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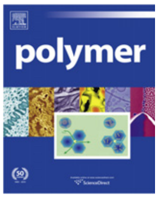

Highly cooperative helix-sense reversal of polyaspartates. Influence of the comonomer of the opposite screw-sense preference

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Poly(L-aspartic acid ester)s are known to form an α-helix in either the right(r)- or left(ℓ)-handed screw depending on the chemical constitution of the ester group in the side chain. While poly(β-benzyl L-aspartate) (PBLA) normally remains in the ℓ-form in conventional helicoidal solution at all temperatures, poly(β-phenethyl L-aspartate) (PPLA) exhibits transformation from the r- to ℓ-form with increasing temperature. In this work, random copolymers comprising residues of the two opposite screw-sense preferences, BLA and PLA, were chosen to investigate the stability of the α-helical hydrogen-bond array in a helix-forming solvent such as chloroform or 1,1,2,2-tetrachloroethane. Sharp S-shape transitions of the helix sense were observed for copolymers in the range PLA > 50%. The minor component BLA strictly obeys the majority rule in these copolymers. ^{2}H NMR studies of partially deuterated samples in the nematic liquid-crystalline (LC) state have proved that the thermally induced r ↔ ℓ helix-sense inversion takes place highly cooperatively along the α-helical backbone: i.e., the cooperativity of the transition was not much affected by the inclusion of substantial amount of weaker points along the chain. These observations unambiguously suggest a zipper-type propagation process for PPLA starting from one terminal to the other. For given copolymer samples, the transition temperatures determined in dilute isotropic solution are nearly identical to those observed in the LC state, suggesting that the transition mechanism remains also similar. In the succeeding paper, relevant experimental data will be interpreted according to the theory consistent with these observations.

1. Introduction

1.1. *Historical background of the helix–helix transition studies of polypeptides*

Poly(L-aspartic acid ester)s $-[\mathrm{NH{-}C^{\alpha}H(CH_2C(O)OX)C(O)}]_n-$ are known to be a unique system that may form an α-helix in either the right(r)- or left(ℓ)-handed screw depending on the chemical constitution of the ester group (X) in the side chain [1]. Poly(β-benzyl L-aspartate) (PBLA) usually takes ℓ-α-helix and does not exhibit any screw-sense inversion in conventional helicoidal solvents [2]. The r-form of α-helical PBLA has been reported under certain limited conditions [3–7]. Reversal of the helix sense in copolymer systems such as those comprising $X_1 =$ benzyl and $X_2 =$ nitro-benzyl residues was first reported by Goodman and his coworkers [8–10]. Hashimoto et al. [11–13] prepared copolymers of BLA (ℓ-preference) in various combinations with L-aspartate carrying $X_2 =$ methyl-, chloro-, cyano-, or nitro-benzyl residue (r-preference), and demonstrated that the screw-sense reversal may be also affected by the chemical nature of the surrounding media. In their Infrared studies on copolymers as a function of comonomer ratios, characteristic amide frequencies such as NH-stretching, amide I, and amide II bands exhibited a gradual shift of the peak position (as a single peak) in the intermediate region where the two types of helices are coexisting in chloroform [13]. The C=O stretching band of the side-chain ester groups was also found to shift gradually over the same transition range.

Bradbury et al. [14] have examined optical rotatory dispersion (ORD) behaviors of random copolymers of BLA with γ-benzyl D-glutamate (BDG) (ℓ-preference) and also with γ-benzyl L-glutamate (BLG) (r-preference). Since both BLA and BDG residues prefer the ℓ-screw-sense, the b_0 value of the copolymer was found to be nearly invariant (400–600) with the comonomer ratio. Oppositely, the helical sense of copoly(BLA-ran-BLG) was largely determined by BLG and the b_0 value remained below −400 over the range BLA content = 0–85%. A sharp transition from the r- to ℓ-regime took place on the extremely lower side of BLG content (<15%). Bradbury et al. [15,16] have also reported that poly(β-*n*-propyl

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L-aspartate) exhibits thermal transition from the r- to ℓ-form in chloroform solution. Tsujita, Takizawa et al. [17–22] have examined the effect of the side-chain length by introducing n-alkyl groups ($n = 2–18$) through the ester-exchange reaction on PBLA. The conformational transitions of these samples observed in solution were found to be fairly consistent with those previously reported in the literature. From the dipole moment measurements on partially ethylated PBLA, they have concluded that the helical conformation is maintained during the r- to ℓ-helix transition in chloroform [18].

Poly(β-phenethyl L-aspartate) (PPLA) forms a very stable r-helix in chloroform, and exhibits a lyotropic cholesteric liquid-crystalline (LC) phase above ca. 20 vol%. Toriumi et al. [23] have reported that PPLA shows a transition from the r- to ℓ-form in chloroform by addition of a small amount of dichloroacetic acid (DCA) (i.e., a solvent-induced transition). Subsequently PPLA was found to show a similar transition in 1,1,2,2-tetrachloroethane (TCE) simply by raising temperature (i.e., a thermally induced transition) [24–26]. The mutual relation of these transitions has been examined on the temperature vs. solvent-composition phase diagram [27–29]. The thermally induced helix-sense reversal was also observed in the moderately concentrated LC state: the transition temperature was found to be unaffected by the concentration. ORD measurements indicate that the macroscopic screw-sense of the cholesteric mesophase concomitantly exhibits a sharp transition from the left- to right-handed arrangements opposite to the chirality of the constituent molecules [25]. Spectroscopic techniques such as ^{2}H NMR have offered epoch-making information in elucidating the molecular mechanism of the helix–helix transition of polyaspartates in the LC state [24]. While the deuterium quadrupolar splitting due to the main-chain ND bond decreases gradually with temperature, those from the side-chain CD bonds exhibit a discrete change at around 70–80 °C [24–31]. The ^{2}H NMR data collected from variously deuterated PPLA samples have been interpreted by the rotational isomeric state (RIS) simulation. The thermodynamic aspects of the transition have been argued on this basis [31,32]. Rewinding of the backbone chain from the r- to ℓ-form is triggered by the change in the conformational mode of the χ_1 bond in the side chain. The orientation of the main chain remains unaffected by the helix inversion, suggesting that the transition may be highly cooperative [32,33]. The intramolecular hydrogen-bond (H-bond) switching between the r- and ℓ-form should take place without perturbing the nematic order.

1.2. Purpose of the present work

In this paper, we wish to report the results of the analysis on random copolypeptides derived from BLA and PLA, i.e., copoly(BLA-ran-PLA)s. BLA and PLA differ by only one methylene unit with each other in the side-chain ester group, yet they exhibit opposite behaviors at room temperature. One of the motivations of the present study originated from the expectation that the cooperativity of the H-bond array might be affected in the copolymers comprising residues of the two opposite screw-sense preferences, and thus the reversal of the helix may take place more frequently along the backbone, facilitating the direct observation of the transition state (i.e., the ℓ/r junction) jointing the two opposite screw regimes. In effect, on the contrary to the above expectation, extensive studies in the dilute isotropic and LC state have provided a convincing proof that the screw-sense reversal of these copolymers also takes place without perturbing the one-dimensional order of the α-helix. In short, the cooperativity of the r ↔ ℓ transition inherent to the PPLA system is hardly affected by the inclusion of the substantial amount of the residues of the opposite screw-sense preference (i.e. weakness to the system). The information gained through these studies has provided a strong support to the Zipper-type transition model of PPLA as previously proposed [32]. Theoretical treatment of the transition described above will be presented in the succeeding paper [34].

2. Experimental

2.1. Preparation of samples

Preparation of PPLA was reported previously [26]. The molecular weight (M) of the standard sample has been estimated to be 6.6×10^4 by the viscosity measurement as described in the later section. Commercial PBLA samples (Sigma Chemical Ltd. $M = 5.99 \times 10^4$) were used as purchased.

All copolymer samples were prepared by the standard amino-acid N-carboxyanhydride (NCA) method [35]. The anhydrides of β-benzyl and β-phenethyl L-aspartate were dissolved separately in dehydrated 1,4-dioxane (or chloroform), appropriate quantities of the two solutions were mixed (ca. 1 g/50 ml), and triethylamine was added to initiate polymerization at room temperature for 2–3 days. The molar ratio of the NCA to the initiator was 50. Copolymers formed were purified by repeated precipitation into a large volume of methanol from a chloroform solution. The molecular weight of a copoly(49BLA-ran-51PLA) sample was estimated to be 3.6×10^4 by the viscosity measurement. In this notation, the numerical figures to the individual comonomers indicate the mole fraction (%) in the copolymer.

L-Aspartic acid-$\beta_R d$ (deuterium content > 90%) was prepared by following the procedure previously reported for deuteration of poly(γ-benzyl L-glutamate) [36]. The esterification of the acid was carried out according to the conventional method [37] by using benzyl and phenethyl alcohol to obtain β-benzyl and β-phenethyl L-aspartate respectively. Partially deuterated copoly(BLA-ran-PLA) samples were prepared by starting from an appropriate mixture of deuterated and protonated NCA monomers.

2.2. Measurements

Circular dichroism (CD) spectra were recorded on a JASCO J-820 spectrometer. The same apparatus was used to investigate the ORD characteristics. The temperature was controlled within ±0.1 °C by using a JASCO PTC-423L.

Viscosity measurements were carried by using a conventional Ubbelohde viscometer. The intrinsic viscosity $[\eta]$ was measured for representative samples in chloroform containing 2 vol% of DCA. The viscosity-average molecular weight of polymer samples was estimated from the relation reported by Hayashi et al. [38]: $[\eta] = 1.36 \times 10^{-2} M^{0.738}$ (dl/g). The expression has been derived from the light-scattering and viscosity studies on fractionated PBLA samples and is valid for the temperature of 25 °C.

^{1}H, ^{2}H, and ^{13}C NMR measurements were performed by using JEOL JNM-LA500. Phase textures were observed by an Olympus polarizing microscope.

3. Results and discussion

3.1. Characterization of copolymer samples

The content of the BLA and PLA residues in given copolymer samples was detected by ^{1}H NMR in chloroform solution (1 wt%) containing a small amount of trifluoroacetic acid (TFA). The chemical shift of ε-protons was well separated from the other peaks and used for the identification of the two residues. The PLA content of copolymers thus determined is plotted against the PLA fraction in the initial NCA mixture in Fig. 1. In view of the similarity in the chemical structures of the two monomers, the rate of chain growth

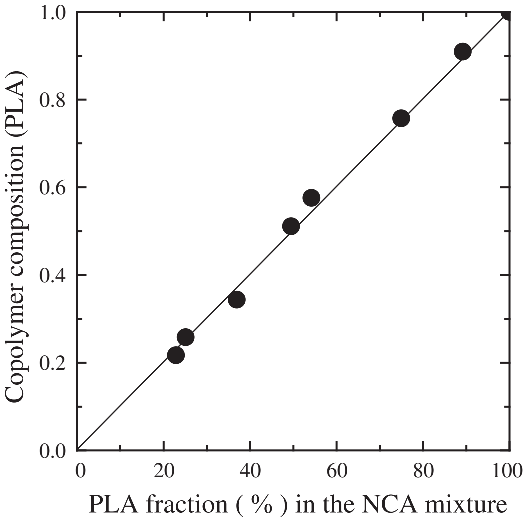

Fig. 1. Copolymer composition of copoly(BLA-ran-PLA) vs. monomer ratio of the initial mixture as expressed in terms of the PLA content.

should be independent of both chain composition and the nature of the adding species [39]. The copolymers synthesized are random in monomer distribution. The experimental points closely follow the diagonal line.

3.2. *Observations in the dilute isotropic solution regime*

3.2.1. *CD and ORD measurements*

Fig. 2 shows CD spectra obtained from copoly(49BLA-ran-51PLA) dissolved in TCE (1 wt%). Variation of the screw-sense with temperature can be followed by the absorption $[\theta]_{225}$ at 225 nm. ORD studies were carried out for polymers in 1 wt% TCE solution, the b_0 term being estimated from the conventional Moffit plot [40–42]. Variation of the specific rotation $[\alpha]_{546}$ as well as the b_0 value at room temperature follows an S-shape trend when plotted against the copolymer composition (PLA content). These results are consistent with the previous observations on copoly(BLA-ran-PLA) as well as copolymers including comonomers other than PLA [8–22].

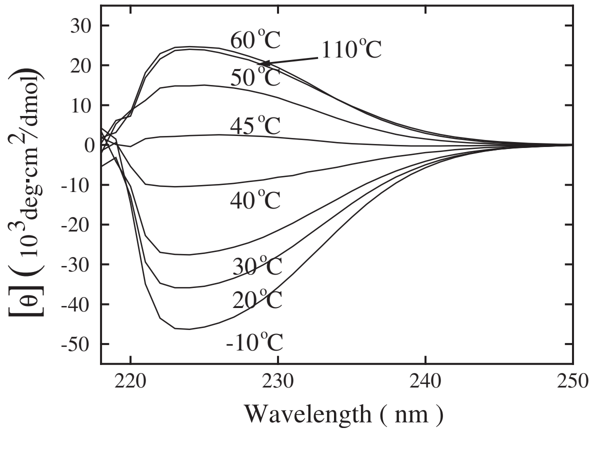

Fig. 2. CD spectra of copoly(49BLA-ran-51PLA) in TCE (1 wt%), temperatures being given to the individual curves.

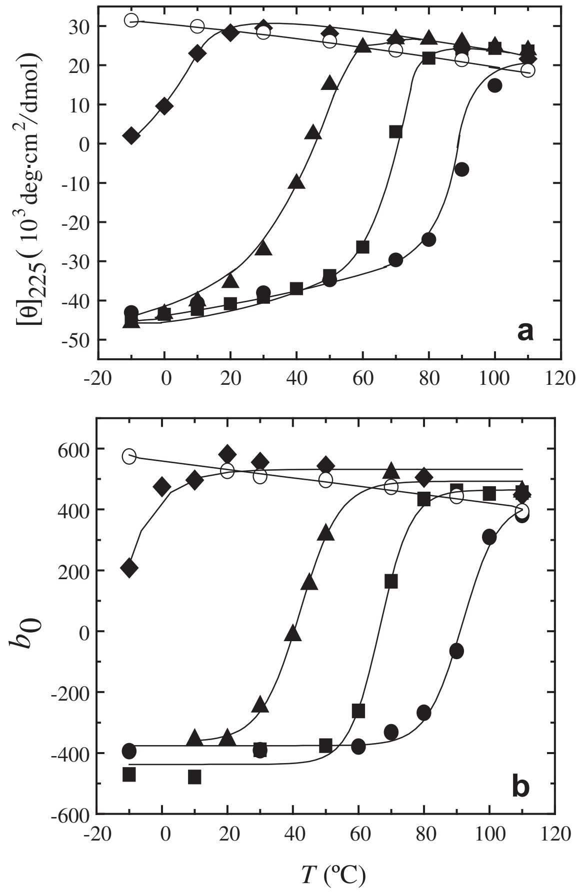

Fig. 3. Variation of (a) CD ($[\theta]_{223}$) and (b) ORD (b_0) as a function of temperature observed for homopolymers and copoly(BLA-ran-PLA)s in TCE (1 wt%). Symbols indicate PPLA (100%PLA) (●), 76%PLA (■), 51%PLA (▲), 26%PLA (◆), and PBLA (0%PLA) (○).

Plotted in Fig. 3 are the representative examples of the transition curves with temperature derived from (a) CD and (b) ORD measurements. PPLA (filled circle) exhibits a sharp change between the r- and ℓ-helix around 90 °C. In contrast, PBLA (open circles) remains in the ℓ-form throughout the temperature range examined. Copolymers of PLA content 0.76 (filled squares) and 0.51 (filled triangles) show a complete r ↔ ℓ transition with some shift in the temperature. In the BLA-rich region (PLA < 0.5), thermal transition curves tend to be somewhat moderate. An example is shown for the copolymer with PLA content 0.26 (filled diamonds). In this region, transition curves could not be followed to the completion due to the poor solubility of copolymers at lower temperatures. The solution tends to be turbid below −20 °C. Transition curves nearly identical to those in Fig. 3 were obtained by plotting the specific optical rotation $[\alpha]_{546}$ as a function of temperature.

Thermal variation of $[\theta]_{225}$ and b_0 values may be used to estimate the transition temperature T_c. In Table 1, the values of T_c obtained from the CD and ORD measurements respectively at

Table 1
Variation of T_c with copolymer composition for a series of copoly(BLA-ran-PLA)s determined by CD ($[\theta]_{225}$) and ORD (b_0).

PLA content	T_c defined for $[\theta]_{225} = 0$ (°C)	T_c defined for $b_0 = 0$ (°C)
1.0 (PPLA)	91.2	90.7
0.91	84.4	83.9
0.76	69.3	66.7
0.51	44.3	41.4
0.34	8.6	−4.8
0.26	−11.0	−12.5
0.0 (PBLA)	–[a]	–[a]

[a] PBLA remains in the ℓ-form without transition down to −20 °C.

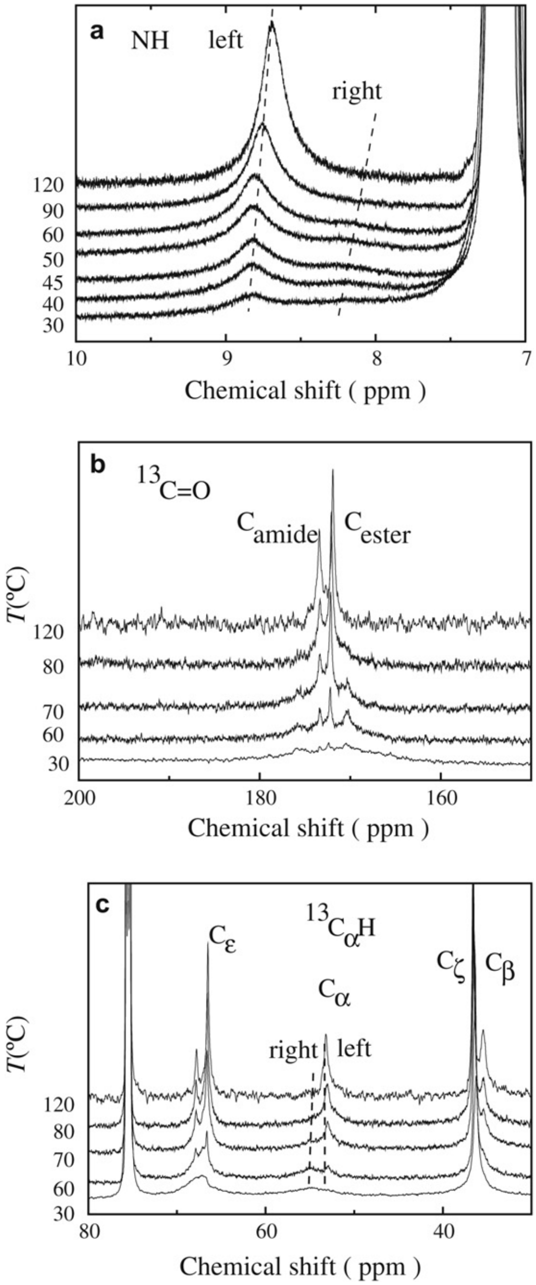

Fig. 4. NMR spectra of the amide group in the dilute isotropic solution in the biphasic region. (a) NH proton of copoly(49BLA-ran-51PLA) in TCE-d_2 (1 wt%), (b) ^{13}C of the amide and ester carbonyl group of copoly(24BLA-ran-76PLA) in TCE-d_2 (8 wt%), and (c) $^{13}C_\alpha$ of the same copolymer in TCE-d_2 (8 wt%).

$[\theta]_{225} = 0$ and $b_0 = 0$ are compared for the homopolymers and copolymers of various compositions. The correspondence between the two measurements is reasonably good except for PLA content 0.34. The source of a significant discrepancy (13.4 °C) noted at this composition is not clear however.

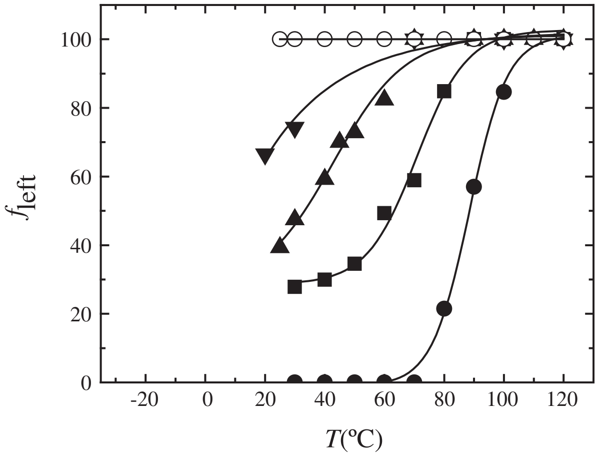

Fig. 5. The r ↔ ℓ transition curves of homopolymers and copoly(BLA-ran-PLA)s as a function of temperature, estimated from the NH proton chemical shift observed in TCE-d_2 (1 wt%). Symbols indicate PPLA (●), 76%PLA (■), 51%PLA (▲), 34%PLA (▼), and PBLA (0%PLA) (○).

3.2.2. ^{1}H and ^{13}C NMR analyses

The inversion of the α-helical screw-sense has also been followed by ^{1}H and ^{13}C NMR in dilute isotropic solution. The NMR analysis may provide further insight about the transition by differentiating the two chiral moieties of the opposite screw-sense in equilibrium. Shown in Fig. 4a (amide NH) are examples of the proton spectra observed for copoly(49BLA-ran-51PLA) in TCE (1 wt%) over the transition region. The rate of transformation can be roughly estimated by measuring the relative intensity of the individual absorption peaks. The fraction of the ℓ-form, $f_{\text{left}} = \ell/(\text{r} + \ell)$, estimated from the NH proton chemical shift at various copolymer compositions is plotted against temperature in Fig. 5. The intensity measurements were found to suffer from the peak broadening at lower temperatures especially for the r-form due to the reduced local mobility (Fig. 4a). With this reason, the results of the ^{1}H NMR analysis at low temperatures should be taken to be only a qualitative indication. In contrast to the transition curves derived from the CD ($[\theta]_{225}$) and ORD (b_0) measurements (Fig. 3), variation of those estimated from the ^{1}H NMR chemical shifts seems to be incomplete for copolymers (PLA content 0.76, 0.51, 0.34) in the low-temperature region. It may be also important to note that the resonance peak characteristic of the ℓ-form remains detectable for these PLA-rich copolymers even at temperatures as low as 30 °C (cf. Fig. 4a). Although quantitative estimate is difficult, the persistence of the ℓ-signal in the low-temperature region may suggest a broad distribution in the copolymer composition.

The ^{13}C NMR spectra should be less affected by the surrounding media. Variations of the chemical shifts due to the carbonyl (Fig. 4b) and C_α carbons (Fig. 4c) were studied for copoly(24BLA-ran-76PLA) in TCE-d_2 (8 mol%). The C_β peaks cannot be distinguished due to the severe overlap with those of C_ζ. Although the broadening of the peaks becomes appreciable at lower temperatures, all signals suggest that a small amount of the ℓ-form persistently remains at temperatures as low as 30 °C.

An important consequence of the ^{1}H and ^{13}C NMR observations described above is that the signals appear as a doublet, one from r and the other from ℓ, over the entire range of transition. This is in contradiction to the previous IR observation of Hashimoto et al. [13] who noted a gradual shift of a single peak in the intermediate region of the transition. More recent FTIR analysis on PPLA in the transition range has however indicated that the deconvoluted amide I stretch band includes two kinds of components, one attributable to the r- and the other to the ℓ-helix in agreement with the aforementioned NMR observations [43].

3.2.3. Viscosity measurements

In order to examine the spatial configuration of copolymers during the course of helix-sense inversion, viscosity measurements were carried out for a dilute TCE solution of copoly(49BLA-ran-51PLA) (0.1 wt%) over the temperature range from 20 to 80 °C. To avoid possible association of solute molecules, a small amount of

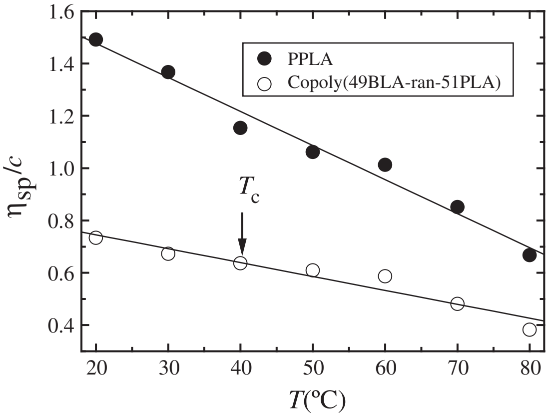

Fig. 6. Temperature dependence of the viscosity number η_{sp}/c of PPLA (●) and copoly (49BLA-ran-51PLA) (○) in TCE (0.1 wt%) containing 2 vol% of DCA. The r ↔ ℓ transition temperature (T_c) of the copolymer estimated by CD and ORD is indicated by the arrow. PPLA remains in the rigid r-form until the transition takes place at around 90 °C.

DCA (2 vol%) was added in solution. Variation of the viscosity number η_{sp}/c thus obtained is plotted as a function of temperature in Fig. 6, where the results obtained for the PPLA homopolymer ($T_c \sim 90\,°C$) are also included for comparison. Within the experimental precision, the observed points align along a straight line over the entire region involving the screw-sense inversion temperature T_c ($\sim 40\,°C$). These results strongly suggest that the copolymer maintains a rigid helical backbone including the transition region where the r- and ℓ-sequences are equally populated in solution.

3.3. Mechanism of the screw-sense reversal elucidated in the lyotropic LC state

3.3.1. Phase texture under the polarizing microscope

The viscosity measurement described above suggests that the spatial configuration of random copolymers of BLA and PLA is possibly rod-like. In agreement with these observations, the copolymers were found to form a lyotropic liquid crystal with the cholesteric texture at higher concentrations in helicoidal solvents such as chloroform or TCE. A polarizing microscopic observation (Fig. 7) has indicated that copoly(49BLA-β_R*d*-ran-51PLA) solution in TCE (25%) reaches the nematic point at around 60 °C, where the cholesteric pitch diverges, while the mid-points of the molecular r ↔ ℓ transition estimated by the CD, ORD, and ^{2}H NMR methods on the same sample are somewhat lower (ca. 40 °C) however.

3.3.2. Deuterium NMR analysis in the LC state

In the conventional NMR magnet (~ 12 T), α-helical polypeptides capable of forming lyotropic liquid crystals exhibit a nematic monodomain with the director lying along the magnetic field [36,44]. The orientational order parameter S can be defined as

$$S = 1 - (3/2)\left\langle \sin^2\psi \right\rangle \tag{1}$$

where angle ψ designates the inclination of the domain axis. The deuterium atom incorporated in the amide group provides the order parameter of the backbone by

$$S = (2/3)\Delta\nu / q_h \tag{2}$$

where $\Delta\nu$ represents the quadrupolar splittings observed for the N–D bond, and q_h is the component of the quadrupolar interaction tensor along the α-helical axis. Quadrupolar splittings due to side-chain C–D bonds include a statistical average ($\langle\rangle$) over all conformations permitted to the side chain:

$$\Delta\nu = (3/2)\left(e^2qQ/h\right)_{CD}\left[3\left(\left\langle \cos^2\theta \right\rangle - 1\right)\Big/2\right]S \tag{3}$$

where $(e^2qQ/h)_{CD}$ is the quadrupolar coupling constant of a given CD bond, and θ indicates the inclination of the CD bond relative to the molecular axis. A combined use of Eqs. (2) and (3) permits an estimation of the orientational order $3(\langle\cos^2\theta\rangle - 1)/2$ for a given C–D bond.

We have learnt from our previous ^{2}H NMR studies [24,30,31] that the substitution of any protons by deuterium at either the main chain or the side chain does not cause any appreciable isotope effect on the helix–helix transition behaviors. Shown in Fig. 8 are the ^{2}H NMR spectra of β-CD observed for copoly(49BLA-β_R*d*-ran-51PLA) in TCE (25 wt%). The assignment of the splittings $\Delta\nu$ to the screw-sense is adopted from our previous work [45]. The copolymer exhibits a discrete transition from r to ℓ by raising temperature.

As illustrated in Fig. 9, the $\Delta\nu_{\beta R}$ of the side-chain β-CD exhibits a drastic change at around 40 °C, while the $\Delta\nu_{ND}$ of the main-chain amide ND (illustrated in the inset) showed only a gradual monotonic decrease. The magnitude of these deuterium quadrupolar splittings is nearly the same as those previously found for PPLA. As the result, Fig. 9 resembles the behavior of PPLA [32] except that the transition temperature is somewhat depressed.

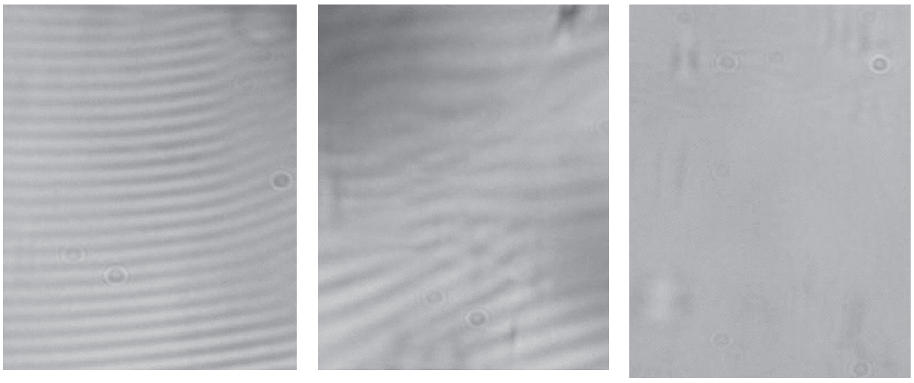
30 °C 45 °C 60 °C

Fig. 7. Polarizing microscopic observations on copoly(49BLA-β_R*d*-ran-51PLA) in TCE (25 wt%) with raising temperature. The cholesteric texture disappears around 60 °C at the nematic point [25].

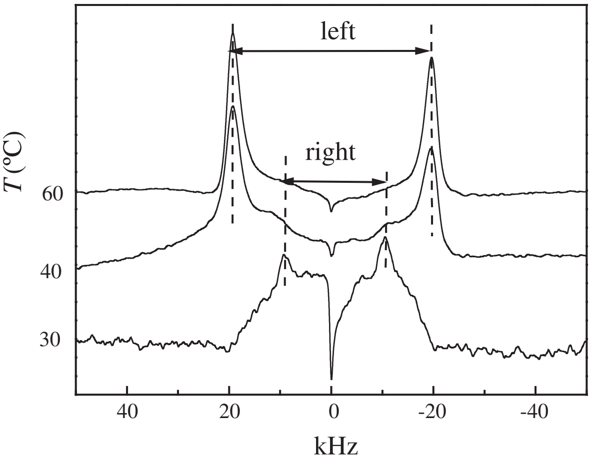

Fig. 8. ^{2}H NMR spectra of copoly(49BLA-β_R*d*-ran-51PLA) in the lyotropic LC state in TCE (25 wt%) at different temperatures. The mid-point of the transition was estimated to be around 37 °C.

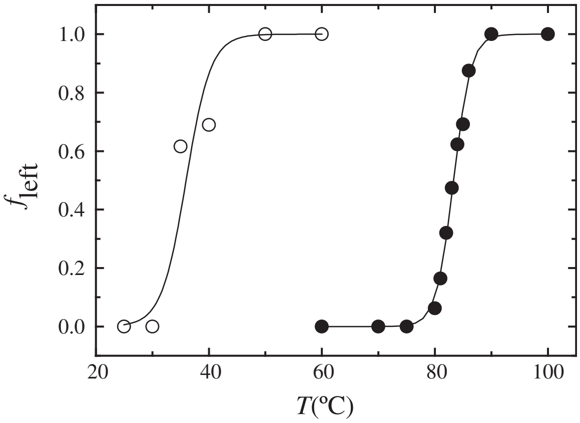

Fig. 10. Comparison of the transition curves of PPLA (●) [26] and copoly(49BLA-β_R*d*-ran-51PLA) (○) derived from the ^{2}H NMR measurements ($\Delta\nu_{\beta R}$) in the lyotropic LC state in TCE (25 wt%). These two curves may be characterized by the parameters such as $T_c = 83.2$ °C and the van't Hoff energy $\Delta H_v/R = 78.9$ K for PPLA, and $T_c = 37.2$ °C and $\Delta H_v/R = 50.4$ K for copoly(49BLA-β_R*d*-ran-51PLA).

As before, these observations suggest a mechanism in which unwinding and rewinding of the α-helical backbone take place while the orientational order of the molecular axis is maintained in the nematic environment. The interconversion between the r- and ℓ-form can be followed by the peak intensity of the β-CD resonance for copoly(49BLA-β_R*d*-ran-51PLA), and the fraction of ℓ-residue ($f_{\rm left}$) thus estimated are shown as a function of temperature in Fig. 10: the van't Hoff energy ΔH_v of the transition may be obtained from the slope [46]. In contrast to the NMR observations in the dilute solution (Fig. 4), the copolymer was found to remain strictly in the r-form at lower temperatures (<30 °C) in the LC state (Fig. 9). The transition curve is slightly sharper than those elucidated for an isolated chain from the CD, ORD, and ^{1}H NMR measurements in the dilute solution. The mid-point of the transition (37.2 °C) is however nearly the same as those (Table 1) found in the dilute solution. Ignoring such trivial differences, the general characteristics of the transition are similar to those found in the isotropic solution (Fig. 3), suggesting that the screw-sense inversion proceeds more or less through the same mechanism.

Also included for comparison in Fig. 10 is the transition behavior of PPLA previously reported by Okamoto [26]. The cooperative nature of the helix–helix transition is little affected by the inclusion of BLA residues. The van't Hoff energies obtained for the two curves are $\Delta H_v/R = 78.9$ K for PPLA and 50.4 K for copoly(49BLA-β_R*d*-ran-51PLA). The ^{2}H NMR observations in the LC state strongly suggest that the helix-sense reversals of the copolymers (up to about 1:1 monomer ratio) follow a zipper-type process starting from one terminal, the same mechanism being proposed for the PPLA homopolymer in our previous work [32,33].

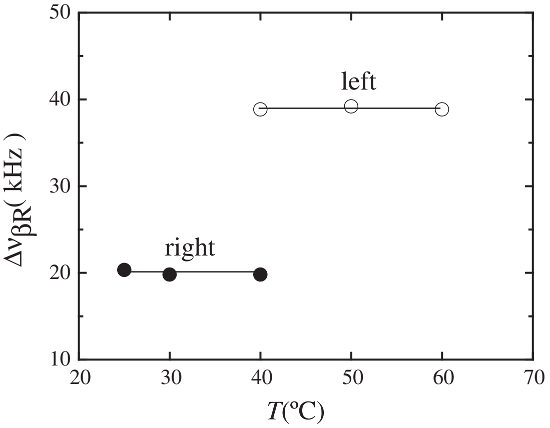

Fig. 9. Variation of the deuterium quadrupolar splitting $\Delta\nu_{\beta R}$ as a function of temperature observed for copoly(49BLA-β_R*d*-ran-51PLA) in TCE (25 wt%). The screw-sense of the α-helix shifts from r (●) to ℓ (○) around 40 °C. As indicated in the inset, the $\Delta\nu_{\rm ND}$ values do not exhibit any discontinuity over the temperature range of the transition, indicating that the orientational order of the backbone remains unaffected.

4. Discussion

The S-shape trend of the b_0 vs. composition curve has been reported for many random copolymers of polyaspartates involving BLA as one of the components. In this work, copoly(BLA-ran-PLA)s were chosen to investigate the molecular mechanism involved in the screw-sense inversion peculiar to these copolyaspartates. As indicated in Fig. 3 and Table 1, the reversal of the helical sense as defined by T_c should become lower than the room temperature for copolymers with PLA content < 0.4. The screw-sense of copolymers below this limit remains in the ℓ-regime at an ordinary temperature.

In Fig. 11, the temperatures T_c estimated according to the definition $[\theta]_{225} = 0$ or $b_0 = 0$ (Table 1) are plotted against the PLA

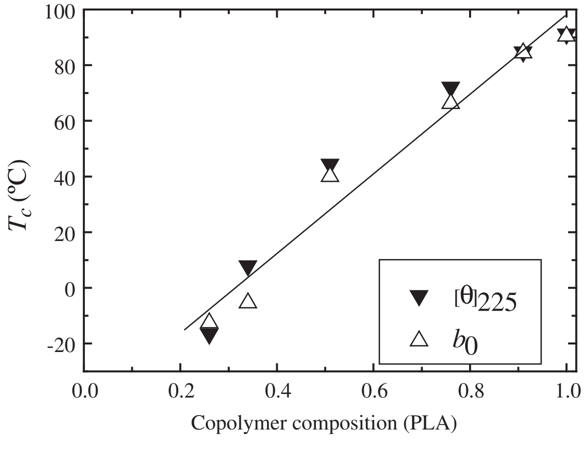

Fig. 11. Variation of the screw-sense inversion temperature T_c estimated from the CD ($[\theta]_{225} = 0$) (▼) and ORD ($b_0 = 0$) (△) as a function of PLA content of copolymers in TCE (1 wt%). The linear fitting indicates a general trend of the composition-dependence of T_c.

content in the range 1.0–0.2. Viscosity measurements in the dilute solution as well as ^{2}H NMR analyses in the LC state indicate that the polymers maintain the rod-like character even at the mid-point of the transition (T_c). The linear extrapolation of the experimental data gives a rough estimate of $T_c =$ ca. $-40\,^\circ$C for PBLA at PLA content $= 0$. This is consistent with the ordinary observation that PBLA α-helix normally exists only in the ℓ-form in conventional helicoidal solvents. PBLA may adopt an r-form at temperatures below $-40\,^\circ$C in TCE. The increment in T_c from PBLA to PPLA amounts to about 120 °C. In the α-helix-to-α-helix transition accompanying the screw-sense reversal, the H-bond exchange takes place between the two well-defined helical states. Since the number of internal H-bonds remains the same before and after the inversion, the energies stabilizing the H-bond network should be nearly identical. The major cause of this discrepancy may be traced back to the difference in the rotational characteristics of the first-articulated χ_1 bond, which plays the key role in the interaction between the main chain and the side chain [45].

In our previous work [31,32], the conformational energies of the side chain involved in PPLA have been estimated for each handedness of the backbone by various techniques. The average energies were elucidated for the individual conformational states, E_ℓ and E_r, by the RIS simulation on the observed ^{2}H NMR data, suggesting that the difference between the ℓ- and r-form, $\Delta E = E_\ell - E_r$, amounts to 1.1 kJ/mole of repeat unit. An MD simulation of the side-chain conformation yielded $\Delta E = 0.6$ kJ/mol. The energy minimization within the RIS framework led to $\Delta E = 3.6$ kJ/mol. The DSC measurements on the helix–helix transition in solution gave an estimate of $\Delta H =$ ca. 2 kJ/mol [26]. If the transition is highly cooperative and a simple van't Hoff expression is approximately valid, the f_{left} vs. T plot shown in Fig. 10 leads to an estimate of the enthalpy difference $\Delta H_v/R$ (per polymer) as given in the legend to the figure. The molecular weight of polymer samples prepared according to our recipe falls into a range of $4–6 \times 10^4$, or DP $= 200–400$. The estimate of ΔH_v for PPLA corresponds to $\Delta H = 2$ kJ/mol of residue in reasonable accord with the DCS data. The ΔH value reduces to 1.4 kJ/mol of residue for the 1:1 copolymer. The strong neighbor-dependent character build up by the H-bond network is responsible for the sharp transition with a small free-energy difference between the two helical states. The values of T_c and $\Delta H_v/R$ (per polymer) estimated from the b_0 data (Fig. 3) in the dilute isotropic solution are $T_c = 90.7\,^\circ$C and $\Delta H_v/R = 22.7$ K for PPLA and $T_c = 41.4\,^\circ$C and $\Delta H_v/R = 13.0$ K for copoly(49BLA-ran-51PLA). Lower values of ΔH_v in the isotropic state relative to the LC state (Fig. 10) may indicate that the cooperativity of the H-bond network is somewhat weaker.

5. Concluding remarks

In the early stage of this experimental work, we thought of two alternative possibilities: (1) the random copolymerization of BLA and PLA may produce higher fraction of the r/ℓ junction along the chain, thus facilitating the direct observation of the molecular arrangement of the transition state, or (2) the cooperativity of the H-bond network may be so strong that the minor component adopts the screw-sense of the majority, leading to a pseudo-first-order transition at T_c. In reality, the latter expectation was the case. The direct observation of the conformational characteristics at the transition site remains unaccomplished.

Experimental observations on a series of copolyaspartates in the LC state have provided a strong support to a zipper-type propagation mechanism starting from one terminal to the other. Theoretical prescription of the phenomenon has been attempted by adopting a Zimm–Bragg two-parameter scheme [47]. This subject will be treated in the following paper [34].

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References

[1] Toniolo C, Falxa ML, Goodman M. Biopolymers 1968;6:1579–603.
[2] Karlson RH, Norland KS, Fasman GD, Blout ER. J Am Chem Soc 1960;82: 2268–75.
[3] Malcolm BR. Nature 1968;219:929–30.
[4] Malcolm BR. Biopolymers 1970;9:911–22.
[5] Giancotti V, Quadrifoglio F, Crescenzi V. J Am Chem Soc 1972;94:297–8.
[6] Kyotani H, Kanetsuna H, Oya M. J Polym Sci Part B Polym Phys 1977;15:1029–36.
[7] Riou SA, Hsu SL, Stidham HD. Biophys J 1998;75:2451–60.
[8] Goodman M, Deber CM, Felix AM. J Am Chem Soc 1962;84:3773–4.
[9] Goodman M, Felix AM, Deber CM, Brause AR, Schwartz G. Biopolymers 1963;1:371–400.
[10] Goodman M, Felix AM, Deber CM, Brause AR. Biopolym Symp 1964;1:409–20.
[11] Hashimoto M, Aritomi. Bull Chem Soc Jpn 1966;39:2707–13.
[12] Hashimoto M. Bull Chem Soc Jpn 1966;39:2713–20.
[13] Hashimoto M, Arakawa S. Bull Chem Soc Jpn 1967;40:1698–701.
[14] Bradbury EM, Downie AR, Elliott A, Hanby WE. Proc Roy Soc (Lond) 1960;A259:110–28.
[15] Bradbury EM, Carpenter BG, Goldman H. Biopolymers 1968;6:837–50.
[16] Bradbury EM, Carpenter BG, Stephens RM. Biopolymers 1968;6:905–15.
[17] Tsujita Y, Fujii N, Imoto Y, Takizawa A, Kinoshita T. J Polym Sci 1984;22:2533–8.
[18] Nishizima R, Tsujita Y, Takizawa A. Polymer 1985;26:379–82.
[19] Kusakawa M, Tsujita Y, Takizawa A. Polymer 1985;26:848–52.
[20] Watanabe T, Tsujita Y, Takizawa A, Kinoshita T. Polymer 1987;28:1809–14.
[21] Tsujita Y. Biophys Chem 1988;31:11–20.
[22] Tsujita Y, Watanabe T, Takizawa A, Kinoshita T. Polymer 1991;32:569–75.
[23] Toriumi H, Saso N, Yasumoto Y, Sasaki S, Uematsu I. Polym J 1979;11: 977–81.
[24] Abe A, Okamoto S, Kimura K, Tamura K, Onigawara H, Watanabe J. Acta Polym 1993;44:54–6.
[25] Watanabe J, Okamoto S, Abe A. Liq Cryst 1993;15:259–63.
[26] Okamoto S. Ph.D. Dissertation, Tokyo Institute of Technology; 1995.
[27] Abe A, Hiraga K, Imada Y, Hiejima T, Furuya H. Biopolym (Peptide Sci) 2005;80:249–57.
[28] Abe A, Imada Y, Hiejima T, Furuya H. In: Blondelle SE, editor. Understanding biology using peptides. American Peptide Society; 2005. p. 700–1.
[29] Abe A, Imada Y, Hiejima T, Furuya H. Chem Today 2006;24:52–4.
[30] Abe A, Furuya H, Okamoto S. Polym Sci Ser A 1996;38:317–23. from Vysokomol Soedin Ser A 1996;38:566–73.
[31] Okamoto S, Furuya H, Abe A. Polym J 1995;27:746–56.
[32] Abe A, Furuya H, Okamoto S. Biopolym (Peptide Sci) 1997;43:405–12.
[33] Ushiyama A, Furuya H, Abe A, Yamazaki T. Polym J 2002;34:450–4.
[34] Abe A, Imada Y, Furuya H. The succeeding paper.
[35] Daly WH, Poche D. Tetrahedron Lett 1988;29:5859–62.
[36] Abe A, Yamazaki T. Macromolecules 1989;22:2138–45.
[37] Hayakawa T, Nishi H, Noguchi J, Ikeda S, Yamashita T, Isemura T. J Chem Soc Jpn Pure Chem Sect (Nippon Kagaku Zasshi) 1961;82:601–4.
[38] Hayashi Y, Teramoto A, Kawahara K, Fujita A. Biopolymers 1969;8:403–20.
[39] Block H. Poly(γ-benzyl l-glutamate) and other glutamic acid containing polymers. New York: Gorden and Breach Sci Pub; 1983. p. 39.
[40] Moffit W, Yang JT. Proc Natl Acad Sci U S A 1956;42:596–603.
[41] Moffit W. Proc Natl Acad Sci U S A 1956;42:736–46.
[42] Moffit W. J Chem Phys 1956;25:467–78.
[43] Yamamoto T, Honma R, Nishio K, Hirotsu S, Okamoto S, Furuya H, et al. J Mol Struct 1996;375:1–7.
[44] Abe A, Yamazaki T. Macromolecules 1989;22:2145–9.
[45] Abe A. In: Kahovec J, editor. Macromolecules 1992. Utrecht: VSP; 1993. p. 221–8.
[46] Van't Hoff JH. Études de Dynamique Chimique. Amsterdam: Frederick Muller & Co.; 1884.
[47] Zimm BH, Bragg JK. J Chem Phys 1959;31:526–35.