Helix sense of copoly(ethyl-L-aspartate-benzyl-L-aspartate)

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Copoly(ethyl-L-aspartate-benzyl-L-aspartate)s with various compositions were prepared by ethylation of poly(β-benzyl-L-aspartate). Helix sense of copolyaspartates in chloroform solution was examined by optical rotatory dispersion and circular dichroism methods as a function of a degree of ethylation and temperature. Copolyaspartates of ethylation less than 35% exhibited the same left handed helix as that of poly(β-benzyl-L-aspartate), while copolymer of ethylation more than 70% assumed the right handed α-helical conformation like poly(β-ethyl-L-aspartate). Copolymers with the intermediate ethyl content of 35–70% indicated the reversal in helix sense from a right handed to a left handed helix with increasing temperature from −20° to 60°C. The transition of reversal was reversibly observed and transition temperature rose with the increase of ethylation. In these intermediate copolymers, the mechanism of the reversal in helix sense was examined by circular dichroism and dielectric measurements. Three possibilities were postulated here for the conformation which should arise at the transition temperature of the reversal; First is the random conformation, second is the equimolar mixture of left and right handed α-helices in a chain, and the third is the equimolar mixture of left and right handed α helix chains. No observational circular dichroism spectra characteristic to random coil conformation and no remarkable change in residue dipole moment strongly suggested the third possibility.

(Keywords: left handed α helix; right handed α helix; helix sense; optical rotatory dispersion; circular dichroism; dipole moment; copolyaspartate)

INTRODUCTION

A left handed α helix is stable in solution and in a solid state of poly(β-benzyl-L-aspartate) (PBLAsp), in contrast to more stable right handed α helix of poly-L-peptide[1]. On the other hand, poly(β-ethyl-L-aspartate) (PELAsp) is in a right handed α helix conformation, although theoretical conformational analysis predicts a left handed helix sense[2]. Copoly(ethyl-L-aspartate-benzyl-L-aspartate) (copoly(ELAsp-BLAsp)) which is composed of an opposite character of helix sense changes the helix sense from a left handed α helix to a right handed α helix in solution and in a solid state with ethylation[3,4]. Bradbury *et al.* reported from optical rotatory dispersion (o.r.d.) studies that the right handed α helix of copoly(ELAsp–BLAsp) with a degree of ethylation, 49%, transformed into the left handed α helix with increasing temperature[3]. It is of interest to investigate in more detail the temperature dependence of the helix sense of copoly(ELAsp–BLAsp) with various compositions and to obtain an information on its structure in the vicinity of the transition of helix sense.

In the present paper, the helix sense of copoly(ELAsp–BLAsp) was examined by o.r.d. and circular dichroism (c.d.) method as a function of ethylation and temperature. The temperature dependence of dipole moment for copoly(ELAsp–BLAsp)s capable of the transition of the helix sense was also measured by a dielectric dispersion and its structure on the transition was discussed.

EXPERIMENTAL

Materials

N-carboxy-β-benzyl-L-aspartate anhydride (NCA) was synthesized by Leuch's method[5]. The well purified NCA was polymerized in 5% (g cm^{-3}) ethylene dichloride solution for about one week, using triethylamine as the initiator. The PBLAsp thus obtained was a viscosity average molecular weight of 2×10^4, using the viscosity molecular weight relation[6]. Seven copoly(ELAsp–BLAsp)s including PBLAsp were prepared by ethylation of PBLAsp following the method reported by Bradbury *et al.*[3] Copoly(butyl-L-glutamate-benzyl-L-glutamate) synthesized by basically the same procedure was concluded to be a random copolymer because of a fairly good agreement of the side chain dispersion temperature of the copolymer with the Gordon–Taylor equation[7]. Furthermore, a relatively narrow side chain dispersion and single dispersion suggested that the copolymer be not block type but random[7]. From these results, copoly(ELAsp–BLAsp) is also considered to be a random copolymer. The degree of ethylation was evaluated from the n.m.r. spectra of the benzyl and ethyl groups in the side chain of copoly(ELAsp–BLAsp). The viscosity average molecular weight of copoly(ELAsp–BLAsp)s was calculated using the degree of ethylation and assuming the same degree of polymerization as PBLAsp.

Measurements

Optical rotatory dispersion measurement was carried out on an automatic spectropolarimeter (ORD/UV-5 manufactured by Japan Spectroscopic Co., Ltd.) at the temperature range from −20° to 60°C and at the wavelength range from 600 to 300 nm. A rectangular quartz cell surrounded by water jacket of a circulating constant temperature was used and the concentration of chloroform solution was 1% (g cm^{-3}).

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Circular dichroism study was performed by an automatic recording spectropolarimeter (J-40 manufactured by Japan Spectroscopic Co., Ltd.) at the temperature range from 25° to 55°C and the wavelength range from 300 to 215 nm. In this experiment, a cylindrical quartz cell of 0.1 mm thick with the water jacket was used.

Dielectric measurements (dielectric constant ε' and dielectric loss ε'') were performed with a mutual inductance bridge (TR-IC), a detector (BDA-IB) and an oscillator (WBG-3G) manufactured by Ando Electric Co., Ltd. A conventional liquid electrode cell with capacity 17.8pF was used. Measurements were carried out in the temperature range from −30° to 50°C and in the frequency range from 30 Hz to 1 MHz. Perfectly solubilized solution by reflux method was prepared for a dielectric measurement. Concentration of solution was $1.48\times10^{-1}\%$ (g cm^{-3}) for PBLAsp and $6.54\times10^{-2}\%$ (g cm^{-3}) for copoly(ELAsp–BLAsp). ε' was obtained after a correction of the capacitance of the leads of cell. In order to get the true ε'' dc conductance due to impurities in solution have to be known. In this study we roughly approximated it to be lower frequency conductance which can be measured. This method provides qualitative discussion on dipole moment of copoly(ELAsp–BLAsp).

RESULTS AND DISCUSSION

Reduced mean residue rotation $[R]$ of copolyaspartate measured over the wavelength λ from 600 to 300 nm was applied to Moffitt's equation[8]. Typical Moffitt plots of $[R](\lambda^2-\lambda_0^2)/\lambda_0^2$ against $\lambda_0^2/(\lambda^2-\lambda_0^2)$ at various temperatures were carried out for PBLAsp and copoly(ELAsp–BLAsp)s. Moffitt parameter b_0 at 25°C was shown as a function of ethylation of copoly(ELAsp–BLAsp) in *Figure 1*. The sign of Moffitt parameter b_0 represents helix sense, that is, positive or negative b_0 means a left or right handedness of helix. The absolute value of b_0 was used to determine the helical content. One can see the transition region from 30 to 70% ethylation at 25°C. The transition region observed here is slightly higher than the result reported by Bradbury *et al.*[3] which may be caused by the difference in molecular weight and/or its distribution of copoly(ELAsp–BLAsp). It was also found that transition region shifted toward higher ethylation range as temperature increased.

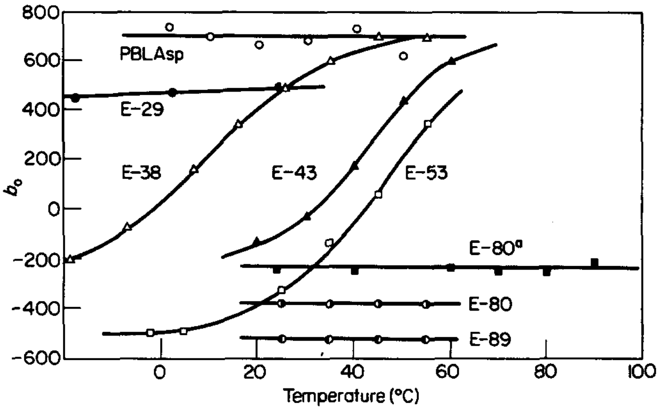

Figure 2 The temperature dependence of Moffitt parameter b_0 for copoly(ethyl-L-aspartate-benzyl-L-aspartate): ○: PBLAsp, ●:E-29 copoly(ELAsp–BLAsp) of 29% ethylation, △:E-38 copoly(ELAsp–BLAsp) of 38% ethylation, ▲:E-43 copoly(ELAsp–BLAsp) of 43% ethylation, □:E-53 copoly(ELAsp–BLAsp) of 53% ethylation, ◑:E-80 copoly(ELAsp–BLAsp) of 80% ethylation, ■:E-80ª copoly(ELAsp–BLAsp) of 80% ethylation (solvent is *m*-cresol, in another system chloroform was used), and ◐:E-89 copoly(ELAsp–BLAsp) of 89% ethylation

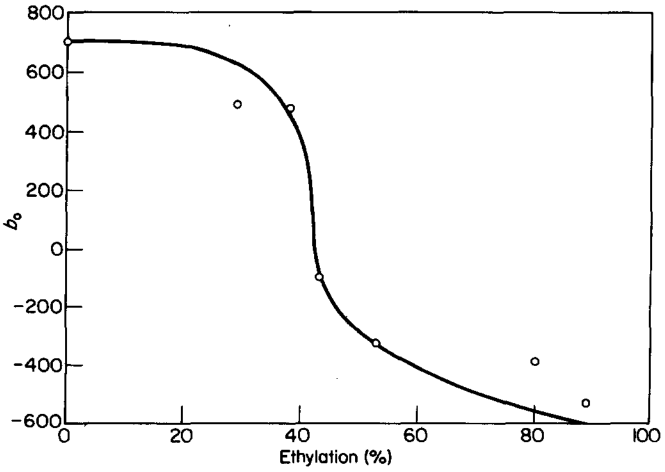

Figure 1 Relation between Moffitt parameter b_0 at 25°C and the degree of ethylation

Figure 2 shows the temperature dependence of Moffitt parameter b_0. Moffitt parameter b_0 of PBLAsp was +700 over the entire temperature range, indicative of helix stability against temperature, although it was much larger than the reference value of +630. The b_0 of 480 for copoly(ELAsp–BLAsp) of 29% ethylation indicates relatively unstable left handed α helix, since the 29% ethyl groups of intrinsically right handedness are incorporated into the left handed α helix consisting of benzyl groups. In this copolymer, no reversal of helix sense has been observed. Copolyaspartates of 38, 43 and 53% ethylation became a right handed α helix at lower temperature range. When they were heated up to higher temperature, the transition of the helix sense from a right to left handedness occurred. The transition point where Moffitt parameter b_0 equals zero, shifted to higher temperature with increasing ethylation. It can be said that the b_0 of copoly(ELAsp–BLAsp) of 38, 43 and 53% closes to that of PBLAsp at higher temperature and the left handed α helix of these copoly(ELAsp–BLAsp)s is almost the same helical content as PBLAsp. Copoly(ELAsp–BLAsp) of 80 and 89% ethylation was in a right handed α helix over the whole temperature range. So far as one uses chloroform as helix solvent, temperatures measured are limited to the boiling point of chloroform, 61°C, and the helix sense above 61°C is not clear. In order to get an information about helix sense above 61°C the o.r.d. measurement was performed for copoly(ELAsp–BLAsp) of 80% ethylation in *m*-cresol solvent. There was no temperature dependence of b_0 up to 100°C and then no reversal in helix sense was confirmed. However, the right handed α helix in *m*-cresol is more unstable than that in chloroform, reflecting a possible formation of hydrogen bond between OH group of *m*-cresol and peptide backbones. As a whole, the right handed α helix of copoly(ELAsp–BLAsp)s is likely to be unstable, when compared to a reference value of −630 for a usual right handed α helix, e.g., poly(γ-benzyl-L-glutamate)[9].

C.d. spectra of copoly(ELAsp–BLAsp) of 53% ethylation is shown in *Figure 3*. One can see c.d. peak and trough at 222 nm which is characteristic to a left handed α helix and right handed α helix, respectively. C.d. spectra changed gradually from trough to peak with increasing temperature. Almost the zero ellipticity appeared at about 45°C, corresponding to the transition point ($b_0 = 0$). In the preceding section we could observe Moffitt parameter $b_0 = 0$ for copoly(ELAsp–BLAsp) of 38, 43 and 53% ethylation. One cannot decide the structure corresponding to zero ellipticity and/or $b_0 = 0$ only by c.d. and o.r.d. method. Three conformations are predictable in the neighbourhood of the transition point: (1) random coil, (2) equimolar mixture of left and right handed α helices in a chain, and (3) equimolar mixture of left and right handed α helix chains. Thereafter each case will be discussed. C.d. spectra of copoly(ELAsp–BLAsp) of 53% ethylation have no characteristic peak around 215 nm which is assigned to the random coil conformation of poly-L-glutamic acid and poly-L-lysine[10]. Furthermore we could not detect the characteristic n.m.r. spectra corresponding to random coil conformation but observed spectra due to α helix conformation, broad backbone and end side chain proton peaks[11]. Therefore the existence of a random coil conformation at the transition point may be denied from c.d. at 45°C in *Figure 3* and n.m.r. spectra. It may be concluded that copoly(ELAsp–BLAsp) at the transition point is not in random coil conformation but in α helix conformation common to the latter two conformations.

The temperature dependence of dipole moment of copoly(ELAsp–BLAsp) of 0, 38 and 43% ethylation was measured by a dielectric dispersion method in order to know which of the latter two conformations are proper at the transition point. Dielectric constants ε_s and ε_∞ at zero frequency and infinite frequency, respectively, have to be determined to get the dipole moment of copoly(ELAsp–BLAsp) in dilute solution, which are evaluated by Cole–Cole plot method. Dielectric constants ε_s and ε_∞ obtained here were applied to Applequist–Mahr equation (1)[11]:

$$\overline{\mu_2^2} = \frac{3kMT}{4\pi NaCfg}\left(\frac{(2\varepsilon_s+1)(\varepsilon_s-n^2)}{2\varepsilon_s+n^2} - \frac{(2\varepsilon_\infty+1)(\varepsilon_\infty-n^2)}{2\varepsilon_\infty+n^2}\right) \quad (1)$$

$$f = \frac{(2\varepsilon_s+1)[1+A(n_2^2-1)]}{3[\varepsilon_s+A(n_2^2-\varepsilon_s)]} \quad (2)$$

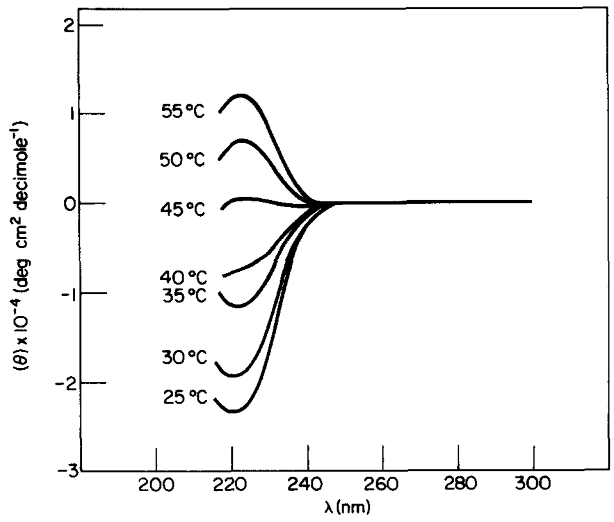

Figure 3 Circular dichroism spectra of copoly(ethyl-L-aspartate-benzyl-L-aspartate) of 53% ethylation at various temperatures

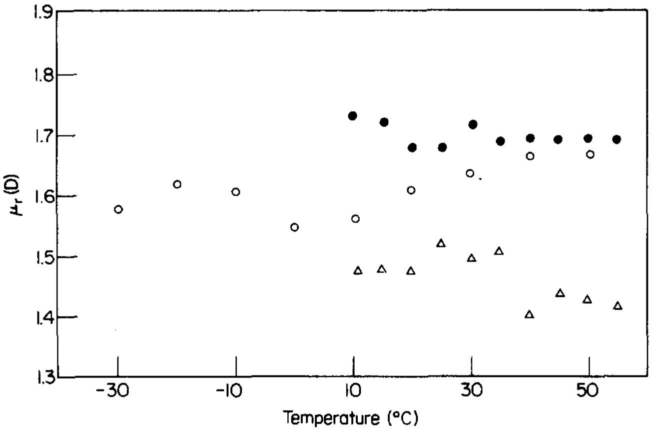

Figure 4 Temperature dependence of residue dipole moment (μ_r) evaluated by Applequist–Mahr equation; ●:PBLAsp, ○:copoly(ELAsp–BLAsp) of 38% ethylation, and △:copoly(ELAsp–BLAsp) of 43% ethylation

$$g = \frac{\varepsilon_s[1+A(n_2^2-\varepsilon_s)]}{\varepsilon_s+A(n_2^2-\varepsilon_s)} \quad (3)$$

where μ_2 is dipole moment, k is Boltzman constant, M is solute molecular weight, T is temperature Na is Avogadro Number, C is concentration (g cm^{-3}), n is index of solvent (CHCl$_3$), n_2 is index of solute and A is a shape factor of solute. In this study on copoly(ELAsp–BLAsp) including PBLAsp, A equals to 0 and then $g = 1$.

Residue dipole moment μ_r evaluated by Applequist–Mahr equation (1) is plotted against temperature in *Figure 4* for PBLAsp and copoly(ELAsp–BLAsp) of 38 and 43% ethylation, which causes the thermal transition in helix sense. Although dipole moment contains a certain error due to an ambiguous determination of dielectric constant ε_s and ε_∞ in the Cole–Cole plot, a relative dipole moment to that of PBLAsp and temperature dependence have a certain meaning when we discuss the structure of copoly(ELAsp–BLAsp) at the transition point. The residue dipole moment of copoly(ELAsp–BLAsp) of 38 and 43% ethylation is not remarkably small but slightly smaller than that of PBLAsp α helix. Temperature independence of residue dipole moment is very similar and there is no remarkable change in the dipole moment around the transition temperature. Existence of random coil conformation also could not be insisted on the thermal transition of the helix sense from these facts. If one assumes a broken α helix consisting of mixture of left and right handed α helices in a chain (conformation (2) mentioned above), a considerable decrease of the residue dipole moment will be expected, but there was, in fact, the slight decrease in the residue dipole moment. Therefore, it is likely that there is no probable formation of such a broken helix. In addition, temperature independence of the residue dipole moment of copoly(ELAsp–BLAsp) of 38 and 43% ethylation is able to deny the formation of mixture of left and right handed α helices in a free chain. Since copoly(ELAsp–BLAsp) is not a block copolymer but a random one as described above, each helix sense in a free chain does not exist in a block type, but either helix sense is expected to be formed as a whole in a chain. Therefore, conformation (2) is not probable from the dielectric study and polymer characterization. Finally, these results might lead to the conclusion of conformation (3), that is, equimolar mixture of left and right handed α helix chains.

CONCLUSION

Copoly(ELAsp–BLAsp), consisting of ethyl-L-aspartate with right handed nature and of benzyl-L-aspartate with left handed nature, is less stable than each homopolyaspartate, PELAsp and PBLAsp. Copoly(ELAsp–BLAsp) of lower ethylation less than 35% and higher ethylation more than 70% is in left and right handed α helices, respectively, over the entire temperature range. On the other hand, copoly(ELAsp–BLAsp) of ethylation from 35 to 70% transforms reversibly from right to left handed α helix with temperature. It might be concluded that conformation at the transition temperature was an equimolar mixture of left and right handed α helix chains.

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