Studies of polymers and copolymers of *O,O'*-dicarbobenzoxy-L-β-3,4-dihydroxyphenyl-L-alanine with γ-benzyl-L- and D-glutamates

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The synthesis of ten different copolymers of *O,O'*-dicarbobenzoxy-L-β-3,4-dihydroxyphenyl-α-alanine (*O,O'*-dicarbobenzoxy-L-Dopa) and γ-benzyl-L- and D-glutamates with degree of polymerization of 40–270 is described. It has been concluded that poly(*O,O'*-dicarbobenzoxy-L-Dopa) and poly(γ-benzyl-L-glutamate) exist as helices with the opposite sense of twist in dioxane on the basis of the following observations. The copolymers of *O,O'*-dicarbobenzoxy-L-Dopa with γ-benzyl-L-glutamate show a linear variation of circular dichroism properties with composition in methylene dichloride, whereas the copolymers do not in dioxane. The results for the copolymers with γ-benzyl-D-glutamate are the reverse in dioxane and methylene dichloride.

INTRODUCTION

In our previous papers we have reported the synthesis and conformational study of poly(*O,O'*-dicarbobenzoxy-L-β-3,4-dihydroxyphenyl-α-alanine) [poly(*O,O'*-dicarbobenzoxy-L-Dopa)][1,2]. The conformation of poly(*O,O'*-dicarbobenzoxy-L-Dopa) is anomalous and depends greatly on the solvent, taking the right-handed helical sense in alkyl halides (chloroform and methylene dichloride) and the left-handed helix in cyclic ethers (tetrahydrofuran and dioxane) or trimethyl phosphate. To study further the anomalous rotation behaviour of poly(*O,O'*-dicarbobenzoxy-L-Dopa) in solution, we widened the experiments on the polypeptide to include γ-benzyl-L- and D-glutamates. In this paper, we report the synthesis and conformational study of a series of random copolymers of *O,O'*-dicarbobenzoxy-L-Dopa with γ-benzyl-L- and D-glutamates. The conformation of the copolymers was studied by circular dichroism (c.d.) and infra-red absorption (i.r.) measurements.

EXPERIMENTAL

Amino-acid N*-carboxyanhydrides (NCA)*

O,O'-Dicarbobenzoxy-L-Dopa (NCA) was prepared from *N,O,O'*-tricarbobenzoxy-L-Dopa and phosphorus pentachloride as described in the previous papers[1,2]. γ-Benzyl-L- and D-glutamate NCA compounds were prepared from the corresponding amino-acids by the usual procedure[3].

Copolymerization

NCA compounds were copolymerized at a concentration of 10% in dioxane with triethylamine as an initiator (A/I = 100). The copolymers were precipitated with water, filtered and dried. The results were shown in *Table 1*.

Table 1 Molecular weight for copolymers of *O,O'*-dicarbobenzoxy-L-Dopa with γ-benzyl-L- and D-glutamates

Glutamate content (mol %)	*O,O'*-Dicarbobenzoxy -L-Dopa NCA (mg)	(mmol)	γ-Benzyl-L-(or D-) glutamate NCA (mg)	(mmol)	Yield (%)	$[\eta]^{25}_{DCA}$	Degree of polymerization	
L-ester:								
0	4730	9.62	–	–	64.7	0.26	60[b]	
9	2460	5.00	132	0.50	70.0	0.20	40	
13	2460	5.00	197	0.75	78.0	0.26	60	
25	2460	5.00	439	1.67	84.3	0.24	60	
50	2460	5.00	1320	5.00	78.9	0.44	170	200[c]
50[a]	2460	5.00	1320	5.00	86.8	0.25	75	105
75	1230	2.50	1980	7.50	86.3	0.45		270
100	–	–	12 900	49.0	95.9	0.64		470
D-ester:								
5	2460	5.00	66	0.25	63.8	0.25	55	
9	2460	5.00	132	0.50	75.9	0.25	60	
13	2460	5.00	197	0.75	75.6	0.23	50	
50	2460	5.00	1320	5.00	87.5	0.26	80	110
75	1230	2.50	1980	7.50	80.0	0.29		150
100	–	–	2630	10.0	98.6	0.85		650

[a] Polymerized in methylene dichloride; [b] calculated from $[\eta] = 3.2 \times 10^{-2} M_w^{0,66}$; [c] calculated from $[\eta] = 2.78 \times 10^{-5} M_w^{0.87}$

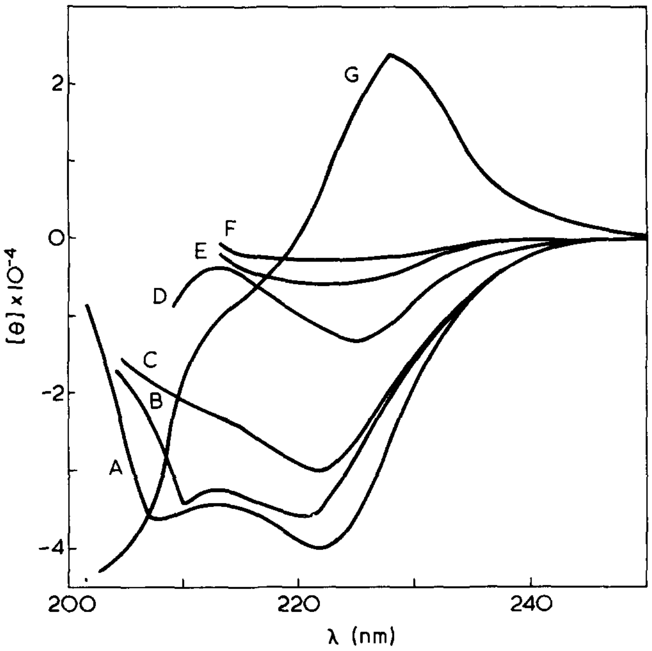

Figure 1 C.d. spectra for copolymers of *O,O'*-dicarbobenzoxy-L-Dopa with γ-benzyl-L-glutamate in dioxane at 25°C. Mol % of *O,O'*-dicarbobenzoxy-L-Dopa in copolymers: A, 0%; B, 25%; C, 50%; D, 75%; E, 87%; F, 91%; G, 100%

Methods

C.d. spectra were measured on a Jasco ORD/UV 5 instrument with a c.d. attachment. Under constant nitrogen flush, cells with path lengths of 0.1–10 mm were used. The concentrations of the samples for c.d. measurements were in the 0.19–0.42% range. The experimental data were expressed in terms of mean residue ellipticity [θ] (degree cm^2/dmol). I.r. spectra were measured on a Jasco IR DS–301 instrument. For the i.r. measurements, the film samples of the copolypeptides were cast from the solution in dioxane.

The intrinsic viscosities were measured in dichloroacetic acid (DCA) at 25°C using an Ubbelohde viscometer. The molecular weights were estimated from empirical equations: $[\eta] = 3.2 \times 10^{-2} M_w^{0.66}$ for poly(*O*-carbobenzoxy-L-tyrosine)[4] and $[\eta] = 2.78 \times 10^{-5} M_w^{0.87}$ for poly(γ-benzyl-L-glutamate)[5] both measured in DCA at 25°C.

RESULTS AND DISCUSSION

The conformation of poly(*O,O'*-dicarbobenzoxy-L-Dopa) was assumed to be the right-handed helix in methylene dichloride and the left-handed helix in dioxane. In order to solve the anomalous rotation behaviour of the polypeptide in solution, we synthesized random copolymers of *O,O'*-dicarbobenzoxy-L-Dopa with γ-benzyl-L- and D-glutamates as listed in *Table 1*. If *O,O'*-dicarbobenzoxy-L-Dopa and γ-benzyl-L-glutamate are helices of the opposite sense of twist, the optical rotation (circular dichroism) of these copolymers should change markedly over a relatively narrow range of copolymer composition, representing a region of transition from one helix sense to the other. As is well known, the helical senses of poly(β-benzyl-L-aspartate)[6], poly(*O*-carbobenzoxy-L-tyrosine)[4], poly(L-tyrosine)[7] and poly(L-tryptophan)[8] were solved by copolymer studies. Unfortunately, the molecular weights of Dopa-rich copolymers in *Table 1* were somewhat low. In general, however,, the degree of polymerization of 40–60 is high enough to measure the optical properties and to discuss the results obtained[9].

Figure 1 shows the c.d. spectra of homo- and copolymers in dioxane. In dioxane, the molar ellipticity value of poly(*O,O'*-dicarbobenzoxy-L-Dopa) is $[\theta]_{228} = 24\,000$ and that of poly(γ-benzyl-L-glutamate) is $[\theta]_{222} = -40\,000$ and $[\theta]_{207} = -36\,000$. Copolymer with 9 mol % γ-benzyl-L-glutamate inverts the ellipticity from positive to negative. The negative ellipticity increases with increasing γ-benzyl-L-glutamate content. Copolymer containing 50 mol % γ-benzyl-L-glutamate enhances the helicity. Copolymer containing 75 mol % γ-benzyl-L-glutamate shows a spectrum of almost right-handed α-helix; that is $[\theta]_{221} = -36\,000$ and $[\theta]_{210} = -34\,000$. These results suggest that the left-handed helix of poly(*O,O'*-dicarbobenzoxy-L-Dopa) is very unstable and that a small amount of γ-benzyl-L-glutamate residue destroys the left-handed helix of poly(*O,O'*-dicarbobenzoxy-L-Dopa).

Figure 2 shows the c.d. spectra of homo- and copolymers in methylene dichloride. In methylene dichloride, the molar ellipticity value of poly(*O,O'*-dicarbobenzoxy-L-Dopa) is $[\theta]_{225} = -18\,900$ and that of poly(γ-benzyl-L-glutamate) is $[\theta]_{222} = -40\,000$. The results in *Figure 2* are easy to understand: the negative ellipticity increases to –40 000 with increasing γ-benzyl-L-glutamate content. Both in methylene dichloride and dioxane, the c.d. spectra of 50 mol % copolymer polymerized in methylene dichloride are approximately the same in magnitude to the copolymer polymerized in dioxane.

Figure 3 shows the dependence of $[\theta]_{222}$ and $[\theta]_{228}$ on the composition of copolymers of *O,O'*-dicarbobenzoxy-L-Dopa with γ-benzyl-L-glutamate (from *Figures 1* and *2*). The ellipticity values of the copolymers in methylene dichloride are linear. The linear relationship between the [θ] values and composition indicates that poly(*O,O'*-dicarbobenzoxy-L-Dopa) in methylene dichloride has the same helical sense as that of poly(γ-benzyl-L-glutamate) which is known to exist as a right-handed α-helix[10]. On the other hand, the ellipticity values of copolymers in dioxane are non-linear and markedly dependent upon the composition of copolymers, particularly above 75 mol % *O,O'*-dicarbobenzoxy-L-Dopa. This implies that *O,O'*-dicarbobenzoxy-L-Dopa and γ-benzyl-L-glutamate

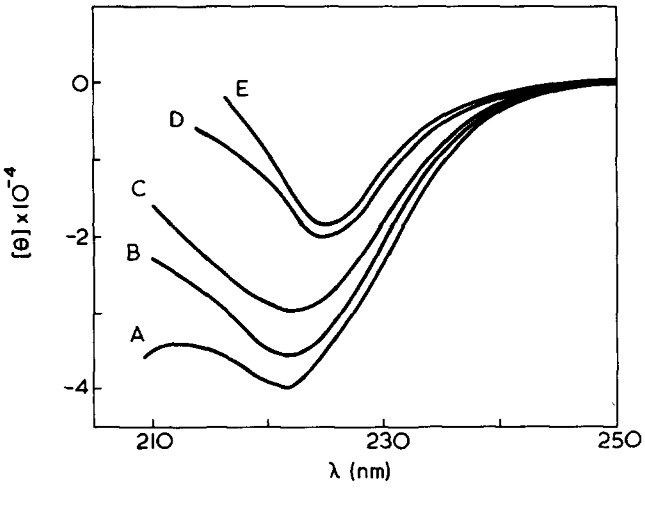

Figure 2 C.d. spectra for copolymers of *O,O'*-dicarbobenzoxy-L-Dopa with γ-benzyl-L-glutamate in methylene dichloride at 25°C. Mol % of *O,O'*-dicarbobenzoxy-L-Dopa in copolymers: A, 0%; B, 25%; C, 50%; D, 75%; E, 100%

Table 2 Helix–coil transition of polypeptides

Polypeptide	Mixed solvents			Reference
	Chloroform–DCA	Chloroform–TFA[a]	Dioxane–DCA	
Poly(*O,O*'-dicarbobenzoxy-L-Dopa)	7[b]			2
		4		2
			40	2
Poly(γ-benzyl-L-glutamate)	69–80			6,11
		25		12
			95–98	13,14

[a] TFA, trifluoroacetic acid; [b] numerals, vol % of acid in mixed solvents

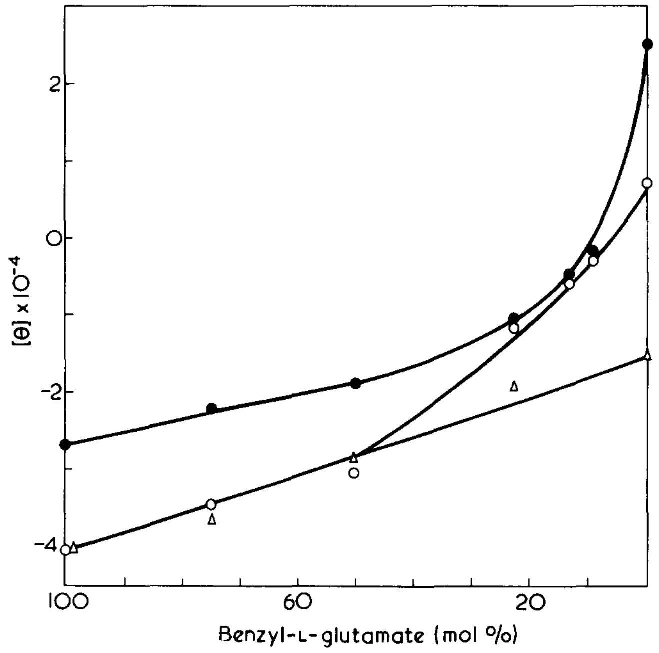

Figure 3 Dependence of the molar ellipticity on the composition of copolymers of *O,O*'-dicarbobenzoxy-L-Dopa with γ-benzyl-L-glutamate; ○, $[\theta]_{222}$ in dioxane; ●, $[\theta]_{228}$ in dioxane; △, $[\theta]_{222}$ in methylene dichloride.

in dioxane are helices of the opposite sense of twist; that is, the former has the left-handed helix. The change in $[\theta]_{228}$ is larger than that in $[\theta]_{222}$ since poly(*O,O*'-dicarbobenzoxy-L-Dopa) shows a sharp peak at 228 nm. *Table 2* gives the evidence to indicate that the γ-benzyl-L-glutamate helix is more stable than the *O,O*'-dicarbobenzoxy-L-Dopa helix. Copolymer containing 50 mol % γ-benzyl-L-glutamate has the right-handed helix. Transition from one helix sense to the other occurs at less than 25 mol % glutamate; that is, either whole chains or parts of chains become left-handed because of local preponderance of *O,O*'-dicarbobenzoxy-L-Dopa. At less than 25 mol % glutamate, of course, the conformation may contain a random coil fraction. The c.d. tendencies of the copolymers with γ-benzyl-D-glutamate are the reverse in dioxane and methylene dichloride.

Table 3 shows the characteristic amide I and II bands for copolymers of *O,O*'-dicarbobenzoxy-L-Dopa with γ-benzyl-L-glutamate. At less than 13 mol % γ-benzyl-L-glutamate, copolymers show the amide I band at 1670 cm^{-1} and the amide II band at 1551 cm^{-1} in films. More than 50 mol % L-glutamate copolymers show the amide bands at 1653–1660 cm^{-1} and 1549–1550 cm^{-1}. It is known that the left-handed helix of polypeptides should show the amide I and II bands at higher frequencies than those of the right-handed helix[15–18]. The i.r. results in *Table 3* fit perfectly with the previous calculations[15] and experiments[16–18] and further support the helical senses discussed in the c.d. experiment above.

Table 3 Characteristic amide bands for copolymers of *O,O*'-dicarbobenzoxy-L-Dopa with γ-benzyl-L-glutamate[a]

L-Glutamate content (mol %)	Amide I (cm^{-1})	Amide II (cm^{-1})
0	1670	1551
9	1670	1551
13	1670	1551
25	1664	1551
50	1660	1550
50[b]	1660	1550
75	1660	1550
100	1653	1549

[a] Film samples cast from dioxane solutions:
[b] Polymerized in methylene dichloride

Thus, the conformation of poly(*O,O*'-dicarbobenzoxy-L-Dopa) must be handled with great caution. Our extensive experimental evidence leads to the conclusion that the helical sense of the polypeptide is solvent-dependent. Though the reason is not clear, it is possible to consider that the solvent affects the delicate balance of the stacking of three aromatic groups of poly(*O,O*'-dicarbobenzoxy-L-Dopa) and causes a reversal of the helical sense in solution. A study of the aromatic effect of the polypeptide is now in progress.

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