Transactions of the Materials Research Society of Japan 31[4] 1053-1056 (2006)

Synthesis of Aromatic Carboxylic Acid Compound Containing Phosphorylcholine Moiety and the Polymer Reaction with Ethyl Cellulose

Yoshinori Tadokoro[1], Yu Nagase[1*], Yasuhiko Iwasaki[2] and Kazuhiko Ishihara[3]
[1*]Department of Applied Chemistry, Graduate School of Engineering, Tokai University, 1117 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan, Fax: +81-463-50-2012, e-mail: yunagase@keyaki.cc.u-tokai.ac.jp
[2] Institute of Biomaterials and Bioengineering, Tokyo Medical & Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan, Fax: +81-3-5280-8027, e-mail: yasu.org@tmd.ac.jp
[3] Department of Materials Engineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan, Fax: +81-3-5841-8647, e-mail: ishihara@bmw.t.u-tokyo.ac.jp

An introduction of phosphorylcholine (PC) group to ethyl cellulose through oxyphenylenecarboxylate linkage was investigated, in order to obtain the improved biocompatible material. For this purpose, a novel aromatic carboxylic acid compound containing PC group, 2-(4-carboxylphenyloxy)ethyl phosphorylcholine (CPC), was synthesized, which could be used for the reagent to introduce the PC group into the polymers containing hydroxyl or amino group via polymer reactions. Thus, polymer reaction between CPC and ethyl cellulose using dicyclohexylcarbodiimide gave ethyl cellulose partially substituted with PC group. The chemical structure of the obtained cellulose with PC moiety was confirmed by ^{1}H-NMR and FT-IR, and the elements of PC group were observed on the film surface by XPS analysis. Furthermore, it was revealed that the obtained polymers containing PC moiety exhibited the higher biocompatibility than the starting ethyl cellulose.

Key words: biomaterial, phosphorylcholine, aromatic carboxylic acid compound, biocompatibility, polymeric reaction.

1. INTRODUCTION

Cellulose membranes are still widely used for hemodialysis because of their good permeability of water and solutes and mechanical strength. However, their blood compatibility must be improved for the hemodialysis. The adsorption of proteins on the membrane surface causes the decrease of the permeability and a series of biochemical reactions, which bring about many complement problems or decrease in their performance during medical therapy. Therefore, the protein adsorption on the membrane surface should be reduced to achieve the biocompatibility[1]. In addition, it is necessary to infuse an anticoagulant such as heparin during hemodialysis to minimize clotting. Because the hemodialysis is regularly done for a long term, various side effects are feared.

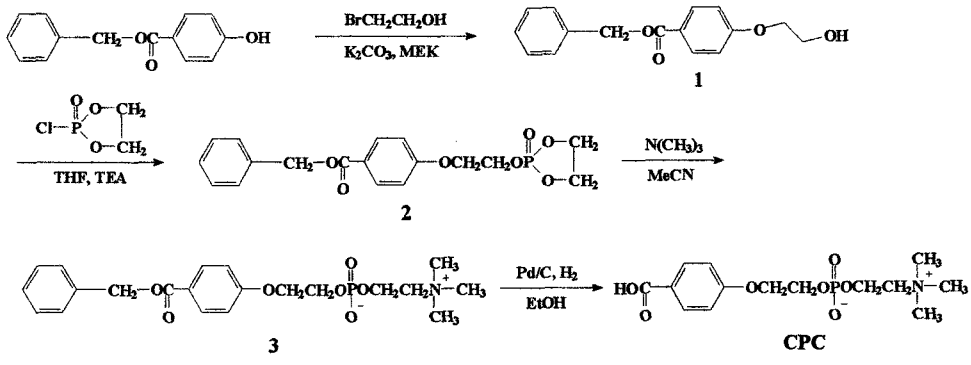
Surface modification is one of the most important techniques for developing biocompatible biomedical devices, when they are used in contact with blood or plasma. Many studies, such as physical adsorption and interpenetrated network formation, have been carried out to modify the surfaces of biomedical devices. However, these surface modification methods would cause the elution of polymer into the blood. On the other hand, a methacrylate with a phospholipids polar group in the side chain, 2-methacryloyloxyethyl phosphorylcholine (MPC), and it's copolymers with various methacrylates and styrene were investigated for new biocompatible materials. Among these polymers, poly(MPC-co-butyl methacrylate) (PMB) exhibited the excellent blood compatibility which derived the reduction of platelet adhesion and aggregation, and the suppression of protein adsorption[2),3)]. In this case, the content of MPC unit in PMB was fixed at 30 mol%, which was enough to exhibit the excellent blood compatibility. Moreover, the modification with MPC copolymers on the surface of cellulose has been reported, where the obtained membranes also showed a decrease in protein adsorption as compared with the original cellulose[4),5)].

In our previous paper, the synthesis of diamine monomer with phosphorylcholine (PC) group has been carried out to prepare polyamide[6)] and poly(urethane-urea)[7)] with PC unit. In the present study, a novel aromatic carboxylic acid compound containing PC group, 2-(4-carboxylphenyloxy)ethyl phosphorylcholine (CPC), was synthesized, in order to introduce the PC group into the polymers containing hydroxyl or amino group by a polymer reaction. This compound, CPC, is expected to be easily prepared in high yield from the commercially available starting materials. Then, the polymer reaction of CPC with ethyl cellulose (Et.Cell-OH) was carried out, because ethyl cellulose was comparatively soluble to common organic solvents as compared with the other cellulose derivatives, and the obtained polymer would be characterized feasibly in the chemical structure and other physical properties. In this paper, the surface property and the biocompatibility of the obtained ethyl cellulose containing PC group (Et.Cell-PC) were also evaluated.

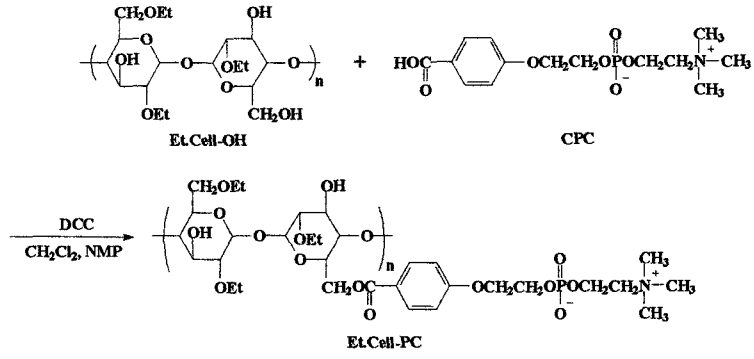
2. RESULTS AND DISCUSSION

2.1 Preparation of CPC

The synthetic route of a novel aromatic carboxylic acid compound containing PC group, 2-(4-carboxylphenyloxy)ethyl phosphorylcholine (CPC), is outlined in Scheme 1. At first, the starting compound, 1, was prepared from benzyl 4-hydroxybenzoate and 2-bromoethanol in the presence of potassium carbonate by the conventional Williamson etherification. Then, the



Scheme 1 Preparation of CPC.



Scheme 2 Preparation of Et.Cell-PC.

phospholane compound, **2**, was prepared from **1** and 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP). The pure product, **2**, was obtained by the extraction with chloroform, because **2** was easily hydrolyzed during purification using column chromatography. Finally, the CPC was obtained in a good yield by ring-opening of the cyclic phosphoric ester moiety of **2** with trimethylamine, followed by the reduction of compound **3** in the presence of palladium catalyst. By using this carboxylic acid compound, CPC, the PC group could be introduced into the polymers containing hydroxyl or amino group by polymer reactions. If the PC group was chemically bonded with the polymer, the elution of PC group or polymer could be avoided during hemodialysis, which would induce the thrombus formation on the polymer surface.

2.2 Polymer reaction and characterizations

The polymer reaction of CPC with ethyl cellulose (ethoxy content = 49 %, Wako Chemical) was carried out using dicyclohexylcarbodiimide (DCC) as a condensation reagent, as shown in Scheme 2, to afford a partially substituted cellulose derivative with PC moiety via the ester linkage (Et.Cell-PC). The desired content of PC group in Et.Cell-PC was fixed at 33 mol%, because 30 mol% of MPC monomer was enough to exhibit the blood compatibility in the case of PMB. This polymer reaction proceeded smoothly in a homogenous system, using a mixture of NMP and dichloromethane. The chemical structure of Et.Cell-PC was confirmed by ^{1}H-NMR and IR spectra. For example, the absorption peaks at 1670 (C=O), 1260 (P=O), 1080 (-P-O-), and 800 cm^{-1} (-C-O-P-) were observed in IR spectrum of Et.Cell-PC. The characterizations of the starting and obtained polymers are summarized in Table 1. The PC content was 11 mol% according to ^{1}H-NMR spectroscopy, which was lower than that in feed. In addition, the number-average molecular weight of Et.Cell-PC was lower than that of the starting Et.Cell-OH, which would be due to the difference of conformations of Et.Cell-OH and

Table 1 Characterizations of polymers and the contact angle of water on the polymer surfaces.

Code	PC content (mol %)		yield (%)	$Mn\times10^{-4}$ c)	Mw/Mn c)	contact angle of water (°)
	in feed a)	in polymer b)				
Et.Cell-OH	-	-	-	15.1	2.15	84
Et.Cell-PC	33	11	63	9.89	1.73	34

a) Molar ratio of CPC and Et.Cell-OH monomer unit.

b) Calculated from the ratio of signal intensities of PC and ethyl group in ^{1}H-NMR spectra.

c) Determined by gel permeation chromatography based on polystyrene standards (Eluent: DMF).

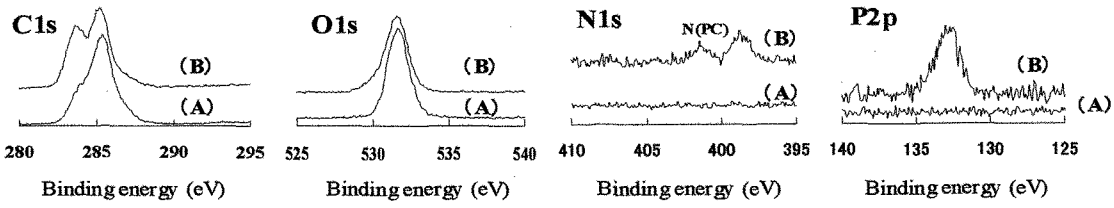


Fig. 1 XPS spectra of Et.Cell-OH (A) and Et.Cell-PC (B) thin films.

Et.Cell-PC in the DMF solution for GPC measurement.

The thin films of the Et.Cell-PC and Et.Cell-OH were prepared by coating and evaporation of the chloroform solutions on poly(ethylene terephthalate) (PET) plates, and the surface analysis of the polymer films was performed. Table 1 also showed the contact angle of water on the surface of polymer films. The contact angle of Et.Cell-PC decreased significantly as compared with Et.Cell-OH, which would be due to the highly hydrophilic property of PC unit. Furthermore, the surface structure of the Et.Cell-OH and Et.Cell-PC thin membranes were analyzed by X-ray photoelectron spectroscopy (XPS), the take-off angle of which was 45°, as shown in Fig. 1. In the case of Et.Cell-OH, XPS intensities attributed to the carbon atoms and oxygen atoms were observed. On the other hand, for Et.Cell-PC, the XPS peaks attributed to nitrogen and phosphorus atoms were also observed at 133 and 402 eV, respectively. From these results of contact angle and XPS analysis, it was obvious that the PC unit was effectively existed in the surface part of Et.Cell-PC membrane.

2.3 Blood compatibility of Et.Cell-PC

Fig. 2 demonstrates the adhesion of blood cells and platelets on the cellulose membranes with and without PC unit after contact with the human whole blood and platelet-rich plasma (PRP) for 60 min. In the case of Et.Cell-PC, no adhesion of blood cells and platelets was observed, whereas many blood cells and platelets were adhered on the original Et.Cell-OH membrane.

Fig. 3 shows the amount of adhered platelets on the polymer films surface after contact with PRP for 60 min. The amount of adhered platelet on Et.Cell-PC was also less than those on Et.Cell-OH, which would be caused by the suppressions of bioreactions, such as platelet adhesion and activation, according to the PC group. Therefore, it is considered that the Et.Cell-PC membrane was covered with the PC unit to improve the biocompatibility on the surface. However, the amount of adhered platelets on Et.Cell-PC was higher than that of PMB (MPC-co-BMA = 30/70), because the mole volume of PC unit in Et.Cell-PC was less than PMB. Thus, it is necessary to increase the PC content of Et.Cell-PC to exhibit the enough biocompatibility, the investigation of which is now in progress.

3. EXPERIMENTAL

3.1 Materials

Ethyl cellulose (ethoxy content = 49%) powder was purchased from the Wako Chemical Co., Tokyo, Japan. The PMB (MPC unit composition = 30 mol%) was prepared by a conventional radical copolymerization technique of MPC and BMA[2].

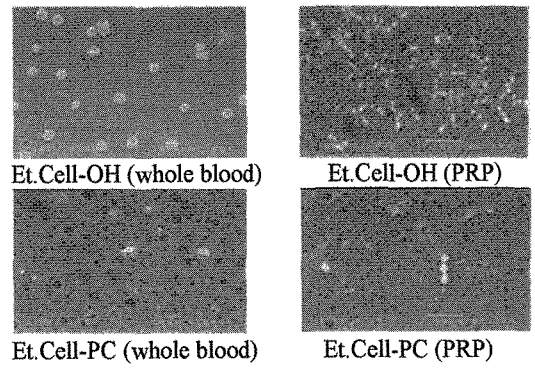


Fig. 2 SEM pictures of polymer film surfaces after contact with human whole blood and PRP for 60 min.

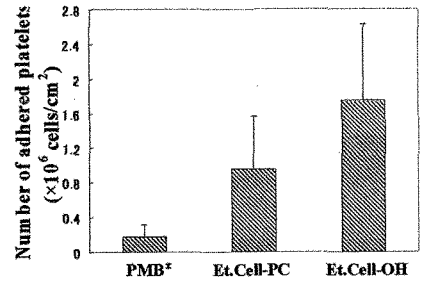


Fig. 3 Amount of adhered platelets on the polymer films after contact with PRP for 60 min.
* Composition of PMB: MPC/BMA = 30/70.

3.2 Synthesis of benzyl 4-hydroxyethylbenzoate (1)

Under an argon atmosphere, 2-bromoethanol (23.3 mL, 329 mmol) was added to the solution of benzyl 2-hydroxybenzoate (15.0 g, 65.7 mmol) and potassium carbonate (45.4 g) dissolved in 400 mL of 2-buthanone (MEK). The reaction mixture was stirred at 100°C for 17 h, and it was poured into an excess of distilled water. The mixture was extracted with chloroform, and the organic layer was dried over sodium sulfonate. After the solvent was evaporated under vacuum, the product was purified by column chromatography on silica gel with hexane/ethyl acetate (3/1 by vol.) to afford 14.5 g of **1** as a white liquid. Yield: 81 %

^{1}H-NMR, δ (400MHz, $CDCl_3$ ppm): 1.25 (1H, t, J = 5.86 Hz), 3.98 (2H, m), 4.13 (2H, m), 5.33 (2H, s), 6.93 (2H, d, 8.79 Hz), 7.31-7.45 (5H, m), 8.03 (2H, d, J = 8.79 Hz).

3.3 Synthesis of 2-(4-benzyloxycarbonylphenyloxyethyl) -2-oxo-1,3,2-dioxaphospholane (2)

Under an argon atmosphere, 2-chloro-2-oxo-1,3,2-dioxaphospholane (7.08 ml, 77.1 mmol) was gradually

added to a solution of **1** (7.00g, 25.7 mmol) and 13.6 mL of triethylamine dissolved in 210 mL of THF at 0°C. After stirring for 2 h at room temperature, the reaction mixture was poured into an excess amount of distilled water and then extracted with chloroform. The obtained organic layer was dried over sodium sulfonate, and the solvent was evaporated under vacuum to afford 8.61 g of **2** as a brown liquid. Yield: 81 %

^{1}H-NMR δ (400MHz, $CDCl_3$, ppm): 4.20-4.53 (8H, m), 5.33 (2H, s), 6.93 (2H, d, J = 9.00 Hz), 7.33-7.45 (5H, m), 8.03 (2H, d, J = 9.00 Hz).

3.4 Synthesis of 2-(4-benzylcarbonylphenyloxy)ethyl phosphorylcholine (**3**)

Under an argon atmosphere, trimethylamine (4.57 ml, 49.2 mmol) was added to a solution of **2** (9.31 g, 24.6 mmol) in 25 mL of acetonitrile at 0°C. Then, the reaction vessel was sealed with a glass cap. After stirring at 60°C for 20 h, the reaction mixture was evaporated to afford 8.68 g of **3** as a white liquid. Yield: 80 %

^{1}H-NMR, δ (400MHz, $CDCl_3$, ppm): 3.19 (9H, s), 3.58 (2H, s), 4.12 (2H, s), 4.21 (2H, s), 4.23 (2H, s), 5.37 (2H, s), 7.13 (2H, d, J = 8.55 Hz), 7.44-7.52 (5H, m), 8.00 (2H, d, J = 8.55 Hz).

3.5 Synthesis of 2-(4-carbonylphenyloxy)ethyl phosphorylcholine (CPC)

5% Pd on charcoal powder (0.04 g, 0.40 mmol by Pd) was suspended in a solution of **3** (8.68 g, 19.8 mmol) dissolved in 100 mL of ethanol. The mixture was degassed under reduced pressure at -78°C, and the vessel was filled with hydrogen gas at over 760 mmHg. After stirring at 50°C for three days, the Pd on charcoal was filtered off washing with ethanol, and the solvent was distilled off under reduced pressure to afford 7.40 g of CPC as a white powder. Yield: 94 %

^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 3.13 (9H, s), 3.73 (2H, m), 3.99 (2H, m), 4.09 (2H, m), 4.17 (2H, m), 7.03 (2H, d, J = 8.79 Hz), 7.88 (2H, d, J = 8.79 Hz).

3.6 Synthesis of ethyl cellulose containing PC group (Et.Cell-PC)

Under an argon atmosphere, DCC (0.45 g, 2.16 mmol) was added to the solutions of ethyl cellulose (ethoxy content = 49%) (1.76 g, 4.16 mmol) and CPC (0.50 g, 1.44 mmol) in 30 mL of dichloromethane and 5.0 mL of NMP. The reaction mixture was stirred at 45 °C for three days. Then the pouring the reaction mixture into excess hexane provided the white precipitate, which was collected by filtration and purified by reprecipitation from its dichloromethane solution into excess hexane. Finally, the product was dried *in vacuo* to afford 1.92 g of Et.Cell-PC as a white powder. Yield: 85 %

^{1}H-NMR, δ (400MHz, $CDCl_3$, ppm): 1.15 (bs), 2.04 (m), 2.90-4.70 (m), 6.87 (bs), 7.48 (bs). IR, ν (KBr neat, cm^{-1}): 3472 (-OH), 2940 (C-H), 1670 (C=O), 1508 (C=C), 1260 (P=O), 1080 (P-O), 1057 (C-O-C), 800 (C-O-P).

3.7 Preparation of polymer coating films

Circular pieces of poly(ethylene terephthalate) (PET) films (diameter: 14 mm, thickness: 0.2 mm) were dipped in 0.5 wt% polymer solutions in chloroform. Then, the solvent was removed slowly at room temperature for 2 h, and then the obtained films were dried *in vacuo*.

3.8 Surface characterization of polymers

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 45°. Contact angles of water on the surfaces of the polymer-coated PET films were measured using an Erma contact-angle microscope at room temperature.

3.9 Evaluation of platelet adhesion on the surface of the polymers

At first, the polymer coating films were equilibrated in phosphate buffered solution (PBS) for overnight. Then, 0.7 mL of human whole blood and platelet-rich plasma (PRP) prepared from healthy donors were added, and the films were incubated for 60 min at 37°C. After the incubation, whole blood and PRP were removed and the films were washed three times with PBS and immersed in PBS containing 2.0 wt% glutaraldehyde to fix the adhered platelets. The surfaces of the polymer films after contact with whole blood and PRP were observed by a scanning electron microscope (SEM, JEOL JSM-5200).

3.10 Measurement of the amount of platelet adsorbed on the polymers surface

After also incubated polymer coating films, PRP was removed and the films were washed 3 times with PBS and transferred into 0.5 wt% aqueous solution of polyethylene glycol mono-*p*-isooctylphenyl ether (Triton X100) to elute the adsorbed platelets. The concentration of platelets in the Triton X100 solution was determined with LHD-Cytotoxic test kit (Wako Chemicals, Osaka, Japan). The amount of protein absorbed on the surface was calculated from their concentration.

Acknowledgment: This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 15550110).

REFERENCES

1) H. Kawano, K. Kurita, Y. Iwasaki, N. Nakabayashi, K. Ishihara, *Kobunshi Ronbunshu*, **55**, 334-343 (1998).
2) T. Ueda, H. Oshida, K. Kurita, K. Ishihara, N. Nakabayashi, *Polym. J.*, **24**, 1259-1269 (1992).
3) K. Ishihara, Y. Iwasaki, N. Nakabayashi, *Mater. Sci. Eng. C*, **6**, 253-259 (1998).
4) K. Ishihara, T. Shinozuka, Y. Hanazaki, Y. Iwasaki, N. Nakabayashi, *J. Biomater. Sci., Polym. Edn.*, **10**, 271-282 (1999).
5) S. H. Ye, J. Watanabe, Y. Iwasaki, K. Ishihara, *J. Membrane Sci.*, **249**, 133-141 (2005).
6) M. Oku, S. Nakajima, Y. Tadokoro, N. Shimoyamada, Y. Nagase, Y. Iwasaki, K. Ishihara, Trans. Mater. Res. Soc., **30** (4), 1037-1040 (2005).
7) S. Nakajima, M. Oku, Y. Nagase, Y. Iwasaki, K. Ishihara, *Proc. Sch. Eng. Tokai Univ.*, **44** (2), 1-8 (2004).

(Received December 10, 2005;Accepted March 30, 2006)