

Conformational Studies of Poly(β -phenethyl L-aspartate)

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ABSTRACT: Poly(β -phenethyl L-aspartate) was synthesized and its conformational transitions in chloroform-dichloroacetic acid and chloroform-trifluoroacetic acid mixed solvents were examined by optical rotatory dispersion, circular dichroism and viscosity measurements. Poly(β -phenethyl L-aspartate), having a right-handed α -helical form in chloroform, undergoes a transition to a left-handed α -helical form by the addition of 2.0% dichloroacetic acid at 21°C. In the range of dichloroacetic acid content between 1.2% and 2.0%, the polymer exhibits a thermally-induced inversion of the helix sense from the right-handed to the left-handed. The transition temperature is dependent on the solvent composition. Furthermore, in the chloroform-trifluoroacetic acid system, the polymer exhibits both the helix-sense inversion and the helix-coil transition when the composition of the mixed solvent is varied. The thermally- and acid-induced inversions of the helix sense are discussed in terms of the change in the side chain conformation.

KEY WORDS α -Helix / Helix-Sense Inversion / Helix-Coil Transition / Poly(β -phenethyl L-aspartate) /

It is well known that the helix sense of ester derivatives of poly(L-aspartic acid) varies with the nature of the side chain, temperature and solvent. Poly(β -benzyl L-aspartate) (PBLA) and poly(β -methyl L-aspartate) take on the left-handed (LH) α -helical form in the chloroform solutions,¹⁻⁴ while other poly(β -alkyl L-aspartate)s (alkyl = ethyl, propyl, *iso*-propyl and butyl) take on the right-handed (RH) α -helical form.^{4,5} Poly(β -propyl L-aspartate) in chloroform undergoes a conformational transition from the RH α -helix to the LH α -helix when the temperature of the solution is raised. Copolymers of β -ethyl L-aspartate and β -benzyl L-aspartate also exhibit the thermally-induced inversion of the helix sense.⁴ Furthermore, the RH α \rightarrow LH α transitions of these copolymers can be induced by the addition of a small amount of dichloroacetic acid (DCA) or trifluoroacetic acid (TFA) to the chloroform solutions.⁶ These observations may indicate that the RH α -helical forms of poly(L-aspartate ester)s are less stable than the LH ones. Infrared studies^{5,7} and calculations of the conformational energies^{8,9} have shown that the side chain conformation is different for the two helical forms of poly(L-aspartate ester)s.

Poly(β -phenethyl L-aspartate) (PPELA) (phen-

ethyl group = $-\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5$) has been shown to take on the RH α -helical conformation in chloroform.^{5,7} In this study, we synthesized high molecular-weight PPELA and examined conformational transitions in chloroform DCA and chloroform TFA mixed solvents.

EXPERIMENTAL

Synthesis of Polypeptide

β -Phenethyl L-Aspartate. L-Aspartic acid (100 g) was dissolved in a mixture of phenethyl alcohol (2-phenyl-ethanol) (250 ml) and sulfuric acid (80%, 65 ml) at 70°C with stirring. Following a reaction period of 4 h, the excess water was distilled off *in vacuo*, and the product was then neutralized with aqueous sodium bicarbonate. The precipitate was collected and recrystallized from a hot aqueous solution.

β -Phenethyl L-Aspartate N-Carboxyanhydride (NCA). Purified β -phenethyl L-aspartate (20 g) was suspended in dry tetrahydrofuran (THF, 600 ml) at 40°C, and trichloromethyl chloroformate (6.0 ml) was added. After the solution became clear, the solvent THF was evaporated *in vacuo*, and then hexane was added. The NCA was purified by

repeated crystallization from the hexane-ethyl acetate system.

PPELA. The polymerization of NCA (10 g) was carried out in chloroform (500 ml) using triethylamine as an initiator. The molar ratio of NCA to initiator was about 50. The reaction was continued at room temperature for 4 days, and the polymer was precipitated with a large excess of methanol. The intrinsic viscosity number $[\eta]$ in chloroform-5% TFA was 0.914 dl/g at 25°C, and the molecular weight¹⁰ estimated was *ca.* 15.2×10^4 .

Measurements

Optical rotatory dispersion (ORD) and circular dichroism (CD) spectra were measured by using a JASCO J-20 Recording Spectropolarimeter. The concentrations of polymer were taken in the range of 0.5–1.0%, and cells with path lengths of 0.1- and 10-mm were used. The ORD parameter, b_0 , was derived from the Moffitt-Yang equation ($\lambda_0 = 212$ nm).¹¹ Infrared (IR) spectra were measured by a JASCO DS-701G Grating Infrared Spectrophotometer using a KRS-5 cell (thallium bromide-thallium iodide mixed crystal) with lead spacer. The viscosity was measured by an Ubbelohde type viscometer.

RESULTS

Solubility of PPELA in Organic Solvents

At room temperature, PPELA was soluble in chloroform and in mixed solvents of chloroform with DCA or TFA. In 1,2-dichloroethane (EDC), dimethylsulfoxide (DMSO) and hexamethyltriamide phosphate (HMTAP), the polymer was dissolved by heating to 50–60°C. When these solutions were cooled to room temperature, the solution in EDC changed into a transparent gel immediately, while the other solutions remained unchanged.

The values of the Moffitt-Yang b_0 parameter for PPELA in these solutions are shown in Table I. Since

Table I. Moffitt-Yang b_0 parameters of PPELA in various solvents

| Solvent | b_0 | Temp/°C |
|-------------------|-------|---------|
| Chloroform | -534 | 25 |
| EDC | -438 | 55 |
| DMSO | -171 | 25 |
| HMTAP | -257 | 25 |
| Chloroform-5% TFA | -295 | 25 |

b_0 values of -630 and +630 have been assigned to the fully RH and LH α -helices, respectively,¹² it appears that PPELA forms the RH α -helix in the chloroform solution. This is further supported by the IR spectrum with the Amide I band at 1656 cm^{-1} , the Amide II band at 1552 cm^{-1} and the carbonyl ester band at 1741 cm^{-1} . The band positions are in agreement with those observed earlier for PPELA and other poly(L-aspartate ester)s in the RH α -helical form.⁵⁻⁷ Similarly, PPELA is predominantly in the RH α -helical form in EDC at 55°C. On the other hand, the random coil conformation is suggested for the polymer in DMSO, HMTAP, and chloroform-5% TFA mixed solvent, on the basis of the fact that PBLA in the random coil conformation in DCA gives an anomalous b_0 value around -250.²

Solvent-Induced Transition

Figure 1 shows the variation in b_0 of PPELA with solvent composition in chloroform-DCA mixed solvents at 21°C. The b_0 value remains constant around -530 up to 1.8% DCA, and then abruptly increases to a positive value of +500 at 2.2% DCA. This change should be ascribed to the inversion of the helix sense from the right-handed to the left-handed. The transition midpoint was found at about 2.1% DCA. In the range of DCA content between 2.2% and 3.3%, the LH α form is more stable than the RH α form. Bradbury *et al.*¹ have reported that, in the case of PBLA, the LH α -helix undergoes a

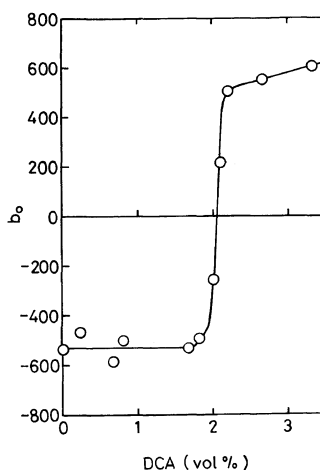


Figure 1. Dependence of the b_0 values of PPELA on DCA content in chloroform-DCA mixed solvents at 21°C.

transition to the random coil conformation when the DCA content exceeds the value of *ca.* 8%. Therefore, it is expected that the helix-coil transition of PPELA can be induced by further addition of DCA. However, this transition could not be observed in this particular solvent system, since precipitation of polymer occurred at about 5% DCA.

Figure 2 shows the ultraviolet CD spectra of PPELA. The sign of the CD band below 250 nm changes from negative to positive when the DCA content increases from 1.8% to 2.1%. The values of

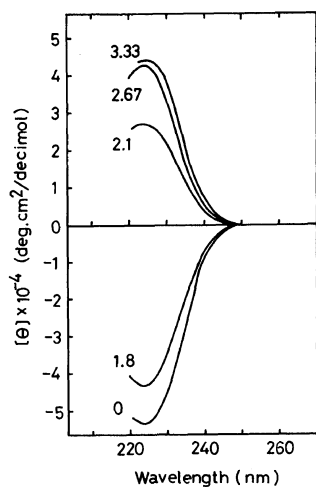


Figure 2. Ultraviolet CD spectra of PPELA in chloroform-DCA mixed solvents. Numerals are the DCA content (vol %).

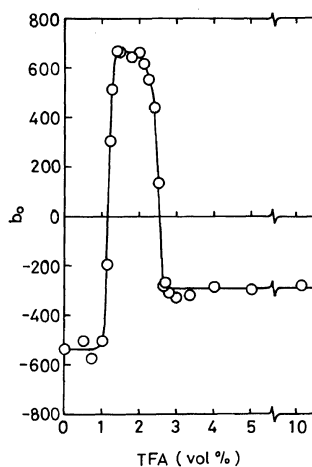


Figure 3. Dependence of the b_0 values of PPELA on TFA content in chloroform-TFA mixed solvents at 25°C.

molar ellipticity at 224 nm are $[\theta]_{224} = -54000$ in chloroform, and $[\theta]_{224} = +45000$ in chloroform-3.3% DCA. The CD data support the conclusion of the ORD study that the helix sense of PPELA inverts from the right-handed to the left-handed.

A similar transition was induced by the addition of TFA to the chloroform solution. This is shown in Figure 3, where the b_0 value measured at 25°C is plotted against the solvent composition in chloroform-TFA mixtures. The b_0 value first increases from -530 to +630 as the TFA content is increased from 1.0% to 1.4%. This is again attributed to the helix-sense inversion. The formation of the LH helical form in chloroform-1.67% TFA was confirmed by the CD measurement; the positive $[\theta]_{224}$ value of +41000 was obtained at 25°C. Further addition of TFA causes a decrease in the b_0 value to -290 at 2.6% TFA, indicating a transition to the random coil conformation. The midpoint of the helix-coil transition of PPELA is at 2.5% TFA, while PBLA requires 3.5% TFA to cause this transition.⁴ If we may use the acid content necessary to induce the helix-coil transition as a measure of the stability of the helix, the LH α -helix of PPELA is then estimated to be somewhat less stable than that of PBLA.

Thermally-Induced Transition

The temperature dependence of $[\theta]_{224}$ for PPELA in chloroform-1.8% DCA mixed solvent is shown in Figure 4. On heating the solution, $[\theta]_{224}$ rapidly increases from -51000 at 25°C to +38000 at 45°C, indicating a thermally-induced transition from the RH α -helical form to the LH one. This transition occurs in a narrow range of temperature around 34°C.

Figure 5 illustrates the temperature dependence of the b_0 value as a function of the solvent composition. In those solutions containing 1.2–2.0% DCA, PPELA undergoes the RH α →LH α transition when the temperature of the solution is raised. As the DCA content increases, the transition shifts to a lower temperature and becomes less sharp. The RH α form in chloroform and the LH α form in chloroform-2.2% DCA are found to be stable throughout the temperature range from 2°C to 55°C.

The temperature dependence of the viscosity η_{sp}/c for PPELA in chloroform-2% DCA and in chloroform-5% TFA is shown in Figure 6. The ORD data in Figure 5 indicate that PPELA in the former

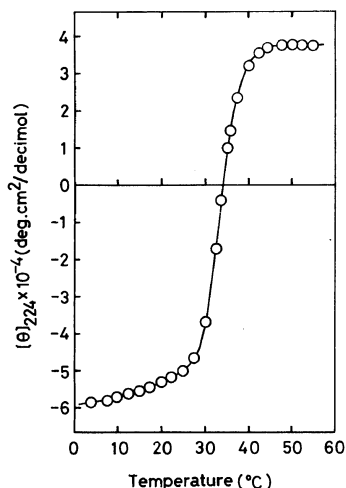


Figure 4. Temperature dependence of the molar ellipticity value at 224 nm for PPELA in a chloroform-1.8% DCA mixed solvent.

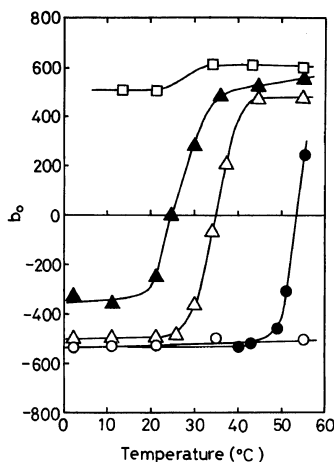


Figure 5. Temperature dependence of the b_0 values of PPELA in chloroform-DCA mixed solvents. DCA contents are (○) 0%, (●) 1.2%, (△) 1.8%, (▲) 2.0%, and (□) 2.2%.

solvent undergoes the RH $\alpha \rightarrow$ LH α transition at 25°C, while η_{sp}/c shows no abrupt change. This is further viewed in the light of the fact that, for the case of the random coil conformation in the latter solvent, the value of η_{sp}/c varies monotonously with temperature at a much lower level around the value of 1.0. As a result, we conclude the absence of the random coil conformation in the transition process from the RH α form to the LH α form. The zero rotatory power at

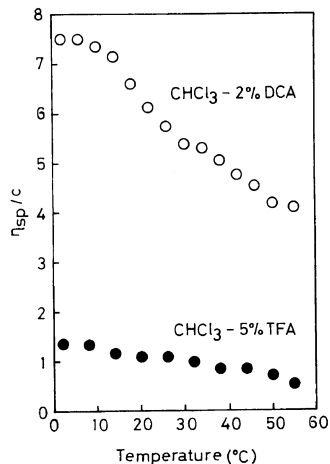


Figure 6. Temperature dependence of the specific viscosity of PPELA: (○) in chloroform-2% DCA and (●) in chloroform-5% TFA.

the transition midpoint can be attributed to an equimolar mixture of the RH and LH α -helices.

DISCUSSION

From the results of the aforementioned study, we can conclude that for PPELA: (i) the RH α -helical form is more stable than the LH α -helical form in the chloroform solution; and (ii) the difference in stability between the two helical forms of PPELA is considerably small; thus, the RH $\alpha \rightarrow$ LH α transition can be induced easily. On the other hand, for PBLA, the LH α -helical form is more stable than the RH α -helical form and exhibits no helix-sense inversion. The conformational stabilities of these two polymers are thus markedly affected by the difference in the primary structure, *i.e.*, PPELA has one additional methylene group in the side chain of PBLA.

It has been shown that the side chain aromatic rings of poly(L-aspartate ester)s are arranged regularly in the RH α form, whereas they are much less regular in the LH α form.^{6,13} This observation may suggest the contribution of the regularity of the side chain conformation to the stability of the RH α form. When the temperature of the solution is raised, the amplitude of librational motion increases and the side chain becomes more flexible. Similarly, small amounts of DCA or TFA added to the chloroform solution of PPELA break down the side chain structure, either by the solvation or by the hydrogen

bond formation with the carbonyl ester group in the side chain of polymer. The presence of such an effect is evidenced by the result of a nuclear magnetic resonance study on PBLA in chloroform; the addition of *ca.* 2% TFA causes a remarkable sharpening of the peaks due to the benzyl-CH₂ and phenyl protons.¹⁴ Furthermore, electric dichroism studies for the phenyl group of poly(γ -benzyl L-glutamate) have revealed a disappearance of the dichroism at 5% TFA.¹⁵ These observations are interpreted in terms of the increased mobility of the side chain. According to the conformational energy calculations by Lotan *et al.*¹⁶ the influence of increased mobility appears mainly on the side-chain nonbonded and torsional energies. Consequently, it may be suggested that both thermally- and acid-induced inversions of the helix sense have similar mechanisms, arising initially from the breaking down of the side-chain regular structure in the RH α -helical form.

This suggestion seems to be also applicable to the inversion phenomena of poly(β -propyl L-aspartate) and poly(β -ethyl L-aspartate-*co*- β -benzyl L-aspartate) in the chloroform solutions.^{4,5} In these cases, the inversion can be induced merely by heating the solutions to 50–60°C, even in the absence of any helix breaking solvent. For PPELA, as can be seen in Figure 5, a temperature increase of about 50°C is not sufficient to induce inversion, and the presence of small amounts of acid (1.2–2.0% DCA) is necessary. From this comparison, the energy difference between the RH and LH α forms of PPELA is

estimated to be slightly greater than those of other two polymers, but much lower than that of PBLA.

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