BIOPOLYMERS VOL. 16, 1593–1607 (1977)

Synthesis and Conformational Study of Poly(0,0′-dicarbobenzoxy-L-β-3,4-dihydroxyphenyl-α-alanine)

HIROYUKI YAMAMOTO and TADAO HAYAKAWA, *Institute of High Polymer Research, Faculty of Textile Science and Technology, Shinshu University, Ueda 386, Japan*

Synopsis

High-molecular-weight poly(0,0′-dicarbobenzoxy-L-β-3,4-dihydroxyphenyl-α-alanine) was prepared by the *N*-carboxyanhydride method. From the results obtained by a study of the optical rotation, nuclear magnetic resonance, and solution infrared absorption, the conformation of poly(0,0′-dicarbobenzoxy-L-β-3,4-dihydroxyphenyl-α-alanine) depended greatly on the solvent taking a right-handed helix with $[\theta]_{225} = -13{,}600 \sim -18{,}900$ in alkyl halides, a left-handed helix with $[\theta]_{228} = 22{,}100 \sim 24{,}800$ in cyclic ethers or trimethylphosphate, and a random coil structure in dichloroacetic acid, trifluoroacetic acid, or hexafluoroacetone sesquihydrate. The polypeptide underwent a right-handed helix-coil transition in chloroform/dichloroacetic acid (or trifluoroacetic acid) mixed solvents and a left-handed helix-coil transition in dioxane/dichloroacetic acid (or trifluoroacetic acid) mixed solvents. The results were compared with those of poly(0-carbobenzoxy-L-tyrosine).

INTRODUCTION

As early as 1911, β-3,4-dihydroxyphenyl-α-alanine (DOPA) was first synthesized by Funk[1] and then was discovered in nature.[2] Later the syntheses of some DOPA derivatives, a series of oligopeptides containing L-DOPA, and poly(DL-DOPA) derivative have been described.[3–6] Optically active poly(L-DOPA) derivative, however, has not yet been reported. In a recent communication[7] we have reported the helical sense of poly(0,0′-dicarbobenzoxy-L-DOPA) in helicogenic solvents. In the present paper we report the synthesis and conformational study of poly(0,0′-dicarbobenzoxy-L-DOPA).

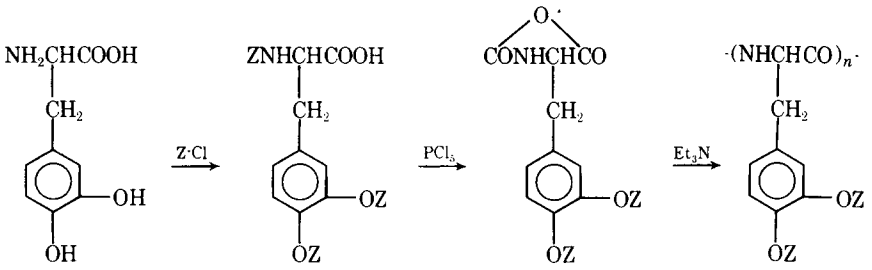
Crude 0,0′-dicarbobenzoxy-L-DOPA *N*-carboxyanhydride (NCA) as a precursor to prepare L-DOPA benzyl ester was described by Felix et al.[6] The description, however, was not complete (no analytical and spectral data) and the polymerization has not been reported. We prepared pure crystalline 0,0′-dicarbobenzoxy-L-DOPA NCA from *N*,0,0′-tricarbobenzoxy-L-DOPA and phosphorus pentachloride at 0°C to about −5°C in anhydrous ether. The acetone solution of the NCA was passed through a dry charcoal-silver oxide column. After removal of the solvent, high-molecular-weight poly(0,0′-dicarbobenzoxy-L-DOPA) was prepared by

polymerizing the NCA in 10% dioxane solution using triethylamine as an initiator. As reported in a previous communication the conformation of poly(0,0′-dicarbobenzoxy-L-DOPA) depended greatly on the solvent taking the right-handed helical sense in alkyl halides and the left-handed helix in cyclic ethers. The conformational transition of the polypeptide in mixed solvents such as chloroform/dichloroacetic acid (Cl_2AcOH) [or trifluoroacetic acid (F_3AcOH)] and dioxane/Cl_2AcOH (or F_3AcOH) were also studied by optical rotation, nuclear magnetic resonance (nmr), and infrared absorption (ir) measurements.

EXPERIMENTAL

Polypeptide

L-DOPA was purchased from the Tokyo Chemical Industry Co., Ltd. Poly(0,0′-dicarbobenzoxy-L-DOPA) was synthesized according to the following scheme:



Z = $C_6H_5CH_2OCO$- Et_3N = triethylamine

N,0,0′-tricarbobenzoxy-L-DOPA. *N*,0,0′-tricarbobenzoxy-L-DOPA was prepared from L-DOPA and carbobenzoxy chloride under nitrogen as described by Felix et al.[6]; yield, 93.7%; mp, 80°C; $[\alpha]_D^{25} = -0.95°$ (c = 2.93, methanol).

ANAL. *Calc. for* $C_{33}H_{29}O_{10}N$: C, 66.10; H, 4.87; N, 2.34. *Found:* C, 66.40; H, 4.63; N, 2.53.

The reported values for the compound are mp, 82–84°C and $[\alpha]_D^{25} = -0.67°$ (c = 5.5, methanol).[6]

0,0′-dicarbobenzoxy-L-DOPA NCA. To a solution of *N*,0,0′-tricarbobenzoxy-L-DOPA (33.0 g) in 300 ml of dry ether, finely pulverized phosphorus pentachloride (13.8 g) was added at 0°C to about −5°C and the solution was vigorously stirred for 20 min. The crystalline NCA started to precipitate. After 30 min, dry *n*-hexane (30 ml) was added to the solution. After stirring at 0°C for an additional 30 min, the crystalline NCA was collected by filtration. The NCA was recrystallized three times from ethyl acetate (30 ml) and *n*-hexane (150 ml); yield, 23.7 g (87.7%); mp, 77°C; ir bands at 1852 and 1788 cm^{-1} (cyclic anhydride).

ANAL. *Calc. for* $C_{26}H_{21}O_9N$: C, 63.54; H, 4.31; N, 2.85. *Found:* C, 63.62; H, 4.59; N, 2.50.

Poly(0,0′-dicarbobenzoxy-L-DOPA). In order to remove trace amounts of the halogen compounds, the above NCA was dissolved in dry acetone and then passed through a dry charcoal-silver oxide column. The acetone was removed to dryness under reduced pressure. The purified NCA (6.5 g) was dissolved in dry dioxane (60 ml) and polymerized for 3 days at room temperature and for 2 days at 45°C using triethylamine as an initiator (A/I = 100).[8] The polymer was precipitated with water, soaked in ethanol to remove the low-molecular-weight compounds, filtered, and dried; yield, 2.8 g (47.3%); ir bands (see below).

ANAL. *Calc. for* $C_{25}H_{21}O_7N$: C, 67.11; H, 4.73; N, 3.13. *Found:* C, 67.17; H, 4.72; N, 3.13.

The polypeptide had an intrinsic viscosity $[\eta]$ of 0.26 dl/g in Cl_2AcOH at 25°C. The molecular weight was estimated to be 28,000 (degree of polymerization = 60) from an empirical equation[9] for poly(0-carbobenzoxy-L-tyrosine) in Cl_2AcOH[9] and from the *N*-terminal titration with perchloric acid in chloroform using crystal violet as an indicator.[10] The polypeptide is soluble in dioxane, tetrahydrofuran (Hyfuran), chloroform, methylene dichloride, trimethylphosphate $[(CH_3)_3P]$, Cl_2AcOH, or F_3AcOH, slightly soluble in carbon tetrachloride or hexafluoroacetone sesquihydrate, and insoluble in 2-chloroethanol, 2,2,2-trifluoroethanol, or 1,1,1,3,3,3-hexafluoro-2-propanol.

Methods

Ultraviolet absorption (UV) spectra were measured on a Shimadzu recording spectrophotometer UV-200. Optical rotatory dispersion (ORD), circular dichroism (CD), and ir spectra were measured on a ORD/UV 5 instrument with a CD attachment and on an IR DS-301 instrument, both made by the Japan Spectroscopic Co., Ltd. Under constant nitrogen flush, cells with path lengths of 0.1–10 mm were used. The concentrations of the sample for rotation measurements were in the (0.20–0.81)% range. The dimension of the rotation is deg cm^2/dmol. The nmr spectra were recorded on a Jeol 60-MHz JNM-c-60HL spectrometer and peak positions were measured relative to internal tetramethylsilane [polypeptide concentration (3–5)%]. The intrinsic viscosity was measured in Cl_2AcOH at 25°C using an Ubbelohde viscometer.

RESULTS

Conformation of Poly(0,0′-dicarbobenzoxy-L-DOPA) in Solution

UV spectra. The UV absorption spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) in dioxane or chloroform are shown in Figure 1. The polypeptide

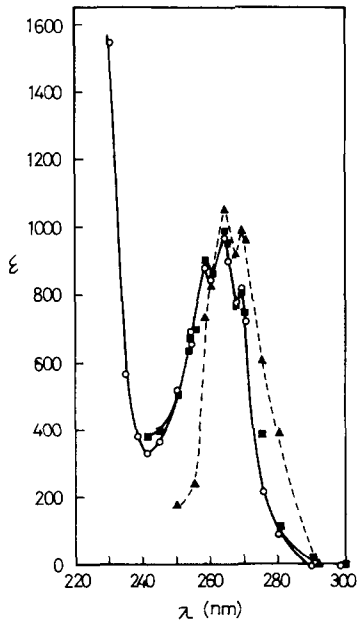


Fig. 1. UV spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) at 25°C: ○, in dioxane; ■, in chloroform; ▲, in F_3AcOH.

showed the same absorption with a same molar extinction coefficient and no significant difference was found in two solvents described above. In F_3AcOH another spectrum was obtained (no peak at 258.5 nm).

ORD spectra. The ORD spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) are shown in Figure 2. In chloroform or methylene dichloride the polypeptide showed a broad peak at 255 nm, a trough at 235 nm, and a strong peak at 220 nm, and in dioxane, Hyfuran, or $(CH_3)_3P$ a broad trough at 270 nm, a peak at 237 nm, and a deep double-humped trough at 223 and 209 nm. In F_3AcOH or hexafluoroacetone sesquihydrate the same curve was obtained showing a peak at 228 nm.

CD spectra. The CD spectra are shown in Figure 3. The molar ellipticity values are $[\theta]_{225} = -13{,}600$ in chloroform, $[\theta]_{225} = -18{,}900$ in methylene dichloride, $[\theta]_{228} = 24{,}800$ in Hyfuran, $[\theta]_{228} = 24{,}000$ in dioxane, $[\theta]_{228} = 22{,}100$ in $(CH_3)_3P$, and $[\theta]_{219} = 16{,}300$ in hexafluoroacetone sesquihydrate. The conformation of poly(0,0′-dicarbobenzoxy-L-DOPA) depends greatly on the solvent taking the right-handed helix in alkyl halides, the left-handed helix in cyclic ethers or $(CH_3)_3P$, and a random coil structure in hexafluoroacetone sesquihydrate. A model compound, *N*,0,0′-tricarbobenzoxy-L-DOPA-glycine ethyl ester, exhibited the same positive CD band in dioxane or chloroform.[7] Figure 4 shows the change of helical sense of poly(0,0′-dicarbobenzoxy-L-DOPA) with the change of solvent composition in dioxane/methylene dichloride mixtures at 25°C. In a 1:1 mixed

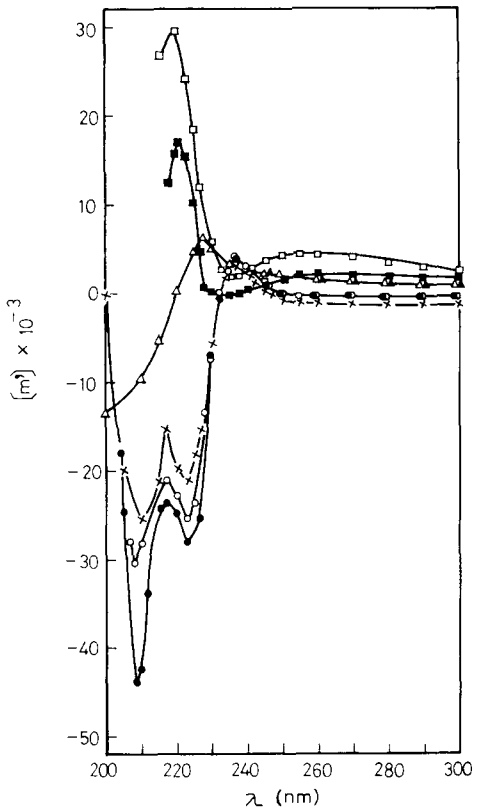


Fig. 2. ORD spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) in the ultraviolet region at 25°C: ○, in dioxane; ●, in Hyfuran; ×, in $(CH_3)_3P$; □, in methylene dichloride; ■, in chloroform; △, in hexafluoroacetone sesquihydrate; ▲, in F_3AcOH.

solvent the CD curve of the polypeptide gave a positive ellipticity band at 228 nm with $[\theta]_{228} = 15{,}800$. This suggests that the helical structure of the polypeptide in dioxane is more stable than that in chloroform.

Nmr spectra. Figure 5 shows the 60-MHz nmr spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) in three solvents at 25°C. In dioxane-d_8 solution (spectrum a), the α-CH resonance appears at 3.95 ppm, whereas in chloroform-d_1 (spectrum b) this proton appears at 4.13 ppm. Spectrum c in F_3AcOH shows the α-CH peak at 4.85 ppm (random coil). These chemical shift trends agree with those reported by Bradbury et al.[11] for the characterization of right- and left-handed helical forms.

Solution ir spectra. The solution ir spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) show amide I and II bands at 1665 and 1545 cm^{-1} in chloroform and at 1670 and 1550 cm^{-1} in dioxane. The amide bands frequencies of the polypeptide in dioxane are higher by 5 cm^{-1} than those in chloroform.

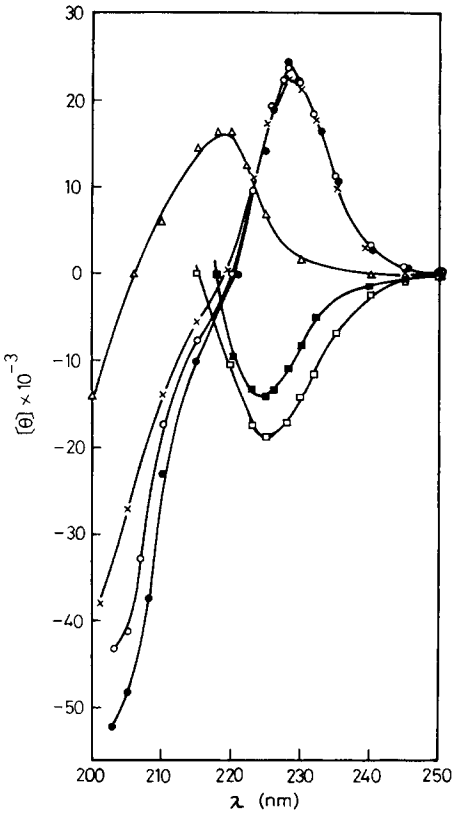


Fig. 3. CD spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) in the ultraviolet region at 25°C: ○, in dioxane; ●, in Hyfuran; ×, in $(CH_3)_3P$; □, in methylene dichloride; ■, in chloroform; △, in hexafluoroacetone sesquihydrate.

Helix-Coil Transition of Poly(0,0′-dicarbobenzoxy-L-DOPA) in Mixed Solvents

ORD spectra. Figure 6 shows the ORD curves above 340 nm in chloroform/Cl_2AcOH [Fig. 6A)] or dioxane/Cl_2AcOH [Fig. 6B)] mixed solvents. When a few percent of Cl_2AcOH or F_3AcOH were added to the chloroform solution, the specific rotation increased markedly, from $[\alpha]_{546} = 55°$ in chloroform to 83° in chloroform/2% Cl_2AcOH. 9% Cl_2AcOH inverted the rotation from *dextro* to *levo*. The polypeptide showed the same behavior in chloroform/F_3AcOH mixed solvents. On the other hand, in dioxane/Cl_2AcOH mixed solvents the rotation of the polypeptide changed anomalously from *levo* ($[\alpha]_{546} = -56°$ in dioxane) to *dextro* (a maximum rotation, $[\alpha]_{546} = 75°$ in a 1:1 mixed solvent), then from *dextro* to *levo* again (above 70% Cl_2AcOH), and finally to a constant value of −23° in Cl_2AcOH. The specific rotations are shown in Figure 7. The parameters, a_0 and b_0, derived from the Moffitt-Yang equation,[12] were also calculated from the ORD curves using $n_0 = 212$ nm as in the case of poly(0-carbobenzoxy-L-tyrosine)[9] and are shown in Figure 8 (see also Table I). The sign of the b_0 values in

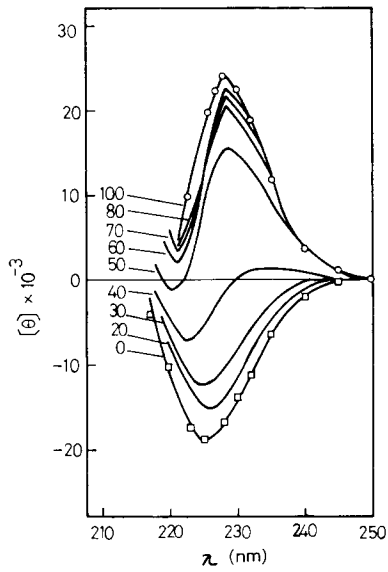


Fig. 4. CD spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) in dioxane/methylene dichloride mixed solvents: O, in dioxane; □, in methylene dichloride; numerals, volume percents of dioxane in methylene dichloride.

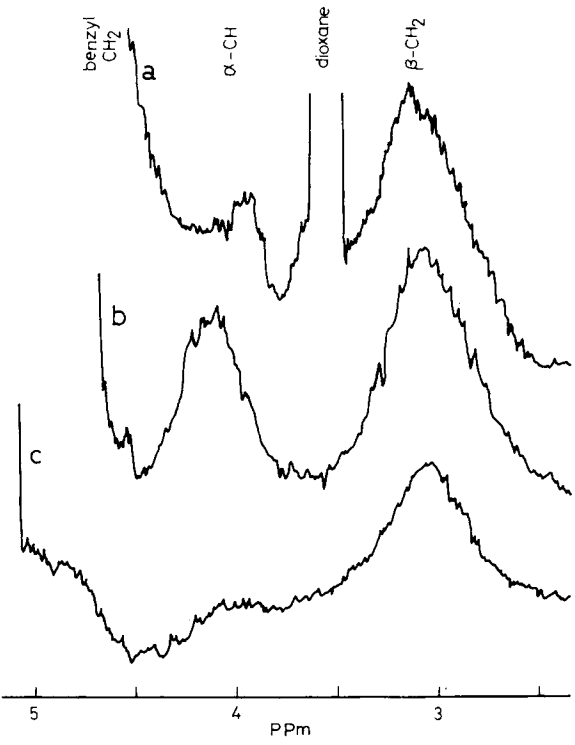


Fig. 5. Nmr spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) at 25°C (no spinning): a) in dioxane-d_8/5% F_3AcOH; b) in chloroform-d_1/1% F_3AcOH; c) in F_3AcOH.

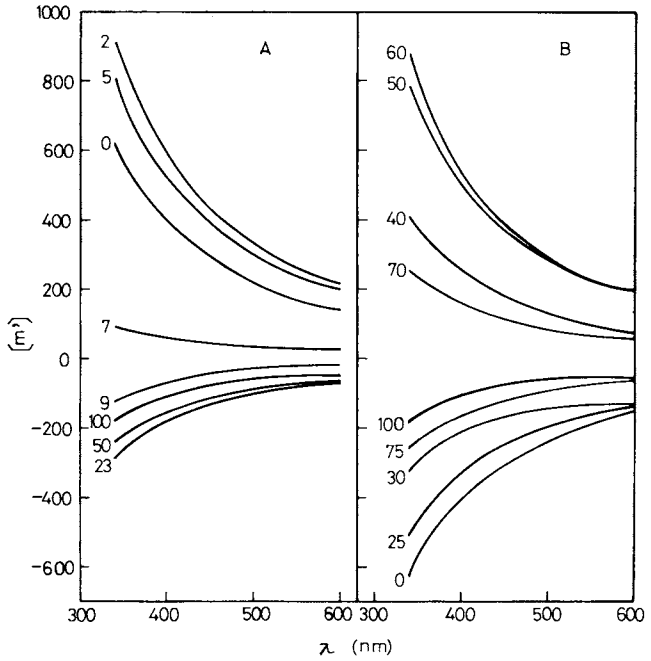


Fig. 6. ORD spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) with the change of solvent composition at 25°C: A) in chloroform/Cl_2AcOH mixed solvents; B), in dioxane/Cl_2AcOH mixed solvents. Numerals, volume percents of Cl_2AcOH in chloroform or dioxane.

chloroform or dioxane again supports the results of the CD spectra. The a_0 values changed greatly suggesting the effects of solvents.

Nmr spectra. Figure 9 shows the behavior of the chemical shift for poly(0,0′-dicarbobenzoxy-L-DOPA) with the change of solvent composition in chloroform/F_3AcOH or dioxane/F_3AcOH mixed solvents at 25°C. The addition of 4% F_3AcOH to the chloroform solution caused a sudden change of the α-CH resonance from 4.13 to 4.70 ppm. It was partly unable to measure the transition since poly(0,0′-dicarbobenzoxy-L-DOPA) decreased its solubility and precipitated at solvent compositions between 23 and 90%

TABLE I
Values of a_0 and b_0 for Poly(0,0′-Dicarbobenzoxy- L-DOPA)

Solvent	a_0 (deg cm²/dmol)	b_0
Chloroform	970	−130
Chloroform/2% Cl_2AcOH	1660	−390
Chloroform/2% F_3AcOH	1400	−220
Dioxane	−1130	220
Cl_2AcOH	−300	−20
F_3AcOH	240	20

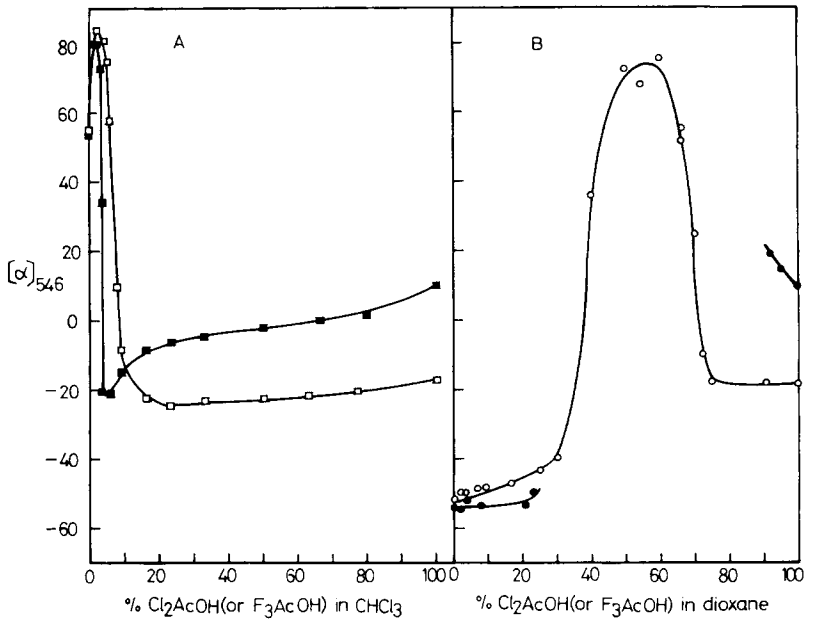


Fig. 7. Specific rotation of poly(0,0′-dicarbobenzoxy-L-DOPA) with the change of solvent composition at 25°C: A) □, in chloroform/Cl_2AcOH; ■, in chloroform/F_3AcOH; B) ○, in dioxane/Cl_2AcOH; ●, in dioxane/F_3AcOH.

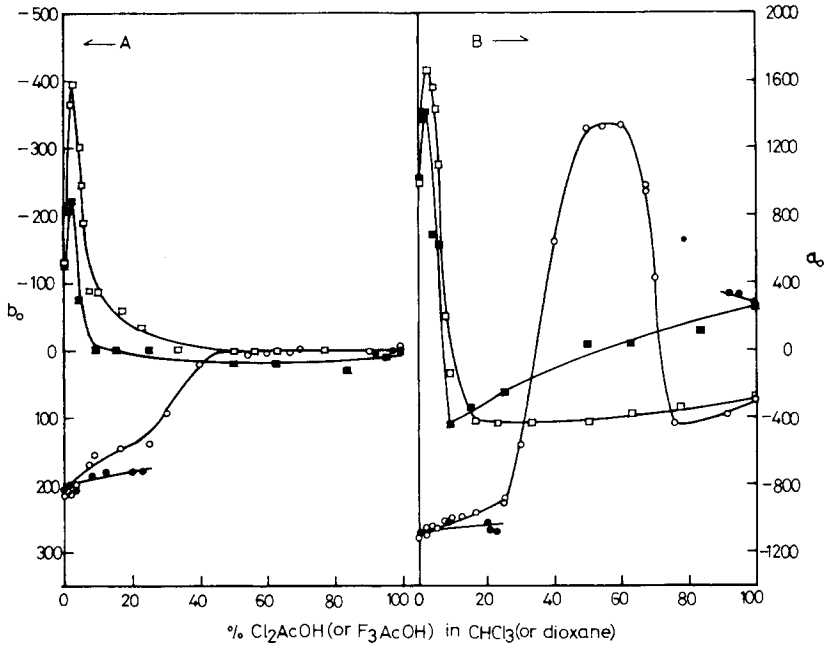


Fig. 8. A) b_0 and B) a_0 values of poly(0,0′-dicarbobenzoxy-L-DOPA) at 25°C: ○, in dioxane/Cl_2AcOH; ●, in dioxane/F_3AcOH; □, in chloroform/Cl_2AcOH; ■, in chloroform/F_3AcOH.

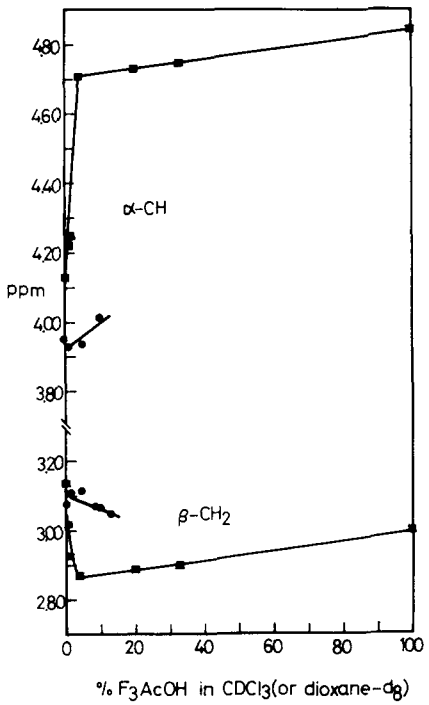


Fig. 9. Nmr peak positions of poly(0,0′-dicarbobenzoxy-L-DOPA) at 25°C: ■, in chloroform-d_1/F_3AcOH; ●, in dioxane-d_8/F_3AcOH.

F_3AcOH in dioxane (between 10 and 95% F_3AcOH in dioxane-d_8). It is clear that the sharp transition in chemical shift is paralleled by the change in conformation.

Solution ir spectra. Figure 10 shows the helix-coil transition for poly(0,0′-dicarbobenzoxy-L-DOPA) in chloroform/F_3AcOH mixed solvents. At less than 4% F_3AcOH the carbonate band at 1775 cm^{-1} and the amide I band at 1665 cm^{-1} were observed, and at more than 4% they shifted to 1785 and 1645 cm^{-1}. Thus, the conformational relationship among the rotation properties, chemical shift, and solution ir in mixed solvents is clearly demonstrated.

DISCUSSION

The ORD and CD behavior of poly(0,0′-dicarbobenzoxy-L-DOPA) in helix-promoting solvents differs completely from those of α-helical poly-(γ-benzyl-L-glutamate) and poly(ϵ-carbobenzoxy-L-lysine) in the same solvents.[13] The CD values of poly(0,0′-dicarbobenzoxy-L-DOPA) are $[\theta]_{225}$ = −13,600 ~ −18,900 in alkyl halides and $[\theta]_{228}$ = 22,100 ~ 24,800 in cyclic ethers or $(CH_3)_3P$. A model compound, *N*,0,0′-tricarbobenzoxy-L-DOPA-glycine ethyl ester, exhibited the same positive CD band with $[\theta]_{224}$

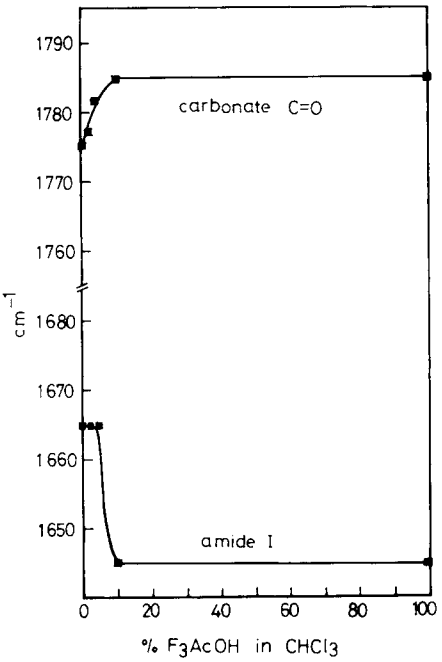


Fig. 10. Carbonate C=O stretching and amide I frequencies of poly(0,0′-dicarbobenzoxy-L-DOPA) in chloroform/F_3AcOH mixed solvents.

= 12,000 in chloroform or dioxane.[7] Thus, the CD behavior of the polypeptide is anomalous. Figure 5 shows the nmr spectra of poly(0,0′-dicarbobenzoxy-L-DOPA) in three solvents. In dioxane-d_8 solution, the α-CH resonance appears at 3.95 ppm, whereas in chloroform-d_1 this proton appears at 4.13 ppm. For left-handed helical poly(β-benzyl-L-aspartate) and right-handed helical poly(β-ethyl-L-aspartate) and copolymers of β-benzyl-L-aspartate with L-alanine, Bradbury et al.[11] reported that the α-CH peak is sensitive to conformation, being at 4.30 ppm for a left-handed helix, 4.40 ppm for a right-handed helix, and 4.85 ppm for a random coil. In another study of random copolymers of β-benzyl-L-aspartate with β-nitrobenzyl-L-aspartate by nmr,[14] the chemical shift of the α-proton was reported to locate at 4.30 and 4.36 ppm for a left-handed and a 60% right-handed α-helix, respectively. The CD investigation of the latter polypeptide agreed with the nmr result and indicated that copolymers containing less than 20-mole % nitroaromatic residues formed left-handed helices in chloroform, while those containing more than 30-mole % nitro residues formed right-handed helices.[15,16] These chemical shift and CD trends agree with those of poly(0,0′-dicarbobenzoxy-L-DOPA) described above. From the solution ir spectra of poly(0,0′-dicarbobenzoxy-L-DOPA), the frequencies of amide I and II bands are observed to be higher in dioxane than in chloroform. As summarized in Table II, the frequencies

TABLE II
Characteristic Amide Bands of Polypeptides

Polypeptide	Method	Helix Sense[a]	Amide I (cm^{-1})	Amide II (cm^{-1})	Reference
Poly(0,0′-Dicarbobenzoxy-L-DOPA)	Chloroform	R.H	1665	1545	This work
	Dioxane	L.H	1670	1550	This work
Poly(0-Carbobenzoxy-L-Tyrosine)	Film	R.H	1665	1547	This work
Poly(L-Alanine)		(L.H)[b]	(1664)	(1553)	17
	Film	R.H	1658	1548	18
Poly(γ-Benzyl-L-Glutamate)	Film	R.H	1653	1550	18,19
Poly(β-Benzyl-L-Aspartate)	Film	L.H	1666	1557	20–22
Poly(β-Methyl-L-Aspartate)	Chloroform (or Film)	L.H	1666	1557	21–23
Poly(β-Ethyl-L-Aspartate)	Film	R.H	1659	1553	21,22

[a] R.H: right-handed; L.H: left-handed.
[b] Calculated by Miyazawa et al. (Ref. 17).

for the left-handed helix are higher by 7–13 cm^{-1} for the amide I band and 4–9 cm^{-1} for the amide II band than for the right-handed helix. These higher frequencies support the normal coordinate calculations of Miyazawa et al.[17] for the differences in frequencies between the left- and right-handed helices of poly(L-alanine).

The CD, solution ir, and nmr results suggest that the conformation of poly(0,0′-dicarbobenzoxy-L-DOPA) depends greatly on the solvent taking the right-handed helical sense in alkyl halides and the left-handed helix in cyclic ethers or $(CH_3)_3P$. Though the nmr spectra are usually run at an almost tenfold higher concentration and the higher concentrations may cause an aggregation of the polypeptide, the helical sense from the nmr study of poly(0,0′-dicarbobenzoxy-L-DOPA) agrees with that obtained from the CD study as in the case of poly(β-alkyl-L-aspartates).[11,14-16] In F_3AcOH, Cl_2AcOH, or hexafluoroacetone sesquihydrate the polypeptide takes a random coil structure.

Let us compare poly(0,0′-dicarbobenzoxy-L-DOPA) with poly(0-carbobenzoxy-L-tyrosine). The conformation of poly(0-carbobenzoxy-L-tyrosine) was reported to have a right-handed α-helix and a random coil structure from the change of b_0 with copolymer composition,[9] the CD spectra in solution,[7,24,25] the α-CH chemical shift,[25] and the X-ray diffraction pattern in the solid state.[26] The CD values of poly(0-carbobenzoxy-L-tyrosine) were -6000 to ~ -8700 (a trough at about 230 nm) in helix-promoting solvents such as chloroform, dioxane, $(CH_3)_3P$, 2-chloroethanol, and 1,1,1,3,3,3-hexafluoro-2-propanol.[25] No left-handed conformation has yet been reported for poly(0-carbobenzoxy-L-tyrosine). The only difference between the two polypeptides is that poly(0-carbobenzoxy-L-tyrosine) has one less 0-carbobenzoxy group at the 3 position in the aromatic side chain. It is possible from this study to suggest that the solvent affects the delicate balance of the stacking of three bulky aromatic groups of poly(0,0′-dicarbobenzoxy-L-DOPA) and causes a reversal of the helix sense in solution.

Previously,[25,27] we have reported the helix-coil transition of poly(0-carbobenzoxy-L-tyrosine). Figures 7 and 8 show a right-handed α-helix-coil transition of poly(0,0′-dicarbobenzoxy-L-DOPA) in chloroform/Cl_2AcOH (or F_3AcOH) mixed solvents with midpoint at 8% (or 4%) Cl_2AcOH (or F_3AcOH). For comparison the transition point for poly(0-carbobenzoxy-L-tyrosine) was located at 9% (or 3%) Cl_2AcOH (or F_3AcOH) in the same mixed solvents. Thus, the introduction of one more 0-carbobenzoxy group to the aromatic group in the side chain shows no influence on the helical stability in chloroform/Cl_2AcOH mixed solvents at 25°C. On the other hand, unlike poly(0-carbobenzoxy-L-tyrosine), which showed a right-handed helix-coil transition in dioxane/Cl_2AcOH (or F_3AcOH) mixtures with midpoint at 62% (or about 35%) Cl_2AcOH (or F_3AcOH), the rotation of poly(0,0′-dicarbobenzoxy-L-DOPA) changed anomalously from *levo* (in dioxane) to *dextro* (in 50% Cl_2AcOH), then from *dextro* to *levo* (above 70% Cl_2AcOH) again, and finally to a constant *levo* rotation (in

Cl_2AcOH) (see Figs. 6 and 7). The specific rotation in a 1:1 dioxane/Cl_2AcOH mixed solvent, about 75°, was in fair agreement with the value of 83° in a chloroform/2% Cl_2AcOH mixed solvent. This may lead to the simplest explanation that the addition of Cl_2AcOH induced two kinds of transitions; firstly from a left-handed helix to a right-handed helix and then from a right-handed helix to a random coil structure. One such transition was the case of poly([Asp(OEt)]^{60} [Asp(OBzl)]^{40}) in chloroform/Cl_2AcOH mixed solvents.[11] However, the possibility was excluded by the b_0 value of zero near 50% Cl_2AcOH in dioxane/Cl_2AcOH mixed solvents (see Fig. 8). The chemical shift in a 1:1 dioxane-d_8/Cl_2AcOH mixed solvent did not show a typical α-CH peak to characterize the conformation. The a_0 values were observed to behave parallel to the change of specific rotation with the left-handed helix-coil transition of poly(0,0′-dicarbobenzoxy-L-DOPA) upon addition of Cl_2AcOH. From these results we consider two conformational possibilities due to the stacking of three aromatic rings: i) an unknown helical conformation (from the specific rotation); and ii) an ordered coil structure (from the a_0 and b_0 values). A β-structure was excluded by the ir result (1673 and 1560 cm^{-1} for the precipitated sample from a 1:1 dioxane/Cl_2AcOH mixed solution). Though we feel that the results of our study do not provide sufficient evidence to explain how solvent interacts, in any case, solvent effects should be emphasized here. Unfortunately, full rotation and nmr data of poly(0,0′-dicarbobenzoxy-L-DOPA) in dioxane [or Hyfuran, $(CH_3)_3P$]/F_3AcOH or dioxane [or Hyfuran, $(CH_3)_3P$]/hexafluoroacetone sesquihydrate mixed solvents are not available since the polypeptide precipitates in these mixed solvents. In addition, the polypeptide has less solubility in deuterated solvents such as dioxane-d_8 and hexafluoroacetone sesquideuterate.

Finally, to study further the anomalous rotation behavior of poly(0,0′-dicarbobenzoxy-L-DOPA) in solution, especially in dioxane or dioxane/Cl_2AcOH (or F_3AcOH) mixed solvents, the syntheses and conformational studies of i) random copolymers of 0,0′-dicarbobenzoxy-L-DOPA with γ-benzyl-L- (or D-)-glutamate to increase the solubility; and ii) poly(0,0′-dimethyl-L-DOPA) to study the aromatic effect are now in progress.

References

1. Funk, C. (1911) *J. Chem. Soc.* **99,** 554–557.
2. Torquati, T. (1913) *Arch. Farmacol. Sper.* **15,** 308–312.
3. Guggenheim, Z. (1913) *Z. Physiol. Chem.* **88,** 276–284.
4. Harington, C. R. & Randall, S. S. (1931) *Biochem. J.* **25,** 1028–1036.
5. Harwood, H. J. & Cassidy, H. G. (1957) *J. Amer. Chem. Soc.* **79,** 4360–4365.
6. Felix, A. M., Winter, D. P., Wang, S.-S., Kulesha, I. D., Pool, W. R., Hane, D. L. & Sheppard, H. (1974) *J. Med. Chem.* **17,** 422–426.
7. Yamamoto, H. & Hayakawa, T. (1976) *Macromolecules* **9,** 532–534.
8. Blout, E. R. & Karlson, R. H. (1956) *J. Amer. Chem. Soc.* **78,** 941–946.
9. Vollmer, J.-P. & Spach, G. (1967) *Biopolymers* **5,** 337–349.
10. Sela, M. & Berger, A. (1955) *J. Amer. Chem. Soc.* **77,** 1893–1898.

11. Bradbury, E. M., Carpenter, B. G., Crane-Robinson, C. & Goldman, H. (1971) *Macromolecules* **4,** 557–564.

12. Moffitt, W. & Yang, J. T. (1956) *Proc. Natl. Acad. Sci. U.S.* **42,** 596–603.

13. Yang, J. T. (1967) in *Poly-α-Amino Acids,* Fasman, G. D., Ed., Dekker, New York, p. 239.

14. Loucheux-Lefebvre, M.-H., Forchioni, A. & Duflot, C. (1974) *Polymer* **15,** 474–478.

15. Toniolo, C., Falxa, M. L. & Goodman, M. (1968) *Biopolymers* **6,** 1579–1603.

16. Loucheux-Lefebvre, M.-H., Duflot, C. & Weill, G. (1975) *Biopolymers* **14,** 469–484.

17. Miyazawa, T., Fukushima, K. & Sugano, S. (1967) in *Conformation of Biopolymers,* Ramachandran, G. N., Ed., Academic, New York, Vol. 2, p. 557.

18. Bamford, C. H., Elliott, A. & Hanby, W. E. (1956) *Synthetic Polypeptides,* Academic, New York, p. 143.

19. Tsuboi, M. (1962) *J. Polym. Sci.* **59,** 139–153.

20. Bradbury, E. M., Brown, L., Downie, A. R., Elliott, A., Hanby, W. E. & McDonald, T. R. R. (1959) *Nature* **183,** 1736–1737.

21. Bradbury, E. M., Carpenter, B. G. & Goldman, H. (1968) *Biopolymers* **6,** 837–850.

22. Bradbury, E. M., Carpenter, B. G. & Stephens, R. M. (1968) *Biopolymers* **6,** 905–915.

23. Goodman, M., Boardman, F. & Listowsky, I. (1963) *J. Amer. Chem. Soc.* **85,** 2491–2497.

24. Goodman, M., Toniolo, C. & Peggion, E. (1968) *Biopolymers* **6,** 1691–1695.

25. Yamamoto, H. (1976) *Polymer* **17,** 1105–1107.

26. Del Pra, A. & Gilli, G. (1975) *Biopolymers* **14,** 1769–1772.

27. Yamamoto, H. & Hayakawa, T. (1976) *Polymer Preprints, Japan* **25,** 257 (in Japanese).

Received June 30, 1976
Accepted November 10, 1976