Synthesis and conformational study of poly(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
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High molecular weight poly(L-β-3,4-dihydroxyphenyl-α-alanine) has been synthesized. Both optical rotatory dispersion and circular dichroism spectra of the polypeptide are anomalous and give little information about its conformation. From the total results obtained by a study of the optical rotation, nuclear magnetic resonance and infra-red absorption, poly(L-β-3,4-dihydroxyphenyl-α-alanine) is most probably right-handed helical in trimethyl phosphate, methanol or water/trimethyl phosphate (1:1 v/v) mixed solvents below pH 10.4. It is in the random coil structure in dimethyl sulphoxide or water/trimethyl phosphate mixed solvents above pH 11. The transition midpoint is pH 10.6 in water/trimethyl phosphate mixed solvents. The results were compared with those of poly(L-tyrosine).

INTRODUCTION

The conformation of poly(L-tyrosine) (polyTyr) has been widely studied in recent years by a variety of physical methods including ultra-violet absorption (u.v.), optical rotatory dispersion (o.r.d.), circular dichroism (c.d.), infra-red absorption (i.r.), nuclear magnetic resonance (n.m.r.), ultracentrifugation, calorimetric measurement, light scattering and potentiometric titration[1–20]. In solvents such as aqueous sodium chloride (below pH 11.5), methanol, dimethylformamide, trimethyl phosphate [$(CH_3O)_3PO$], quinoline, water/ethanol mixed solvents, dimethyl sulphoxide (Me_2SO)/dichloroacetic acid (DCA) mixed solvents, Me_2SO/D_2O mixed solvents and $Me_2SO/(CH_3O)_3PO$ mixed solvents polyTyr assumes an α-helical conformation[1,3,4,6,10,11,19,20]. In strongly basic aqueous solution or Me_2SO polyTyr is a random coil[2–5,9,12,13,15–17,19,20]. If the pH is lowered very slowly, a transition from coil to antiparallel β-conformation is observed in water and in the prevailing aqueous region[9,12,13,17,18].

Optically inactive poly(DL-β-3,4-dihydroxyphenyl-α-alanine), which has one more hydroxyl group at the 3-position in the aromatic side-chain than poly(DL-tyrosine), has been synthesized by Harwood and Cassidy[21]. However, poly(L-β-3,4-dihydroxyphenyl-α-alanine) (polyDopa) has not yet been reported. Previously, we have reported the synthesis and conformational study of poly(*O,O'*-dicarbobenzoxy-Dopa)[22,23]. In the present paper we report the synthesis and conformational study of polyDopa. The conformation and the helix–coil transition of polyDopa in pure and mixed solvents were followed by u.v., o.r.d., c.d., i.r. and n.m.r. measurements. Although we were not able to solve completely the helical sense of polyDopa, we think that our preliminary experimental data for the polypeptide contribute to the understanding of its complicated conformation and conformational transition.

EXPERIMENTAL

Materials

PolyDopa was prepared by polymerization of *O,O'*-dicarbobenzoxy-Dopa *N*-carboxyanhydride[22–24] and subsequent removal of the protecting group by the following two kinds of reagents[21]. (i) Alcoholic sodium hydroxide; yield, 95%. Calculated for $(C_9H_9O_3N)_n$: C = 60.33%; H = 5.06%; N = 7.82%. Found: C = 60.35%; H = 5.06%; N = 7.56%. (ii) 2N hydrogen bromide in a 1:2 dioxane/glacial acetic acid mixed solvent; yield, theoretical. Found: C = 60.42%; H = 5.08%; N = 7.47%.

The degree of polymerization (*DP*) of polyDopa was estimated to be 60 from the intrinsic viscosity (0.26 dl/g) of the starting poly(*O,O'*-dicarbobenzoxy-Dopa) in DCA at 25°C[23]. PolyDopa (*DP* = 60) is soluble in water (above pH 10.5), $(CH_3O)_3PO$, Me_2SO or pyridine. It is insoluble in water (below pH 10.5), chloroform, acetic acid, dioxane, 2-chloroethanol, DCA, trifluoroacetic acid, trifluoroethanol, hexafluoroacetone sesquihydrate, hexamethyl phosphoramide or hexafluoro-2-propanol. The low molecular weight sample (*DP* = 35) is soluble in methanol, ethanol, formic acid or dimethylformamide.

PolyDopa (*DP* = 60), which was prepared by use of anhydrous hydrogen bromide, was used throughout the measurements except the measurements in methanol. The polypeptide in water at high alkaline pH is rapidly oxidized into a deep red coloured polyquinone-type derivative and is difficult to measure its spectroscopic properties. $(CH_3O)_3PO$ was added to suppress an oxidation reaction.

The deuterated compounds such as Me_2SO-d_6, CD_3OD, D_2O and NaOD were Merck, Sharp and Dohme products and were used directly from fresh ampoules or bottles.

Methods

U.v. spectra were measured on a Shimadzu recording spectrophotometer UV-200. O.r.d., c.d. and i.r. spectra were measured on an ORD/UV 5 instrument with a c.d. 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 0.10–0.40% range. The experimental data were expressed in terms of specific rotation [α], reduced mean residue rotation [*m'*] (degree cm^2/dmol) for o.r.d., mean

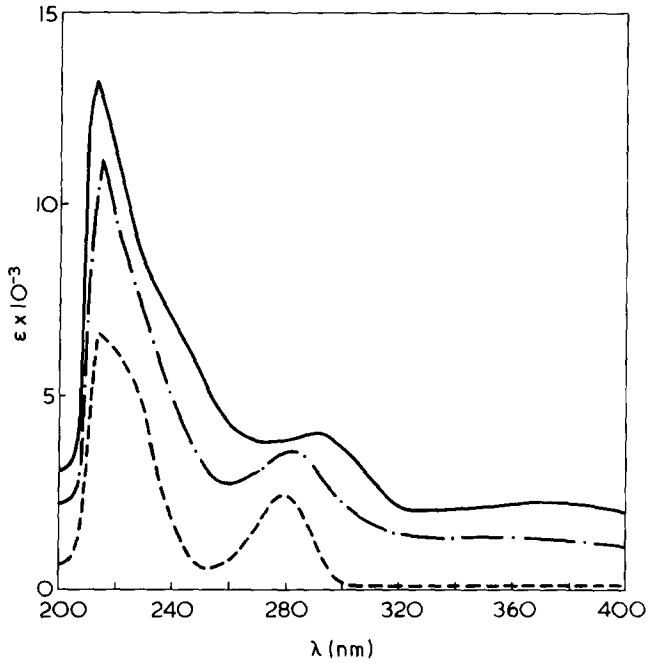

Figure 1 U.v. spectra of polyDopa, *DP* = 60, in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) at 25°C: – – – –, at pH 3.2; –·–·–, at pH 10.7; ——, at pH 13.2

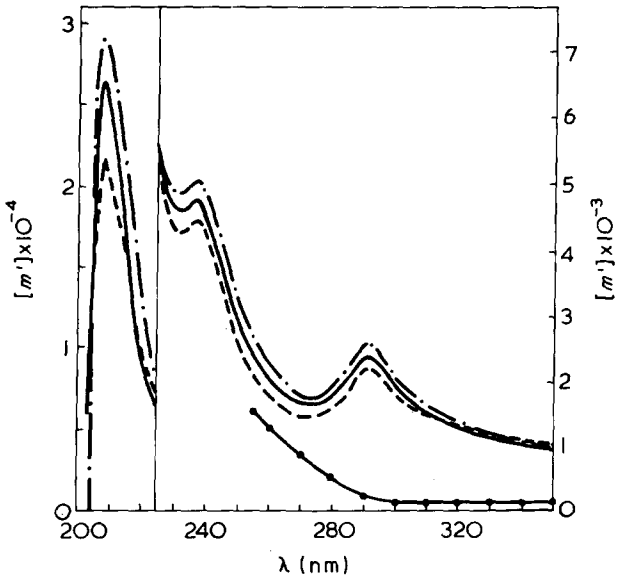

Figure 2 O.r.d. spectra of polyDopa, *DP* = 60, in the ultra-violet region at 25°C: ——, in $(CH_3O)_3PO$; –·–·–, in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v); ●, in Me_2SO; – – – –, in methanol (*DP* = 35)

residue ellipticity $[\theta]$ (degree cm^2/dmol) for c.d. or molar extinction coefficient ϵ for u.v. The parameters, a_0 and b_0, derived from the Moffitt–Yang equation were calculated from the o.r.d. curves using $\lambda_0 = 212$ nm as in the case of polyTyr[1,3,9,25]. The n.m.r. spectra were recorded on a Jeol 60-MHz JNM-c-60HL spectrometer and peak positions were measured relative to internal tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulphonate and the polypeptide concentrations were in the 1–2% range. pD was considered to be equal to the apparent pH value + 0.4 unit[26].

RESULTS

In order to avoid a rapid oxidation by air and to increase the solubility, we chose a 1:1 water/$(CH_3O)_3PO$ mixed solvent to measure the optical properties. The u.v. spectra of polyDopa in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) mixed solvents at pH 3.2, 10.7 and 13.2 are shown in *Figure 1*. As shown below, pH 10.7 is near the transition point of a charge-induced conformational transition. PolyTyr was reported to have $\epsilon_{277} = 1610$ and $\epsilon_{282} = 1080$ at pH 11.2 (mostly unionized phenolic group) and $\epsilon_{294} = 2250$ at pH above 13 (ionized)[3]. The u.v. behaviour of polyDopa is much simpler. The ϵ values for polyDopa are $\epsilon_{280} = 2400$ at pH 3.2, $\epsilon_{285} = 3350$ at pH 10.7 and $\epsilon_{287} = 3950$ at pH 13.2.

Figure 2 shows the o.r.d. spectra in organic solvents such as methanol, $(CH_3O)_3PO$ and Me_2SO. Approximately, the same o.r.d. spectrum to 200 nm is obtained in methanol, $(CH_3O)_3PO$ or 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v). Multiple Cotton effects are seen in this wavelength range. A positive Cotton effect with a maximum at 290 nm is observed with a mean residue rotation $[m']_{290} = 2500$ in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v). The second positive peak is observed at 238 nm with $[m']_{238} = 5000$. The third large positive Cotton effect is observed at 208 nm with $[m']_{208} = 28\,000$. A different spectrum is observed in Me_2SO. In Me_2SO the measurement at lower wavelengths could not be made owing to high absorption of the solvent.

Figure 3 shows the effect of pH on the three Cotton effects of polyDopa in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v). The change in o.r.d. curves in going from pH 3.4–13.5 represents probably a helix–coil transition. The positive peak at 290 nm remains relatively unchanged on raising the pH from 3.4 to 10.4. However, at pH 10.6 the peak shifts to 295 nm and the magnitude lowers. Above pH 11.5 the position shifts to 300 nm and the magnitude is much smaller ($[m']_{300} = 850$ at pH 13.5). The second positive peak at 238 nm diminishes a little at pH 10.4 and disappears

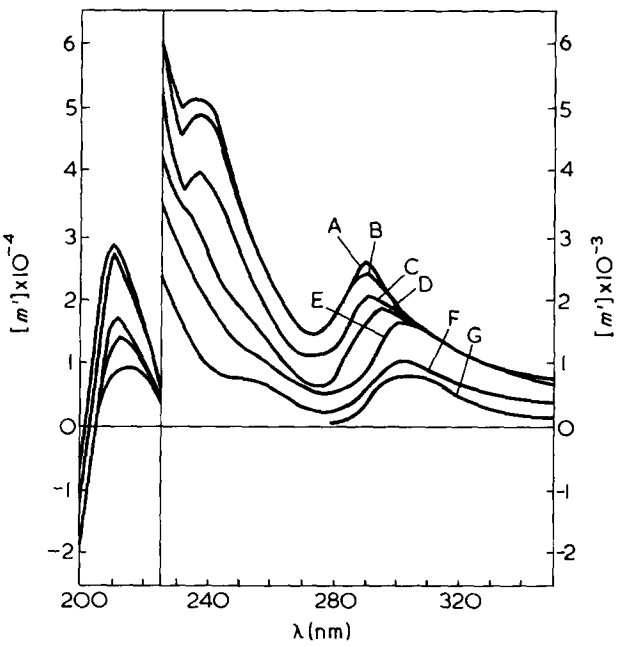

Figure 3 O.r.d. spectra of polyDopa, *DP* = 60, as a function of pH in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) at 25°C. A, pH 3.4; B, pH 8.5; C, pH 10.4; D, pH 10.6; E, pH 11.5; F, pH 11.8; G, pH 13.5

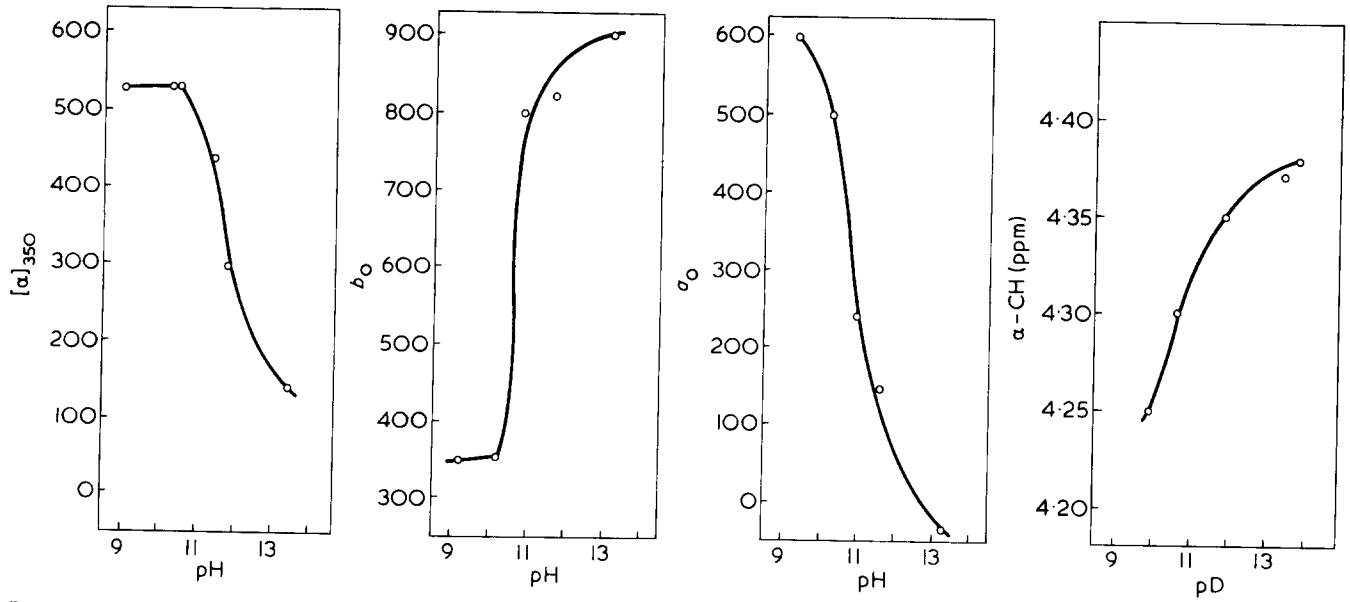

Figure 4 Specific rotation, b_0, a_0 and α-CH chemical shift values of polyDopa, *DP* = 60, as a function of pH (or pD) at 25°C: $[\alpha]_{350}$, b_0 and a_0 in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) and α-CH in D_2O

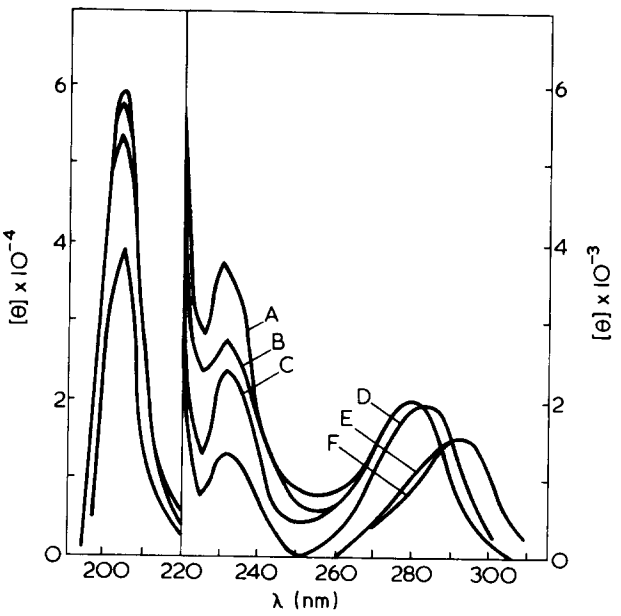

Figure 5 C.d. spectra of polyDopa, *DP* = 60, as a function of pH in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) at 25°C. A, pH 3.4; B, pH 8.5; C, pH 10.4; D, pH 10.6; E, pH 11.5; F, pH 13.5

above pH 10.6. The peak of the Cotton effect at 208 nm decreases gradually with increasing pH and cannot be measured above pH 11.5 owing to high absorption.

Figure 4 shows the a_0 and b_0 values, the specific rotation and the α-CH chemical shift for polyDopa. The b_0 values of the polypeptide in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) mixed solvents were calculated for the 400–600 nm wavelengths and were 350 at pH 3.4, 360 at pH 10.3 and 900 at pH 13.5. *Figure 4* shows also a plot of $[\alpha]_{350}$ values *versus* pH in the same mixed solvents. The transition as indicated by the two parameters b_0 and $[\alpha]_{350}$ is identical. The helix–coil conformational transition depends on the degree of ionization as in the case of polyTyr.

Figure 5 shows the pH dependence of the c.d. spectra for polyDopa in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) mixed solvents. The spectra comprise three dichroic bands at 280–295, 230 and 204 nm. Like polyTyr, the assignment of the three dichroic bands of polyDopa is very difficult. In this case, the sign and magnitude differs completely from poly(L-glutamic acid) and poly(L-lysine) which are typically right-handed α-helices[27]. As anticipated from the o.r.d. data, the positive dichroic band at 280 nm remains unchanged on raising the pH from 3.4 to 10.4. At pH 10.6 the band at 280 nm shifts to 285 nm and above pH 11.5 it shifts to 295 nm. The second and third bands do not shift but decrease their ellipticities on raising the pH.

Additional information on the conformational behaviour of the polypeptide was obtained from n.m.r. study. *Figure 6* shows the n.m.r. spectra of polyDopa. In Me_2SO-d_6 or at pD 13.5 in D_2O the peak assignments are 7.93 ppm NH, 6.52 ppm aromatic H, 4.36 ppm α-CH and 2.69 ppm β-CH_2 protons. It is apparent that the spectrum is very sharp in these two solvents. In CD_3OD or at pD 10.0 in D_2O several changes in the spectrum are observed. Firstly, the amide proton NH peak becomes too broad to be observed. Secondly, several peaks show a transition of the chemical shift which parallels the change of optical rotation. On lowering the pD from 13.5 to 10.0 the α-CH peak shifts upfield by 0.12 ppm. Thirdly, the β-CH_2 protons shift from 2.69 ppm (at pD 13.5) to 2.85 ppm (at pD 10.0).

Table 1 shows the 1600–1700 cm^{-1} region of i.r. spectra of polyDopa in Me_2SO-d_6, $(CH_3O)_3PO$, CD_3OD and $D_2O/(CH_3O)_3PO$ mixed solvents (1:1 v/v). Like polyTyr, the amide I frequency is unusually high. The amide I band appears at 1665 cm^{-1} in $(CH_3O)_3PO$ or CD_3OD and the conformation of polyDopa can be assumed to be helical in these solvents. The same band of the spectrum in Me_2SO-d_6 lies at 1670 cm^{-1} and the conformation can be assumed to be a random coil structure. The i.r. spectra in $D_2O/(CH_3O)_3PO$ mixed solvents (1:1 v/v) were measured. Below pD 10.0, the amide I band of polyDopa appears at 1665 cm^{-1}. Above pD 11.0, the same band lies at 1670 cm^{-1}. At pD 10.5, the polypeptide assumes two different conformations showing the amide I band at 1670 and 1665 cm^{-1}.

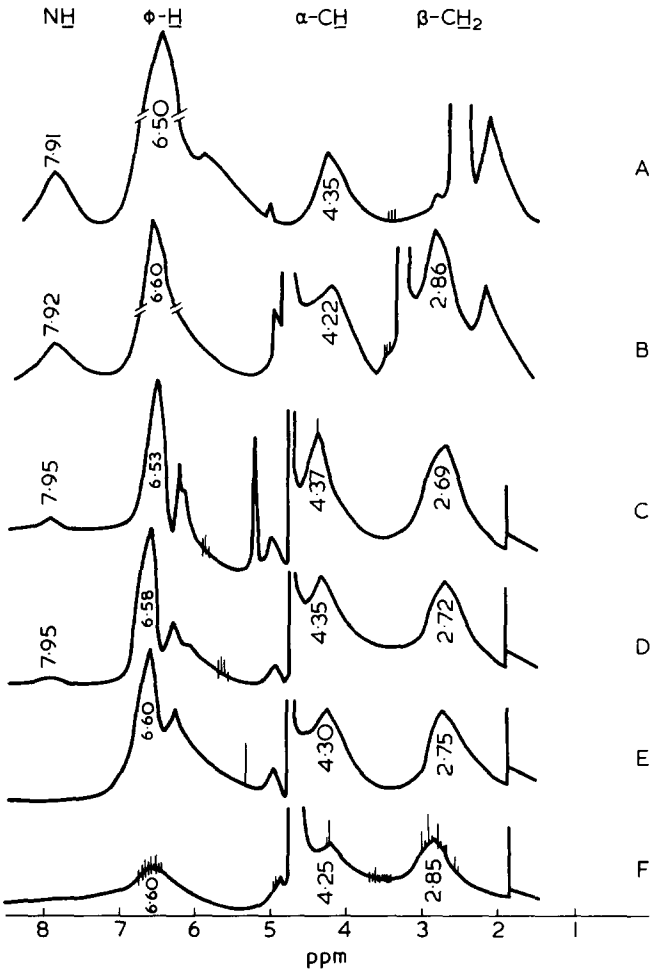

Figure 6 N.m.r. spectra of polyDopa, *DP* = 60, in Me_2SO-d_6, D_2O (at pD 10.0, 10.7, 12.0 and 13.5) and CD_3OD (*DP* = 35). A, Me_2SO-d_6; B, CD_3OD; C, pD 13.5; D, pD 12.0; E, pD 10.7; F, pD 10.0

DISCUSSION

The interpretation of the anomalous o.r.d. and c.d. behaviour of newly synthesized polyDopa requires further experimental and theoretical evidence. The basic question concerns the meaning of the three positive ellipticity bands observed in the c.d. spectrum in the ultra-violet region. At least two dichroic bands overlap in the ultra-violet region and we do not know how the side-chain band shifts when the polypeptide assumes the ordered conformation. The pure n–π^* peptide contribution cannot be separated. In the case of the absence of chromophores other than the peptide group in this region the n–π^* band at 222 nm immediately related to the α-helix conformation and the helical sense and content can be assigned. But in polyDopa it is evident that the experimental results cannot allow any conformation to be attributed since the n–π^* transition involves the contribution of the ^1L_a and ^1L_b transitions of the substituted benzenes in the Platt notation[28–30].

If we follow a conformational assignment for polyTyr first done by Beychok and Fasman[4] and assign the 230 nm c.d. band and the 205 nm c.d. band to the n-π^* transition and the parallel-polarized π-π^* exciton transition of the peptide group, the conformation of polyDopa could be assumed to be a left-handed helical conformation. However, this approach is very doubtful since the o.r.d. spectra in *Figures 2* and *3* show a positive 208–210 nm peak. If polyDopa does take a left-handed conformation, the peak should be negative. Indeed the o.r.d. spectrum of right-handed helical polyTyr showed a large positive peak at 208 nm in $(CH_3O)_3PO$. Furthermore, if we follow an assignment based on the b_0 value, polyDopa takes a left-handed helical structure again since the b_0 values of polyDopa are 360 at pH 10.3 and 900 at pH 13.5 (*Figure 4*) and those of polyTyr are 540–630 below pH 11.4 and 410–430 above pH 11.6[1,3,9]. Clearly, the 290 nm (o.r.d.) and the 280 nm (c.d.) peaks of polyDopa are conformation-dependent. These o.r.d. and c.d. data suggest that polyDopa is in the random coil form in water/$(CH_3O)_3PO$ (1:1 v/v) mixed solvents above pH 11 or Me_2SO, while the polypeptide is most probably helical in $(CH_3O)_3PO$, methanol or water/$(CH_3O)_3PO$ mixed solvents below pH 10.4. The transition midpoint is pH 10.6 in 0.2 M sodium chloride/$(CH_3O)_3PO$ (1:1 v/v) mixed solvents. We cannot decide the helical sense from the measurement of optical property.

Infra-red spectroscopy has been of increasing use in conformational studies of polypeptide and, especially, the conformation of polyTyr in solution and in the solid state has extensively been studied by the i.r. method (see *Table 1*)[9–13,15,19]. I.r. results can give an indication of helix sense[15,22,23,31–33]. The frequencies 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 those for the right-handed helix. As shown in *Table 1*, the amide I frequency of helical polyDopa lies at 1665 cm^{-1} suggesting a left-handed helical sense. However, the question arises as to the helix sense. In our previous papers we have reported that the solution i.r. spectra of poly(*O*,*O*′-dicarbobenzoxy-Dopa), which is the starting polypeptide to prepare polyDopa, show unusually high amide I frequencies: at 1665 cm^{-1} in chloroform (right-handed helix) and at 1670 cm^{-1} in dioxane (left-handed helix)[22,23]. Such high frequency is also true for right-handed α-helical poly(*O*-carbobenzoxy-L-tyrosine) showing the band at 1665 cm^{-1} [23]. Thus, polyDopa is most probably right-handed helical on the basis of its chemically related aromatic polypeptides.

Additional information on the conformational behaviour of polyDopa has been obtained from n.m.r. studies. The 60 MHz spectra in Me_2SO-d_6, CD_3OD or D_2O are shown in *Figure 6*. As the pD is varied through the helix–coil transition region, the α-CH peak shifts, broadens, weakens and finally disappears. An upfield shift of the α-CH peak from 4.37 ppm at pD 13.5 to 4.25 ppm at pD 10.0 is observed. Shift, broadening and loss of area occur also for the side-chain methylene protons but are not marked as for the α-carbon proton. Such peak broadening and loss of area are qualitatively similar to those shown by poly(L-lysine) and

Table 1 Characteristic amide band of polypeptides

Polypeptide	Conformation	Amide I (cm^{-1})	Solvent	Reference
PolyDopa	Helix	1665	$(CH_3O)_3PO$	This work
		1665	CD_3OD	This work
		1665	pD 3.0	This work
		1665,1670	pD 10.5[a]	This work
	Random coil	1670	Me_2SO-d_6	This work
		1670	pH 13.5	This work
PolyTyr	Helix	1657	$(CH_3O)_3PO$	9,11,15
	Random coil	1663	Me_2SO	10,15,19

[a] Mixture of both forms

poly(L-glutamic acid)[34]. As the helix content increases over the transition, upfield shifts of 0.10 ppm are observed for the α-CH peak of poly(L-lysine) (from 4.35 ppm at pD 3.7 to 4.25 ppm at pD 13) and poly(L-glutamic acid) (from 4.30 ppm at pD 5.75 to 4.20 ppm at pD 4.4). Thus, the n.m.r. behaviour of polyDopa in the region of the conformational transition is similar to the right-handed α-helix random coil transition.

In summary, both o.r.d. and c.d. spectra of polyDopa are very anomalous and give little information about its conformation. PolyDopa behaves like polyTyr, even though there are some differences between the experimental results for the two polypeptides. The spectroscopic evidence for polyDopa strongly indicates that the transition from the helix to a random coil occurs. From the i.r. frequencies for the amide I band and the n.m.r. α-CH chemical shifts, the helical sense is most probably right-handed. As found for copolymers of β-benzyl-L-aspartate with γ-benzyl-L-glutamate and of L-tyrosine with L-glutamic acid[2,35], the copolymer study now in progress will solve the screw sense of polyDopa.

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