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Rotatory Dispersion and Circular Dichroism of Low-Molecular-Weight Poly-γ-benzyl-L-glutamate

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Synopsis

Rotatory dispersion and circular dichroism of low-molecular-weight poly-γ-benzyl-L-glutamate, which was prepared from the *N*-carboxyanhydride by *n*-hexylamine initiation at [A]/[I] 3, 4, and 8, have been measured in ethylene dichloride and dioxane at different concentrations. The rotatory properties of the polypeptides are all characterized by a trough at 233 mμ of a negative Cotton effect or by a negative circular dichroic band at 223 mμ. With increasing A/I value or concentration, dextrorotation increases and the negative dichroic band becomes deeper. Both the trough magnitude and the negative ellipticity are linearly dependent on the content of β-structure, and the rotatory parameters for the pure β-structure are estimated by extrapolation of the linear relations. Circular dichroism and infrared spectra of the polypeptides have also been measured in trifluoroethanol, and the effect of solvents on the polypeptide conformation is discussed.

INTRODUCTION

Low-molecular-weight poly-γ-benzyl-L-glutamate has been shown to have at least two conformations in ethylene dichloride and dioxane.[1] The two conformations can be identified and distinguished from each other by means of infrared spectroscopic method. One is designated as the σ-form, which has an amide I band around 1670 cm^{-1} and is predominant for lower A/I polymers and at lower concentrations. The other is the β-form characterized by an amide I band around 1630 cm^{-1} and is more stable for higher A/I polymers and at higher concentrations. Here A/I denotes the anhydride-to-initiator molar ratio used when the polymer has been prepared from its *N*-carboxyanhydride by *n*-hexylamine initiation, and ranges from 3 to 8. The content of β-structure, which is defined as the fraction of residues in the β-structure, can be determined by an analysis of infrared spectra.

In the present work, rotatory dispersion and circular dichroism of low-molecular-weight poly-γ-benzyl-L-glutamates are measured in ethylene dichloride and dioxane at the ultraviolet region, and the results are correlated with the conformation and further with the content of β-structure. Circular dichroism and infrared spectra of these polypeptides are also measured in trifluoroethanol, and the effect of solvents on the polypeptide conformation is discussed.

EXPERIMENTAL

Materials

All the samples of low-molecular-weight poly-γ-benzyl-L-glutamate were the same as previously used.[1] They were prepared from the *N*-carboxyanhydride with *n*-hexylamine initiator at A/I 3, 4, and 8, and the latter two polymers were obtained after fractionation by formic acid. The average degree of polymerization was shown to be almost equal to the A/I value.

Ethylene dichloride and dioxane were purified as previously described.[1] Trifluoroethanol was purchased from Aldrich Chemical Co., Inc.

Measurements

Rotatory dispersion and circular dichroism of the polypeptides in ethylene dichloride and dioxane were measured on a Jasco ORD/UV 5 Spectropolarimeter with a CD attachment over the wavelengths from 300 to 205 mμ. Circular dichroism of the polypeptides in trifluoroethanol was measured on a Jasco J-20 Circular Dichrograph Apparatus down to 190 mμ. Path length of quartz cells was chosen 0.064 mm for solutions of concentration, 1 to 8 g/dl, 1 mm for 0.1 to 1 g/dl, and 1 cm for more dilute solutions. Temperature was about 25°C. Residue rotation, $[m]$, and residue ellipticity, $[\theta]$, were calculated from the observed rotation and circular dichroism by using values of residue molecular weight such as previously defined,[1] in which the contributions of terminal hexylimino and formate groups were evenly distributed over all residues.

Infrared spectra were measured on a Jasco DS-402 G Spectrophotometer at 25°C, as previously described.[1] Procedure to obtain the optical density of dissolved polymer was also given there. Residue extinction coefficient ϵ, was calculated from the optical density by using the path length and the residue molecular weight. The content of β-structure, f_β, was estimated from an analysis of extinction coefficients of two amide I bands around 1630 and 1670 cm^{-1}, as previously discussed.[1]

RESULTS

Rotatory dispersion of the three polypeptides in ethylene dichloride is shown in Figure 1. As the A/I ratio is higher, dextrorotation increases over all the wavelength region. A negative Cotton effect with a trough at 233 mμ and a peak around 220 mμ is observed. Circular dichroism of the same solutions is shown in Figure 2. Corresponding to the negative Cotton effect in rotatory dispersion, a negative circular dichroic band is manifest at 222–224 mμ. However, the negative residue ellipticity is larger as the A/I ratio is higher. To reconcile the increasing dextrorotation with the increasing negative ellipticity, a large positive Cotton effect or circular dichroic band must exist at a wavelength shorter than 220 mμ, and its

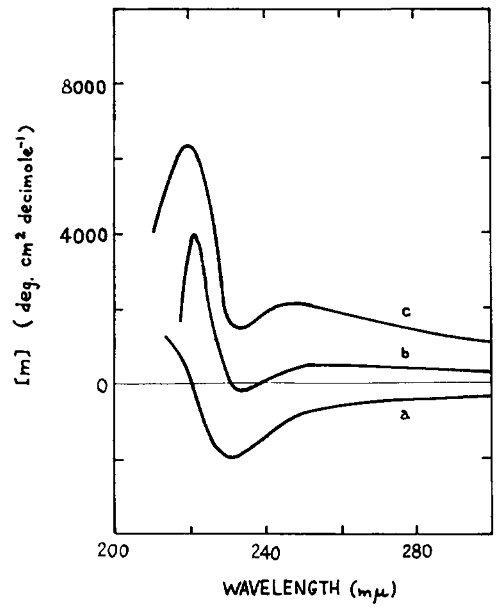

Fig. 1. Rotatory dispersion of poly-γ-benyzl-L-glutamate in ethylene dichloride. (*a*) A/I 3, 3.95 g/dl; (*b*) A/I 4, 3.83 g/dl; (*c*) A/I 8, 3.78 g/dl.

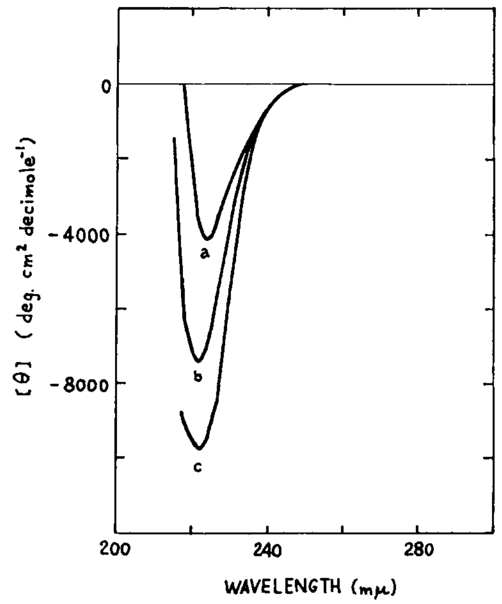

Fig. 2. Circular dichroism of poly-γ-benzyl-L-glutamate in ethylene dichloride. (*a*) A/I 3, 3.95 g/dl; (*b*) A/I 4, 3.83 g/dl; (*c*) A/I 8, 3.78 g/dl.

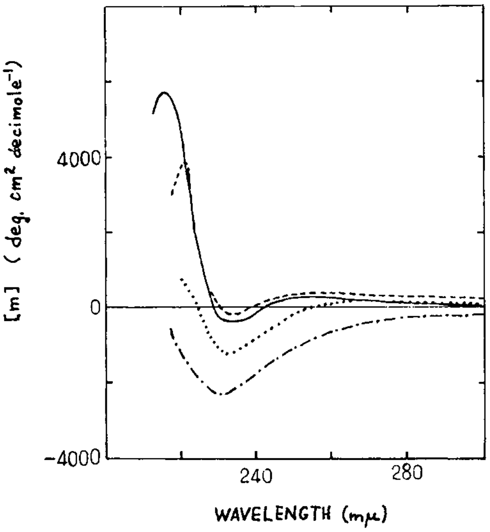

Fig. 3. Rotatory dispersion of poly-γ-benzyl-L-glutamate A/I 4 in ethylene dichloride. -·-·-·-, 0.19 g/dl; ······ 0.61 g/dl; ——, 1.97 g/dl; - - -, 3.83 g/dl.

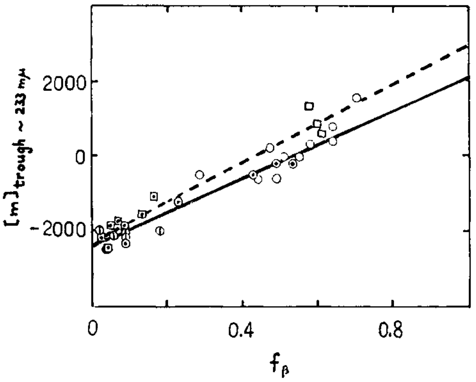

Fig. 4. Relation of residue rotation, $[m]_{233}$, with the content of β-structure, f_β. —— and circles, ethylene dichloride; - - - and squares, dioxane. ⦶, A/I 3; ⊙⊡, A/I 4; ○□, A/I 8.

magnitude must be larger as the A/I ratio is larger, although its observation would never be accomplished because of strong absorption of the solvent. In dioxane, similar dispersion and dichroism were observed.

In these two solvents both rotatory dispersion and circular dichroism vary with concentration. Figure 3 illustrates the concentration dependence of rotatory dispersion of the A/I 4 polymer in ethylene dichloride. Dextrorotation at 233 mμ and at longer wavelengths increases as the concentration is higher. This observation can be extended to the visible region, where the same behavior was reported by other workers.[2-4] It was also

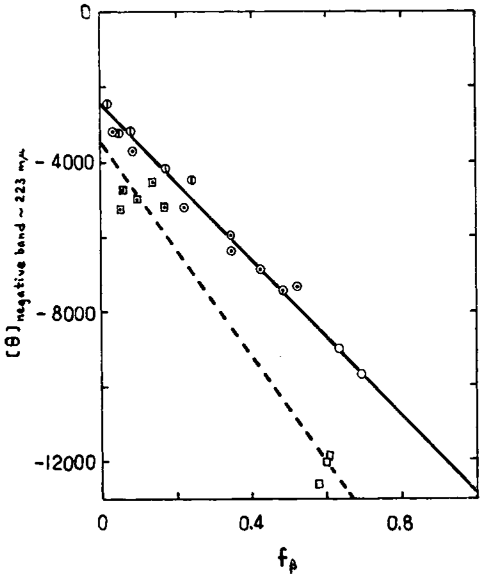

Fig. 5. Relation of residue ellipticity, $[\theta]_{223}$, with the content of β-structure, f_β. —— and circles, ethylene dichloride; - - - and squares, dioxane. ⦶, A/I 3; ⊙⊡, A/I 4; ○□, A/I 8.

found that the negative circular dichroic band at 223 mμ is deeper at higher concentrations. For all the other polymers, similar concentration dependences were noticed in both rotation and ellipticity.

It was shown in the previous paper[1] that the content of β-structure increases with increasing A/I value or concentration in both of those solvents. Then it can be expected that the observed dependences of the rotatory properties on the A/I value and the concentration are closely related to the content of β-structure, f_β.

Figure 4 shows the residue rotation of the trough at 233 mμ as a function of the content of β-structure, and Figure 5 shows the residue ellipticity of the band at 223 mμ as plotted against the content of β-structure. Each of these relations in ethylene dichloride can be approximated by a linear relation. Similar linear relations appear to hold for the data in dioxane, although data are somewhat meager. Assuming the observed linear relations, the rotatory parameters, $[m]_{233}$ and $[\theta]_{223}$, for the pure β- and σ-forms in those two solvents can be evaluated by extrapolation. These values are given in Tables I and II. They are compared with the values observed for high molecular weight poly-γ-benzyl-L-glutamate in ethylene dichloride, $[m]_{233} = -20{,}800$ and $[\theta]_{220} = -41{,}200$, which was prepared by sodium methoxide initiation and confirmed to be α-helical in ethylene dichloride by means of infrared spectra.

Figure 6 shows circular dichroism of the polypeptides measured in trifluoroethanol. A negative dichroic band is observed at 198–207 mμ, the

TABLE I
Residue Rotation of the Trough Around 233 mμ

	In ethylene dichloride		In dioxane	
	λ, mμ	[m]	λ, mμ	[m]
σ-Form	233	−2450	237	−2300
β-Form	233	2150	233	3000

TABLE II
Residue Ellipticity of the Circular Dichroic Band Around 223 mμ

	In ethylene dichloride		In dioxane	
	λ, mμ	[θ]	λ, mμ	[θ]
σ-Form	222.5	−2,400	230	(−3,500)
β-Form	222.5	−12,800	221	(−17,700)

position shifting to longer wavelength for higher A/I polymers. Another smaller negative band is manifest around 223 mμ for the A/I 4 and 8 polymers. Both of these bands are deeper as the A/I value is larger. These characteristics of circular dichroism are quite similar to those observed for a series of oligo-γ-ethyl-L-glutamate in trimethyl phosphate or trifluoroethanol.[5]

Infrared spectra of the polypeptides in trifluoroethanol are shown in Figure 7. The amide I band is almost single, but its wave number shifts from 1670 to 1663 cm^{-1}, as the A/I value changes from 3 to 8. The ester

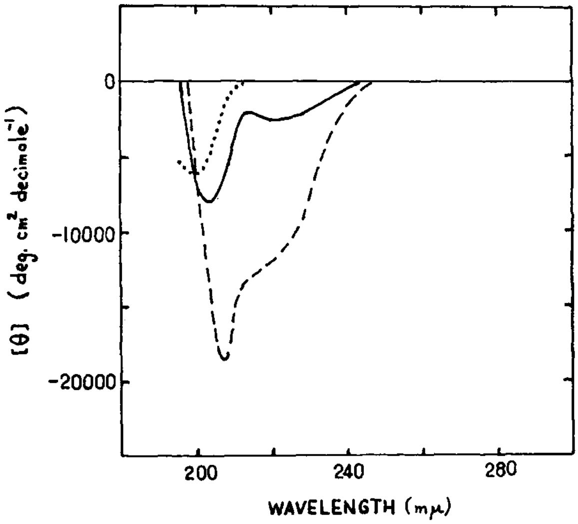

Fig. 6. Circular dichroism of poly-γ-benzyl-L-glutamate in trifluoroethanol. ······, A/I 3, 0.0256 g/dl; ——, A/I 4, 0.0253 g/dl; - - -, A/I 8, 0.0255 g/dl.

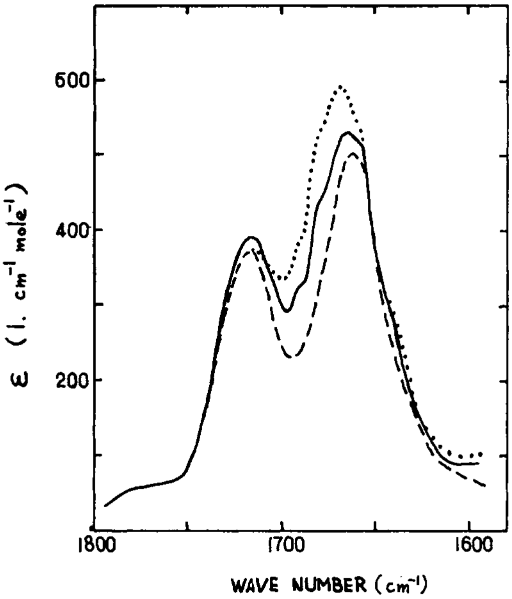

Fig. 7. Infrared spectra of poly-γ-benzyl-L-glutamate in trifluoroethanol. ······, A/I 3, 1.03 g/dl; ——, A/I 4, 0.96 g/dl; - - -, A/I 8, 0.96 g/dl.

carbonyl band lies at 1717 cm^{-1}. The location of these bands indicates that the polypeptide conformation is scarcely distinguishable from the σ-form as previously defined.[1,6]

DISCUSSION

In contrast to the coil-to-helix transition of polypeptides,[7] the σ-to-β transition of low-molecular-weight poly-γ-benzyl-L-glutamate causes the rotation of the trough at 233 mμ to increase, but this increase in dextrorotation can be ascribed to the effect of a positive Cotton effect at a shorter wavelength, which should be larger as the content of β-structure is increased. Thus the change of ellipticity at 233 mμ accompanying the σ-to-β transition is in the same direction as that of the coil-to-helix transition.[8]

The increases in dextrorotation of the 233 mμ trough and in the negative ellipticity at 223 mμ, both observed in the σ-to-β transition, are rather similar to those found in the coil-to-β transition of poly-S-carboxymethyl-L-cysteine[9] and poly-S-carboxyethyl-L-cysteine[10] in aqueous solutions. The negative residue ellipticity of the σ-form obtained at 223 mμ by extrapolation suggests that the σ-form of poly-γ-benzyl-L-glutamate is different from the random coil such as defined for poly-L-glutamic acid and poly-L-lysine[11] but is much similar to the random coil formed by the poly-L-cysteine derivatives.[9,10]

The negative circular dichroic band at 223 mμ is clearly associated with the *n*–π transition of peptides groups. It has been observed that the *n*–π transition confers different rotatory characteristics on the β-conformations of different polypeptides. Specifically, the location of circular dichroic band for the *n*–π transition permits us to divide the polypeptide β-conformations into two classes, as was already done from film studies.[12,13] The β-conformation of poly-L-lysine in aqueous solution belongs to a class exhibiting the dichroic band at 217–218 mμ,[14,15] while poly-L-serine[16] and two homologous poly-L-cysteine derivatives[9,10] form another class of β-conformation having the dichroic band at 222–226 mμ. The β-conformation formed by poly-γ-benzyl-L-glutamate in ethylene dichloride or in dioxane adds another example to the second class.

In the previous paper[1] it was demonstrated that the A/I 8 polymer is in the intramolecularly hydrogen bonded β-structure at low concentrations in ethylene dichloride or even at high concentrations up to 10% in dioxane. Otherwise, the β-conformations formed by the polypeptides including lower A/I polymers are all intermolecularly hydrogen bonded and associated. The intramolecular β-structure can not be discriminated by infrared spectra alone, but its formation can be manifested by an analysis of combined data on the amounts of both hydrogen bonding and molecular association. We must now examine whether or not the observed rotatory properties reflect any difference or transition between the intra- and intermolecularly hydrogen bonded β-structures. If the rotatory properties were able to distinguish the two β-structures, a deviation from the linear relation of $[m]_{233}$ or $[\theta]_{223}$ with f_β should be observed for the data in ethylene dichloride and dioxane, respectively. However, it is difficult to observe such a deviation in Figures 4 and 5, if the precision of the present experiments is taken into account.

Finally it is pertinent to discuss the conformation of poly-γ-benzyl-L-glutamate in trifluoroethanol. Trifluoroethanol is known to be a helix-forming solvent for poly-γ-methyl-L-glutamate,[11] while it has been shown that the formation of β-conformation of poly-L-serine in water is promoted by addition of this solvent.[16] The observed infrared spectra indicate that trifluoroethanol stabilizes the σ-conformation of low-molecular-weight poly-γ-benzyl-L-glutamates and prevents them from forming the β-conformation. It has been noticed that poly-γ-benzyl-L-glutamate can assume the α-helix only when its degree of polymerization exceeds some critical value.[17] The observed circular dichroic spectra, along with those for oligo-γ-ethyl-L-glutamate,[5] suggest that the onset of helix formation locates around the chain length of 8. The shift in frequency of amide I band can also be interpreted as an indication of partial formation of helix with increasing chain length. These results are in contrast with those in ethylene dichloride and dioxane. In those solvents, the β-conformation is induced more on poly-γ-benzyl-L-glutamate with increasing chain length or concentration, either before or concomitant with helix formation. The critical chain length for the helix formation could then depend on concentration as well as on solvent.

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