Block copolymerization of styrene using azo-containing poly(γ-benzyl L-glutamate) initiator and interaction of the block copolymers with blood

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Poly(γ-benzyl L-glutamates) containing an azo group (Azo-PBLG) were synthesized. They were shown to take an α-helical conformation in solid phase and in dimethylformamide solution, when the degree of polymerization was higher than ten. The rate constants of decomposition and initiation of styrene polymerization by Azo-PBLG were found to be affected by the degree of polymerization of Azo-PBLG. The rate of polymerization of styrene with Azo-PBLG in dimethylformamide solution at 120°C was proportional to $[\text{styrene}]^{2.83}$ and $[\text{Azo-PBLG}]^{0.45}$, which suggests the occurrence of chain-transfer reaction to solvent. With increasing degree of polymerization of Azo-PBLG, the overall rate constant increased, which suggests the participation of styrene–Azo-PBLG interaction in the polymerization. The block copolymerization yielded poly(γ-benzyl L-glutamate)–polystyrene–poly(γ-benzyl L-glutamate) (A–B–A-type) block copolymers, whereas the block copolymerization in the presence of n-hexylmercaptane gave A–B-type block copolymers. *In vitro* blood-clotting test of the block copolymers showed that the block copolymer containing *ca.* 15 mol% of γ-benzyl L-glutamate unit was most antithrombogenic and the adsorption of plasma proteins to this block polymer caused a low degree of denaturation.

(Keywords: azo-containing poly(γ-benzyl L-glutamate); initiator efficiency; block copolymerization; styrene; *in vitro* blood clotting test; protein adsorption)

INTRODUCTION

Block and graft copolymers of poly(α-amino acids), which are usually polar, hydrophilic and crystalline, with vinyl polymers, which are usually non-polar, hydrophobic and amorphous, have been of interest in the application of biocompatible materials. On the microphase-separated surface of the block or graft copolymer films of this kind, adsorption of plasma proteins and activation of platelet are controllable, leading to the antithrombogenicity[1,2].

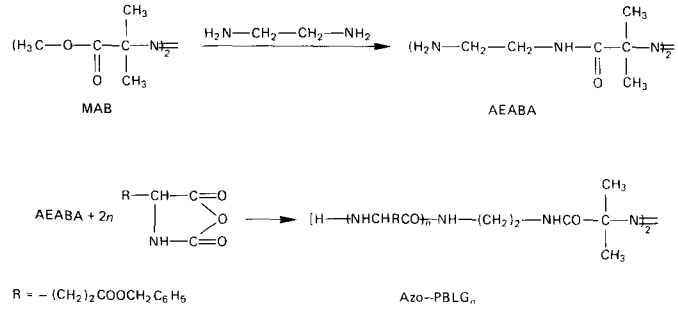
Block copolymers of α-amino acid and vinyl compounds have been synthesized by radical polymerization initiated by the terminal-group activation of poly(α-amino acids)[3] or by α-amino acid *N*-carboxyanhydride (NCA) polymerization initiated by the terminal active group of vinyl polymers[4–10]. In the present investigation we explored a new method for preparing block copolymers of poly(α-amino acids) (A-segment) and vinyl polymers (B-segment), in which azo-containing poly(γ-benzyl L-glutamates) (Azo-PBLG$_n$) having various degrees of polymerization (n) were used to initiate the radical polymerization of styrene. Block copolymers have been prepared by the same method previously[11], but characterization of the polymer initiator and the product copolymers was not detailed.

In this paper, the preparation and properties of Azo-PBLG$_n$, kinetics of radical polymerization of styrene with Azo-PBLG$_n$, and the blood compatibility of the block copolymers will be described.

EXPERIMENTAL

Synthesis of Azo-PBLG$_n$

Azo-PBLG$_n$ was synthesized by the scheme shown below.



Dimethyl 2,2′-azobisisobutyrate (MAB) (5 g) was dissolved in a small amount of diethylether, and the solution was added dropwise to 1,2-diaminoethane (50 ml, 34.5 eq) under stirring. The mixture was stirred for 4 days and the solvent was distilled off to leave a white solid. The product was dissolved in a small amount of methanol and hydrochlorinated. 1,2-Diaminoethane dihydrochloride, which is insoluble in methanol, was removed. The filtrate was poured into ethyl acetate and the insoluble material was recovered. The product was treated with an anion-exchange resin, Amberlite IRA-

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400, and made free from hydrogen chloride. The yield of bis(2-aminoethyl)-2,2'-azobisisobutyramide (AEABA) was 4.97 g (80%). Elemental analysis, calculated for $C_{12}H_{26}N_6O_2$: C, 50.35%; H, 9.09%; N, 29.37%. Found: C, 49.48%; H, 9.16%; N, 29.10%. The structure of AEABA was identified by the i.r. spectrum: 1630 cm^{-1} (amide I), 1530 cm^{-1} (amide II), and the absence of 1740 cm^{-1} (ester carbonyl). The structure of AEABA was identified by proton nuclear magnetic resonance (^1H n.m.r.) spectrum, too. U.v. spectrum of AEABA showed an absorption maximum at 375 nm ($\varepsilon = 26.04$). AEABA was titrated with 0.1 M $HClO_4/CH_3OH$ using bromophenol blue as an indicator, showing an incorporation of terminal amino groups in the molecule to be complete.

γ-Benzyl L-glutamate [Glu(OBzl)] was synthesized from benzyl alcohol and L-glutamic acid, and purified by recrystallization from hot water. Glu(OBzl) was reacted with trichloromethyl chloroformate in tetrahydrofuran to yield Glu(OBzl) NCA, which was recrystallized from ethyl acetate/n-hexane: m.p. 95–96°C (lit. 93–94°C)[12].

Polymerization of Glu(OBzl) NCA using AEABA as an initiator was carried out in dichloroethane at room temperature. Completion of the polymerization was confirmed by the disappearance of i.r. absorptions at 1780 and 1840 cm^{-1} which are characteristic of NCA carbonyl groups, and by the appearance of new absorptions at 1650 and 1550 cm^{-1} which are due to amide I and II linkages, respectively.

Molecular weight (M_n) of Azo-PBLG$_n$ was determined by vapour pressure osmometry in dimethylformamide ($HCONMe_2$) solution at 85°C using Corona 117 molecular weight apparatus. Conformation of Azo-PBLG$_n$ in $HCONMe_2$ solution was investigated by circular dichroism (c.d.) spectrum and n.m.r. spectroscopy, hexamethyl disilane (HMDS) being used as internal standard, and conformation of Azo-PBLG$_n$ film by *FT*i.r. spectroscopy using Digilab FTS 15 E/D apparatus.

Kinetic investigation of styrene polymerization by AEABA

The rate of decomposition of Azo-PBLG$_n$ at 120°C and azobisisobutyronitrile (AIBN) at 60°C was determined by measuring the time course of N_2 evolution[13] from $HCONMe_2$ solution. The decomposition rate constant (k_d) was calculated according to equation (1),

$$\ln[V_\infty/(V_\infty - V_t)] = k_d t \quad (1)$$

where V_t and V_∞ represent the volume of N_2 evolved during time t and infinite time, respectively.

The rate of initiation of styrene polymerization by Azo-PBLG$_n$ at 120°C and AIBN at 60°C in $HCONMe_2$ solution was determined by measuring the induction period (τ) in the presence of $FeCl_3$[14]. The initiation rate constant (k_i) was calculated according to equation (2),

$$\tau = [FeCl_3]_0/2k_i[C]_0 \quad (2)$$

where $[FeCl_3]$ and $[C]_0$ represent the initial concentrations of $FeCl_3$ and the initiator, respectively.

The initiator efficiency (I) in styrene polymerization of Azo-PBLG$_n$ at 120°C and of AIBN at 60°C in $HCONMe_2$ solution was determined as a ratio of k_i against k_d.

The overall rate of styrene polymerization by Azo-PBLG$_n$ at 120°C and AIBN at 60 and 120°C in $HCONMe_2$ solution was determined using a dilatometer. The square of thermal rate of polymerization was subtracted from the square of total rate of polymerization to obtain the square of the catalysed rate of polymerization. The overall rate constant and the kinetic orders with reference to monomer and initiator concentrations in the catalytic polymerization were determined.

Synthesis of block copolymers

Block copolymerization of styrene with Azo-PBLG$_n$ as initiator was carried out in $HCONMe_2$ solution at 90°C in vacuum. After a suitable time lapse, solution was poured into methanol. Methanol-insoluble product was extracted with cyclohexane and acetic acid to remove polystyrene (PST) and poly(γ-benzyl L-glutamate) {P[Glu(OBzl)]}, respectively. Sometimes, the block copolymerization was carried out in the presence of n-hexylmercaptane to increase the content of P[Glu(OBzl)] in the block copolymer.

The M_n of block copolymers was determined by vapour pressure osmometry in $HCONMe_2$ solution using Corona 117 molecular weight apparatus. The degree of polymerization (DP) of P[Glu(OBzl)] segment of the block copolymer can be calculated from the molecular weight and the N% of elemental analysis.

In vitro *blood clotting test and plasma protein adsorption to block copolymers*

$HCONMe_2$ solution (0.1 wt%) of block copolymer was eluted on both sides of a CaF_2 plate, and a film was cast under irradiation of i.r. lamp and vacuum-dried overnight.

Bovine serum albumin (BSA, 4.5 g dl^{-1}), bovine γ-globulin (BγG, 1.6 g dl^{-1}) or bovine plasma fibrinogen (BPF, 0.3 g dl^{-1}), was dissolved in physiological salt solution (pH 7.4, 0.01 M Tris-HCl buffer). The block copolymer film was immersed in the plasma protein solution for 1 h, washed with the buffer solution for 5 min, vacuum-dried over P_2O_5 overnight, and subjected to investigation by *FT*i.r. spectroscopy. The amount of plasma protein adsorbed to the film and the conformation of adsorbed protein were determined as reported previously[15]. The experiment-to-experiment variations of the protein adsorption were 6% for BSA, 7% for BγG and 5% for BPF in the present investigation.

For *in vitro* blood clotting test, fresh canine blood was used. To freshly collected blood (100 ml), ACD solution (15 ml), which contained D-glucose (2.45 g), sodium citrate dihydrate (2.20 g) and citric acid monohydrate (0.80 g) in distilled water (100 ml), was added, and the mixture was stored in a refrigerator. The blood-clotting test was carried out within 5 h after the sampling of blood. The ACD blood (200 μl) was put on a polymer film in the atmosphere thermostatted at 37°C, and 0.1 M aqueous $CaCl_2$ solution (20 μl) was added to start the clotting. Thereafter the time course of thrombus formation was followed according to the method reported by Imai and Nosé[16]. The range of deviation in the present experiments was ±9%.

Table 1 Polymerization of γ-benzyl L-glutamate NCA[a]

Molar ratio [NCA]/[Amine]	Conv. of NCA (%)	M_n[b]	DP[c]
10	88	4700	10
30	91	12000	27
40	95	16800	37
40	82	16900	38

[a] [NCA] = 1.7 mol l^{-1}
[b] Measured by vapour pressure osmometry ($HCONMe_2$, 85°C)
[c] Number of peptide units added to each amino terminal of AEABA

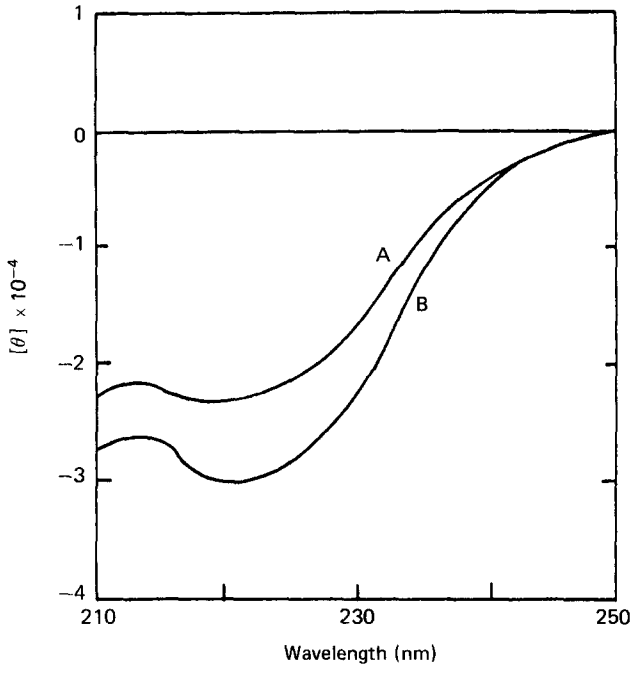

Figure 1 Circular dichroism spectra of Azo-PBLG$_n$ in dioxane solution at 25°C. A, $n = 10$; B, $n = 27, 37$

RESULTS AND DISCUSSION

Synthesis of Azo-PBLG$_n$

The experimental results on the polymerization of Glu(OBzl) NCA by AEABA are shown in *Table 1*. Three different molar ratios of the NCA concentration against the amine concentration were employed in the present experiments. Since the *DP* of the product Azo-PBLG$_n$ agrees nearly with the [NCA]/[amine] molar ratio in the feed, it is concluded that the polymerization was initiated by the nucleophilic addition of the terminal amino groups of AEABA initiator to the 5-carbonyl group of Glu(OBzl) NCA[17]. Therefore, the structure of Azo-PBLG$_n$ should be as expected above.

Conformation of Azo-PBLG$_n$

C.d. spectra of Azo-PBLG$_n$ in dioxane solution at 25°C are shown in *Figure 1*. All spectra show the pattern characteristic of α-helical conformation. However, the molar ellipticity at 222 nm of Azo-PBLG$_{10}$ was only -2×10^4, suggesting a partial involvement of randomly coiled conformation.

Transmission *FT*i.r. spectra of Azo-PBLG$_n$ film are shown in *Figure 2*. The pattern of amide absorption in i.r. spectra is independent of n of Azo-PBLG$_n$ and characteristic of α-helical conformation.

These spectroscopic investigations clearly show that Azo-PBLG$_n$ takes mainly the α-helical conformation either in solution or in solid phase.

As will be described later, kinetic investigations of radical polymerization of styrene by Azo-PBLG$_n$ were carried out at 120°C. Therefore, the conformation of Azo-PBLG$_n$ at higher temperatures was investigated by n.m.r. spectroscopy in $HCONMe_2$-d_7. The ^1H n.m.r. spectrum of Azo-PBLG$_{37}$ at 120°C is shown in *Figure 3*. Allowing for the fact that the half-life of Azo-PBLG$_{37}$ at 120°C is

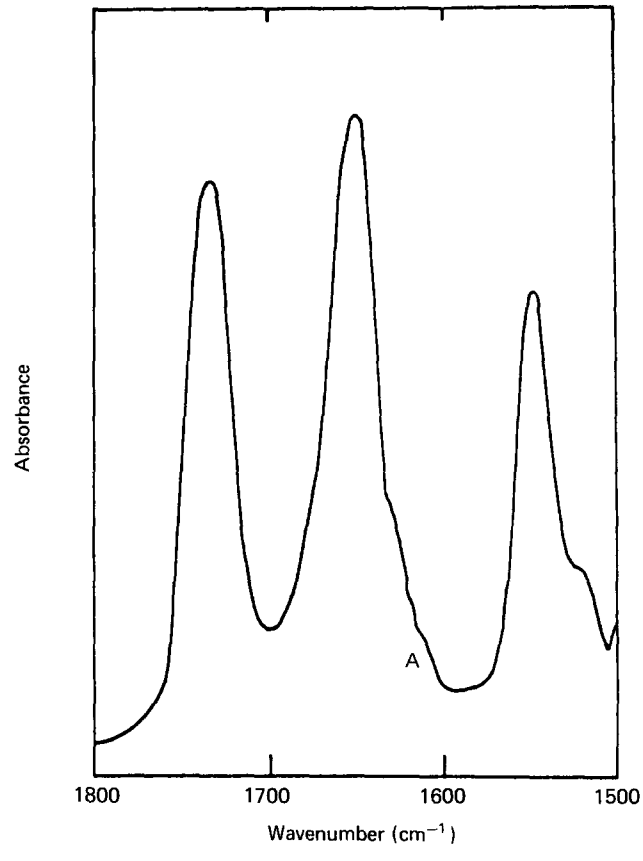

Figure 2 Amide absorption region in transmission *FT*i.r. spectra of Azo-PBLG$_n$ films cast from $HCONMe_2$ solution. A, $n = 10, 27, 37$

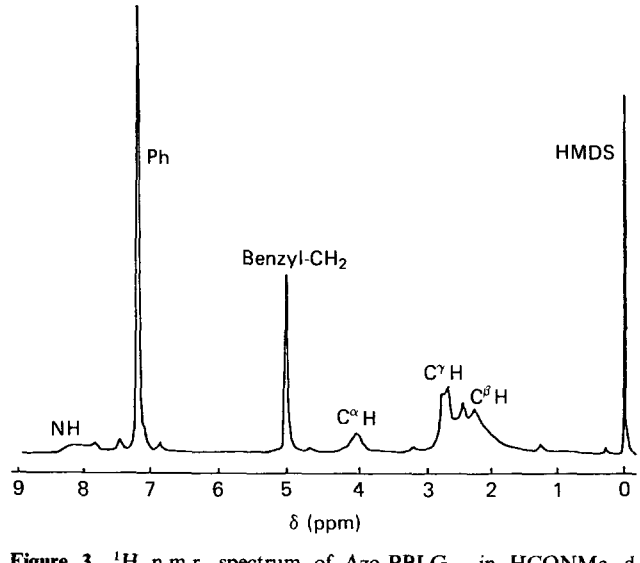

Figure 3 ^1H n.m.r. spectrum of Azo-PBLG$_{37}$ in $HCONMe_2$-d_7 solution at 120°C, hexamethyl disilane (HMDS) being used as internal standard

about 3 min, Azo-PBLG$_{37}$ may have partly decomposed during the measurement. However, the disappearance of azo group may not affect the conformation of PBLG$_{37}$, so that we can discuss the conformation of Azo-PBLG$_{37}$ in $HCONMe_2$ at 120°C. The chemical shift of $C^{\alpha}H$ and NH signal indicates that Azo-PBLG$_{37}$ takes the α-helical conformation[18]. The spectrum in $HCONMe_2$ did not change over a temperature range from 30 to 120°C. The spectrum at 30°C did not change on the addition of trifluoroacetic acid up to 20 vol%. Therefore, it can be concluded that α-helical conformation of Azo-PBLG$_n$ is stable under these conditions.

Decomposition of Azo-PBLG$_n$

$HCONMe_2$ solution of Azo-PBLG$_{10}$ was heated at 120°C and the nitrogen evolution was determined. The plot of $\ln[V_\infty/(V_\infty - V_t)]$ against t according to equation (1) gave a straight line (seven points). Values of k_d determined from the slope of the straight line are listed in *Table 2* for various aliphatic azo compounds.

In *Table 2* is included the k_d value for AIBN at 60°C, which is very close to $8.45 \times 10^{-6}\ s^{-1}$ reported for AIBN at 60°C in benzene[19], confirming the validity of the experimental method employed in the present experiment.

It is shown that k_d for Azo-PBLG$_n$ increases with increasing n. This could be due to an increased strain on the azo group with increasing chain length of polypeptide.

Initiation of radical polymerization of styrene by Azo-PBLG$_n$

The induction period τ of styrene polymerization initiated by four different concentrations of Azo-PBLG$_{10}$ in the presence of $FeCl_3$ was determined, and plotted against the initiator concentration. The plot was linear and k_i was determined from the slope of the straight line according to equation (2). The k_i values are listed in *Table 2* for various aliphatic azo compounds.

The k_i value for AIBN at 60°C is also included in *Table 2*, which agrees very well with $7.0 \times 10^{-6}\ s^{-1}$ reported previously[14]. The agreement shows the accuracy of the present experimental procedure.

It is shown that k_i for Azo-PBLG$_n$ decreases with increasing n. This could be due to a diffusion of polypeptide radical produced in thermal decomposition of Azo-PBLG$_n$ outside the cage being more difficult with increasing chain length of polypeptide.

Initiator efficiency of Azo-PBLG$_n$

The initiator efficiency of various aliphatic azo compounds in styrene polymerization was calculated from k_i and k_d values and is shown in *Table 2*. The initiator efficiency for AIBN is 100%, which is very different from about 60% reported for the styrene polymerization in benzene[20]. The disagreement could be due mainly to rather rough experimental procedures taken by us, and partly to different methods employed for the determination of the efficiency by us and by Bevington[20]. Bevington used ^{14}C-labelled AIBN to initiate the polymerization of styrene in benzene at 60°C. He determined the kinetic chain length after necessary corrections were made on the concentration of initiator dropping during polymerization, some fractionation of the polymer during its recovery, and termination of reaction chains with radicals derived directly from the initiator. If the overall rate of polymerization is divided by the kinetic chain length, the rate of initiation was found. Comparison of the rate of initiation with the rate of production of radicals from the initiator gave the efficiency of initiation. On the other hand, in our determination, these corrections were not made. The disagreement could be due partly to errors involved in our method of determination.

The initiator efficiency of Azo-PBLG$_n$ at 120°C is still much smaller than that of AIBN at 60°C, and decreases with increasing n. These results could have arisen from a slow diffusion of polypeptide radical outside the cage.

Overall rate of styrene polymerization by Azo-PBLG$_n$

The polymerization of styrene initiated by Azo-PBLG$_n$ was immeasurably slow either at 60 or at 80°C. Therefore, we had to raise the polymerization temperature to 120°C, where we observed moderate polymerizations. The experimental results are summarized in *Table 3*. The results in the comparative experiments for AIBN are also included in *Table 3*. The polymerization temperature of 120°C for styrene polymerization with AIBN is much higher than usual, but we had to use this for comparison. It has been reported[21] that the rate of styrene polymerization by AIBN in $HCONMe_2$ at 60°C is

Table 2 Decomposition rate constant (k_d), initiation rate constant (k_i) and initiator efficiency (I) in styrene polymerization initiated by Azo-PBLG$_n$ or AIBN in $HCONMe_2$

Azo compound	Temperature (°C)	k_d (s^{-1})	k_i (s^{-1})	I (%)
Azo-PBLG$_{10}$	120	2.4×10^{-3}	7.3×10^{-4}	30.4
Azo-PBLG$_{27}$	120	3.0×10^{-3}	4.7×10^{-4}	15.7
Azo-PBLG$_{37}$	120	3.2×10^{-3}	4.4×10^{-4}	13.8
AIBN[a]	60	7.6×10^{-6}	7.6×10^{-6}	100

[a] Literature values for AIBN at 60°C, $k_d = 8.45 \times 10^{-6}\ s^{-1}$ (in benzene)[19], $k_i = 7.0 \times 10^{-6}\ s^{-1}$ (in $HCONMe_2$)[14], $I = 60\%$ (in benzene)[20]

Table 3 Polymerization of styrene initiated by Azo-PBLG$_n$ or AIBN in $HCONMe_2$; $R_p = k[M]^a[C]^b$

Styrene (mol l^{-1})	Initiator		Temperature (°C)	k ($l\ mol^{-1}\ s^{-1}$)	a	b
	Nature	mmol l^{-1}				
2.60–5.19	Azo-PBLG$_{10}$	0.86–2.23	120	2.0×10^{-5}	3.08	0.43
1.73–6.06	Azo-PBLG$_{27}$	0.37–5.52	120	$4.7 \pm 2.5 \times 10^{-5}$	2.60	0.42
2.60–6.06	Azo-PBLG$_{37}$	0.24–1.19	120	$2.2 \pm 0.1 \times 10^{-4}$	2.80	0.50
2.60–6.06	AIBN	0.55–1.22	120	2.8×10^{-4}	2.19	0.45
1.97–6.69	AIBN	25.1–83.0	60	$5.8 \pm 0.4 \times 10^{-5}$	0.94	0.46

Lit.[21] $R_p = 5 \times 10^{-5}\ [M]^{1.05}\ [C]^{0.45}$ (AIBN, 60°C)

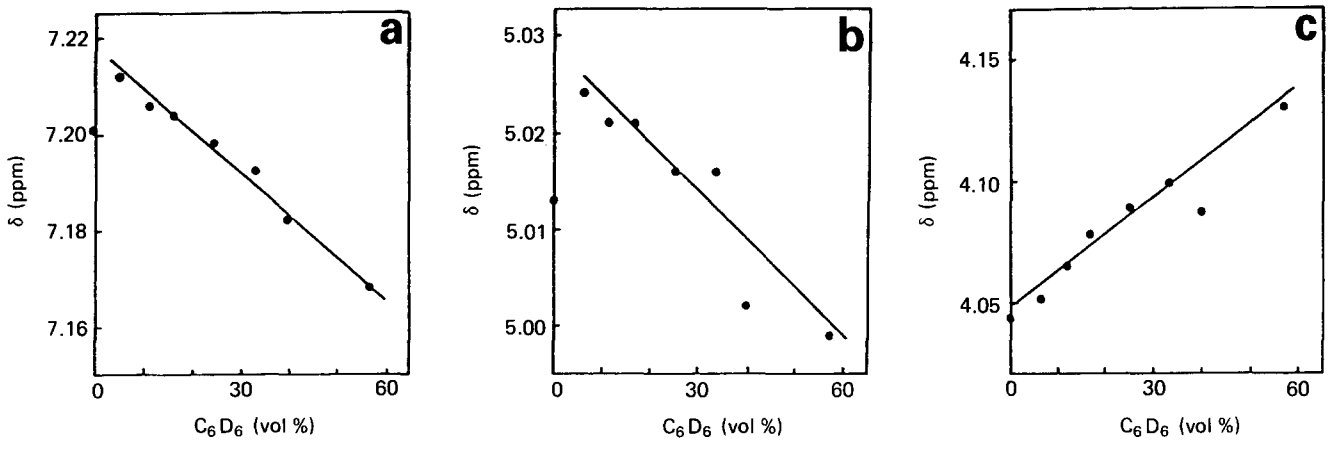

Figure 4 Effect of benzene-d_6 addition on ^{1}H n.m.r. chemical shift of Azo-PBLG$_{37}$ in HCONMe$_2$-d_7 solution at 120°C. (a) Ph, (b) benzyl-CH$_2$, (c) C$^\alpha$H

Table 4 Block copolymerization of styrene initiated by Azo-PBLG$_n$ in HCONMe$_2$ at 90°C

Block copolymer	*DP* of Azo-PBLG$_n$	Azo-PBLG$_n$ (mg)	Styrene (g)	Mercaptane (μl)	HCONMe$_2$ (ml)	Styrene conv. (%)	Block copolymer yield (g)
P[Glu(OBzl)]/PST-2.3	38	45	2.72	0	3	32.5	0.927
P[Glu(OBzl)]/PST-4.2	38	90	2.72	0	3	32.6	0.968
P[Glu(OBzl)]/PST-5.0	38	135	2.72	0	8	37.4	1.130
P[Glu(OBzl)]/PST-9.3	10	300	7.25	100	8	44.4	0.856
P[Glu(OBzl)]/PST-13.0	10	300	7.25	10	8	22.9	0.811
P[Glu(OBzl)]/PST-14.6	27	300	7.25	10	8	27.9	0.524
P[Glu(OBzl)]/PST-20.2	27	300	7.25	300	8	33.7	0.553
P[Glu(OBzl)]/PST-24.2	27	300	7.25	100	8	33.6	0.476

Efficiency of block copolymn. (%)		Block copolymer		
Azo-PBLG$_n$	Styrene	M_n ($\times 10^{-4}$)[a]	Glu(OBzl) wt%[b]	*DP* of P[Glu(OBzl)][c]
96.6	32.5	11.1	4.7	24
91.4	32.6	9.48	8.5	37
83.7	37.4	7.05	10.0	32
50.8	9.7	0.55	17.8	4
64.6	8.5	1.13	23.9	12
46.1	5.3	1.18	26.4	14
64.1	5.0	0.61	34.8	10
64.3	3.9	0.69	40.5	13

[a] Determined by vapour pressure osmometry
[b] Determined by elemental analysis
[c] Determined by number average molecular weight and P[Glu(OBzl)] content

proportional to $[M]^{1.05}$ and $[C]^{0.45}$ and the overall constant is 5×10^{-5} M^{-1} s^{-1}. These literature values are in excellent agreement with the present values. Nevertheless, for the above reasons, the kinetic measurements must be subject to large errors.

In all of the polymerizations in *Table 3*, the kinetic order with reference to the concentration of initiator is almost unchanged around 0.5, indicating the absence of chain transfer reactions to solvent[22]. On the other hand, the kinetic order with reference to the concentration of monomer in the styrene polymerization by AIBN at 120°C is higher than that at 60°C, and that in the styrene polymerization by Azo-PBLG$_n$ at 120°C is much higher than that by AIBN. The higher kinetic order with reference to the monomer concentration indicates the initiator efficiency depending on the monomer concentration[23]. The dependence of the initiator efficiency on the monomer concentration may be related to the cage effect, which also affected the relation between k_i and n of Azo-PBLG$_n$.

In *Table 3* it is shown that the overall rate constant of the polymerization increased with increasing n of Azo-PBLG$_n$. In highly dipolar HCONMe$_2$, styrene and Azo-PBLG$_n$ may have aromatic–aromatic interactions. This type of interaction may lead to a concentration of styrene along the chain of primary radical or growing radical, hence leading to rate enhancement. If the concentration effect is more remarkable with longer polypeptide chains, the increased rate of polymerization with longer chain initiators can be explained by this view point.

To confirm the occurrence of styrene/P[Glu(OBzl)] interaction, the effect of the addition of benzene-d_6 to ^{1}H n.m.r. spectrum of the HCONMe$_2$-d_7 solution of Azo-PBLG$_{37}$ was investigated. The experimental results are shown in *Figure 4*. The addition of benzene-d_6 caused upfield shift of the phenyl-H signal and benzyl-CH$_2$ signal and downfield shift of C$^\alpha$H signal. The chemical shift change is caused undoubtedly by the magnetic anisotropic effect of benzene in the neighbourhood of Azo-PBLG$_n$ chain. Therefore, it is very likely that in the polymerization of styrene by Azo-PBLG$_n$ styrene has some interaction with P[Glu(OBzl)] chain.

Synthesis of block copolymers

The experimental results are shown in *Table 4*. In the designation of block copolymers, P[Glu(OBzl)]/PST-2.3, for example, represents a P[Glu(OBzl)]/PST block copolymer, the content of the P[Glu(OBzl)] segment being 2.3 mol% on the basis of Glu(OBzl) residue. If the *DP* of P[Glu(OBzl)] segment of block copolymers is twice as large as that of initiator Azo-PBLG$_n$, the formation of P[Glu(OBzl)]–PST–P[Glu(OBzl)] [A–B–A- type) block copolymer should be concluded. On the other hand, if both are equal, we should conclude the formation of A–B-type block copolymer. In the present block copolymers, the *DP* of P[Glu(OBzl)] segment is always smaller than that of initiator Azo-PBLG$_n$. However, the *DP* of P[Glu(OBzl)] segment of the block copolymers obtained in the absence of n-hexylmercaptane is nearly twice as large as those obtained in the presence of n-hexylmercaptane. Therefore, we may conclude that A–B–A-type block copolymers were obtained in the polymerization without n-hexylmercaptane and A–B-type block copolymers in the presence of n-hexylmercaptane. The reason for lower *DP* of P[Glu(OBzl)] segment in block copolymers than expected is not known, but should be related to inaccurate determination of the molecular weight of block copolymers.

The efficiency of block copolymerization of Azo-PBLG$_n$ is much higher than expected from the initiator efficiency of Azo-PBLG$_n$ shown in *Table 2*. This must be due to an incomplete extraction of P[Glu(OBzl)] from the product of block copolymerization.

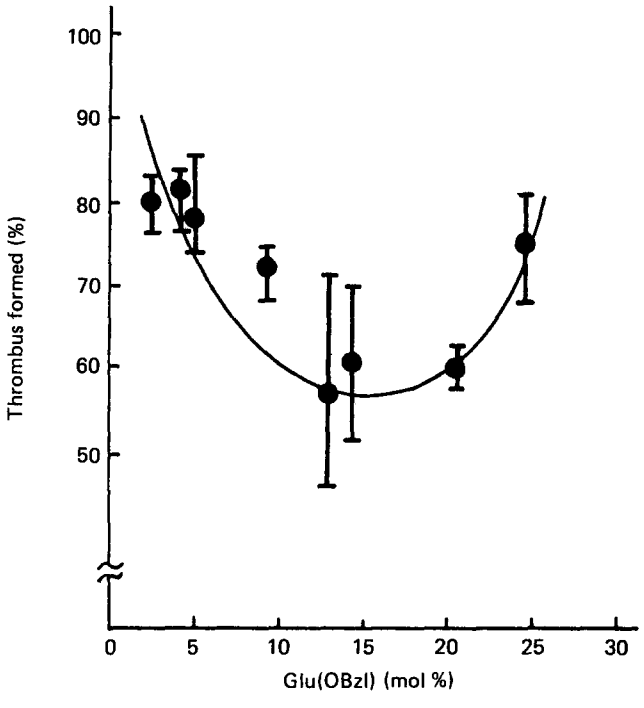

Figure 5 Dependence of thrombus formation rate on the composition of P[Glu(OBzl)]/PST block copolymer

In vitro blood clotting test of block copolymers

The weights of thrombus formed by contact of blood with the block copolymers for 15, 20 and 25 min were averaged, and the average value relative to the weight of thrombus formed in 20 min contact of blood with a glass plate was calculated and plotted against Glu(OBzl) mole per cent in the block copolymers in *Figure 5*.

Since the thrombus formation by Azo-PBLG$_{10}$ reaches 80–90%, the P[Glu(OBzl)]/PST block copolymers are more thromboregistant than Azo-PBLG$_{10}$. It is very interesting that the rate of thrombus formation takes the minimum value at the Glu(OBzl) content of 15 mol%. The conclusion that with P[Glu(OBzl)]/PST block copolymers the best antithrombogenicity is reached at a relatively low content of P[Glu(OBzl)] segment has been obtained in our previous work[3,24].

Table 5 Amount and conformation of plasma proteins adsorbed to block copolymers

Block copolymer	BSA				Bγ G				BPF			
	Amount of adsorption ($\mu g\ cm^{-2}$)			Degree of denaturation (%)	Amount of adsorption ($\mu g\ cm^{-2}$)			Degree of denaturation (%)	Amount of adsorption ($\mu g\ cm^{-2}$)			Degree of denaturation (%)
	Native	Total	Denatured		Native	Total	Denatured		Native	Total	Denatured	
P[Glu(OBzl)]/PST-2.3	3.22	6.02	2.80	46.5	2.12	6.67	4.55	68.2	1.78	2.86	1.08	37.8
P[Glu(OBzl)]/PST-4.2	4.71	8.77	4.06	46.3	2.35	8.22	5.87	71.4	2.08	3.55	1.47	41.4
P[Gly(OBzl)]/PST-5.0	6.30	10.04	4.17	39.8	5.60	12.88	7.28	56.5	5.12	6.41	1.29	20.1
P[Glu(OBzl)]/PST-9.3	12.14	12.14	0	0	1.08	2.42	1.34	55.4	2.41	2.83	0.42	14.8
P[Glu(OBz))]/PST-13.0	7.83	7.83	0	0	1.09	2.53	1.44	56.9	2.08	2.37	0.29	12.4
P[Gly(OBzl)]/PST-14.6	10.81	10.81	0	0	1.27	2.77	1.50	54.2	1.78	2.08	0.30	14.4
P[Glu(OBzl)]/PST-20.2	14.42	18.52	4.10	22.1	1.78	4.53	2.75	60.7	1.62	1.92	0.30	15.6
P[Glu(OBzl)]/PST-24.2	9.05	14.31	5.26	36.8	1.86	4.87	3.01	61.8	1.91	2.25	0.34	15.1

Adsorption of plasma proteins to block copolymers

The experimental results are shown in *Table 5*.

When plasma proteins are adsorbed to the surface of block copolymer films, they are denatured more or less. The denaturation upon adsorption was most extensive with BγG, and less extensive with BSA and BPF. Among three kinds of plasma proteins, the denaturation of albumin upon adsorption was most strongly dependent on the composition of block copolymer. It is very interesting that with any plasma protein the degree of denaturation took the minimum value upon adsorption to block copolymers containing P[Glu(OBzl)] segment of 9.3–14.6 mol%. It should be noted that the minimum degree of protein denaturation upon adsorption to a block copolymer corresponds to the minimum degree of thrombus formation on the block copolymer. This correspondence has been observed with P[Glu(OBzl)]/PST block copolymers in our previous work[3,24]. Therefore, it is a firm conclusion that a controlled surface property of P[Glu(OBzl)]/PST block copolymer suppresses the plasma protein denaturation upon adsorption which in turn suppresses thrombus formation. The mechanism by which the protein denaturation activates the thrombogenic system is to be investigated in future.

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