The Structure and Properties of Copolyaspartate I. Copoly(butyl-L-aspartate-benzyl-L-aspartate)

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ABSTRACT: The α helices of copoly(butyl-L-aspartate-benzyl-L-aspartate) prepared by substituting some of the benzyl groups in the side chains of poly(β -benzyl-L-aspartate) by butyl groups, were transformed, throughout the entire range of butylation tested, into ω helices with a tetragonal packing when heated up to 140°C. The α - ω transition temperature was about 100°C for copolyaspartates of butylation less than 30% and near 130°C for copolyaspartates of butylation more than 30%. In the case of more than 30% butylation the α - ω transition was furthermore accompanied by a change from the right handed α helix to the left handed ω helix. The α - ω transition resulted in a remarkable change in the magnitude of the dynamic modulus and an exothermic peak. It was concluded that the ω helix of copoly(butyl-L-aspartate-benzyl-L-aspartate) was based on the formation of stacks between benzyl and butyl groups as well as the ω helix consisting of stacks between the benzyl groups of poly(β -benzyl-L-aspartate).

KEY WORDS α - ω Transition / ω -Helix / Helix Sense / Stacks /
Copoly(butyl-L-aspartate-benzyl-L-aspartate) /

It is well known that a left handed helix is stable in solution and in a solid state of poly(β -benzyl-L-aspartate) (PBLAsp), in contrast to a right handed α helix of poly-L-peptides.¹ The α helix of PBLAsp transforms into ω helix only in a solid state by heat treatment (near 140°C). Bradbury *et al.*² have shown that the molecule in the ω helix forms a structure of the type described earlier by Bragg, Kendrew, and Perutz.³ This structure of the ω helix has four residues per turn of a helix and the ω helices are tetragonally packed. Thus, the α - ω transition was accompanied by a change from the right handed α helix to the left handed ω helix and the transformation from hexagonal to tetragonal packing. The exothermic peak⁴⁻⁶ and the volume contraction⁷ have been observed for the α - ω transition.

Malcolm^{8,9} has reported that a right handed α helix of PBLAsp prepared by monolayer technique also transforms into a left handed ω helix by heating to *ca.* 160°C; that is, both a reversal of the helix sense and the transformation from hexagonal to

tetragonal packing occurred in the α - ω transition. Furthermore, for poly(β -chlorobenzyl-L-aspartate), such a reversal in the solid state was confirmed by Tsuboi *et al.*¹⁰ Reversal of the helix sense occurs easily for a certain polyaspartate on the α - ω transition.

Poly(β -butyl-L-aspartate) is known to be a right handed α helix, contrary to the helix sense of PBLAsp. However, it was found that this compound does not transform into the ω helix.^{11,12} Thus it may be expected that copoly(butyl-L-aspartate-benzyl-L-aspartate) (copoly(BuLAsp-BeLAsp)) retains either a left handed or right handed α helix, depending on the degree of butylation and that copoly(BuLAsp-BeLAsp) at a low degree of butylation transforms into the ω helix on heat treatment.

In the present paper, the formation of the ω helix is studied for copoly(BuLAsp-BeLAsp). The α - ω transition of copoly(BuLAsp-BeLAsp) was investigated on the basis of the change from hexagonal to tetragonal packing and reversal of helix sense.

Furthermore, the viscoelastic and thermal behavior in the α - ω transition region along with the ω - β transformation were studied.

EXPERIMENTAL

Materials

N-Carboxy- β -benzyl-L-aspartate anhydride (NCA) was synthesized by Leuch's method with β -benzyl-L-aspartate produced from aspartic acid.¹³ The purified NCA was polymerized in about a 5% (w/v) ethylene dichloride solution, using triethylamine as the initiator. The PBLAsp thus obtained had an intrinsic viscosity of $[\eta]=0.426$ in dichloroacetic acid, which gave a viscosity average molecular weight of 6.6×10^4 as calculated from the viscosity molecular weight relation of Doty *et al.*¹⁴ for poly(γ -benzyl-L-glutamate). Copoly(BuLAsp-BeLAsp) was prepared by butylation of PBLAsp following the method reported by Bradbury *et al.*¹⁵ Six samples with various degrees of butylation as shown in Table I were produced with different conditions of reaction, *i.e.*, content of concentrated sulfuric acid and *n*-butyl alcohol and reaction time. The degree of butylation was evaluated from the NMR spectra of the benzyl and butyl groups.

Solid films 0.1 mm in thickness were prepared by casting from *ca.* 5% (w/v) chloroform solution at room temperature. For infrared absorption spectra measurements, thin films with 0.01–0.03 mm thick were prepared by the same method.

Measurements

NMR spectra were recorded on a 100 MHz spectrometer (Japan Electron Optics Laboratory Co. Ltd.) at room temperature in the τ region from

0 to 10 ppm. The polypeptide solution of tri-fluoroacetic acid had concentration of 10% (w/v) and tetramethyl silane was used as an internal standard.

Dynamic viscoelastic measurements were carried out on a Rheovibron DDV-II (Toyo Baldwin Co. Ltd.) at a frequency of 110 Hz over a temperature range from -100 to 230°C at the heating rate of $1^\circ\text{C}/3$ min.

Infrared absorption spectra were recorded in the wave number range from 400 to 4000 cm^{-1} with an infrared grating spectrophotometer (Japan Spectroscopic Co. Ltd.). The wave numbers of all spectra were calibrated using a polystyrene film as a standard specimen.

X-Ray photographs were taken on flat films with Cu- $K\alpha$ ($1.54\text{ \AA}$) radiation with a Rigaku Denki X-ray diffractometer. The distance from specimen to camera film (6.896 cm) was calibrated by silicon powders.

The DTA measurements were carried out on a differential thermal analyser with 10 mg of sample at a heating rate of $4^\circ\text{C}/\text{min}$. The temperature was calibrated on the basis of the melting points of benzoic acid, indium, and tin.

RESULTS AND DISCUSSION

Infrared Absorption Spectroscopy

The relationships between the helix conformation and characteristic band positions for PBLAsp are summarized in Table II. Tables III, IV, and V give characteristic band positions observed for the as-cast films, and the films annealed at 140°C and at 210°C , respectively. The copoly(BuLAsp-BeLAsp) obtained here was designated as Bu-1—Bu-6, de-

Table I. Conditions of butylation

Sample	Content of PBLAsp	Content of CHCl_3	Content of H_2SO_4	Reaction time	<i>n</i> -Butyl alcohol	Degree of butylation
	g	ml	ml	h	ml	%
Bu-1	0.4	100	0.3	72	30	17.9
Bu-2	0.4	100	0.3	72	50	20.6
Bu-3	0.4	100	0.4	100	55	32.5
Bu-4	0.4	100	0.4	145	60	39.1
Bu-5	0.4	100	1.0	190	70	69.3
Bu-6	0.4	100	1.5	214	80	86.7

pending on the degree of butylation as shown in Table I. Comparing band positions (Amide I and II bands) in Table II and III, it was found that the Amide I and II band positions of the as-cast films of

Table II. Band position of PBLAsp (cm^{-1})^a

	Ester C=O	Amide I	Amide II
Left handed α helix	1735 ± 2	1666 ± 2	1557 ± 2
Right handed α helix	1741 ± 2	1659 ± 2	1553 ± 2
ω Helix	1731 ± 2	1675 ± 2	1536 ± 2
β Form	1726	1636	1529

^a Based on Green *et al.*⁴ and Bradbury *et al.*¹¹

Table III. Band positions of as-cast films (cm^{-1})

	Ester C=O	Amide I	Amide II
Bu-1	1740	1665	1555
Bu-2	1740	1668	1556
Bu-3	1739	1659	1550
Bu-4	1738	1659	1550
Bu-5	1740	1660	1550
Bu-6	1739	1659	1549

Table IV. Band positions of films annealed at 140°C (cm^{-1})

	Ester C=O	Amide I	Amide II
PBLAsp (ω)	1730	1676	1533
Bu-1	1723	1667	1532
Bu-2	1726	1670	1532
Bu-3	1726	1667	1533
Bu-4	1725	1670	1533
Bu-5	1726	1666	1533
Bu-6	1723	1666	1531

Table V. Band positions of films annealed at 210°C (cm^{-1})

	Ester C=O	Amide I	Amide II
PBLAsp (β)	1725	1634	1530
Bu-1	1723	1634	1532
Bu-2	1724	1634	1529
Bu-3	1728	1634	1530
Bu-4	1728	1634	1530
Bu-5	1724	1634	1530
Bu-6	1726	1634	1530

Bu-3—6 were lower in wave number than Bu-1,2 and that the as-cast films of Bu-1, 2 as well as those of PBLAsp consisted of left handed α helices and that those of Bu-3—6 consisted of right handed α helices. With an increase in the degree of butylation, left handed α helices transformed into right handed α helices.

By annealing at 140°C , both left handed and right handed α helices of the as-cast films (Bu-1—6) transformed into ω helices. Since the steric conditions of the main chain and side chain of copoly(BuLAsp-BeLAsp) are unfavorable to the formation of the ω helix with the right handed screw sense,¹⁶ the ω helix obtained here is considered to be of the left handed helix sense, as in the case of PBLAsp's helix. That is, in Bu-3—6, the α - ω transition may simultaneously be accompanied with the change from a right handed to left handed helix, in contrast to the α - ω transition of Bu-1,2 and PBLAsp. The ω - β transformation, (ω helix to β form) by heating to 210°C , occurred in all samples (Bu-1—6), as shown in Table V (specially Amide I band).

X-Ray Diffraction Analysis

The spacings calculated from X-ray diffraction photographs for the as-cast films of all copolyaspartates (Bu-1—6) are given in Table VI. Comparing the X-ray diffraction photographs of the as-cast films of copoly(BuLAsp-BeLAsp) with that of PBLAsp,⁴ the as-cast films were found to consist of α helical chains of low degree of crystallinity. The spacings of the as-cast films of copoly(BuLAsp-BeLAsp) tend to become shorter as the degree of butylation increases, since the length

Table VI. Spacings ($\text{\AA}$) observed in X-ray diffraction photographs of PBLAsp and copoly(BuLAsp-BeLAsp)

	As-cast films	Films annealed at 140°C	Films annealed at 210°C
PBLAsp	13.2	13.9	17.1
Bu-1	13.2	13.9	16.7
Bu-2	13.2	14.0	17.0
Bu-3	13.1	14.0	16.6
Bu-4	13.0	13.8	16.7
Bu-5	12.8	13.8	16.8
Bu-6	12.7	13.8	16.6

Table VII. Spacings (Å) observed in X-ray diffraction photograph of Bu-4

As-cast film		Films annealed at 140°C				Films annealed at 210°C	
d_{obsd}	Int.	d_{obsd}	Int.	d_{calcd}^a	hkl	d_{obsd}	Int.
13.00	vvs	13.79	vvs	13.80	100	16.66	vs
		6.85	w	6.90	200		
		6.15	w	6.18	210		
		5.30	s				
		4.95	vs	4.88	220		
		4.62	s	4.60	300	4.69	s
		4.31	s	4.36	310		
		4.19	s	4.26	201		
		3.99	s	4.07	211		
		3.42	w	3.45	400		
		3.34	w	3.35	410		
		3.06	w	3.09	420		

^a d_{calcd} is based on the tetragonal unit cell with $a=b=13.80$ Å and $c=5.42$ Å.

of the *n*-butyl group is somewhat shorter than that of the benzyl group.

The spacings of the films (Bu-4) annealed at 140°C were the same as those of the ω helix of PBLAsp reported by Green *et al.*⁴ The α - ω transition of copoly(BuLAsp-BeLAsp) was also accompanied by a transformation from hexagonal to tetragonal packing. Although the spacings of the as-cast films (α helix) become shorter as the degree of butylation increases, the spacings of the films annealed at 140°C (ω helix) were approximately the same for all copolyaspartates, as shown in Table VI. These facts suggest that stacks between the benzyl and butyl groups can be formed in the ω helix and that conformation of a side chain in the butyl-L-aspartate takes on a somewhat extended form. This explanation may probably be extended to the co-crystallization or isomorphous replacement observed in copolyamide. Edgar and Hill¹⁷ noted, from calculations based on normal bond lengths and angles, that the distance between the carboxyl groups in terephthalic and adipic acid is almost the same. This was observed for the crystalline part of copoly(hexamethylene adipamideterephthalamide). Thus, it is reasonable to suggest that stacks between the benzyl and butyl groups take place in the ω helix.

The films annealed at 210°C transformed into the β form with spacings of 16.6–17.1 Å and 4.6–4.7 Å, as shown in Tables VI and VII. The former

is considered to correspond to the intersheet spacing of the β form and the latter, to the backbone spacing in the sheet.

Dynamic Viscoelastic Behavior

The dynamic modulus E' and $\tan \delta$ at 110 Hz are shown as functions of temperature in Figure 1. Viscoelastic behavior may be classified into two patterns, one for Bu-1, 2 and the other for Bu-3–6. These two patterns correspond to the different respective transformations from a left handed α helix to a left handed ω helix in Bu-1, 2 and from a right handed α helix to a left handed ω helix in Bu-3–6 as described in the section of IR absorption spectroscopy and X-ray diffraction analyses.

The dynamic modulus of Bu-1, 2 in room temperature region was lowered to some extent by butylation and increased to the modulus value of the ω helix of PBLAsp above *ca.* 100°C, the α - ω transition temperature. A steep decrease based on the transformation to the β form was observed near 200°C. The $\tan \delta$ dispersion near 60°C is considered to result from side chain motion.¹⁸

For Bu-3–6, remarkable changes in E' and $\tan \delta$, corresponding the α - ω transition accompanied by a reversal from a right handed α helix to left handed ω helix, were observed near 130°C. The change in E' was probably due to opening of hydrogen bonds and then refolding to the opposite helix accompanied by the formation of the ω helix.

Figure 1. Dynamic modulus E' and $\tan \delta$ at 110 Hz as functions of temperature for PBLAsp and Bu-1—6: —, PBLAsp; ---, Bu-1; ·····, Bu-2; — · —, Bu-3; ·····, Bu-4; — · —, Bu-5; —, Bu-6.

Figure 2 shows a dynamic modulus and $\tan \delta$ for Bu-2, 5 of the as-cast films and films annealed at 140°C and 210°C. By annealing at 140°C, the ω helix was formed and no dispersion was observed up to 140°C in either Bu-2 or Bu-5. It was thus confirmed that the α - ω transition is irreversible and that the change in E' near 100°C with the $\tan \delta$ dispersion near 60°C for Bu-2, and the change in E' near 130°C for Bu-5 observed for the as-cast films are due to the α - ω transition with the same helix sense and a reversal in helix sense, respectively. The β form was prepared by annealing at 210°C. The dynamic modulus of the β form is much smaller than those of the α and ω helices. Both Bu-2 and Bu-5 show the $\tan \delta$ dispersion near 120°C, corresponding possibly to the side chain motion of the β form. The ω - β transformation was also found to be irreversible by an annealing experiment.

Thermal Analysis

The DTA thermograms of all copoly(BuLAsp-

BeLAsp) are shown in Figure 3. The exothermic peak (the α - ω transition) and the endothermic peak (the ω - β transformation) appeared for all samples. The number in the thermogram is the transition temperature. The thermograms were also classified into the two patterns described above, one for Bu-1, 2 and the other for Bu-3—6.

The exothermic peak of Bu-1, 2 shifts from the transition temperature of PBLAsp, 127.3°C to *ca.* 100°C with a small degree of butylation. These thermal results correspond to the viscoelastic behavior of Bu-1, 2.

The exothermic peak of Bu-3—6 appeared at nearly the same temperature as that of PBLAsp and became considerably sharper and larger compared with the peak of PBLAsp given in Table VIII. This fact along with the IR absorption spectroscopy and X-ray diffraction analysis may be an adequate basis for concluding that this large sharp peak is ascribable to the transition from a right handed α helix to a left handed ω helix. Kyotani and Kanetsuna have

Figure 2. Dynamic modulus E' and $\tan \delta$ at 110 Hz as functions of temperature for Bu-2 and Bu-5. As cast film: ———, Bu-2; ———, Bu-5. 140°C annealing: - - - -, Bu-2; - - - -, Bu-5; 210°C annealing: ·····, Bu-2; ·····, Bu-5.

Figure 3. DTA thermograms for PBLAsp and Bu-1—6. The numbers in the figure are the transition temperatures.

Table VIII. The α - ω transition enthalpy

	$\Delta H/\text{kcal mol}^{-1}$
PBLAsp	0.18
Bu-1	0.28
Bu-2	0.32
Bu-3	1.46
Bu-4	1.44
Bu-5	1.28
Bu-6	0.90

studied the relation of the α - ω - β transition with molecular weight⁶ and found that cast films with a molecular weight of 25×10^4 show the left handed α helix-left handed ω helix- β form transition and cast films with a molecular weight of 3,300 show the right handed α helix-left handed ω helix- β form transition. The α - ω transition of low molecular weight cast film involves a reversal of the helix sense and exhibits an exothermic peak larger than that of high molecular weight cast film. Our results agree well with those of these authors in regard to helix sense. It is reasonable to suggest that in Bu-3—6, the reversal from a right handed to a left handed helix causes the exothermic peak to be sharp and large. Therefore, the following three mechanisms may possibly occur simultaneously near 130°C for Bu-3—6: the α helix to ω helix transition, the chain packing transformation from hexagonal to tetragonal packing, and the reversal from a right handed to a left handed helix. The enthalpy of the α - ω transition obtained by the DSC (Table VIII) becomes smaller with increasing butylation degree for Bu-3—6. This fact suggests that the stacking energy between butyl groups is smaller than that between benzyl groups.

The endothermic peak accompanied by a ω - β transformation, appeared for all copoly(BuLAsp-BeLAsp). The ω - β transformation may be classified into two patterns, one for Bu-1, 2 and the other for

Bu-3—6. The former transformation temperature is about 10 degree higher than the latter. Similarly, two types of dynamic viscoelastic behavior may be designated, although the transformation temperatures are different. These results suggest that the ω helix which does not undergo a reversal in helix sense is much more stable than that which does so.

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