Conformational Analysis by IR and Birefringence Measurements for Poly(β -phenylpropyl L-aspartate) Exhibiting a Helical Sense Inversion in the Solid State

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Normally L-polypeptide exists as a right-handed α -helix (α_R), but poly(L-aspartate) (PLA) can also assume a left-handed helix.^{1–5} Formation of helices with both handedness in PLA indicates that the energy difference between right-handed and left-handed helices is relatively small.

In such a system with a small energy difference between the two skeletons, the handedness of helices is easily affected by several factors such as the side chain, solvent, pressure and temperature. Thus, at a specific case, the helix inversion takes place merely with the variation of temperature. This is interesting, since the systematic studies on mechanism and thermodynamics of helical sense inversion, can be more easily performed as a function of temperature. In a previous work on poly(β -phenylethyl L-aspartate) (2PLA), it was reported that the transition between two α -helical forms takes place reversibly in the dilute solutions of tetrachloroethane at around 80°C.⁶ Furthermore, the lyotropic liquid crystals in tetrachloroethane also showed the reversible transition in a somewhat wide range of temperature of 80–100°C.^{6–9} In this case, the helical sense inversion of polymer backbone simultaneously resulted in the sense inversion of macroscopic helix of cholesteric liquid crystal.⁷ More interesting result is that even in the solid state poly(β -phenylpropyl L-aspartate) (3PLA), poly(β -phenylbutyl L-aspartate) (4PLA) and poly(β -phenylpentyl L-aspartate) (5PLA) underwent the reversible and first order transition from α_R to the left-handed ω -helix (ω_L).^{10–12} The essentially common result obtained in these solid systems is that the uniaxial orientation of the helical axes is maintained on the transition. In other words, the transition should be of the helix–helix transition which does not include the coil form. It was also supported by the DSC isothermal kinetic analyses according to the Avrami equation, which show that the transformation was a type of homogeneous nucleation and one-dimensional growth.¹³ The hexagonal chain packing is nearly identical before and after the transition and so the enthalpy change observed on the helix–helix transition must reflect the energy difference between the right-handed and left-handed regimes. It was estimated around 0.5 kcal mol^{–1}, indicating the small energy difference between the two skeletons.

In this small energy difference system, it is likely that

the side chain conformation may play a key part in triggering the helix–helix transition. Examples are abundantly known for conventional polymers in which the conformational asymmetry involved in the side chain largely affects the spatial configuration of the skeletal chain.^{14–16} In an asymmetric polymer, the side chain conformation varies more or less with the configuration of the main chain. Since bond conformations are often neighbor-dependent along the polymer chain, small free energy differences accumulated in the individual side chain may become a driving force for a large deformation of the skeletal chain.

In the present work, with the objective of investigating the change of side chain conformation that would take place on the screw sense inversion of the back bone in the solid state, we performed infrared (IR) dichroism and birefringence measurements for the oriented films of a representative 3PLA. The results simply indicate that considerable difference exists in the side chain conformations between the α_R and ω_L forms.

EXPERIMENTAL

3PLA was synthesized by the NCA method using triethylamine as an initiator in chloroform solution.¹⁰ The molecular weight of 3PLA was estimated to be 26000 from the viscosity measurement in a mixture of dichloroacetic acid and chloroform.¹⁷ Oriented films were obtained by shearing unidirectionally the liquid-crystalline solutions in chloroform on a glass plate, so that the orientation axis corresponds to the helical axis.^{10,18,19} IR dichroism was measured with a JASCO MFT-2000 Fourier transform-infrared spectrophotometer. The birefringence measurement was performed by an Olympus BX 50 polarizing microscope equipped with a Berek compensator. The temperature was regulated within $\pm 1^\circ\text{C}$ with a Mettler FP 82 hot stage.

RESULTS AND DISCUSSION

3PLA exhibits the helix–helix transition at about 160°C on heating and 145°C on cooling.¹⁰ Figures 1a and 1b give the polarized IR spectra observed at 50 and 170°C, respectively. In Figure 1a, the average amide frequencies of 3302, 1659, and 1553 cm^{–1} for amides A, I,

Figure 1. Polarized infrared spectra of an oriented 3PLA solid film observed for (a) the right-handed α -helix at 50°C and (b) the left-handed ω -helix at 170°C. Solid and dotted curves were observed with the electric vector of the polarized infrared radiation parallel and perpendicular to the oriented axis, respectively.

and II, respectively, show it to be in the α_R form, while in Figure 1b those of 3315, 1671, and 1549 cm^{-1} can be attributed to the ω_L form.^{4,10–12,18–20} These variations were observed reversibly on repeated heating and cooling cycles.

The degree of orientation in thin films is so high to result in the clear dichroism. We can thus estimate the angles (θ) of the transition moments with respect to the helical axis according to the following equations.^{18,19,21–24}

$$f = (R - 1)(R_0 + 2) / \{(R_0 - 1)(R + 2)\}$$

$$R = \{f \cos^2 \theta + (1/3)(1 - f)\} / \{(1/2)f \sin^2 \theta + (1/3)(1 - f)\}$$

Here, the angle of the transition moments (θ) is related to the observed dichroic ratio (R) and the fraction of polymer oriented (f). The angle of the transition moment for amide A in α_R has been estimated to be about 20° for a perfectly oriented sample, where the dichroic ratio R_0 is 15 : 1.^{18,19} In this study, thus, f was estimated to be 0.86 in the α_R form by using $R = 8.19$ observed for the amide A band. We have known from X-ray measurement that the helix-helix transition occurs along the polypeptide backbone without disturbing the uniaxial

orientation of helical axes.¹⁰ In fact, the dichroic ratio of the amide absorption bands is not changed by the transformation to the ω_L form on heating nor by the retransformation to the α_R form on cooling. Thus, the same value of f was used for the analyses of all data, allowing a comparison to be made of the side chain conformation between the two helices. The observed R and calculated θ are listed in Table I.

The main absorption bands associated with the side chains are from the ester C=O stretching vibration at 1741 cm^{-1} , and the ester C–O–C antisymmetric stretching vibration at 1174 cm^{-1} for α_R . They are observed at 1736 cm^{-1} and 1165 cm^{-1} , respectively, for ω_L . From a comparison of spectra in Figures 1a and 1b, one can find that the dichroism for these bands is extremely different before and after the helical sense inversion. Furthermore, it is sure from Figures 2a–2d that the drastic change of the frequency and dichroic ratio takes place just on the helix-helix transition. The ester C=O stretching band has the highly perpendicular dichroism for α_R , while the ester C–O–C antisymmetric stretching band has the parallel dichroism. On the other hand, the ester C=O and C–O–C bands in ω_L turn to have nondichroic and perpendicular dichroism, respectively. As given in Table I, the angles, θ , are 69° for the ester C=O vibration and 43° for the ester C–O–C vibration in α_R , while in ω_L the angles are 53° and 68°.²⁵ It should be noted that Bradbury and his coworker^{18,19} also examined the effect of the helix sense on the side chain conformation in poly(β -benzyl L-aspartate) and reported the similar orientation.

As reported in our previous paper, the oriented 3PLA film was shrunk along the fiber axis on the transition of α_R to ω_L .^{10–12} The shrinkage is due to the decrease of the residue height of repeating unit along the helical axis from 1.5 Å of α -helix to 1.27 Å of ω -helix.^{10–12} It is interesting that at the same time the birefringence was weakened enormously. This phenomenon may also be related to the side chain conformation, whose orientation affects the anisotropy of the polarizability. Figure 3 shows the temperature dependence of the birefringence $|\Delta n|$. Here, $|\Delta n|$ represents $|n_{\parallel} - n_{\perp}|$, the difference in the refractive indices of the parallel and perpendicular components with respect to the helical axis. It is obvious that $|\Delta n|$ in α_R gradually decreases with the increasing temperature and suddenly reduces to be one-twentieth on the transformation into ω_L . Some increment of $|\Delta n|$ observed just before the transformation may be due to the pretransition.¹⁰ $|\Delta n|$ in α_R and ω_L were estimated as 0.023 (at 50°C) and 0.001 (at 170°C), respectively. From Table I, we know that the orientation angles of amide bands are similar between both helices and so it is likely that the contribution of the backbone to the polarizability is nearly similar for both helices. Thus, this reduction should be mainly attributed to the change of side chain conformation. Possibly, the parallel polarizability of the main chain may be cancelled by the perpendicular polarizability of the side chains.

The other homologues of polyaspartates, 4PLA and 5PLA, which exhibit the reversible helix-helix transition,¹¹ gave the similar data with respect to IR spectra and birefringence. Thus we reach the simple conclusion that there is a specific side chain conformation preferred

Table I. Frequencies of characteristic IR bands and their transition moment directions with respect to helix axis

Band	Position ($\parallel$) / cm^{-1}	Position ($\perp$) / cm^{-1}	Dichroic ratio	$\theta / ^\circ$ ^a
Right-handed α -helix (α_R) at 50°C				
Amide A	3301	3303	8.19	20
Amide I	1659	1659	2.51	40
Amide II	1553	1552	0.19	77
Ester C=O	1739	1742	0.39	69
Ester C-O-C	1173	1175	2.03	43
Left-handed ω -helix (ω_L) at 170°C				
Amide A	3314	3317	7.34	22
Amide I	1673	1670	2.70	37
Amide II	1548	1550	0.26	78
Ester C=O	1736	1736	1.09	53
Ester C-O-C	1164	1167	0.44	68

^a $f = 0.86$ was used for estimation (see the text). **Figure 2.** Temperature dependence of the position and dichroic ratio for the ester bands of side chain; (a) and (b) for C=O band, and (c) and (d) for the C-O-C band. The closed and open circles were collected on heating and cooling, respectively. **Figure 3.** Temperature dependence of the birefringence $|\Delta n|$ for an oriented 3PLA solid film. The closed and open circles were collected on heating and cooling, respectively.

to each α_R or ω_L skeleton, and suggest that the helix-helix transition may be forced by the small free energy difference accumulated in the side chains. The precise conformations of the side chains to satisfy the present data are being sought with a cooperation of the computer calculation for the rotation energy around $C_\alpha-C_\beta$ and $C_\beta-C(=O)$ bonds in side chain.^{26,27}

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