Circular dichroism on copolyaspartates containing a long alkyl side chain

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Copoly(β-stearyl L-aspartate-β-benzyl L-aspartate)s (copoly(SLAsp–BLAsp)s) with various compositions were synthesized by ester exchange reaction of parent poly(β-benzyl L-aspartate) (PBLAsp). Circular dichroism (c.d.) studies were performed in chloroform solutions and for solid films as a function of degree of stearylation and temperature. The left-handed α helix of copoly(SLAsp–BLAsp) transformed to right-handed with increasing degree of stearylation at room temperature. A temperature-induced reversal of α helix sense from right to left was observed in chloroform solution of copoly(SLAsp–BLAsp) of certain composition, e.g. degree of stearylation 41 and 63%. On the other hand, two kinds of conformational behaviour were observed in solid films with respect to composition. At low degree of stearylation (0–30%), an α–ω transition occurred, while at degree higher than 40%, the α helix sense reversed from right-handed to left-handed as the temperature increased. All the conformational behaviours observed in solid films were irreversible, but they were reversible in solution.

(Keywords: α helix; helix sense; ω helix; copolyaspartates; circular dichroism; long alkyl side chain)

INTRODUCTION

It is well known that polyaspartates take an unstable α helix form, since their peptide chains cannot form perfect hydrogen bonds among neighbouring peptide residues along α helices, which is due to competitive hydrogen bonding between NH groups in the main chain and CO groups in the side chains[1].

Conformational analysis of polyaspartates calculated by Scheraga *et al.*[2,3] led to the conclusion that the α helix of copolyaspartates was less stable compared to other polypeptides. Bradbury *et al.*[4,5] studied the optical rotatory dispersion (o.r.d.) on copoly(β-alkyl L-aspartate-β-benzyl L-aspartate). They found that the methyl group in the side chain of copolyaspartate favoured the left-handed helix and longer ethyl, propyl and butyl groups formed the right-handed helix.

In our previous work[6–8], o.r.d. and c.d. studies were performed on the helix sense of copolyaspartates with various alkyl side chain lengths. It was found that copoly(β-ethyl L-aspartate–β-benzyl L-aspartate) and copoly(β-propyl L-aspartate–β-benzyl L-aspartate) changed their conformations from left- to right-handed α helix with increasing degree of alkylation. Furthermore, copolyaspartates with longer alkyl side chains such as hexyl, nonyl and dodecyl groups were studied[9]. They also changed from left- to right-handed α helix with increasing degree of alkylation. Highly alkylated copoly(β-hexyl L-aspartate–β-benzyl L-aspartate) and copoly(β-dodecyl L-aspartate–β-benzyl L-aspartate) showed a small circular dichroism trough around 230 nm. This was explained as being due to the formation of an ordered structure in the alkyl side chain region.

PBLAsp cast quickly from chloroform solution at room temperature and atmospheric pressure is in a left-handed α helix. After heating to 140°C, it takes a left-handed ω helix induced by the stacking formation of benzyl groups; and after heating up to 210°C, the β sheet structure resulting from the formation of intermolecular hydrogen bonds appears. Bradbury *et al.*[5] reported from i.r. study that introduction of methyl L-aspartate residues into PBLAsp prevents the formation of the ω helix. On the other hand, we found the formation of the ω helix in copoly(β-butyl L-aspartate–β-benzyl L-aspartate) with relatively fewer butyl groups[8]. This might be explained in terms of capable incorporation of butyl groups into the benzene stacking of the ω helix.

Helix stability of copolyglutamates, which form more rigid α helices, was also studied[10]. Copoly(γ-stearyl L-glutamate–γ-methyl L-glutamate) (copoly(SLG–MLG)) in the solid state showed a reversible change in the screw sense of the α helix with temperature. It is of interest that one could discuss not only the effect of the side-chain length but also the effect of the formation of an ordered side-chain structure of copolyglutamates.

In the present study, the conformational behaviours of copoly(β-stearyl L-aspartate–β-benzyl L-aspartate)s (copoly(SLAsp–BLAsp)s) were examined both in solution and in the solid state by c.d. as a function of degree of stearylation and temperature. The helix stability and its structure on transitions were discussed.

EXPERIMENTAL

The parent polymer, PBLAsp, was obtained by polymerization of well purified N-carboxy-β-benzyl L-aaspartate anhydride (NCA)[11] in 0.05 g cm^{-3} ethylene dichloride solution for about one week, using triethylamine as the initiator. The PBLAsp thus prepared had a viscosity-average molecular weight of 3.2×10^4 as

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calculated from the viscosity molecular weight relation of Hayashi *et al.*[12] Copoly(SLAsp–BLAsp) was prepared by stearylation of PBLAsp according to the method of Bradbury *et al.*[4] Stearyl alcohol of special grade was used in this reaction. The copolypeptides synthesized were purified thoroughly by several precipitations from chloroform solution into methanol and then by soxhlet extraction with methanol for about one week to remove unreacted stearyl alcohol. The conditions of the ester exchange reaction are shown in *Table 1*, in which degree of stearylation was controlled by varying the reaction time at constant molar ratio of reactants. The abbreviation 'SA-X' in which X represents the degree of stearylation of copoly(SLAsp–BLAsp) determined by elemental analysis, is used in the following discussion.

Copolymers were dissolved in chloroform at concentration 0.005 g cm^{-3} and subsequently the solutions were cast quickly on quartz plates (15 × 10 × 1 mm) at ambient temperature and atmospheric pressure in order to get thin solid films. The thickness of the films was adjusted from 0.2 to 0.5 μm. The film was left on the quartz plate for ease of handling. The films on the quartz plate are transparent and are likely to have no unidirectional orientation, but random planar orientation.

Circular dichroism measurements were carried out for chloroform solutions and solid films using a Jasco J-40C spectropolarimeter equipped with a J-DPZ data processor (manufactured by Japan Spectroscopic Co. Ltd) in the wavelength range 300–219 nm. As for chloroform solutions, a cylindrical quartz cell of 0.1 mm thickness surrounded by a jacket circulating methanol at constant temperature was used in the temperature range from −20 to 60°C. The c.d. of a thin polypeptide film was measured in the temperature range 30–180°C, which was controlled by a home-made brass temperature controller. To avoid linear dichroism of solid films, we checked the effect of the linear dichroism by rotation of the films in the plane perpendicular to the light beam. No rotation effect could be observed for all the copolyaspartate solid films. Well defined c.d. spectra were obtained by eight times integration using the data processor.

RESULTS AND DISCUSSION

Copolyaspartates in solution

Circular dichroism spectra of copolyaspartates with various degrees of stearylation in chloroform solution at 20°C are shown in *Figure 1*. Molar ellipticity $[\theta]$ of the spectra exhibited a peak or a trough at 222 nm, indicative of left- or right-handed α helix, respectively. The absolute value of the molar ellipticity of copolyaspartates is slightly small compared to that of the other stable polypeptide, reflecting a less stable helix of polyaspartates. The helix sense of copoly(SLAsp–BLAsp) transformed from left- to right-handed with increasing stearylation, similar to the other copolyaspartates previously studied[6–9]. The maximum molar ellipticity $[\theta]_{max}$ obtained from *Figure 1* is plotted against the degree of stearylation in *Figure 2*. The $[\theta]_{max}$ changed from positive through zero to negative, that is, the helix sense changed from left- to right-handed with increasing stearylation, as mentioned above, although the α helix itself is not perfectly stable. The transition in helix sense from left- to right-handed, at which $[\theta]_{max}$ equals zero, is estimated to occur at a degree of stearylation of ~40%. In our previous work[6–9] on copolyaspartates containing ethyl, hexyl, nonyl and dodecyl groups, the transitions of

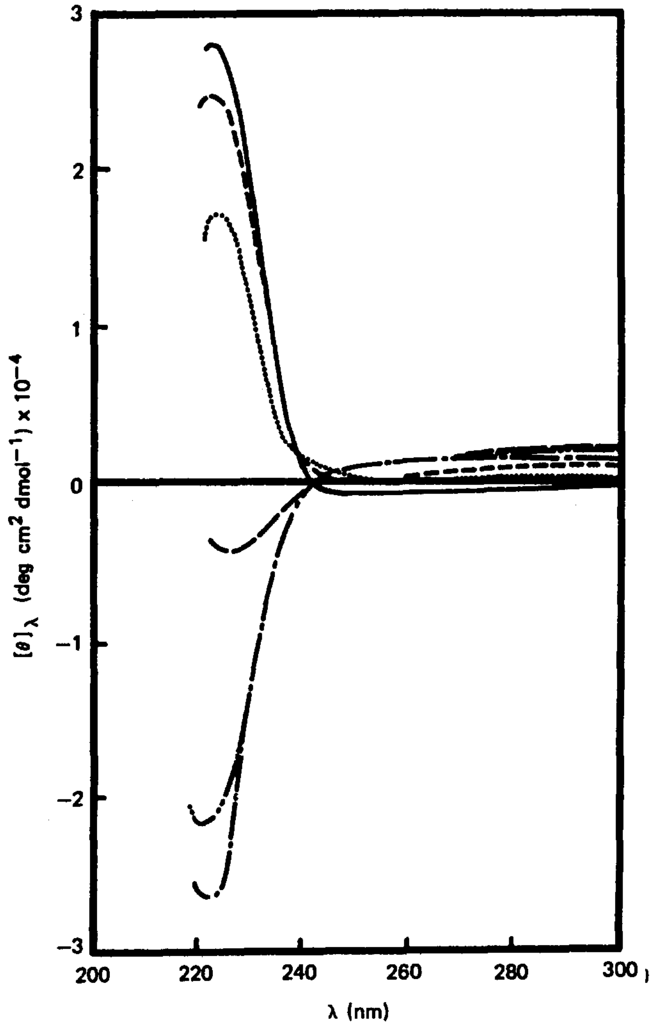

Figure 1 C.d. spectra of copoly(SLAsp–BLAsp) with various degrees of stearylation in chloroform solution at 20°C: ———, SA-0 (PBLAsp); -----, SA-8; ·····, SA-26; – – –, SA-41; —··—, SA-63; —·—·—, SA-72

Table 1 Conditions of ester exchange reaction

Sample	PBLAsp (g)	$CHCl_3$ (cm^3)	Ste-OH (g)	H_2SO_4 (cm^3)	Temp. (°C)	Time (h)	Ave. of M_w/res.
SA-0							205
SA-8	1.0	200	100	0.2	63	93	219
SA-26	1.0	200	100	0.2	63	334	248
SA-41	1.0	200	100	0.2	63	407	272
SA-63	0.5	100	50	0.1	63	504	308
SA-72	1.0	200	100	0.2	63	982	322

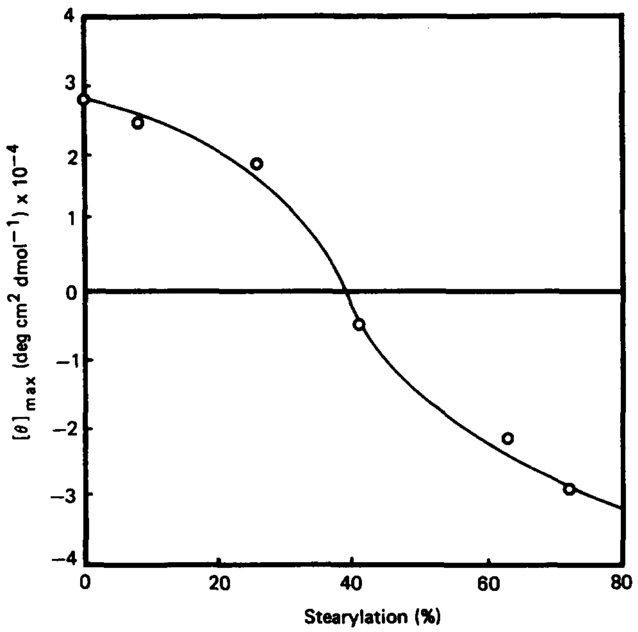

Figure 2 Plots of $[\theta]_{max}$ against degree of stearylation

helix sense from left- to right-handed in chloroform solutions at 20°C were at compositions of 40, 50, 70 and 10%, respectively. The helix stability of the latter two copolyaspartates could not be discussed, since they form an ordered structure in the side chain region as found[9] by the c.d. trough around 230 nm. One can consider that a stearyl L-aspartate residue has a similar tendency to be right-handed as have the ethyl or hexyl L-aspartates studied.

Figure 3 shows c.d. spectra of SA-41 at various temperatures. The sign of $[\theta]$ changed from negative to positive with increasing temperature, representing a reversal in the helix sense from right- to left-handed. SA-63 also showed a similar temperature dependence of the c.d. spectra. *Figure 4* shows the temperature dependences of $[\theta]_{max}$ of each copolyaspartate studied. The $[\theta]_{max}$ of copolyaspartates with a low degree of stearylation, such as SA-8 and SA-26, decreases gradually with temperature. Thus left-handed α helices of these copolyaspartates tend to become unstable as the temperature increases. This implies α helix unstabilization due to the introduction of stearyl L-aspartate residue with right-handed helix nature to benzyl L-aspartate residue with left-handed nature. SA-41 and SA-63 caused a reversal in helix sense from right- to left-handed with increase of temperature, as mentioned above. The transition point of the reversal in helix sense, at which $[\theta]_{max}$ equals zero, shifts to higher temperature with increase of the degree of stearylation. The α helices of SA-63 and SA-72 with higher degree of stearylation of the copoly(SLAsp–BLAsp) prepared are comparatively stable below 20 and 40°C, respectively, irrespective of temperature, although $[\theta]_{max}$ increases gradually towards zero at higher temperature. The transition point of helix sense would be expected to shift towards a temperature higher than the boiling point of the solvent, chloroform.

Copolyaspartates in solid films

PBLAsp cast quickly at ambient temperature and atmospheric pressure takes a left-handed α helix. PBLAsp is well known[13–15] to shown an α–ω thermal transition around 140°C. *Figures 5a* and *5b* show the c.d. spectra of SA-0 (PBLAsp) in a solid film at 25 and 140°C, respectively. The c.d. intensity observed did not depend upon rotation of the specimen in the film plane. Therefore, an orientation effect of PBLAsp is not likely to appear. An irreversible and remarkable increase of $[\theta]_{max}$ and a slight blue shift were observed around 140°C as

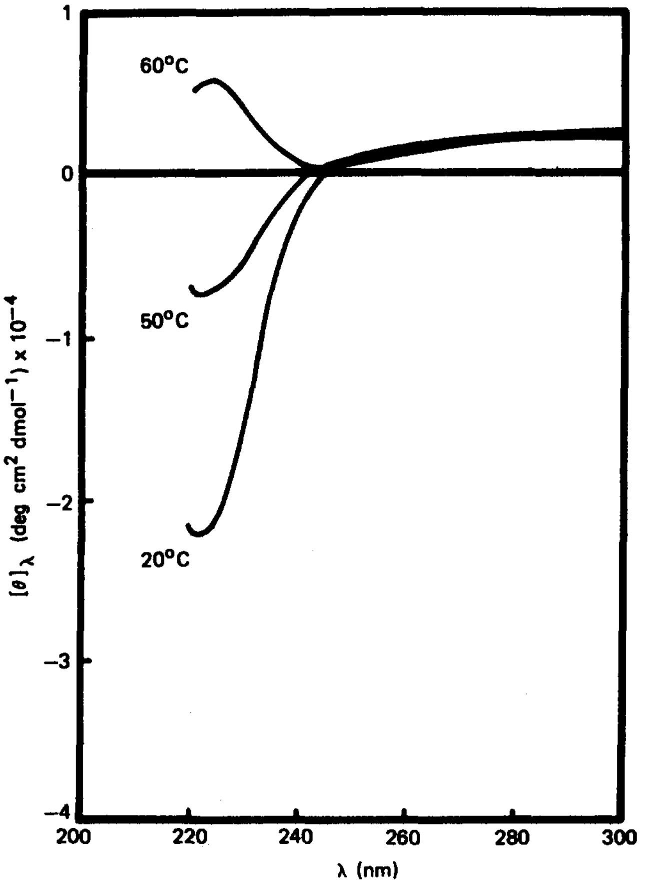

Figure 3 C.d. spectra of SA-41 in chloroform solution at various temperatures

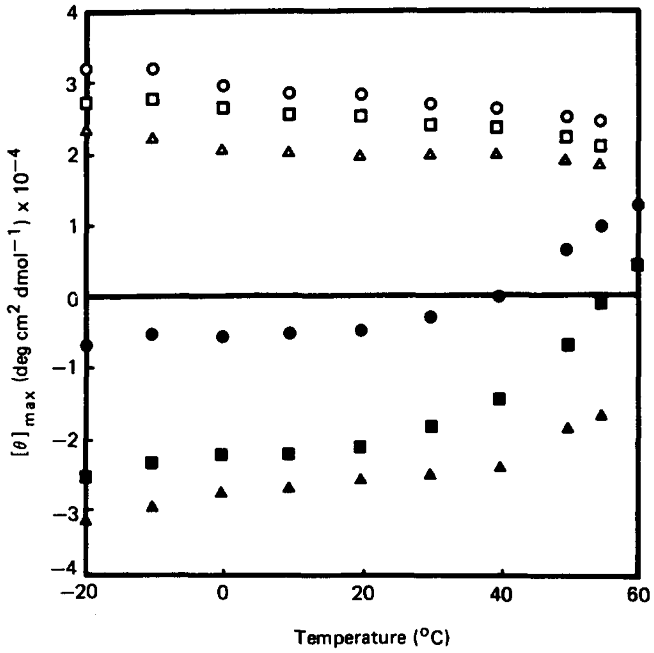

Figure 4 Temperature dependence of $[\theta]_{max}$ of copoly(SLAsp–BLAsp) with various degrees of stearylation in chloroform solution: ○, SA-0; □, SA-8; △, SA-26; ●, SA-41; ■, SA-63; ▲, SA-72

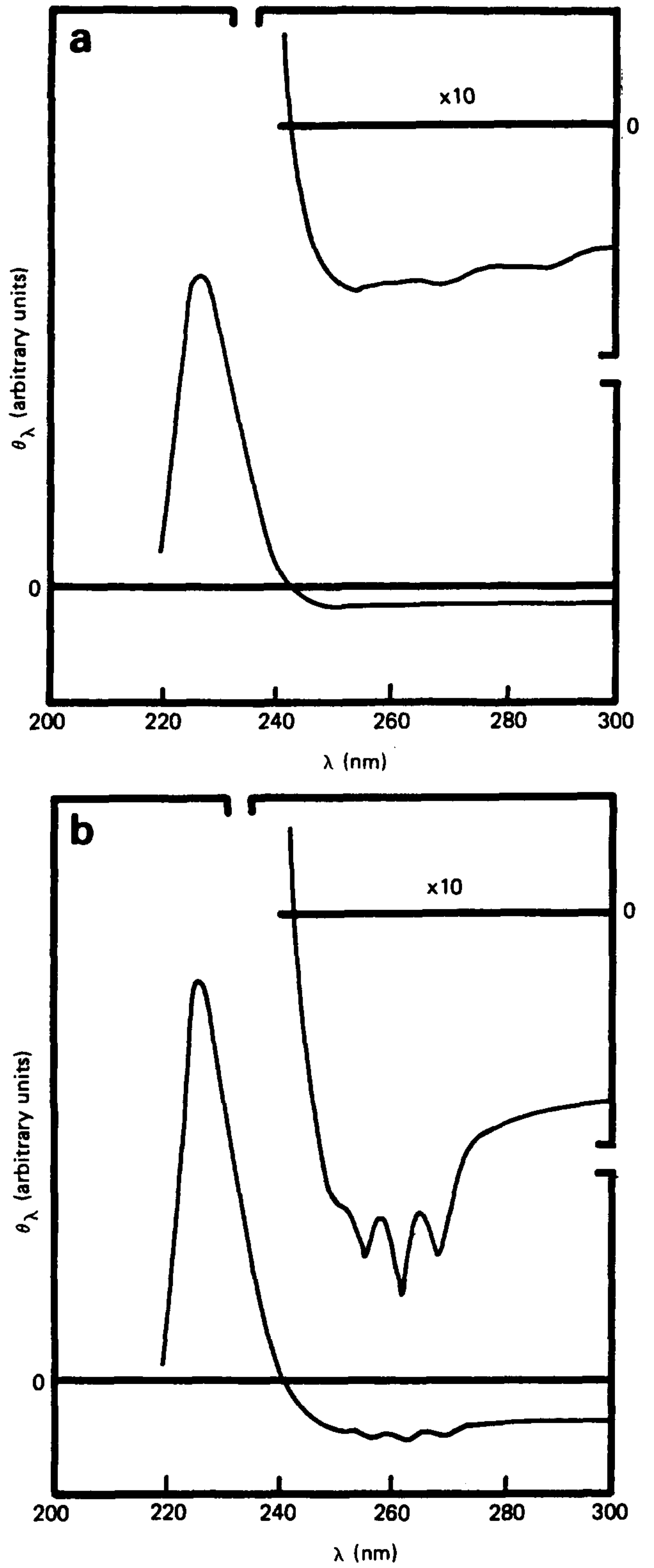

Figure 5 C.d. spectra of SA-0 (PBLAsp) in solid film at (a) 25°C and (b) 140°C

temperature increased, where the α–ω transition would occur. *Figure 5* also includes an enlarged c.d. spectra at 25 and 140°C. Weak c.d. troughs around 260 nm appeared for the ω helix, which could be attributed to induced c.d. of the ordered stacked benzene ring of the ω helix. The α–ω transition was also confirmed by shifts of characteristic i.r. absorption bands: ester CO, 1735 (α helix)→1731 cm^{-1} (ω helix); amide I, 1666 (α helix)→1675 cm^{-1} (ω helix); amide II, 1557 (α helix)→1536 cm^{-1} (ω helix)[5,14,15]. So we consider that the c.d. spectra above 140°C are characteristic of an ω helix induced by the stacking formation of side-chain benzyl groups. The same behaviour as for PBLAsp was observed for copoly(SLAsp–BLAsp) with low degree of stearylation, e.g. SA-8 and SA-26, also indicative of ω helix formation irrespective of the presence of a few stearyl groups. The temperature dependence of $[\theta]_{max}$ for SA-0, SA-8 and SA-26 is plotted in *Figure 6*. One can see that the α–ω transition temperature shifts to lower temperature for SA-8 and SA-26, around 70 and 40°C, respectively, and the change in $[\theta]_{max}$ accompanied by the α–ω transition decreased considerably with increasing degree of stearylation. It is considered that about 26% degree of stearylation could be the higher limit for forming an ω helix of copoly(SLAsp–BLAsp). The existence of more stearyl groups might disturb the formation of stacking and they cannot form the ω helix. In other words, more stearyl groups in the side chain cannot be incorporated into the benzene ring stacking in the ω helix. This behaviour is different from the effect of introduction of butyl groups into PBLAsp[8]. It is probably due to the similar relative size of the butyl groups and the benzyl groups.

As-cast copolyaspartates such as SA-41, SA-63 and SA-72 would be expected to take a right-handed α helix from *Figure 1*. *Figure 7* shows the c.d. spectra of SA-41 solid film at various temperatures. As the temperature was increased, the negative c.d. trough indicative of a right-handed α helix transformed irreversibly to a positive peak around 70°C. The positive c.d. peak is considered to be either left-handed α helix or left-handed ω helix. Since the stacking formation between benzyl groups may not occur in copolyaspartates with high degree of stearylation, the positive c.d. spectra are considered to represent the left-handed α helix. It was also again confirmed from a rotation measurement that there is no orientation effect in the c.d. spectra. The value of $[\theta]_{max}$ for SA-41, SA-63 and SA-72 solid films is plotted against temperature in *Figure 8*. The $[\theta]_{max}$ of SA-41 in the temperature range from 100 to 150°C is quite high, indicative of a stable left-handed α helix. Right-handed α helix nature in the low-temperature

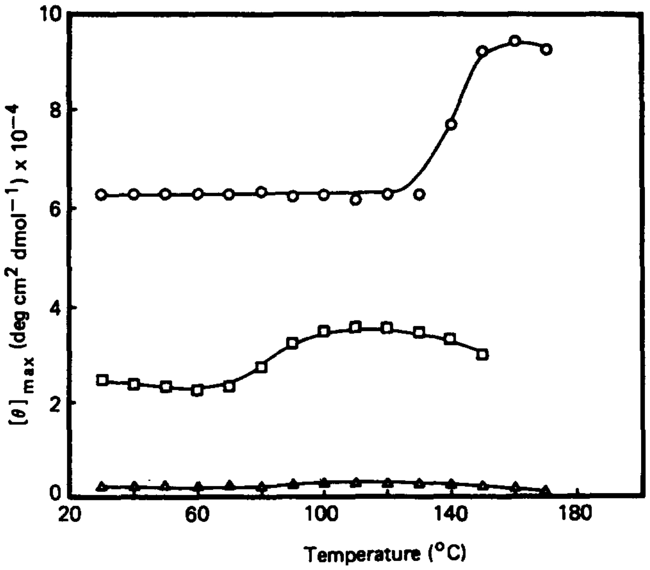

Figure 6 Temperature dependence of $[\theta]_{max}$ of SA-0, SA-8 and SA-26 in solid film: ○, SA-0; □, SA-8; △, SA-26

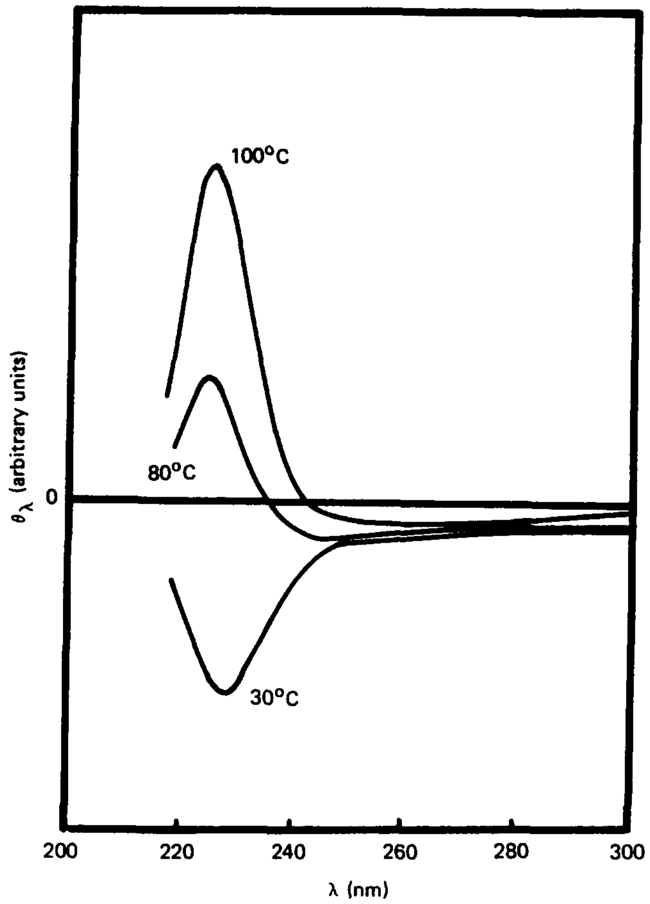

Figure 7 C.d. spectra of SA-41 in solid film at various temperatures

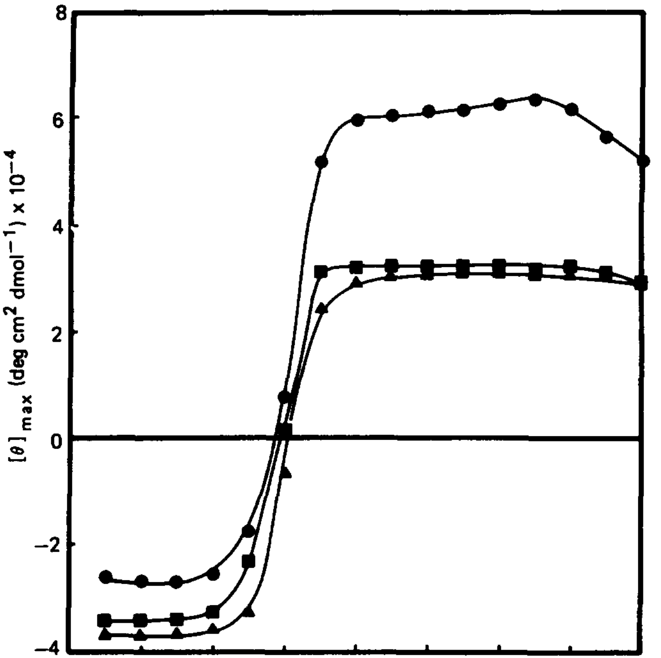

Figure 8 Temperature dependence of $[\theta]_{max}$ of SA-41, SA-63 and SA-72 in solid film: ●, SA-41; ■, SA-63; ▲, SA-72

range is also considered to be more stable with increasing degree of stearylation, reflecting higher shifts of helix-sense transition temperature. The transitions of helix sense of highly stearylated copolyaspartate solid films are similar to those of solution. It is noted that the helix-sense transition occurs in a narrow temperature range compared with that in solution. The transition was irreversible, different from that of copoly(SLG–MLG)[10]. These facts might be explained in terms of differences of interaction on the helix-sense transition. The helix-sense transition of copoly(SLAsp–BLAsp) might be induced by a subtle change in the interaction in the side-chain region; on the other hand, that of copoly(SLG–MLG) might be induced by a change in the interaction due to melting and crystallization of the stearyl side-chain groups. The latter is a possible cause of the reversible transition.

The helix sense of SA-0, SA-8 and SA-26 is left-handed as seen from *Figure 5* and *6*, while that of SA-41, SA-63 and SA-72 is right-handed in the low-temperature range from 30 to 70°C as seen from *Figures 7* and *8*. So, the change of the helix sense from left to right occurs with increase of the degree of stearylation in the solid state. In the higher temperature range, the conformational change from left-handed ω helix to left-handed α helix would be expected from *Figures 6* and *8* as the degree of stearylation increases.

CONCLUSIONS

The conformational behaviours of copoly(SLAsp–BLAsp)s discussed above are summarized in *Figure 9*. In contrast to conformational changes of copoly(SLG–MLG)[10], the helix sense of the present copoly(SLAsp–BLAsp) is characteristic of its helix instability. A reversible change in the sense of the α helix occurs in solution as the degree of stearylation and temperature increase. Such a conformational change is common to all the copolyaspartates consisting of alkyl L-aspartate and benzyl L-aspartate.

The left-handed α helix transforms into the left-handed ω helix without a reversal in helix sense with increasing

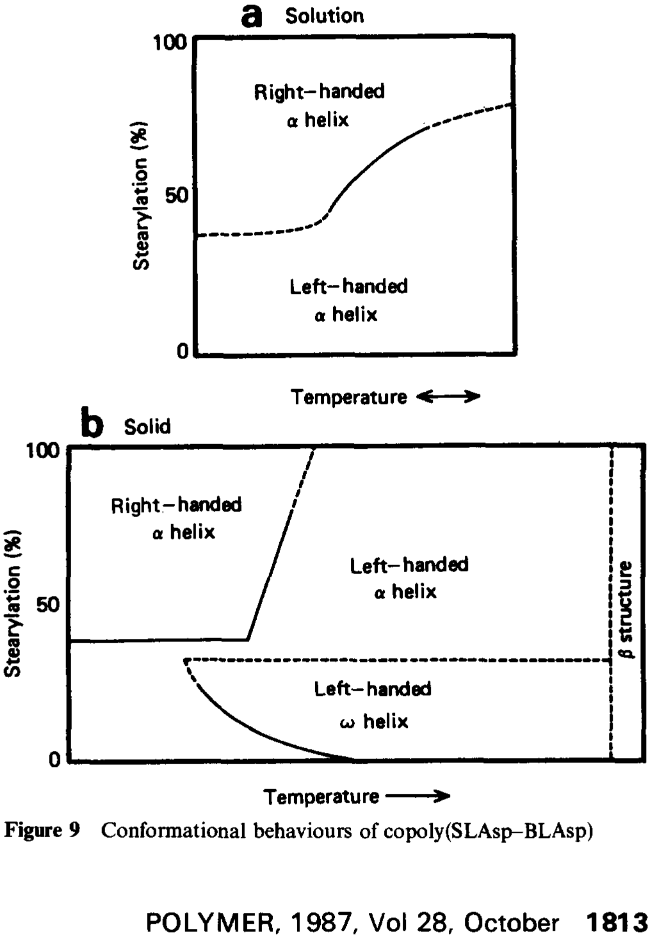

Figure 9 Conformational behaviours of copoly(SLAsp–BLAsp)

temperature for copoly(SLAsp–BLAsp) solid films with a low degree of stearylation, while a reversal in α helix sense from right- to left-handed occurs for those with a high degree of stearylation with increasing temperature. No α–ω transition was observed. The helix sense change from left-handed α helix to right-handed α helix occurs in the low-temperature range and, on the other hand, the conformational change from left-handed ω helix to left-handed α helix does occur in the high-temperature range as the degree of stearylation increases. The reversal in helix sense in the solid phase is irreversible, contrary to the solid state of copoly(SLG–MLG).

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