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## Preparation and Properties of Phospholipid-Modified Polypeptide

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**ABSTRACT:** Poly(sodium glutamate) (DP = 160) was modified by sheep brain L- $\alpha$ -phosphatidylethanolamine with about 65% substitution in side chains via the active ester method. The structure of the modified polypeptide was characterized by its IR and <sup>1</sup>H NMR spectral data and elemental analysis. The  $\pi$ -A isotherms of the modified polypeptide at different temperatures and pH were measured. Reproducible Y-type deposition, with a deposition ratio of 0.9 Y-type Langmuir–Blodgett films (30 layers) of the modified polypeptide were also prepared, and lamellar structure, together with an average 32 Å thick monolayer was observed by transmission electron microscopy. The formation of  $\beta$ -structure but no  $\alpha$ -helix structure for cast film of the modified polypeptide was observed by circular dichroism spectroscopy. A wide-angle X-ray diffraction pattern investigation for the cast film showed a small peak at  $2\theta = 5.7^\circ$  and a big peak at  $2\theta = 20.1^\circ$ , corresponding to  $d$  spacing of 15.5 and 4.4 Å, respectively. In addition, investigation of Na<sup>+</sup> ion and K<sup>+</sup> ion transport behavior for cast film of the modified polypeptide showed that Na<sup>+</sup> ion permeating through the cast film was easier than K<sup>+</sup> ion, and both Na<sup>+</sup> ion and K<sup>+</sup> ion permeated through the film easier under acidic conditions (pH 4) than that under neutral conditions (pH 7).

## Introduction

The synthesis and property investigations of polymers with lipids and proteins are of increasing interest, because the lipids and proteins are principal components of biological membranes. Among three types of lipids found in membranes (phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin as predominant phospholipids; neutral lipids, particularly cholesterol; and glycolipids), phospholipids are the most abundant.<sup>1</sup> Accordingly, it is particularly interesting to prepare polymers with phospholipid analogs, to investigate their properties, and to obtain polymers with more desirable properties. Over the past twenty years a great number of studies concerning the synthesis and properties of polymeric phospholipid analogs have been reported.<sup>2–7</sup>

On the other hand, proteins are macromolecular polymers composed of L- $\alpha$ -amino acids linked by peptide chains.<sup>8</sup> Due to the amphiphilic properties of phospholipids, they do not form macromolecular analogs to the proteins, but form highly organized structures of macromolecular dimensions. Therefore it seemed of particular interest to prepare synthetic polypeptides containing phospholipid units in the side chains and to investigate their properties.

Many approaches to the modification of polypeptides have been performed. Nakaya et al.<sup>9</sup> introduced phosphatidylcholine analogs into poly( $\gamma$ -methyl L-glutamate). Hanabusa et al.<sup>10</sup> introduced the (4-hydroxyphenyl)porphyrin moieties into poly(L-glutamic acid)s and also found that the introduction of large amounts of porphyrin moieties into poly(L-glutamic acid)s tends to prevent  $\alpha$ -helix formation. Maeda et al.<sup>11</sup> reported a pH-induced reversible conformational change in the transmembrane polypeptide domain of the synthetic membrane, which was prepared from butyl methacrylate (backbone)–L-aspartic acid (branch) graft copolymer. Particularly, the

use of modified polypeptides in Langmuir–Blodgett (LB) films has been extensively investigated.<sup>12–14</sup> These interesting studies suggest that modified polypeptides with a rigid-rodlike backbone and flexible side chains, so-called “hairy rods”, are capable of forming stable monomolecular films at an air/water interface. And the monolayer can be further transferred to substrates by LB technique. The resulting LB films are homogenous and stable and exhibit unique properties.<sup>15</sup>

In the study reported herein, in order to construct a stable biological membrane model system having ordered oriented multilayers of the macromolecules, we synthesized the polypeptide containing phospholipid side chains and also prepared LB films of the phospholipid-modified polypeptide. L- $\alpha$ -Phosphatidylethanolamine from sheep brain was selected as a model phospholipid and introduced it into a polypeptide. In addition, we describe the  $\pi$ -A isotherms and transmission electron microscopy (TEM) analysis for LB films, CD spectroscopy measurements, wide-angle X-ray diffraction patterns, and Na<sup>+</sup> ion as well as K<sup>+</sup> ion transport behavior for obtained polypeptide cast films.

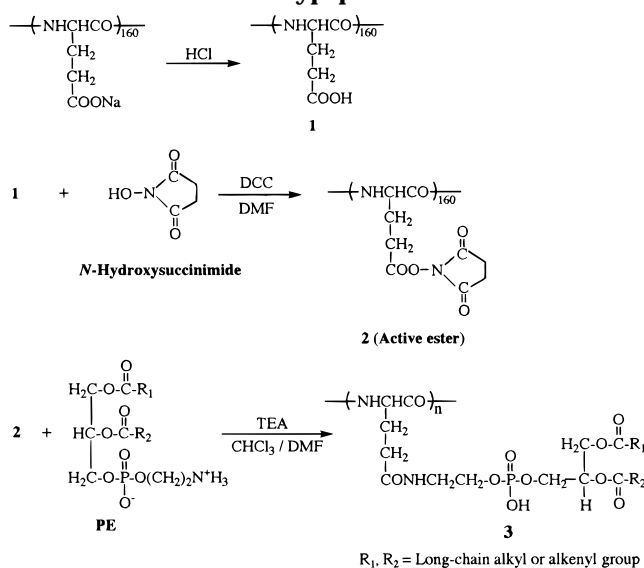
## Experimental Section

**General Methods.** The solvents and reactants, such as hydrochloride acid, *N*-hydroxysuccinimide, *N,N*-dicyclohexylcarbodiimide (DCC), and triethylamine (TEA), were of the best commercial grade available and were used without further purification unless noted. *N,N*-dimethylformamide (DMF) was distilled over calcium hydride. Chloroform was dried by distillation from phosphorus pentoxide. Ethanol was distilled in the presence of magnesium ethoxide to ensure dryness. Poly(sodium glutamate) (DP = 160) was a gift from Ajinomoto Co. Inc. L- $\alpha$ -Phosphatidylethanolamine (PE; type II-S) originated from sheep brain and was obtained from Sigma Co.

IR spectra were run on a Jasco A202 spectrometer. Absorption values are expressed in wavenumber (cm<sup>-1</sup>). <sup>1</sup>H NMR was taken on a 400 MHz  $\alpha$  FT NMR spectrometer JNM-A400, chemical shifts ( $\delta$ ) are in parts per million (ppm) relative to tetramethylsilane (TMS). TEM measured for the LB film of phospholipid-modified

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**Scheme 1. Preparation of Phospholipid-Modified Polypeptide**

polypeptide consisted of 30 layers. The picture was magnificated 250 000 times, and the modified polypeptide LB multilayers on PET were embedded in epoxy resin (Araldite) at 60 °C for 24 h. Ultrathin sections of the embedded specimens with a thickness of 80 nm were obtained with an ultramicrotome (Sorvall, Model MT-6000). The sections were stained with a 1 wt % aqueous solution of uranyl acetate and were examined with the aid of a high-resolution TEM (Hitachi, Model H-800) operated at an acceleration voltage of 200 kV. CD spectra were taken with a Jasco J-720 spectropolarimeter. Wide-angle X-ray diffraction patterns were taken with a Rigaku Geigerflex (35 kV, 25 mA, Cu K $\alpha$  line).

**Preparation of Poly(glutamic acid) (1).** The reaction Scheme 1 was utilized to prepare poly(glutamic acid) and phospholipid-modified polypeptide. To an aqueous solution of poly(sodium glutamate) (2.42 g, 0.1 mmol) in an ice bath under stirring was slowly added dilute hydrochloride acid until the "pH" was adjusted to 2, the mixture was kept in the refrigerator overnight. After the top layer was discarded by decantation, the residue was filtered and washed thoroughly with cooling water, and then it was kept in desiccator and fully dried under vacuum to give the poly(glutamic acid) in quantitative yield.

**Synthesis of Poly(glutamic acid *N*-hydroxysuccinimide ester) (2).** In a round-bottom flask equipped with a magnetic stir bar and a rubber septum attached to a nitrogen line and a bubbler, poly(glutamic acid) (0.35 g; 0.017 mmol), which was fully dried before use, *N*-hydroxysuccinimide (2.0  $\times$  excess to each unit; 0.62 g, 5.39 mmol) and *N,N*-dicyclohexylcarbodiimide (2.2  $\times$  excess to each unit; 1.23 g, 5.96 mmol) were dissolved in 35.0 mL of dry DMF. The flask was kept at room temperature and stirred for 4 days. After the formed needle crystalline, byproduct, dicyclohexylcarbodiurea, was filtered off, the filtrate was concentrated and reprecipitated from ethanol. And after the top layer was discarded by decantation, the residue was filtered and washed thoroughly with ethanol, and then it was kept in a desiccator and fully dried under vacuum to afford active ester **2** as a white powder: yield 0.38 g (62%); IR (neat) 1780 cm<sup>-1</sup> C=O stretch (succinimidyl ester end group); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.81 ppm (s, succinimide, 4H). The formed dicyclohexylcarbodiurea was filtered off and

fully dried with vacuum at 40 °C; 3.74 g active ester substitution yield, 98%.

**Synthesis of Polypeptide (3).** To a solution of poly(glutamic acid *N*-hydroxysuccinimide ester) (**2**; 0.35 g, 0.01 mmol) in dry DMF (50 mL) was added L- $\alpha$ -phosphatidylethanolamine (2.14 g) and triethylamine (0.63 g, 6.22 mmol) previously dissolved in dry chloroform (150 mL) under stirring. After the mixture was kept at room temperature for 2 days with magnetic stirring, the solvents were removed under vacuum. The residue was dissolved in the minimum amount of chloroform and reprecipitated from a large amount of ethanol. And after the top layer was discarded by decantation, the residue was filtered and washed thoroughly with ethanol, and then it was kept in a desiccator and dried under vacuum to obtain modified polypeptide **3** as a yellow brown solid: yield 0.64 g; IR (neat) 2915, 2850 (CH<sub>2</sub>), 1650 (CONH), 1240 (P=O), 1070 cm<sup>-1</sup> (POCH<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.88 (t, 6H, CH<sub>3</sub>, *J* = 6.8 Hz), 1.26 (m, 54H, CH<sub>2</sub>), 2.0 (m, 4H, =CHCH<sub>2</sub>), 2.75 (t, 6H, CH<sub>2</sub>CO), 3.4–4.5 (m, 9H, CONCHCON, POCH<sub>2</sub>CH, CH<sub>2</sub>OCO, NHCH<sub>2</sub>CH<sub>2</sub>O), 5.35 ppm (m, 3H, >CHOCO, CH=CH). Elemental analysis P (2.35%). Substitution degree with PE was estimated to be about 65%.

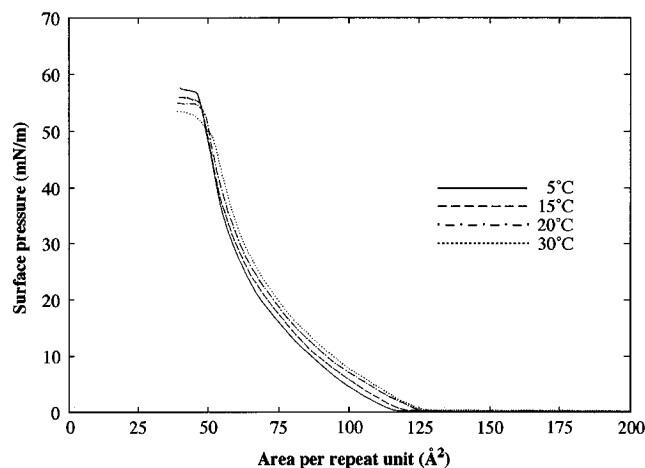
**Langmuir Isotherms and Film Deposition.** The  $\pi$ -*A* isotherms were measured for a monolayer (by using a toluene solution of polymer **3** in a concentration of 2.0 mg/mL) on a subphase of water at 5, 15, 20, and 30 °C and pH 7, as well as at pH 5, 7, 9, and 11 and 20 °C by Lauda Filmbalance (Model, FW-2, Lauda Co.), at a speed of 61.8 cm<sup>2</sup>/min for 15 min, respectively.

LB multilayers was deposited onto a hydrophobic poly(ethylene terephthalate) (PET) film plate. Deposition was generally carried out at 35 mN/m, a dipping speed of 1 cm/min, and an immersion time of 1 min. An LB multilayers (30 layers) of the modified polypeptide **3** was deposited onto the PET substrate.

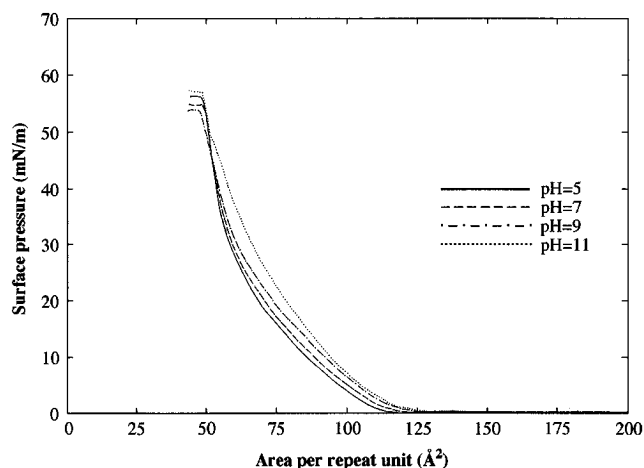
**Na<sup>+</sup> Ion and K<sup>+</sup> Ion Transport Across the Film Experiment.** The modified polypeptide **3** was dissolved in chloroform and was cast on a Teflon-brand plate to obtain the film by solvent evaporation. The film (24  $\mu$ m thick) determined by micrometer (Mitutoyo, no. 293-401) was fixed vertically in the center of a diaphragm cell (diameter, 14 mm). To investigate the relationship between the cation permeability and pH, both sides of the cast film were immersed in 1  $\times$  10<sup>-4</sup> N HCl solution at pH 4 (acidic condition) or in distilled water at pH 7 (neutral condition) for 3 h, respectively, and then 50 mL of 0.025 M NaCl and KCl aqueous solution was placed into the left side, and 50 mL of distilled water was poured into the right side, simultaneously. Both sides were kept at 25 °C. Samples (0.1 mL) were taken from both sides and diluted with 2 mL of distilled water every hour to determine the ion concentration of the two sides by atomic absorption spectrometry.

## Results and Discussion

**Polypeptide Synthesis.** The phospholipid-modified polypeptide was prepared as shown in Scheme 1. To obtain poly(glutamic acid) (**1**), poly(sodium glutamate) (DP = 160) was dissolved in water and treated with hydrochloride acid. Successively, according to the methods described by Gewehr et al.<sup>16</sup> and Yang et al.,<sup>17</sup> the obtained **1** after being fully dried was reacted with *N*-hydroxysuccinimide in the presence of DCC in dry DMF to afford the active ester, poly(glutamic acid *N*-hydroxysuccinimide ester) (**2**). The substituted yield of *N*-hydroxysuccinimide into polypeptide was almost



**Figure 1.**  $\pi$ - $A$  isotherms of phospholipid-modified polypeptide **3** at different temperatures. All isotherms were measured at pH 7.

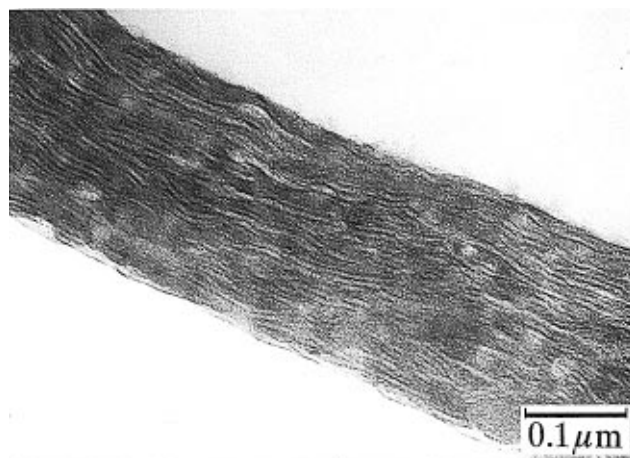


**Figure 2.**  $\pi$ - $A$  isotherms of phospholipid-modified polypeptide **3** at different pH. All isotherms were measured at 20 °C.

quantitative, determined by the amount of byproduct dicyclohexylcarbodiurea. The IR measurement for active ester **2** shows the absorption bands at  $1780\text{ cm}^{-1}$  owing to the C=O stretch (succinimidyl ester end group), and the  $^1\text{H}$  NMR spectrum for active ester **2** shows the peak at 2.81 ppm due to the succinimide. The desired polypeptide **3** containing phospholipid side chains was prepared by reaction of active ester **2** with L- $\alpha$ -phosphatidylethanolamine (PE) in the presence of triethylamine in a mixture of dry DMF and chloroform (volume ratio 1/3). The obtained phospholipid-modified polypeptide **3** was soluble in chloroform but insoluble in trimethyl phosphate. The characterizations of the synthetic polypeptide were based on its IR and  $^1\text{H}$  NMR spectral data and elemental analysis. The IR spectrum of modified polypeptide **3** showed the absorption bands due to P=O at  $1240\text{ cm}^{-1}$  and POCH<sub>2</sub> at  $1070\text{ cm}^{-1}$ .  $^1\text{H}$  NMR (in CDCl<sub>3</sub>) showed the presence of saturated and/or unsaturated long alkyl chains at 0.88 (CH<sub>3</sub>), 1.26 (CH<sub>2</sub>), and 5.35 ppm (CH=CH); for details, see experimental procedures. The elemental analysis showed that the phosphorus content for modified polypeptide **3** was 2.35%. These results suggest that about 65% of the PE has been successfully incorporated into polypeptide.

**Measurements of  $\pi$ - $A$  Isotherms and LB Multilayers.** Initially, a monolayer of modified polypeptide **3** was prepared [by using a toluene solution of the modified polypeptide **3** in concentration of 2.0 mg/mL on a subphase of water at 5, 15, 20, and 30 °C and pH 7 (Figure 1), as well as at pH 5, 7, 9, and 11 and 20 °C (Figure 2)], and their surface pressure ( $\pi$ )-area ( $A$ ) isotherms were measured by Lauda Filmbalance, respectively. The collapse pressures,  $\pi_c$ , are greater than 53 mN/m as shown in Figures 1 and 2. The following points are obvious from a comparison of the various isotherms. The nature of the isotherms at different temperatures and pH generally makes little difference to the shape and position of the isotherms. The areas per repeating unit measured at surface pressures of 30 mN/m,  $A_{30}$ , were in the range of 56–61 Å<sup>2</sup> for Figure 1 and 58–66 Å<sup>2</sup> for Figure 2. While the areas per repeating unit measured at collapse pressures were in the range of 39–47 Å<sup>2</sup> for Figure 1 and 43–52 Å<sup>2</sup> for Figure 2.

The isotherms at different temperatures in Figure 1 show that as temperature is increased, the areas per repeating unit are also increased. The isotherms at different pH in Figure 2 show that as the pH is

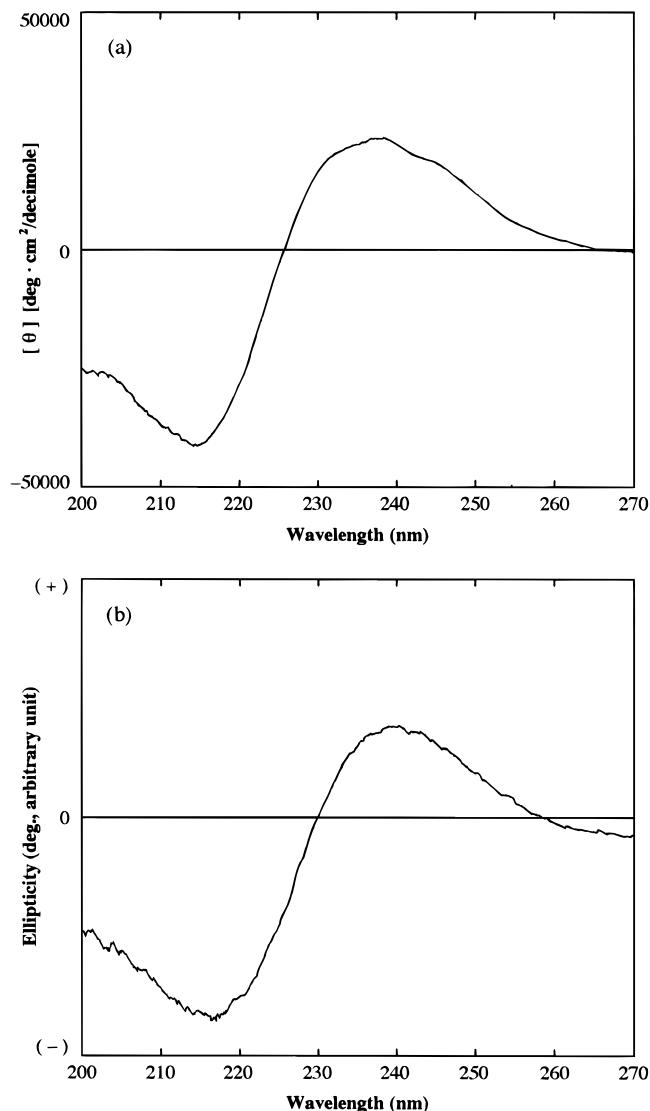


**Figure 3.** Transmission electron micrograph of a cross-sectional view of the phospholipid-modified polypeptide **3** LB films (30 layers) stained on a PET film plate and imaged at an original 250 000 $\times$  magnification.

increased, the areas per repeating unit are also increased. This may be due to extension of the polymer backbone resulting from the anion electric charge repulsion between the phosphate parts.

The phospholipid-modified polypeptide **3** that formed a stable monolayer on water could be transferred at surface pressures of 35 mN/m onto a hydrophobic poly(ethylene terephthalate) (PET) film plate. A reproducible Y-type deposition, with a deposition ratio of 0.9 LB film, was obtained. Sufficient thick films were deposited (30 layers) to allow TEM measurements to be carried out.

**Transmission Electron Microscopy Studies of LB Films.** The morphology of the LB multilayers of modified polypeptide **3** was investigated by high-resolution TEM. Figure 3 represents a typical transmission electron micrograph of a cross-sectional view of the modified polypeptide LB multilayers. The LB films consisted of 30 layers and the picture was magnified 250 000 $\times$ . This figure shows not only a regular lamellar structure but also a compact film of uniform thickness without pinholes. These findings indicate that a monolayer of modified polypeptide could be transferred from a water subphase onto ordered multilayers of precise thickness by the LB deposition technique. The dark region in the photograph corresponds to the polar group domains of the polymer stained with uranyl acetate, while the white region corresponds to their hydrophobic

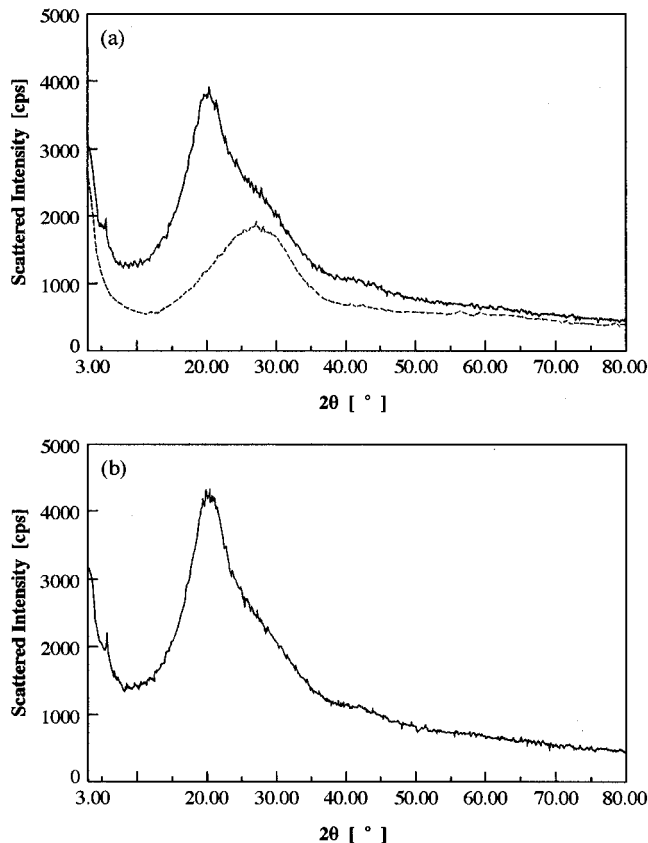


**Figure 4.** Circular dichroism spectra: (a) Poly(glutamic acid *N*-hydroxysuccinimide ester) (**2**) in trimethyl phosphate; (b) phospholipid-modified polypeptide **3** film cast from chloroform solution.

alkyl group domains. The TEM shows layered structures with microphase separation. According to the method described by Duda et al.<sup>18</sup> and from the photograph, an average monolayer is estimated to be 32 Å thick, because the thickness of the arbitrary 20 layers is 640 Å. These findings suggest that the structure of LB films of phospholipid-modified polypeptide **3** agrees with the model of polymeric lipids.<sup>19</sup>

#### Structural Analysis by Circular Dichroism (CD).

The CD spectra of polypeptides give important information on the conformation of the polymer backbone in solution.<sup>20</sup> The CD spectra in the 200–270 nm region for **2** in trimethyl phosphate (TMP) solution (Figure 4a) and for phospholipid-modified polypeptide **3** cast film (Figure 4b) were measured. In Figure 4a, the negative maximum peak at 214.4 nm, together with the positive maximum peak at 238.0 nm, was observed. Moreover, a smaller positive maximum peak at 247 nm was also observed. Although modified polypeptide **3** was soluble in chloroform, its CD measurement could not be carried out in chloroform solution below 250 nm. Also, the modified polypeptide **3** was insoluble in TMP. In order to measure the modified polypeptide's CD spectrum, the cast film of **3** was prepared from a chloroform solution

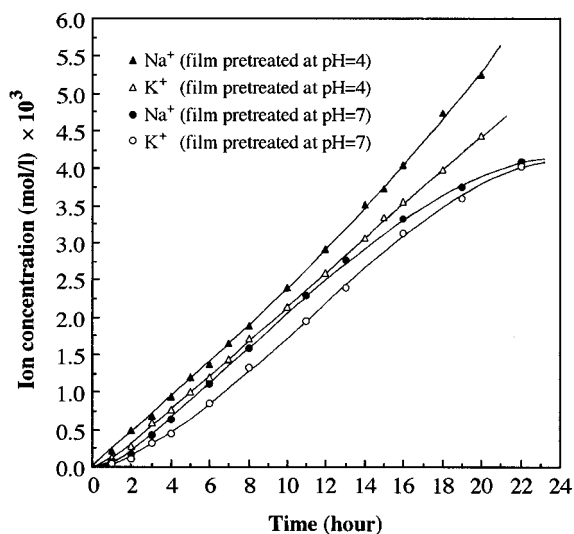


**Figure 5.** Wide-angle X-ray diffraction patterns for cast film of phospholipid-modified polypeptide **3**: (a) diffractograms of cast film of **3** (—) and the glass holder (---); (b) diffractogram of cast film of **3** at a right angle to (a).

on a quartz plate (5 mm thick). As shown in Figure 4b, the positive peak at 239.6 nm and negative peak at 216.2 nm indicate the formation of  $\beta$ -structure; however,  $\alpha$ -helix structure was not observed. These results suggest that the introduction of a large amount of phospholipid moieties into the polypeptide tends to prevent  $\alpha$ -helix formation. These findings are similar to the results of Hanabusa et al.<sup>10</sup>

#### Structural Analysis by Wide-Angle X-ray for Cast Film.

The structure of the cast film of **3** was also investigated by wide-angle X-ray diffraction analysis (WXR). The cast film was prepared from a chloroform solution of modified polypeptide **3** on a glass plate. The WXR was measured at room temperature from two directions at right angles, and the diffractograms are illustrated in Figure 5. As a reference, the X-ray measurement of the glass holder, which was used to prepare the cast film, was also carried out, and its diffraction scans were shown in Figure 5a. The diffractogram of **3** shows a small peak at  $2\theta = 5.7^\circ$  ( $d = 15.5$  Å) and a big peak at  $2\theta = 20.1^\circ$  ( $d = 4.4$  Å) in Figure 5a, while it shows a small peak at  $2\theta = 5.7^\circ$  ( $d = 15.5$  Å) and a big peak at  $2\theta = 19.7^\circ$  ( $d = 4.5$  Å) in Figure 5b (right angle to Figure 5a). Based on the results of WXR, it is assumed that the alkyl groups in side chains of modified polypeptide **3** are in a crystalline state, giving the strong reflection at  $4.4$ – $4.5$  Å<sup>-1</sup>. Its interplanar spacings are little different at two directions, perhaps due to the manifold hydrophobic side chains. However, the small peaks with spacing of 15.5 Å in Figure 5 are not clear. The peaks at  $2\theta = 20.1^\circ$  in Figure 5a and  $2\theta = 19.7^\circ$  in Figure 5b are unsymmetrical; it is thought to come from the influence of the



**Figure 6.** Changes in the concentration of Na<sup>+</sup> ion and K<sup>+</sup> ion on the right side of the cast film at 25.0 °C: initial concentration of Na<sup>+</sup> ion and K<sup>+</sup> ion on the left side, 0.025 M. A diaphragm cell that consisted of two detachable parts with the volume for 50 mL was used. The transported concentration of Na<sup>+</sup> ion and K<sup>+</sup> ion was measured by atomic absorption spectrometer.

glass holder, which shows a broad peak in the range of 23–32° centered at 27.5°.

#### Permeability of Na<sup>+</sup> and K<sup>+</sup> Ions to Cast Film.

Some papers<sup>21,22</sup> on the transport properties of small molecules through synthetic polypeptides of rodlike,  $\alpha$ -helical structure have been published. The studies on poly(*n*-alkyl-L-glutamate)<sup>21,22</sup> indicate that the diffusion of small molecules in polypeptide occurs through the side-chain region between helices. Moreover, biomembranes consist of a continuous nonpolar hydrocarbon matrix from the phospholipid bilayer substantially impermeable to most polar substances and protein molecules capable of reversibly binding specific substrates and of transporting them across the membrane even against a concentration gradient. The protein molecules may undergo reversible conformational changes to create a "hole" or a "channel" in the membrane for the specific substrate transported.<sup>23</sup> In order to mimic the function of a biomembrane, i.e., specific, facilitated, or active transport, numerous studies on model systems have been reported, most of which examined the transport through fluid membranes or lipid bilayer membranes by the aid of naturally occurring and synthetic ionophores.<sup>24</sup> However, such mobile carrier-mediated transports appear not to be very common in natural systems, and neither liquid membranes nor lipid bilayer membranes are satisfactory as model systems and for possible practical applications because of their low stability.

To investigate the ion permeability of the phospholipid-modified polypeptide film, a preliminary experiment of Na<sup>+</sup> and K<sup>+</sup> ion transport across the cast film of modified polypeptide **3** was carried out. Before the measurement was carried out, the cast film was immersed into a solution of pH 4 or pH 7 for 3 h. The relationship between the time and ion concentration change are shown in Figure 6. The results show that Na<sup>+</sup> ion permeating through the film was easier than K<sup>+</sup> ion, and both Na<sup>+</sup> ion and K<sup>+</sup> ion permeate through the film more easily under acidic (pH 4) than under neutral conditions (pH 7). From these results, it is considered that continuous phases of poly(glutamic

acid) domain are formed in the membrane and foundation as permeating pathways, or "transmembrane channels", for Na<sup>+</sup> ion and K<sup>+</sup> ion. Under acidic conditions (pH 4), the transport rates of Na<sup>+</sup> ion and K<sup>+</sup> ion increased, compared with that under neutral conditions (pH 7), which is considered to be ascribed, at least partly, to the pH-induced conformational change of the poly(glutamic acid) domain. In other words, the ion permeability is related to the decomposing behavior of POH.

On the basis of the above results, the membrane of the phospholipid-modified polypeptide may be regarded as a good model of a biomembrane; the transmembrane polypeptide domain undergoes the pH-dependent reversible conformational change, with the impermeable polymer domain as the stable matrix.

#### Conclusions

Our results have shown that the polypeptide has been successfully modified with about 65% substitution of phospholipid in the side chains. This modification was carried out by using poly(sodium glutamate) (DP = 160) and L- $\alpha$ -phosphatidylethanolamine from sheep brain as materials in the presence of dicyclohexylcarbodiimide and triethylamine. The structure of modified polypeptide was characterized by its IR and <sup>1</sup>H NMR spectral data and elemental analysis. The  $\pi$ -A isotherms of modified polypeptide were measured at different temperatures and pH. Furthermore, reproducible Y-type deposition with a deposition ratio of 0.9 Y-type Langmuir-Blodgett film (30 layers) of the modified polypeptide were also prepared, and lamellar structure, together with an average 32 Å thick of monolayer, were observed by transmission electron microscopy. The formation of  $\beta$ -structure but no  $\alpha$ -helix structure for cast film of the modified polypeptide was observed by circular dichroism spectroscopy. Wide-angle X-ray diffraction pattern investigation for the cast film showed a small peak at  $2\theta = 5.7^\circ$  and a big peak at  $2\theta = 20.1^\circ$ , correspond to *d* spacing of 15.5 and 4.4 Å, respectively. In addition, investigation of Na<sup>+</sup> and K<sup>+</sup> ion transport behavior for cast film of the modified polypeptide showed that Na<sup>+</sup> ion permeating through the cast film was easier than K<sup>+</sup> ion, and both Na<sup>+</sup> and K<sup>+</sup> ions permeated through the film more easily under acidic (pH 4) than under neutral conditions (pH 7). Although the  $\alpha$ -helix behavior was not as clear, this investigation has provided some information on the  $\beta$ -structure and morphology of LB multilayers of polypeptide with phospholipid side chains. From the biomembrane mimic point of view, these phospholipid-modified polypeptide multilayers could probably be utilized as ultrathin membranes of biomedical sensors or devices.

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