

Characterization of copolyaspartates containing a long alkyl side chain

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Copoly(β -stearyl L-aspartate- β -benzyl L-aspartate) with various degrees of stearylation was synthesized and secondary and higher ordered structure in solid state was examined from the standpoint of composition and temperature of the copolyaspartates. Copolyaspartate with degree of stearylation <30% transformed in solid state from left-handed α helix to right-handed α helix with increasing temperature. Copolyaspartate with degree of stearylation >30% caused the reverse transformation, from right-handed α helix to left-handed α helix. The transition from α helix to β form was observed at higher temperature for all copolyaspartates studied. The transitions of helix sense and α helix to β form were irreversible. Copolyaspartate with degree of stearylation >20% could furthermore cause an intramolecular side chain crystallization of a long alkyl chain. The side chain crystal of such a copolyaspartate melted but a helix backbone was still stable. The helix surrounded by a molten long side chain which plays a role as a solvent could be considered for construction of a thermotropic liquid crystalline phase. Copolyaspartate with a long alkyl side chain was compared with copolyglutamate and the difference of helix stability and side chain crystalline part was interpreted in terms of the competitive hydrogen bonding between NH groups in the backbone and CO groups in the side chain formed in the copolyaspartate.

(Keywords: copolyaspartate; characterization; α helix; ω helix; β form; helix sense; long side chain; side chain crystallization; liquid crystal)

INTRODUCTION

It is well known that polyaspartate takes an unstable α helix compared to the stable α helix of polyglutamate since important intramolecular hydrogen bonding for α helix formation is disturbed by competitive hydrogen bonding between NH groups in the backbone and CO groups in the side chain¹. Therefore, the secondary and higher ordered structure of copolyaspartate is influenced easily by environmental conditions such as temperature and species of groups in the side chain and so on. Scheraga *et al.*²⁻⁴ calculated the conformational energy of polypeptides and discussed the helix instability of polyaspartate. In addition, Bradbury *et al.*^{5,6} and our colleagues⁷⁻¹⁵ studied experimentally infrared spectroscopy, optical rotatory dispersion and circular dichroism spectra of copolyaspartate having various alkyl esters. We confirmed a reversal in helix sense with degree of alkylation and temperature⁷⁻¹⁵.

Polypeptide is very interesting in the formation of higher ordered structure. Quick-cast film of poly β -benzyl L-aspartate (PBLAsp) is left-handed α helix and takes two-dimensional pseudo hexagonal array while slow-cast film or film annealed at 140°C are left-handed ω helix and takes two-dimensional tetragonal array (ω form). It is well known that the ω form of PBLAsp can form an intramolecular benzene ring stacking in the side chain region. When the benzyl group in PBLAsp was substituted by alkyl groups, except for butyl groups of very similar size to the benzyl group, copolyaspartates with $\geq 30\%$ alkylation cannot form ω form and its helix

sense is transformed from right- to left-handed α helix with increasing temperature. One of the present authors¹⁶ studied higher ordered structure of copolyglutamate with long alkyl side chain which is stable α helix in solution and solid state. The long alkyl side chain of the copolyglutamate can crystallize intermolecularly between α helices. Reproducible transition of crystallization and melting of the copolyglutamate with long alkyl side chain was observed. Watanabe *et al.*¹⁷⁻²¹ also studied poly γ -alkyl L-glutamate and concluded that the poly γ -alkyl L-glutamate with alkyl groups >10 methylenes could crystallize in the long alkyl side chain region and form thermotropic liquid crystalline phase. Similarly, side chain crystallization is expected for copolyaspartate with stearyl groups. Polyaspartates are in general unstable, as mentioned above. The conformation depends strongly upon the interaction in the side chain region. It is interesting that the formation of side chain crystal might influence conformation of copolyaspartate with stearyl groups.

In the present article copoly(β -stearyl L-aspartate- β -benzyl L-aspartate) (copoly(SLAsp-BLAsp)), which is less stable α helix than copolyglutamate or polyglutamate, was synthesized by ester exchange reaction from PBLAsp. Characterization of secondary structure such as α and ω helices and β form and higher ordered structure like side chain crystalline phase and liquid crystalline phase was examined as a function of the degree of stearylation and temperature.

EXPERIMENTAL

The parent polymer, PBLAsp, was obtained by polymer-

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ization of well purified *N*-carboxy- β -benzyl L-aspartate anhydride (NCA)¹⁴. The PBLAsps thus prepared had viscosity average molecular weights of 31 900 and 33 100. Copoly(SLAsp-BLAsp) was prepared by stearylation of PBLAsp according to the method of Bradbury *et al.*²². The degree of stearylation was controlled by varying the reaction time at constant molar ratio of reactants. The abbreviation SA-*x*, representing the degree of stearylation (*x*) of copoly(SLAsp-BLAsp) determined by elemental analysis, is used in the following discussion. Copoly(SLAsp-BLAsp) was quick cast at 25°C from chloroform solution (conc. $\approx 0.005 \text{ g cm}^{-3}$).

Infrared absorption spectra (Jasco IR-700) of copoly-(SLAsp-BLAsp) of 15 μm for the wavenumber range 4000–400 cm^{-1} and temperature range 25–160°C. The infrared dichroic ratio of an oriented copoly(SLAsp-BLAsp) prepared by stroking out concentrated chloroform solution on KBr plate was measured by using a polarizer (PL-81).

Differential scanning calorimetry (d.s.c.) (SSC 560S, Seiko electronics Co. Ltd) measurements were made under N_2 atmosphere at a heating rate of 5°C min^{-1} in the temperature range –30–250°C.

A dilatometer was prepared containing dry copoly-(SLAsp-BLAsp) and mercury. The dilatometry method is described in detail elsewhere²³.

X-ray diffraction patterns were obtained by a 20 \times integration of scattered intensity at 2θ range from 1.2 to 40° using an X-ray diffractometer (RAD-RC, Rigaku). X-ray photographs of through and edge views were taken by a Laue flat camera.

RESULTS AND DISCUSSION

Bradbury *et al.*^{5,6} and one of the present authors^{7,8} have summarized the assignment of infrared (i.r.) characteristic bands of backbone peptide bonds and side chain groups. The wavenumber of the characteristic bands of copoly-(SLAsp-BLAsp) as cast is plotted as a function of the degree of stearylation in Figure 1. For comparison, the wavenumbers of left-handed (L) and right-handed (R) α helices and ω helix are also depicted in Figure 1. Reversal in helix sense from L to R α helices at 25°C is observed as the degree of stearylation increases: copoly-(SLAsp-BLAsp) with a low degree of stearylation takes L α helix at 25°C while that with a high degree of stearylation takes R α helix. This i.r. behaviour corresponds to the results of circular dichroism (c.d.) reported previously¹⁴.

The temperature dependence of the wavenumber of the characteristic bands of SA-71 is shown in Figure 2. The wavenumber of the characteristic bands varies drastically around 80°C, indicative of the reverse in helix sense from R α helix to L α helix. Although it is not clear which conformation, L α helix or ω helix, is stable at high temperature, L α helix is a probability according to c.d. results¹⁴.

The orientation of α helix backbone and side chain was examined using polarized i.r. spectroscopy. Figure 3 shows polarized i.r. spectra of SA-71 as cast. Parallel dichroic spectra are observed especially for NH stretching, amide I and ester C–O bands, while perpendicular dichroic spectra appear for ester C=O and amide II bands. Results are summarized in Tables 1 and 2, for SA-71 as cast and SA-71 annealed at 100°C for 1 h,

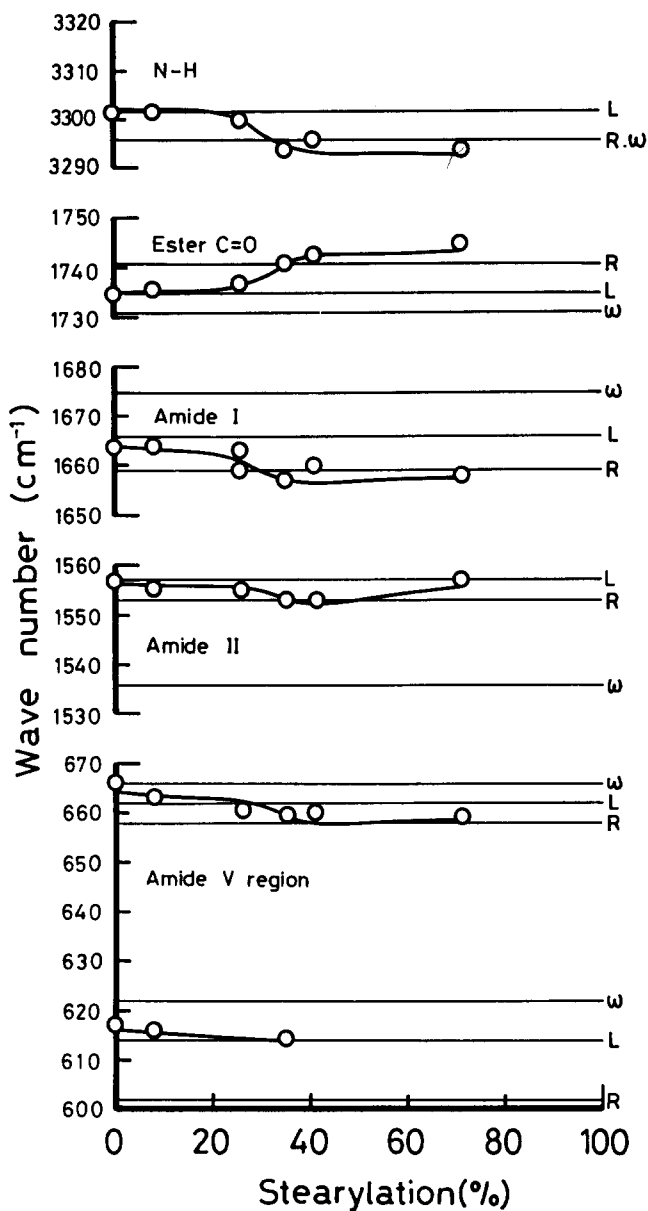


Figure 1 Wavenumber of characteristic bands of copoly(SLAsp-BLAsp) as cast: L, left handed α helix; R, right handed α helix; ω , ω helix

respectively. The orientation factors of α helix axis of SA-71 as cast and SA-71 annealed were calculated to be 0.355 and 0.343 respectively, using dichroic ratio of NH stretching. It was found that the orientation of NH transition moment in both L and R α helices was the same irrespective of reversed α helix sense.

The orientation of the side chain is different in L and R α helices. The angle θ between α helix axis and ester C=O and ester C–O of SA-71 as cast of R α helix was 87.2° and 42.7°, respectively, and that of SA-71 annealed of L α helix was 52.3° and 58.6°. Since ester C=O orientation of R α helix is nearly perpendicular to the α helix axis, structural c.d. spectra based on ester C=O were not observed, while the structural c.d. spectra of L α helix appeared because of a finite C=O transition moment along the α helix axis ($\theta = 52.3^\circ$)¹⁴. Thus it is concluded that the side chain of copoly(SLAsp-BLAsp) forms a relatively regular structure between α helices and such a side chain structure is a general feature of copolyaspartate.

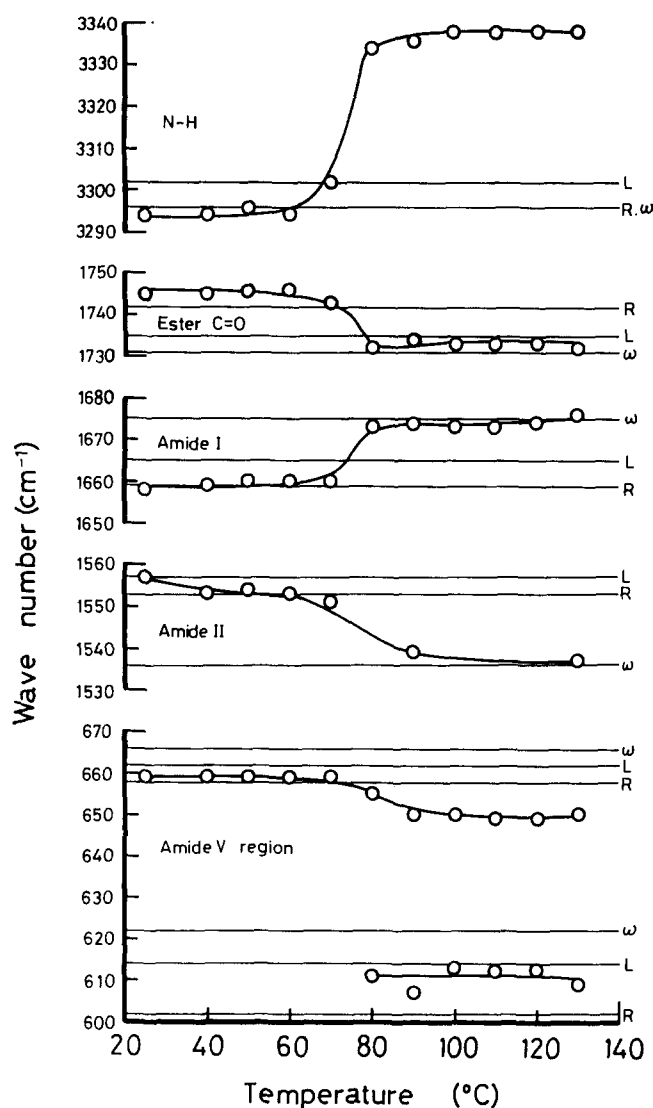


Figure 2 Temperature dependence of wavenumber of characteristic bands of SA-71: L, left handed α helix; R, right handed α helix; ω , ω helix

The higher ordered structure of copoly(SLAsp-BLAsp) was studied as a function of the degree of stearylation and temperature. X-ray diffraction patterns of copoly(SLAsp-BLAsp) as cast with various degrees of stearylation are shown in Figure 4. One can observe the diffraction peak corresponding to the helical spacing [100] of copoly(SLAsp-BLAsp) as cast. Figure 5 shows a plot of the spacing [100] versus degree of stearylation. The spacing increases markedly from 14 (PBLAsp) to 35 Å* (SA-8) and then decreases down to 31 Å as the degree of stearylation increases. Copoly(SLAsp-BLAsp) with <30% stearylation demonstrates a broad peak indicative of a disordered arrangement of less stable α helix, while that with >30% gives rise to a relatively sharp diffraction peak as shown in Figure 4. Note that the appearance of an ordered α helical arrangement above 30% stearylation corresponds to the reversal in helix sense of copoly(SLAsp-BLAsp). In other words, R α helix is more rigid and is able to arrange more regularly than L α helix.

An X-ray diffraction photograph, edge-viewed, of SA-71 is shown in Figure 6. Two equatorial diffractions appear: one is the diffraction from an α helical arrangement and the other is the diffraction from the crystallized side chain of a stearyl group. The latter corresponds to the spacing of the side chain of copoly(stearyl L-glutamate-methyl L-glutamate), although the diffraction of copolyglutamate was observed on the longitude²⁴. From these X-ray patterns one can draw a schematic representation of an intramolecular side chain crystalline region of copoly(SLAsp-BLAsp) as shown in Figure 7a. For comparison, the schematic structural model of copolyglutamate with a long side chain is shown in Figure 7b. A long side chain in copolyaspartate is considered to crystallize intramolecularly along the α helix axis, while that in copolyglutamate is arranged intermolecularly. This is also attributed to the difference in the interaction

* 1 Å = 10^{-1} nm

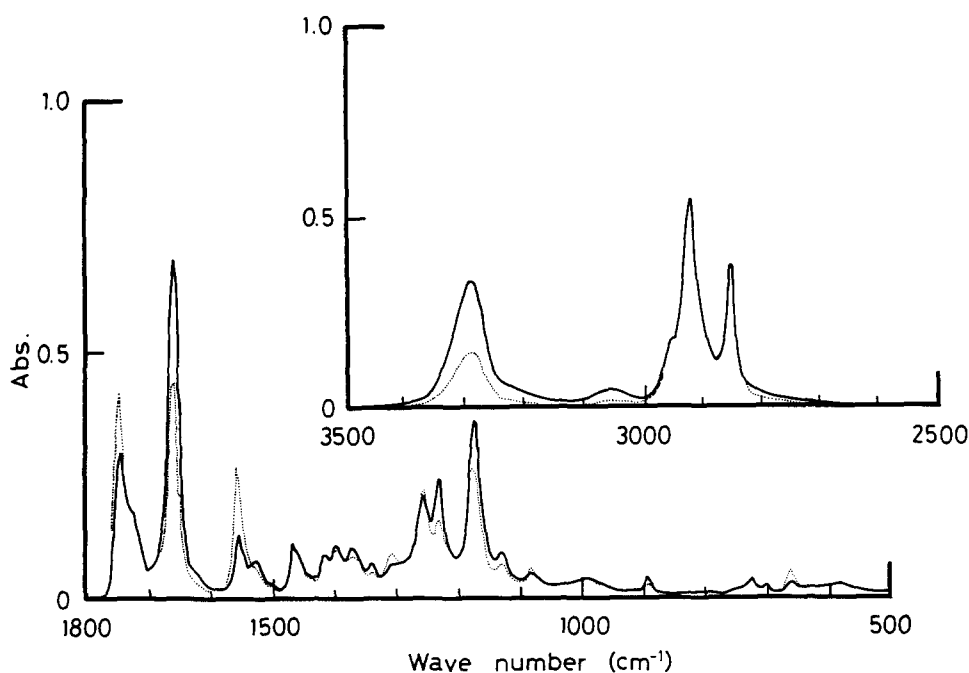


Figure 3 Polarized infrared absorption spectra of SA-71 as cast; —, parallel; ···, perpendicular

Table 1 Band position, absorbance, and dichroic ratio of the characteristic band and angle θ between α helix axis and group transition moment assigned to characteristic band of SA-71 as cast

Assignment	Band position (cm^{-1})		Absorbance		Dichroic ratio, R	Angle θ (deg)
	\parallel	\perp	\parallel	\perp		
N-H stretch	3290	3292	0.336	0.150	2.24	18
C-H stretch						
ν as	2918	2920	0.553	0.549	1.01	
ν s	2850	2850	0.382	0.383	1.00	
Ester C=O	1743	1746	0.299	0.416	0.55	87.2
Amide I	1659	1659	0.687	0.441	1.56	37.6
Amide II	1554	1555	0.135	0.266	0.60	
Ester C-O	1172	1175	0.359	0.262	1.37	42.7

Orientation factor, $f = 0.355$

Table 2 Band position, absorbance, and dichroic ratio of the characteristic band and angle θ between α helix axis and group transition moment assigned to characteristic band of SA-71 annealed at 100°C for 1 h

Assignment	Band position (cm^{-1})		Absorbance		Dichroic ratio, R	Angle θ (deg)
	\parallel	\perp	\parallel	\perp		
N-H stretch	3332	3332	0.251	0.115	2.18	18
C-H stretch						
ν as	2918	2918	0.598	0.572	1.05	
ν s	2848	2848	0.436	0.402	1.09	
Ester C=O	1732	1735	0.343	0.322	1.07	52.3
Amide I	1673	1663	0.499	0.310	1.61	35.6
Amide II	1540	1540	0.108	0.201	0.54	
Ester C-O	1172	1171	0.224	0.247	0.91	58.6

Orientation factor, $f = 0.343$

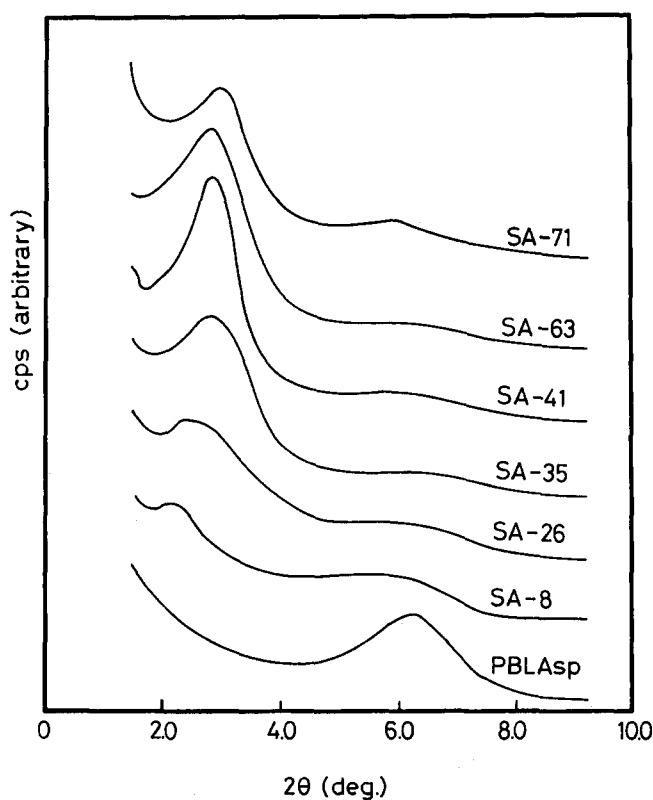


Figure 4 X-ray diffraction patterns of as cast copoly(SLAsp-BLAsp) with various degrees of stearylation

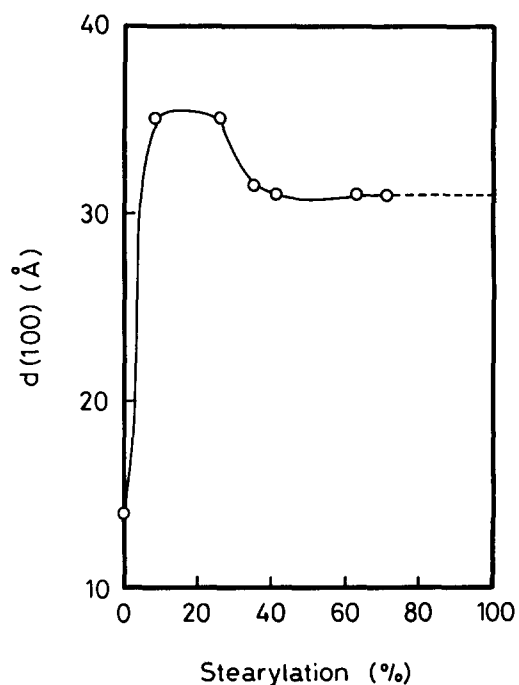


Figure 5 Plot of spacing [100] against degree of stearylation

between NH groups in the main chain and CO groups in the side chain.

Figure 8 shows a volume-temperature relationship of SA-9 obtained by dilatometry. SA-9 exhibited the



Figure 6 X-ray diffraction photograph, edge-viewed, of SA-71

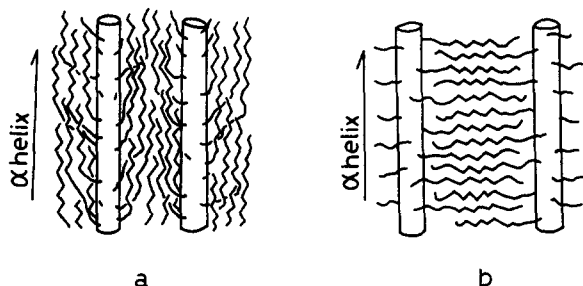


Figure 7 Schematic representation of (a) side chain crystalline region of copoly(SLAsp-BLAsp) and (b) copolyglutamate with long alkyl side chain

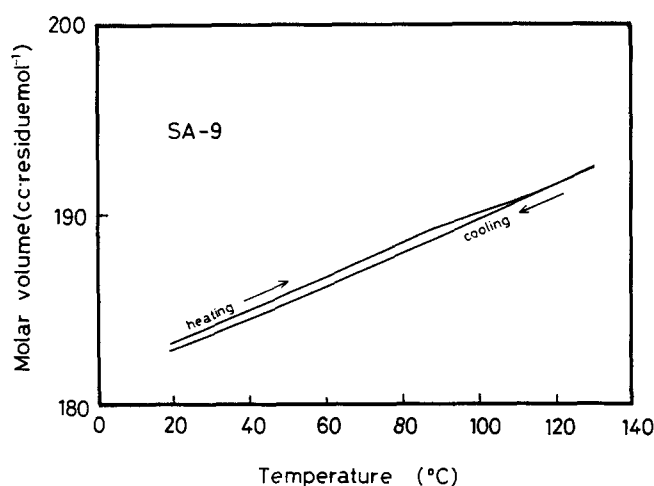


Figure 8 Volume-temperature relationship of SA-9 as cast

α - ω transition and resulting volume decrease as well as PBLAsp (SA-9 $\Delta V_{\alpha-\omega} = 0.33 \text{ cm}^3 \text{ mol}^{-1}$, PBLAsp $\Delta V_{\alpha-\omega} = 0.70 \text{ cm}^3 \text{ mol}^{-1}$). This fact is interpreted in terms of a disturbance for the formation of benzene ring stacking due to the presence of a small amount of stearyl groups. The volume increase of SA-9 on ω - β transition also decreased compared with PBLAsp (SA-9 $\Delta V_{\omega-\beta} = 2.5 \text{ cm}^3 \text{ mol}^{-1}$, PBLAsp $\Delta V_{\omega-\beta} = 4.0 \text{ cm}^3 \text{ mol}^{-1}$), al-

though the results are not demonstrated in the figure. The volume-temperature relationship of SA-35 is shown in Figure 9. Volume decrease is observed at ≈ 80 and $\approx 120^\circ\text{C}$, the former being attributed to the transition to the reverse helix sense and the latter being the α - ω transition. The side chain region of R α helix is, therefore, considered to be coarser than that of L α helix, which takes a transverse conformation of side chain.

Figure 10 shows d.s.c. thermograms of as cast copoly(SLAsp-BLAsp). PBLAsp, SA-8 and SA-26 demonstrated an exothermic peak assigned to the α - ω transition around 110 - 140°C and an endothermic peak of the ω - β transition around 200°C . Note that the α - ω transition temperature did not shift to lower temperature with increase of stearylation as well as the result of dilatometry, contrast to the transition of conformation itself. D.s.c. thermograms of copoly(SLAsp-BLAsp) with $>41\%$ stearylation demonstrated entirely different behaviour. An enlarged d.s.c. thermogram is shown as a typical example of copoly(SLAsp-BLAsp) with $>41\%$ in Figure 11 and the peaks are labelled T_1 - T_4 as temperature increases. The peak T_1 was assigned to the melting of stearyl side chain and appeared near 42°C for SA-41 and SA-63. In addition, the thermogram of SA-71 shows two endothermic peaks at 41 and 57°C . This suggests two types of side chain crystals, probably intramolecular and intermolecular side chain crystals. The peak T_1 (41°C) is due to the melting of intramolecular side chain crystals and is independent of stearylation, characteristic of copolyaspartate with a long alkyl side chain. On the other hand, the peak of higher temperature (57°C) might be assigned to the melting of intermolecular side chain crystals, which depends upon stearylation. In fact the peak T_1 of the copolyglutamate with a long alkyl side chain depended upon stearylation according to our previous report²⁴. It can be said that copoly(SLAsp-BLAsp) can crystallize mainly intramolecularly for low stearylation and crystallize both intra and intermolecularly for high stearylation such as 71% , although intermolecular crystallization is relatively less.

Figure 12 shows a plot of enthalpy of melting ΔH_m against stearylation. The extrapolation to $\Delta H_m = 0$

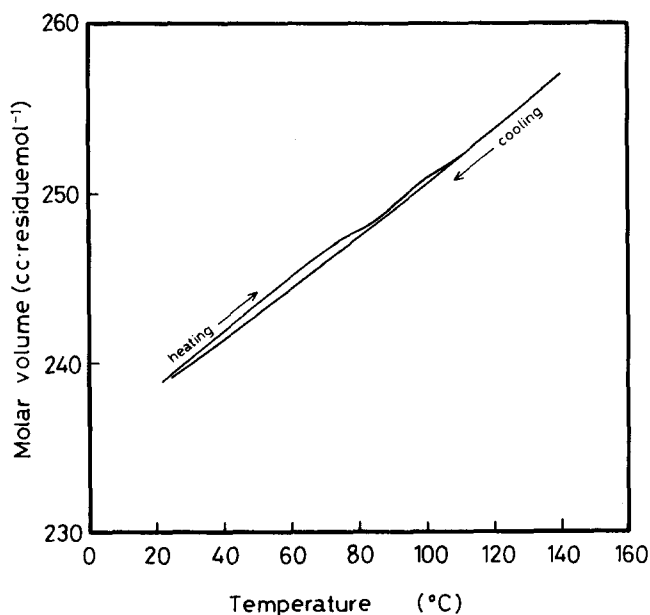


Figure 9 Volume-temperature relationship of SA-35 as cast

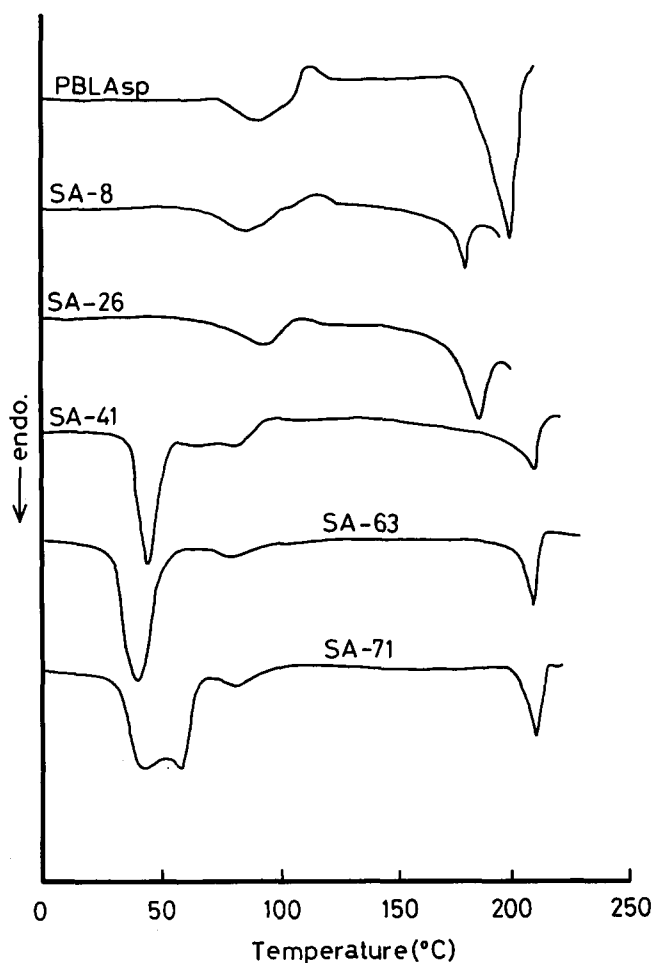


Figure 10 D.s.c. thermograms of as cast copoly(SLAsp-BLAsp) with various degrees of stearylation

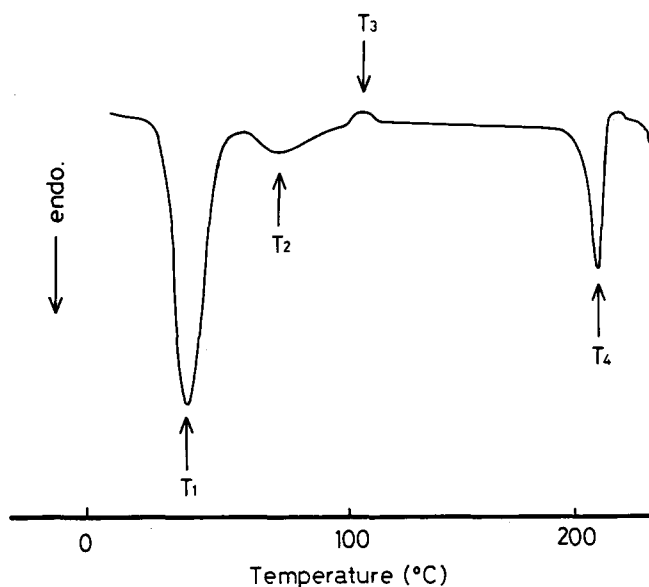


Figure 11 D.s.c. thermograms of as cast SA-63: peaks are labelled T_1 - T_4 from lower to higher temperature

indicates stearylation of 21% for the initial stearylation of side chain crystallization. Extrapolation to stearylation of 100% gives a value $\Delta H_m = 5.9 \text{ kcal mol}^{-1}$, namely ΔH_m of poly(β -stearyl L-aspartate). This means that one third of the whole stearyl side chain (about six CH_2 units) crystallizes, using the standard ΔH_m of n-alkane per

methylene unit ($1.0 \text{ kcal mol}^{-1} \text{ CH}_2$ unit). On the other hand, about nine CH_2 units of the stearyl side chain can crystallize for copolyglutamate with a stearyl side chain. These differences of crystallizable sequence between copolyaspartate and copolyglutamate are mainly based on the mode of crystallization, namely intramolecular and intermolecular, respectively. Side chain melting was observed irrespective of main chain conformation such as L α helix, R α helix and β form. The melting temperature and enthalpy of melting of β form were higher than those of L α helix and R α helix.

The peaks T_2 and T_4 were confirmed to be the transition to the reverse helix sense and ω - β transition^{7,8}, as found by c.d. spectra and X-ray diffraction analysis.

The X-ray diffraction pattern of SA-71 was measured as a function of temperature to clarify the peak T_3 and is shown in Figure 13. The spacing [100] of the helix of copolyaspartate is observed at 25°C at $2\theta \approx 3.5^\circ$ as described above. The intensity of the spacing [100] increased and the spacing shifted slightly to smaller angle around 110°C , which corresponds to the temperature of the peak T_3 , as temperature increases. It is probable that higher ordered structure of SA-71 could be formed around 110°C . Apparently, liquid-like fluidity and a homogeneous light field of the polarizing microscope were observed above 110°C . The peak T_3 is tentatively assigned to crystal-liquid crystal transition (thermotropic liquid crystal). Although a selective reflection characteristic of cholesteric liquid crystal has to appear in visual region of c.d. spectra, it could not be observed for copolyaspartate. In contrast, copolyglutamate with a long alkyl side chain can take well defined thermotropic cholesteric liquid crystal, which demonstrates the selective reflection^{17,20,21,25}. The side chain higher ordered structure caused by the difference in intra or intermolecular interactions might be important to construct well defined liquid crystalline structure. Higher ordered structure of copolyaspartate with higher stearylation has to be examined, furthermore, from the point of cholesteric liquid crystal.

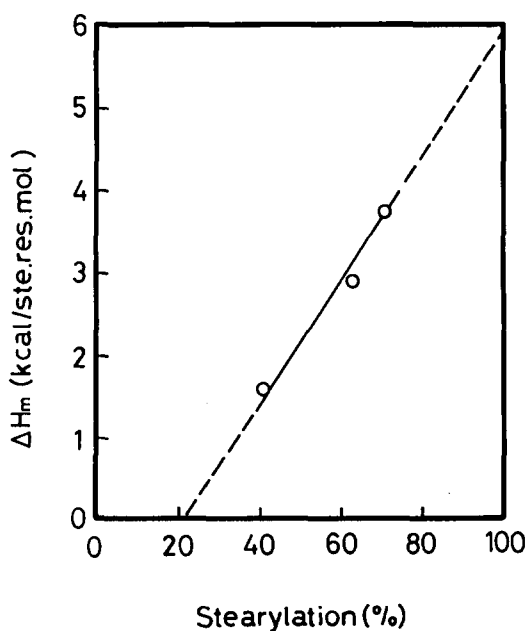


Figure 12 Plot of enthalpy of melting of side chain crystals of copoly(SLAsp-BLAsp) against degree of stearylation

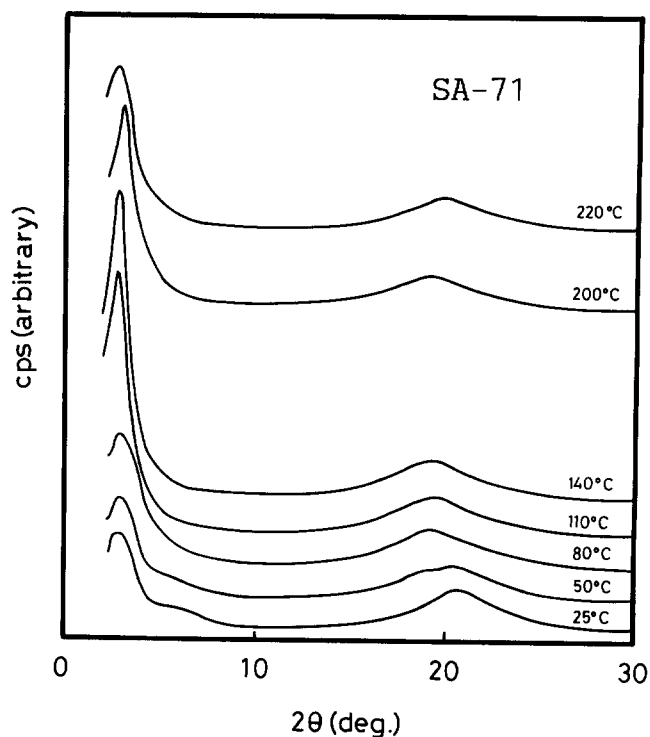


Figure 13 X-ray diffraction patterns of SA-71 at various temperatures

CONCLUSIONS

Copolyaspartate transformed helix sense from L to R with increasing stearylation. Copolyaspartate with a high degree of stearylation transformed helix sense from R to L with increasing temperature. The copolyaspartate backbone was able to orient parallelly by stroking out of concentrated solution. Ester C=O in the side chain of R and L α helices was arranged nearly perpendicularly and parallelly, respectively.

X-ray helical spacing levelled off after a maximum as stearylation increased. The arrangement of helical rod and crystalline side chain were nearly parallel.

Volume change and enthalpy change on the side chain melting transition (α - ω , α - β and ω - β) and reversal in helix sense were observed from dilatometry and d.s.c., respectively.

The stearyl group with >21% stearylation in the side chain of copolyaspartate could crystallize in the side

chain region and only one third of the stearyl group took part in the side chain crystal. Side chain conformation such as transverse conformation of left-handed α helix and longitudinal conformation of right-handed α helix was confirmed and could determine higher ordered structure and properties of copoly(SLAsp-BLAsp).

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