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Studies of Poly-β-benzyl-L-aspartate Helix. II. The Circular Dichroism and Optical Rotatory Dispersion of Copolymers of β-*p*-Methyl, Chloro, or Cyanobenzyl-L-aspartate with β-Benzyl-L-aspartate

By Masahisa HASHIMOTO

Research Laboratory, Dainippon Pharmaceutical Co., Ltd., Ebie, Fukushima-ku, Osaka

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The observation of the circular dichroism (240—290 mμ) due to the aromatic side chain indicates that, in a chloroform solution, even the side-chain aromatic rings of the copolyaspartate chain in the right-handed helix are in a regular arrangement to form an outer helix. On the contrary, for the copolyaspartate chain in the left-handed helix, the aromatic rings are much less regular. The ORD measurements indicate that, in a dimethylformamide solution, the poly-β-benzyl-L-aspartate chain is essentially in a random coil form, whereas the para-substituted derivatives are essentially in a right-handed helix. Accordingly, for each series of copolymers, the transition from a random coil form to a right-handed helix is observed as the molar content of the para-substituent is increased. The copolypeptide chain of β-benzyl-L-aspartate and β-(*p*-methylbenzyl)-L-aspartate (molar ratio, 1 : 1) takes either the left-handed helix in a chloroform solution or the right-handed helix in a dimethylformamide solution. Accordingly, for this copolypeptide, the transition from the left to right-handed conformation is observed at the mixing volume ratio, 90 : 10, of the chloroform-dimethylformamide solution.

Goodman et al.[1] have reported that the left-handed helix of poly-β-benzyl-L-aspartate (PBLA) is reversed to the right-handed sense by the introduction of the nitro group in the para-position of the side-chain aromatic ring. They have proposed that this right-handed conformation is stabilized by an electronically-coupled nitroaromatic side-chain helix rigidly arrayed about the main chain helix. In the previous paper,[2] the present author and Aritomi have revealed that the helix reversal

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[1] M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause and G. Schwartz, *Biopolymers*, **1**, 371 (1963).

[2] Part I of this series: M. Hashimoto and J. Aritomi, This Bulletin, **39**, 2707 (1966).

is also brought about by the introduction of the methyl, chloro, or cyano groups at the para-position of the benzyl group. This is quite clear since, for each series of the copolymers of β-benzyl-L-aspartate (BLA) with β-(p-methyl, chloro, or cyanobenzyl)-L-aspartate (MeBLA, ClBLA, or CNBLA), a sharp change in b_0 values³⁾ has been observed with the variation in the molar contents of the para-substituent.

In this paper, the author will present; (1) the results of the optical rotatory dispersion (ORD) and circular dichroism (CD) measurements of the above copolymers in a chloroform solution, in the absorption region (around 240—290 mμ) of the side-chain aromatic rings, and (2) the results of the ORD measurements of the copolymers in a dimethylformamide solution in the visible and near-ultraviolet regions. The evidence obtained to establish the conformations of the side-chain aromatic rings of the copolymers in a chloroform solution will be presented, and the conformations of the copolymer chains in dimethylformamide will be discussed.

Experimental

The samples of the copolymers used in the present study are the same as those used in the previous report²⁾ except for PClBLA (C1102), which was synthesized in a similar way but by adopting anhydride to the initiator ratio, 50.

Chloroform obtained from E. Merck AG was treated with molecular sieves (4A, Tomoe Kogyo Co., Tokyo) to remove a small amount of ethanol. The dimethylformamide (DMF) used was purified by distillation, and the dichloroacetic acid (DCA) was purified by vacuum distillation.

Ultraviolet ORD and CD measurements were carried out through the courtesy of Professor Kazutomo Imahori of College of General Education, The University of Tokyo, on a JASCO, model ORD/UV5, optical rotatory dispersion recorder with a CD attachment equipped with a xenon light source at 30°C. The CD measurements were made at the minimum scanning speed (10 mμ/10 min.). Fused-quartz cells 10 mm. and 1 mm. long were used. Identical cells and solutions were used in both ORD and CD measurements; the solutions were prepared to be around 2 in optical density. ORD and CD data are expressed in terms of $[m']$ and θ respectively, and their units are both deg.cm^2/decimole. The refractive index was estimated from the Sellmeir equation, using the indices of chloroform observed at 434 and 589 mμ.

The ultraviolet absorption measurements were carried on a Hitachi EPS-2U spectrophotometer.

Optical rotatory dispersion measurements in the near-ultraviolet and visible regions were carried through the courtesy of Professor Tatsuo Miyazawa and Dr. Kozo Hamaguchi of the Institute for Protein Research, Osaka University, on a Rudolph, model 200-S, photoelectric polarimeter using a mercury lamp as the light source. The values of b_0 and λ_c were calculated from measurements at five wavelengths, from 5780 to 3650 Å; the concentration was 0.5% w/v, and the path length was 2 dm. In the Moffitt plot, the dispersion of the refractive index is neglected, using the values at the sodium D line.

Results and Discussion

Ultraviolet Rotatory Dispersions and Circular Dichroisms of Copolymers of β-Benzyl-L-aspartate with β-p-Methyl, Chloro, and Cyanobenzyl-L-aspartate in a Chloroform Solution.—*a) Copolymers of BLA with CNBLA.*—The copolymer of BLA with CNBLA containing 60 mol.-% cyano residues had b_0 values of -401 in a chloroform solution.²⁾ The ORD curve (Fig. 1a) of the cpolymer showed a negative plain curve, and no definite Cotton effect was found in the region from 260 to 290 mμ. However, the CD measurements for this copolymer gave, in this region, a distinct negative CD peak involving fine vibrational structures. This dichroism is evidently associated with the side-chain aromatic rings of the copolymer. This negative ellipticity decreased remarkably in a chloroform solution containing 10% dichloroacetic acid (Fig. 1d). Therefore, it is clear that the CD of this copolymer in a chloroform solution is not essentially due to an optically active chromophore, which still gives rise to CD in a random coil form. (For poly-L-tyrosine, it has been reported that one of the CD bands, observed at 245 mμ in a helical conformation, persists in a random conformation.⁴⁾) Accordingly,

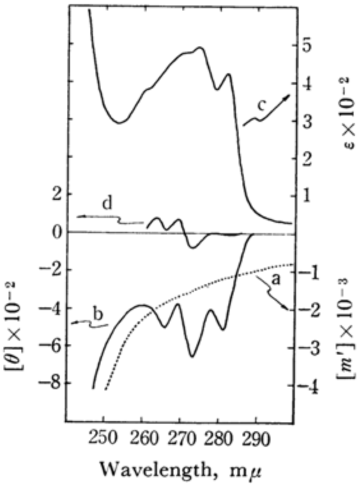

Fig. 1. Curves of ORD (a), CD (b), and UV (c) in chloroform, and CD (d) in chloroform containing 10%v/v dichloroacetic acid for the copolymer of BLA with CNBLA containing 60 mole per cent cyano residues.

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3) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956); W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.* **42**, 596 (1956).

4) G. D. Fasman, E. Bodenheimer and C. Lindblow, *Biochemistry*, **3**, 1665 (1964); S. Beychok and G. D. Fasman, ibid., **3**, 1675 (1964).

the results may be attributed to the fact the negative CD curve involving fine vibrational structures evidently arises from a regular arrangement of the side-chain aromatic rings of this copolymer in a helical conformation. The increasing negative ellipticity with the decrease in the wavelength below 250 mμ observed for the copolymer may suggest that the copolymer has a negative ellipticity for the n-π^* transition near 220 mμ, which is associated with a right-handed α-helix.[5] The above results are consistent with the negative b_0 values (-401).[2]

The ORD and CD curves of the copolymer of BLA with CNBLA containing 30 mol.-% cyano residues are shown in Figs. 2a and 2b respectively. In the region from 250 mμ to 280 mμ, a very weak positive ellipticity was found. The increasing positive ellipticity with a decrease in the wavelength below 250 mμ suggests that this copolymer exists in a left-handed α-helix which is opposite in the helical sense to that of the copolymer containing 60 mol.-% cyano residues. This result is also consistent with the positive b_0 values $(+626)$ observed for this copolymer.[2]

The CD curve of poly-β-benzyl-L-aspartate (PBLA) shows a small ellipticity involving fine structures (Fig. 3b). The magnitude of this ellipticity is rather small; however, the results were reproducible. The peaks in CD curves either in Figs. 2b or 3b do not correspond to those in UV spectra; however, these two CD curves are fairly similar to each other, both in their shape and in the position of the peaks. A comparison of the magnitude of their ellipticity may be meaningless because of the weakness in their ellipticity. However, the fact that the absolute magnitude of the ellipticity of PBLA associated with the aromatic group is smaller than that of the copolymer in Fig. 1b may indicate that the side-chain aromatic rings of PBLA are packed with much less regularity. The same conclusion can be obtained for the side-chain of the copolyaspartate containing 30 mol.-% cyanobenzyl residues, since the magnitude of the CD peak is far less than that of the copolymer in Fig. 1b.

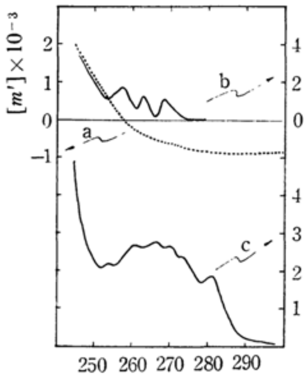

Fig. 2. Curves of ORD (a), CD (b), and UV (c) in chloroform for the copolymer of BLA with CNBLA containing 30 mole per cent cyano residues.

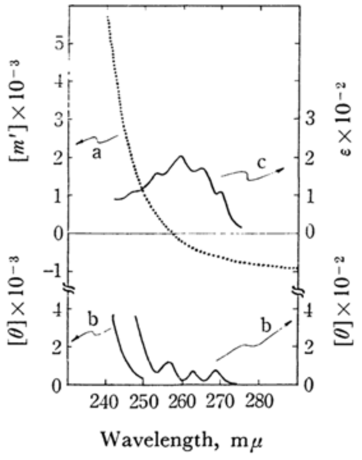

Fig. 3. Curves of ORD (a), CD (b), and UV (c) for PBLA in chloroform solution.

The CD curve of poly-γ-benzyl-L-glutamate (PBLG) may be seen in Fig. 4a. This polypeptide showed no significant ellipticity in the region of the absorption of the aromatic group. This finding indicates that the aromatic rings in PBLG are not in a regular orientation in a chloroform solution, although in oriented solid film Tsuboi has elucidated, from his infrared studies, that the aromatic rings of this polypeptide are held in a regular orientation.[6]

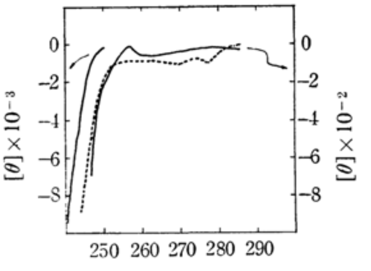

Fig. 4. CD of PBLG (a), ——, and the copolymer of BLA with ClBLA containing 50 mole per cent chloro residues (b), ……, in chloroform.

b) Copolymers of BLA with MeBLA.—The UV, ORD, and CD curves of copolymers of BLA with MeBLA containing 80 and 60 mol.-% methyl residues in a chloroform solution are shown in Figs.

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[5] G. Holzwarth, W. B. Gratzer and P. Doty, *J. Am. Chem. Soc.*, **84**, 3194 (1962).

[6] M. Tsuboi, *J. Poly. Sci.*, **59**, 139 (1962).

5 and 6 respectively, while the results concerning a homopolymer, poly-β-(p-methylbenzyl)-L-aspartate (PMeBLA), are shown in Fig. 7. The ellipticity observed for PMeBLA is to some extent larger than that of the copolymer containing 80 mol.-% methyl residues. The negative CD curves observed for these polypeptides are different in magnitude and in the wavelength of their maximum ellipticity. However, the wavelengths of the CD peaks of these polypeptides are in good agreement with the wavelengths of their UV absorption peaks. These results may indicate that both the methylbenzyl and benzyl groups are regularly arranged and contribute to the ellipticity. On the contrary, for the copolymer containing 60 mol.-% methyl residues the CD curve (Fig. 6b) is almost the same as that of PBLA (Fig. 3b). This result indicates that this copolymer exists in a left-handed helix and that the methylbenzyl group does not contribute to the ellipticity. Therefore, the side-chain aromatic rings of this copolymer may be said to be much less regularly packed as in PBLA.

c) Copolymers of BLA with ClBLA.—The UV, ORD, and CD curves in a chloroform solution of a copolymer of BLA with ClBLA containing 60 and 40 mol.-% chloro residues are shown in Figs. 8 and 9 respectively, while the results concerning a homopolymer, poly-β-(p-chlorobenzyl)-L-aspartate (PClBLA), are shown in Fig. 10. The

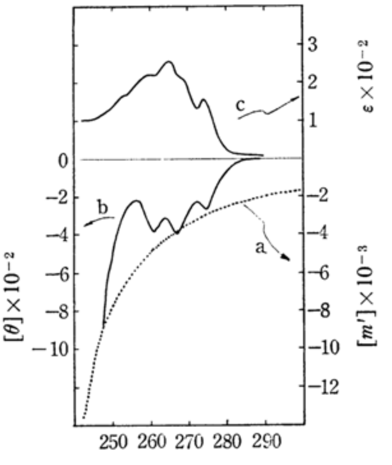

Fig. 5. Curves of ORD (a), CD (b), and UV (c) in chloroform for the copolymer of BLA with MeBLA containing 80 mole per cent methyl residues.

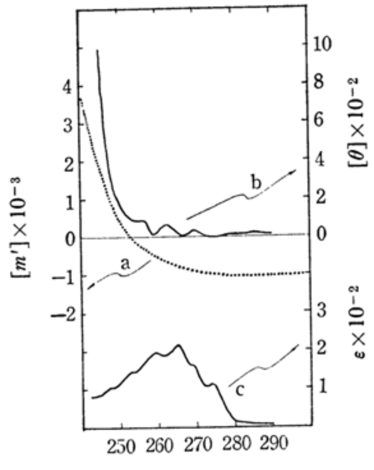

Fig. 6. Curves of ORD (a), CD (b), and UV (c) in chloroform for the copolymer of BLA with MeBLA containing 60 mole per cent methyl residues.

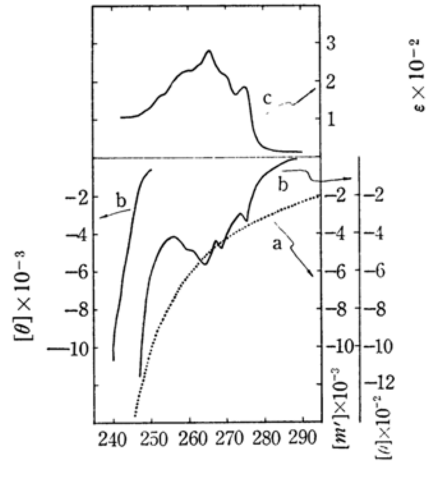

Fig. 7. Curves of ORD (a), CD (b), and UV (c) in chloroform for PMeBLA.

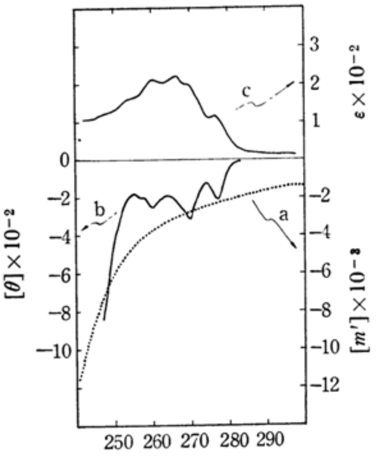

Fig. 8. Curves of ORD (a), CD (b), and UV (c) in chloroform for the copolymer of BLA with ClBLA containing 60 mole per cent chloro residues.

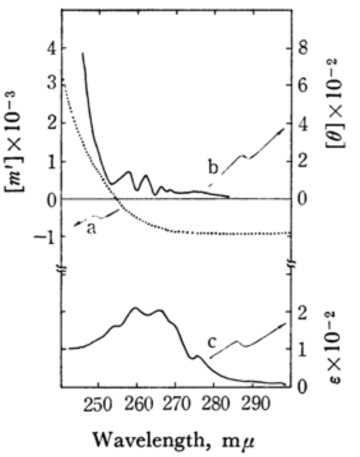

Fig. 9. Curves of ORD (a), CD (b), and UV (c) in chloroform for the copolymer of BLA with ClBLA containing 40 mole per cent chloro residues.

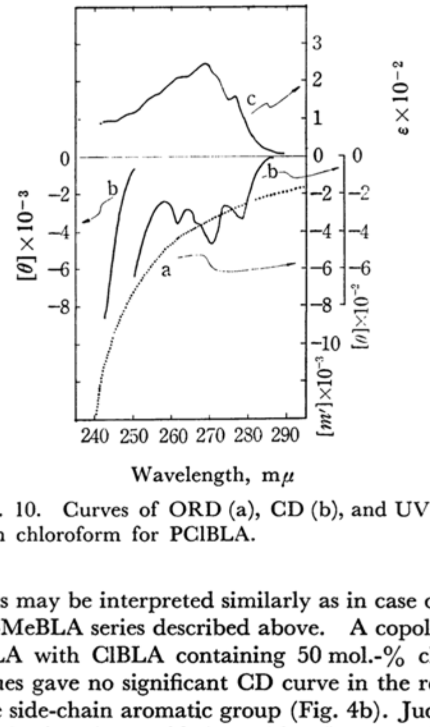

Fig. 10. Curves of ORD (a), CD (b), and UV (c) in chloroform for PClBLA.

results may be interpreted similarly as in case of the BLA-MeBLA series described above. A copolymer of BLA with ClBLA containing 50 mol.-% chloro residues gave no significant CD curve in the region of the side-chain aromatic group (Fig. 4b). Judging from the b_0 value, −93, in a chloroform solution,[2] the copolymer exists essentially in a random coil. As is evident from Figs. 7b and 8b, the ellipticity associated with the aromatic rings is comparatively large in magnitude if the backbone is folded in the right-handed helix, while the left-handed helix exhibits a negligible amount of ellipticity for the side-chain chromophore. Thus, if equal amounts of the left- and right-handed helices exist, the CD value due to the right-handed helix would exceed that of the left-handed one, and the resulting CD curve should be one similar in shape to, and a half the magnitude of, that of the right-handed helix (Fig. 10). However, since no significant peak was detected in the CD curve of the copoly-(BLA-ClBLA) (50 : 50), as may be seen in Fig. 4b, the assumption of the mixture of the left- and right-handed helices for this copolymer should be discarded; rather, the copolymer has been proved to exist in a random coil. The amount and the sense of the helical conformation judging from CD measurements of PClBLA and three copolymers (chlororesidue contents; 40, 50, and 60 mol.-%) are consistent with those obtained from b_0 analysis.[2]

A pronounced increase in negative ellipticity and negative ORD with a decrease in the wavelength below 250 mμ was observed for both PMeBLA and PClBLA. This result indicates that both PMeBLA and PClBLA have a negative ellipticity for the n-π^* transition near 220 mμ; it leads to the conclusion that both polypeptides exist in the right-handed helix, although the ultraviolet absorption of chloroform prevents the direct measurement of the n-π^* transition around 220 mμ. On the other hand, a positive ellipticity and a positive ORD curve were observed for PBLA, which exists in the left-handed α-helix. In each series of copolymers, the transition from the left- to the right-handed helix, depicted by the change in the signs of ellipticity and optical rotation parameter, takes place in a narrow range of composition.

The ellipticities observed for PMeBLA and PClBLA at 250 mμ, are about twice as large as that observed for PBLG, although this comparison is only qualitative because their ellipticity steeply changes around 250 mμ. The ellipticity around 240 mμ observed for PMeBLA and PClBLA in chloroform is also about twice as large as that for poly-γ-methyl-L-glutamate, as estimated from the results of Holtzwarth et al.[7] The ORD curve of PBLA in chloroform in the present study is in accordance with that in methylene dichloride reported by Blout.[8] However, for PClBLA and PMeBLA the observed optical rotations at 250 mμ are about twice and one-and-a-half times as large, respectively, as that for poly-α, L-glutamic acid in a water solution (pH, 4.3; the helical form), as estimated from the results of Blout et al.[9] The above comparison indicates that, for PClBLA and PMeBLA, the CD and ORD around 240 mμ are both larger in magnitude than the ordinary polypeptides in the right-handed α-helix. These results may be due to the contributions to CD and ORD of the aromatic absorption band around

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7) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

8) E. R. Blout, *Biopolymers*, **Symp. No. 1,** 397 (1964).

9) E. R. Blout, I. Schmier and N. S. Simmons, *J. Am. Chem. Soc.*, **80**, 1259 (1958).

220 mμ[10] and/or of the ester-carbonyl absorption around 220—230 mμ resulting from the regular arrangement of the side-chain group. However, qualitatively the above results do not change the conclusion obtained as to the helical sense of the polypeptides.

In the present study, the negative CD bands were found in the 250—290 mμ region, whereas no definite Cotton effect was observed in this region, for the polypeptide chains forming the right-handed helix. The Kronig-Kramers transform relating ellipticity to rotation has been shown to lead to the following relation when the circular dichroism is in a Gaussian form:[11]

$$[\theta^\circ] = \pm 0.82\frac{n_\lambda^2 + 2}{3}([m'_{max}] - [m'_{min}])$$

where $[\theta^\circ]$ is the maximum ellipticity of the band, and $[m']$ is the reduced mean residue rotation. This relation suggests that Cotton effects should be observed in the 250—290 mμ region for the present, right-handed polypeptides. (Even when the CD curve deviates from a Gaussian curve, the relation should hold qualitatively.) Furthermore, essentially the same selection rules hold on ORD and CD even in the case of a vibration-coupled optically active chromophore.[12] The largest observed ellipticity associated with the aromatic chromophore in the present study is -650 (Fig. 1b). The value of $(n_\lambda^2+2)/3$ in the above formula is about 0.7 in the region from 260 mμ to 290 mμ; thus, the value of the $0.82(n_\lambda^2+2)/3$ term, is about 1.2. Therefore, if this maximum ellipticity (-650) is completely due to a band, the above relation leads to an estimation that a trough, about 280 in magnitude, should appear in the ORD curve. However, as is evident from Fig. 1b, the three peaks of CD lie close together. In addition, the contribution of the large ellipticity (ca. -1.5×10^4) of the n-π^* transition around 220 mμ to this region cannot be excluded completely. Therefore, if one assumes that the net maximum ellipticity for the band is about a half of the observed value after the correction of the contributions from the other bands, this ellipticity may, practically speaking, not be so large as to give rise to a definite Cotton effect. This situation is less profound in the other polypeptides in the present study.

The results of the ORD and CD measurements described above indicate qualitatively but strongly that a reversal in the helical sense really takes place in each series of copolymers, and that PClBLA and PMeBLA in a chloroform solution exist in the right-handed α-helix. The results also lead to the conclusion that, in the right-handed helix, the side-chain aromatic rings of the copolyaspartate chain are arranged regularly to form an outer helix, whereas in the left-handed helix the aromatic rings are much less regular.

From the results in the present study, it can not be determined whether or not an outer helix formed by the side-chain aromatic rings contributes to the stability of the right-handed helix of the main chain.

The Optical Rotatory Dispersion in Dimethylformamide.—In a chloroform solution PBLA is known to exist in a left-handed α-helix,[13,14] whereas in a dimethylformamide (DMF) solution PBLA has been estimated to be essentially in a random conformation from the studies of the copolymers of BLA and *p*-nitrobenzyl-L-aspartate.[1]

The results of the ORD measurements in the present study of homopolymers and copolymers of BLA with CNBLA, ClBLA, or MeBLA are listed in Tables I—III respectively.

In the series of copolymers of BLA and CNBLA, the specific rotations at 546 mμ are ca. -100 for the copolymers containing cyanoresidues of more than 30 mol.-% (C4101—C4041); the value sharply decreases to ca. -40 with a decrease in the cyanoresidue content, as Table I shows. (PBLA itself and the copolymers up to a 30 mol.-% cyanoresidue content were less soluble in DMF, and 0.25% solutions were used for the measurements instead of a 0.5% solution.) The results indicate that there is a transition.

A similar change are found in the b_0 value, just as in case of specific rotation. The b_0 values of

TABLE I. COPOLYMERS OF β-*p*-CYANOBENZYL-L-ASPARTATE WITH β-BENZYL-L-ASPARTATE IN DMF*

Polymer No.	Mol.-% of cyanobenzyl residues	b_0	$[\alpha]_{546}$	λ_c
C0014	0	-315	-40	283
C4011	10.0	-307	-26	292
C4021	20.0	-276	-58	252
C4031	30.0	-422	-97	249
C4041	39.9	-533	-107	251
C4051	50.0	-568	-115	247
C4061	60.0	-548	-110	249
C4071	76.6	-580	-109	248
C4081	80.0	-490	-99	249
C4091	89.9	-496	-100	250
C4101	100	-483	-105	251

* At 30°C; the concentration was 0.5% w/v for C4041-C4101, and 0.25% w/v for the others.

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[10] L. C. Jones, Jr., and L. W. Taylor, *Anal. Chem.*, **27**, 235 (1955).

[11] J. A. Schellman and C. Schellman, "The Proteins," II, Ed. by H. Neurath, Academic Press, New York (1964), p. 84; A. Moscowitz, "Optical Rotatory Dispersion," Ed. by C. Djerassi, McGraw-Hill, New York (1960), p. 150.

[12] W. Moffitt and A. Moscowitz, *J. Chem. Phys.*, **30**, 648 (1959).

[13] R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2268 (1960).

[14] E. M. Bradbury, A. R. Downie, A. Elliott and W. E. Hanby, *Proc. Roy. Soc.*, **A259**, 110 (1960).

−480—−580 for the copolymers (C4101—C4041) become sharply less (ca. −300) for the rest of the copolymers (C4021—C0014). As has been reported in the previous paper,[2] the b_0 value of PBLA in a chloroform solution was +659 (left-handed helix), whereas the value of PClBLA or PMeBLA was −680 (right-handed helix). Karlson et al. have shown that the b_0 value of ca. −250 is attributable to the residual contribution for PBLA in a random chain.[13] If this residual contribution exists in the left- and right-handed helical conformations of poly-L-aspartate esters, the b_0 values should differ not only in sign but also in magnitude according to the sense of the helix. The small difference in b_0 value between the above-mentioned left- and right-handed poly-L-aspartate esters leads to the conclusion that the residual contribution to the b_0 value is small for these polypeptides in a helical solvent like chloroform. Therefore, the b_0 value of ca. −660 can possibly be expected for poly-L-aspartate esters in a DMF solution if they exist in the right-handed helix.

On the other hand, the b_0 values of PBLA were observed to be −315 and −280 in DMF and DCA solutions respectively. The similarity in the b_0 values of the two solvents suggests that PBLA exists essentially in a random coil in a DMF solution because DCA has been revealed as a random coil solvent for PBLA by Karlson et al.[13] This fact seems to be an exception for PBLA, because DMF is usually considered to be a helix-promoting solvent.

As for λ_c values observed for this series of copolymers in a DMF solution, no sharp change in λ_c was found as the molar content of the cyano residue varied. λ_c values of 251 and 285 were observed for PCNBLA and PBLA respectively, both in a DMF solution. These values are ordinally correlated with the right-handed helix,[15] since the constant, λ_c, usually has a value of 210 or less for the random-coil form (for poly-γ-benzyl-L-glutamate[16] and poly-γ-methyl-L-glutamate[17] in a DCA solution, 180 and 187 were reported as the respective λ_c values). The plot for λ_c of PBLA in a DCA solution was nonlinear, but it could roughly be estimated to be 280—310. Considering that both values of b_0 and λ_c obtained for PBLA in a DMF solution coincide with those for a DCA solution, it may be deduced that these unusually high values should be correlated with a random coil form. Another example of anomaly is found for poly-L-tyrosine (λ_c=330) in its coiled form.[18] Thus, both λ_c and b_0 strongly suggest that PBLA exists in a random form in a DMF solution.

On the other hand, the b_0 values of −580—−480 observed for the copolymers of BLA and CNBLA containing cyano residues more than 30 mol.-% in a DMF solution are somewhat smaller than the value for PClBLA or PMeBLA in a chloroform solution, but considerably larger than the value for PBLA in a DMF solution. The evidence that some copolymer in a DMF solution is in a right-handed helix may be derived from the fact that a sharp change in b_0 value from ca. −600 to ca. +600 was found for a copoly-L-aspartate ester (BLA-MeBLA 1 : 1) in chloroform-DMF solutions, as will be described later. Consequently, the b_0 values of −580—−480 indicate that the copolymers are mainly in a right-handed helix, and possibly partially in a random coil form also. The nonlinear change in b_0 and $[\alpha]_{546}$ values (Table I) observed for a series of copolymers of BLA and CNBLA in DMF solution reflect a transition from a right-handed helix to a random-coil form, a transition which occurs as the molar content of the cyano residue is decreased. There is a possibility that the transition also affects the solubilities of the copolymers in DMF.

The results of the ORD measurements of the copolymers of BLA with ClBLA and with MeBLA are shown in Tables II and III. For these series the results are essentially the same as with a series of copolymers of BLA and CNBLA; PClBLA and PMeBLA themselves exist mainly in a right-handed helix, and in the case of copolymers with BLA transitions, from a right-handed helix to a random-coil form, were found at 15—30 and 30—40 mol.-% of chloro and methyl residue contents respectively.

The above finding that poly-β-(*p*-substituted benzyl)-L-aspartates exist in a right-handed helix in a DMF solution was confirmed by another piece of evidence that PClBLA showed a helix-to-coil transition in a DMF-DCA solvent system (as is shown in Fig. 11). When DCA was added to the PClBLA solution in DMF up to a 24% v/v of DCA,

TABLE II. COPOLYMERS OF β-*p*-CHLOROBENZYL-L-ASPARTATE WITH β-BENZYL-L-ASPARTATE IN DMF*

Polymer No.	Mol.-% of chlorobenzyl residues	b_0	$[\alpha]_{546}$	λ_c
C1021	15.1	−299	−60	258
C1031	30.0	−489	−91	259
C1041	40.0	−553	−103	258
C1051	49.9	−534	−103	258
C1061	60.0	−572	−111	257
C1071	70.1	−569	−117	258
C1081	79.9	−562	−122	257
C1091	90.0	−609	−122	260
C1101	100	−554	−110	253

* At 27.5°C; the concentration was 0.5% w/v except C1021 (0.25% w/v).

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15) Ref. 11 (The Proteins), p. 78.

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TABLE III. COPOLYMERS OF β-p-METHYLBENZYL-L-ASPARTATE WITH β-BENZYL-L-ASPARTATE IN DMF*

Polymer No.	Mol.-% of methylbenzyl residues	b_0	$[\alpha]_{546}$	λ_c
C3011**	9.9	—	—	—
C3021	19.8	−266	−53	253
C3031	29.9	−298	−80	241
C3041	39.9	−496	−110	251
C3051	49.9	−532	−105	250
C3061	59.9	−525	−111	248
C3071	70.0	−576	−120	250
C3081	80.0	−614	−123	250
C3091	90.1	−544	−118	251
C3101	100	−491	−111	251

* At 30°C; the concentration was 0.5% w/v for C3051-C3101, and 0.25% w/v for the others.

** Not observed because of its poor solubility in DMF.

the solution became turbid; the further addition of DCA caused the precipitation of PClBLA. The same phenomenon occurred in the reversed procedure when DMF was added to a PClBLA solution in DCA up to a 70% v/v of DMF. The small magnitude of the observed optical rotation of PClBLA in DMF or DCA solutions resulted in the somewhat scattered b_0 plot of Fig. 11. However, the nonlinear change in the b_0 value in Fig. 11 evidently indicates that a helix-to-coil transition takes place as the DCA content in DMF increases.

Finally, a clear-cut transition from the left- to the right-handed helix should be mentioned for a copoly-BLA: MeBLA (molar ratio, 1 : 1). This copolypeptide chain takes either the left-handed helix in a chloroform solution or the right-handed helix in a DMF solution (b_0 value; +602 in $CHCl_3$, −532 in DMF). For this copolymer in chloroform-DMF solutions, the observed b_0 values as a function of the solvent composition are illustrated in Fig. 12. The sharp change in b_0 value from ca. +600 to ca. −600 obviously indicates that the transition from the left- to right-handed helix occurs at the mixing volume ratio, 90 : 10, of the chloroform-DMF solution. Therefore, it may also be expected that a transition from the right-handed helix conformation to a random-coil form can be observed in the DMF-DCA solvent system. Such experiments were, indeed, attempted, but unfortunately the transition was not observed because of the precipitation of the copolymer when DCA was added in about a 10% v/v ratio to its DMF solution.

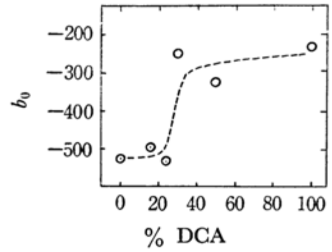

Fig. 11. b_0 Values of PClBLA versus solvent composition (DMF-DCA).

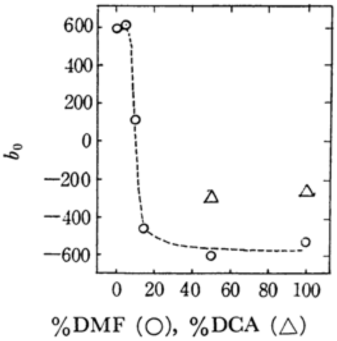

Fig. 12. b_0 Values of the copolymer of BLA with MeBLA containing 50 mole per cent methyl residues versus solvent composition (○ chloroform-DMF; △ DMF-DCA).

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