Synthesis, Structure, and Mechanical Properties of A-B-A Tri-Block Copolymers Consisting of Poly(ϵ -*N*-benzyloxycarbonyl-L-lysine) as the A Component and Polybutadiene as the B Component

Kouhei KUGO, Toshio HAYASHI, and Akio NAKAJIMA

*Department of Polymer Chemistry, Kyoto University,
Sakyo-ku, Kyoto 606, Japan.*

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ABSTRACT: A-B-A type tri-block copolymers composed of ϵ -*N*-benzyloxycarbonyl-L-lysine as the A component and butadiene as the B component were prepared. The synthesis could be carried out by allowing the *N*-carboxy anhydride of the α -amino acid to react with the amine-terminated polybutadiene. The structure of these block copolymers was estimated using the relation between the helix-coil transition temperature and the number-average molecular weight. The morphology of the membrane specimens was investigated by wide-angle X-ray diffraction and electron microscopy. It was found that the dynamic mechanical spectra can be well explained by the microheterophase structure observed under electron microscope. By minimizing the Gibbs free energy ΔG per unit volume for the micelle formation, the domain size was estimated for each type of micelle. The shape and size calculated were in accord with those observed by electron microscope.

KEY WORDS Tri-Block Copolymer / Poly(ϵ -*N*-benzyloxycarbonyl-L-lysine) / Polybutadiene / Microheterophase Structure / Dynamic Mechanical Properties /

Recently, Gallot *et al.*^{1,2} carried out a study on the synthesis and structure determination of A-B type di-block copolymers consisting of polypeptides and polymers such as vinyl polymers and polybutadiene.

In previous studies,³⁻⁵ we synthesized A-B-A type tri-block copolymers consisting of γ -benzyl L-glutamate as the A component and butadiene as the B component, and characterized the bulk properties of their membranes: microheterophase structure, mechanical properties, and water permeability, etc. The results of circular dichroism measurements in ethylene dichloride solution and infrared spectra measurements in the solid state showed that the polypeptide chain in the copolymer assumes the same α -helical conformation as the homopolypeptide chain. The microheterophase structure in membranes cast from different solvents was examined by electron microscope. The micelle dimensions evaluated from thermodynamic considerations, taking into account the conformational parameters of the

component block chains, were in good agreement with those obtained from electron micrographs. The dynamic mechanical spectra for the block copolymer membranes predicted accurately the morphology of spherical and cylindrical micelles when analyzed by Takayanagi's equivalent mechanical model.⁶ Moreover, from the experimental results on the hydraulic permeability of water, it was clarified that the interfacial region between the α -helical polypeptide chain and the amine-terminated polybutadiene chain is responsible for the water permeability.

In order to confirm whether these findings are general characteristics of block copolymers consisting of a polypeptide block and a polybutadiene block, we studied the preparation, structure, and properties of A-B-A type tri-block copolymers (LBL) composed of poly(ϵ -*N*-benzyloxycarbonyl-L-lysine) (PBCL) as the A component and polybutadiene (PB) as the B component. The results are described in this paper. The surface properties of

these block copolymer membranes will be reported in the following paper.

EXPERIMENTAL

Synthesis and Purification of Materials Amine-Terminated Polybutadiene

The preparation and purification of the middle block, a cycloaliphatic secondary amine-terminated polybutadiene (ATPB), are described in a previous paper.³ The ATPB was high in *trans* isomers, having a number-average molecular weight, M_n , of 3600.

ϵ -N-Benzylloxycarbonyl-L-lysine N-Carboxy Anhydride

The monomer *N*-carboxy anhydride of ϵ -N-benzylloxycarbonyl-L-lysine (ϵ -BCL-NCA) was prepared according to the method proposed by Blout and Karlson,⁷ and purified by recrystallization from an ethyl acetate solution with the addition of petroleum ether. Recrystallization was repeated from two to three times.

Synthesis of Block Copolymers

Calculation was made of the amount of ϵ -BCL-NCA and ATPB necessary for obtaining the desired degree of polymerization of the polypeptide block. The polymerization was carried out in the absence of moisture, at room temperature, in a dioxane-methylene dichloride mixture (1:2, v/v) at a total concentration of ϵ -BCL-NCA and ATPB, 3%. After 48 h, the polymerization was terminated, and the copolymer was precipitated by 5 volumes of cold methanol. This method of precipitation eliminated any traces of ϵ -BCL-NCA still present and the oligopeptide formed by autopolymerization.⁸ The copolymer was then dried *in vacuo* at 50°C.

The results of the copolymerization are listed in Table I.

Characterization. Molecular Weights and Molecular Weight Distributions

The molecular weights of the tri-block copolymers were estimated from elemental analysis using the number-average molecular weight, $M_n = 3600$, of ATPB. The molecular weights obtained are, therefore, expected to be the number-average molecular weights. The number-average molecular weight of PBCL was determined from the limiting viscosity number $[\eta]$ in *m*-cresol measured with a Ubbelohde type viscometer; the $[\eta]$ -number-average molecular weight M_n relationship⁹ proposed for PBCL was used. The limiting viscosity numbers $[\eta]$ of the block copolymers were measured using the same viscometer under the same conditions. The limiting viscosity numbers $[\eta]$ and molecular weights obtained are summarized in Table I. The molecular weight evaluated from elemental analysis was checked in *N,N*-dimethylformamide at 25°C by using a MOM Type-3170-b ultracentrifuge. Molecular weight distributions of these polymers were examined by sedimentation patterns.

Composition of Block Copolymers

The molar composition of the LBL block copolymers was determined by elemental analysis and the number-average molecular weight of ATPB. The results are listed in Table I.

Conformation and Helix-Coil Transition of the Polypeptide Blocks

Circular dichroism (CD) spectra were measured at $25 \pm 0.5^\circ\text{C}$ on a JASCO J-20 CD/ORD spectropolarimeter equipped with a quartz cell having a

Table I. Molecular characterization of samples prepared

Designation	$[\eta]$	Butadiene mol%	$[\theta]_{222}^c/[\theta]_{222}^0$	P_A	$M_n \times 10^{-4}$
	dl g ⁻¹ (<i>m</i> -cresol, 25°C)				
LBL-1	0.33	52.3	0.47	28	1.8
LBL-2	0.39	42.3	0.57	42	2.6
LBL-3	0.47	23.7	0.76	98	5.5
LBL-4	0.48	16.2	0.81	158	8.7
LBL-5	0.49	11.9	0.88	226	12.2
PBCL	1.83	0.0	1.00	795	41.7

path length of 1 mm. The optical rotatory dispersion (ORD) spectra were measured to examine the helix-coil transition, using a Yanagimoto OR-100 Type spectropolarimeter.

Wide-Angle X-Ray Diffraction and Electron Microscopy

Wide-angle X-ray diffraction (WAXD) profiles were obtained by using Ni-filtered $\text{CuK}\alpha$ radiation, setting a flat surface of the membrane normal to the beam. Films for WAXD measurements were cast from a 10:1 (v/v) mixture of chloroform (CF) and 2,2,2-trifluoroethanol (TFE). In observing the microheterophase structure with a Hitachi H-500 transmission electron microscope, thin membranes cast from the same solvent mixture as that for WAXD measurements were stained with osmium tetroxide vapor.

Mechanical Property

For mechanical studies, films of *ca.* 50 μm in thickness were prepared by casting from the same solvent as that for the X-ray diffraction experiments. The dynamic mechanical relaxation behavior was measured with a DDV-II Rheovibron at a frequency of 110 Hz and a heating rate of 0.3°C min^{-1} over a temperature range from -20 to 100°C.

RESULTS AND DISCUSSION

Molecular Characterization

The pertinent data on polymerization are summarized in Table I. From the circular dichroism (CD) spectra in 1,2-dichloroethane (DCE), the ratios of the residue ellipticity $[\theta]_{222}$ of LBL block copolymers to that of PBCL homopolymer, $[\theta]_{222}^{\text{copolymer}}/[\theta]_{222}^{\text{PBCL}}$, were evaluated and are listed in the third column of Table I. The value of the ratio is in accord with that of molar composition obtained from elemental analysis. This fact shows that the helical content of the L component in the LBL block copolymers is equal to that of the PBCL homopolymer in DCE solution. In Table I, P_A is the degree of polymerization of the A block chain.

The sedimentation patterns of LBL-5 and LBL-3 block copolymers and the PBCL homopolymer exhibit a single peak and no tailing on either side of the peak, as shown in Figure 1. This behavior was observed for all block copolymers studied.

Figure 1. Sedimentation patterns of samples in DMF at 25°C under the following conditions: speed of rotation, 50,000 rpm, Schlieren angle, 65°, and concentration, 0.5 g dl^{-1} ; (1) LBL-5 (70 min); (2) LBL-3 (85 min); (3) PBCL (80 min).

The number-average molecular weight M_n of LBL-5 was examined by ultracentrifugation for comparison with the M_n evaluated from elemental analysis. For a polydisperse system, the apparent weight-average molecular weight M_w^{app} and the apparent *z*-average molecular weight M_z^{app} are determined as functions of the polymer concentration. We assumed that the small amount of the polybutadiene portion would not effect the thermodynamic nonideality. From the LBL-5 measurements, we obtained $M_w^{\text{app}} = 14.4 \times 10^4$ at $C_0 = 0.06 \text{ g dl}^{-1}$, 13.8×10^4 at $C_0 = 0.14 \text{ g dl}^{-1}$, 14.1×10^4 at $C_0 = 0.15 \text{ g dl}^{-1}$, and 13.9×10^4 at $C_0 = 0.22 \text{ g dl}^{-1}$, in which C_0 is the polymer concentration before centrifugation. By extrapolation to infinite dilution, we obtain the weight-average molecular weight, $M_w = 14.5 \times 10^4$. Furthermore, the apparent *z*-average molecular weight M_z^{app} at $C_0 = 0.22 \text{ g dl}^{-1}$ was

evaluated, and then we obtained $M_z^{app}/M_w^{app} = 1.15$. We assumed that M_z^{app}/M_w^{app} is nearly equal to M_z/M_w , and obtained $M_n = 12.3 \times 10^4$ for the number-average molecular weight of LBL-5 from these data, assuming the generalized exponential distribution function, for which the relation $M_n : M_w : M_z = (b+1) : (b+2) : (b+3)$ holds. The molecular weight (12.2×10^4) evaluated from elemental analysis is in accord with this M_n (12.3×10^4) value, which supports the conclusion that the molecular weights evaluated from elemental analysis using the number-average molecular weight, $M_n = 3600$, of polybutadiene can be regarded as the number-

average molecular weights.

The thermal helix-coil transition of PBCL and the LBL block copolymers was investigated in a 36:64 (v/v) mixture of dichloroacetic acid (DCA) and DCE. Figures 2 and 3 show the temperature dependence of the Moffitt parameter for PBCL and the LBL block copolymers, respectively. It is obvious from these figure that the helix-coil transition temperature T_i shifts toward higher temperature as the molecular weight of PBCL decreases.

Lifson and Roig,¹⁰ and Nagai¹¹ have expressed T_i of a homopolypeptide in powers of the reciprocal number-average molecular weight, M_n^{-1} ,

Figure 2. Temperature dependence of the Moffitt parameter b_0 for PBCL homopolymers of different molecular weights in a 36:64 DCA-DCE mixture. The number-average molecular weights M_n are (1), 34.5×10^4 , (2), 20.8×10^4 , (3), 13.9×10^4 , (4), 9.7×10^4 , and (5), 7.0×10^4 .

Figure 3. Temperature dependence of the Moffitt parameter b_0 for the LBL block copolymers in a 36:64 DCA-DCE mixture.

Figure 4. Helix-coil transition temperatures of LBL copolymers (○) and PBCL homopolymers (●) plotted against the reciprocal of the number average molecular weights, M_n^{-1} .

$$T_i = T_i^\infty + K'/M_n + O(1/M_n^2) \quad (1)$$

where T_i^∞ is the value of T_i for infinite molecular weight and K' is a constant independent of M_n . Teramoto *et al.*¹² have applied the above equation to the case in which a polypeptide molecule has an intrachain defect which will not allow it to participate in the formation of a helix. In such a case, T_i is expressed as,

$$T_i = T_i^\infty + 2\beta K'/M_n + O(1/M_n^2) \quad (2)$$

where β is a parameter associated with the chemical nature of the defect, and should take a value of unity if the defect acts in the same way as the chain end of the molecule in the formation of the helix.

The LBL block copolymers may be regarded as once-broken rods which may be treated by eq 2 with $\beta=1$. The values of T_i for PBCL and LBL obtained from Figures 2 and 3 are plotted against M_n^{-1} in Figure 4. The experimental points for the PBCL homopolymers follow a straight line. The broken straight-line has a slope twice as large as that for the PBCL homopolymers. The experimental points for the LBL block copolymers fall well on this broken straight-line. This suggests that the LBL block copolymers are of the A-B-A tri-block type, consisting of α -helical rods (A) of equal length linked to the ends of a random coil chain (B).

Figure 5. Wide-angle X-ray diffraction profiles of PBCL and LBL block copolymers.

Structure of Copolypeptides in Solid State

Figure 5 illustrates the WAXD patterns for the LBL block copolymers and the PBCL homopolymer. The first main reflection of PBCL is very sharp and corresponds to an intermolecular spacing of the α -helical chains, which is 15.0 Å. Assuming that the α -helical rods form the hexagonal packing ($a=b$) in the films, we could calculate the radius of the α -helical rod containing the side-chain and was to be 8.7 Å, which is in good agreement with the literature data¹³ obtained from small-angle X-ray scattering for poly(ϵ -N-benzyloxycarbonyl-L-lysine) in solution. Regarding the diffraction patterns of the LBL block copolymers, the first main reflections are at the same position as that of PBCL, and become broader as the amount of butadiene increases. Apparently in

this case, microheterophase separation occurred, and the crystallinity or orientation of the α -helical rods diminished.

Morphology

The morphology of the LBL block copolymers in a membrane was investigated by electron microscopy. The electron micrographs of the LBL membranes show clear microheterophase structures, as illustrated in Figure 6. The dark region in these photographs corresponds to the polybutadiene domain (B domain) stained with osmium tetroxide (OsO_4) vapor. The B domains of LBL-5, LBL-4 and LBL-3, and LBL-2 and LBL-1 are spherical, cylindrical, and lamella-like micelle, respectively. The shapes and dimensions of the domains are discussed in detail in the succeeding sections.

Figure 6. Electron micrographs of the LBL copolymer membranes cast from a 10:1 (v/v) mixture of CF and TFE at 25°C. The dark portions correspond to PB domains stained by osmium tetroxide.

Dynamic Mechanical Properties

The dynamic mechanical properties of a two-phase system have been successfully described by using Takayanagi's mechanical equivalent model,^{6,14,15} which is the simple model composed of series and parallel elements. Dickie¹⁶ has shown that Takayanagi's model equation is equivalent to Kerner's equation¹⁷ assuming that the inclusion phase has the shape of spherical particles and that the Poisson's ratios of the inclusion and matrix phases are equal to 0.5. The dynamic Young's modulus E_c^* for Takayanagi's model is given by,

$$\frac{1}{E_c^*} = \frac{\psi}{\lambda E_2^* + (1-\lambda)E_1^*} + \frac{1-\psi}{E_1^*} \quad (3)$$

where E_1^* and E_2^* are the complex moduli of the matrix and the inclusion phase, respectively, λ and ψ are the parameters representing the mixing state, and $\lambda\psi$ is equivalent to the volume fraction ϕ of the inclusion phase, *i.e.*, $\phi = \lambda\psi$. Under the above conditions, λ and ψ are expressed as follows¹⁴:

$$\lambda = \frac{2+3\phi}{5} \quad (4)$$

$$\psi = \frac{5\phi}{2+3\phi} \quad (5)$$

The log E' vs. temperature relations for the LBL-3 block copolymer and the PBCL homopolymer are shown in Figure 7. The dynamic modulus E' of polybutadiene in this temperature range was estimated according to the literature value.¹⁸ In this system, the polybutadiene domain (domain 2) is dispersed in the matrix phase of the PBCL domain (domain 1) as was shown by electron microscopy. The volume fraction ϕ of the polybutadiene component in the LBL-3 block copolymer membrane was estimated to be 0.237 from the results of molecular characterization, assuming that the polybutadiene chains in the solid membrane are in an unperturbed state, *i.e.*, the expansion coefficient α is 1.0.^{5,19} The solid curves in Figure 7, except for those of PBCL and polybutadiene, were calculated by a trial-and-error method. By minimizing the deviation between experimental and calculated curves, $\lambda=0.56$ and $\psi=0.42$ were obtained for the best fitting case (Figure 7). When the shape of the inclusion phase is spherical, we obtain $\lambda=0.54$ from eq 4 using $\phi=0.237$. The best-fit value, 0.56, obtained for the LBL-3 block copolymer, is somewhat larger than that for spherical inclusion. This sug-

Figure 7. Temperature dependences of log E' for the LBL-3 block copolymer. Calculated curves 1—5 are compared with experimental data ($\circ$). The curve C denotes the experimental results on PBCL homopolymer. The curve B denotes the literature value for polybutadiene.¹⁸

gests that the dispersed phase takes on a more extended shape, *i.e.*, the ellipsoidal or cylindrical inclusion phase is expected for the polybutadiene component in the LBL-3 block copolymer membrane; this is in good agreement with observation by electron microscope.

Thermodynamic Investigation for Micelle Dimension

The micelle formation of the A-B-A type block copolymer consisting of an α -helical A component and a random coil B component was clarified in the previous paper⁴ by taking into account the conformational parameters of the component block chains. The equations derived therein should be pertinent to this study, since the LBL block copolymers consist of an α -helical block and a random coil block. By minimizing the Gibbs free energy ΔG per unit volume for the micelle formation, we obtained the equilibrium micelle dimensions for each type of micelle models, *i.e.*, sphere $D_{s,eq}$, cylinder $D_{c,eq}$, and lamella L_{eq} ⁴:

Table II. Conformational parameters and dimensions of domains (Å)

Designation	ϕ_B	$\frac{N}{\text{\AA}^{-3}}$	$D_{s,eq}$	$D_{c,eq}$	L_{eq}	D_{EM} (Micelle)
LBL-1	0.653	3243×10^{-8}			257	350 (Lamella)
LBL-2	0.557	2764×10^{-8}			301	380 (Lamella)
LBL-3	0.350	1738×10^{-8}		357		400 (Cylinder)
LBL-4	0.250	1243×10^{-8}		422		460 (Cylinder)
LBL-5	0.189	940×10^{-8}	421			460 (Sphere)

$$D_{s,eq} = \left(\frac{8\Delta W \langle r_{B/2}^2 \rangle}{kTN} \right)^{1/3} \quad (6)$$

$$D_{c,eq} = \left(\frac{16\Delta W \langle r_{B/2}^2 \rangle}{3\phi_B^{1/2} kTN} \right)^{1/3} \quad (7)$$

$$L_{eq} = \left(\frac{8\Delta W \langle r_{B/2}^2 \rangle}{3\phi_B^2 kTN} \right)^{1/3} \quad (8)$$

ΔW is the interfacial free energy per unit area of A/B interface, $\langle r_{B/2}^2 \rangle$ is the mean-square end-to-end distance of the B/2 chain, k is the Boltzmann constant, T is the absolute temperature, N is the number of junction points between A and B block per unit volume of micelle, *i.e.*, twice the number of block copolymers per unit volume of micelle, and ϕ_B is the volume fraction of the domain occupied by B blocks relative to the total volume occupied by the copolymer. In this study, we obtained $\Delta W = 21.1 \times 10^{-16}$ erg $\text{\AA}^{-2}$ for the PBCL-PB interface.²⁰ Table II summarizes the data for the microheterophase structure of the LBL block copolymers. All calculations were made using eq 6, 7, and 8 in the same manner as described in our previous paper.⁴ D_{EM} is the observed micelle dimensions, and the shape of micelles as determined by electron microscope is listed in the last column of Table II. It is seen that the shape and size of the micelles calculated thermodynamically agree with the electron microscope observation.

In summary, we synthesized A-B-A type block copolymers consisting of poly(ϵ -N-benzyloxy-carbonyl-L-lysine) as the A component and polybutadiene as the B component, with the hope of acquiring more information on the A-B-A type block copolymers of α -amino acid and butadiene components. We characterized the samples and established their microheterophase structure by dynamic mechanical measurements and thermodynamic considerations.

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