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The Structure of Copolymers of L-Proline with γ-Benzyl-L-glutamate in Organic Solvents

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The equilibrium conformations of three copolymers of L-proline with γ-benzyl-L-glutamate were studied in 1,2-dichloroethane, *m*-cresol, and dichloroacetic acid by measuring the optical rotatory dispersion and the infrared absorption spectra. In *m*-cresol and dichloroacetic acid, the proline residues take the *trans*-configuration. In *m*-cresol their incorporation into copolymers destroys the right-handed-α-helical conformation of glutamate residues. In 1,2-dichloroethane, the proline residues assume the *cis*-configuration if the sequence of proline residues is long enough. If it is short, they are in the *trans*-configuration. The poly-L-proline-I-helix can never coexist with the α-helix.

Poly-γ-benzyl-L-glutamate (PBLG) can assume the right-handed α-helix in helicogenic solvents, such as 1,2-dichloroethane (DCE), chloroform, and *m*-cresol, while it is a random coil in dichloroacetic acid (DCA) or trifluoroacetic acid.1) Its helix-coil transition has been extensively investigated by changing the solvent or the temperature.2) Poly-L-proline (PLP) has two types of conformations.3) The one, known as the form I, is a right-handed helix with all the peptide bonds in the

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1) P. Urnes and P. Doty, "Advances in Protein Chemistry, XVI," Academic Press, New York (1961), p. 401.

2) B. H. Zimm, P. Doty and K. Iso, *Proc. Natl. Acad. Sci. U. S.*, **45**, 1601 (1959).

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cis-configuration,[4] $[\alpha]^{25}_{\mathrm{D}}=+50°$ [5] (immediately after the dissolution of polyproline into acetic acid). The other, the form II, takes a left-handed helix with *trans*-imide bonds,[6] $[\alpha]^{25}_{\mathrm{D}}=ca.\ -500°$, and is stable in acetic acid, formic acid, DCA, and water. The mutual conversion between the forms I and II has also been elucidated in relation to the nature of the solvent. It is known that the PLP-I-helix can be readily transformed into the PLP-II-helix by dissolving it in organic acids, water, and *m*-cresol, and that it can be recovered from these solvents by the addition of aliphatic alcohols.[5]

It is of interest to study the stable conformation of the copolymers of L-proline and γ-benzyl-L-glutamate (γBLG) in various environments. DCE favors the right-handed α-helix of PBLG, it seems also to favor the right-handed PLP-I-helix, because the conformation of PLP in DCE-acetic acid (99.5—0.5%) (DCE-AA) is the form I.[7] *m*-Cresol also favors the right-handed α-helix of PBLG, but folds PLP into a left-handed PLP-II-helix. While DCA disrupts the PBLG helix into the random coil, it keeps PLP in the left-handed PLP-II-helix.[8]

We have previously reported the synthesis and some properties of the copolymers.[7] The copolymers studied were copoly-3:1-(L-proline, γBLG) (Copoly-3:1), copoly-1:1-(L-proline, γBLG) (Copoly-1:1), and copoly-1:3-(L-proline, γBLG) (Copoly-1:3).

In the present investigation, studies have been made of the conformation of these copolymers in three different solvents, DCE, *m*-cresol, and DCA, by measuring the optical rotatory dispersion and the infrared absorption spectra of films cast from those solvents.

Experimental

Materials. The *m*-cresol and DCA used in the present study were twice redistilled in a vacuum. The DCE was distilled under atmospheric pressure. The acetic acid was a JIS special grade acid from Wako Pure Chemical Industries, Ltd., and was used without further purification. All the polymers were the same as those used in the previous investigation.[7]

Optical Rotatory Dispersion (ORD). The ORD of the poly-peptides in *m*-cresol and DCA solutions was measured at room temperature with a Rudolph and Sons Model 200S photoelectric spectropolarimeter. The ORD of the DCE solutions was measured by a Perkin-Elmer Model 141 photoelectric polarimeter. The ORD results were analysed by the Moffitt-Yang equation,[9] assuming λ_0 to be 212 mμ. The a_0 and b_0 are constants. PLP and the copolymers exhibited mutarotation in a *m*-cresol solution, although less mutarotation was observed with copolymers of lower proline contents. All the ORD curves shown below were obtained at room temperature after the mutarotation had been completed. For the measurements, polymer solutions were put in a cell with a 10-cm path.

Infrared Absorption Spectra. The infrared absorption spectra of films cast from the solutions on KBr plates were measured on a Perkin-Elmer Model 221 spectrophotometer at a relative humidity of 40% and at 20°C.

Results

DCE Solutions. The ORD curves of the polymers are shown in Fig. 1, while the plots of a_0 and b_0 against the polymer composition in DCE are shown in Fig. 2.

The values of a_0 and b_0 for PBLG in DCE were found to be 202° and −630° respectively, in good agreement with the values observed by Moffitt and Yang.[9] Those values for PLP in DCE-AA were found to be −280° and +285°. For PLP in DCE-AA, the specific rotation at 589 mμ (the D-line of sodium) was almost the same value as $[\alpha]_{578}=-36°$, which lies between the $[\alpha]_{\mathrm{D}}=+50°$ for the form I and $[\alpha]_{\mathrm{D}}=-100°$. The form IA of PLP was discovered and thus denoted by Sasisekharan;[10] it was later identified as the form I by Traub and Shmueli.[4] Therefore, the conformation of PLP in DCE-AA seems to be the form I. This is supported by the infrared study of a film cast from the solution. A prominent

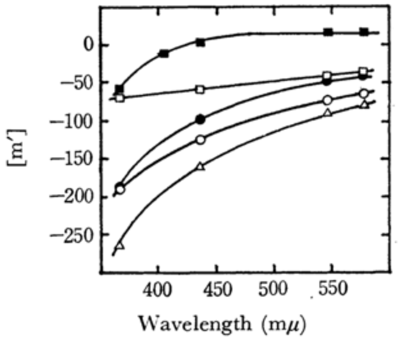

Fig. 1. The optical rotatory dispersion curves of the solutions of polymers in DCE.

□ PLP (in DCE-AA)　○ Copoly-3:1
△ Copoly-1:1　● Copoly-1:3
■ PBLG

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[5] I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela and E. Katchalski, *J. Am. Chem. Soc.*, **82**, 5263 (1960).

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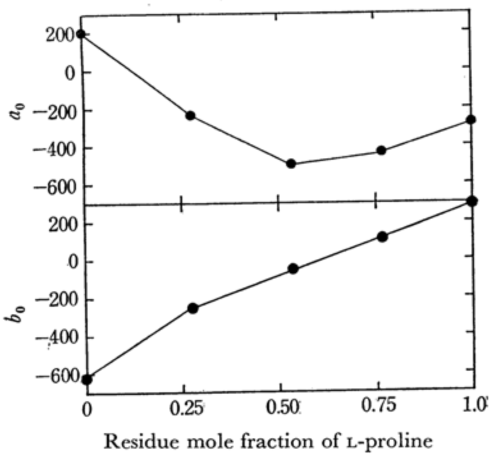

Fig. 2. The relation of a_0 and b_0 in DCE or DCE-AA to the content of proline.

feature of the copolymers is the fact that a minimum a_0 value is found at the proline content of 50%. This feature was also observed for $[m']_{546}$.⁷⁾ The inference to be drawn from this observation will be discussed below. As the residue mole fraction of proline increased from 0 to 0.25, the value of b_0 increased more sharply than it did on going from 0.25 to 1.0.

The infrared absorption spectra of polymers cast from DCE or DCE-AA are shown in Fig. 3.

In the spectrum of PLP, the CO stretching frequency of the amide (amide I) was observed at 1653 cm^{-1}, while the characteristic frequencies of the form I were observed at 960 and 1360 cm^{-1}. The last two absorptions clearly indicate that the PLP-I-helix is predominant in the DCE-AA solution of PLP. An NH stretching was observed at about 3300 cm^{-1}, and a CO stretching of the γ-ester, at about 1740 cm^{-1}, together with amide I and II bands, as in the spectrum of PBLG.¹¹⁾ In the neighborhood of 1360 cm^{-1}, however, no absorption was observed. For the copolymers the ratio of the intensity of the amide I to that of the absorption at 1740 cm^{-1}, defining the existence of glutamate, increased with the proline content. The absorption at 960 cm^{-1} was observed for Copoly-3:1, and the absorption at 1360 cm^{-1}, for Copoly-3:1 and Copoly-1:1. These facts suggest that the proline parts in Copoly-3:1 and Copoly-1:1 take the same conformation as in the form I of PLP. The frequencies of the amide I of the polymers are listed in Table 1.

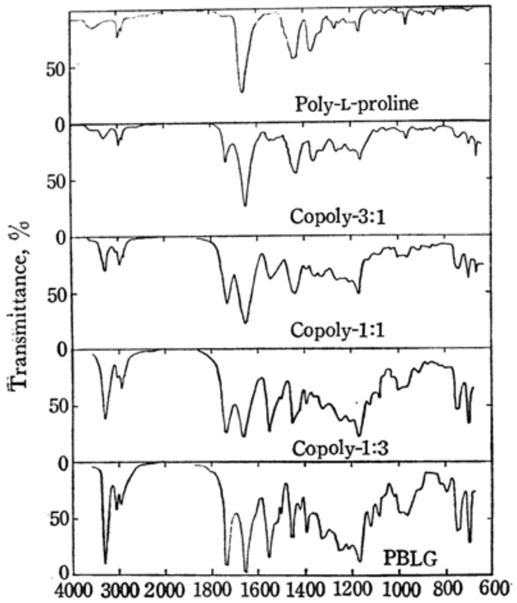

Fig. 3. Infrared absorption spectra of polymers cast from DEC of DCE-AA.

TABLE 1. THE AMIDE I FREQUENCIES OF POLYMERS CAST FROM *m*-CRESOL AND DCE

Polymer	Solvent	
	m-Cresol cm^{-1}	DCE cm^{-1}
PLP	1637	1653
Copoly-3:1	1642	1650
Copoly-1:1	1661 1650 1642	1653
Copoly-1:3	1658	1661
PBLG	1655	1656 1650

The frequencies of Copoly-1:3 were higher than that of PBLG. The frequency of the amide I is known to be higher than that of the right-handed α-helix if ordinary polypeptides take the left-handed helix.¹²⁻¹⁵⁾ Therefore, Copoly-3:1 might contain some left-handed helicies.

***m*-Cresol Solutions.** The ORD curves of the polymers in *m*-cresol are shown in Fig. 4, while the plots of a_0 and b_0 against the polymer composition in *m*-cresol are shown in Fig. 5.

The values of a_0 and b_0 for PBLG in *m*-cresol were +350° and −530° respectively. These values were not compatible with those reported by Cassim and Taylor¹⁶⁾ (a_0=223°, b_0=−650°). Although

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13) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature*, **183**, 1736 (1959).

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15) E. M. Bradbury, B. G. Carpenter and R. M. Stephans, *Biopolymers*, **6**, 905 (1968).

16) J. Y. Cassim and E. W. Taylor, *Biophys. J.*, **5**, 553 (1965).

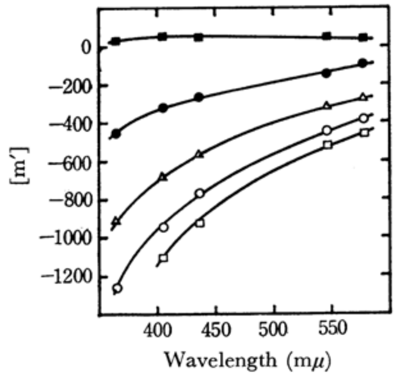

Fig. 4. The optical rotatory dispersion curves of the solutions of polymers in *m*-cresol.
The symbols are the same as in Fig. 1.

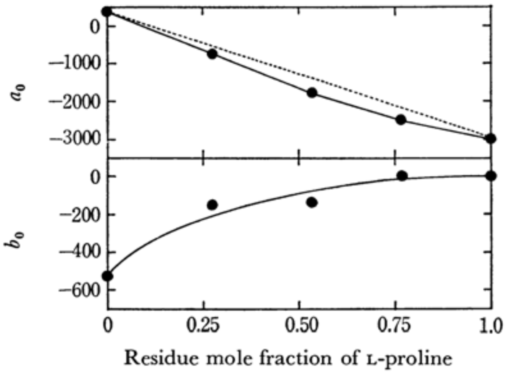

Fig. 5. The relation of a_0 and b_0 in *m*-cresol to the content of proline.

the reason for this discrepancy is not clear, the values fall in the same category as those of PBLG in helicogenic solvents. PLP had an $[\alpha]_{546}$ value of $-538°$ in *m*-cresol, and was found to have the conformation of the form II. The values of a_0 and b_0 of PLP were $-3000°$ and 0° respectively. The plot of a_0 was concave against the residue mole fraction of proline. The plot of b_0 was convex.

In the infrared absorption spectra of polymers cast from *m*-cresol, no absorption band near 1360 cm^{-1} was observed. This fact is to be expected for PLP, for the conformation of PLP in *m*-cresol is the form II and this conformation would be retained in a solution even after regeneration into the solid state. The frequencies of the amide I of polymers cast from *m*-cresol are listed in Table 1. The frequencies increased from PBLG to Copoly-3:1 and Copoly-1:1. This also suggests that Copoly-1:3 and Copoly-1:1 cast from *m*-cresol consist of some parts of the left-handed helices.

DCA Solutions. The ORD curves of the polymers in DCA are shown in Fig. 6, while the plots of a_0 and b_0 against the polymer composition in DCA are shown in Fig. 7.

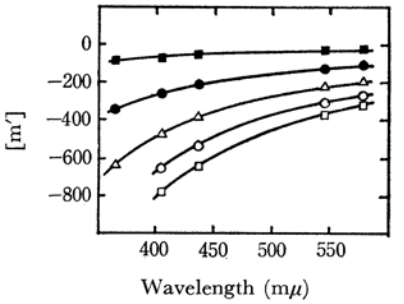

Fig. 6. The optical rotatory dispersion curves of solutions of polymers in DCA.
The symbols are the same as in Fig. 1.

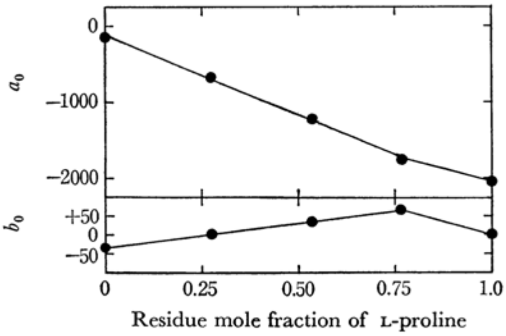

Fig. 7. The relation of a_0 and b_0 in DCA to the content of proline.

The a_0 and b_0 values of PBLG in DCA were $-160°$ and 35°. PBLG is randomly coiled in DCA. PLP in DCA is known to be in the form II,[8] and in the present experiment it was found that $[\alpha]_{546}=-373°$. The observed value was less negative than that for PLP in formic acid or in acetic acid, but the deviation could be caused by some solvent effect or by the smaller molecular weight of our sample. The a_0 and b_0 values of PLP were $-2100°$ and zero respectively. The b_0 value was equal to that in *m*-cresol. The plot of a_0 is concave against the increase of the residue mole fraction of proline, as is shown in Fig. 7. The b_0 value increased linearly as the proline fraction increased from zero to 0.75. However, these b_0 values are nearly zero when the ambiguity of the experiment is considered.

Discussion

The conformation of the copolymers of L-proline with γBLG will be considered as a function of the composition in different solvents, based on the following assumptions. First, the rotatory properties observed for copolymers can be reduced to those of the constituent residues. Second, the a_0 value reflects the configuration of the L-proline residue. Although it ranges from $-160°$ for the random coil to 400° for the α-helix in PBLG, the a_0 value of PLP changes much more, *i. e.*, from $-300°$ for the form I to $-3000°$ for the form II. Therefore, the second assumption is roughly justified. Third, the b_0 value depends on the conformation of the benzyl glutamate residue alone. The right-handed α-helical conformation of the glutamate residue has $b_0=-600°$, while its random coil conformation has $b_0=0$. Finally, it is assumed that the conformation of copolymers regenerated from solutions is not distinguishable from that in the original solutions when the solvent is either DCE or *m*-cresol. For DCA this assumption is not necessarily true.[17]

DCE Solutions. The observed gradual change in a_0 with the increase in proline content seems to indicate that any peptide bond associated with a proline residue assumes the *cis*-configuration if the proline content is more than 50%. This is supported by the infrared measurements, which reveal the appearance of absorption bands at 1360 and 960 cm^{-1}. In proline-rich copolymers proline residues occur consecutively more frequently, and in such sequences proline have the *cis*-configuration. On the other hand, in the copolymers with proline contents of less than 50%, the chance of finding more than two or three proline residues in a sequence is smaller and more proline residues would be isolated from the others. The change in a_0 with the proline content suggests that the proline residue tends to take the *trans*-configuration. This is consistent with the results on oligomers and low-molecular-weight polymers of proline.[18] *N*-Carbobenzoxy-di-, tri-, and tetra-L-proline had more negative specific rotations at 546 mμ in methanol or acetic acid than the monomer. Mutarotation studies of PLP dodecylamides (PPDA) of different molecular weights in organic acids have also shown that, in the initial conformation, more proline residues exist in the *trans*-configuration as the degree of polymerization decreases. Further, in a mixed solvent, benzene-alcohol, PPDA of a higher degree of polymerization existed in the PLP-I-helix. Even in this solvent, however, PPDA of a lower degree of polymerization had a higher content of *trans*-peptide bonds. Consequently, in a sequence composed of a small number of proline residues in the copolymers, the proline residues will be in the *trans*-configuration, even if they are in DCE, which stabilizes the *cis*-configuration of the peptide bond between prolines, or the form I. Proline residues take the *cis*-configuration in the PLP-I-helix-solvent only if they are included in a long sequence of proline residues.

The b_0 value increases rather markedly even if a small quantity of proline residues is incorporated into PBLG. The right-handed α-helix formed by glutamate residues is partly broken or converted into the left-handed helix by the occasional occurrence of proline residues. The latter suggestion is supported by the infrared spectra, which show a rather high amide I band frequency for the copolymer with a 27% proline content, since the high amide I frequency has thus far been attributed to the left-handed helix.[12-15]

***m*-Cresol Solutions.** All the a_0 values observed for the copolymers are more negative than those calculated from the weighted sum of the proline-II-helix and the glutamate helix. *m*-Cresol is a solvent supporting both the PLP-II-helix and α-helix. Consequently, the proline residues tend to assume the *trans*-configuration. The infrared spectra of the solid film regenerated from *m*-cresol solutions do not have any absorption around 1360 cm^{-1}. This fact also indicates that the *cis*-configuration is absent in the copolymers. The b_0 value increases rather abruptly with as the proline content increases to 27%. Considerable sections of the right-handed helix have been broken in this composition. In the same way as has been discussed in the case of DCE solution, the glutamate residues in the copolymers are considered to be folded together into a helix of the same kind as that of the proline residues, *i. e.*, into a left-handed helix.

DCA Solutions. Proline residues have the *trans*-configuration, and most benzyl glutamate residues are in random coils. The experimental curve for a_0 is lower, *i. e.*, more negative, than that expected for the simple sum of random coil glutamate residues and *trans* proline residues. This can be most adequately interpreted by assuming that the glutamate residues are folded into a PLP-II-helix or a left-handed α-helix. Since, however, b_0 does not change appreciably with the composition, the formation of a left-handed α-helix can be rejected.

In the PLP-II-solvent, proline residues take the *trans*-configuration and interrupt the formation of the right-handed α-helix conformation specific for the glutamate-rich parts in helicogenic solvents. In the PLP-I-solvent, proline residues assume a *cis*-configuration if the series of proline residues is long enough, although they take a *trans*-configuration if the number of proline residues in a sequence is small. Over the whole range of mole fractions of proline to benzyl glutamate, the right-handed α-helix of the glutamate residues and the right-handed PLP-I-helix can never coexist in the copolymers.

The authors wish thank Professor G. D. Fasman, Brandeis University, for his many helpful comments.

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