound **1** as a colorless viscous liquid. Yield: 3.65 g (98.0%). IR (KBr): 1650 (vs; –CONH–, $\nu_{C=O}$), 1625 (m; CH₂=CH–, $\nu_{C=C}$), 1260–1280 (s; –O–P=O, $\nu_{P=O}$), 1050 cm^{–1} (vs; –P–O–C–, ν_{C-O}). ¹H-NMR (400 MHz, CD₃OD): δ 4.31 (m; 4H, –OCH₂CH₂O–), 4.71 (s; 2H, –CONHCH₂–), 5.83 (m; 1H, CH=CHCO–, trans), 6.41 (m; 1H, CH₂=CHCO–), 6.45 ppm (m; 1H, CH=CHCO–, cis).

1-(Acrylamidomethyl)-2-(trimethylammonio)ethyl Phosphate (2). Into a 300 mL glass pressure bottle were placed 3.65 g (17.6 mmol) of **1**, 100 mL of dry acetonitrile, and 5.20 g (88.0 mmol) of trimethylamine. The pressure bottle was closed and then transferred into a thermostat maintained at 50 °C for 8 h. After it had been cooled to room temperature, the pressure bottle was opened and the reacted solution was found to separate into two layers. The upper layer was filtered off, and the filtrate was evaporated. Then the obtained pale

Table 1. Summary of Physical Properties for the Synthesized Amphiphilic Copolymers

| copolymer | state | mp (°C) | yield (%) | elemental analyses (wt %) | | |
|------------|-------------|---------|-----------|---------------------------|-------|------|
| | | | | C | H | N |
| AMP1–LOAAm | white solid | 85–97 | 79.8 | 62.90 | 10.59 | 5.91 |
| AMP1–SOAAm | white solid | 106–125 | 78.4 | 64.22 | 10.86 | 5.68 |
| AMP1–DOAAm | white solid | 110–130 | 74.6 | 64.01 | 10.65 | 5.80 |
| AMP4–LOAAm | white solid | >250 | 84.3 | 62.90 | 10.59 | 5.91 |
| AMP5–SOAAm | white solid | >250 | 81.5 | 64.22 | 10.86 | 5.68 |
| AMP5–DOAAm | white solid | >250 | 80.7 | 64.01 | 10.65 | 5.80 |

yellow viscous liquid was washed with dry acetone three times. Upon the removal of solvent under vacuum, compound **2** as a hygroscopic pale yellow highly viscous wax was obtained. Yield: 3.38 g (72.0%). IR (KBr): 1655 (vs; –CONH–, $\nu_{C=O}$), 1630 (m; CH₂=CH–, $\nu_{C=C}$), 1470 (m; –CH₂–, δ_{C-H}), 1230 (vs; O–P=O, $\nu_{P=O}$), 1050–1080 cm^{–1} (vs; –P–O–C–, ν_{C-O}). ¹H-NMR (400 MHz, CD₃OD): δ 3.09 (s; 9H, –N⁺(CH₃)₃), 4.21 (m; 2H, –CH₂N⁺–), 4.24 (m; 2H, –POCH₂–), 4.80 (s, 2H, –CONHCH₂–), 5.85 (m; 1H, CH=CHCO–, trans), 6.44 (m; 1H, CH₂=CHCO–), 6.45 ppm (m; 1H, CH=CHCO–, cis). ¹³C-NMR (100.40 MHz, CD₃OD): δ 167.56 (–CONH–), 129.37 (CH₂=CH–), 126.53 (CH₂=CH–), 67.93 (–CH₂N⁺–), 64.38 (–CONHCH₂–), 62.97 (–POCH₂–), 53.06 ppm (–N⁺(CH₃)₃).

Anal. Calc for C₉H₁₉N₂PO₅ (266.23): C, 40.60; H, 7.19; N, 10.52. Found: C, 40.54; H, 7.17; N, 10.39.

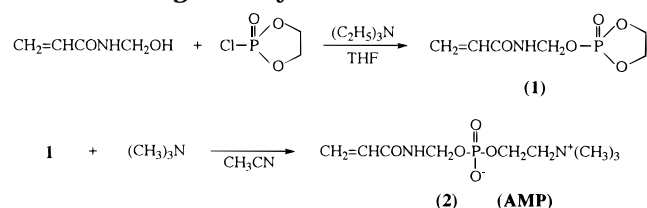
Copolymerization Procedures. Monomer **2** and monomers R_1R_2 AAm were dissolved into a mixed solvent of anhydrous benzene and methanol with 10% [wt (g)/v (mL)] concentration, together with α, α' -azobis(isobutyronitrile) (AIBN) (1.5% mol of total monomers) as an initiator. The solutions were transferred into sealable ampules and were flushed with nitrogen for 10 min. Then the ampules were sealed and shaken at 70 °C for 20 h. After the ampules were opened, the polymerization solutions were concentrated and poured into an amount of dry benzene to give white precipitates. The reprecipitations were repeated three times, and the obtained precipitates were washed with dry methanol three times. The precipitates were filtered off and then dried in vacuum to afford copolymers AMPn– R_1R_2 AAm as white solids. The copolymers were characterized by their IR (KBr) and FT-NMR (CDCl₃ and CD₃OD) spectral data, while their compositions were determined by elemental analysis. The results are listed in Table 1.

Surface Pressure–Area (π -A) Isotherm Measurements. A computer-controlled film balance containing a Wilhelmy pressure pickup system was used for measuring surface pressure as a function of occupied area per repeat unit of copolymers. The trough area is 927 cm², and the temperature of the aqueous subphase were maintained at ± 0.1 °C, centering a set temperature during the measurement. The concentration of the spreading solution was 3.0–3.5 mg/10 mL in the mixture of toluene and methanol (9:1, v/v). After 0.1 mL of the solution was spread, the monolayer film was incubated for 15 min and then compressed by a barrier at a rate of 0.5 mm/s. The surface pressure (π) vs area/repeat unit plots were recorded at a certain temperature for the amphiphilic copolymers.

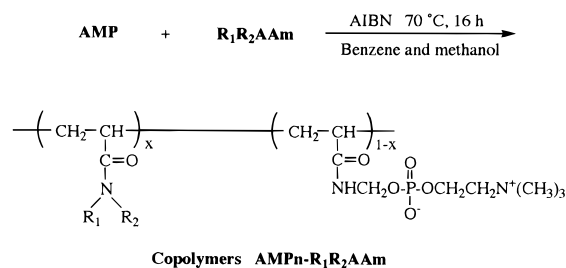
LB Film Preparations. Langmuir–Blodgett multilayers were prepared by means of a commercially available film balance (Lauda) on pure aqueous subphases at 20 °C. Multilayers were deposited on poly(ethylene terephthalate) (PET) wafers, using the vertical dipping method. The hydrophobic support material PET plates were cleaned by ultrasonication in G.R. grade chloroform, washed with G.R. grade diethyl ether, and then rinsed with clean water several times. LB depositions were performed at surface pressure π = 30 mN/m with downward and upward speeds of 10 mm/min. Between subsequent dips, the deposited layers were allowed to dry in the air for 15 min, to avoid retransferring of the last deposited monolayer to the water surface.

Contact Angle Measurement. The water contact angles of obtained LB films of the copolymers were measured at room temperature after drying the films in a desiccator for 24 h in the presence of silica gel as a drying agent. The quoted values

Scheme 1. Synthesis of Phosphatidylcholine Analogous Acrylamide Monomer AMP



Scheme 2. Copolymerizations of Phosphatidylcholine Analogous Acrylamide Monomer AMP with Two Long Chain Acrylamide Monomer R₁R₂AAm



| Copolymer | n | R ₁ | R ₂ | x |
|------------|---|----------------|----------------|------|
| AMP1-DOAAm | 1 | Oleyl group | Docosyl group | 0.46 |
| AMP5-DOAAm | 5 | Oleyl group | Docosyl group | 0.17 |
| AMP1-SOAAm | 1 | Oleyl group | Stearyl group | 0.48 |
| AMP5-SOAAm | 5 | Oleyl group | Stearyl group | 0.16 |
| AMP1-LOAAm | 1 | Oleyl group | Lauryl group | 0.54 |
| AMP4-LOAAm | 4 | Oleyl group | Lauryl group | 0.21 |

are the average of 10 measurements of each sample taken during 20 s contact with the water droplet, using a face contact angle meter.

Results and Discussion

Syntheses of Hydrophilic Monomer and Amphiphilic Copolymers. The synthesis of a hydrophilic acrylamide monomer containing a phosphatidylcholine group was achieved according to the reaction in Scheme 1.

In the presence of triethylamine, 1-(hydroxymethyl)-acrylamide was reacted with 2-chloro-2-oxo-1,3,2-dioxaphospholane in dry THF at -20 – 0°C . After the triethylamide hydrochloride as a white solid was filtered off, the filtrate was evaporated to give 2-(acrylamidomethoxy)-2-oxo-1,3,2-dioxaphospholane (**1**). Then compound **1** was ring-opened by trimethylamine in dry acetonitrile at 50°C to afford the purposed hydrophilic monomer 1-(acrylamidomethyl)-2'-(trimethylammonio)-ethyl phosphate (**2**) (AMP) as a pale yellow highly viscous wax. Monomer AMP is highly hygroscopic. It is easily soluble in water or methanol but almost insoluble in chloroform or acetone.

The copolymerizations of monomer AMP with a series of hydrophobic acrylamide monomers R₁R₂AAm containing a saturated and an unsaturated long chain alkyl group were performed by using the mixture of dry benzene and methanol as a solvent, and AIBN as an initiator (Scheme 2). The polymerization products were purified by reprecipitation in benzene and then washed with methanol three times. In these processes, the monomers R₁R₂AAm still remained in benzene but were not precipitated together with the copolymers, while the monomer AMP and its homopolymer were removed due to the easy solubility in methanol. In addition, it has been found that the homopolymerizations of monomers

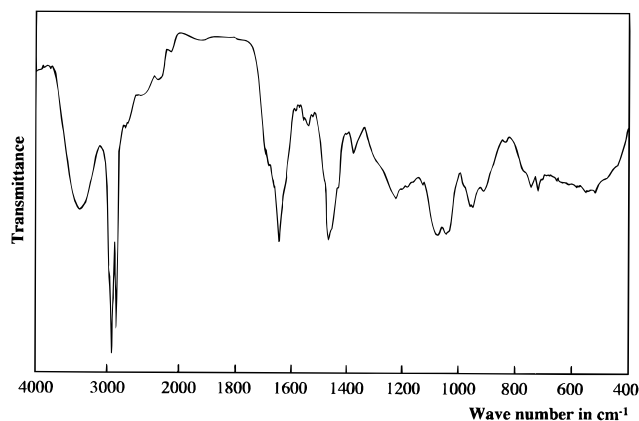


Figure 1. IR spectrum of copolymer AMP1-DOAAm.

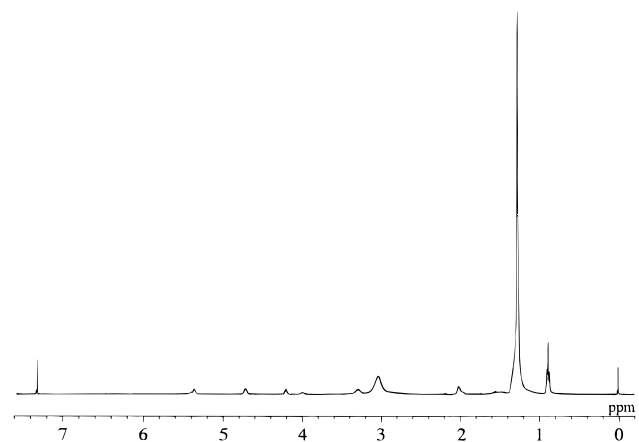
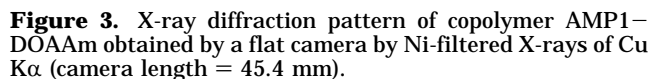


Figure 2. ¹H-NMR spectrum of copolymer AMP1-DOAAm.

R₁R₂AAm almost could not be carried out owing to the large steric hindrance.¹³ These copolymers as white solids are soluble in the mixture of chloroform and methanol, but almost insoluble in pure chloroform or pure methanol. They were characterized by IR and ¹H-NMR spectra, elemental analysis, and melting points, respectively (Table 1).

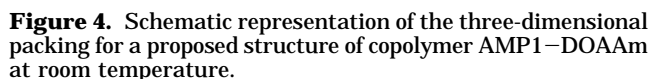
The characterizations of these copolymers were based on their IR and ¹H-NMR spectral data. As an example, the IR and ¹H-NMR spectra of copolymer AMP1-DOAAm are shown in Figures 1 and 2. In the IR spectrum, the introduction of monomer R₁R₂AAm (DOAAm) is determined by the peaks at 2850 and 2915 cm^{-1} , owing to the stretching vibration of $-\text{CH}_2-$ of long chain alkyl groups. The strong absorption bands at 1230 and 1050–1085 cm^{-1} are attributed to the existence of phosphate groups. In the ¹H-NMR spectrum, the characteristic peak at $\delta = 1.26$ ppm comes from the long chain alkyl groups, while the peak at $\delta = 3.04$ ppm corresponds to the $-\text{N}^+(\text{CH}_3)_3$ group. The peaks of the double bonds of the oleoyl group appear at 5.35 ppm. In addition, the peaks of the vinyl group of $\text{CH}_2=\text{CHCO}-$ could not be observed in this spectrum. From these spectral data, it can be proved that the copolymerization of monomer AMP with monomer DOAAm was achieved indeed.

Structures of Condensed Phases of the Copolymers. The structure of the condensed phase of copolymer AMP1-DOAAm was investigated by the X-ray diffraction (XRD) method at room temperature. In its XRD pattern, a strong ring at 64.4 \AA^{-1} together with strong rings at 32.2 \AA^{-1} , 21.5 \AA^{-1} , and 16.1 \AA^{-1} , which are the second, third, and fourth reflections of that at 64.4 \AA^{-1} , respectively, in the small-angle region, and



On the basis of the XRD results, the copolymer AMP1-DOAAm is suggested to be constructed of a stacked bilayer structure, and a proposed model of the molecular packing of the copolymer is shown in Figure 4. The copolymer has two hydrocarbon chains as a hydrophobic part and a bulky polar head group as a hydrophilic part. The rough calculated value of the thickness of the hydrocarbon chain part is about 48 Å when the packing and conformations of the two hydrocarbon chains, i.e. docosyl group and oleoyl group, are taken as shown in Figure 4. The torsion angle around the cis bond should be set so as to give a linear conformation to the oleic chain, which otherwise is bent, as observed in the crystalline state of oleic acid.¹⁹ The similar linear conformation of oleic chains is also reported for the crystalline state of egg yolk lecithin.¹⁸ The polar head group is proposed to lie on a bilayer plane just the same as that of egg yolk lecithin in the crystalline state.¹⁸ The thickness of one polar head group part is estimated to be about 8 Å.¹⁷ The whole thickness of the extended hydrophobic part and hydrophilic part is calculated as about 64 Å. The observed long spacing 64.4 Å is equal to the calculated value. Therefore, the structure proposed for the condensed phase of copolymer AMP1-DOAAm is basically constructed from alternately stacked bilayers with hydrophilic and hydrophobic side chains. The main chain is arranged on the border between the hydrophobic region and hydrophilic region, and the two kinds of side chains stand out alternately toward opposite directions from the main chain. Consequently, lattice parameters of the present model are $a = 9.60$ Å, $b = 8.32$ Å, and $c = 64.4$ Å and the system is orthorhombic. These values give the calculated density, $\rho_{\text{calc}} \approx 1.16$ g/cm³.

From these results of X-ray analysis, the amphiphilic copolymer in the condensed phase is determined to exist in a bilayer structure similar to that in lipid bilayers. Therefore, it is predictable that not only lyotropic but also thermotropic phase transitions will occur in this



Thermal Properties. In the previous work,¹¹ we reported that some amphiphilic phospholipid analogous polymers which contained long chain alkyl groups in the side chains displayed polymorphic phase transitions in the condensed phase with the variation of temperature. These phase transitions were found to correspond to the transformation from the crystalline state to the liquid-crystalline state and the following disappearance of the liquid-crystalline state. In this study, we also investigated the thermal properties for copolymer AMP1-DOAAm by DSC measurement and POM observation. As shown in Figure 5, two endothermic peaks around 36.4 and 128.2 °C, together with an unclear endothermic peak in the temperature range from 165 to 198 °C, were observed from the DSC trace on heating. Depending on the XRD result in the wide-angle region at room temperature, it could be suggested that the endothermic peak around 36.4 °C corresponds to a phase transition process in which the arrangement of hydrocarbon chains in the stacked bilayers disappeared. Referring to the melting point (copolymer AMP1-DOAAm melted slowly in the temperature range of 110–130 °C), the endothermic peak around 128.2 °C may indicate the melting process of this copolymer. Furthermore, according to the results of Kishore et al.^{22,23} and our early studies,^{11,24} the unclear endothermic peak observed from 165 to 198 °C corresponds to the process of pyrolysis of the phosphates.

The smectic liquid-crystalline arrangements are essential for biomembranes because the substance trans-

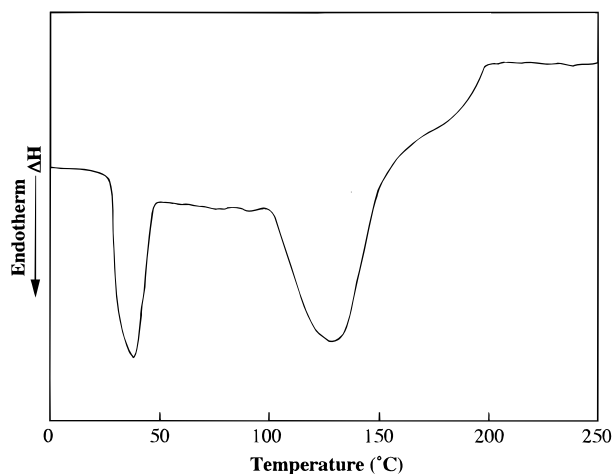


Figure 5. DSC curve of copolymer AMP1-DOAAm.



Figure 6. Polarizing microscope photo of copolymer AMP1-DOAAm at room temperature.

ports between the inside and the outside of membranes are almost carried out in the liquid-crystalline state. Therefore, the polarizing microscope observation of copolymer AMP1-DOAAm was carried out with a micro melting point apparatus. The copolymer was observed to exhibit clear polarizing behavior from room temperature to 38 °C and melt in a temperature range of 110–130 °C. This observation agreed with the results of the DSC measurement mentioned above. Moreover, the polarizing optical microscope (POM) photo was taken by use of a 530 nm sensitive color plate at room temperature after the copolymer was handled orientationally (Figure 6).

Viscosity Measurements. Measurements of solution viscosity of synthesized copolymers were conducted in a Ubbelohde-type viscometer in a mixture of chlorobenzene and methanol (2:1, v/v) at 25 °C. For different concentrations, reduced viscosity (η_{sp}/C) of copolymers was established, and the plots of these η_{sp}/C versus polymer concentrations are shown in Figure 7. The η_{sp}/C for all copolymers was found to increase with the reduction of concentration, especially at the range of low concentrations (<0.33 g/dL). These viscosity plots are similar to those of some phospholipid analogous copoly-(acrylamide)s containing the same hydrophobic side chains and different hydrophilic side chains in the side chains,¹³ and some acrylamide homopolymers bearing both long chain alkyl groups and phosphatidylcholine analogues in their side chains.²⁵ This result suggests that the present copolymers having phosphatidylcholine moieties in the side chains show properties similar to

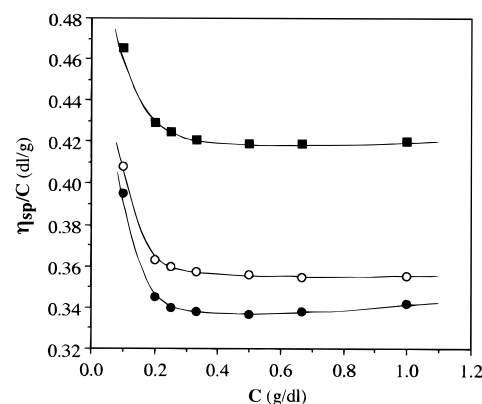


Figure 7. Reduced viscosity of copolymers AMP5-DOAAm (●), AMP5-SOAAm (○), and AMP4-LOAAm (■) in the mixture of chlorobenzene and methanol (2:1, v/v) at 25 °C.

those of usual polyelectrolytes. These phenomena may result from the mutual repulsion between N^+ and N^+ , particularly the possible chain expansion at low concentrations.

Spreading Behavior of Copolymers. The monolayers of these amphiphilic copolymers were prepared at the air–water interface, and their π - A isotherms were investigated at different temperatures, resulting in the formation of stable monolayers. Figure 8a–c shows the π - A isotherms of copolymers AMP4-LOAAm, AMP5-SOAAm, and AMP5-DOAAm measured at 10, 20, and 30 °C, respectively. In all experiments, no surface pressure was measurable when the occupied area was higher than 150 Å²/unit. Upon further compression, an obvious increase in surface pressure was observed with the decrease of occupied area at all conditions investigated. The first-order phase transition from a liquid-expanded phase to a liquid-condensed phase was determined for copolymers AMP4-LOAAm and AMP5-SOAAm at all measured temperatures and for copolymer AMP5-DOAAm at 20 and 30 °C. In the case of copolymer AMP5-DOAAm at 10 °C, only a liquid-condensed phase was observed, probably due to the more ordered arrangement at low temperature for this copolymer which contains long saturated alkyl groups in the side chains. It has been revealed that the liquid analogous phase is thermodynamically unstable compared to the condensed phase.²⁶ The collapse pressures for all these copolymers were found to reduce gradually as the temperature increased. These results indicated that the stability of the monolayers increases with the decrease of measured temperature, particularly for copolymer AMP5-DOAAm which showed a high sensitivity to temperature. Comparing the isotherms of the three copolymers (Figure 8a–c), one can see that the collapse pressure increases with increasing length of the saturated alkyl group in the side chain because the interaction among the hydrocarbon chains is stronger for long alkyl chains than for short alkyl chains.

The π - A isotherms of copolymers AMP1-SOAAm and AMP1-DOAAm at 10, 20, and 30 °C are shown in Figure 9a,b, and the temperature dependence of collapse pressure was obvious. Both copolymers show a first-order phase transition at 20 and 30 °C but show a monotonic increase of surface pressure at 10 °C. It could be considered that the arrangement of hydrocarbon chains on the surface of water is more ordered and the monolayer is more stable at low temperature, giving only a liquid-condensed phase. Moreover, the collapse pressure for copolymer AMP1-SOAAm is lower than that of the copolymer AMP1-DOAAm, as well as those

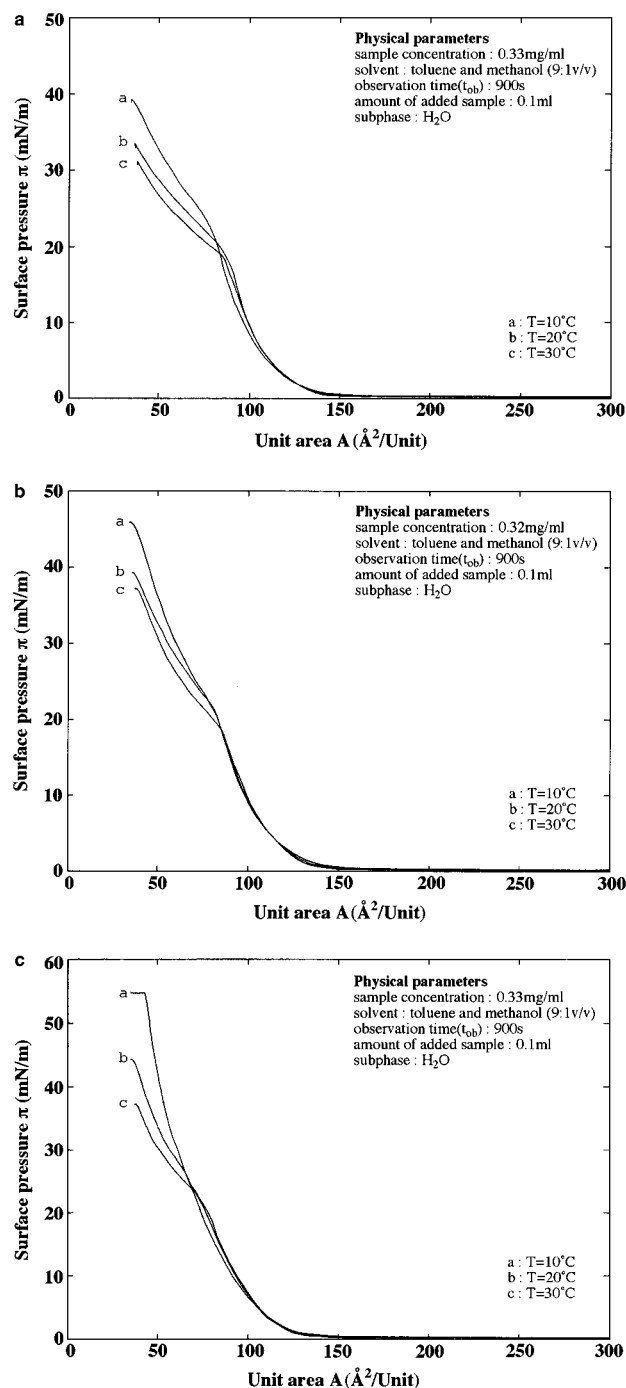


Figure 8. π - A isotherms of copolymers (a) AMP4-LOAAm, (b) AMP5-SOAAm, and (c) AMP5-DOAAm at 10, 20, and 30 °C.

observed in Figure 8b,c. Comparing the copolymers AMP5-R₁R₂AAm with the copolymers AMP1-R₁R₂AAm, one clearly finds that the rising of surface pressure is slower and the collapse pressure is higher for copolymers AMP1-R₁R₂AAm than for copolymer AMP5-R₁R₂AAm. It may come from the closely ordered arrangement of hydrocarbon chains when the molar ratio of hydrophobic and hydrophilic groups is 1 to 1.

The dependence of observation time was investigated for the monolayers of copolymer AMP1-DOAAm, and the π - A isotherms measured at 20 °C for 120, 900, and 7200 s are shown in Figure 10. The isotherms at different observation times look similar except in the collapse region. That is to say the monolayer shows the same stability for different observation times before it

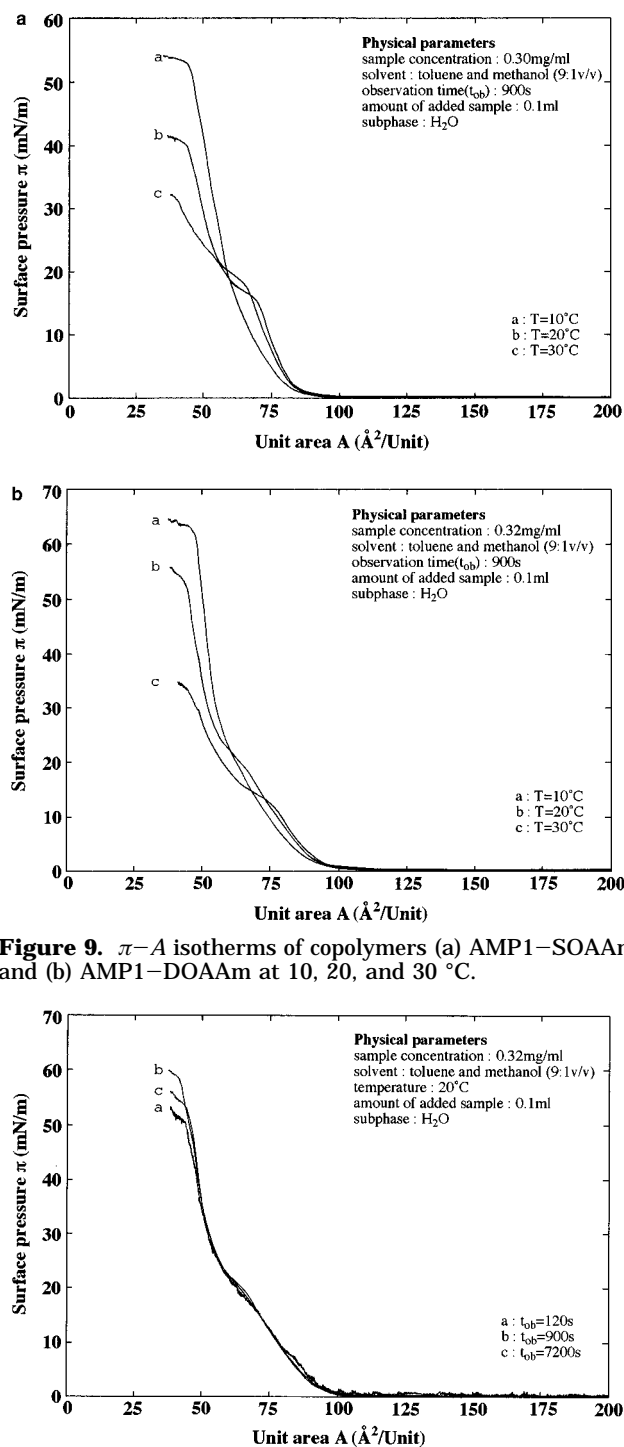


Figure 9. π - A isotherms of copolymers (a) AMP1-SOAAm and (b) AMP1-DOAAm at 10, 20, and 30 °C.

Figure 10. π - A isotherms of copolymer AMP1-DOAAm at different observation times.

begins to collapse. On the other hand, the collapse pressure is the highest for 900 s and is the lowest for 120 s. It is considered that the ordered arrangement of macromolecules cannot be formed when the time of observation is shorter than the relaxation time. On the contrary, the monolayer is not stable when the observation time is far longer than the relaxation time.

Based on these investigations of monolayers of the synthesized copolymers by changing the measured temperature and observation time, the best conditions to prepare LB films could be given as $T = 20$ °C and $\pi = 30$ mN/m because the stable and close arrangement of alkyl chains could be formed at this temperature, in a liquid condensed state, and for a long deposition time.

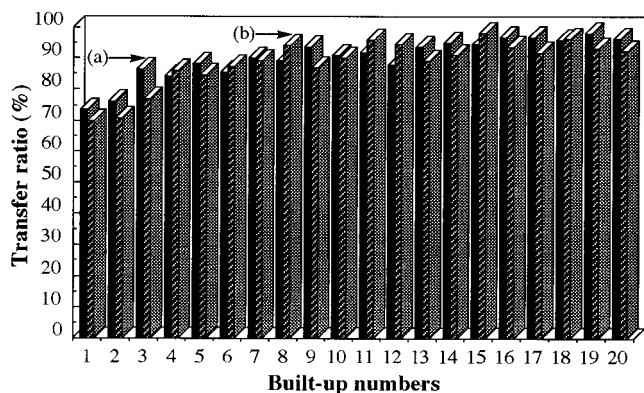


Figure 11. Transfer ratio of LB films of copolymers (a) AMP1-DOAAm and (b) AMP1-SOAAm.

Langmuir-Blodgett Films of Copolymers. It is well recognized that LB multilayers show a great potential for various applications. Most LB films prepared from low molecular weight amphiphiles are high ordered, but they are not sufficiently stable or robust for use in commercial devices. Therefore two approaches may be used for the preparation of polymeric LB films. First, multilayers could be obtained by polymerization of monomeric amphiphiles after deposition. Not surprisingly, polymeric films produced using this approach tend to crack and craze,²⁷ though this is not always the case. The second approach is to prepare the LB films directly from preformed polymers. However, some preformed polymers, such as polyacrylates,²⁸ polymethacrylates,²⁸⁻³⁰ poly(vinyl ester)s,³¹ and poly(vinyl ether)s,³¹ contain relatively weak hydrophilic groups, and the preparation of ordered LB multilayers is not always easy.^{28,29} For our approach, the synthesized amphiphilic polymers hold relatively strong hydrophilic phosphatidylcholine groups in the polar heads, and their LB films were prepared and investigated.

All the copolymers that formed stable monolayers on the surface of water could be transferred at surface pressures of 30 mN/m onto hydrophobic PET wafers with 10 mm/min as the downward and upward speeds. The deposition ratios (%) (deposited area/dipped area of wafer) for these copolymers were obtained by recording the amount of the monolayers consumed during the successive up and down strokes of the wafers. The odd layers were deposited on the down strokes, and the even layers were deposited on the up strokes. It has been found that all copolymers deposited well on both the odd and even layers, suggesting Y-type depositions. The deposition ratios of the LB films for copolymers AMP1-SOAAm and AMP1-DOAAm are shown in Figure 11. Both copolymers started to deposit with 0.70–0.76 ratios, but their deposition ratios on both the down and up strokes increased gradually. After three to four cycles, the transferred polymers showed reproducible deposition ratios as 0.90–0.98 to give good Y-type multilayers. These results suggest that the amphiphilic copolymers are suitable for designing and constructing synthetic phospholipid membranes.

Contact Angles for Obtained LB Films. The water contact angle is a common evaluator of the hydrophilicity–hydrophobicity of the surface of film materials. After the LB films were dried in a desiccator for 24 h in the presence of silica gel as a driver, the water contact angles of obtained LB films for copolymers were measured at room temperature. They are found to be 87.4° for copolymer AMP1-SOAAm and 91.0° for copolymer AMP1-DOAAm. As a referrer, the contact

angle of the PET wafer with pure water was also measured (81.0°). From these results, it can be seen that the LB multilayers obtained show a homogeneous and hydrophobic surface constituted by long hydrocarbon chains, and the surface hydrophobicity for the synthesized copolymers is higher than that of PET wafers.

The present work may be helpful to construct a synthetic biomembrane because not only the synthesized copolymers exist in a stacked bilayer structure in solid state but also stable monolayers and multilayers could be obtained from these copolymers.

Conclusion

The amphiphilic copolymers containing a saturated and an unsaturated long alkyl chain as the hydrophobic groups and phosphatidylcholine analogues as the hydrophilic groups in the side chains were prepared and characterized. The copolymers were found to display solution viscosity behavior similar to that of usual polyelectrolytes. The structure of the condensed phase of copolymer AMP1-DOAAm was proposed to be constituted from stacked bilayers. The stable monolayers of synthesized copolymers show obvious temperature dependence and length dependence of saturated alkyl groups in the side chains, and the first-order phase transitions have been observed for all copolymers. Furthermore, the high-quality Y-type multilayers were successfully obtained on PET wafers, and the hydrophobicity increased after the copolymers were deposited onto the wafers.

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