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SYNTHESIS AND PROPERTIES OF PHOSPHOLIPID ANALOGOUS POLY(ACRYLAMIDE) CONTAINING VITAMIN E MOIETY

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ABSTRACT

An amphiphilic acrylamide monomer containing a vitamin E moiety as the hydrophobic group and a phosphatidylcholine analogue as the hydrophilic group was newly synthesized in high yield. The radical polymerization was carried out in the presence of α, α'-azobisisobutyronitrile as an initiator. The obtained monomer and homopolymer were characterized by ^{1}H NMR, IR, and melting points. A stacked bilayer structure was proposed for homopolymer from x-ray diffraction analysis. Furthermore, monolayers of the homopolymer were prepared on the surface of water and were investigated at different temperatures.

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INTRODUCTION

Recently, polymeric phospholipid analogues have been extensively studied in syntheses, packing structures, monolayers, liposomes, and Langmuir–Blodgett multilayers [1–9]. This is because phospholipid is a potential component of biomembranes and is physiologically active. Phospholipids possess two hydrophobic chains attached to a hydrophilic head-group. Depending on this amphiphilic character of phospholipid, a stable bilayer structure can be freely formed by itself in a condensed phase or in an aqueous solution. Most biological membranes are constructed of such phospholipid bilayers. Accordingly, it is highly important to investigate the structures and properties of amphiphilic phospholipid analogues in order to understand the biological functions of membranes in greater detail.

On the other hand, several decades of vitamin E research have shown that it is probably essential for the maintenance and function of all animal cells. Vitamin E acts as an antioxidant in biological membranes because it inhibits peroxidation of membrane-bound unsaturated fatty acids [10]. From this point of view, it seems to be highly important to investigate the properties of polymers containing the vitamin E moiety. The synthesis of a polymethacrylic phospholipid analogue with a vitamin E moiety as the hydrophobic group was reported in our early work [11]. To obtain a phospholipid analogue polymer having a stacked bilayer structure similar to that found for lipid bilayers for use in this paper, we prepared a novel amphiphilic polymer containing a vitamin E moiety as the hydrophobic part and a phosphatidylcholine analogue together with a acrylamide group as the polar head part in the side chains. The present paper is mainly concerned with the synthesis, structure, and properties of such a polymer.

EXPERIMENTAL

Materials

Chloroform, chlorobenzene, and benzene were distilled over phosphorus pentoxide. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride. Anhydrous methanol was obtained by distillation in the presence of magnesium and iodine. Acetone was dried by distillation from anhydrous potassium carbonate. *N,N*-Dimethylformamide (DMF) was distilled from calcium hydride. [(*N,N*-Dimethylamino)propyl]acrylamide was obtained from Kohjin Co., Japan. All other solvents and chemicals were of extra pure reagent quality and used without further purification. All reagents were purchased from Nacalai Chemical Co. unless otherwise noted.

2-Chloro-2-oxo-1,3,2-dioxaphospholane (**1**), bp (1.0 mbar) 102.5–105.0°C, was prepared according to the method of Lucas et al. [12] and Edmundson [13].

The synthesis and characterization of 2-[2*R*,4′*R*,8′*R*-2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyltridecyl)chroman-6-yloxy]-2-oxo-1,3,2-dioxaphospholane (**2**) were described in detail previously [11].

Synthesis of Monomer

2-[(3-Acrylamide Propyl)dimethylammonio]ethyl 2*R*,4′*R*,8′*R*-2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltridecyl)chroman-6-yl Phosphate (3)

After 10.0 g (0.02 mol) of **2** in 50 mL of dry DMF was placed into a 300-mL glass pressure bottle, 5.82 g (0.04 mol) of [(*N,N*-dimethylamino)propyl]acrylamide

dissolved in 50 mL of dry DMF was quickly added into the same bottle. The pressure bottle was closed and then shaken in a thermostat at 70°C for 48 hours. After the reaction mixture was cooled to room temperature, the pressure bottle was opened. The solution was concentrated to give a crude product as a yellow liquid. The crude product was purified by adsorption chromatography through a silica gel column with a mixture of chloroform and methanol (1:2, v/v) to give pure compound 3 as a pale yellow viscous liquid. Yield: 15.6 g (68.4%).

IR (KBr): 1658 (vs: CONH, $\nu_{C=O}$); 1625 (s: $CH_2=CH-$, $\nu_{C=C}$); 1230 (s: O—P=O, ν_{P-O}) and 1050–1080 cm^{-1} (vs: P—O—C, ν_{C-O}).

^1H NMR ($CDCl_3$): δ = 0.85–2.62 (bm: 49H, tocopheryl and 2H, CONH-CH_2CH_2), 3.28 (s: 6H, $N^+(CH_3)_2$), 3.40 (m: 2H, CONHCH_2), 3.62–3.95 (m: 6H, $CH_2^+NCH_2CH_2$), 5.55 (dd: J = 10.24 and 1.46, 1H, C*H*=CCONH, *trans*), 6.24 (dd: J = 17.08 and 1.95, 1H, C=C*H*—CONH), 6.42 (dd: J = 17.07 and 10.24, 1H, C*H*=CCONH, *cis*), and 9.12 ppm (bs: 1H, CON*H*)

$C_{39}H_{69}O_6N_2P \cdot H_2O$ (710.98). Calculated: C, 65.88; H, 10.07; N, 3.94%, Found: C, 66.02; H, 10.24; N, 3.60%.

Polymerization Procedure

The homopolymerization of monomer 3 was carried out in a sealed ampule in a mixed solvent of chlorobenzene and methanol (4:1, v/v) with α, α'-azobisisobutyronitrile (AIBN) (2% mol of monomer) as an initiator by shaking at 70°C for 16 hours. After opening the ampule, the solution was poured into anhydrous acetone to precipitate the homopolymer. Then the crude product was purified by reprecipitation with a mixture of acetone, chloroform, and methanol (10:1:1, v/v/v) three times. The pure homopolymer (4) was obtained as a white solid. Yield, 70.2%; mp, 125–160°C.

Surface Pressure–Area (π–A) Isotherms Measurements

A computer-controlled film balance apparatus was used for measuring surface pressure as a function of unit area for homopolymer. The trough area was 927 cm^2, and the temperature of the aqueous subphase was maintained at ±0.1°C, centering a set temperature during the measurement. The concentration of the spreading solution was 5.0 mg/10 mL in a mixture of chlorobenzene and methanol (4:1, v/v). After spreading 0.1 mL of the solution, the monolayer was incubated for 15 minutes and then compressed at a rate of 0.5 mm/s.

Characterizations

Proton ^1H-NMR spectra were recorded on a JEOL α-400 (400 MHz) spectrometer. 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, double doublet dd, multiplet m, and broad multiplet bm. Infrared (IR) spectra (KBr) were obtained using a Jasco A 202 spectrometer and were reported in wavenumbers (4000–400 cm^{-1}). In the IR data presentation, bracketed s and vs indicate the extent of absorption as strong and very strong. The viscosity measurement was performed with a Ubbelohde-type viscometer in a mixture of chlorobenzene and methanol (4:1, v/v) at 25°C. For an x-ray diffraction

measurement, the specimen was completely sealed with mica in the sample holder. The specimen was stable during x-ray diffraction measurement as judged from the reproducibility of the diffraction pattern. The x-ray powder diagram was photographed with nickel-filtered CuKα radiation (37.5 kV, 20 mA) using a flat-plate camera of 66.9 mm passage at room temperature. Melting point measurement and polarizing optical observation of homopolymer were carried out by using a micro melting-point apparatus (Yanaco MP-J3). Thermal properties were determined by differential scanning calorimetry (DSC), using a Rigaku Thermoflex apparatus DSC-8230B. The sample quantity was 10 mg with a 10°C/min rate of heating from room temperature to 200°C. π-A isotherms were measured by a computer-controlled apparatus for film-balance measuring (Lauda).

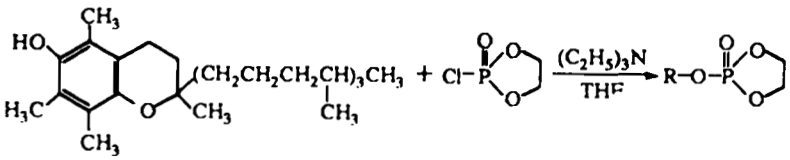
RESULTS AND DISCUSSION

The synthetic procedure of the amphiphilic acrylamidic monomer **3** and homopolymer **4** is outlined in Scheme 1.

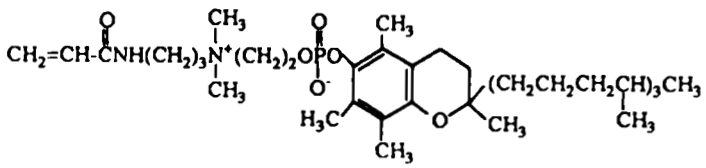
2-Chloro-2-oxo-1,3,2-dioxaphospholane (**1**) was prepared according to the procedure described by Lucas et al. [12] and Edmundson [13]. 2-[2*R*,4′*R*,8′*R*-2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltridecyl)chroman-6-yloxy]-2-oxo-1,3,2-dioxaphospholane (**2**) was obtained by the reaction of **1** with *R*,*R*,*R*-α-tocopherol [11]. Following the method of Thoung and Chabrier [14], the reaction of **2** with [(*N*,*N*-dimethylamino)propyl]acrylamide was carried out in anhydrous DMF at 70°C for 48 hours to give 2-[(3-acrylamide propyl)dimethylammonio]ethyl 2*R*,4′*R*,8′*R*-2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyltri-decyl)chroman-6-yl phosphate (**3**) in good yield. The monomer was confirmed by its IR, ^{1}H-NMR spectral data, and elemental analysis. Monomer **3** is a hygroscopic pale yellow liquid, soluble in a mixture of chloroform and methanol at room temperature, but almost insoluble in acetone or diethyl ether.

Monomer **3** was homopolymerized with AIBN as an initiator at 70°C for 16 hours to obtain the corresponding homopolymer **4**. The homopolymer was purified by precipitation and reprecipitation from the mixture of anhydrous acetone, chloroform, and methanol (10:1:1, v/v/v) three times. Homopolymer **4** was investigated by its IR, ^{1}H-NMR spectra, and melting point measurement. The IR spectrum shows that the absorption bands of C=C double bonds are nonexistent, while the absorption bands of other groups appear as well as monomer **3**. In the ^{1}H-NMR spectrum, only the peaks of C=C double bonds of acrylamide group cannot be observed, and the other peaks are similar to those of monomer **3**.

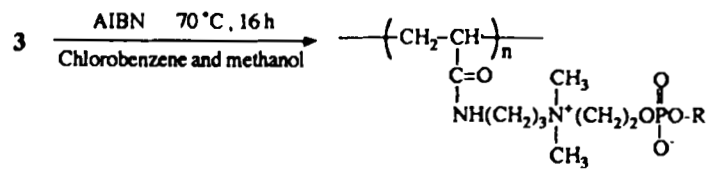
The obtained homopolymer is a white solid and its solubility is similar to monomer **3**. The viscosity measurement for homopolymer **4** was performed at 25°C in a mixture of chlorobenzene and methanol (4:1, v/v). As shown in Fig. 1, the reduced viscosity (η_{sp}/C) of homopolymer **4** was found to increase with the dilution of polymer concentration. This result suggests that the homopolymer displays properties similar to normal polyelectrolytes during the detection of its viscous behavior in a polar solvent. This phenomenon may come from the mutual repulsion between N^+ and N^+, particularly the possible chain expansion at low concentrations. Moreover, this result of viscosity measurement is in agreement with some amphiphilic



(R-OH) (1) (2)



(3)



(4)

SCHEME 1. Synthesis route for monomer **3** and homopolymer **4**.

phospholipid analogous poly(acrylamide)s which contain the same or a similar polar head in their side chains [6, 7].

The structure of homopolymer **4** was investigated by an x-ray diffraction (XRD) method at room temperature. In the XRD pattern, a strong ring at (71.1 Å)$^{-1}$ together with a ring at (35.6 Å)$^{-1}$, which is the second-order diffraction of that at (71.1 Å)$^{-1}$ in the small-angle region, are observed as shown in Fig. 2. In addition, a weak diffuse ring with spacing of 4.6 Å, the spacing of which is reported to come from an arrangement of hydrocarbon chains in a liquid crystalline state [15], is also observed in the wide-angle region.

The XRD results in the small-angle region suggest that the structure of homopolymer **4** in the condensed phase is basically constructed from alternately stacked bilayers. The bilayers are constructed with the main chains together with the hydrophilic and hydrophobic regions of the side chains as shown in Fig. 3. The side chain of homopolymer **4** has one bulky polar head and one hydrophobic hydrocarbon chain. The length of the extended polar head group and of the hydrophobic group in the side chain are estimated as about 10 and 25 Å, respectively. The resulting

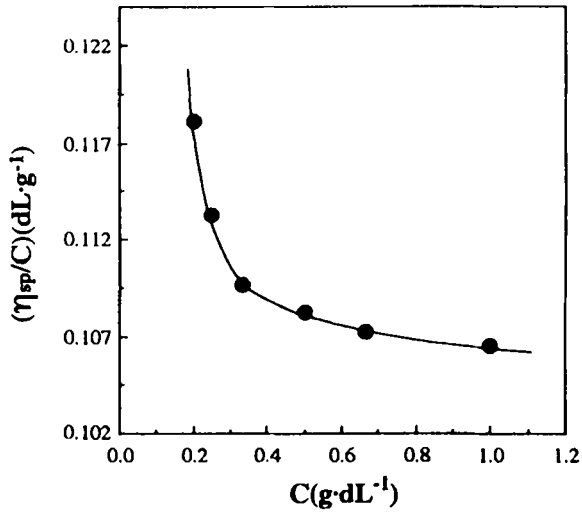


FIG. 1. Reduced viscosity of homopolymer **4** at 25°C in the mixture of chlorobenzene and methanol (4:1, v/v).

total length of a side chain, i.e., 35 Å (=10 Å + 25 Å), is too small to explain the observed value of the long period, and the twice the total length, i.e., 70 Å (35 Å × 2), is nearly equal to the observed long spacing, 71.1 Å. Therefore, in the proposed model, the main chain is arranged in the middle of hydrophilic region where the polar head groups of side chains stand out alternately in opposite directions from the main chain, giving a bilayer structure with a thickness of about 70 Å. The side-by-side packing of hydrophobic group of side chains in the bilayer is in a liquid crystalline state judging from the observed diffuse ring in the wide-angle region of XRD measurement. In contrast to a phospholipid analogous homopolymer which

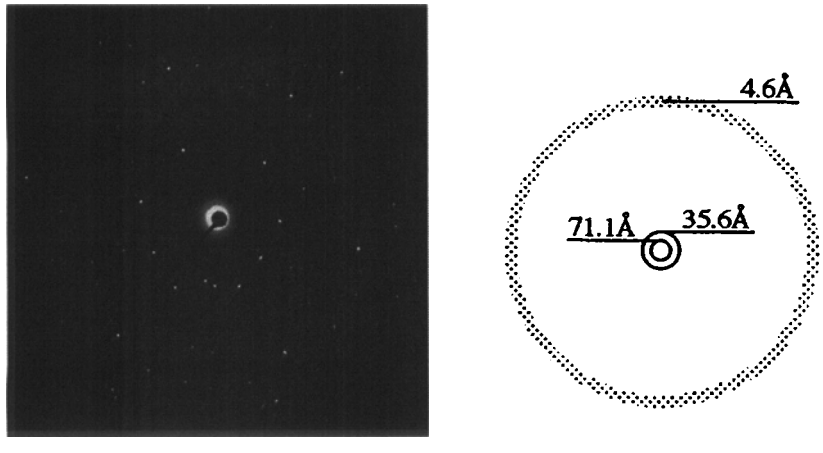


FIG. 2. X-ray diffraction pattern of homopolymer **4** obtained by a flat camera by Ni-filtered x-rays of CuKα (camera length = 66.9 mm).

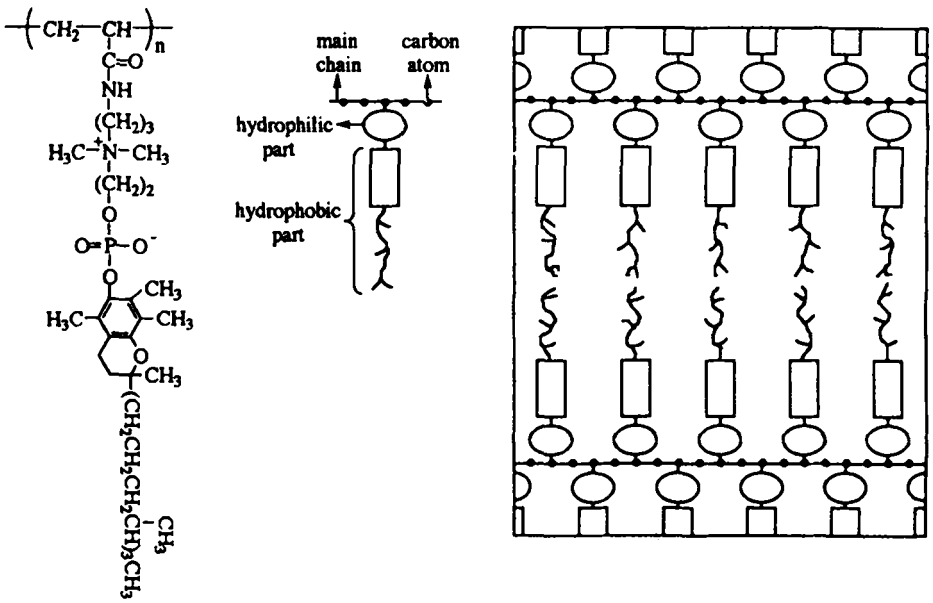


FIG. 3. Schematic representation of the two-dimensional packing for a proposed structure of hompolymer 4 at room temperature.

bears the same polar head and a eicosyl group in the side chain [6], the hydrophobic groups of homopolymer 4 are arranged only toe-to-toe and are not interdigitated with each other. This may come from the influence of the three methylene groups existing in the hydrophobic region of the side chains. Furthermore, homopolymer 4 shows a liquid crystalline state but not a crystalline state at room temperature perhaps due to the disordered hydrophobic groups in the side chains.

To investigate the thermal properties, DSC measurement of the polymer was performed from 20 to 200°C on heating. From its DSC plot, a broadly large endothermic peak was observed in the 60–165°C temperature range and an unclear endothermic peak was shown from 170 to 185°C. Referencing to the previous studies and the results of x-ray analysis, polarizing optical observation (polarizing optical behavior was observed from room temperature to about 155°C), and melting point measurement (homopolymer melts at 125–160°C), the large endothermic peak may correspond to the disordering of the stacked layer structure constructed from side chains and main chains in the lipid-crystalline state and following the melting process of this polymer. In addition, the unclear endothermic peak is thought to indicate pyrolysis of the phosphates. Similar thermal properties have been observed for an amphiphilic phospholipid analogous polymer bearing a cholesterol moiety as the hydrophobic part [16].

Monolayers of homopolymer 4 were prepared on the surface of pure water at different temperature (10, 20 and 30°C), and their surface pressures were measured. The π-A isotherms at the three temperatures are shown in Fig. 4; they result in the formation of stable monolayers. No surface pressure was measurable in all experiments when the occupied area was higher than 120 $Å^2$/repeat unit. Upon

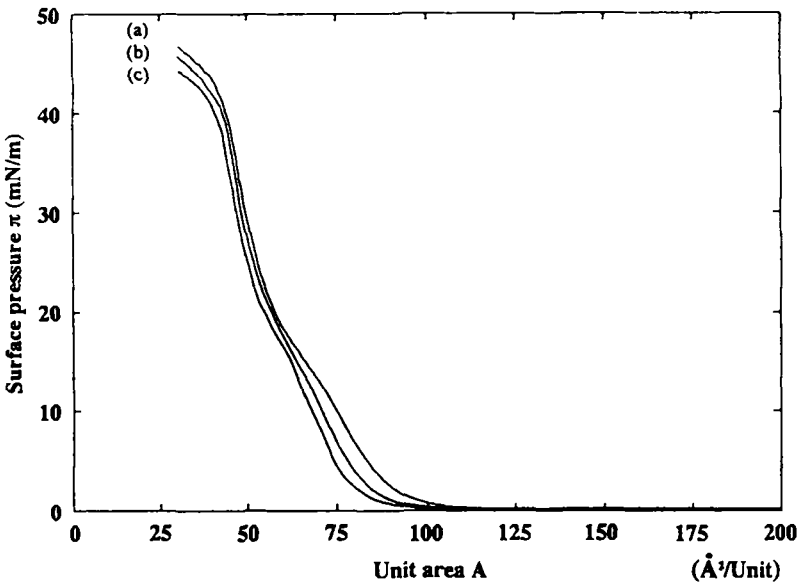


FIG. 4. π–A isotherms of homopolymer **4** at different temperatures: (a) 10°C, (b) 20°C, (c) 30°C.

further compression, a monotonic increase in surface pressure was observed at all conditions investigated. These isotherms at different temperatures look similar except that the initial rise of surface pressure occurs slowly and the collapse pressure (46.8, 45.6, and 44.1 mN/m for 10, 20, and 30°C) decreases gradually as the temperature increases.

The synthesized amphiphilic phospholipid analogous polyacrylamide containing vitamin E moiety in side chains may be well applied as a biomaterial due to its stacked bilayers structure in the condensed state and the formation of a stable monolayer on the surface of water.

CONCLUSION

2-[(3-Acrylamide propyl)dimethylammonio]ethyl 2*R*,4′*R*,8′*R*-2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyltridecyl)chroman-6-yl phosphate as an amphiphilic acrylamide monomer was synthesized and homopolymerized in high yield. The obtained monomer and homopolymer were confirmed based on their ^{1}H-NMR, IR spectral data, and melting points. From the XRD results the amphiphilic homopolymer in condensed phase was suggested to exist as an alternately stacked bilayers structure which was constructed from the main chains together with the hydrophilic and hydrophobic regions of the side chains. The side-by-side packing of a hydrophobic group of side chains in the bilayer is in a liquid crystalline state as judged from x-ray diffraction analysis. On the other hand, stable monolayers of the homopolymer were obtained at different temperatures on the surface of pure water, and the temperature dependence of the collapse pressure was observed.

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