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ORIGINAL ARTICLE

Helix–coil transformation of poly(γ-benzyl-L-glutamate) with polystyrene attached to the N or C terminus in trifluoroacetic acid–chloroform mixtures

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The helix–coil transformations of block copolymers composed of poly(γ-benzyl-L-glutamate) (PBLG) and polystyrene (PS), in which PS was attached to either the N terminus ($PBLG_n$-N-PS_m) or the C terminus (PS_n-C-$PBLG_m$) of PBLG, were investigated in trifluoroacetic acid (TFA)–chloroform mixtures with TFA concentrations in the range of 0.7–10 vol%. The helical content (f_H) estimated from ^1H nuclear magnetic resonance measurements indicated that the PBLG segment in $PBLG_{55}$-N-PS_{160} underwent a gradual helix–coil transformation from $f_H=1$ to $f_H=0.75$ over the range of 3.6–5.7 vol% TFA and then a drastic transformation to $f_H=0$ at 9.5 vol% TFA, in a manner similar to that of the $PBLG_{55}$ homopolymer. In PS_{120}-C-$PBLG_{55}$, the helical deformation of the PBLG segment was observed by adding a very small amount of TFA (0.7–3.6 vol%), followed by gradual and drastic transformations at higher TFA concentrations. The results indicate that the conformational stability of the C terminus in a PBLG chain differs from that of the N terminus. Transformations of PBLG/PS block copolymers with various molecular weights and compositions were also studied.
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Keywords: block copolymer; chain terminus; chloroform; helix–coil transformation; poly(γ-benzyl-L-glutamate) (PBLG); polystyrene; trifluoroacetic acid (TFA)

INTRODUCTION

Block copolymers composed of polypeptide and synthetic polymer segments have received much interest because of their great potential to yield novel materials with the combined properties of the two segments.[1,2] One of the attractive features of block copolymers is that the self-assembly of peptide/synthetic block copolymers is driven not only by microphase segregation but also by aggregation of α-helical rod segments.[3] The helical structure can be changed by external stimuli such as pH, temperature, ion strength and solvent conditions, and this structural change can be accompanied by the formation, collapse or dissociation of the self-assembled structures.[4] Therefore, peptide/synthetic block copolymers are expected to be applied in drug delivery, gene transfer therapy and tissue regeneration.[1,2]

Some properties of the self-organized structure of peptide/synthetic block copolymers, including size, stability and drug-loading capacity, depend on the conformation of the polypeptide segment.[4–7] It is well known that the helical content depends on the molecular weight of peptide homopolymers.[8] For peptide/synthetic block copolymers, in addition, effects of the synthetic polymer segments on the helical structure have been reported. Cammas *et al.*[9] found that a left-handed helix of poly(β-benzyl-L-aspartate) (PBLA) was stabilized by the attachment of poly(ethylene oxide) (PEO) to form a PBLA/PEO diblock copolymer. Similar observations have been reported for block copolymers composed of α-helical polypeptide and PEO segments in water or hydrophobic solvents.[10–13] In bulk, Lecommendoux *et al.*[6,7] reported that the attachment of an oligomeric polystyrene (PS) to an oligomeric poly(γ-benzyl-L-glutamate) (PBLG) resulted in a significant stabilization of the helical structure. In contrast, Kugo *et al.*[14] reported that the helical content of PBLG in solid-state PBLG/PEO/PBLG triblock copolymers decreased gradually as the polymers swelled in the presence of water. We also observed a decrease in the helical content of poly(β-phenethyl-L-aspartate) (PPLA) after the attachment of PS to the C terminus in a study of thermally induced conformational transformations of PPLA in 1,1,2,2-tetrachloroethane.[15] These reports indicate that the effects of block copolymerization on the helical structure of a polypeptide segment are complicated.

PBLG is well known to undergo a conformational transformation between an ordered right-handed α-helical structure and a disordered random-coil structure, a transition that is induced by the addition of an organic acid (such as trifluoroacetic acid (TFA)) to a polymer solution in an organic solvent such as chloroform.[16–21] Although the helix–coil transformation of PBLG in a PBLG/PS block copolymer in

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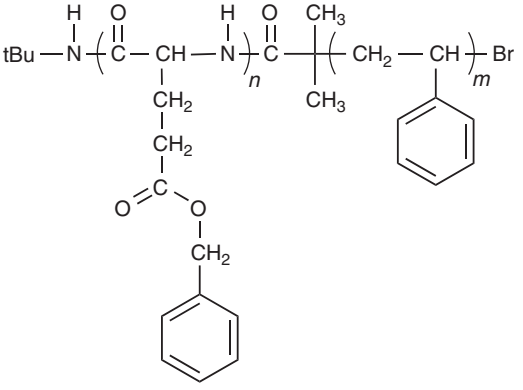
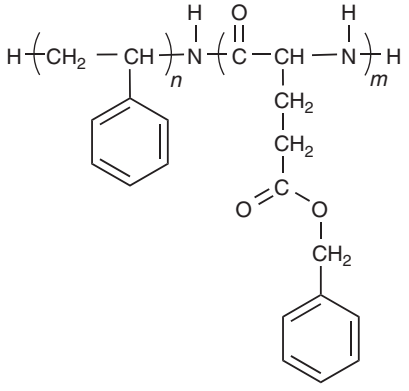


PBLG$_n$-N-PS$_m$



PS$_n$-C-PBLG$_m$

Figure 1 Chemical structure of PBLG$_n$-N-PS$_m$ and PS$_n$-C-PBLG$_m$. PBLG, poly(γ-benzyl-L-glutamate); PBLG$_n$-C-PS$_m$, polystyrene attached to the C terminus; PBLG$_n$-N-PS$_m$, polystyrene attached to the N terminus.

solution was studied using small-angle neutron scattering,[22] the effects of molecular weight and the peptide terminus to which the PS is attached remain unclear. As PBLG block copolymers are often used as drug-delivery carriers and gene vectors,[23] further study of the conformational behavior is required.

In this study, we investigated the effects of the addition of TFA and the attachment of PS on PBLG conformation in PBLG/PS block copolymers in TFA–chloroform mixtures. Block copolymers in which a PS segment was attached to the N terminus (PBLG$_m$-N-PS$_n$) or the C terminus (PS$_n$-C-PBLG$_m$) of a PBLG chain (Figure 1) were synthesized by the combination of the atom transfer radical polymerization of styrene and the ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxylic anhydride (BLG-NCA). The helix–coil transformation of the PBLG segment was monitored using ^{1}H-nuclear magnetic resonance (^{1}H NMR) measurements.

EXPERIMENTAL PROCEDURES

Materials

Triethylamine, tetrahydrofuran (THF), dimethylformamide, chloroform and styrene (Nacalai Tesque, Kyoto, Japan, 98%) were purified using conventional methods before use. 2-Bromoisobutyryl bromide (Sigma-Aldrich, St Louis, MO, USA, 98%), (1-bromoethyl)benzene (Tokyo Chemical Industry, Tokyo, Japan, 95%), *tert*-buthylamine (Tokyo Chemical Industry, 98%), CuBr (Wako Pure Chemical Industries, Osaka, Japan, 95%), (–)-sparteine (Tokyo Chemical Industry, 95%), phthalimide potassium salt (Nacalai Tesque, 98%), hydrazine monohydrate (Nacalai Tesque, 80%), anisole (Wako Pure Chemical Industries, 95%), L-glutamic acid (Wako Pure Chemical Industries, 99%), benzylalcohol (Wako Pure Chemical Industries, 99%), triphosgene (Tokyo Chemical Industry, 98%) and TFA (Nacalai Tesque, 99%) were used as received.

Methods

Molecular weight (M_n) and molecular weight distribution (M_w/M_n) of the PS precursors for synthesis of PS$_n$-C-PBLG$_m$ block copolymers were determined using gel-permeation chromatography on a Jasco–Borwin system (version 1.50; Jasco, Tokyo, Japan) equipped with PS-calibrated Tosoh TSKgel (G3000H$_{HR}$, G4000H$_{HR}$ and G6000H$_{HR}$; Tosoh, Tokyo, Japan) using THF as an eluent. The gel-permeation chromatography spectra of PBLG homopolymers and PBLG$_n$-N-PS$_m$ and PS$_n$-C-PBLG$_m$ block copolymers were also detected using TSKgel G4000H$_{XL}$ with dimethylformamide as an eluent. The ^{1}H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 spectrometer (Bruker, Rheinstein, Germany). The M_n values of PBLG homopolymers and block copolymers were determined based on ^{1}H NMR measurements taken in 20% TFA–$CDCl_3$ solution. For conformational studies, 400 µl of $CDCl_3$ solutions of 2% PBLG segments was analyzed after the repeated addition of TFA up to 10%.

Synthesis of PBLG$_n$-N-PS$_m$

A PBLG$_{55}$ homopolymer (M_n=12 000) was synthesized by a standard ring-opening polymerization of *N*-carboxy-γ-benzyl-L-glutamate anhydride (BLG-NCA) in dry chloroform at room temperature using *tert*-butylamine as an initiator.[24] 2-Bromoisobutyryl bromide (1.0 ml, 4.3 mmol) was then added dropwise to the ice-cooled dry chloroform solution of the PBLG$_{55}$ homopolymer (1.2 g, 0.19 mmol) and triethylamine (4.2 ml, 17.3 mmol). The reaction was warmed to room temperature and stirred for 12 h. The mixture was concentrated by evaporation and poured into methanol. The precipitate was purified by repeated reprecipitations, thoroughly dried and collected quantitatively as a white powder, which was used as a macroinitiator for the following atom transfer radical polymerization of styrene.

A mixture of styrene (2.5 g, 24 mmol), the macroinitiator (1.0 g, 0.08 mmol), CuBr (12 mg, 0.08 mmol), (–)-sparteine (38 mg, 0.16 mmol) and anisole (17 g) was placed in a glass tube and degassed. The tube was sealed off under vacuum and heated at 110 °C for 2 h. The polymeric mixture was diluted with THF and passed through neutral alumina. PBLG$_{55}$-N-PS$_{160}$ was precipitated and thoroughly dried. ^{1}H NMR (20% TFA–$CDCl_3$): δ=7.9 (*s*, N*H*, 1H), 7.4–7.1 (*m*, aromatic *H*), 5.1 (*t*, benzyl, 2H), 4.6 (*s*, C$^{\alpha}$*H*, 2H), 2.5 (*s*, CγH_2, 2H), 2.1 (*t*, C$^{\beta}H_2$, 2H) for the PBLG segment and 7.4–7.1 (*m*, aromatic *H*), 6.6 (br, aromatic *H*, 2H), 1.9 (br, CH_2–C*H*, 1H), 1.5 (br, C*H*$_2$–CH, 2H) for the PS segment.

Synthesis of PS$_n$-C-PBLG$_m$

A bromo-terminated PS homopolymer (PS$_{120}$-Br) (M_n=12 000, M_w/M_n=1.13) was synthesized by the atom transfer radical polymerization of styrene.[25] A solution of PS$_{120}$-Br (3.0 g, 0.25 mmol) and phthalimide potassium salt (0.27 g, 1.5 mmol) in dimethylformamide (80 ml) was stirred at room temperature for 24 h. The mixture was concentrated by evaporation. The residue was dissolved in chloroform and washed two times with aqueous 5% NaOH solution and two times with water. The chloroform solution was dried with $MgSO_4$, filtered, concentrated and poured into methanol. The resulting polymer was purified by repeated reprecipitations.

The polymer was dissolved in a mixture of THF (100 ml) and hydrazine monohydrate (60 ml). The mixture was stirred at 45 °C for 24 h. After evaporation of the THF to precipitate the polymer, the residual hydrazine monohydrate was removed by decantation. The product was dissolved in THF and purified by repeated reprecipitations in methanol. The resultant PS$_{120}$-NH_2 was further purified by column chromatography (yield: 2.2 g (0.18 mol, 73%)).

A mixture of BLG-NCA (1.2 g, 5.5 mmol), PS$_{120}$-NH_2 (1.0 g, 0.083 mmol) and dry chloroform (80 ml) was placed in a flask and stirred for 40 h. The PS$_{120}$-C-PBLG$_{55}$ was purified by repeated reprecipitations in diethyl ether (yield: 2.0 g (0.083 mmol, 90%)). ^{1}H NMR (20% TFA–$CDCl_3$): δ=7.9 (*s*, N*H*, 1H), 7.4–7.1 (*m*, aromatic *H*), 5.1 (*t*, benzyl, 2H), 4.6 (*s*, C$^{\alpha}$*H*, 2H), 2.5 (*s*, CγH_2, 2H), 2.1 (*t*, C$^{\beta}H_2$, 2H) for the PBLG segment and 7.4–7.1 (*m*, aromatic *H*), 6.6 (br, aromatic *H*, 2H), 1.9 (br, CH_2–C*H*, 1H), 1.5 (br, C*H*$_2$–CH, 2H) for the PS segment.

RESULTS AND DISCUSSION

Synthesis of PBLG/PS block copolymers

Two types of PBLG/PS block copolymers were produced, one with PS at the N terminus of PBLG and the other with PS at the C terminus: these are denoted $PBLG_n$-N-PS_m and PS_n-C-$PBLG_m$, respectively. These polymers were synthesized following the method presented in our previous report on block copolymers composed of PS and PPLA,[15] using *N*-carboxy-γ-benzyl-L-glutamate anhydride (BLG-NCA) instead of β-phenethyl-L-aspartate-NCA. For synthesis of $PBLG_n$-N-PS_m, a $PBLG_n$ macroinitiator that was prepared by the ring-opening polymerization of BLG-NCA,[24] followed by amidation of the N-terminal amine with 2-bromoisobutyryl bromide was used for the atom transfer radical polymerization of styrene.[25] PS_n-C-$PBLG_m$ was prepared by the ring-opening polymerization of BLG-NCA with a PS_n-NH_2 macroinitiator, the initiating amino group of which was attached to the chain end by a reaction of the ω-terminal bromide of PS_n-Br with potassium phthalimide and hydrazine monohydrate. Gel-permeation chromatography charts for $PBLG_n$-N-PS_m and PS_n-C-$PBLG_m$ showed unimodal peaks at higher molecular weights than those of their corresponding macroinitiators. In the ^{1}H NMR spectra, all signals for the block copolymers in a mixture of 20 vol% TFA in $CDCl_3$ were assignable to protons in the PS and PBLG segments. The compositions of the segments were determined by calculating the relative integrals of the peaks in the ^{1}H NMR spectra. The PBLG/PS block copolymers used in this study are summarized in Tables 1 and 2.

Helix–coil transformation of $PBLG_n$-N-PS_m

The helix–coil transformation of PBLG was induced by adding TFA repeatedly to chloroform solutions of the block copolymers. The C^αH proton signals in NMR spectra are useful for characterizing the conformation of PBLG.[17–21] Figure 2 shows the dependence of C^αH proton signals on vol% TFA in $CDCl_3$ for $PBLG_{55}$-N-PS_{160}. A $CDCl_3$ solution of the copolymer showed a signal at 4.0 p.p.m., indicating that the PBLG segment had a helical structure. Another signal at 4.6 p.p.m. assignable to a random coil appeared at concentrations of TFA above 4.3 vol%. The helix signal diminished with further addition of TFA and completely disappeared above 9.5 vol% TFA, accompanied by an increase in the coil signal. This result indicates that the PBLG segment in $PBLG_{55}$-N-PS_{160} underwent a helix–coil transformation upon addition of TFA to the $CDCl_3$ solution.

Figure 3 shows vol% TFA dependence of the extent of the helical fraction based on the C^αH peak areas ($f_H=P_H/(P_H+P_C)$) for the $PBLG_{55}$-N-PS_{160}, $PBLG_{55}$-N-PS_{320} and $PBLG_{310}$-N-PS_{320} block copolymers. For comparison, the f_H values for the corresponding PBLG homopolymers, $PBLG_{55}$ and $PBLG_{310}$, are also plotted in the figure. Although the helical content of the PBLG segment in $PBLG_{55}$-N-PS_{160} remained at $f_H=1$ below 2.9 vol% TFA, a gradual decrease in the f_H value to 0.75 was observed in the range of 3.6–5.7 vol% TFA. Straight-line approximations of the plotted relationships gave a slope of $k=-12$. With further addition of TFA up to 9.5 vol%, the helical content dropped steeply to $f_H=0$ with $k=-29$. $PBLG_{55}$-N-PS_{320}, which was prepared from the same $PBLG_{55}$ macroinitiator, had a similar f_H value at each TFA concentration. For $PBLG_{310}$-N-PS_{320} block copolymers, which have a high-molecular-weight PBLG segment, the value of $f_H=1$ was maintained up to 7.0 vol% TFA and dropped steeply to $f_H=0$ at 9.5 vol% TFA with $k=-47$.

The f_H values for the $PBLG_{55}$-N-PS_m and $PBLG_{310}$-N-PS_{320} block copolymers are in good agreement with those for the corresponding homopolymers, $PBLG_{55}$ and $PBLG_{310}$, respectively, as shown in Figure 3. This result indicates that the PS segment attached to the N terminus did not affect the conformation of the PBLG segment. Therefore, the helix–coil transformation observed for $PBLG_n$-N-PS_m block copolymers can be explained only by the behavior of the PBLG segments. It is known that the helix–coil transformation depends on the molecular weight of the polypeptides because residues near the terminus of the chain have a greater probability of forming coils than those in the middle of a chain.[19,26] For $PBLG_{55}$-N-PS_m block copolymers, the gradual transformation observed over the range of 3.6–5.7 vol% TFA is attributed to the low-molecular-weight PBLG segment.

Table 1 Characterizations of $PBLG_n$-N-PS_m block copolymers prepared by the ATRP of styrene at 110 °C in anisole with a $PBLG_n$ macroinitiator (n=55 or 310)

Block copolymer	$M_{n,PBLG}$	$M_{n,PS}$
$PBLG_{55}$-N-PS_{160}	12 000	16 000
$PBLG_{55}$-N-PS_{320}	12 000	32 000
$PBLG_{310}$-N-PS_{320}	69 000	32 000

Abbreviations: ATRP, atom transfer radical polymerization; ^{1}H NMR, ^{1}H nuclear magnetic resonance; $M_{n,PBLG}$, molecular weight of a PBLG segment determined by ^{1}H NMR in 20% TFA-$CDCl_3$; $M_{n,PS}$, molecular weight of a PS segment determined by ^{1}H NMR on the basis of $M_{n,PBLG}$;. PBLG, poly(γ-benzyl-L-glutamate); $PBLG_n$-N-PS_m, polystyrene attached to the N terminus; PS, polystyrene; TFA, trifluoroacetic acid.

Table 2 Characterizations of PS_n-C-$PBLG_m$ block copolymers prepared by the ring-opening polymerization of BLG-NCA in dry chloroform with a PS_n-NH_2 macroinitiator (n=48 or n=120) at room temperature

Block copolymer	$M_{n,PS}$	$M_{n,PBLG}$
PS_{48}-C-$PBLG_{59}$	4800	13 000
PS_{48}-C-$PBLG_{120}$	4800	27 000
PS_{120}-C-$PBLG_{55}$	12 000	12 000

Abbreviations: BLG-NCA, γ-benzyl-L-glutamate *N*-carboxylic anhydride;^{1}H NMR, ^{1}H nuclear magnetic resonance; $M_{n,PS}$, molecular weight of a PS segment determined by GPC measurements for the PS_n-Br precursors; $M_{n,PBLG}$, molecular weight of PBLG segment determined by ^{1}H NMR measurement in 20% TFA-$CDCl_3$ on the basis of $M_{n,PS}$; PBLG, poly(γ-benzyl-L-glutamate); $PBLG_n$-C-PS_m, polystyrene attached to the C terminus; PS, polystyrene; TFA, trifluoroacetic acid.

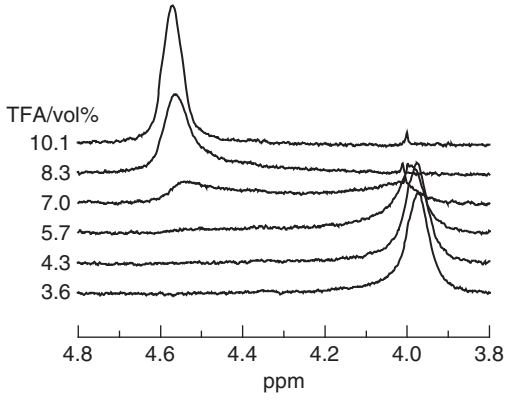


Figure 2 C^αH proton signals for $PBLG_{55}$-N-PS_{160} in various concentrations of TFA–chloroform. PBLG, poly(γ-benzyl-L-glutamate); TFA, trifluoroacetic acid.

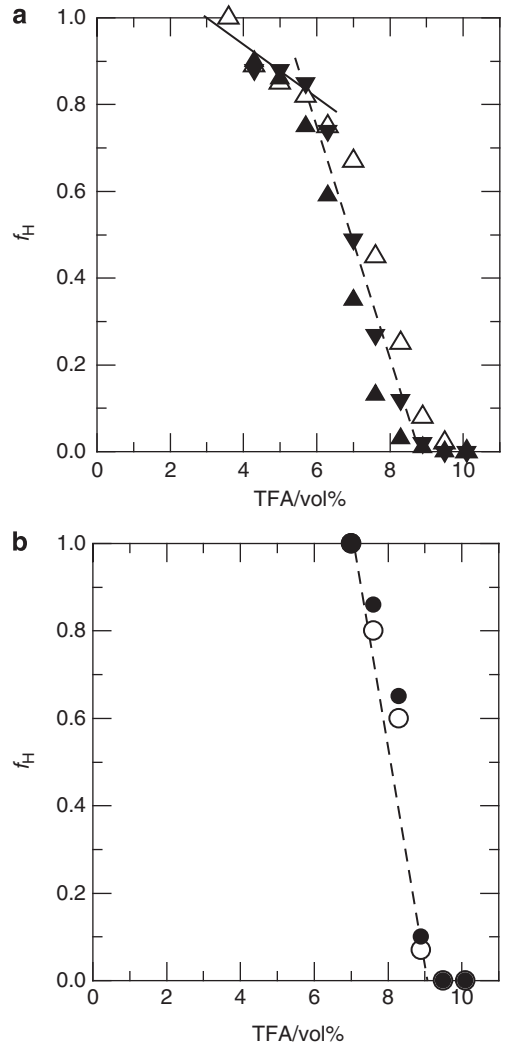


Figure 3 TFA–$CDCl_3$ concentration dependence of the ratio of the integral of helix $C^{\alpha}H$ proton peaks to integral of all $C^{\alpha}H$ proton peaks (f_H) for (**a**) the $PBLG_{55}$ homopolymer (open triangles), $PBLG_{55}$-N-PS_{160} (solid triangles) and $PBLG_{55}$-N-PS_{320} (solid inverse triangles) and (**b**) the $PBLG_{310}$ homopolymer (open squares) and $PBLG_{310}$-C-PS_{320} (solid squares). The solid and dashed lines denote the straight-line approximations of the plotted relationships for the gradual and drastic transformation regions, respectively. PBLG, poly(γ-benzyl-L-glutamate); TFA, trifluoroacetic acid.

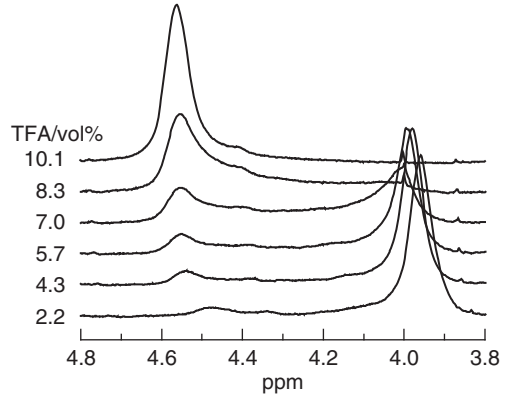


Figure 4 $C^{\alpha}H$ proton signals for PS_{120}-C-$PBLG_{55}$ in various concentrations of TFA–chloroform. PBLG, poly(γ-benzyl-L-glutamate); TFA, trifluoroacetic acid.

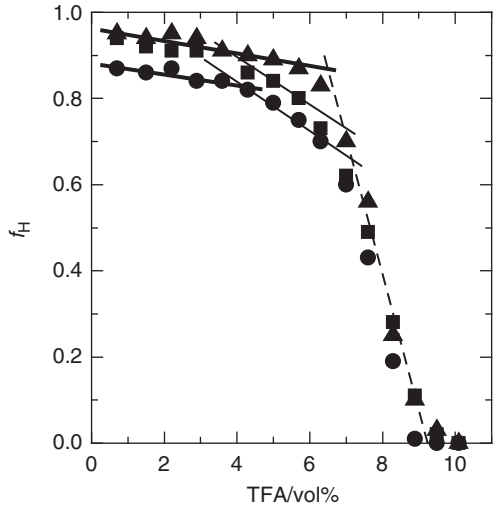


Figure 5 TFA–$CDCl_3$ concentration dependence of f_H for PS_{48}-C-$PBLG_{120}$ (triangles), PS_{48}-C-$PBLG_{59}$ (squares) and PS_{120}-C-$PBLG_{55}$ (circles). The bold, solid and dashed lines denote the straight-line approximations of the plotted relationships for partial deformation and the gradual and drastic transformations, respectively. PBLG, poly(γ-benzyl-L-glutamate); TFA, trifluoroacetic acid.

Helix–coil transformation of PS_n-C-$PBLG_m$

Figure 4 shows vol% TFA dependence of $C^{\alpha}H$ signals in the ^1H NMR spectra for $CDCl_3$ solutions of PS_{120}-C-$PBLG_{55}$. A small coil signal at 4.6 p.p.m. was visible in 2.2 vol% TFA along with a helix signal at 4.0 p.p.m. The small shoulders at 4.2 and 4.4 p.p.m. were assigned to the terminal residues of the helical structure and those residues interconverting between the helix and coil forms;[18–21] these shoulders were not observable for $PBLG_{55}$-N-PS_{160} because of noise. The helix–coil transformation was completed at 9.5 vol% TFA.

Figure 5 shows plots of f_H vs the TFA concentration for PS_n-C-$PBLG_m$ block copolymers. The helical structure of the PBLG segment of PS_{120}-C-$PBLG_{55}$ was partly deformed, with f_H=0.87, at 0.7 vol% TFA. The helical deformation proceeded slowly to f_H=0.84 at 3.6 vol% TFA. The straight-line approximation revealed a weak dependence of the helical deformation on the TFA concentration, with a very gentle slope of k=−1. Moreover, extrapolation of the plots to 0 vol% TFA did not yield an intercept of f_H=1 but of f_H=0.88. This result indicates that the attached PS had a much greater effect on the helical deformation than TFA addition did.

One might expect that the N-terminal amino group would bind to a TFA molecule to generate an ionic species at the N terminus of PS_n-C-$PBLG_m$ block copolymers, leading to enhancement of the helical deformation. However, no differences in the helix–coil transformation between $PBLG_n$-N-PS_m, which has a protected N-terminal amino group, and the corresponding $PBLG_n$ homopolymer, which has an unprotected N-terminal amino group, were observed, as shown in Figure 3. When the N-terminal amino group in PS_{120}-C-$PBLG_{55}$ was protected with 2-bromoisobutyryl bromide to form an amide group, the f_H values were in good agreement with those for unprotected PS_{120}-C-$PBLG_{55}$ at each TFA concentration. We have no evidence that ionization of the N-terminal residue made a major contribution to the helical deformation observed below 3.6 vol% TFA.

A partial deformation of the helical structure was observed for all PS_n-C-$PBLG_m$ block copolymers in this study. However, $PBLG_n$ homopolymers and $PBLG_n$-N-PS_m block copolymers did not exhibit

any coil content below 3.6 vol% TFA. Accordingly, the results strongly suggest that the PBLG segment in PS_{120}-C-$PBLG_{55}$ underwent helical deformation preferentially at the C-terminal side rather than at the N-terminal side and that this deformation was induced by the PS attachment rather than the addition of TFA. In the same TFA concentration range of 0.7–3.6 vol%, PS_{48}-C-$PBLG_{59}$ exhibited partial deformation of the helix with $f_H=0.91\pm0.03$, which is 0.04–0.07 larger than that for PS_{120}-C-$PBLG_{55}$. The smaller extent of the helical deformation was likely the result of the relatively short PS segment.

In the TFA concentration range of 4.3–5.7 vol%, gradual helix–coil transformations were observed for PS_{120}-C-$PBLG_{55}$ and PS_{48}-C-$PBLG_{59}$, whereas PBLG in PS_{48}-C-$PBLG_{120}$ did not undergo this gradual transformation. The molecular weight dependence is in good agreement with the observations for the $PBLG_n$ homopolymers and the $PBLG_n$-N-PS_m block copolymers. In addition, the slopes of the gradual transformations observed for PS_{120}-C-$PBLG_{55}$ and PS_{48}-C-$PBLG_{59}$ were $k=-12$ and $k=-10$, respectively, which are similar to those for the $PBLG_{55}$ homopolymer and the $PBLG_{55}$-N-PS_m block copolymers. This result indicates that the attachment of PS to the C terminus of PBLG did not make any effective contribution to the helical deformation in 4.3–5.7 vol% TFA. Moreover, this raises the possibility that the gradual helical deformations observed for PS_{120}-C-$PBLG_{55}$ and PS_{48}-C-$PBLG_{59}$ preferentially took place in the end of the molecule to which PS was not attached; that is, TFA addition could induce greater deformation of the helical structure at the N-terminal side than at the C-terminal side in PBLG segments.

After addition of more TFA, solutions of the PS_n-C-$PBLG_m$ block copolymers underwent a drastic transformation in the range of 6.3–9.5 vol% TFA with $k=-25\pm2$. All PBLG homopolymers and block copolymers investigated in this study completed the helical deformation at the same TFA concentration of 9.5 vol%. In the drastic transformation region, inherent deformation of the helical structure throughout the PBLG chain overwhelmed the effects of molecular weight and PS attachment.

Effect of PS attachment to PBLG on helix–coil transformation

Cammas *et al.*[9] reported for PEO/PBLA block copolymers that the helical content of the PBLA segment increased after attachment of the PEO due to hydrogen-bonding interactions between the two segments in organic solvent. Such an increase was also observed for other peptide/synthetic block copolymers because of the aggregation of polypeptide segments in micelles[10–13] and in microphase-segregated structures.[6,7] In PS_n-C-$PBLG_m$, however, there was a decrease in the helical content of the PBLG segment in 0.7–3.6 vol% TFA after attachment of a PS segment to the C terminus. As TFA and $CDCl_3$ are good solvents for both PS and PBLG, it can be assumed that solvation of the two segments hindered not only intermolecular aggregation but also intramolecular interactions between the two segments. The molecular motion of the directly attached PS segment would disturb some residues near the C terminus.

In contrast, the effect of PS attachment at the N terminus on the helical structure was negligible in $PBLG_n$-N-PS_m block copolymers. In contrast to the results for PS_n-C-$PBLG_m$, this result suggests that the residues near the N terminus were hardly disturbed by the directly attached PS segment. The result is in good agreement with those of our previous study of the conformational transformation of block copolymers composed of PPLA and PS in 1,1,2,2-tetrachloroethane.[15] PPLA homopolymer in solution is known to undergo a reversible helix-sense inversion from a right-handed helix to a left-handed helix in response to temperature elevation. When PS was attached to the N terminus of PPLA, the helical structure was not deformed during the course of the helix-sense inversion. In contrast, a PPLA segment the C terminus of which was attached to a PS segment no longer maintained a helical structure at elevated temperature. The result was partly explained by the difference in helix capping between the N and C termini.[27] For α-helical polypeptides and proteins, because the first four NH groups and the last four CO groups of a helix necessarily lack intrahelical hydrogen bonds, the polar side chains near the terminal ends are often able to form hydrogen bonds to these unbonded groups.[27] For PBLG, irrespective of PS attachment, it is well known that side-chain benzyl ester CO groups interact with free main-chain NH groups. Helix capping might alter the conformational free energy of the residues near the N terminus, resulting in the negligible effect of PS attachment in the case of $PBLG_n$-N-PS_m block copolymers.

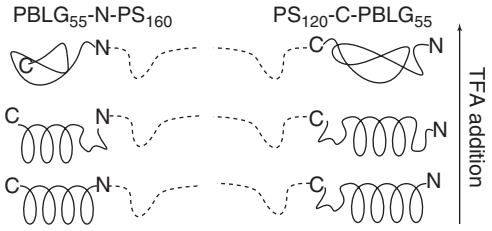


Figure 6 A schematic illustration of the helix–coil transformations for $PBLG_{55}$-N-PS_{160} and PS_{120}-C-$PBLG_{55}$. Solid and dashed lines denote the PBLG and PS segments, respectively. C and N denote the C and N termini of the PBLG segment, respectively. PBLG, poly(γ-benzyl-L-glutamate); PS, polystyrene; TFA, trifluoroacetic acid.

In the TFA concentration range of 4.3–5.7 vol%, gradual helical deformation was observed for low-molecular-weight PBLG homopolymers and $PBLG_n$-N-PS_m and PS_n-C-$PBLG_m$ block copolymers. In spite of the helix capping, some residues with free NH groups at the N terminus of the helix might be strongly solvated by additional TFA. Although further study is required, it is possible that the helical structure of residues on the N-terminal side of low-molecular-weight PBLGs is more likely to deform than that of C-terminal residues and residues in the middle of the helical structure regardless of the point of PS attachment.

CONCLUSIONS

The helix–coil transformation of PBLG in block copolymers in which PS was attached to the N or C terminus of the PBLG was investigated in TFA–chloroform solutions. The results reveal the non-equivalent sensitivity of the PBLG conformation at the N and C termini in response to attachment of a PS and the addition of TFA. Deformation of the helical structure at the C-terminal side was predominantly caused by PS attachment, whereas the effect of TFA addition on the helical structure was greater on the N-terminal side, as schematically illustrated in Figure 6. Although the helix–coil transformation of PBLG is a well-investigated subject, to the best of our knowledge, this is the first experimental study to reveal a difference in conformational stability between the N and C termini in the PBLG chain. Although the reason remains unclear at present, it seems reasonable to assume that the interaction of free NH groups with side-chain CO groups at the N terminus of the helix is responsible for the different sensitivities of the PBLG chain to PS attachment and TFA addition at the N and C termini.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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