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A HIGH RESOLUTION ^{13}C NMR STUDY OF SOLID POLY(β-BENZYL-L-ASPARTATE) BY THE CROSS POLARIZATION - MAGIC ANGLE SPINNING METHOD. DISTINCTION OF THE RIGHT-HANDED α-HELIX, LEFT-HANDED α-HELIX, ω-HELIX, AND β-SHEET FORMS BY CONFORMATION-DEPENDENT ^{13}C CHEMICAL SHIFTS

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High resolution ^{13}C NMR spectra of solid poly(β-benzyl-L-aspartate) taking the right-handed α-helix, left-handed α-helix, ω-helix, and β-sheet conformations were recorded by the cross polarization-magic angle spinning method. ^{13}C chemical shifts of the right-handed α-helix form were significantly displaced as compared with those of the left-handed α-helix and ω-helix forms.

We have previously shown that ^{13}C chemical shifts of amino acid residues in solid polypeptides[1-3] and proteins[4] are significantly displaced taking particular conformations. In particular, ^{13}C chemical shifts of the C_α, C_β, and carbonyl carbons are displaced as large as 7 ppm between the right-handed α-helix (α_R-helix) and β-sheet forms.[1-4] It appears that ^{13}C chemical shifts might vary with the dihedral angles (ϕ and ψ),[1-6] as suggested by our previous theoretical calculation of ^{13}C chemical shifts utilizing the finite perturbation-INDO (FPT-INDO) molecular orbital theory.[7] Our view is that ^{13}C chemical shifts of individual amino-acid residues in polypeptides and proteins are mainly determined by the local conformation which governs the electronic state of the residue under consideration. As an alternative explanation of the aforementioned conformation-dependent ^{13}C chemical shifts, Tonelli[8] attempted to utilize the concept of the effect of the gauche arrangement of the γ-substituents (γ-effect).

In order to ascertain which mechanism is operative for the characteristic conformation-dependent ^{13}C shifts, it is essential to accumulate experimental ^{13}C chemical shifts which are specifically displaced for particular conformations in the solid state as many as possible. For this purpose, it is obvious that poly(β-benzyl-L-aspartate) [$(Asp(OBzl)_n$] provides one excellent source of the experimental data, because this molecule has four kinds of polymorphs depending on the condition of the crystallization as well as molecular weights: left-handed α-helix (α_L-helix), right-handed α-helix (α_R-helix), ω-helix, and β-sheet forms.[9-15] Initially, it was shown that this polymer normally forms the α_L-helix rather than the α_R-helix.[9-10] However, the α_R-helix is formed either at an air-water interface[13,14] or in films prepared from chloroform solution.[15] Thus, it is very important to examine how and to what extent ^{13}C chemical shifts of the α_L- and ω-helices are displaced relative to those of the α_R-helix and β-sheet forms.

In this communication, we demonstrate that these four forms are easily distinguishable by ^{13}C chemical shifts as determined by the cross polarization-magic angle spinning (CP-MAS) method.[16]

Higher molecular weight $(Asp(OBzl))_n$ (sample 1; M_v 14000) was a generous gift from Kyowa

Hakko Company, Japan. This sample was found to take the α_R-helix form as judged from the infrared spectra (1660 and 1552 cm^{-1}).[15] Lower molecular weight polymer (sample 2) was obtained by polymerization of β-benzyl-L-aspartate N-carboxyanhydride (NCA) in ethyl acetate using water as an initiator. Oligomers of $(Asp(OBzl))_n$ with various molecular weights were obtained by polymerization of β-benzyl-L-aspartate NCA in acetonitrile using butylamine as an initiator and the mole ratios of the monomer to the initiator 5 and 15 (samples 3 and 4, respectively). The number-average degree of polymerization ($\overline{DP}_n$) was determined by comparison of the peak-intensity of the CH_3 signal at the C-terminal residue with that of the $C_\beta H_2$ signal from 300 MHz ^1H NMR spectra taken in CF_3COOD solution. In order to obtain the α_L-helical sample, films were dried quickly from $CHCl_3$ solution.[15] The α_L-helix form was verified by the infrared spectra (1665 and 1550 cm^{-1}, converted from sample 1). The α_R-helical sample 1 was converted to the ω-helical form by heating the disk sample at 150 °C for 1 h. This conversion was checked by examining the characteristic change of the infrared spectra (1670 and 1535 cm^{-1}).[11] Further heating the ω-helical sample at 170 °C for 5 h resulted in the conversion to the β-sheet form (1630 and 1530 cm^{-1}).[17]

Single contact ^{13}C CP-MAS NMR spectra were recorded by a Bruker CXP-300 spectrometer operating at 75.46 MHz equipped with a CP-MAS accessory. Samples were placed in an Andrew-Beams rotor machined from perdeuterated poly(methyl methacrylate) and spun as fast as 3-4 kHz. A contact time of 800 μs was chosen not as optimal but to avoid a buildup of signals from the rotor and probe assembly. Chemical shifts were calibrated through external benzene and converted to the value from tetramethylsilane (TMS).

Figs. 1A-1D show the ^{13}C CP-MAS NMR spectra of the four forms of higher molecular weight polymer (sample 1) in the solid state. Interestingly, the C_α and carbonyl ^{13}C chemical shifts of the α_L-helix are displaced upfield by 2.5 and 3.8 ppm, respectively, relative to those of the α_R-helix, although the C_β signal is unchanged by going from the α_R-helix to α_L-helix (see Table 1). We found that sample 2 gave the ^{13}C NMR spectra of the α_R-helix and that

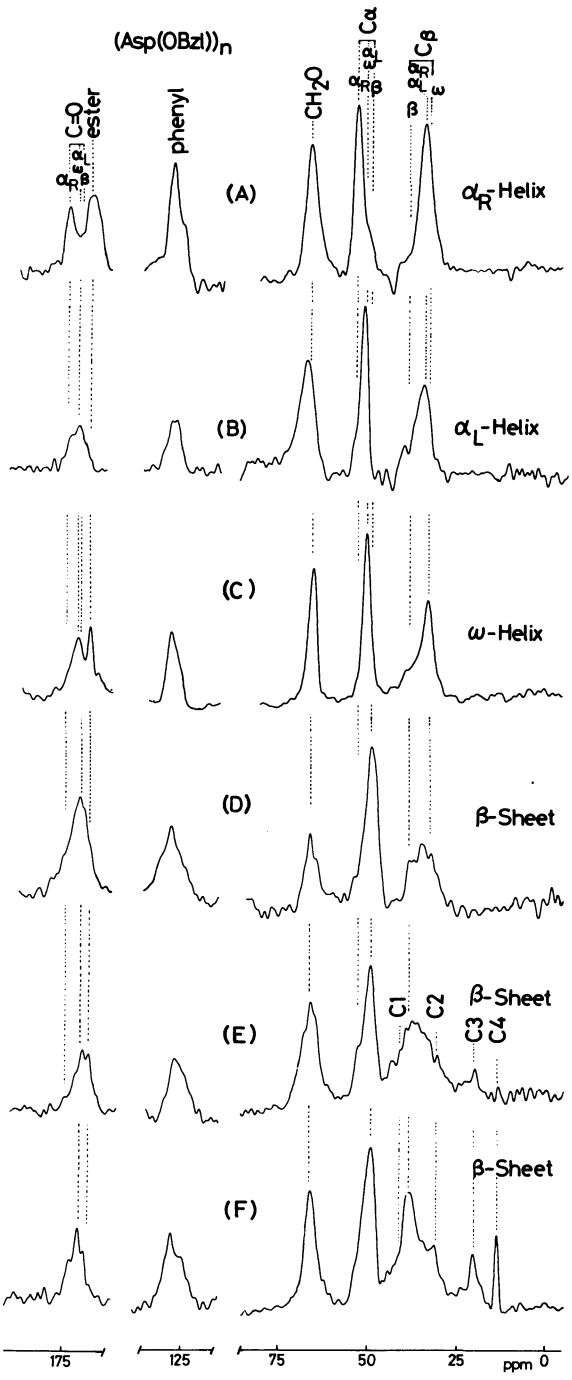

Fig. 1. 75.46 MHz ^{13}C CP-MAS NMR spectra of solid $(Asp(OBzl))_n$. (A)-(D) four conformations of the sample 1. For the procedure of conversion, see text. (E) sample 4 ($\overline{DP}_n$ 10), (F) sample 3 ($\overline{DP}_n$ 5). Spectral width 30 kHz; data points 4 K; recycle time 2 s. Number of transients 2000-3000.

Table 1. ^{13}C chemical shifts of various conformations of $(Asp(OBzl))_n$ in the solid state (ppm from TMS; ± 0.5 ppm)

Sample	Conformation	C_α	C_β	C=O (amide)	C=O (ester)	Phenyl	CH_2O
Polymer (Sample 1)	α_R-helix[a]	53.4	33.8	174.9	167.0	129.8	65.7
	α_L-helix	50.9	33.8	171.1	169.0[b]	129.2	66.1
	ω-helix	50.5	32.9	171.3	167.8	129.0	65.3
	β-sheet	49.2	38.2; 35.1	169.6	169.5[b]	129.2	65.9
Oligomers							
(DP_n 5; Sample 3)	β-sheet	49.1	38.0	169.8	168.2	129.0	65.5
($\overline{DP}_n$10; Sample 4)	β-sheet	49.5	36.8	169.6	168.0	128.4	65.9

a) Chemical shifts were also recorded in ref. 19. b) Shoulder at the amide carbonyl peak.

the films from chloroform solution gave mixture of the α_R- and α_L-helices (spectra not shown). The ^{13}C chemical shifts of the α_L-helix form are rather close to those of the ω-helix, because the dihedral angles of both forms are at proximate position in the conformational map (α_L, 47.0° and 57.2°; ω-helix, 64.4° and 55.4°).[18] Nevertheless, these two forms might be distinguishable by careful examination of the peak-position of the C_β signal (Table 1). In the β-sheet form, the C_β signal splits into three peaks and the linebroadening takes place simultaneously (Fig. 1D). The highermost peak might be ascribed to the small amount of the unchanged ω-helical form, as judged from the peak-position of the C_β signal. To make this point clear, we recorded the ^{13}C CP-MAS NMR spectra of oligomers of β-benzyl-L-aspartate with $\overline{DP}_n$ 10 and 5, taking the β-sheet conformation, as shown in Figs. 1E and 1F, respectively. The peaks designated by the C1-C4 are ascribed to butyl-amide group at the C-terminal residue. Naturally, these peaks of the oligomer with $\overline{DP}_n$ 10 are decreased (Fig. 1E). Obviously, the ^{13}C chemical shifts of the β-sheet form of the higher molecular-weight are identical to those of the oligomers, except for the C_β signal (Fig. 1). It appears that the C_β signal of the oligomer with $\overline{DP}_n$ 10 (36.8 ppm; Fig. 1E) is rather close to that of the most intense peak of the polymer (35.1 ppm; Fig. 1D). Previously, we showed that the C_β signal of the β-sheet poly(γ-benzyl-L-glutamate) $[(Glu(OBzl))_n]$ is considerably broadened and displaced to some extent as a result of disordering effect of the side-chain moiety.[3] Therefore, it is probable that the displaced C_β signal of Figs. 1D and 1E could be ascribed to the presence of disordered side-chain in the β-sheet form.

As summarized in Table 1, the displacement of the ^{13}C chemical shifts by going from the α_R-helix to α_L-helix (or ω-helix) is 2.5 (2.9), 0 (0.9), and 3.8 (3.6) ppm for the C_α, C_β, and carbonyl carbons, respectively. This observation is very similar to that observed between the α_R-helix of poly(L-alanine) and D- or L-alanine residues incorporated into the right-handed or left-handed α-helix form in the copoymers of L- and D-alanines, respectively.[2,7] As pointed out previously,[3] it is now possible to examine whether or not the α-helix form achieved in the solution state is identical to that of the solid state. In contrast to the case of $(Glu(OBzl))_n$,[3,19,20] there appears significant displacement of peaks in the C_α and carbonyl region between the data in Table 1 and solution state,[21,22] probably because of unstable nature of the α_L-helix in solution. Further, the displacement of the peaks between the α_R-helix and β-sheet form is 4.2, -4.4, and 5.3 ppm for the C_α, C_β, and carbonyl carbons, respectively, which is in consistent with our previous values.[1-4] Naturally, the ^{13}C chemical shifts arising from the benzyl side-chain do not exhibit the conformation-dependent change.

It is surprising to note that the C_α and carbonyl ^{13}C chemical shifts of the α_L-helix and ω-helix forms are very close to those of the β-sheet form, although the C_β signal is unchanged from that of the α_R-helix form. As pointed out above, a similar situation occurred in the copolymers of L- and D-alanines.[2,7] Itoh et al. showed that D-alanine or L-alanine residues incorporated into the right-handed or left-handed α-helices exhibit the conformational behavior of the α_L-helix.[23] In consistent with this view, we showed that the ^{13}C chemical shifts of D- and L-alanine residues in the copolymers are well reproduced by the calculated contour map of ^{13}C chemical shifts of N-acetyl N'-methyl-L-alanine amide as a model of $(Ala)_n$ utilizing the FPT-INDO method.[7] In contrast, it was difficult to explain the changes of ^{13}C chemical shifts by the concept of the γ-effect.[7] It is emphasized that our preliminary theoretical calculation of ^{13}C chemical shifts of $(Asp(OBzl))_n$ is in good agreement with the experimental displacement of shifts (unpublished).

In summary, we found that four kinds of conformation of $(Asp(OBzl))_n$ are easily distinguished by the characteristic displacements of the conformation-dependent ^{13}C chemical shifts, although chemical shifts of the α_L- and ω-helices are very close reflecting the proximate position in the conformational map. These ^{13}C chemical shifts are very valuable in understanding the behavior of the conformation-dependent ^{13}C chemical shifts of polypeptides as well as role of the diagnostic purpose in elucidation of the conformational behavior.

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