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Studies of Poly-β-benzyl-L-aspartate Helix. III.[*1] Infrared Spectra of Copolymers of β-Benzyl-L-aspartate with β-*p*-Methyl, Chloro, Cyano, or Nitrobenzyl-L-aspartate in a Chloroform Solution

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Infrared studies of the copolymers in a chloroform solution have revealed that, in each series of copolymers, the transition from the left- to the right-handed α-helix simultaneously brings about sharp changes in the characteristic amide frequencies. The frequencies of the NH-stretching, amide I, and amide II bands of the copolymers in the left-handed α-helix are higher by *ca.* 13 cm^{-1}, *ca.* 10 cm^{-1}, and *ca.* 5 cm^{-1} respectively than those in the right-handed α-helix. The amide bands of the copolymers in the right-handed form lie in the normal frequency ranges as established for α-helical polypeptides. On the contrary, the amide frequencies of poly-β-benzyl-L-aspartate or the copolymers in the left-handed form are anomalous. The anomalous amide frequencies of the left-handed helix are to be ascribed to the reversal in the helical sense, but not to the particular aspartate group, since the copolyaspartate esters in the right-handed helix exhibit normal amide frequencies. The transition in the helical sense is reflected also in the C=O stretching bands of the side-chain ester groups.

Polypeptide have been known to take various chain conformations, and the infrared spectra reflect the influence of their conformations on the characteristic amide bands. The correlations between various conformations of polypeptides and their characteristic amide bands have been analyzed.[1,2] Poly-β-benzyl-L-aspartate (PBLA) has been known to take the left-handed α-helical conformation.[3,4] In this conformation, the amide I and amide II frequencies have been found to be abnormally higher than those of normal L-polypeptides in the α-conformation.[5,6] However, it has not been clarified whether these anomalous amide frequencies of the PBLA chain are associated with its unusual left-handed helix or with a particular aspartic side chain, in which the carbonyl group is attached directly to the β-carbon atom. Previous papers[7,8] have revealed that poly-β-(*p*-substituted benzyl)-L-aspartates (substituents: methyl, chloro, or cyano) take the right-handed helical conformation, as does poly-β-(*p*-nitrobenzyl)-L-aspartate.[9] In this paper infrared studies of the copoly-L-aspartate esters in the left- or right-handed helical conformations in a chloroform solution will be presented.

Experimental

Materials. The samples polypeptides used in the present study are the same as those used in the previous report[7] except for poly-γ-benzyl-L-glutamate and poly-β-phenethyl-L-aspartate. Poly-γ-benzyl-L-glutamate was prepared according to the same procedure as was used for PBLA[7] (Found: C, 65.62; H, 5.97; N, 6.29%). β-Phenethyl-L-aspartate was prepared in a manner similar to that employed for β-benzyl-L-aspartate: mp 189—190°C (decomp.); $[\alpha]^{23}_{\mathrm{D}}$ +22.5° (*c* 1, glacial acetic acid). β-Phenethyl- L -aspartate- *N* -carboxyanhydride: mp 108—108.5°C; $[\alpha]^{24}_{\mathrm{D}}$ −33.9° (*c* 1.6, ethyl acetate). The NCA was polymerized, with a triethylamine initiation ($A/I=100$), to give poly-β-phenethyl-L-aspartate.

Found: C, 65.34; H, 6.16; N, 6.42%. Calcd for $C_{12}H_{13}O_3N$: C, 65.74; H, 5.98; N, 6.39%.

p-Substituted benzyl acetates were prepared by refluxing the corresponding *p*-substituted benzyl chlorides and potassium acetate; they were purified by distillation or by recrystallization, and were checked by elementary analysis.

Infrared Spectra. The infrared spectra of the polypeptides were recorded in the region from 5 μ to 10 μ

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1) T. Miyazawa, *J. Chem. Phys.*, **32**, 1647 (1960).

2) T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).

3) E. M. Bradbury, A. R. Downie, A. Elliott and W. E. Hanby, *Proc. Roy. Soc.*, **A 259**, 110 (1960).

4) R. H. Karlson, K. S. Norland, C. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2268 (1960).

5) T. Miyazawa, "Polyamino Acids, Polypeptides, and Proteins," ed. by M. A. Stahmann, Univ. of Wisconsin Press, Madison, Wisconsin (1962), p. 201.

6) E. M. Bradbury, L. Brown, A. R. Downie A Elliott, R. D. B. Fraser and W. E. Hanby, *J. Mol. Biol.*, **5**, 230 (1962).

7) M. Hashimoto and J. Aritomi, This Bulletin, **39**, 2707 (1966).

8) M. Hashimoto, *ibid.*, **39**, 2713 (1966).

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

on a Perkin-Elmer model 21 spectrophotometer equipped with a sodium chloride prism. The concentrations of the solutions were about 0.02 mol/l, and the cell thickness was 0.5 mm. In the 3 μ region, the measurements were carried out on a Perkin-Elmer model 521 grating infrared spectrophotometer used through the courtesy of Professor Tatsuo Miyazawa of the Institute for Protein Research, Osaka University.

Results and Discussion

The infrared spectra of poly-β-benzyl-L-aspartate (PBLA), poly-β-(p-methylbenzyl)-L-aspartate (PMeBLA), poly-β-(p-chlorobenzyl)-L-aspartate (PClBLA), and poly-β-phenethyl-L-aspartate (PPELA), together with poly-γ-benzyl-L-glutamate (PBLG) in a chloroform solution, were measured. The observed frequencies of the amide I, amide II, and NH stretching bands, together with the C=O stretching bands of the side-chain ester groups, are listed in Table 1. For PBLA, the observed frequencies of the amide I and amide II bands are higher by *ca.* 10 cm^{-1} and 5 cm^{-1} respectively than those of the other polyaspartate esters (PMeBLA, PClBLA, and PPELA). The frequencies of the amide I and amide II bands of poly-β-benzyl-L- or D-aspartate have been remarked to be higher than have so far been reported in α-helical polypeptide spectra.[8,9] The NH stretching band of PBLA appeared at 3298 cm^{-1}, whereas those of the other polyaspartate esters were observed at 3285—3290 cm^{-1}.

TABLE 1. OBSERVED FREQUENCIES (cm^{-1}) OF THE NH, ESTER C=O, AMIDE I, AND AMIDE II BANDS OF POLY-L-ASPARTATE ESTERS IN CHLOROFORM SOLUTION*

Substance	ν_{NH}	$\nu_{C=O}$ (ester)	Amide I	Amide II
PBLA	3298	1735	1668	1560
PMeBLA	(3286)**	1744	1656	1555
PClBLA	3285	1746	1657	1556
PPELA	3290	1741	1657	1554
PBLG	3287	1732	1651	1549

* PCNBLA and PNBLA were insoluble in chloroform.

** Estimated from Fig. 3.

Amide Bands of Main-chain Groups. Figures 1 and 2, respectively, show the amide I and amide II frequencies observed for the copolymers of β-benzyl-L-aspartate (BLA) with β-(p-methylbenzyl)-L-aspartate (MeBLA), β-(p-chlorobenzyl)-L-aspartate (ClBLA), β-(p-cyanobenzyl)-L-aspartate (CNBLA), or β-(p-nitrobenzyl)-L-aspartate (NBLA). In the series of the copolymers of BLA with CNBLA or NBLA, the copolymers containing residues of more than 60 mol% cyanobenzyl or 40 mol% nitrobenzyl are, unfortunately, insoluble in chloroform. In all the series of copolymers, the amide I frequencies change sharply as the molar contents of the p-substituents are varied, as can be seen in Fig. 1.

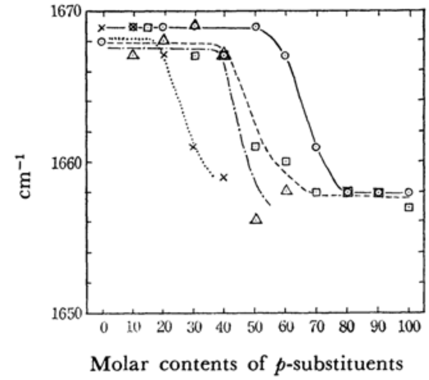

Fig. 1. Amide I frequencies of copoly-L-aspartate esters in a chloroform solution.
⊙—⊙—⊙, copolymers of BLA with MeBLA
⊡---⊡---⊡, copolymers of BLA with ClBLA
△—△—△, copolymers of BLA with CNBLA
×---×---×, copolymers of BLA with NBLA

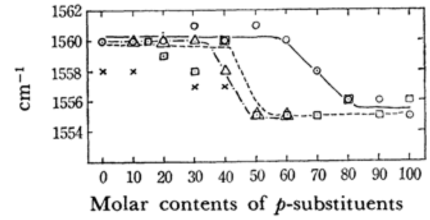

Fig. 2. Amide II frequencies of copoly-L-aspartate esters in a chloroform solution. Symbols as in Fig. 1.

These molar contents of p-substituents where the amide I frequencies are changing sharply are in good agreement with those of copolymers at the transitions from the left- to the right-handed helix found in the previous studies[7,8]; in the series of the copolymers of BLA with MeBLA, ClBLA, CNBLA, or NBLA, for instance, transitions occur at *ca.* 70, *ca.* 50, 40—50, and 20—30 mol% respectively. Accordingly, it may be concluded that, in each series of copolymers, the transition from the left- to the right-handed α-helix simultaneously brings about a sharp change in the amide I frequency. It should be noted that the amide I bands of these copolymers, in several kinds of p-substituents, and in p-substituents of various contents, lie in a narrow frequency range if the copolymers exist in the same helical sense.

Thereofre, the amide I frequencies of poly- or copoly-L-aspartate esters are *ca.* 1668 cm^{-1} and *ca.* 1658 cm^{-1} in the left- and the right-handed α-helices respectively. The amide I frequencies of solid films of α-helical poly-L-amino acids reported in the literature[10,11] lie in the range of 1652—

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[10] C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York (1956), p. 157.

[11] Y. Masuda, *Kobunshi Kagaku* (*Chemistry of High Polymers, Japan*), **20**, 206 (1963).

1658 cm^{-1}. Therefore, the amide I bands of poly- or copoly-L-aspartate esters in the right-handed α-helix lie in the normal frequency range, in contrast with the higher frequencies in the left-handed helix.

For the amide II band, frequency changes similar to those for the amide I band were observed, though they are less clear, since the frequency difference in the bands in the left- and right-handed senses is small as compared with that of the amide I band. The amide II frequencies of poly- or copoly-L-aspartate esters are 1560 cm^{-1} and 1555 cm^{-1} in the left- and right-handed α-helices respectively.

The amide II frequencies of α-helical poly-L-amino acids have been reported to be *ca.* 1550 cm^{-1}.[10,11] Therefore, the amide II frequencies of copoly-L-aspartate esters in the right-handed form are *ca.* 5 cm^{-1} higher than the normal amide II frequencies. However, this difference is not large. It may, therefore, be concluded that the amide II bands of copoly-L-aspartate esters in the right-handed form lie essentially in the normal frequency range.

For the NH stretching band, sharp frequency-changes were found, as can be seen in Fig. 3. The NH stretching frequencies of poly- or copoly-L-aspartate esters in the left- and the right-handed α-helices are 3298 cm^{-1} and 3285 cm^{-1} respectively. The NH stretching band of PBLG was observed at 3287 cm^{-1}. Therefore, for the NH stretching band, the poly- or copoly-L-aspartate esters in the right-handed form exhibit normal frequencies, and those in the left-handed form display frequencies higher by *ca.* 13 cm^{-1}.

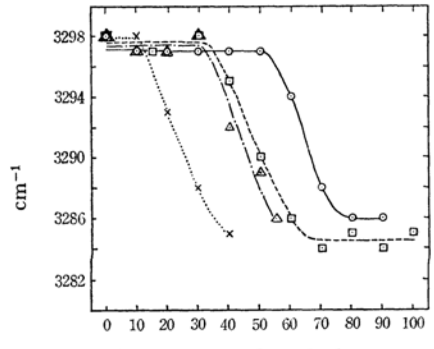

Fig. 3. NH stretching frequencies of copoly-L-aspartate esters in a chloroform solution. Symbols as in Fig. 1.

The amide III bands of these copolymers showed far less remarkable behavior. This may be due to the hybridization of the C–N stretching, N–H in-plane bending, and C–C_α stretching modes for this band.[12]

The NH stretching, amide I, and amide II frequencies of poly- or copoly-L-aspartate esters in the left-handed α-helix are higher by *ca.* 13 cm^{-1}, *ca.* 10 cm^{-1}, and *ca.* 5 cm^{-1} respectively than those in the right-handed form. These amide frequencies are anomalous, since amide bands of poly- or copoly-L-aspartate esters in the right-handed α-form lie in the normal frequency ranges as established for α-helical polypeptides.

These unusually higher amide frequencies of poly- or copoly-L-aspartate esters are to be ascribed to the reversal in the helical sense, but not to the particular aspartate group. These anomalous frequency shifts, from the right- to the left-handed helix, can not be interpreted simply in terms of the weakening of the intrachain N–H···O=C hydrogen bonds, since then the higher frequency shifts in the NH stretching and amide I bands and the simultaneous lower frequency shift in the amide II band should have been observed. X-Ray diffraction studies of PBLA have reported that PBLA in the α-form exhibits a layer line corresponding to 5.4 Å as has been observed for all α-helix structures.[6] Miyazawa *et al.*[13] have recently calculated the characteristic amide frequencies of poly-L-alanine in the rignt- and the left-handed α-conformations: the amide I and amide II frequencies for the left-handed form were found to be higher by 8 cm^{-1} and 2 cm^{-1} respectively than those for the right-handed form. They suggested that the frequency differences for the left- and right-handed helices are to be ascribed, at least in part, to the difference in the atomic arrangements of the β-carbon atoms with respect to the main chain. This is in harmony with the present results.

The C=O Stretching Band of Side-chain Ester Groups. The C=O stretching frequencies observed for the copolymers are shown in Fig. 4. In each series of copolymers the sharp frequency-changes with the variation in the molar contents of the *p*-substituent correspond well to such changes

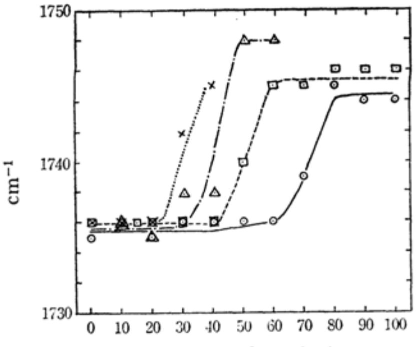

Fig. 4. Ester C=O stretching frequencies of copoly-L-aspartate esters in a chloroform solution. Symbols as in Fig. 1.

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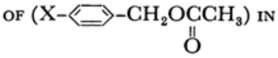
[12] Y. Miyazawa, T. Shimanouchi and S. Mizushima, *J. Chem. Phys.*, **29**, 611 (1958).

[13] T. Miyazawa, K. Fukushima, S. Sugano and Y. Masuda, International Symposium on Conformation of Biopolymers, Madras, January, 1967.

found for the amide bands described above. The helix reversal is found, therefore, to bring about a frequency difference in the C=O bands of copoly-L-aspartates. However, Fig. 4 appears to indicate that, for the copolymers in the right-handed helix, the C=O frequencies depend, even though weakly, upon the *p*-substituents.

Therefore, the infrared spectra of *p*-substituted benzyl acetates were examined as models of the side-chains of polyaspartate esters[14] (Table 2).

TABLE 2. OBSERVED C=O STRETCHING FREQUENCIES (cm^{-1}) OF (X-⟨⟩-CH$_2$OCCH$_3$) IN SOME SOLVENTS



$\mathrm{(X{-}C_6H_4{-}CH_2O\underset{\underset{O}{\|}}{C}CH_3)}$

Substance X =	Solvent		
	Chloroform	Carbon tetrachloride	Carbon disulfide
OCH_3	1736	1743	1741
CH_3	1738	1744	1746
H	1733	1747	1749
Cl	1738	1743	1747
CN	1739	1750	1749
NO_2	1739	1752	1751
(benzyl propionate)	1734	1743	1744

These C=O frequencies were found to depend upon the *p*-substituent, since a roughly linear relation was found between these frequencies and the Hammett's σ values of the *p*-substituents.[15]

For each series of *p*-substituted copolymers, the C=O frequencies of the copolymers in the right-handed helix in a chloroform solution are almost the same as those of the corresponding *p*-substituted benzyl acetate in nonpolar solvents, carbon tetrachloride, or carbon disulfide. On the contrary, for PBLA or the copolymers in the left-handed helix, the frequencies are lower than that of benzyl acetate in nonpolar solvents and are rather similar to that in chloroform.

This might be explained by side-chain solvations. Molecular model examinations can, however, not reasonably account for this difference in the solvation of the side-chain carbonyl groups in the left- and right-handed helices. In addition, a lower C=O stretching frequency was also found for a solid film of PBLA (1736 cm^{-1}) than in the other poly-L-aspartates (1739—1745 cm^{-1}).[16] Another possibility in accounting for these facts may be a difference of the internal rotations about the C_β–C_γ (C=O) bond between the left- and right-handed conformations; such a difference is, in fact, found for the solid films of poly-L-aspartate esters (the gauche and trans forms about the C_β–C_γ bond in the left- and right-handed helices respectively.[16]) It is highly likely that these conformations in solid films persist in chloroform solutions. Remarkable differences (*ca.* 20 cm^{-1}) in the C=O stretching frequencies have, indeed, been established for internal-rotation isomers of halogenated acetic acid alkyl esters, though these are primarily due to the dipolar field effects.[17]

From the characteristic amide frequencies (Table 1), PPELA was found to exist in the right-handed conformation (this polymer has an additional methylene group between the benzyl group and the ester group of PBLA). Its C=O stretching band, observed at 1741 cm^{-1}, is higher than that of PBLA. This C=O frequency is also consistent with its right-handed conformation, assuming the effects on the C=O frequency of the additional methylene group to be negligible.

The ester C–O stretching bands showed less remarkable changes in frequency than the C=O bands. The intensities of all the bands described above were also examined; however, no remarkable changes were found.

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[14] The corresponding benzyl propionate is considered to be preferable to the acetate as a model compound, as this compound involves a C–C bond adjacent to the CO–C bond. However, these acetates may make possible an estimate of the substitution effects in this case, though there seems to exist a slight difference in C=O frequency between benzyl acetate and propionate, as is shown in Table 2.

[15] A roughly linear relation between the C=O stretching frequencies and the σ values was found except for the chlorine substitution. NMR studies of these acetates also indicate that the chemical shifts of the benzylic methylene groups depend on the σ values of the *p*-substituents. Therefore, a linear relation holds between the C=O stretching frequencies and the chemical shifts of the methylene groups, including the chlorine group. These findings evidently indicate that the substituents bring about frequency shifts even in the C=O stretching bands.

[16] M. Hashimoto and S. Arakawa, to be published.

[17] M. L. Josien and C. Castinel, *Bull. Soc. Chim. France*, **25**, 801 (1958).