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Tadao Nakaya[a]; Hajime Shoji[a]; Minoru Imoto[a]
[a] Department of Applied Chemistry Faculty of Engineering, Osaka City University, Sumiyoshi-ku, Osaka, Japan

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NOTE
POLYMERIC PHOSPHOLIPID ANALOGS. XXII. A SYNTHETIC POLYPEPTIDE WITH PHOSPHOLIPID ANALOGS

TADAO NAKAYA, HAJIME SHOJI, and MINORU IMOTO

Department of Applied Chemistry
Faculty of Engineering
Osaka City University
Sumiyoshi-ku, Osaka 558, Japan

INTRODUCTION

Within the past 15 years, a considerable amount of our effort has been directed toward the syntheses and properties of the polymers containing phosphatidylethanolamine [1, 2] and choline [3-5] analogs in the side chains and phosphatidylcholine analogs in the main chains [6]. On the other hand, it is well known that biological membranes are formed of lipids and proteins composed of polypeptide chains [7]. Therefore, it seemed to be of great interest to prepare a synthetic polypeptide containing phospholipid analogs in the side chains.

EXPERIMENTAL

Materials

Most of the reagents were commercially obtained and used without further purification. Thionyl chloride, phosphorus pentoxide, trimethylamine hydrochloride, 1,20-eicosanedioic acid, *p*-toluenesulfonic acid, and oxygen were commercially obtained and used without further purification. Poly-(γ-methyl *L*-glutamate) in 10 wt% aqueous solution of dichloroethane/tetrachloroethylene (volume ratio 7/3) (Ajicoat A-2000, $\bar{M}_w$ = 100 000)

was supplied by Ajinomoto Co. THF and diethyl ether were dried by distillation from lithium aluminum hydride. DMF was distilled from calcium hydride to ensure dryness. Dichloromethane, chloroform, and benzene were dried by distillation from phosphorus pentoxide. 2-Chloro-1,3,2-dioxaphospholane, bp 45.4-46.5°C/15 torr (Ref. 8: bp 45.5-47.0°C/15 torr), was prepared in 65% yield by the method of Lucas et al. [8]. 2-Chloro-2-oxo-1,3,2-dioxaphospholane, bp 102.5-105.0°C/1 torr (Ref. 9: bp 79°C/0.4 torr), was prepared in 95% yield by oxidation of 2-chloro-1,3,2-dioxaphospholane with oxygen, following the method of Edmundson [9]. Trimethylamine was prepared by reaction of trimethylamine hydrochloride with 40% sodium hydroxide, following the procedure of Adams and Brown [10]. 1,20-Eicosanediol was obtained by the lithium aluminum hydride reduction of 1,20-eicosanedioyl chloride which had been prepared by heating 1,20-eicosanedioic acid with thionyl chloride, as described previously [11].

Copoly(γ-20-(Hydroxy)eicosyl *L*-Glutamate, γ-Methyl *L*-Glutamate) (I)

Into a 300-mL round-bottomed flask, equipped with a reflux condenser with a drying tube, were placed 0.3 g (2.1 mmol) poly(γ-methyl *L*-glutamate), 2 g (6.4 mmol) 1,20-eicosanediol, 0.017 g (0.09 mmol) *p*-toluenesulfonic acid, 100 mL dichloromethane, and boiling chips [12]. The mixture was refluxed at 70°C for 15 h. Then the mixture was concentrated to one-third of its original volume under reduced pressure. The concentrate was poured into 500 mL methanol. The white precipitate was filtered off, washed three times with 150 mL of hot methanol to remove the excess unreacted 1,20-eicosanediol, and dried *in vacuo* to give 0.16 g (17.9%) of a white polymer. The nitrogen content of the resulting polymer was found to be 7.07%. From this result the extent of the ester exchange reaction, x, was estimated to be 0.85. IR (KBr) showed absorptions at 3250 (OH), 2930 (CH_2), and 1736 cm^{-1}.

Copoly(γ-20-[2-(Trimethylammonium)ethylphosphatidyl] eicosyl *L*-Glutamate, γ-Methyl *L*-Glutamate) (II)

Into a thoroughly dried 300-mL three-necked round flask, equipped with a mechanical stirrer, calcium chloride drying tube, and dropping funnel, were placed 2.3 g (5.4 mmol) I and 0.7 g (6.5 mmol) triethylamine in 180 mL dichloromethane. After cooling the solution in a Dry Ice/methanol bath (-30°C), 0.6 g (5.4 mmol) 2-chloro-2-oxo-1,3,2-dioxaphospholane in 50 mL dichloromethane were added slowly to the stirred solution over a period of 0.5 h. The reaction mixture was maintained at -10 to 0°C during the addi-

tion and then allowed to warm up to 5°C. After being kept at this temperature for 1 h, the reaction mixture was evaporated *in vacuo* in a stream of nitrogen for 1 h to give copoly(γ-20-[2-oxo-1,3,2-dioxaphospholan-2-yloxy]-eicosyl *L*-glutamate, γ-methyl *L*-glutamate) and triethylamine hydrochloride as white solids. Into a 300-ml glass pressure bottle (Top Model E, Type A) were rapidly placed the solid product and 70 mL dry DMF. The pressure bottle was closed and then shaken for 25 h in a thermostat maintained at 70°C. Then its contents were concentrated to one-third of their original volume under reduced pressure with a stream of nitrogen for 1 h. The concentrate was poured into 300 mL of methanol. The white precipitate was filtered, washed with 100 mL methanol, and dried *in vacuo* to give 3.0 g (98.9%) of a white polymer. The nitrogen content of the resulting copolymer was found to be 5.26%. From this result, x was estimated to be 0.84. IR (KBr) showed absorptions at 2930 (CH_2), 1740 (C=O), 1230, and 1060 cm^{-1} ($PO-O^-$).

Polarization Microscopy

This was carried out with a polarizing optical microscope with a Yanaco Model heating stage.

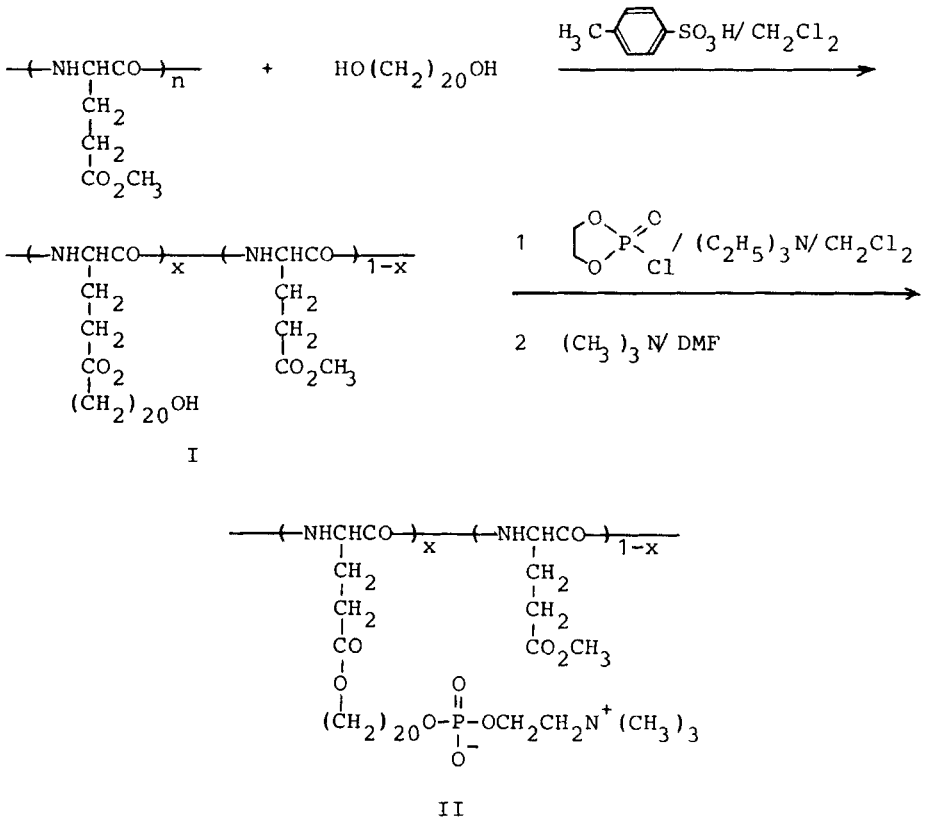
IR Measurements

The measurements were carried out with a Jasco Model IR-G spectrometer.

RESULTS AND DISCUSSION

Copoly(γ-20-[2-(trimethylammonium)ethyl phosphatidyl]eicosyl *L*-glutamate, γ-methyl *L*-glutamate) (II) was prepared as shown in Scheme 1.

Copoly[γ-20-(hydroxy)eicosyl *L*-glutamate, γ-methyl *L*-glutamate] (I) was prepared by ester exchange of poly(γ-methyl *L*-glutamate) with 1,20-eicosanediol [12]. In the IR spectrum of the reaction product (I), new absorption bands due to the stretching vibration of the hydroxy group and to the methylene groups appeared at 3250 and 2930 cm^{-1}, respectively. Copoly[γ-20-(2-oxo-1,3,2-dioxaphospholan-2-yloxy)eicosyl *L*-glutamate, γ-methyl *L*-glutamate], synthesized from I, was characterized by its IR spectrum, in which absorption band due to P=O stretching appeared at 1300 cm^{-1} and the band due to the $-P-OCH_2-$ bond appeared at 1220 and 960 cm^{-1}



SCHEME 1.

The resulting product was reacted with trimethylamine according to the method of Thoung and Chabrier [13] to afford the corresponding II as a white polymer. Product II was characterized by elemental analysis and its IR spectrum in which the absorption bands due to the PO–O^{-1} bond appeared at 1230 and 1060 cm^{-1}. The polymer was soluble in DMF, but almost insoluble in chloroform and acetone, and it exhibited a liquid crystalline phase from room temperature to 170°C. The same polymer was insoluble even in DMF when dried completely *in vacuo*.

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