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Syntheses of a Novel Diamine Monomer and Aromatic Polyamides Containing Phosphorylcholine group

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The synthesis of novel aromatic diamine compounds containing phosphorylcholine (PC) group was carried out, which gave the high molecular weight polyamides with PC group by polycondensation. The obtained polymers exhibited the excellent biocompatibility, which would be due to the surface structure derived from PC side chain. In addition, the obtained polymers showed the high thermal stability up to 250℃, at which the thermal degradation of PC moiety occurred. Therefore, these polymers are expected as tough biocompatible materials for the use of biomedical devices.

Key words: Biomaterial / Biocompatibility / Diamine monomer / Phosphorylcholine / Polyamide

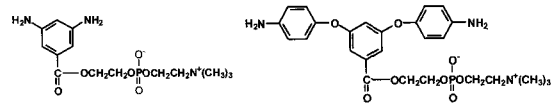
1. INTRODUCTION

Recently, various polymeric materials are used in the medical fields as not only medical instruments but also artificial organs which contact with living body, especially blood. For example, segmented polyurethane, polydimethylsiloxane, and polytetrafluoroethylene are used as artificial heart, artificial lung, and artificial blood vessel, respectively. However, when the surface of these polymers was contacted with blood components, a lot of platelets are adhered on the surface, therefore, these materials have been applied only for temporary uses.

On the other hand, the ideal blood-compatible surface is the blood vessel inner surface, in other words, a biomembrane. Based on the idea of which imitate the structure of biomembrane, the biomimetic polymer, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, was developed [1]. MPC polymer contains the phosphorylcholine (PC) group, which is one of the phospholipid polar head groups. MPC polymer exhibits a surface property that resists nonspecific protein adsorption and cell adhesion. Then, MPC polymer has been used in many biomaterial fields, and applied research were widely proceeded to expand the use of it [2-8]. However, MPC polymer have not enough durability to alcohol [9]. In addition, it is thought that the mechanical property and the heat-resistance is inadequate, which is due to the main chain structure consisted of methacrylate units.

In our previous study, we have synthesized the aromatic copolyamides containing PC group by polycondensation of 2-(3,5-diaminophenylcarbonyloxy)-ethyl phosphorylcholine (DAPC in Fig. 1) and other comonomer with acid chloride [10,11]. It was confirmed that the obtained copolyamides exhibited the excellent blood-compatibility. However, the molecular weight and the PC content was not enough to produce a self-standing film and to exhibit the higher biocompatibility, which would be due to the low reactivity and also the highly hygroscopic property of DAPC.

The purpose of our study is the syntheses of novel polymer compounds in order to create the practical biomaterials for several applications, which exhibit the excellent biocompatibility in addition to the processability, the durability to solvents, the thermal stability and the mechanical strength. For this purpose, we designed a new diamine monomer containing PC group, 2-[3,5-Bis(4-aminophenoxy)phenylcarbonyloxy]-ethylphosphorylcholine (BAPPC in Fig. 1), to solve these problems of DAPC. BAPPC is expected to show the higher reactivity in the polymerization than DAPC, which would be due to the relatively higher reactive amino groups on *p*-position of phenoxy groups. In this paper, the synthetic procedure of BAPPC and the preparations of high molecular weight copolyamides by using BAPPC will be described. Furthermore, the characteristics of the obtained polyamides were discussed for the applications as biomedical devices, where the solubility, the blood compatibility, the thermal properties and the surface properties were investigated in detail to reveal the possibility of a durable biocompatible polymer material.



DAPC BAPPC

Fig. 1 Chemical structures of diamine monomers containing PC group.

2. EXPERIMENTAL

2.1 Materials

Tetrahydrofuran (THF) and triethylamine (TEA) were purified by distillation on Na and CaH_2, respectively. 2,2-Bis[4-(aminophenyloxy)phenyl]-propane (DA) was prepared according to the literature [11]. Other chemical reagents were used without further purification.

2.2 Synthesis of methyl 3,5-dihydroxybenzoate (1)

Under an argon atmosphere, 3.33 ml of conc. H_2SO_4 were added to a solution of 3,5-dihydroxybenzoic acid

(10.0 g, 64.9 mmol) in 100 ml of methanol. After the mixture was refluxed for 18 h, the saturated aqueous solution of $NaHCO_3$ was added and it was extracted with ethyl acetate. Then, the solvents were evaporated to afford 10.0 g of **1** as a white powder. Yield: 92.7 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 3.80 (3H, s), 6.44 (1H, t, *J*=2.44Hz), 6.83 (2H, d, *J*=2.44Hz), 9.53 (2H, s).

2.3 Synthesis of methyl 3,5-bis(4-nitrophenyloxy)-benzoate (**2**)

Under an argon atmosphere, 4-fluoronitrobenzene (8.39 g, 59.5 mmol) and K_2CO_3 (8.22 g, 59.5 mmol) were added to a solution of **1** (5.00g 29.7 mmol) in 50 ml of dimethylacetoamide. After stirring at 85℃ for 5 h, the mixture was poured into an excess amount of distilled water. The precipitated solid was filtered and dried *in vacuo* to afford 10.4 g of **2** as a white powder. Yield: 85.4 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 3.85 (3H, s), 7.27 (4H, m), 7.32 (1H, s), 7.53 (2H, s), 8.28 (4H, m).

2.4 Synthesis of 3,5-bis(4-nitrophenyloxy)benzoic acid (**3**)

Under an argon atmosphere, **2** (10.4 g, 25.4 mmol), acetic acid (75 ml), sulfuric acid (30 ml) and distilled water (20 ml) were mixed and the mixture was stirred at 120℃ for 18 h. Then, it was poured into an excess amount of distilled water. The precipitated solid was filtered and dried *in vacuo* to afford 9.28 g of **3** as a white powder. Yield: 92.3 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 7.26 (4H, d, J=8.78Hz), 7.29 (1H, s), 7.51 (2H, s), 8.28 (4H, d, J=8.78Hz), 12.2 (1H, s).

2.5 Synthesis of 2-hydroxyethyl 3,5-bis(4-nitrophenyloxy) benzoate (**4**)

Under an argon flow, the mixture containing **3** (14.7 g, 37.1 mmol), 150 ml of thionyl chloride and 0.2 ml of dimethylformamide was refluxed for 6 h. After the excess thionyl chloride was distilled off, the obtained solid was dissolved in 130 ml of THF. Then, the mixture of ethylene glycol (20.6 ml, 371 mmol) and 10.3 ml of TEA in 290 ml of THF was gradually added to the solution at 0℃. The reaction mixture was stirred at room temperature for 20 h, and it was poured into an excess amount of distilled water. The mixture was extracted with chloroform, and the product was purified by recrystallization from chloroform to afford 9.18 g of **4** as a yellow powder. Yield: 56.0 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 3.70 (2H, q, *J*=5.04Hz), 4.29 (2H, t, *J*=4.63Hz), 4.93 (1H, t, *J*=5.61Hz), 7.26 (4H, d, *J*=9.27Hz), 7.29 (1H, s), 7.63 (2H, d, *J*=1.95Hz), 8.27 (4H, d, *J*=9.27Hz).

2.6 Synthesis of 2-[2-{3,5-bis(4-nitrophenyloxy)-benzoyloxy}ethyl]-2-oxo-1,3,2-dioxaphospholane (**5**)

Under an argon atmosphere, 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP, 8.60 ml, 91.4 mmol) was gradually added to a solution containing **4** (9.00 g, 20.4 mmol) and 12 ml of TEA dissolved in 110 ml of THF at 0℃. After stirring for 2 h at r.t., the reaction mixture was poured into an excess amount of distilled water and then extracted with chloroform. Then, the solvent was evaporated *in vacuo* to afford 10.9 g of **5** as a pink powder. Yield: 97.7 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 4.36 (6H, m), 4.47 (2H, d, *J*=4.89Hz), 7.25 (4H, m), 7.27 (1H, s), 7.60 (2H, s), 8.28 (4H, d, *J*=9.27Hz).

2.7 Synthesis of 2-[3,5-bis(4-nitrophenyloxy)-benzoyloxy]ethyl phosphorylcholine (**6**)

Under an argon atmosphere, trimethylamine (3.54 ml, 39.9 mmol) was added to a solution of **5** (10.9 g, 20.0 mmol) in 130 ml of acetonitrile at 0℃, then the reaction vessel was sealed with a glass cap. After stirring at 60℃ for 20 h, the reaction mixture was evaporated, and the obtained product was purified by recrystallization from acetonitrile to afford 11.3 g of **6** as a white powder. Yield: 93.1 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 3.16 (9H, s), 3.59 (2H, s), 4.01 (2H, s), 4.10 (2H, s), 4.42 (2H, s), 7.22 (1H, m), 7.27 (4H, d, *J*=9.27Hz), 7.58 (2H, d, *J*=2.44Hz), 8.27 (4H, d, *J*=9.27Hz).

2.8 Synthesis of 2-[3,5-bis(4-aminophenyloxy)benzoyl-oxy]ethyl phosphorylcholine (BAPPC)

5% Pd on charcoal powder (0.62 g, 0.29 mmol by Pd) was suspended in a solution of **6** (8.87 g, 14.7 mmol) in 70 ml of methanol. The mixture was degassed under reduced pressure at -78℃, and the vessel was filled with H_2 gas at over 760 mmHg. After stirring for 20 h at r. t., the Pd on charcoal was filtered off washing with THF, and the solvent was evaporated under reduced pressure. Then, the residue was poured into an excess amount of diethyl ether, and the product was filtered and dried *in vacuo* to afford 7.81 g of BAPPC as a yellow powder. Yield: 97.7 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 3.15 (9H, s), 3.33 (4H, bs), 3.52 (2H, t, *J*=4.63Hz), 3.94 (2H, t, *J*=8.78Hz), 4.06 (2H, s), 4.33 (2H, t, *J*=4.88Hz), 6.58 (4H, m), 6.61 (1H, s), 6.78 (4H, d, *J*=8.78Hz), 7.02 (2H, d, *J*=1.95Hz).

IR, ν (KBr, cm^{-1}): 3219 (-NH_2), 1717 (C=O), 1240 (P=O), 1209 (C-O-C).

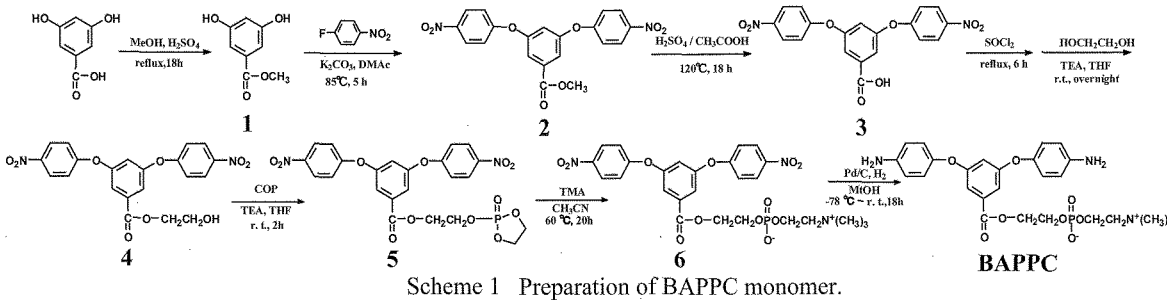
2.9 General procedure of polymerization

The preparation of CPAPC-1 listed in Table 1 is given as a representative example.

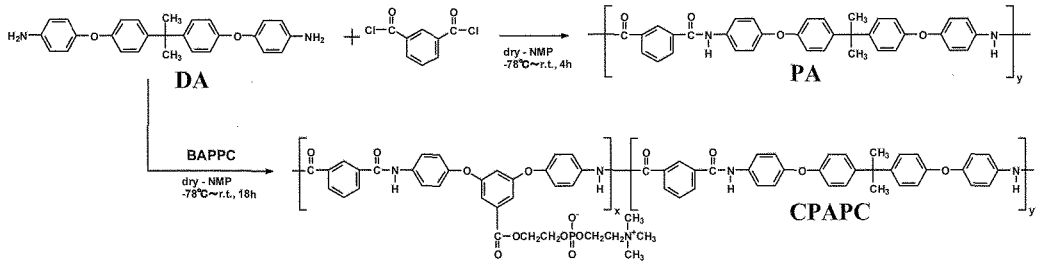
Under an argon atmosphere, BAPPC (0.40 g, 0.74 mmol), DA (0.91 g, 2.22 mmol) and isophthaloyl chloride (0.5 g, 2.46 mmol) were mixed in 3.28 ml of NMP at -78℃. The mixture was stirred for 18 h with increasing temperature from -78℃ to r. t. Then, the mixture was poured into excess methanol to precipitate the polymer, and it was filtered and purified by reprecipitation from its NMP solution to methanol. Finally, the product was dried *in vacuo* to afford 1.31 g of CPAPC-1 as a brown powder. Yield: 91.8 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 1.65 (6H, s), 3.11 (9H, s), 3.53 (2H, s), 4.01(2H, s), 4.10 (2H, s), 4.36 (2H, s), 6.89 (4H, d, *J*=6.83Hz), 6.99 (4H, d, *J*=6.83Hz), 7.08 (5H, s), 7.20 (6H, m), 7.63 (1H, s), 7.80 (4H, d, *J*=8.29Hz), 7.86 (4H, s), 8.13 (2H, d, *J*=7.32Hz), 8.56(1H, s), 10,39 (2H, d, *J*=12.68Hz).

IR, ν (KBr, cm^{-1}): 3219 (-NH_2), 1717 (C=O), 1240 (P=O), 1209 (C-O-C).



Scheme 1 Preparation of BAPPC monomer.



Scheme 2 Preparation of PA and CPAPC

Table 1 Results of polymerizations.

Code	Composition (mol%) BAPPC/BPADA	x/y[a)]	Mn[b)] (x10^4)	Mw/Mn[b)]	Yield (%)
CPAPC-1	30/70	24/76	18.2	4.03	91.8
CPAPC-2	50/50	46/54	57.9	2.01	89.7
CPAPC-3	70/30	54/46	59.1	2.05	54.5

a) The copolymer composition, x/y, was determined by ^{1}H-NMR.
b) Mn and Mw were estimated by GPC using DMF as eluent.

2.10 Characterizations

^{1}H-NMR spectra were conducted with a JEOL NM-TH5SK 400MHz FT-NMR spectrometer, and Infrared (IR) spectra were recorded with a Shimadzu FTIR-8400 spectrometer. The molecular weights of polymers were estimated by Tosoh gel permeation chromatography (GPC) system equipped with a pump of CCPD, three columns of TSK gels Multipore HXL-M, a column oven of CO-8010 and RI detector of RI-8010 in DMF eluent at 45℃. Average molecular weights were evaluated by polystyrene standards.

The surfaces of the polymer films were analyzed with an X-ray photoelectron spectroscope (ULVAC-PHI Quantum 2000 XPS). The take-off angle of photoelectrons was adjusted to be 90 degree. The thermo gravimetric analysis was conducted with Seiko Instruments Inc. TG/DTA 6200, which was carried out under N_2 flow at a heating rate of 10 ℃/min. The differential scanning calorimetry was performed with Seiko Instruments DSC 6200, which was carried out under N_2 flow at a heating rate of 10℃/min.

2.11 Evaluation of blood compatibility

Circular pieces of poly(ethylene telephthalate) (PET) films (diameter: 14 mm, thickness: 0.2 mm) were dipped in 0.5 wt% polymer solutions in NMP for 30 min. Then, the solvent was removed slowly at 60℃ for 2 h, and the films were dried *in vacuo*. After the polymer coating films were immersed in phosphate buffered solution (PBS) for overnight and in 0.7 ml of human whole blood or platelet-rich plasma (PRP) prepared from healthy donor. Then, the films were incubated for 60 min at 37℃, and PRP was removed and the films were washed with PBS. The surfaces of the polymer films were observed by a scanning electron microscope (SEM) using JEOL JSM-5200, and the number of adhered platelets was estimated by the procedure written in our literature [11].

3. RESULTS AND DISCUSSION

3.1 Preparations of BAPPC monomer and polyamides

The synthetic route of the novel diamine monomer containing PC group, BAPPC, is outlined in Scheme 1. All of the reaction steps proceeded smoothly in high yields. The chemical structure of BAPPC was confirmed by IR and ^{1}H-NMR spectra. This novel diamine compound, BAPPC, would be useful for the synthesis of various aromatic polymers which has PC group in the side chain.

The synthesis of copolyamide (CPAPC) was carried out by the polycondensation of BAPPC with acid chloride and another diamine comonomer, as shown in Scheme 2. As the comonomer, 2,2-Bis[4-(amino-phenyloxy)phenyl]propane (DA) was used to make the polymer soluble in some solvents. On the other hand, a polyamide without PC group (PA) was prepared from DA and isophthaloyl chloride to compare the physical property with CPAPC.

Table 1 summarizes the results of polymerizations. Three copolyamides with different contents of PC unit were prepared by changing the amount of BAPPC in the feed of polymerization. The obtained polyamides had the number-average molecular weights (*M*n) more than 10^5. It suggested that this novel diamine monomer containing PC group has high reactivity in the polymerization, and this diamine monomer may be useful when synthesize another polymer such as polyurea, polyimide and poly(urethane-urea) which contains PC group.

3.2 Characterization of Polyamides

The CPAPCs were soluble in aprotic polar solvents at room temperature, whereas they were insoluble in water, methanol, ethanol, acetone and other ordinary organic solvents. This solubility in specific solvents is advantageous in the processing for medical devices, and

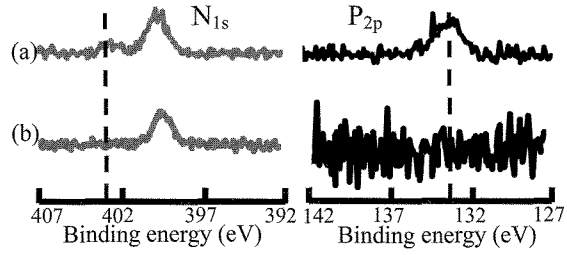


Fig. 2 XPS spectra of (a) CPAPC-2 and (b) PA film surfaces.

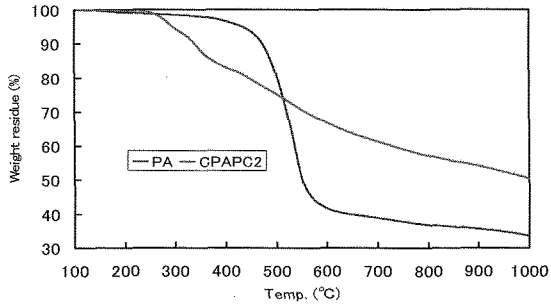


Fig. 3 TGA curves of PA and CPAPC-2

the insolubility in other solvents enables the material durable to the solvents.

The surface chemical structure of the CPAPC membrane was analyzed by X-ray photoelectron spectroscope (XPS), as shown Fig. 2. At the CPAPC film surface, nitrogen and phosphorus peaks which were attributed to the ammonium group ($-N^+-$) and the phosphorus in phosphate group were observed at 402.5 and 133.0 eV, respectively. It was suggested that the CPAPC film surface was covered with the polar PC group.

Fig. 3 shows the thermo gravimetric analysis (TGA) curves of PA and CPAPC film under N_2 flow. The thermal degradation of PA started at about 400 ℃ but the CPAPC degraded at about 250 ℃. This would be due to the degradation in polymer side chain, which consisted of the PC or spacer moiety. In nitrogen atmosphere, the aromatic backbone would be carbonized, which resulted in the residue more than 30 wt.% at 1000℃, and the phosphate moiety of CPAPC would be incombustible. However, the heat resistance of CPAPC until 250℃ is enough to use for biomaterial devices.

On the other hand, glass transition temperature (T_g) and melting temperature (T_m) were not observed in DSC analyses in the range between -100℃ and 250℃, which suggested that these polyamides were glassy polymers.

3.3 Blood compatibility of CPAPC

Fig. 4 and Fig. 5 shows the number of adhered platelets and SEM pictures of PA and CPAPC film surfaces after contact with human platelet-rich plasma (PRP) for 60 min, respectively. As seen in SEM picture of PA in Fig. 5, it was found that the large aggregates of the human platelets occurred, where a lot of adhered platelets on PA film were observed. On the other hand, CPAPC film surfaces resisted the adhesion of platelets, which were clearly shown in Fig. 4, where the number of adhered platelets of CPAPC3 was reduced in one-sixth amount as compared with that of PA. These results indicate that CPAPC exhibited the excellent blood compatibility and the PC unit is an important element

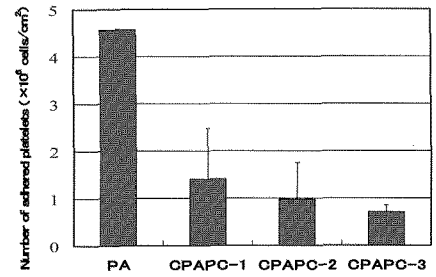


Fig. 4 Number of adhered platelets on polymer surfaces.

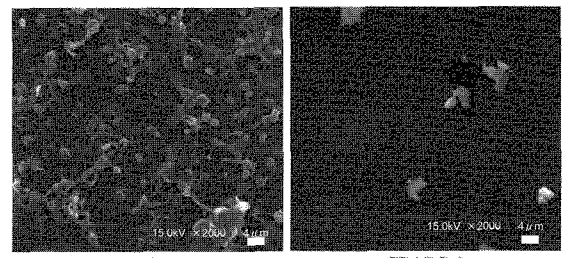


Fig. 5 SEM pictures of polymer film surface after contact with human PRP for 60 min.

for the blood compatibility of the polymers. Furthermore, according to the increase of the PC unit of CPAPC, the number of adhered platelets decreased. Thus, the composition of the PC unit was a dominant factor in the reduction of the blood cell and platelet adhesion.

Consequently, the novel aromatic diamine compound containing PC group would be very useful to create such high functional copolyamides and a new generation of biomedical devices, which showed the durability and the higher thermal stability with addition to the excellent biocompatibility.

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