Synthesis and structure of vinyl polymer–poly(α-amino acid) graft copolymers

Yukio Imanishi, Taeko Kimura and Toshinobu Higashimura
Department of Polymer Chemistry, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606, *Japan*
(*Received* 2 *September* 1980; *revised* 23 *October* 1980)

N-(2-Carbobenzyloxyaminoethyl)acrylamide (CAEA) which is a vinyl compound carrying a protected aliphatic primary amino group in the side chain has been synthesized and its homopolymerization and copolymerization induced by radical initiators have been investigated. CAEA was found to possess good copolymerizability with vinyl monomers having large *Q* values such as styrene, 2-vinyl pyridine, acrylonitrile and methyl methacrylate. Vinyl polymers containing a small amount of aliphatic primary amino group in the side chain were produced by the treatment of vinyl polymers containing a small amount of CAEA unit with HBr/AcOH or H_2/Pd. Using the former polymers as initiators for the polymerization of *N*-carboxyanhydrides of (*RS*)-Phe and (*S*)-Glu(OBz), vinly polymer (trunk)–poly-(α-amino acid) (branch) graft copolymers were synthesized. These copolymers were investigated by transmission electron microscopy, and the development of domain structure due to microphase separation was observed.

INTRODUCTION

Vinyl polymers with pendant primary amino groups have proved useful for the variety of reactions which they can undergo through the reactive amino groups[1]. A number of attempts have been made to synthesize vinyl polymers carrying pendant primary amino groups[2], but the effective and quantitative incorporation of primary amino groups into the polymer side chain has not been totally successful. Recently, Dawson *et al.*[3] have reported the efficient synthesis of poly(vinyl amine hydrochloride) by acid hydrolysis of poly(*N*-vinyl acetamide).

Among the many possible reactions of primary amino group, the polymerization of α-amino acid *N*-carboxyanhydride (NCA) is particularly interesting. When the primary amino group is aliphatic, the polymerization of NCA is effectively initiated by the nucleophilic addition of the amino group to the NCA and the resulting poly(α-amino acid) carries the initiator fragment at the *C* terminal[4]. If a vinyl polymer carrying a pendant primary amino group is used as an initiator for the NCA polymerization, a graft copolymer should be formed in which the vinyl polymer constitutes the trunk and the poly(α-amino acid) forms the branch.

This type of copolymer, i.e. vinyl–peptide graft copolymer, is interesting because the trunk and the branch are of different natures. In one aspect, it should be useful as an enzyme model polymer[5] because the hydrophobic segment (usually vinyl polymers) and the hydrophilic and functional segment (usually poly(α-amino acids)) are covalently bound in one large molecule. In another aspect, it is interesting as a biomedical material because the formation of hydrophilic–hydrophobic domain structure is considered to give an excellent antithrombogenicity[6].

The synthesis of specially-characterized copolymers of vinyl compounds and α-amino acid has been achieved by block copolymerization. For example, Yamashita *et al.*[7] polymerized γ-methyl (R)-glutamate NCA with polystyrene having terminal glycyl groups and obtained 'peptide–vinyl–peptide' block copolymers. Gallot *et al.*[8–10] synthesized polystyrene and polybutadiene-carrying primary amino groups at one end by living anionic polymerization and subsequent reactions. They polymerized (*S*)-lysine NCA with γ-benzyl (*S*)-glutamate NCA and obtained several vinyl–peptide block copolymers.

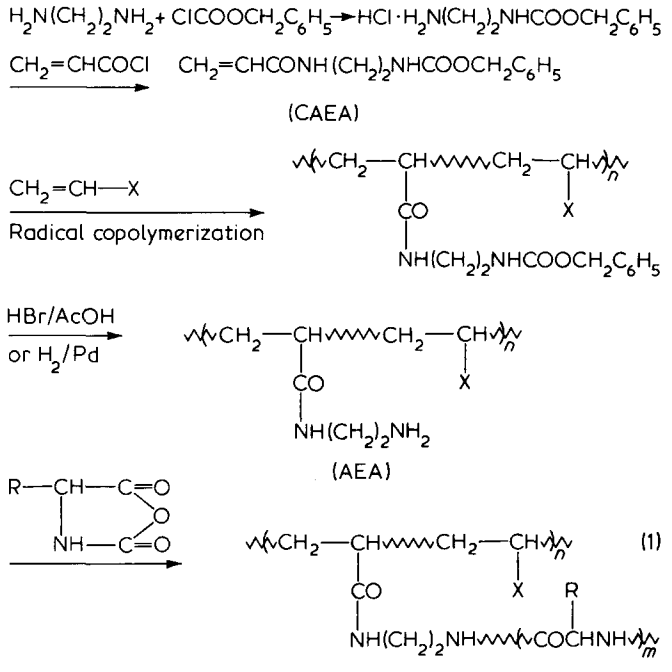
We have synthesized a vinyl compound carrying a protected aliphatic primary amino group in the side chain, and found that it is copolymerizable with various kinds of vinyl monomers by a radical mechanism. By deblocking the resulting copolymer, free primary amino groups have been produced along the polymer side chain which initiated the polymerizations of different α-amino acid NCAs to obtain vinyl–peptide graft copolymers. We believe that this method of graft copolymerization is of general use and produces graft copolymers having widely different properties. Here, we will describe the synthetic route to the vinyl–peptide graft copolymers and (to a lesser extent) the microstructure of the resulting copolymers.

0032-3861/81/101407-08$02.00

EXPERIMENTAL

General procedure for the synthesis of vinyl polymer–poly(α-amino acid) graft copolymers

Vinyl polymer (trunk)–poly(α-amino acid) (branch) graft copolymers were synthesized as described below.



Ethylene diamine blocked at one end with a carbobenzyloxy group was synthesized according to Lawson *et al.*[11], m.p. 171°C (literature[11] 172°–173°C), yield 10.3%.

N-Carbobenzyloxyethylenediamine hydrochloride was dissolved in aqueous NaOH solution kept at 0°–10°C, and a CH_3CN solution of acrylic chloride was added dropwise to obtain N-(2-carbobenzyloxyaminoethyl)acrylamide (CAEA). CAEA was recrystallized from water, m.p. 123.5°C, yield 12.8%. Analysis, calculated for $C_{13}H_{16}N_2O_3$: C, 62.89%; H, 6.50%; N, 11.28%. Found: C, 62.73%; H, 6.45%; N, 11.42%. CAEA is soluble in MeOH, Me_2CO, CCl_4, $CHCl_3$, CH_2Cl_2, AcOEt, slightly soluble in MePh, and insoluble in n-C_6H_{14}.

Using radical initiators, CAEA was polymerized and copolymerized with styrene (X = Ph), 2-vinylpyridine (X= ) acrylonitrile (X = CN), methyl methacrylate (X = CH_3, $COOCH_3$), and *N*-vinylpyrrolidone (X= ) Treatment of the copolymers with HBr/AcON or H_2/Pd resulted in the formation of deblocked copolymers[12]. Thus the CAEA residues in the copolymers were converted into *N*-(2-aminoethyl)acrylamide (AEA) residues. Copolymers having AEA residues were used to initiate polymerizations of γ-benzyl (*S*)-glutamate NCA (R = $C_6H_5CH_2$–$OCOCH_2CH_2$–) and (*RS*)-phenylalanine NCA (R = $C_6H_5CH_2$–) to obtain vinyl polymer (trunk)–poly(α-amino acid) (branch) graft copolymers.

α-Amino acid NCA was synthesized by the reaction of α-amino acid and trichloromethyl chloroformate[13]. The progress of the graft copolymerization was checked by the change in absorption due to the carbonyl group of NCA.

Radical polymerization of CAEA

500 mg (2.01 mmol) of CAEA was dissolved in 10 ml of $HCONMe_2$ and 10 mg (0.0609 mmol) of azobisisobutyronitrile added. The solution was sealed in a glass ampoule under vacuum and heated at 100°C for 60 h. After the solvent had been distilled off, the residual mass was dissolved in $CHCl_3$ and reprecipitated with MeOH. Poly(CAEA) was obtained as a white powder, yield 100%. Analysis, found: C, 60.75%; H, 6.55%; N, 11.39%. Poly(CAEA) is soluble in $HCONMe_2$, $CHCl_3$, tetrahydrofuran, but insoluble in H_2O, Me_2CO, dioxane, MeOH, EtOH, and benzene.

To determine the relative reactivities of CAEA involved in the radical polymerization, mixtures of CAEA–styrene with nine different molar ratios (the total weight was always 1 g) were dissolved in 10 ml of $HCONMe_2$. The appropriate amount of azobisisobutyronitrile was added and the solution sealed in a glass ampoule under vacuum; copolymerization was allowed to proceed at 100°C. Copolymers were collected at roughly constant conversion (~10%) and subjected to elemental analysis. The copolymer composition was determined by taking an average of the compositions calculated according to C% and N%.

Synthesis of styrene-(RS)-phenylalanine graft copolymer

0.7 ml (6.07 mmol) styrene and 0.3 g (1.21 mmol) CAEA were dissolved in 7 ml of MePh, and 0.007 g (0.0043 mmol) of azobisisobutyronitrile were added. The mixture was sealed in a glass ampoule under vacuum and heated at 100°C for 73 h. The product was precipitated and washed with MeOH and dried under vacuum: yield, 0.538 g (57.3%), $[\eta]$ (benzene solution at 30°C), 0.183 dl g^{-1}. The CAEA content of the copolymer [copoly(CAEA–ST)] was 13.8 mol % represented the average value of the copolymer compositions determined on the basis of C% and N% in the elemental analysis.

0.5 g of copoly(CAEA–ST) were dissolved in 30 ml of MePh, and treated with 7 ml of 25% HBr/AcOH for 30 min at room temperature. The solvent was distilled to dryness and the residual mass was washed repeatedly with aqueous $NaHCO_3$ solution at 0°C, washed with water, and dried: yield, 0.420 g, $[\eta]$ (benzene solution at 30°C), 0.142 dl g^{-1}. Titration of a tetrahydrofuran solution of the product by N/100 HCl showed the presence of 8.5×10^{-4} mmol mg^{-1} amino groups. The deblocked copoly(CAEA–ST) will be designated as copoly(AEA–ST) hereafter.

200 mg (1.7×10^{-4} mol amino group) of copoly(AEA–ST) and 649 mg (3.4×10^{-3} mol) of (*RS*)-Phe NCA were dissolved in 50 ml of CH_2Cl_2, and the mixed solution was allowed to react at room temperature for 50 h. When the i.r. absorptions characteristic of the NCA disappeared, the reaction solution was poured into petroleum ether to separate the product: yield, 0.665 g (95.1%). Extraction with benzene yielded 89.0% insoluble and 1.2% soluble product. The graft copolymers of styrene and (*RS*)-Phe thus formed will be represented as copoly(AEA–ST–Phe).

Synthesis of 2-vinylpyridine-(RS)-phenylalanine graft copolymer

0.4 g (37.9 mmol) of 2-vinyl pyridine and 0.472 g (1.90 mmol) of CAEA were dissolved in 40 ml of MePh, and 0.04 g (0.244 mmol) of azobisisobutyronitrile were added. The mixture was sealed in a glass ampoule under vacuum

and heated at 100°C for 67 h. The solution was poured into petroleum ether to separate the product. The product was dissolved in benzene and reprecipitated by petroleum ether: yield, 1.61 g (36.0%), [η] (MeOH solution at 30°C), 0.101 dl g^{-1}. The content of CAEA unit in the copolymer [copoly(CAEA–VP)] was 18.6 mol % which was determined by elemental analysis for N%.

0.5 g of copoly(CAEA–VP) was dissolved in 80 ml of MePh and treated with 10 ml of 30% HBr/AcOH at room temperature for 30 h. The solid mass which separated from the solution during the reaction was recovered and suspended in MeOH. The treatment of the suspension with IRA-400 ion exchanger resulted in a homogeneous solution. After filtering the ion exchanger, the solvent was distilled off to dryness. The residue was dissolved in water and treated again with IRA-400 for 30 min, after which time precipitation occurred. The precipitate was extracted from the mixture using $CHCl_3$. $CHCl_3$ was evaporated to obtain the reaction product: yield, 0.161 g. Titration of the MeOH solution of the product with N/100 HCl showed 1.4×10^{-4} mmol mg^{-1} amino groups. The deblocked copolymer will be denoted as copoly(AEA–VP) hereafter.

0.141 g (1.98×10^{-5} mol amino group) copoly(AEA–VP) and 0.757 g (3.96 mmol) (*RS*)-Phe NCA were dissolved in 60 ml of CH_2Cl_2 and the mixture allowed to react at room temperature for 210 h. After the disappearance of the i.r. absorptions due to the NCA, the solution was poured into petroleum ether to separate the product: yield, 0.616 g (85.0%). Extraction by benzene yielded soluble (41.8%) and insoluble (58.2%) fractions. The graft copolymers of 2-vinyl pyridine and (*RS*)-Phe thus formed will be represented as copoly(AEA–VP–Phe).

Synthesis of acrylonitrile-γ-benzyl (S)*-glutamate graft copolymer*

3.24 g (61.6 mmol) acrylonitrile and 0.758 g (3.05 mmol) CAEA were dissolved in 20 ml $HCONMe_2$, and 0.04 g (0.244 mmol) azobisisobutyronitrile were added. The mixture was sealed into a glass ampoule under vacuum and heated for 24 h at 50°C. The solution was poured into water to separate the reaction product: yield, 3.04 g (76.3%), [η] ($HCONMe_2$ solution at 25°C), 0.642 dl g^{-1}. The CAEA content in the copolymer [copoly(CAEA–AN)] was 6.78 mol %, which was determined by averaging the copolymer compositions calculated on the basis of C% and N% from elemental analysis.

0.708 g of copoly(CAEA–AN) were dissolved in 30 ml of $HCONMe_2$ containing ~1 g of Pd-black, and hydrogen gas was bubbled through the solution for 166.5 h. After filtering off the Pd-black, the solution was poured into MeOH to precipitate the reaction product. The product was washed with MeOH and dried: yield, 0.646 g. The $HCONMe_2$ solution of the reaction product was mixed with 1 ml of N/100 HCl and back-titrated with N/100 NaOH. The product was found to contain 0.129×10^{-3} mmol mg^{-1} amino groups. The deblocked copolymer will be represented as copoly(AEA–AN).

0.363 g (0.0464 mmol amino group) copoly(AEA–AN) and 0.241 g (0.914 mmol) γ-benzyl (*S*)-glutamate NCA were dissolved in 25 ml of $HCONMe_2$, and the mixture was allowed to react at room temperature for 142 h. The solution was poured into MeOH to precipitate the reaction product which was washed with MeOH and dried: yield, 0.477 g (84.6%). The extraction of the product with $CHCl_3$ left 89.0% of insoluble fraction. The insoluble fraction was a graft copolymer which will be represented as copoly[AEA–AN–Glu(OBz)].

Synthesis of methyl methacrylate–γ-benzyl (S)*-glutamate graft copolymer*

2.68 g (26.7 mmol) methyl methacrylate and 1.33 g (5.34 mmol) CAEA were dissolved in 20 ml of $HCONMe_2$, and 0.02 g (0.122 mmol) of azobisisobutyronitrile were added. The mixture was sealed in a glass ampoule under vacuum, and heated at 100°C for 114.5 h. The solution was poured into MeOH to separate the reaction product: yield, 2.013 g The product was precipitated from $CHCl_3$ solution with MeOH: [η] (benzene solution at 25°C), 0.152. The CAEA content of the copolymer [copoly(CAEA–MMA)] was 14.6 mol %, determined by averaging the compositions calculated on the basis of C% and N% of the elemental analysis. It should be mentioned that from the filtrate 0.743 g of MeOH-soluble product was recovered. The soluble product was fractionated into 0.136 g benzene-soluble and 0.376 g benzene-insoluble fractions. By i.r. spectroscopy the former proved to be copoly(CAEA–MMA) and the latter to be unreacted CAEA. Thus, the overall yield of copoly(CAEA–MMA) was 2.15 g (53.7%). The copolymer was completely soluble in benzene but partly soluble in MeOH. I.r. spectra of MeOH-soluble and -insoluble copolymers were nearly the same.

0.631 g copoly(CAEA–MMA) (MeOH-insoluble) was dissolved in 30 ml $CHCl_3$ and treated with 3 ml of 25% HBr/AcOH at room temperature for 30 min. The solution was poured into petroleum ether to precipitate the reaction product, which was washed with dilute NH_4OH and water and then dried: yield, 0.467 g. The solution of the product in a $CHCl_3$–MeOH mixture was titrated with N/100 HCl, indicating the presence of 3.12×10^{-4} mmol mg^{-1} of amino group. The deblocked copolymer is represented as copoly(AEA–MMA).

25.0 mg (7.79×10^{-3} mmol amino group) copoly(AEA–MMA) and 41.6 mg (0.158 mmol) γ-benzyl (*S*)-blutamate NCA were dissolved in 5 ml of $CHCl_3$, and the mixture was allowed to react at room temperature for 21 h. Having confirmed the disappearance of i.r. absorptions characteristic of NCA, the reaction solution was poured into petroleum ether to separate the reaction product: yield 20.6 mg (34.6%). Extraction with acetone yielded 79.6% insoluble and 6.87% soluble product. The graft copolymer of methyl methacrylate and (*S*)-Glu(OBz) is represented as copoly[AEA–MMA–Glu(OBz)].

Synthesis of styrene-γ-benzyl (S)*-glutamate graft copolymer having different degrees of polymerization*

The synthesis of styrene–(*S*)-Glu(OBz) graft copolymer was carried out using copoly(AEA–ST) as an initiator for the polymerization of (*S*)-Glu(OBz) NCA. Copoly(AEA–ST) used was that described in the synthesis of copoly(AEA–ST–Phe) and the degree of polymerization (*DP*) was ~200. In order to synthesize a graft copolymer in which the trunk polymer has a higher *DP*, emulsion copolymerization of styrene and CAEA was carried out.

8.2 ml (71 mmol) styrene and 1.77 g (7.1 mmol) CAEA were placed in 16.4 ml of water; 82 mg (0.303 mmol) $K_2S_2O_8$, 82 mg of Na_2HPO_4, and 328 mg of $C_{12}H_{25}SO_3Na$ were added, and the mixture flushed with nitrogen. The mixture was heated under a nitrogen atmosphere at 70°C for 2 h and subsequently at 95°C for 2 h. Aqueous

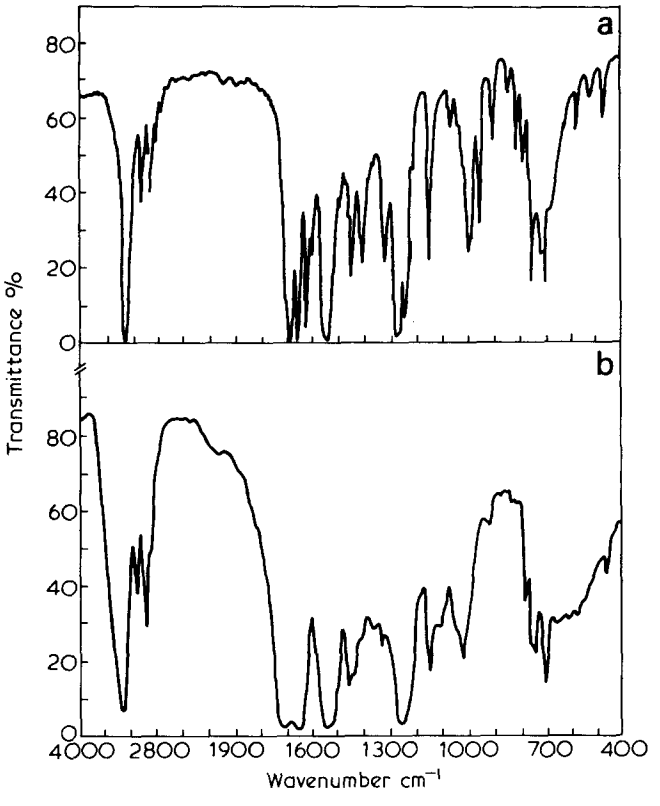


Figure 1 Infra-red spectra: KBr disc: (a) CAEA; (b) poly(CAEA)

potassium alum solution was added to the emulsion to separate the copolymer: yield, 50.6%, $[\eta]$ (benzene solution at 30°C), 3.90 dl g^{-1}. The content of CAEA in the copoly(CAEA–ST) was determined to be 5.96 mol % on the basis of N% from elemental analysis. If this were polystyrene, an $[\eta]$ value of 3.90 corresponds to the molecular weight of 2.38×10^6. The emulsion polymerization thus produced a trunk polymer having *DP* as high as $\sim 2 \times 10^4$.

Copoly(CAEA–ST) was dissolved in $CHCl_3$ and treated with HBr/AcOH. The titration of the resulting copoly(AEA–ST) revealed that it contained 3.92×10^{-7} mmol mg^{-1} of amino group. Assuming complete debenzylation, this amino group content corresponds to 4.10 mol % AEA units in copoly(AEA–ST). The AEA content determined from elemental analysis for N% was 4.33 mol %. Both values are in good agreement.

250 mg (9.8×10^{-5} mol amino group) of copoly(AEA–ST) and 2.46 g (9.8 mmol) of γ-benzyl (*S*)-glutamate NCA were dissolved in $CHCl_3$, and the resulting solution allowed to react at room temperature for 110 h. Pouring the reaction solution into acetone a trace amount of unreacted NCA was removed and the reaction product recovered: yield, 77%. Extraction of the product with CF_3COOH (TFA) yielded TFA-soluble and -insoluble fractions.

Synthesis of N-*vinyl pyrrolidone–CAEA copolymer*

1.38 g (12.4 mmol) *N*-vinyl pyrrolidone and 0.618 g (2.49 mmol) CAEA were dissolved in 15 ml of water, and 0.025 ml of 30% aqueous H_2O_2 solution and 0.02 ml of aqueous ammonia solution were added. The mixture was sealed in a glass ampoule under vacuum and heated at 50°C for 24 h As polymerization proceeded, the solution became heterogenous and precipitation was observed. After filtration, the filtrate was evaporated to dryness. The yields of water-soluble and water-insoluble products were 0.676 g (33.8%) and 0.720 g (36.0%), respectively. The former proved to be poly(*N*-vinyl pyrrolidone) and the latter a copolymer of *N*-vinyl pyrrolidone and CAEA by i.r. spectroscopy. The intrinsic viscosity of the copolymer in $HCONMe_2$ solution at 25°C was 1.00. However, C% and N% of the copolymer from the elemental analyses were lower than values for either poly(*N*-vinyl pyrrolidone) or poly(CAEA). This may have been caused by the strong hydrophilicity of the copolymer. The copolymer compositions were, therefore, not determined.

The strong hydrophilicity of the copolymer complicated the subsequent treatment with HBr/AcOH for debenzylation. The tendency to form homopolymer in the binary copolymerization suggests non-randomness in the copolymer. For these reasons the synthesis of *N*-vinyl pyrrolidone-α-amino acid graft copolymer was not attempted.

RESULTS

Radical polymerization and copolymerization of CAEA

The infra-red spectrum of poly(CAEA), produced by the radical polymerization of CAEA is shown in *Figure 1* together with that of monomeric CAEA. Absorptions at 900–1000 cm^{-1} due to unsaturated bonds in CAEA disappeared from the i.r. spectrum of poly(CAEA). Instead, absorptions at 1450 cm^{-1} due to the methylene group are strengthened in the spectrum of poly(CAEA).

In order to discover the relative reactivity of CAEA in radical copolymerization, CAEA(M_2) was copolymerized with styrene(M_1). The copolymer compositions are shown in *Figure 2*. The monomer reactivity ratios were determined according to the cross-section method as $r_1 = 2.37 \pm 0.90$ and $r_2 = 1.05 \pm 0.37$. The monomer reactivity ratios indicate that CAEA is approximately as reactive as styrene. It follows that CAEA may easily be copolymerized with vinyl monomers having a large conjugated system (large *Q* values) such as styrene ($Q = 1.0$). Therefore, vinyl–peptide graft copolymers may be

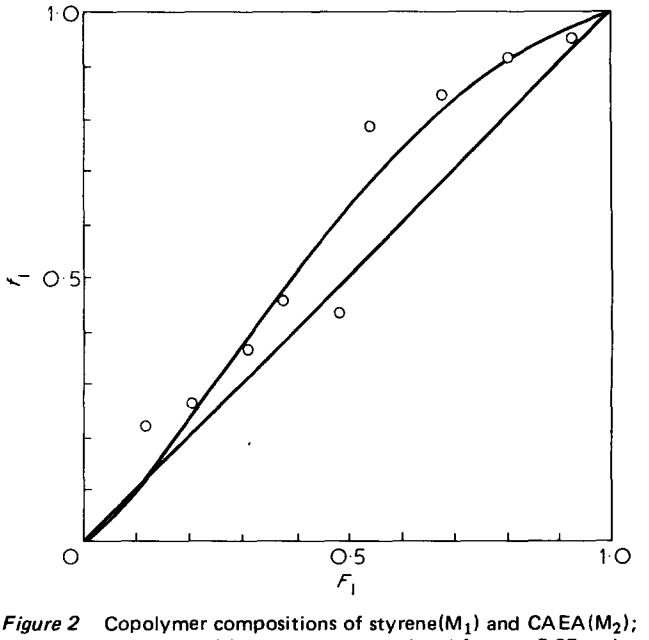


Figure 2 Copolymer compositions of styrene(M_1) and CAEA(M_2); the copolymer composition curve was calculated for $r_1 = 2.37$ and $r_2 = 1.05$

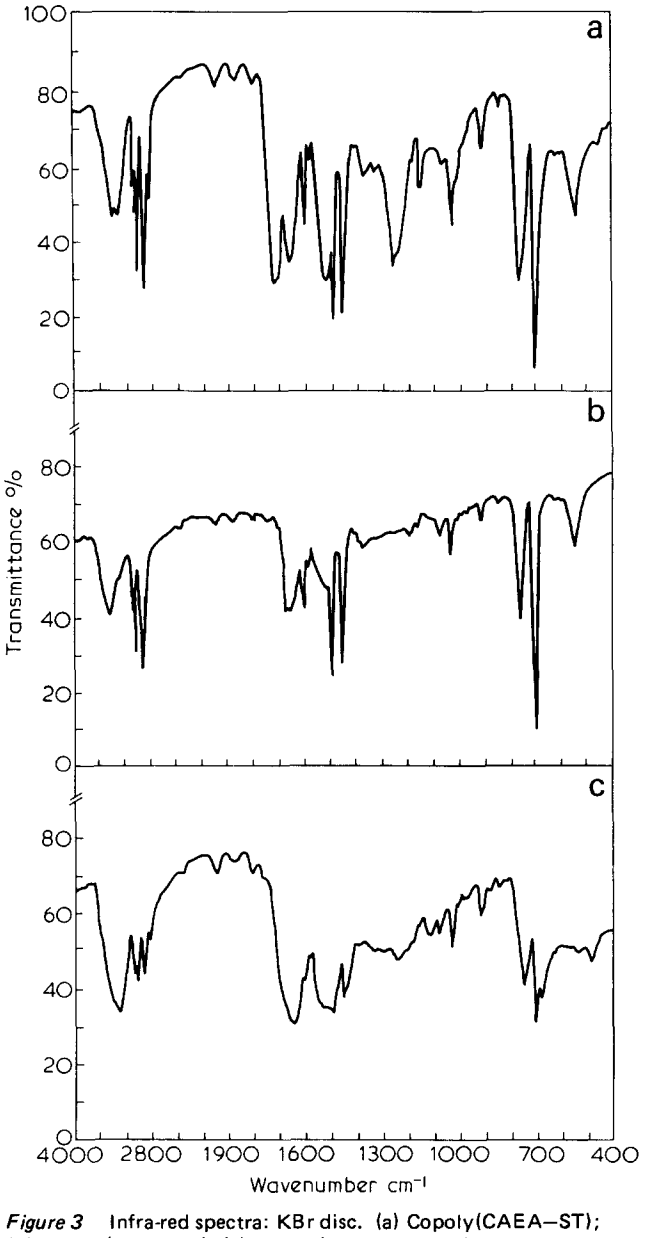


Figure 3 Infra-red spectra: KBr disc. (a) Copoly(CAEA–ST); (b) copoly(AEA–ST); (c) copoly(AEA–ST–Phe), benzene-insoluble part

synthesized in which the trunk vinyl polymer carries a conjugated side chain.

Styrene-(RS)-phenylalanine graft copolymer

The i.r. spectrum of copoly(CAEA–ST), shown in *Figure 3a*, is different from those of poly(CAEA) and polystyrene. It exhibits absorptions at 1710 cm^{-1} (ester C=O), 1250 cm^{-1} (ester OC–O–C), and 1650 and 1530 cm^{-1} (amide C=O) which are characteristic of poly(CAEA). It also exhibits absorptions at 1460 and 1490 cm^{-1} (CH_2) which are observed strongly in polystyrene. These findings indicate that the product is a true copolymer of styrene and CAEA.

The i.r. spectrum of copoly(AEA–ST) is shown in *Figure 3b*. The treatment with HBr/AcOH made the absorptions at 1710 and 1250 cm^{-1} due to the carbamate group disappear, which proves the complete deblocking of the pendant primary amino group and the conversion of the CAEA residue in the copoly(CAEA–ST) into AEA residue in the copoly(AEA–ST).

The i.r. spectrum of the benzene-insoluble product from copoly(AEA–ST–Phe) is shown in *Figure 3c*. The absorptions at 1650 and 1530 cm^{-1} (amide C=O), and that at 3300 cm^{-1} (amide NH) become stronger after the copolymerization of (*RS*)-Phe NCA. Both the benzene-insoluble portion and the benzene-soluble portion gave an essentially similar spectrum, and both showed absorptions characteristic of either the styrene unit or the phenylalanyl unit. These observations indicate the formation of a graft copolymer.

2-Vinylpyridine–(RS)-phenylalanine graft copolymer

The i.r. spectrum of copoly(CAEA–VP) was different from those of poly(CAEA) and poly(2-vinylpyridine), but showed absorptions at 1710 cm^{-1} (ester C=O), 1250 cm^{-1} (ester OC–O–C), 1650 and 1530 cm^{-1} (amide C=O) which are characteristic of poly(CAEA), and absorptions at 1565 and 1590 cm^{-1} (pyridyl group) and 1430 and 1470 cm^{-1} (CH_2) which are characteristic of poly(2-vinylpyridine). These observations indicate that the product is a true copolymer of CAEA and 2-vinyl pyridine.

After deblocking copoly(CAEA–VP), the i.r. spectrum of the reaction product did not show any absorptions at 1710 and 1250 cm^{-1} due to the carbamate group and at 700 cm^{-1} due to phenyl group. Therefore, the conversion of CAEA units into AEA units by deblocking was considered complete. The reaction product should be copoly(AEA–VP).

In the reaction product of the Phe NCA polymerization initiated by copoly(AEA–VP), the i.r. absorptions at 1650 and 1530 cm^{-1} (amide C=O) and 3000 cm^{-1} (amide NH) were much stronger than before the NCA polymerization, and new absorptions appeared at 700 and 750 cm^{-1} (phenyl group). These absorptions are characteristic of poly(phenylalanyl) units and appear either in benzene-soluble or in benzene-insoluble fractions of the product. the i.r. absorptions were also observed at 1430 and 1470 cm^{-1} (CH_2 group) and 1565 and 1590 cm^{-1} (pyridyl group) either in benzene-soluble or in benzene-insoluble fractions. These absorptions are characteristic of 2-vinyl pyridyl units. These observations indicate the formation of a graft copoly(AEA–VP–Phe). The characteristic absorption of the 2-vinyl pyridine unit appeared stronger in the benzene-soluble fraction than in the benzene-insoluble fraction. Copoly(AEA–VP–Phe) containing more 2-vinyl pyridine units should be more soluble in benzene.

The absorptions due to phenylalanyl units observed in the benzene-soluble fraction are due to the phenylalanyl residue in the graft copolymer. However, those observed in the benzene-insoluble fraction could be due partly to poly(phenylalanines) which were produced in the polymerization initiated by basic 2-vinyl pyridyl units of copoly(AEA–VP). The tertiary amine initiator initiates the Phe NCA polymerization according to the activated NCA mechanism, and consequently does not enter into the resulting polypeptides[13]. The separation of poly(phenylalanine), if formed, was attempted but proved unsuccessful. By extraction with MeOH, in which poly(2-vinyl pyridine) is soluble, of either benzene-soluble or benzene-insoluble fractions, MeOH-soluble and MeOH-insoluble fractions were obtained. I.r. spectra of both fractions were nearly identical regardless of the different solubilities in benzene. The solubility differences in copoly(AEA–VP–Phe) are therefore probably due to

variations in composition which the activated NCA-type polymerization may influence. The activated NCA mechanism leads to a functional intermediate carrying an NCA terminal which can couple with primary amines (AEA units) as well as NCA anions, producing polypeptide-rich graft copolymers.

Acrylonitrile-γ-benzyl (S)*-glutamate graft copolymer*

The i.r. spectrum of copoly(CAEA–AN) was different from polyacrylonitrile and poly(CAEA), but showed absorptions at 1710 cm^{-1} (ester C=O), 1250 cm^{-1} (ester OC–O–C), 1660 and 1530 cm^{-1} (amide C=O), and 750 and 700 cm^{-1} (phenyl group) which are characteristic of CAEA units, and an absorption at 2230 cm^{-1} which is characteristic of acrylonitrile. Since the product contained no $CHCl_3$-soluble fractions, the CAEA units in the reaction product must have been incorporated by copolymerization. These observations indicate the formation of a true copolymer.

The infra-red spectrum of the product of debenzylation by H_2/Pd was compared with that obtained from copoly(CAEA–AN), and was found to be nearly the same. It was considered that only some of the CAEA units in copoly(CAEA–AN) were deblocked into AEA units by H_2/Pd, and the difficulty of debenzylation of protected polymer side chains by H_2/Pd has previously been reported[12]. The debenzylation of copoly(CAEA–AN) by HBr/AcOH was also unsuccessful. The incompletely debenzylated copolymer [copoly(AEA–AN)] was used for the further reactions.

The i.r. spectrum of the $CHCl_3$ insoluble fraction in the graft copolymerization product is shown in *Figure 4*, where the absorptions at 1650 and 1540 cm^{-1} (amide C=O), 695 and 740 cm^{-1} (phenyl group), and 3050 cm^{-1} (phenyl group) appear more strongly than in copoly(AEA–AN). An absorption at 1730 cm^{-1} due to carbonic ester C=O is observable in *Figure 4*, which is close to but definitely different from an absorption at 1710 cm^{-1} due to carbamate C=O group which exists because of incomplete deblocking. In *Figure 4*, an absorption appears at 2230 cm^{-1} which is ascribable to acrylonitrile units. Thus the i.r. spectrum of the $CHCl_3$-insoluble product reveals the existence of acrylonitrile units and γ-benzyl glutamate units, and the reaction product is therefore a true graft copolymer copoly[AEA–AN–Glu(OBz)].

Methyl methacrylate–γ-benzyl (S)*-glutamate graft copolymer*

The i.r. spectrum of copoly(CAEA–MMA) was different from those of poly(CAEA) and poly(MMA). It showed absorptions at 1660 and 1535 cm^{-1} (amide C=O) and 3050 and 700 cm^{-1} (phenyl group) which are characteristic of CAEA units, and absorptions at 1390 cm^{-1} (CH_3), 1480 cm^{-1} (CH_2, CH_3), and 1190 cm^{-1} (not assignable) which are characteristic of MMA units. These observations indicate the formation of a true copolymer.

After the treatment of copoly(CAEA–MMA) with HBr/AcOH the i.r. absorptions at 3050 and 700 cm^{-1} due to phenyl groups disappeared. This change indicates the debenzylation of CAEA units in the copolymer and the formation of copoly(AEA–MMA).

Both acetone-soluble and acetone-insoluble fractions of the graft copoly[AEA–MMA–Glu(OBz)] exhibited i.r. absorptions due to γ-benzyl (*S*)-glutamate units and methyl methacrylate units. Since poly[γ-benzyl (*S*)-glutamate] is insoluble and poly(MMA) is soluble in acetone, the formation of a true graft copolymer is apparent. Both acetone-insoluble and acetone-soluble fractions of the graft copolymer gave similar infra-red spectra, suggesting a higher content of MMA units in the latter. This difference of copolymer composition explains the solubility difference.

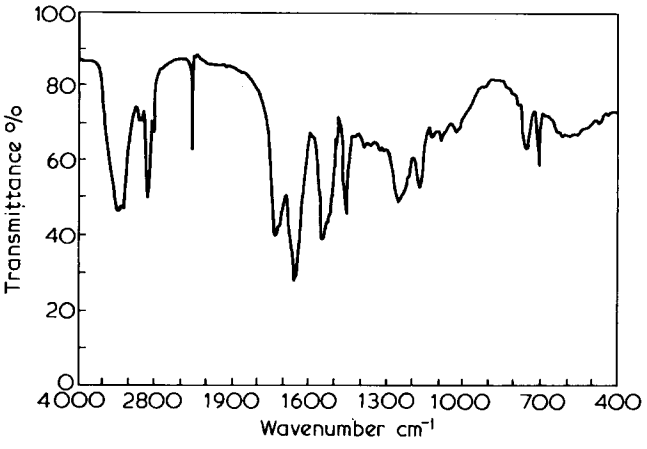


Figure 4 Infra-red spectrum of copoly[AEA–AN–Glu(OBz)], $CHCl_3$-insoluble product: KBr disc

Styrene–α-amino acid graft copolymers having different numbers of branches

The synthesis of vinyl polymer (trunk)–poly(α-amino acid) (branch) graft copolymers having different numbers of branches can easily be achieved by controlling the composition of the vinyl compound–CAEA copolymers. For example, mixtures of styrene and CAEA having different feed compositions were polymerized using radical initiators and styrene–CAEA copolymers having different compositions were produced. The resulting copolymers were subjected to debenzylation and subsequently used as initiators for the graft polymerization of α-amino acid NCA. The details of the procedures are essentially the same as described above. The experimental results are summarized in *Table 1*.

Decreasing portions of CAEA in the feed made its content in copoly(CAEA–ST) lower, and accordingly the content of the primary amino group in copoly(AEA–ST) lower. It should be noted here that when the molar ratio of styrene against CAEA in the feed was higher than twenty, the content of the primary amino group in the copolymer after debenzylation was too low to initiate the polymerization of α-amino acid NCA. This difficulty may have arisen from the imperfect debenzylation process, which remains to be overcome. When (*RS*)-Phe NCA was graft-copolymerized, the copolymer was extracted with benzene. When (*S*)-Glu(OBz) NCA was graft-copolymerized, the copolymer was extracted with cyclohexane. In both cases, both soluble- or insoluble-fractions were recovered. Both fractions showed i.r. absorptions characteristic of styrene and α-amino acid. Since polystyrene is soluble in benzene and cyclohexane but poly(α-amino acids) are insoluble in both, the formation of true copolymers is apparent. It is now established that the number of branches in the graft copolymer can be controlled by the control of the radical copolymerization of styrene and CAEA.

Table 1 Preparation of styrene–α-amino acid graft copolymers having different numbers of branches

Radical copolymerization[a]										
Feed	Copolymer		Debenzylation[d]		NCA grafting[f]			Graft copolymers			
CAEA: styrene, molar ratio	CAEA mol %[b]	$[\eta]^c$ 100 ml g^{-1}	Amine mmol g^{-1}[e]	$[\eta]^c$ 100 ml g^{-1}	Kind of NCA	[NCA]/[Amine]	Conversion %	Solubility	Yield %	α-Amino acid	I.r. absorption styrene
1:5	12.8	0.183	0.85	0.142	(*RS*)-Phe	20	95.1	C_6H_6-sol.	1.2	0	0
								C_6H_6-insol.	89	0	0
1:10		0.216	0.60		(*RS*)-Phe	20	87.1	C_6H_6-sol.	trace	0	0
								C_6H_6-insol.	75.8	0	0
1:20	9.6	(0.216)	0.175	0.175	(*RS*)-Phe	30	60.8	C_6H_6-sol.	4.4	0	0
								C_6H_6-insol.	51.7	0	0
1:20	3.6	(0.216)	0.267	0.210	(*S*)-Glu(OBz)	20	83.4	C_6H_{12}-sol.	trace	0	0
								C_6H_{12}-insol.	75.8	0	0
1:30		0.218	<0.1		(*RS*)-Phe	100	trace				
1:40	2.9	0.219	<0.1	0.158	(*RS*)-Phe	20	trace				
1:50	1.7	0.216	<0.1		(*RS*)-Phe	10	trace				

[a] In PhMe by AIBN at 100°C;
[b] by elemental analysis;
[c] in benzene solution at 30°C;
[d] in PhMe by HBr/AcOH at room temperature;
[e] titrated with N/100 HCl;
[f] in CH_2Cl_2 at room temperature

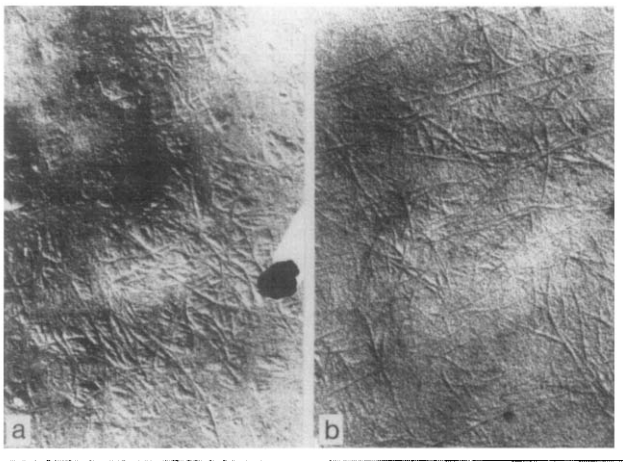


Figure 5 Transmission electron microscope photograph of polymer film cast from 0.1% $C_2H_2Cl_4$ solution. (a) Poly[(*RS*)-phenylalanine], *DP* 550, one-step replica; (b) copoly(AEA–ST–Phe), *DP* of trunk polymer 300, *DP* of branch polymer = 20, number of branches 18 ~ 19, shadowed with Pt/Pd

Styrene-γ-benzyl (S)*-glutamate graft copolymer having different degrees of polymerization*

The regulation of graft copolymer *DP* can be achieved by controlling the *DP* of either trunk polymer or branch poly(α-amino acid). The *DP* of the branches formed by the normal amine-type polymerization of NCA is determined by the ratio of molar concentrations: [α-amino acid NCA]/[primary amine][14]. The regulation of the *DP* of trunk polymer, which is produced by radical polymerization, is more difficult. The possibility of obtaining a trunk polymer having a higher *DP* by emulsion polymerization than by the usual solution polymerizations was tested.

Starting with copoly(CAEA–ST) having a molecular weight as high as 2.38×10^6, γ-benzyl (*S*)-glutamate NCA was grafted to copoly(AEA–ST). The TFA-soluble and TFA-insoluble fractions of the reaction product showed infra-red absorptions characteristic of styrene and γ-benzyl (*S*)-glutamate. Since polystyrene is insoluble in TFA and poly[γ-benzyl (*S*)-glutamate] is soluble in TFA, both the TFA-soluble and TFA-insoluble fractions of the product are true graft copolymers.

Since CAEA is somewhat hydrophilic, a greater amount of emulsifier than usual had to be used to undertake an emulsion polymerization. The *DP* and the number of branches from the trunk polymer were $\sim 2 \times 10^4$ and $\sim 10^3$, respectively, and the conditions for graft copolymerization was so chosen that the *DP* of each branch was 10^2. The total molecular weight of the resulting copoly[AEA–ST–Glu(OBz)] should therefore be $\sim 2.5 \times 10^7$. We have thus succeeded in obtaining a vinyl polymer–poly(α-amino acid) graft copolymer having an extremely high *DP* by emulsion polymerization.

Microstructure of vinyl polymer–poly(α-amino acid) graft copolymer

Since vinyl polymers and poly(α-amino acids) are usually poorly compatible, their graft copolymers are expected to cause microphase separation and thus to form some domain structure. This interesting point has been thoroughly investigated by Professor H. Kawai. The experimental results on the microstructure of vinyl polymer–poly(α-amino acid) graft copolymers are briefly summarized below.

The development of domain structure in vinyl polymer–poly(α-amino acid) graft copolymer and also the development of higher-order organization in poly(α-amino acid) vary regularly with the conditions under which the samples are prepared. Since, here, a crystalline polymer and an amorphous polymer are graft-copolymerized, the mode of phase separation was somewhat different from that of graft copolymers between amorphous polymers. Detailed analysis of the phase separation was therefore difficult, but the following trend was observed.

In *Figure 5a* a transmission electron micrograph of poly[(*RS*)-phenylalanine] film cast from a tetrachloroethane solution one-step replica of, shows the

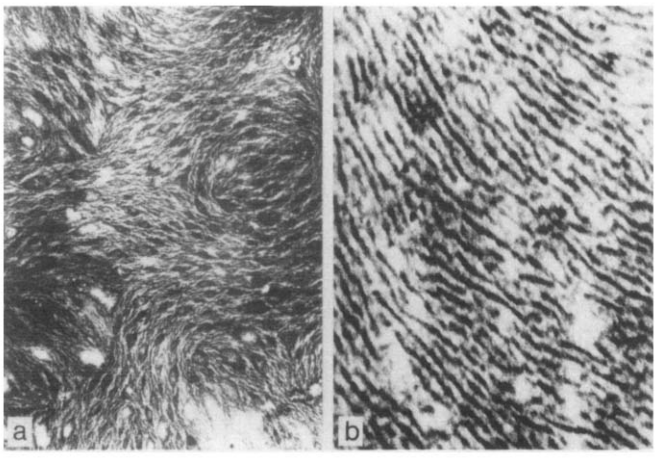


Figure 6 Transmission electron microscope photograph of polymer film cast from 0.1% $C_2H_2Cl_4$ solution and stained with tungstophosphoric acid. (a) Poly[γ-benzyl (*S*)-glutamate], *DP* = 500; (b) copoly[AEA–ST–Glu(OBz)], *DP* of trunk polymer = 225, *DP* of branch polymer = 20, number of branches 6 ~ 7

formation of needle-like textures, ~50 Å wide. Copoly(AEA–ST–Phe) film shadowed with Pt/Pd showed the same sort of texture, shown in *Figure 5b*. In these cases, no effect of grafting on the formation of domain structure was observed.

In *Figure 6a* a transmission electron microscope photograph of poly[γ-benzyl (*S*)-glutamate] film cast from a tetrachloroethane solution and stained with phosphotungstic acid shows the formation of crystalline, rope-like textures, ~600 Å wide. However, in copoly[AEA–ST–Glu(OBz)] film treated similarly, a crystalline portion made up of poly[γ-benzyl (*S*)-glutamate] branches formed rod-like textures, ~150 Å wide, placed in an amorphous material made up or trunk polystyrenes, shown in *Figure 6b*. In this case, a definite effect of grafting on the development of higher-order structure was clearly observed.

DISCUSSION

By the synthesis and reaction of vinyl copolymers containing suitable numbers of CAEA units, vinyl polymer–poly(α-amino acid) graft copolymers were successfully synthesized. The method has the following features: (i) different kinds of vinyl polymers can be used as trunk polymers; (ii) different kinds of poly(α-amino acid) can be connected as branch polymers; (iii) the *DP* of trunk polymer and the *DP* of branch polymer are variable; (iv) the number of branches is changeable; (v) the distance of the amino group which produces the poly(α-amino acid) branch from the backbone of trunk polymer is controlled by n–$H_2N(CH_2)_nNH_2$ used in reaction (1) (this is not experimentally shown here). Apparently this method has wide applicability and a general use as a means of preparing vinyl polymers or copolymers carrying pendant primary amino groups.

The only limitation to the use of CAEA is that it is only slightly copolymerizable with vinyl compounds having small Q values. In view of its good copolymerizability with styrene ($Q=1$), methyl methacrylate ($Q=0.74$), acrylonitrile ($Q=0.60$), and 2-vinyl pyridine ($Q=1.30$), the unsuccessful copolymerization with *N*-vinyl pyrrolidone ($Q=0.14$) is easily understandable[15]. This is in contrast to a recent paper by Carpino *et al.*[16] who reported a copolymer of *N*-vinyl pyrrolidone and 2-cyanoethyl *N*-vinyl carbamate which is a source of pendant primary amino group in the copolymer.

A polymer and copolymers of AEA could be used as supports for the immobilization of biologically-active substances such as enzymes, hormones, drugs, etc. We are more interested in the biological activity of vinyl polymer–poly(α-amino acid) graft copolymers, and in particular, the use of the graft copolymers as antithrombogenic materials.

ACKNOWLEDGEMENT

The authors are deeply indebted to Professor H. Kawai and Dr T. Hashimoto of this department for transmission electron microscope experiments and the discussions on the heterogeneous surface structure of the graft copolymers.

REFERENCES

1 Okawara, M. 'Chemical Reactions of Polymers', Kagaku Dojin, Kyoto, 1972
2 Jones, G. D., Zomlefer, J. and Hawkins, K. *J. Org. Chem.* 1944, **9**, 500
3 Dawson, D. J., Gless, R. D. and Wingard, Jr., R. E. *J. Am. Chem. Soc.* 1976, **98**, 5996
4 Ballard, D. G. H. and Bamford, C. H. *Proc. Roy. Soc.* (*A*) 1954, **223**, 495
5 Imanishi, Y. 'Enzymes and Polymer Catalysts', Kagaku Dojin, Kyoto, 1972
6 Lyman, D. J., Knutson, K., McNeill, B. and Shibatani, K. *Trans. Am. Soc. Artif. Int. Organs* 1975, **21**, 49
7 Yamashita, Y., Iwaya, Y. and Ito, K. *Makromol. Chem.* 1975, **176**, 1207
8 Billot, J-P., Douy, A. and Gallot, B. *Makromol. Chem.* 1976, **177**, 1889
9 Perly, B., Douy, A. and Gallot, B. *Makromol. Chem.* 1976, **177**, 2569
10 Billot, J-P., Douy, A. and Gallot, B. *Makromol. Chem.* 1977, **178**, 1641
11 Lawson, W. B., Leafer, Jr., M. D., Tewes, A. and Rao, G. J. S. *Hopp-Seyler's Z. Phys. Chem.* 1968, **349**, 251
12 Hart, R. *J. Polym. Sci.* 1958, **29**, 629
13 Bamford, C. H. and Block, H. 'Poly-α-Amino Acids, Polypeptides and Proteins', Univ. of Wisconsin Press, Madison, Wisc., 1962
14 Bamford, C. H., Elliott, A. and Hanby, W. E. 'Synthetic Polypeptides', Academic Press, New York, 1956
15 Young, L. J. *J. Polym. Sci.* 1961, **54**, 411
16 Carpino, L. A., Hettrich, G. M. and Ringsdorf, H. *Makromol. Chem.* 1978, **179**, 2877