Grafting of Oligopeptide on Poly(aminostyrene)s and Characterization of the Polymers

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ABSTRACT: Graft copolymers that have oligopeptides (OP) as graft molecules were prepared through the coupling reaction of the carboxylic acids of OP with the amino groups of poly(aminostyrene)s (PAS). The three OPs are Boc-Gly, Z-Gly-Pro, and Z-Gly-Pro-Leu-Gly-Pro, where Boc and Z are, respectively, the *t*-butyloxycarbonyl group and benzyloxycarbonyl group as protective groups on nitrogen. The two PASs are poly(4-vinylphenylamine) and poly(*N*-isopropyl-4-vinylbenzylamine). These polymers that have narrow molecular weight distributions were prepared via anionic living polymerization. The coupling reaction to form the peptide bonds was mediated by dicyclohexylcarbodiimide in a mixed solvent of *N,N′*-dimethylformamide and methyl chloride. The degree of grafting (DOG) on PAS was determined from the ^{1}H-NMR spectrum. The dependence of the reaction time (0–8 h) on the DOG, the dependence of the reaction temperature (0–45°C) on the DOG, and the dependence of the molar ratio of OP to the amino group of PAS (1–4 times) on the DOG were studied. By alternating the reaction time and the molar ratio, the DOG values of PAS could be controlled in the range from 0 to 100%. DOG seems to be independent of the molecular weight of OP and the degree of basicity of PAS. The contact angle of the resultant graft copolymers was measured and the preliminary nonthrombogenic test was also performed. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 1558–1567, 2000

Key words: graft copolymers; poly(aminostyrene)s-*graft*-oligopeptides; coupling reaction; degree of grafting; blood compatibility

INTRODUCTION

Poly(aminostyrene)s (PAS)[1] provide an interesting example of stable intermediates of final polymers, because they can be used as the backbone chains of graft copolymers,[1,2] as the prepolymer of crosslinked polymers,[3,4] and for introducing other functions.[5,6] When PAS is used as a backbone molecule of high-performance graft copolymers, two problems must be overcome.

The first problem concerns the preparation of a well-defined PAS that has a narrow molecular weight distribution. For this purpose, we have studied the anionic living polymerization of three tertiary aminostyrenes[7] and a secondary aminostyrene[8] that has a protective group on the amino group. Nakahama et al.[9,10] and the present authors[8] have performed the anionic living polymerization of primary aminostyrenes that have a protective group on the amino group. Based on these

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results, two well-defined PASs were prepared by the anionic living mechanism: poly(*N*-isopropyl-4-vinylbenzylamine) (PBA),[8] —[(CH_2—CH)—C_6H_4 $CH_2NHCH(CH_3)_2]_n$—, and poly(4-vinylphenylamine) (PPA),[8–10] —[(CH_2—CH)—$C_6H_4NH_2]_n$—. The first problem appears to be overcome at the present time.

The second problem concerns the coupling reaction of the amino groups of PAS with the acid derivatives acting as graft molecules. The representative acid derivatives are alkyl halides and carboxylic acids. The alkyl halides can react with the amines of PAS to form the salts. This reaction is well known as the Menschutkin reaction.[11,12] For preparing water-soluble polymers and crosslinked polymers of PAS, the Menschutkin reaction of PAS with alkyl halides has been studied by the present authors.[4] On the other hand, the carboxylic acids should react with the amines of PAS to form an amide and/or peptide bond. This coupling reaction is expected to be useful for preparing high-performance graft copolymers that have oligopeptides (OPs) and liquid crystals acting as graft molecules, because the peptide bond is formed by a covalent bond, thereby becoming stabler than the quaternized salts.[4] However, few studies have been conducted on the coupling reaction. Because of this, the coupling reaction that forms the peptide bonds acting as a link between backbone and graft molecules is studied in this work.

The resultant graft copolymer will be applied to biocompatible materials,[13] biomedical polymers,[14] polymer catalysts with enzyme-like activities,[15] and model liquid crystalline side chain polymers. Before preparing the polymer catalysts and the new type liquid crystalline side chain polymers, we performed a systematic study on the coupling reaction of PAS with N-protected and C-free OPs. Three OPs were used: *t*-butyloxycarbonyl-glycine (Boc-Gly), benzyloxycarbonyl-glycyl-proline (Z-Gly-Pro), and benzyloxycarbonyl-glycyl-L-prolyl-L-Leucyl-glycyl-L-proline (Z-Gly-Pro-Leu-Gly-Pro), where Boc [$(CH_3)_3C$—O—CO—] and Z (C_6H_5—CH_2—O—CO—) are the amino protecting groups of OP. These OPs contain glycine, proline and glycyl-prolyl forms, which are the main components of collagen.[18,19] The purpose of this study is to control the degree of grafting (DOG) of OP on PAS. We also performed two preliminary experiments for blood compatibility[20] of the resultant graft copolymers, namely, the contact angle[20,21] and nonthrombogenic tests[14,20,22] on the resultant graft copolymers.

EXPERIMENTAL

Reagents

Three OPs were purchased from the Peptide Institute, Inc. (Osaka, Japan)[23]: Boc-Gly, Z-Gly-Pro, and Z-Gly-Pro-Leu-Gly-Pro. They were used for the coupling reaction without further purification. Dicyclohexylcarbodiimide (DCC), C_6H_{11}—N═C═N—C_6H_{11}, that is an efficient, nonacidic dehydrating agent was purchased and used without further recrystallization. Tetrahydrofuran (THF), *N,N′*-dimethylformamide (DMF) and methylene chloride (CH_2Cl_2) were also purchased. They were distilled and dried over sodium-wire.

Polymers

PPA was prepared via three steps[8,9]: 1. the monomer synthesis, 2. the polymerization, and 3. the deprotection of the protective group. First, the monomer was prepared as follows: *p*-aminophenethyl alcohol (100 g, 0.73 mol) was dehydrated by heating at 200°C under a pressure of 1 mmHg with potassium hydroxide (300 g, 5.35 mol) to yield 4-vinylphenylamine (PA) (70 g, 0.59 mol; yield, 81%; bp, 72°C/1 mmHg). PA (64 g, 0.54 mol) was reacted with trimethylsilyl chloride (40 g, 0.37 mol) in the presence of excess hexamethyldisilazane (400 g, 2.48 mol) at a reflux temperature for 2 h.[8,9] Ethylmagnesium bromide (117 g, 0.88 mol) dissolved in THF was added to the monosilyl chloride (84 g, 0.44 mol) in THF at room temperature for 2 h. Trimethylsilyl chloride (191 g, 1.76 mol) was added to the solution at room temperature overnight to yield *p-N,N*-bis(trimethylsilyl)-4-vinylphenylamine (SPA), CH_2═CH—C_6H_4—N[—$Si(CH_3)_3]_2$ (50 g, 0.19 mol; yield, 43%; bp, 60°C/0.5 mmHg). Second, after drying SPA over calcium hydride, SPA was sequentially purified using octylbenzophenone sodium and *sec*-butylmagnesium bromide. SPA was anionically polymerized by *n*-butyllithium in THF at −78°C under a pressure of 10^{-5} mmHg to prepare poly(SPA). The anionic polymerization techniques are almost the same as those used in previous studies.[24,25] Third, the complete deprotection of the trimethylsilyl-protecting group for poly(SPA) (4.2 g) was performed using 2*N* HCl (42 mL) at room temperature for a few minutes to yield poly(4-vinylphenylamine hydrochloride).

Table I Anionic Polymerization of *N,N-bis*(trimethylsilyl)-4-vinylphenylamine (SPA) and *N*-Isopropyl-*N*-trimethylsilyl-4-vinylbenzylamine (SBA) by *n*-BuLi in THF at −78°C

Monomer		Initiator	Solvent	Polymerization	Conversion	Polymer with Protecting Group (10^{-4} M_n)				Polymer Without Protecting Group		
Name	mmol	(mmol)	(mL)	Time (h)	(%)	Name	Calc[a]	Obs[b]	M_w/M_n[b]	Name	10^{-4} M_n[b]	M_w/M_n[b]
SPA[c]	16	0.18	40	1	100	Poly(SPA)	2.3	2.4	1.04	PPA	1.1	1.05
SBA[c]	17	0.066	57	24	100	Poly(SBA)	6.4	6.0	1.05	PBA	4.9	1.05

[a] Molecular weight calculated from the amounts of monomer and initiator.
[b] Determined from GPC measurements.
[c] SPA and SBA were polymerized to yield Poly(SPA) and Poly(SBA), respectively. PPA and PBA were, respectively, obtained by the removal of the trimethylsilyl-protective group from Poly(SPA) and Poly(SBA).

Sequential neutralization of the resultant polymer with methanolic KOH solution produced PPA (1.9 g; yield, 100%).

PBA was prepared according to the same procedure as those used in a previous study: 1. the preparation of *N*-isopropyl-*N*-trimethylsilyl-4-vinylbenzylamine (SBA), CH_2═CH—C_6H_4—CH_2—N[—Si$(CH_3)_3$][—CH—$(CH_3)_2$], 2. the anionic polymerization of SBA, and 3. the deprotection of the protecting trimethylsilyl group for poly(SBA). Table I shows the molecular characteristics of the two PASs used in this study.

Coupling of OP with PAS

DCC (3.0 w/v %) in a mixed solvent of DMF and $CH_2Cl_2$was added to a solution of OP (2.5 w/v %) in the mixed solvent at 0°C and allowed to react for 1 h at 0°C.[26,27] To the resultant solution, a solution of PAS (1.5 w/v %) in the mixed solvent was added to react PAS with OP at 0°C and 45°C for 0.5–8 h. *N,N′*-dicyclohexylurea (DCUrea),[26,27] C_6H_{11}—NH—CO—NH—C_6H_{11}, immediately precipitated after the reaction started and the amount of DCUrea increased with the reaction time. After a certain reaction time, DCUrea was removed from the solution by filtration. The solution was then left standing in a refrigerator for a few hours. DCUrea reprecipitated from the solution and was subsequently removed again. These procedures were repeated several times until no precipitate of DCUrea was detectable. After evaporating the mixed solvent, the resultant polymer was washed with ethyl acetate and dried *in vacuo*.

Molecular Characterization

All polymer samples were tested using a gel permeation chromatograph (Model HLC-803) with high resolution columns of GMH6 + G4000H8 (7.8 mm i.d. × 60 cm; Tosoh Co., Kyobashi 3-24, Chuohku, Tokyo 104-0031, Japan) using standard poly(styrene)s (TSK poly(styrene)s; Tosoh Co.) to estimate the molecular weights and molecular weight heterogeneity (M_w/M_n values). The molecular characteristics of the two polymers are described in Table I.

The ^1H and ^{13}C chemical shifts of the nuclear magnetic resonance (JNM-GX270, JEOL Ltd. (Musashino 3-1-2, Akishima, Tokyo 196-8558, Japan)) were measured in CD_3OD at 30°C to estimate the DOG of OP on PAS. Trimethylsilane was used as an internal standard of the chemical shift for the spectrum.

Preliminary Experiments for Blood Compatibility

Contact angle determination and nonthrombogenic tests of the graft copolymers were performed for blood compatibility. The contact angle was obtained by gently placing a drop of water on the polymer surface with a syringe by a sessile drop method with a contact-angle meter manufactured by Kyowa Kagaku Co., Nishi-Nippori 5-36-3, Arakawaku, Tokyo 116-0013, Japan. The contact angle was measured with a goniometer at several different points on the same surface and was averaged to determine the mean value.

Nonthrombogenic tests were performed as follows. A Pyrex test tube (an inner diameter of 0.8 cm) was filled with the THF solution of the graft copolymers (0.2 w/v %) and the solution was poured out of the test tube by inclining. The test tube was dried over *in vacuo* for 72 h to prepare the testing test tube (TTT); the inside surface of TTT was coated with the film of the corresponding graft copolymers. Human whole blood (HWB) was collected from a vein of the human arm by a syringe. When a fresh HWB was introduced into

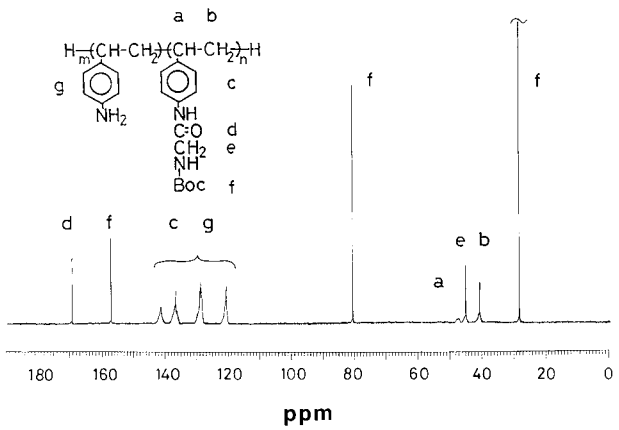

Figure 1 ^{13}C-NMR spectrum of PPA-*g*-(Gly-Boc) prepared with [Boc-Gly]/[PA] = 1 at 0°C for 2 h. Signals are assigned in the figure.

the syringe, a stopwatch was started. The fresh HWB of 1 mL was introduced into each of the two TTTs from the syringe at 37°C. After the two TTTs were left standing at 37°C for 5 min under an N_2 atmosphere, the first TTT was tipped to observe whether HWB flows at 37°C at intervals of 30 seconds. When HWB was found not to flow by tipping the first TTT, tipping of the second TTT was started by the same manner. When the second HWB was found not to flow by tipping the second TTT, the stopwatch was stopped. The resultant time was evaluated as a Lee–White clotting time.[28–30] The Lee–White clotting time was measured five times and averaged to determine a mean Lee–White clotting time. By dividing the mean Lee–White clotting time of the samples by that of a Pyrex glass, the Lee–White relative clotting time (L-WRCT) was determined.

RESULTS AND DISCUSSION

Preparation of Poly(4-vinylphenylamine)-*graft*-(oligopeptides)

Boc-Gly was reacted with PPA to yield poly(4-vinylphenylamine)-*graft*-(Gly-Boc) [PPA-*g*-(Gly-Boc)] at 0°C for 2 h. Although the N-terminal of Gly should be written at the left side of the chemical formula of Gly such as Boc-Gly, the C-terminal of Gly is written at the left side of Gly such as Gly-Boc for the resultant graft copolymer. This unusual chemical formula is convenient to express that a peptide bond is formed between the amine of PPA and the carboxylic acid of the N-protected and C-free Gly. Similar chemical formulas of the graft copolymers will appear in the following text.

Figure 1 shows the ^{13}C-NMR spectrum of the resultant polymer; all NMR signals in Figure 1 were assigned to the corresponding carbons as described in the figure. A ^{13}C signal of the α-carbon of Boc-Gly was observed at 42.5 ppm, whereas the corresponding signal of the resultant polymer was observed not at 42.5 ppm, but at 45 ppm. A ^{13}C signal of the carbonyl group of Boc-Gly was observed at 173.5 ppm, whereas the corresponding signal of the resultant polymer was observed not at 173.5 ppm, but at 169 ppm, which is attributed to the ^{13}C signal of the amide group. These results suggest that a peptide bond was formed between the amino group of PPA and the carboxylic acid of Boc-Gly, thereby yielding a graft copolymer. Boc-Gly that was not allowed to react with PPA was not found in the final product.

The DOG of OP on a PAS molecule was defined as follows:

$$\mathrm{DOG}/\% = ([\mathrm{OP}]/[\mathrm{AS}]) \times 100 \qquad (1)$$

where [OP] and [AS] are, respectively, the molar concentrations of OP and of the aminostyrene monomer unit of PPA for the graft copolymer. To determine the DOG of PPA-*g*-(Gly-Boc), a ^{1}H-NMR spectrum of PPA-*g*-(Gly-Boc) was measured. Figure 2 shows the ^{1}H-NMR spectrum; all NMR signals in the figure were assigned to the corresponding protons as described in the figure.

Particular attention should be paid to the ^{1}H signals of the aromatic protons.[31] The 4H aromatic protons of polystyrene derivatives have

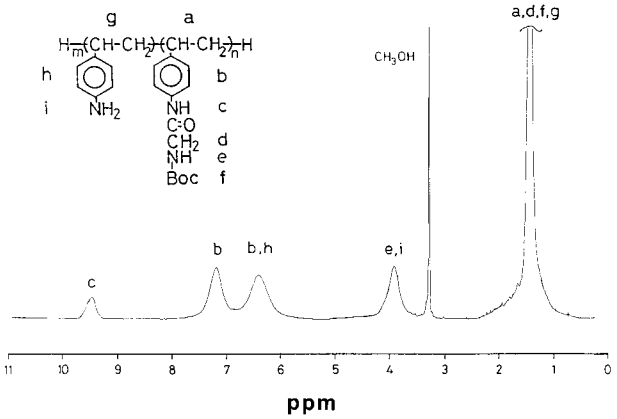

Figure 2 ^{1}H-NMR spectrum of PPA-*g*-(Gly-Boc) prepared with [Boc-Gly]/[PA] = 1 at 0°C for 2 h. Signals are assigned in the figure.

been found separately in the vicinity of 6.5 ppm (2H) and 7.0 ppm (2H)[31]: the 5H aromatic protons of polystyrene were found at 7.05 ppm (3H) and 6.60 ppm (2H), and the 4H aromatic protons of PBA were found at 6.95 ppm (2H) and 6.47 ppm (2H).[8] In contrast, the 4H aromatic protons of PPA were found as a single signal at 6.41 ppm, according to electron-releasing property of the nitrogen directly attached to the phenyl group.[8,9] Taking into account the facts mentioned above, the aromatic ^{1}H signal at 6.41 ppm in Figure 2 is assigned to the sum of the 4H protons of the phenylamino styrene unit (ASU) and 2H protons of the grafted phenylamino styrene unit (grafted ASU) having Boc-Gly, and the aromatic ^{1}H signal at 7.20 ppm in Figure 2 is assigned to the 2H protons of the grafted ASU. When the molar numbers of ASU and grafted ASU for PPA-*g*-(Gly-Boc) are x mol and y mol, respectively, the signal intensity at 6.41 ppm can be represented by $4kx + 2ky$ and the signal intensity at 7.20 ppm can be represented by $2ky$, where k is a numeral constant. From the signal intensities at 6.41 and 7.20 ppm, the kx and ky values could be calculated. Hence, the DOG value can be determined as $ky/(kx + ky)$.

The ^{1}H signal at 3.92 ppm is assigned to the amino protons of PPA[8,9] and Boc-Gly, namely, the signal intensity should be represented by $2kx + ky$. The observed intensity is in fair agreement with the calculated value of $2kx + ky$ obtained using the resultant kx and ky values. The ^{1}H signal at 1.37 ppm is assigned to the sum of the methylene and methine protons of the backbone molecule, the methylene protons of Gly, and the *t*-butyl protons of Boc, namely, the signal intensity should be represented by $3kx + 14ky$. The observed intensity is in fair agreement with the calculated value of $3kx + 14ky$. These results suggest that DOG can be determined by a combination of the two signal intensities from the four signal intensities at 7.20, 6.41, 3.92, and 1.37 ppm. The experimental error was found to be less than 3%. Taking into account the contamination of Boc-Gly and DCUrea to the final product, both peaks of the aromatic protons at 7.20 and 6.41 ppm are easier to handle when determining the DOG.

On the other hand, the DOG value of PPA-*g*-(Gly-Boc) that was sufficiently dried *in vacuo* was determined via gravimetric analysis, weighing the difference between PPA and PPA-*g*-(Gly-Boc). The resultant DOG values were found to be consistent with those determined by the NMR measurement. However, it takes both time and effort to isolate PPA-*g*-(Gly-Boc) from a mixture of the product and reactants. Hence, the NMR measurements of the aromatic protons were performed for the following systematic determination of the DOG values of PPA-*g*-(Gly-Boc).

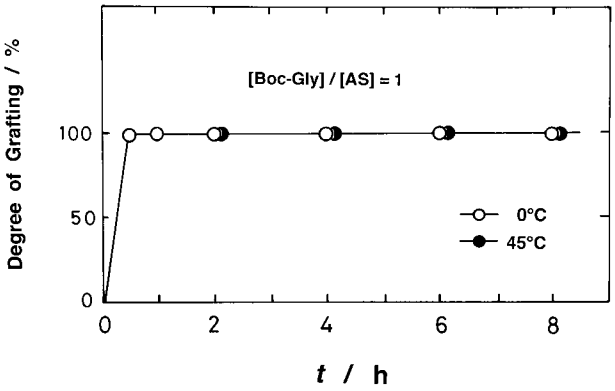

Figure 3 Reaction time dependence of the degree of grafting for PPA-*g*-(Gly-Boc) with [Boc-Gly]/[PA] = 1 at 0°C (—○—) and 45°C (—●—).

The coupling reaction was performed at 45°C with the molar ratio of [Boc-Gly]/[AS] to be 1.0 in the reactant. As shown in Figure 3, the DOG value of PPA-*g*-(Gly-Boc) approached 100% after 2 h and remained fairly constant even though the reaction time was 2 h longer. To produce PPA-*g*-(Gly-Boc) with the DOG value of less than 100%, the coupling reaction was performed at 0°C for the reaction time of less than 2 h. However, the DOG value approached 100% after 0.5 h and remained fairly constant even though the reaction time was 0.5 h longer. The coupling reaction using DCC was found to be fast even at 0°C and quantitatively proceeded.

The coupling reaction was performed at 0°C for 2 h with a change in the molar ratio of [Boc-Gly]/[AS] from 0.5 to 4. As shown in Figure 4(A), the DOG value increases in proportion to the [Boc-Gly]/[AS] value from 0 to 1, and remained constant at 100% even when increasing the [Boc-Gly]/[AS] value from 1 to 4. This result indicates that the coupling reaction quantitatively proceeds.

Z-Gly-Pro was reacted with PPA to yield poly(4-vinylphenylamine)-*graft*-(Pro-Gly-Z) [PPA-*g*-(Pro-Gly-Z)] with [Z-Gly-Pro]/[AS] = 1 at 0°C for 2 h. Figure 5 shows the ^{13}C-NMR spectrum of the resultant PPA-*g*-(Pro-Gly-Z). A ^{13}C signal of Pro of the reactant (Z-Gly-Pro) was observed at 61.6 ppm, whereas the corresponding signal of the resultant

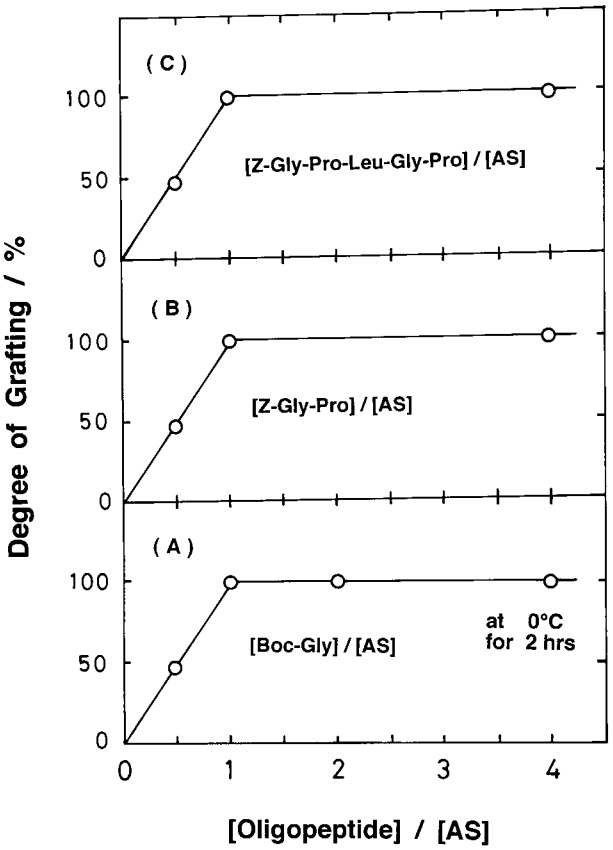

Figure 4 Plots of the degree of grafting vs molar ratios of (A) Boc-Gly, (B) Z-Gly-Pro, and (C) Z-Gly-Pro-Leu-Gly-Pro to the monomer unit for PPA in the reaction mixtures at 0°C for 2 h.

polymer was observed not at 61.6 ppm, but at 63.0 ppm. A ^{13}C signal of the carbonyl group of Z-Gly-Pro was observed at 175.5 ppm, whereas the corresponding signal of the resultant polymer was observed not at 175.5 ppm but at 172.5 ppm, which is attributed to the ^{13}C signal of the amide group. These results suggest that the peptide bond was formed from the amino group of PPA and the carboxylic acid of Z-Gly-Pro, thereby yielding the graft copolymer.

Figure 6 shows the ^{1}H-NMR spectrum of PPA-*g*-(Pro-Gly-Z). Taking into account the ^{1}H-NMR spectra of PPA and Z-Gly-Pro, the aromatic signal at 6.41 ppm is assigned to the sum ($4kx + 2ky$ mol) of the 4H protons of ASU (x mol) and 2H protons of the grafted ASU (y mol), and the aromatic signal at 7.23 ppm is assigned to the sum ($7ky$ mol) of the 2H protons of the grafted ASU and 5H protons of Z group of Z-Gly-Pro acting as a graft. From the signal intensities at 6.41 ppm and 7.23 ppm, the kx and ky values could be

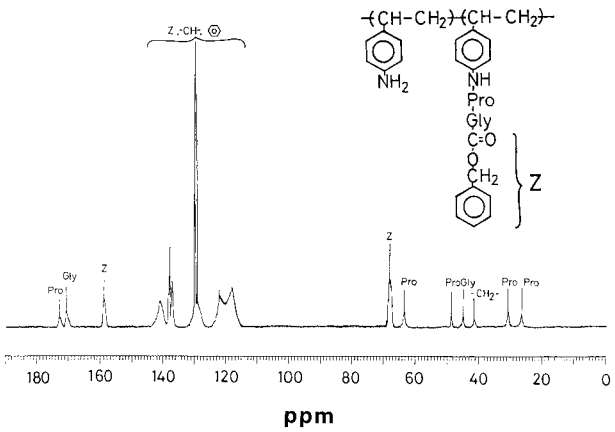

Figure 5 ^{13}C-NMR spectrum of PPA-*g*-(Pro-Gly-Z) with (Z-Gly-Pro)/(PA) = 1 at 0°C for 2 h. Signals are assigned in the figure.

calculated. Hence, the DOG value can be determined as $ky/(kx + ky)$.

The grafting reaction was conducted at 0°C for 2 h with a change in the molar ratio from 0.5 to 4 for [Z-Gly-Pro] to [AS]. As shown in Figure 4(B), the DOG value increases in proportion to the [Z-Gly-Pro]/[AS] value from 0 to 1, and remained constant at 100% even with increasing the [Z-Gly-Pro]/[AS] value from 1 to 4. This result indicates that the coupling reaction quantitatively proceeds.

Z-Gly-Pro-Leu-Gly-Pro reacted with PPA to yield poly(4-vinylphenylamine)-*graft*-(Pro-Gly-Leu-Pro-Gly-Z) [PPA-*g*-(Pro-Gly-Leu-Pro-Gly-Z)] at 0°C for 2 h. As shown in Figure 4(C), the coupling reaction quantitatively proceeds; PPA-*g*-

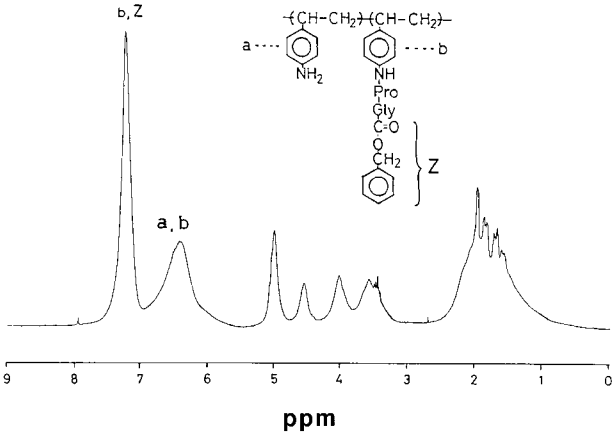

Figure 6 ^{1}H-NMR spectrum of PPA-*g*-(Pro-Gly-Z) prepared with (Z-Gly-Pro)/(PA) = 1 at 0°C for 2 h. Signals are assigned in the figure.

(Pro-Gly-Leu-Pro-Gly-Z) was found to be quantitatively prepared. The DOG value of the resultant graft copolymer could be controlled by changing the molar ratio of [Z-Gly-Pro-Leu-Gly-Pro]/[AS].

Preparation of Poly(*N*-isopropyl-4-vinylbenzylamine)-*graft*-(Gly-Boc)

Boc-Gly was reacted with PBA having a secondary amino group to yield poly[*N*-isopropyl-4-vinylbenzylamine)-*graft*-(Gly-Boc)] [PBA-*g*-(Gly-Boc)]. It was difficult to isolate the resultant graft copolymer from the reaction mixture, because the solubility of DCUrea was similar to that of PBA. Fractional liquid chromatography is expected to be useful to isolate PBA-*g*-(Gly-Boc) from the mixture. Before performing the isolation of the final product, the respective experiments of the fractional liquid chromatography for PBA, Boc-Gly, and DCUrea were performed. Each of the three products was observed at different elution times. Therefore, the fractional liquid chromatography of the mixture was performed; three chromatographic peaks were observed at the corresponding elution times of the model experiments. After evaporating the carrier solvent, the corresponding three products were obtained. The ^{1}H-NMR measurements of these three products were performed. From the NMR spectra and elution times of the fractional liquid chromatography, the first, the second, and the third chromatographic peaks were assigned, respectively, to PBA-*g*-(Gly-Boc), the symmetrical anhydride[16,17] of Boc-Gly, and DCUrea, in the order of increasing elution time. DOG could be determined from the three peak intensities detected by the refractive index increment. DOG could also be determined from the ^{1}H-NMR spectrum of PBA-*g*-(Gly-Boc) obtained as the first chromatographic peak. Both DOG values fairly agreed with each other. These results are shown in Figure 7. When Boc-Gly was reacted with PBA at 0°C for 2 h, the DOG value increased in proportion to the [Boc-Gly]/[AS] value from 0 to 1, and remained constant at 100% even when increasing the [Boc-Gly]/[AS] value from 1 to 2. This result indicates that the coupling reaction quantitatively proceeds.

The four graft copolymers having OP acting as the grafts were prepared. The DOG value could be controlled in the range of 0 to100% by changing the molar ratio of [OP] to [AS]. DOG was found to be independent of the length of the oligopeptide (from monopeptide to pentapeptides) and to be independent of the differences in both of these

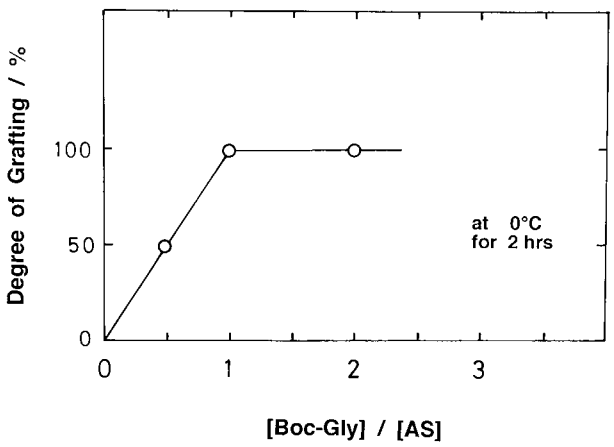

Figure 7 Plot of the degree of grafting *vs*. molar ratio of Boc-Gly to the monomer unit for PBA in the reaction mixture at 0°C for 2 h.

polymers (PPA with the primary phenylamine and PBA with the secondary benzylamine).

We have reported preparing two model graft copolymers by a "grafting onto process"[1] (coupling reaction) and a "grafting from process"[2] (backbone initiation) via an anionic living technique. In the case of a "grafting onto process" where polyisoprenyllithium (PIs^-) reacts with polystyrene-*block*-poly(4-vinylphenyl)dimethylvinylsilane to yield polystyrene-*block*-{poly[(4-vinylphenyl)dimethylvinylsilane]-*graft*-polyisoprene}, the degree of grafting (the spacing of the graft points on a backbone molecule) could not approach a value of 100% but approached a value of less than 32%.[1] The nonquantitative coupling was due to steric hindrance and electro-static repulsion between the PIs^- carbanions and the negative charges of the backbone molecule. In case of a "grafting from process" where ethylene oxide is polymerized by polystyrene-*block*-poly(*p*-styrylpotassium hydroxide)-*block*-polystyrene to yield polystyrene-*block*-[poly(*p*-hydroxystyrene)-*graft*-poly(ethylene oxide)]-*block*-polystyrene, the degree of grafting could not approach a value of 100% but approached a value of less than 50%.[2] The nonquantitative metallation of the backbone initiator was due to electro-static repulsion between the negative charges of the initiator (cumyl potassium) and the resultant potassium hydroxide on the backbone molecule. The anionic living technique appears to be unfavorable for preparing the graft copolymers with a DOG value of 100%.

On the other hand, ester formation between carboxylic acids and alcohols is known to be an equilibrium reaction; the ester formation should

Table II Contact Angle of the Samples for a Drop of Water

Sample	Contact Angle
PSt[a]	91
PPA	83
PPA-*g*-(Gly-Boc)	78
PPA-*g*-(Pro-Gly-Leu-Pro-Gly-Z)	73[b]

[a] PSt was measured as a reference.
[b] After 10 min, the contact angle attained a constant value of 36°.

not be used for completing the coupling reaction. In contrast, as shown in Figures 3, 4, and 7, the formation of the peptide bond between the carboxylic acids of OP and the amino groups of PAS seems to quantitatively proceed and to yield the graft copolymers with a DOG value of 100%. We know that when discussing the coupling reaction, the length of the graft molecule should be considered an important factor. However, the coupling reaction using DCC appears to be suitable for preparing the graft copolymers by a "grafting onto process" compared with the anionic living mechanism.

Preliminary Experiments for Blood Compatibility

Many biomedical applications of polymers have been reported in the literature.[14,20] From a clinical point of view, blood compatibility appears to be the most important characteristic for applying the sample to biomedical applications. Two tests should be conducted as the preliminary experiments for blood compatibility: one is the contact angle determination and the other is a characterization of nonthrombogenicity at surfaces of the samples.

Hydrophilicity might play an important role to improve blood compatibility of the samples.[20] PPA was insoluble in methanol but PPA-*g*-(Gly-Boc) was soluble in methanol. An introduction of Boc-Gly to PPA was found to hydrophilize PPA. To understand the difference in hydrophilicity of the samples, the contact angle of the samples was measured. The results are listed in Table II. Because PPA has a phenylamine group, PPA shows an increase in hydrophilicity in comparison with polystyrene (PSt). An introduction of OPs to PPA shows an increase in hydrophilicity in the sequence of Gly-Boc and Pro-Gly-Leu-Pro-Gly-Z in comparison with PPA.

PPA-*g*-(Pro-Gly-Leu-Pro-Gly-Z) exhibited a time dependence of the contact angle, which has never been observed for PPA and PPA-*g*-(Gly-Boc). After 10 min, the contact angle approached a constant value of 36°,[21] which is a 50% decrease in the value shown in Table II. When the film was dipped in water, however, the film was not soluble in water and did not swell for 2 weeks. Tecoflex™ is a cycloaliphatic poly(ether urethane) derivative, which is already in use for catheter system.[20] The contact angle of Tecoflex is reported to be 55°.[20] PPA-*g*-(Pro-Gly-Leu-Pro-Gly-Z) that was in contact with water for 10 min appears to have a good hydrophilic property in comparison with Tecoflex.

The most important parameters to characterize blood compatibility are thrombocyte adhesion and thrombocyte number. Before determining these two parameters, a preliminary nonthrombogenic test for the four samples shown in Table II was conducted. Lee–White clotting time[28–30] of a Pyrex glass as a reference was not influenced by an atmosphere of air or N_2 over the human whole blood, and was measured to be 11–12 min. The resultant values of the Lee–White relative clotting time (L-WRCT)[28–30] of the samples are shown in Figure 8. As shown in Figure 8, PSt indicates an L-WRCT value of 2.3. Poly(vinyl chloride) was also measured to indicate a L-WRCT value of 2.0–2.5.[32] Many block copolymers for biomedical applications have been prepared; for example, charge-mosaic membranes[32,33] which is prepared from pentablock copolymers of ABACA type by selectively introducing anion and

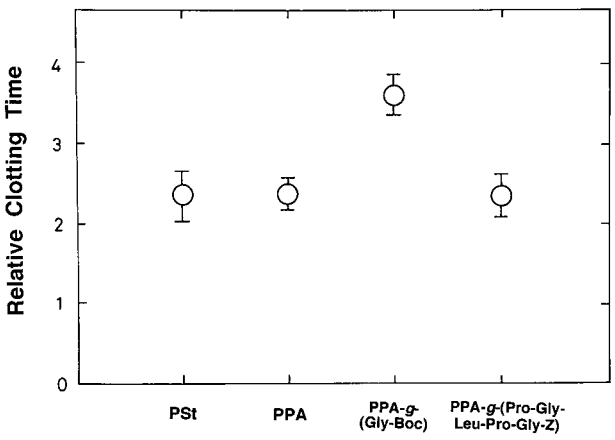

Figure 8 Lee–White Relative clotting time (L-WRCT) of polystyrene, PPA, PPA-*g*-(Gly-Boc), and PPA-*g*-(Pro-Gly-Leu-Pro-Gly-Z) having a DOG value of 100%. The L-WRCT values were determined from the Lee–White method using human whole blood.[28–30]

cation exchange group into the micro-separated phase and poly(amino acid) derivative-*block*-polystyrene block copolymers.[34] Even in the case of these block copolymers that have the special groups depressing thrombocyte adhesion, they indicated the L-WRCT values of 2.0–3.0.[32–34]

As shown in Figure 8, PPA and PPA-*g*-(Pro-Gly-Leu-Pro-Gly-Z) having a DOG value of 100% indicate L-WRCT values of 2.5. Especially, PPA-*g*-(Gly-Boc) was found to indicate an L-WRCT value of 3.7. Taking into account L-WRCT of the common polymers[20,32] and the specially prepared block copolymers,[32–34] the graft copolymers prepared in this study might show the higher qualities of the nonthrombogenicity. A mechanism of modifying the nonthrombogenic property[35,36] of PPA-*g*-(Gly-Boc) is not well known at the moment. Further study of the nonthrombogenic property of the graft copolymers having OP is in progress.

CONCLUSION

The N-protected and C-free oligopeptides were reacted with the amino groups of PPA to yield the corresponding graft copolymers. This coupling reaction was found to be fast even at 0°C and quantitatively proceeded when DCC was used as a dehydrating agent; namely, the coupling reaction was independent of the length of OPs (from monopeptide to pentapeptides). The DOG values of the graft copolymers could be controlled in the range of 0 to 100% by changing the molar ratio of [OP] to [AS]. The coupling reaction using DCC appears to be suitable for preparing the graft copolymers by a "grafting onto process" compared with the anionic living mechanism. The resultant graft copolymers exhibited the higher qualities of the hydrophilicity and nonthrombogenicity in comparison with PSt and PPA.

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