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Reversal in helix sense of copoly(β-alkyl-L-aspartate-β-benzyl-L-aspartate)

Yoshiharu Tsujita

Department of Materials Science and Engineering, Polymeric Materials Course, Nagoya Institute of Technology,
Gokiso, Showa-ku, Nagoya 466, Japan

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The conformation of copoly(β-alkyl-L-aspartate-β-benzyl-L-aspartate), in which the alkyl group is ethyl, propyl, butyl, hexyl, nonyl, dodecyl, or stearyl, was studied in solution and the solid state by optical rotatory dispersion and circular dichroism methods. The helix sense of the copolyaspartate studied here is transformed from a left-handed to right-handed α-helix as the degree of alkylation increases. Reversal in helix sense occurs, i.e., the left-handed α-helix based on the handedness of poly(β-benzyl-L-aspartate) is transformed into a right-handed α-helix with increase in alkyl groups with right-handed nature. Reversal in helix sense is also observed for copolyaspartates with an intermediate or high degree of alkylation as temperature rises. Copolyaspartates with hexyl, nonyl, or dodecyl groups exhibit an induced circular dichroism around 230–238 nm and can form an ordered side chain structure which is broken down at high temperature. One has to consider the conformation of the ω-helix and β-form of the copolyaspartates in the solid state in addition to the reversal in helix sense. Copolyaspartates with a low degree of alkylation are in the α-helical conformation over the low temperature range and adopt the ω-helical conformation in the high temperature range, indicative of a thermal α-ω transition. A small number of alkyl groups can be incorporated into the benzene ring stacking of the ω-helix, but not a large number. All the copolyaspartates can assume the β-form at high temperatures. The helix conformation is not significantly affected by the formation of side chain crystals of the copolyaspartate with a large number of stearyl groups, in contrast to copolyglutamate.

1. Introduction

It is well known that polyaspartates adopt an unstable α-helix conformation, since their peptide groups cannot form perfect hydrogen bonds among neighboring peptide residues along the α-helices, due to competitive hydrogen bonding between NH groups in the main chain and CO groups in the side chain [1]. A conformational analysis of polyaspartates carried out by Scheraga et al. [2,3] led to the conclusion that the α-helix of polyaspartates was less stable compared to other polypeptides such as polyglutamates and polyleucines. Bradbury et al. [4,5] have reported the α-helix sense of copoly(β-alkyl-L-aspartate-β-benzyl-L-aspartate) determined by the optical rotatory dispersion (ORD) method. They found that the methyl group in the side chain of copolyaspartates favored the left-handed α-helix and longer ethyl, propyl, and butyl groups formed the right-handed α-helix. Our group [6–14] has also studied the helix sense of copolyaspartates composed of short or long alkyl and benzyl group side chains by means of circular dichroism (CD). In addition to the results of Bradbury et al. [4,5], it was found that alkyl side chains such as hexyl, nonyl, dodecyl, and stearyl groups give rise to the character of the right-handed α-helix, in contrast to the left-handed α-helix of poly(β-methyl-L-aspartate) and poly(β-benzyl-L-aspartate) (PBLAsp) [13,14]. Moreover, alkyl side chains of intermediate length, e.g., hexyl, nonyl, and dodecyl groups, could form a relatively ordered side chain structure as indicated by an induced CD trough around 230–238

Correspondence address: Y. Tsujita, Department of Materials Science and Engineering, Polymeric Materials Course, Nagoya Institute of Technology, Gokiso, Showa-ku, Nagoya 466, Japan.

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nm [13]. Copolyaspartates of the longer alkyl side chain of the stearyl group, however, cannot form an ordered side chain structure.

A left-handed solid PBLAsp α-helix is well known to be transformed into a left-handed ω-helix by heat treatment up to approx. 140 °C, reflecting an unstable helix [15–19]. The ω-helix of PBLAsp is characterized by benzene ring stacking in the side chain region. The introduction of an alkyl-L-aspartate residue into the benzyl-L-aspartate residue of PBLAsp generally prevents formation of the ω-helix. However, copoly(β-butyl-L-aspartate-β-benzyl-L-aspartate) (copoly(BuLAsp-BLAsp)) can form the ω-helix, incorporating the butyl-L-aspartate residue into the benzyl-L-aspartate residue as a result of the almost equal size of both residues [6]. Copolyaspartates including PBLAsp can be transformed into β-form at high temperatures, around 200 °C, due to intermolecular hydrogen bonding [6,7,9,14].

Thus, the helix sense and helix stability of copolyaspartates vary considerably depending on the degree of alkylation and temperature. Here, conformational behavior such as the helix sense and helix stability of copolyaspartates are summarized both in solution and in the solid state.

2. Experimental

N-Carboxy-β-benzyl-L-aspartate anhydride, synthesized according to the method of Leuch [20], was polymerized to obtain the parent polyaspartate, PBLAsp, in 5% (g cm^{-3}) ethylene dichloride solution for about 1 week, using tetraethylamine as the initiator. The copolyaspartates studied here were prepared by alkylation of PBLAsp using ethyl, propyl, butyl, hexyl, nonyl, dodecyl, or stearyl alcohols of special grade. The copolyaspartates synthesized were purified thoroughly by conventional methods. Purification of copoly(β-stearyl-L-aspartate-β-benzyl-L-aspartate) (copoly(SLAsp-BLAsp)) was performed by several steps of precipitation from chloroform solution into methanol and then by soxhlet extraction with methanol for about 1 week to remove unreacted stearyl alcohol. The degree of alkylation was controlled by varying the reaction time at constant molar ratio of reactants and was determined by NMR or elemental analysis.

Chloroform solutions of concentration 1% (g cm^{-3}) and 0.2–0.5 μm thick solid films were used for measurements. ORD and CD measurements of solution were carried out at 20 °C in the wavelength range 600–300 nm with an automatic spectropolarimeter (ORD/UV-5, Japan Spectroscopic) and over the temperature range −20 to 60 °C in the wavelength range 260–219 nm by an automatic recording spectropolarimeter with a data processor (J-40C, Japan Spectroscopic), respectively. A cylindrical quartz cell of 0.1 mm thickness surrounded by a jacket of circulating methanol was used for chloroform solutions. The CD of a thin copolyaspartate film was measured in the temperature range 20–180 °C, which was monitored by a home-made brass temperature controller. To avoid linear dichroism of solid films its effect was checked by rotation of the films in the plane perpendicular to the light beam. No rotation effect could be observed for all copolyaspartates solid films studied here.

3. Results and discussion

3.1. Copolyaspartates in solution

Optical rotation of copoly(β-ethyl-L-aspartate-β-benzyl-L-aspartate) (copoly(ELAsp-BLAsp)) and copoly(β-propyl-L-aspartate-β-benzyl-L-aspartate) (copoly(PLAsp-BLAsp)) of various compositions was measured in the wavelength range 600–300 nm [11,12]. Optical rotatory dispersion of copoly(PLAsp-BLAsp) (degree of propylation, 86%) at various temperatures is shown in fig. 1. The reduced mean residue rotation [*R*] of copoly(ELAsp-BLAsp) and copoly(PLAsp-BLAsp) was substituted in the Moffitt equation [21]. Moffitt plots of copoly(PLAsp-BLAsp) of 86% propylation at various temperatures are shown as typical examples in fig. 2 [11]. The Moffitt parameter b_0 evaluated from the slope of the Moffitt plot provides a measure of the helix sense and helical content. The Moffitt parameter b_0 at 25 °C is shown as a function of the degree of ethylation of copoly(ELAsp-BLAsp) in fig. 3 [12]. A positive

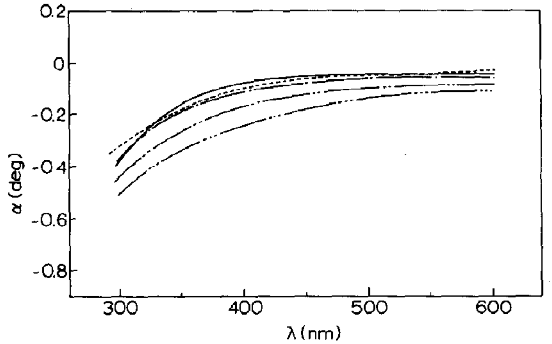

Fig. 1. Optical rotatory dispersion of copoly(PLAsp-BLAsp) of 86% propylation at various temperatures: (———) 10 °C, (------) 30 °C, (·–·–·) 40 °C, (·–··–·) 50 °C, (–···–) 55 °C.

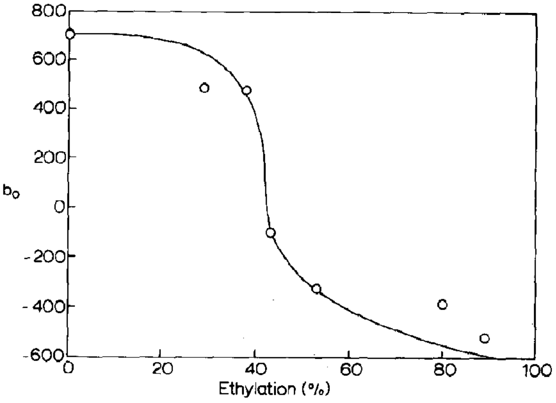

Fig. 3. The relationship between the Moffitt parameter b_0 at 25 °C and the degree of ethylation of copoly(ELAsp-BLAsp).

value for the Moffitt parameter b_0 indicates left-handedness of a helix, a negative value signifying right-handedness. The left-handed α-helix reverses the helix sense to the right-handed α-helix as the degree of ethylation increases. The helix sense of poly(ELAsp) is suggested to be right-handed, contrary to the theoretical proposition of Scheraga et al. [2]. One can observe a transition region from 30 to 70% ethylation at 25 °C. The b_0 values of the other copolyaspartates are demonstrated in fig. 4. Basically, a similar trend in the relation of b_0 and degree of alkylation is observed for all copolyaspartates except for copoly(β-nonyl-L-aspartate-β-benzyl-L-aspartate) (copoly(NoLAsp-BLAsp)) and copoly(β-dodecyl-L-aspartate-β-benzyl-L-aspartate) (copoly(DoLAsp-BLAsp)) which form an ordered side chain structure, as described later. It was found that copolyaspartates with shorter alkyl side chains cause a rather sharper reversal in helix sense than those with longer chains. Molar ellipticity $[\theta]_{\max}$ can give an estimate of the helix sense and helical content as well as the Moffitt parameter b_0. CD spectra of copoly(SLAsp-BLAsp) at 20 °C are shown in fig. 5 [14]. Molar ellipticities of the spectra exhibit a

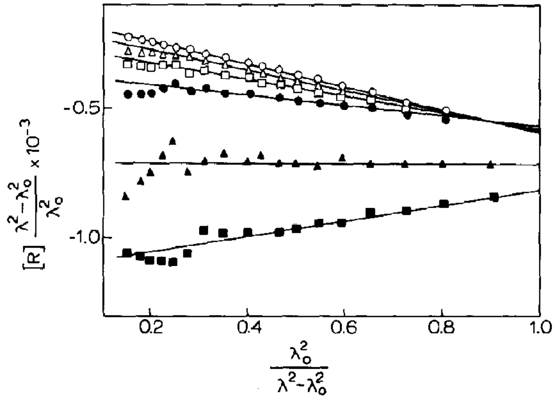

Fig. 2. Moffitt plot of copoly(PLAsp-BLAsp) of 86% propylation at various temperatures: (○) 10 °C, (△) 20 °C, (□) 30 °C, (●) 40 °C, (▲) 50 °C, (■) 55 °C.

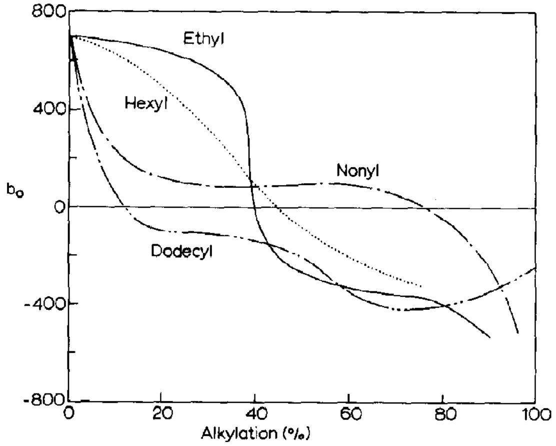

Fig. 4. The relationship between the Moffitt parameter b_0 at 25 °C and the degree of alkylation of copolyaspartates: (———) copoly(ELAsp-BLAsp), (······) copoly(HeLAsp-BLAsp), (·–·–·) copoly(NoLAsp-BLAsp), (·–··–·) copoly(DoLAsp-BLAsp).

peak or trough at 222 nm, corresponding to positive or negative b_0, indicative of a left- or right-handed α-helix, respectively. The molar ellipticity at a peak or trough, $[\theta]_{\max}$, of copolyaspartates with propyl, hexyl, and stearyl groups is plotted vs. the respective degree of alkylation in fig. 6. A right-handed α-helix is likely to be stable with increase in alkyl side chain length. These facts suggest that the competitive hydrogen bonding between the NH group in the main chain and the CO group in the side chain is weakened by an active molecular motion of the longer alkyl side chain.

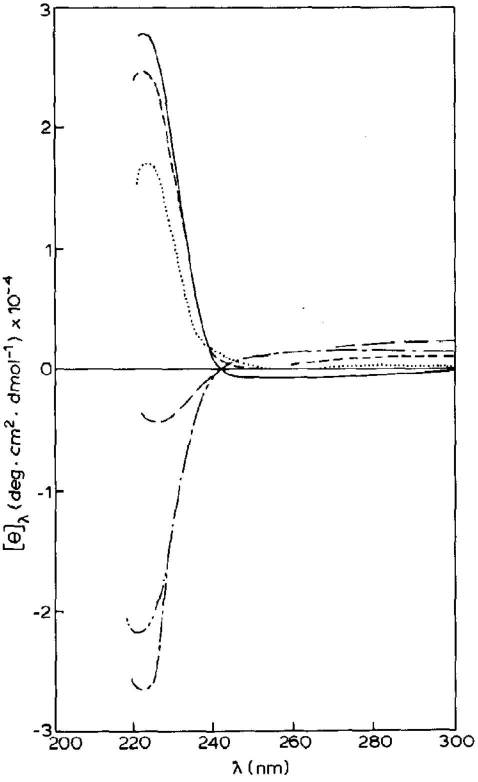

Fig. 5. CD spectra of copoly(SLAsp-BLAsp) of various degrees of stearylation in chloroform solution at 20 °C: (———) PBLAsp, (- - - - -) copoly(SLAsp-BLAsp), 8% stearylation; (· · · · · ·) copoly(SLAsp-BLAsp), 26% stearylation; (— — —) copoly(SLAsp-BLAsp), 41% stearylation; (·—··—·) copoly (SLAsp-BLAsp), 63% stearylation; (·—·—·) copoly(SLAsp-BLAsp), 72% stearylation.

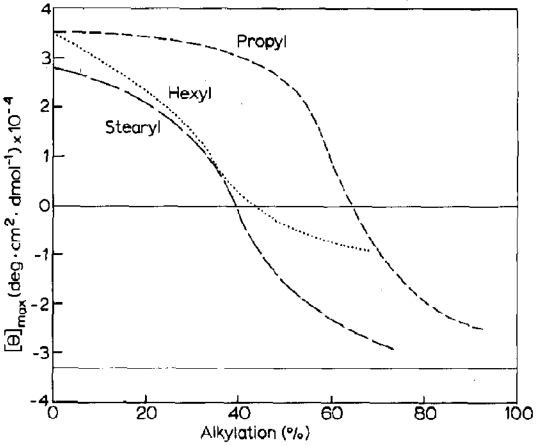

Fig. 6. Plot of molar ellipticity $[\theta]_{\max}$ vs. degree of alkylation: (— — —) copoly(PLAsp-BLAsp), (· · · · · ·) copoly(HeLAsp-BLAsp) and (———) copoly(SLAsp-BLAsp).

Next, the temperature dependence of the handedness of the α-helix of copolyaspartates was studied. The temperature dependence of the parameter b_0 of copoly(ELAsp-BLAsp) and copoly(PLAsp-BLAsp) of various compositions is illustrated in figs. 7 and 8 [11,12]. The b_0 values of copoly(ELAsp-BLAsp) samples with lower and higher degrees of ethylation do not change considerably with respect to temperature, remaining positive and negative, respectively. However, a reversal in b_0 is noted for copoly(ELAsp-BLAsp) with an intermediate degree of ethylation. A reversal in the helix sense from right- to left-handed occurs with increasing temperature. On the other hand, the temperature dependence of b_0 of copoly(PLAsp-BLAsp) with a higher degree of propylation is very similar to that of copoly(ELAsp-BLAsp) of intermediate ethylation. We confirm that these two copolyaspartates cause the reversal in helix sense by changing the degree of alkylation and temperature.

The temperature dependence of $[\theta]_{\max}$ of copolyaspartates with a rather higher degree of alkylation is shown in fig. 9. $[\theta]_{\max}$ of copolyaspartates with a lower degree of alkylation decreases very gradually with temperature similar to PBLAsp. Thus, left-handed α-helices of these copolyaspartates tend to become gradually unstable as the temperature rises. This implies destabilization of the α-helix due to the introduction of alkyl-L-aspartate with a right-handed helix into

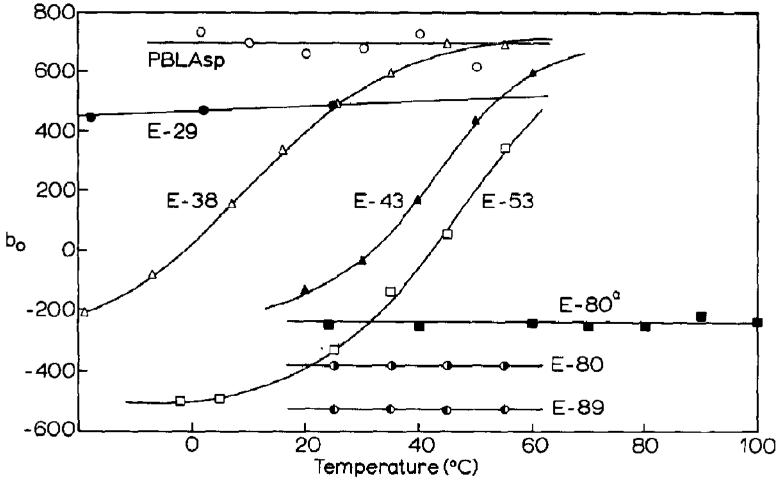

Fig. 7. Temperature dependence of the Moffitt parameter b_0 for copoly(ELAsp-BLAsp): (○) PBLAsp; (●: E-29) copoly(ELAsp-BLAsp), 29% ethylation; (△: E-38) copoly(ELAsp-BLAsp), 38% ethylation; (▲: E-43) copoly(ELAsp-BLAsp), 43% ethylation; (□: E-53) copoly(ELAsp-BLAsp), 53% ethylation; (◑: E-80) copoly(ELAsp-BLAsp), 80% ethylation; (■: E-80^a) copoly (ELAsp-BLAsp), 80% ethylation (solvent, *m*-cresol; in another system chloroform was used); (◐: E-89) copoly(ELAsp-BLAsp), 89% ethylation.

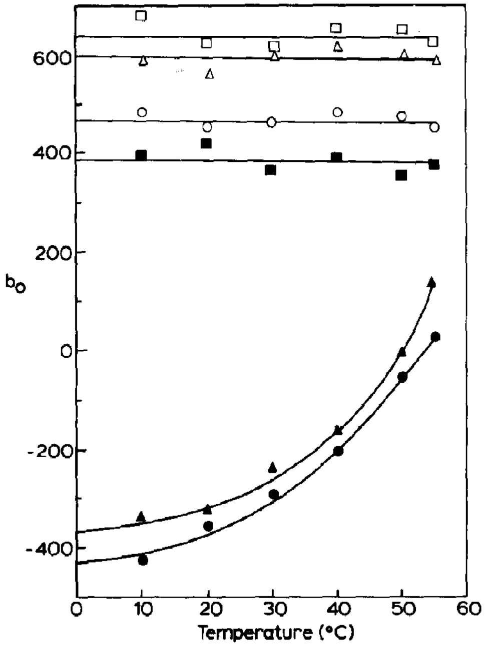

Fig. 8. Temperature dependence of the Moffitt parameter b_0 for copoly(PLAsp-BLAsp) of different degrees of propylation: (□) PBLAsp; (△) copoly(PLAsp-BLAsp), 12% propylation; (○) copoly(PLAsp-BLAsp), 41% propylation; (■) copoly(PLAsp-BLAsp), 50% propylation; (▲) copoly(PLAsp-BLAsp), 86% propylation; (●) copoly(PLAsp-BLAsp), 93% propylation.

benzyl-L-aspartate residues with a left-handed nature. On the other hand, $[\theta]_{\max}$ of copolyaspartates with a higher degree of alkylation increases

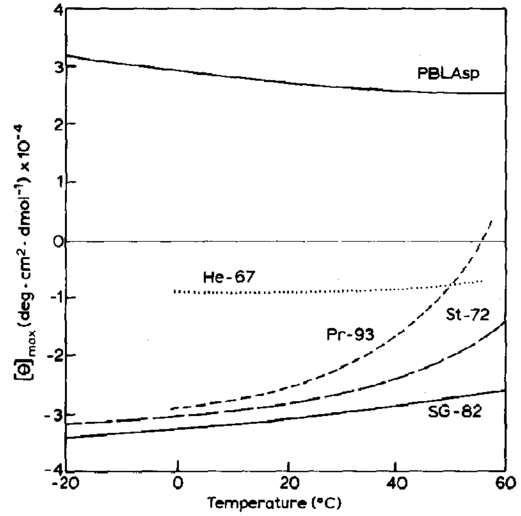

Fig. 9. Temperature dependence of molar ellipticity $[\theta]_{\max}$ of copolyaspartates: (———) PBLAsp; (— — —) copoly-(PLAsp-BLAsp), 93% propylation; (······) copoly(HeLAsp-BLAsp), 67% hexylation; (— —) copoly(SLAsp-BLAsp), 72% stearylation; (———) copoly(SG-MG), 82% stearylation.

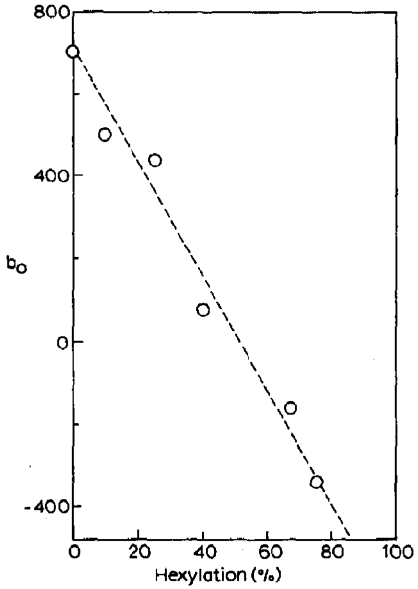

Fig. 10. Moffitt parameter b_0 of copoly(HeLAsp-BLAsp) at 20 ° C as a function of the degree of hexylation.

from negative values to zero or positive values with temperature, suggesting reversal in the helix sense. Such α-helices of the copolyaspartates are comparatively stable at low temperatures as compared to the temperature dependence of $[\theta]_{\max}$ of stable copoly(γ-stearyl-L-glutamate-γ-methyl-L-glutamate) of 82% stearylation (SG-82) (fig. 9).

The ORD and CD spectra of copoly(β-hexyl-L-aspartate-β-benzyl-L-aspartate) (copoly(HeLAsp-BLAsp), copoly(NoLAsp-BLAsp) and copoly(DoLAsp-BLAsp) exhibited peculiar behavior [13]. Fig. 10 shows plots of the Moffitt parameter b_0 vs. degree of hexylation. It is found that the left-handed α-helix is transformed into the right-handed form with increasing hexylation, although b_0 changes linearly with respect to the degree of hexylation. This behavior is slightly different from that shown in figs. 3 and 4. The CD spectra of copoly(HeLAsp-BLAsp) of 76% hexylation are abnormal and do not exhibit any trough at 222 nm which is characteristic of an α-helix as shown in fig. 11. This polymer is considered to adopt the right-handed α-helix as expected from fig. 10. The temperature dependence of $[\theta]_{\max}$ of copoly (HeLAsp-BLAsp) is shown in fig. 12. The left-handed α-helix of copoly(HeLAsp-BLAsp) of 39% hexylation is transformed into the right-handed form at approx. 40 ° C on raising the temperature, indicative of a reversal in helix sense. The temperature dependence of $[\theta]_{\max}$ of copoly(HeLAsp-BLAsp) of 67% hexylation is almost the same as that of the other copolyaspartates.

Copolyaspartates with relatively high degrees of hexylation, nonylation and dodecylation can form an additional ordered side chain structure even in solution [13]. This is confirmed by an induced CD spectrum appearing at 230–238 nm due to the ordered side chain structure. The CD spectra of copoly(NoLAsp-BLAsp) of 92% nonylation and poly(DoLAsp) at various temperatures are shown in figs. 13 and 14. A trough around 235 nm is observed up to 40 ° C, signifying that the dodecyl side chain is arranged in a right-handed helix up to 40 ° C. The induced CD disappears and the CD trough characteristic of the right-handed α-helix appears at 50 ° C. The change in the induced CD spectra of poly(DoLAsp) demonstrates a breakdown only of the ordered dodecyl side chain structure between 40 and 50 ° C. Breakdown of the

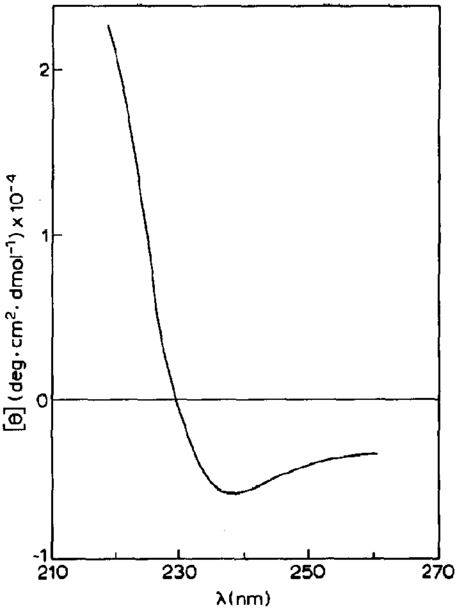

Fig. 11. CD spectrum of copoly(HeLAsp-BLAsp) of 76% hexylation at 0 ° C.

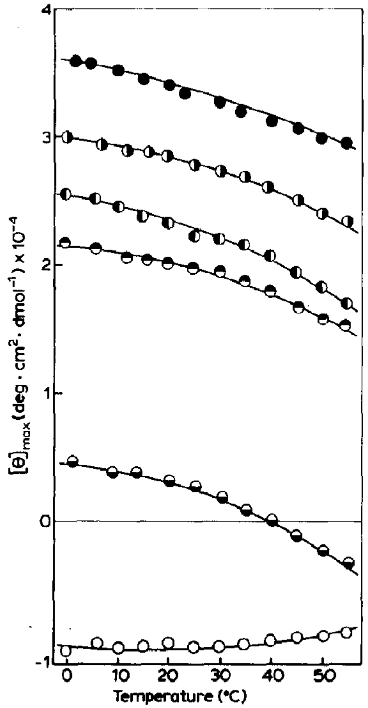

Fig. 12. Molar ellipticity of copoly(HeLAsp-BLAsp) of various degrees of hexylation at 222 nm vs. temperature: (●) PBLAsp; (◑) copoly(HeLAsp-BLAsp), 9% hexylation; (◐) copoly(HeLAsp-BLAsp), 15% hexylation; (◓) copoly(HeLAsp-BLAsp), 25% hexylation; (◒) copoly(HeLAsp-BLAsp), 39% hexylation; (○) copoly(HeLAsp-BLAsp), 67% hexylation.

ordered nonyl side chain structure does not occur at 55 °C. The intensity of the CD spectra of copoly(NoLAsp-BLAsp) of 92% nonylation decreases with temperature, indicative of a probable breakdown at higher temperature. The ability to form ordered side chain structures is greater for the nonyl than for the dodecyl side chain. This means that an adequate side chain length is necessary for formation of an ordered side chain structure attached to a helix.

3.2. Copolyaspartates in the solid state

Since solid copolyaspartates are cast from solution, as cast solid copolyaspartates, in general, retain the α-helix conformation. The helix conformation of copoly(β-butyl-L-aspartate-β-benzyl-L-aspartate) (copoly(BuLAsp-BLAsp)) in the solid state is almost the same as that in solution [6]. The dynamic viscoelastic properties, such as the dynamic modulus and tan δ, have been discussed in relation to the conformational change of copoly(BuLAsp-BLAsp) [6]. The dynamic modulus E' and tan δ at 110 Hz are plotted vs. temperature for PBLAsp and copoly(BuLAsp-BLAsp)s of 21 and 69% butylation which have left- and right-handed α-helix conformations, respectively (fig. 15). Interestingly, a butyl group

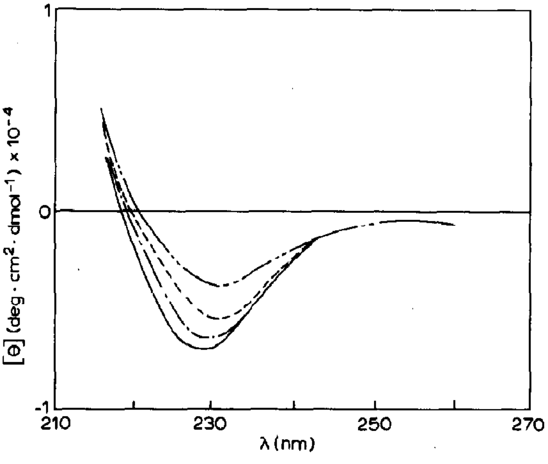

Fig. 13. CD spectra of copoly(NoLAsp-BLAsp) of 92% nonylation at various temperatures: (———) 0 °C, (·–·–·) 20 °C, (·-----·) 40 °C, (·–··–·) 55 °C.

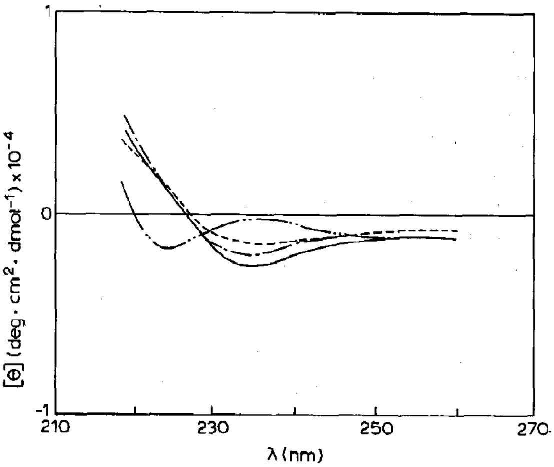

Fig. 14. CD spectra of poly(DoLAsp) at various temperatures: (———) 0 °C, (·–·–·) 20 °C, (·-----·) 40 °C, (·—··—·) 50 °C.

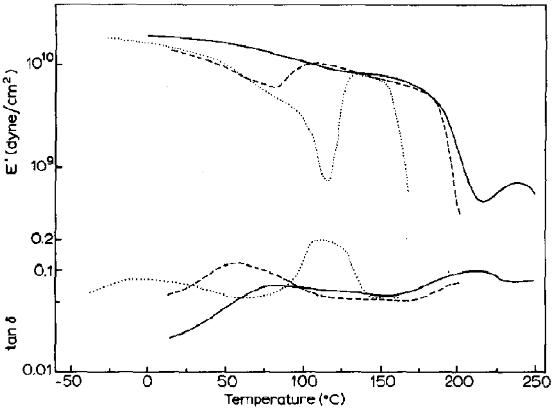

Fig. 15. Temperature dependence of dynamic modulus E' and tan δ at 110 Hz of copoly(BuLAsp-BLAsp): (———) PBLAsp; (- - - - - -) copoly(BuLAsp-BLAsp), 21% butylation; (· · · · · ·) copoly(BuLAsp-BLAsp), 69% butylation.

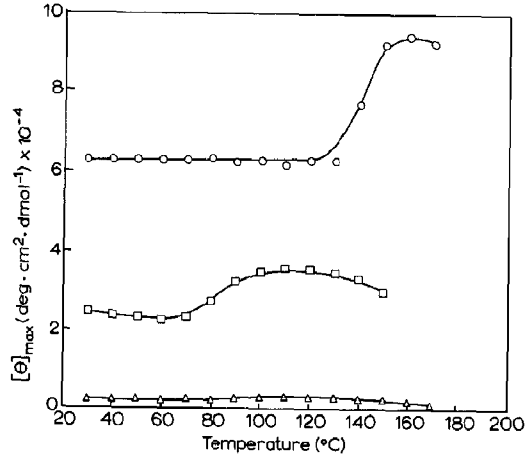

Fig. 16. Temperature dependence of $[\theta]_{max}$ of copoly(SLAsp-BLAsp): (○) PBLAsp; (□) copoly(SLAsp-BLAsp), 8% stearylation; (△) copoly(SLAsp-BLAsp), 26% stearylation.

can be incorporated into the benzene ring stacking of the ω-helix, irrespective of the degree of butylation. This means that the size of each residue is almost the same, especially butyl and benzyl groups. The modulus E' of copoly(BuLAsp-BLAsp) of 21% butylation increases to the modulus value of the ω-helix of PBLAsp above approx. 100 °C, the α-ω transition. Copoly(BuLAsp-BLAsp) of 69% butylation exhibits more complex viscoelastic behavior around the α-ω transition. Remarkable changes in E' and tan δ are observed near 130 °C, corresponding to the α-ω transition accompanied by a reversal in helix sense from right-handed α-helix to left-handed ω-helix. Furthermore, the ω-β transition can be observed with a marked drop in E'.

The temperature dependence of $[\theta]_{max}$ of solid copoly(SLAsp-BLAsp) with a lower degree of stearylation is shown in fig. 16 [14]. One can see a change in $[\theta]_{max}$ of solid copoly(SLAsp-BLAsp)s of 0 and 8% stearylation at the α-ω transition without reversal in helix sense. The temperature dependence of $[\theta]_{max}$ of solid copoly(SLAsp-BLAsp)s of 41, 63 and 72% stearylation is shown in fig. 17. The reversal in helix sense from right- to left-handed in the solid state is also observed; this reversal occurs over a narrower temperature range compared to that in solution. The stearyl side chain of these copolyaspartates can crystallize at room temperature and can melt above it, depending upon the degree of stearylation. Such melting behavior does not affect the temperature dependence of $[\theta]_{max}$, and is different from the case of copoly(γ-stearyl-L-glutamate-γ-methyl-L-gluta-

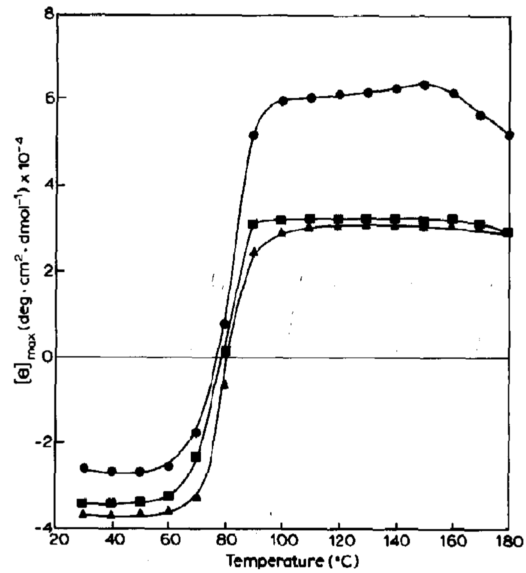

Fig. 17. Temperature dependence of $[\theta]_{max}$ of copoly(SLAsp-BLAsp) in solid film: (●) copoly(SLAsp-BLAsp), 41% stearylation; (■) copoly(SLAsp-BLAsp), 63% stearylation; (▲) copoly(SLAsp-BLAsp), 72% stearylation.

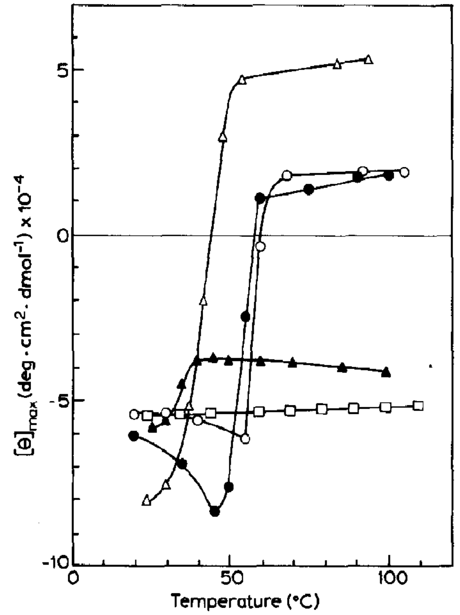

Fig. 18. Temperature dependence of molar ellipticity, $[\theta]_{max}$, of solid film of copoly(SG-MG): (□) copoly(SG-MG), 16% stearylation: (▲) copoly(SG-MG), 52% stearylation; (△) copoly(SG-MG), 67% stearylation; (●) copoly(SG-MG), 70% stearylation (using stearyl alcohol of guaranteed grade); (○) copoly(SG-MG), 82% stearylation.

mate) (copoly(SG-MG)). The temperature dependence $[\theta]_{max}$ of copoly(SG-MG) is shown in fig. 18 [22]. The drastic change in $[\theta]_{max}$ from negative to positive values with temperature occurs at the melting temperature of the stearyl side chain crystal of copoly(SG-MG). The reason for this is not clear as yet and will be further examined. The drastic change in $[\theta]_{max}$ of copoly(SLAsp-BLAsp) with a higher degree of stearylation is caused by the reversal in helix sense, not by the side chain melting as observed in copoly(SG-MG).

4. Conclusion

The conformational behavior of the copolyaspartates is summarized in fig. 19. Many kinds of helices are characteristic of the copolyaspartates due to helix instability. The reversal in helix sense of the copolyaspartates studied here is reversible in solution as the degree of alkylation and temperature increase. Copoly(HeLAsp-BLAsp), copoly(NoLAsp-BLAsp) and copoly(DoLAsp-BLAsp) with a higher degree of alkylation can give rise to an ordered side chain structure even in solution.

One must consider the left-handed ω-helix and the β-form in the solid state in addition to the α-helix in solution. The left-handed α-helix is transformed into the left-handed ω-helix without reversal in helix sense on raising the temperature for solid copolyaspartates with a low degree of alkylation, while the reversal in α-helix sense from right- to left-handed occurs for those with a high degree of alkylation with increasing temperature. No α-ω transition is observed. The reversal in helix sense from left- to right-handed α-helix occurs over the low temperature range, however, the α-ω transition without reversal in helix sense does occur over the high temperature range as the degree of alkylation increases.

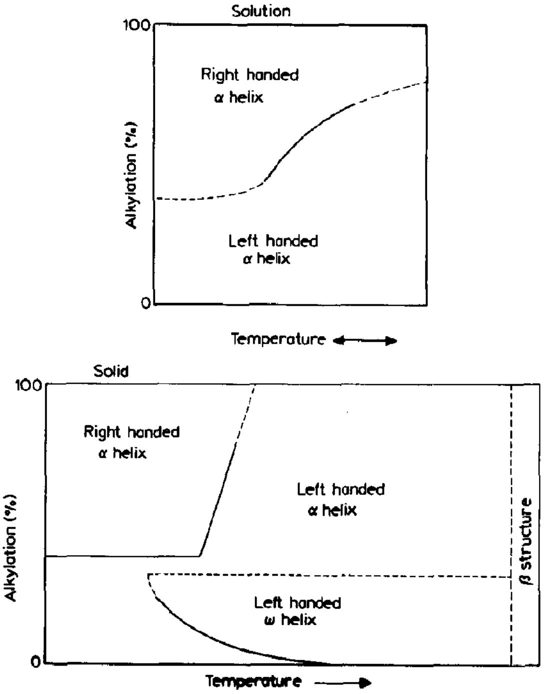

Fig. 19. Conformational behavior of copoly(β-alkyl-L-aspartate-β-benzyl-L-aspartate) in solution and in the solid state.

References

1 J.T. Yang, Protein models for conformational studies, ed. G.D. Fasmann (Marcell Dekker, New York, 1967) ch. 6, p. 249.
2 J.F. Yan, G. Vanderkooi and H.A. Scheraga, J. Chem. Phys. 49 (1968) 2713.
3 J.F. Yan, F.A. Momany and H.A. Scheraga, J. Am. Chem. Soc. 92 (1970) 2713.
4 E.M. Bradbury, B.G. Carpenter and H. Goldman, Biopolymers 6 (1968) 837.
5 E.M. Bradbury, B.G. Carpenter and R.M. Stephens, Biopolymers 6 (1968) 905.
6 Y. Tsujita, M. Fukagawa and I. Uematsu, Polym. J. 14 (1982) 773.
7 Y. Tsujita, M. Fukagawa and I. Uematsu, Polym. J. 14 (1982) 781.
8 Y. Tsujita, H. Nakahara, R. Nishizima and A. Takizawa, Sen-i Gakkaishi 39 (1983) T-475.
9 Y. Tsujita, N. Satomi and A. Takizawa, J. Macromol. Sci. Phys. B22 (1983–1984) 619.
10 Y. Tsujita, Y. Murafuji, K. Yano and A. Takizawa, J. Macromol. Sci. Phys. B23 (1984) 311.
11 Y. Tsujita, N. Fujii, Y. Imoto, A. Takizawa and T. Kinoshita, J. Polym. Sci. Polym. Chem. Ed. 22 (1984) 2533.
12 R. Nishizima, Y. Tsujita and A. Takizawa, Polymer 26 (1985) 379.
13 M. Kusakawa, Y. Tsujita and A. Takizawa, Polymer 26 (1985) 848.
14 T. Watanabe, Y. Tsujita, A. Takizawa and T. Kinoshita, Polymer 28 (1987) 1809.
15 E.M. Bradbury, L. Brown, A.R. Downie, A. Elliott, R.D.B. Fraser and W.E. Hanby, J. Mol. Biol. 5 (1962) 230.
16 D.B. Green, F. Happey and B.M. Watson, Eur. Polym. J. 6 (1970) 7.
17 H. Obata and H. Kanetsuna, J. Polym. Sci. A2, 9 (1971) 1977.
18 H. Kyotani and H. Kanetsuna, J. Polym. Sci. A2, 10 (1972) 1931.
19 Y. Tsujita, Sen-i Gakkaishi 33 (1977) P-45.
20 H. Leuchs, Berichte 39 (1906) 857.
21 W. Moffitt, J. Chem. Phys. 25 (1956) 467.
22 Y. Tsujita, R. Ojika, K. Tsuzuki, A. Takizawa and T. Kinoshita, J. Polym. Sci. Polym. Chem. Ed. 25 (1987) 1041.