Nitrogen-15 Chemical Shift Tensors and Conformation of Poly(β-benzyl L-aspartate) in the Solid State by NMR

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ABSTRACT: The relationship between the ^{15}N isotropic chemical shift (δ_{iso}) or ^{15}N chemical shift tensor components (δ_{11}, δ_{22}, and δ_{33}) and the main-chain conformation—such as the right-handed α-helix (α_R-helix), left-handed α-helix (α_L-helix), left-handed ω-helix (ω_L-helix), and antiparallel β-sheet (β-sheet) forms of ^{15}N-labeled poly(β-benzyl L-aspartate) [Asp*(OBzl)]$_n$ in the solid state—was studied using solid-state ^{15}N NMR methods. We have found that the δ_{iso} and δ_{22} of Asp*(OBzl) residue were significantly displaced depending upon conformation: δ_{iso} (α_R-helix, 98.9; α_L-helix, 96.8; ω_L-helix, 96.3; β-sheet, 100.0 ppm) and δ_{22} (α_R-helix, 52.8; α_L-helix, 48.3; ω_L-helix, 47.4; β-sheet, 56.4 ppm). Thus, it became apparent that the δ_{iso} and δ_{22} are very useful barometers for the conformational analysis, especially for the determination of the helical sense of [Asp*(OBzl)]$_n$ in the solid state.

Introduction

High-resolution and solid-state ^{15}N NMR spectroscopy offers many possibilities for the investigation of the structure and dynamics in polypeptides, proteins, and biopolymers.[1−19] In our previous investigations,[13−17] it was demonstrated that the ^{15}N isotropic chemical shifts (δ_{iso}) of solid polypeptides are sensitive to the nature of individual amino acid residues as well as the secondary structure (main-chain conformation) such as right-handed α-helix (α_R-helix) and antiparallel β-sheet (β-sheet), poly(glycine) I (PGI) and II (PGII), silk I and II, and collagen-like triple helix (triple helix) forms. The δ_{iso} determined from the cross-polarization−magic angle spinning (CP-MAS) method in an α_R-helical polypeptide is generally upfield in comparison with that in a β-sheet polypeptide, which was supported by the theoretical calculation of ^{15}N shielding constants (chemical shifts) utilizing the finite perturbation INDO (FPT-INDO) theory.[13] The calculation of the ^{15}N shielding constants was applied also to PGI and PGII forms, and we found that our experimental results were qualitatively explained by the theoretical calculation.[17]

The ^{15}N chemical shift tensor components (δ_{11}, δ_{22}, and δ_{33}) determined from the CP-static (powder pattern) spectra, on the contrary, provide more detailed information about the conformation of polypeptides.[14] It is clear, in particular, that the δ_{22} (the direction of which is assumed to be perpendicular to the peptide plane[20−23]) is sensitive mainly to the secondary structure of polypeptides, and the difference of the δ_{22} values between the α_R-helix and β-sheet forms is generally larger than that of the δ_{iso}. Furthermore, the ^{15}N chemical shift tensor components seem to provide extensive information about the neighboring amino acid sequence of peptides in the solid state.[14−17] The ^{15}N chemical shift tensor components could become extremely useful for the structural analysis of polypeptides and proteins in the solid state.

Using the ^{15}N chemical shift data of peptides, polypeptides, and proteins in solution,[24,25] it is possible to study the correlation between the ^{15}N chemical shifts and the conformational parameters such as main-chain dihedral angles. Le and Oldfield[25,26] recently reported the empirical correlation between the ^{15}N isotropic chemical shift ($\delta^{(i)}$) of the ith amino acid residue and the dihedral angles $\phi^{(i)}$ (C′−N−C$_\alpha$−C′) of the ith residue and $\psi^{(i-1)}$ (N− C$_\alpha$ −C′−N) of the (i − 1)-th residue. However, it is unfortunate that they did not show any data for ^{15}N chemical shift tensor components, which are very important structural parameters to follow and evaluate whether their conclusion is correct or not. To clarify this, however, it is necessary to accumulate the experimental ^{15}N chemical shift tensors data for various kinds of conformation of polypeptides in the solid state. Furthermore, the correlation between the ^{15}N chemical shifts (including chemical shift tensor components) and the structural parameters describing the conformational feature is not clarified yet.

To clarify this, therefore, it is necessary to accumulate the ^{15}N chemical shift data for various kinds of conformations of polypeptides in the solid state. For this, poly(β-benzyl L-aspartate) [Asp(OBzl)]$_n$ is a quite interesting sample, because it is well-known to form four types of conformations, the α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms, by appropriate treatment.[27] Therefore, we have prepared the ^{15}N-labeled poly(β-benzyl L-aspartate) [Asp*(OBzl)]$_n$ adopting the four types of conformations and obtained their ^{15}N chemical shifts by the ^{15}N CP-MAS and CP-static NMR methods. Moreover, we have compared their ^{15}N chemical shift data, which have the polar side-chain esters with those of the other specific amino acid residue with nonpolar side chains.

Experimental Section

Materials. The ^{15}N-labeled β-benzyl L-aspartate (Asp*(OBzl); 99 at. % of isotope purity) was purchased from ISOTEC, Inc. The Asp*(OBzl)-*N*-carboxy anhydride (NCA) was synthesized by phosgenation of Asp*(OBzl) and recrystallized from ethyl acetate/*n*-hexane and then from chloroform/*n*-hexane.

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The ^{15}N-labeled poly(β-benzyl L-aspartate) [Asp*(OBzl)]$_n$ was prepared by polymerization of Asp*(OBzl)−NCA in 1,2-dichloroethane at 30 °C using triethylamine as an initiator. The contents of the Asp*(OBzl) against natural abundance of Asp(OBzl) in the polypeptide is about 20 mol %. The molar ratio of the Asp*(OBzl)−NCA (A) to the initiator (I), A/I, was 150. The reaction mixture was poured into methanol, and the precipitated [Asp*(OBzl)]$_n$ was filtered, washed with methanol and acetone, and then dried in vacuo.

The conformation of the [Asp*(OBzl)]$_n$ sample as polymerized was the α_R-helix form ([Asp*(OBzl)]$_n$-1). The α_L-helical sample ([Asp*(OBzl)]$_n$-2) was obtained from the [Asp*(OBzl)]$_n$-1 by precipitation from the concentrated chloroform solution of the sample [Asp*(OBzl)]$_n$-1. The sample [Asp*(OBzl)]$_n$-1 was also converted to the ω_L-helix form ([Asp*(OBzl)]$_n$-3) and to the β-sheet form ([Asp*(OBzl)]$_n$-4) by heating at 150 °C for 3.5 h and 220 °C for 3 h, respectively, in vacuo. The conformational characterization of these samples was made on the basis of the conformation-dependent ^{13}C chemical shifts determined using CP-MAS NMR and also by the characteristic bands in the infrared (IR) spectra.

Measurements. The solid-state ^{15}N and ^{13}C NMR measurements were performed using a JEOL EX-270W spectrometer operating at 27.25 and 67.80 MHz, respectively, equipped with a CP-MAS probe. The contact time was 2−5 ms (for both ^{15}N and ^{13}C), and the repetition time was 5 s (for both ^{15}N and ^{13}C). The 90° pulse width was typically 6.3 μs for both ^{15}N and ^{1}H under CP conditions and 4.4 μs for both ^{13}C and ^{1}H. The spectral width was 20 kHz (for ^{15}N CP-MAS), 200 kHz (for ^{15}N CP-static), and 27 kHz (for ^{13}C CP-MAS), and the data points were 8K (for CP-MAS) and 16K (for ^{15}N CP-static). The spectra were accumulated ca. 300−3800 (for ^{15}N CP-MAS) and 2200−7200 (for ^{13}C CP-MAS) to achieve a reasonable signal-to-noise ratio for samples. The ^{15}N CP-static (powder pattern) spectra were recorded without spinning and were accumulated 9000−15 000 times to achieve a reasonable signal-to-noise ratio for samples. The ^{15}N chemical shifts were calibrated indirectly by external $^{15}NH_4Cl$ (δ 18.0) relative to saturated $^{15}NH_4NO_3$ solution (δ 0) in H_2O. The ^{13}C chemical shifts were calibrated indirectly by external adamantane (29.5 ppm relative to tetramethylsilane, $(CH_3)_4Si$). The experimental errors in the data for the isotropic ^{15}N and ^{13}C chemical shift values were estimated to be less than ±0.3 ppm.

The ^{15}N chemical shift tensor components (δ_{11}, δ_{22}, and δ_{33} from the downfield side) were obtained from powder pattern spectra. The values of δ_{22} can be read directly from the observed powder pattern (CP-static) spectra, with the error being less than ±0.5 ppm. The remaining two components (δ_{11} and δ_{33}) were estimated in order to satisfy the following equation: $\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3$, where the values of δ_{iso} and δ_{22} were read directly from the observed CP-MAS and powder pattern spectra. So, the error limits of δ_{11} and δ_{33} (less than ±2 ppm) were larger than those of δ_{iso} and δ_{22}.

Results and Discussion

Conformational Characterization of [Asp*-(OBzl)]$_n$ Samples. To clarify the relation between the ^{15}N chemical shifts (δ_{iso}, δ_{11}, δ_{22}, and δ_{33}) and the main-chain conformation (α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms) of [Asp*(OBzl)]$_n$ samples, it is first necessary to characterize their conformations in the solid state. The conformational characterization of the [Asp*(OBzl)]$_n$ samples was made on the basis of the conformation-dependent ^{13}C chemical shifts.

Figure 1 shows 67.80 MHz ^{13}C CP-MAS NMR spectra of [Asp*(OBzl)]$_n$ adopting four different kinds of conformations (α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms). The assignment of the peaks for each carbon was determined on the basis of reference data reported previously.[28,29] The ^{13}C isotropic chemical shifts determined from these spectra are listed in Table 1.

For [Asp*(OBzl)]$_n$-1, the ^{13}C chemical shift values of the CO (amide), CO (ester), C$_\alpha$, and C$_\beta$ signals are 175.0, 168.9, 54.1, and 34.8 ppm, respectively, which are characteristic of the α_R-helix form. For [Asp*(OBzl)]$_n$-2, the ^{13}C chemical shift values were observed at 172.5 (CO (amide and ester)), 51.5 (C$_\alpha$), and 34.5 ppm (C$_\beta$), which are characteristic of the α_L-helix form. These values are in good agreement with those obtained for films cast from chloroform solution.[28] For [Asp*(OBzl)]$_n$-3, the ^{13}C chemical shift values of the CO (amide and ester), C$_\alpha$, and C$_\beta$ signals are 172.1, 51.8, and 34.1 ppm, respectively, which are characteristic of the ω_L-helix form. As shown in Figure 1c, the peaks assigned to the α_R-helix form were not detected, suggesting complete conformational conversion from α_R-helix to ω_L-helix in the solid state. This result was also confirmed by characteristic bands (amide I, 1675 cm^{-1}; amide II, 1533 cm^{-1}) in the IR spectra.[30,31] For [Asp*(OBzl)]$_n$-4, finally, the CO (amide and ester), C$_\alpha$, and C$_\beta$ peaks appeared at 171.0, 51.1, and 39.6 and 35.3 ppm (doublet), respectively. These values are in good agreement with reference data[28] in [Asp(OBzl)]$_n$ (β-sheet) and for β-benzyl L-aspartate oligomers (β-sheet). The peaks corresponding to the α_R-helix, α_L-helix, or ω_L-helix form were not observed in Figure 1d, indicating that most of the [Asp*(OBzl)]$_n$-1 (α_R-helix) was transformed into [Asp*(OBzl)]$_n$-4 (β-sheet).

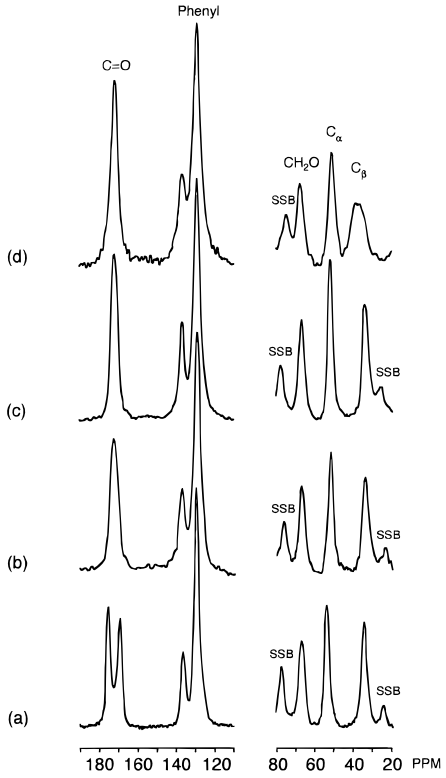

Figure 1. The 67.80 MHz ^{13}C CP-MAS NMR spectra of [Asp*(OBzl)]$_n$ with (a) α_R-helix, (b) α_L-helix, (c) ω_L-helix, and (d) β-sheet forms in the solid state.

From these results, it was confirmed that the samples having the desired conformation were successfully prepared, and these samples contain negligibly small amounts of other conformations.

^{15}N Isotropic Chemical Shift (δ_{iso}) of [Asp*-(OBzl)]$_n$. Figure 2 shows 27.25 MHz ^{15}N CP-MAS NMR spectra of [Asp*(OBzl)]$_n$ adopting the α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms. The δ_{iso} determined from the observed spectra are listed in Table 2, and a diagram of the δ_{iso} of [Asp*(OBzl)]$_n$ adopting α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms is shown in Figure 3

Table 1. ^{13}C Chemical Shifts and Conformational Characterization of [Asp*(OBzl)]$_n$ in the Solid State

	^{13}C chemical shift,[a] δ (ppm)							
sample	C═O (amide)	C═O (ester)	phenyl (C_1)	phenyl (C_{2-6})	CH_2O	C_α	C_β	conformation
[Asp*(OBzl)]$_n$-1	175.0	168.9	135.9	128.9	66.8	54.1	34.8	α_R-helix
[Asp*(OBzl)]$_n$-2	172.5	sh[b]	136.6	128.8	66.2	51.5	34.5	α_L-helix
[Asp*(OBzl)]$_n$-3	172.1	sh[b]	136.8	129.4	66.3	51.8	34.1	ω_L-helix
[Asp*(OBzl)]$_n$-4	171.0	171.0	136.3	128.9	66.7	51.1	39.6, 35.3	β-sheet

[a] ±0.3 ppm from TMS. [b] Abbreviation "sh" indicates shoulder at the C═O (amide) peak.

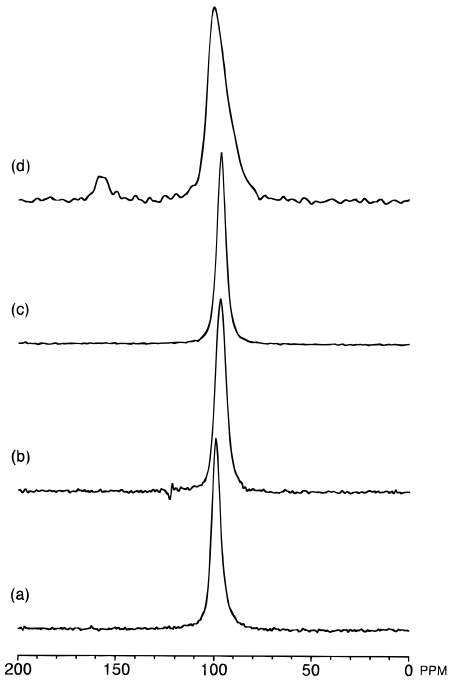

Figure 2. The 27.25 MHz ^{15}N CP-MAS NMR spectra of [Asp*(OBzl)]$_n$ with (a) α_R-helix, (b) α_L-helix, (c) ω_L-helix, and (d) β-sheet forms in the solid state.

Table 2. ^{15}N Chemical Shift (δ_{iso}) and ^{15}N Chemical Shift Tensors (δ_{11}, δ_{22}, δ_{33}) of [Asp*(OBzl)]$_n$ Characteristic in α_R-Helix, α_L-Helix, ω_L-Helix, and β-Sheet Forms

		^{15}N chemical shift,[a] δ (ppm)				
sample	conformation	δ_{iso}	δ_{11}	δ_{22}	δ_{33}	$\delta_{11} + \delta_{33}$
[Asp*(OBzl)]$_n$-1	α_R-helix	98.9	205	52.8	39	244
[Asp*(OBzl)]$_n$-2	α_L-helix	96.8	201	48.3	41	242
[Asp*(OBzl)]$_n$-3	ω_L-helix	96.3	202	47.4	40	242
[Asp*(OBzl)]$_n$-4	β-sheet	100.0	203	56.4	41	244

[a] Experimental errors were estimated to be less than ±0.3 ppm for δ_{iso}, ±0.5 ppm for δ_{22}, and ±2 ppm for δ_{11} and δ_{33} (from $^{15}NH_4NO_3$).

together with the reference data of δ_{iso} for some other homopolypeptides adopting the α_R-helix and β-sheet forms. The δ_{iso} values of [Asp*(OBzl)]$_n$ adopting the α_R-helix and β-sheet forms appear at 98.9 and 100.0 ppm, respectively, in agreement with our previous data for natural abundance of [Asp(OBzl)]$_n$ within experimental error.[16] We have also confirmed that the δ_{iso} of low molecular weight [Asp(OBzl)] (β-sheet, 100.4 ppm) was consistent with that of [Asp*(OBzl)]$_n$-4 within experimental error.[16] Thus, the difference of the δ_{iso} of [Asp*(OBzl)]$_n$ between α_R-helix and β-sheet forms is small (1.1 ppm) with respect to those of [Ala*]$_n$ (3.4 ppm) and [Leu*]$_n$ (10.0 ppm) with hydrophobic (nonpolar hydrocarbon) side chains.[14−16] It is noteworthy that the δ_{iso} of the Asp*(OBzl) residue adopting the α_R-helix form appears downfield relative to those of other amino acid residues and is even close to those of β-sheet polypeptides. In contrast, the δ_{iso} of the Asp*(OBzl) residue adopting the β-sheet form appears upfield relative to those of other amino acid residues and close to those of Glu(OBzl) (99.5 ppm) and Glu(OMe) (99.5 ppm) with polar side-chain esters.[13,16] Since the differences of the δ_{iso} between α_R-helix and β-sheet forms in both [Glu(OBzl)]$_n$ and [Glu(OMe)]$_n$ are also small (1.9 ppm), such chemical shift displacements may be ascribed to a characteristic feature in amino acid residues with polar side-chain esters.

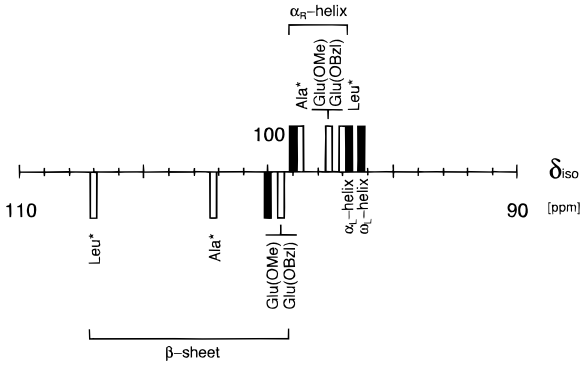

Figure 3. Diagram of the ^{15}N isotropic chemical shifts (δ_{iso}) of [Asp*(OBzl)]$_n$ with four different kinds of conformations (closed bars), together with some other amino acid residues in homopolypeptides (open bars) in the solid state. Abbreviations: Ala*, ^{15}N-labeled L-alanine; Leu*, ^{15}N-labeled L-leucine; Glu(OBzl), γ-benzyl L-glutamate; and Glu(OMe), γ-methyl L-glutamate residue.

Next, we consider the relation between the δ_{iso} and the helix sense of [Asp*(OBzl)]$_n$. As Figure 4 shows, the δ_{iso} of the α_L-helix [Asp*(OBzl)]$_n$-2 (96.8 ppm) is displaced upfield by 2.1 ppm with respect to that of the α_R-helix one (98.9 ppm). Similar chemical shift displacement in δ_{iso} has been observed for the Ala residue incorporated into the α_L-helix of poly(D-alanine), which was published previously.[15] This result indicates that the δ_{iso} is sensitive to the helical sense of polypeptides and that the left-handed helix form appears upfield with respect to the right-handed helix form. Therefore, the ^{15}N chemical shift is superior to the ^{13}C chemical shift in analyzing the helix sense such as right-handed or left-handed helix conformation.

In addition, the δ_{iso} value of the α_L-helix [Asp*(OBzl)]$_n$-2 (96.8 ppm) is close to that of the ω_L-helix form (96.3 ppm). This can be understood by considering the similar dihedral angles (ϕ and ψ) of polypeptide backbone between these two conformations (α_L-helix, ϕ = 57.2° and ψ = 47.0°; ω_L-helix, 64.4° and 55.4°, respectively[31−35]), which was supported also by the ^{13}C chemical shift data of α_L-helix and ω_L-helix, as shown in Table 1. From the theoretical calculation of ^{15}N shielding constants (chemical shifts) for *N*-acetyl L-alanine methylamide as a model peptide utilizing the finite perturbation INDO (FPT-INDO) theory,[13] it was shown that the δ_{iso} is conformation-dependent of polypeptides in the solid state. Consequently, it is concluded that the δ_{iso}

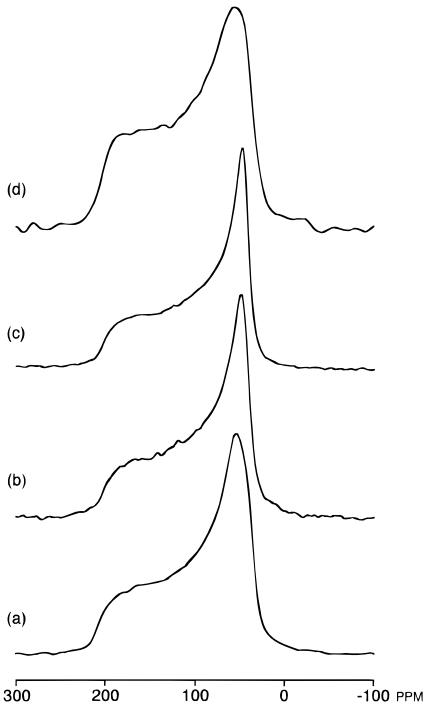

Figure 4. The 27.25 MHz ^{15}N CP-static (powder pattern) NMR spectra of [Asp*(OBzl)]$_n$ with (a) α_R-helix, (b) α_L-helix, (c) ω_L-helix, and (d) β-sheet forms in the solid state.

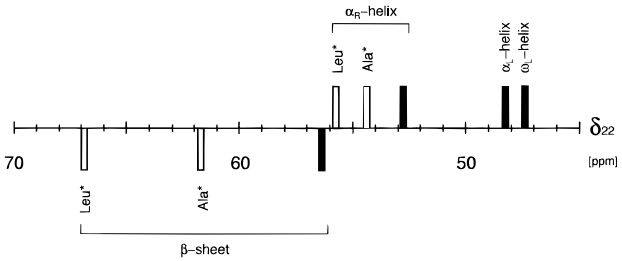

Figure 5. Diagram of the principal value δ_{22} of the ^{15}N chemical shift tensors of [Asp*(OBzl)]$_n$ with four different kinds of conformations (closed bars), together with some other amino acid residues in homopolypeptides (open bars) in the solid state. Abbreviations: Ala*, ^{15}N-labeled L-alanine; Leu*, ^{15}N-labeled L-leucine.

of [Asp*(OBzl)]$_n$ depends mainly on main-chain conformation (including the helix sense) of polypeptides in the solid state.

^{15}N Chemical Shift Tensor Components (δ_{11}, δ_{22}, and δ_{33}) of [Asp*(OBzl)]$_n$. Figure 4 shows 27.25 MHz ^{15}N CP-static (powder pattern) NMR spectra of [Asp*(OBzl)]$_n$ adopting the α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms. The ^{15}N chemical shift tensor components (δ_{11}, δ_{22}, and δ_{33}) determined from the powder pattern spectra are listed in Table 2, and a diagram of the δ_{22} of [Asp*(OBzl)]$_n$ adopting α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms in the solid state is shown in Figure 5, together with the reference data of δ_{22} for some homopolypeptides adopting the α_R-helix and β-sheet forms.

We consider, at first, the δ_{22} values of the Asp*(OBzl) residues. The δ_{22} of the β-sheet [Asp*(OBzl)]$_n$ (56.4 ppm) is observed downfield with respect to that of α_R-helix form (52.8 ppm). For the β-sheet samples, we analyzed and determined this powder pattern spectra very carefully, because this peak is broader than other spectra (Figure 4). This conformational dependency of the δ_{22} observed in [Asp*(OBzl)]$_n$ is the same result as that of the Ala* and Leu* residues, indicating that the δ_{22} of the homopolypeptides[14−16] is useful for the conformational analysis of polypeptides in the solid state. However, the chemical shift difference of δ_{22} between the α_R-helix and β-sheet forms is 3.6 ppm, which is larger than that of δ_{iso}. This feature is the same result as those obtained for [Ala*]$_n$ and [Leu*]$_n$, but the chemical shift difference of the δ_{22} is smaller than that of [Ala*]$_n$ (7.3 ppm) and [Leu*]$_n$ (11.2 ppm).[14−16] It is noteworthy that the δ_{22} of the Asp*(OBzl) residue adopting the α_R-helix appears upfield relative to those of the Ala* and Leu* residues, which is quite different from the δ_{iso}. On the contrary, the δ_{22} of the Asp*(OBzl) residue adopting the β-sheet appears also upfield relative to those of the Ala* and Leu* residues, which is nearly the same as the δ_{iso}. Thus, the δ_{22} of the Asp*(OBzl) residue seems to be characteristic of the nature of amino acid residues with polar side-chain esters and is very useful for the conformational analysis of [Asp*(OBzl)]$_n$ in the solid state.

Second, as shown in Figure 5, the δ_{22} of the α_L-helix form (48.3 ppm) of [Asp*(OBzl)]$_n$ is displaced upfield by 4.5 ppm with respect to that of the α_R-helix form (52.8 ppm). This chemical shift displacement of the δ_{22} (4.5 ppm) between the α_R-helix and α_L-helix forms is large with respect to that of the δ_{iso} (2.1 ppm), suggesting that the δ_{22} is more useful than δ_{iso} for distinction of the helix sense such as the right-handed or left-handed α-helix. However, this is a quite different result from the δ_{22} of the Ala* residue incorporated into the left-handed α-helix form of poly(D-alanine).[15] As reported previously,[15,16] in the case of [Ala*,D-Ala]$_n$ (Ala* content is 5%), the δ_{22} value (57.1 ppm) of the Ala* residue (α_L-helix form) incorporated into [D-Ala]$_n$ (left-handed α-helix) is displaced downfield by 2.7 ppm with respect to that of α_R-helix [Ala]$_n$ (54.4 ppm). This difference in the chemical shift displacement of the δ_{22} may be caused by the neighboring amino acid sequence effect in the case of [Ala*,D-Ala]$_n$ (Ala* content is 5%).[15,16] Therefore, it is noteworthy that δ_{22} depends on both the copolymer content and conformation of polypeptides in the solid state, whereas δ_{iso} depends only on conformation and is independent of the copolymer content, which is consistent with our previous papers.[14−17]

Third, the δ_{22} of the ω_L-helix form (47.4 ppm) is displaced upfield (by 5.4 ppm) with respect to that of the α_R-helix, but it is displaced very little upfield (by 0.9 ppm) with respect to that of the α_L-helix [Asp*(OBzl)]$_n$. Accordingly, it may be assumed that the δ_{22} of the Asp*(OBzl) residue is very useful for conformational analysis and especially for the helix sense determination of polypeptides. In addition, the characteristic values for δ_{22} of the α_L-helix and ω_L-helix form of [Asp*(OBzl)]$_n$ in the solid state are now clarified.

Finally, we discuss the sum of δ_{11} and δ_{33} values ($\delta_{11} + \delta_{33}$). As shown in Table 2, the displacement of $\delta_{11} + \delta_{33}$ is very small with respect to that of δ_{22}, so that $\delta_{11} + \delta_{33}$ is almost independent of the conformation. Therefore, it may be concluded that the displacement in δ_{iso} comes mainly from that of δ_{22}. A similar relationship has already been pointed out for the ^{13}C chemical shift tensors of the carbonyl carbon in these peptides.[36,37] Although the reason is not clear yet, this finding seems to be useful for understanding the nature of the chemical shift tensors.

Concluding Remarks

We have synthesized ^{15}N-labeled poly(β-benzyl L-aspartate), [Asp*(OBzl)]$_n$, and successfully prepared the samples adopting four different kinds of conformations (α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms) by appropriate treatment. The conformational characterization of these samples was made on the basis of the conformation-dependent ^{13}C NMR chemical shifts and also by the characteristic bands in the IR spectra, and it was confirmed that the samples having the desired conformation were successfully prepared. The relationship between the ^{15}N chemical shifts (δ_{iso}, δ_{11}, δ_{22}, and δ_{33}) and the secondary structure (main-chain conformation)—α_R-helix, α_L-helix, ω_L-helix, and β-sheet forms of [Asp*(OBzl)]$_n$—was studied using solid-state ^{15}N NMR methods. We have found that the δ_{iso} and δ_{22} of the Asp*(OBzl) residue were significantly displaced depending on conformation: δ_{iso} (α_R-helix, 98.9; α_L-helix, 96.8; ω_L-helix, 96.3; β-sheet, 100.0 ppm) and δ_{22} (α_R-helix, 52.8; α_L-helix, 48.3; ω_L-helix, 47.4; β-sheet, 56.4 ppm).

It should be emphasized in this study that the ^{15}N isotropic chemical shift (and ^{15}N chemical shift tensors) are superior to the ^{13}C isotropic chemical shift in analyzing the helix sense such as right-handed or left-handed helix conformation. Thus, it became apparent that the δ_{iso} and δ_{22} are very useful for the conformational analysis (including the helix sense determination) of [Asp*(OBzl)]$_n$ in the solid state.

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References and Notes

(1) Förster, H. G.; Müller, D.; Kricheldorf, H. R. *Int. J. Biol. Macromol.* **1983**, *5*, 101−105.
(2) Huang, T. H.; Bachovchin, W. W.; Griffin, R. G.; Dobson, C. M. *Biochemistry* **1984**, *23*, 5933−5937.
(3) Stewart, P. L.; Valentine, K. G.; Opella, S. J. *J. Magn. Reson.* **1987**, *71*, 45−61.
(4) Fields, G. B.; Fields, C. G.; Petefish, J.; Van Wart, H. E.; Harold, E.; Cross, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1384−1388.
(5) Garbow, J. R.; Jacob, G. S.; Stejskal, E. O.; Schaefer, J. *Biochemistry* **1989**, *28*, 1362−1367.
(6) Ketchem, R. R.; Hu, W.; Cross, T. A. *Science* **1993**, *261*, 1457−1460.
(7) Nicholson, L. K.; Asakura, T.; Demura, M.; Cross, T. A. *Biopolymers* **1993**, *33*, 847−861.
(8) Asakura, T.; Demura, M.; Hiraishi, Y.; Ogawa, K.; Uyama, A. *Chem. Lett.* **1994**, 2249−2252.
(9) Naito, A.; Tuzi, S.; Saito, H. *Eur. J. Biochem.* **1994**, *224*, 729−734.
(10) North, C. L.; Barranger-Mathys, M.; Cafiso, D. S. *Biophys. J.* **1995**, *69*, 2392−2397.
(11) Lazo, N. D.; Hu, W.; Cross, T. A. *J. Magn. Reson. B* **1995** *107*, 43−50.
(12) Asakura, T.; Demura, M.; Date, T.; Miyashita, N.; Ogawa, K.; Williamson, M. P. *Biopolymers* **1997**, *41*, 193−203.
(13) Shoji, A.; Ozaki, T.; Fujito, T.; Deguchi, K.; Ando, I. *Macromolecules* **1987**, *20*, 2441−2445.
(14) Shoji, A.; Ozaki, T.; Fujito, T.; Deguchi, K.; Ando, S.; Ando, I. *Macromolecules* **1989**, *22*, 2860−2863.
(15) Shoji, A.; Ozaki, T.; Fujito, T.; Deguchi, K.; Ando, S.; Ando, I. *J. Am. Chem. Soc.* **1990**, *112*, 4693−4697.
(16) Shoji, A.; Ando, S.; Kuroki, S.; Ando, I.; Webb, G. A. In *Annual Reports on NMR Spectroscopy*; Webb, G. A., Ed.; Academic Press: London, 1993; Vol. 26, p 55.
(17) Shoji, A.; Ozaki, T.; Fujito, T.; Deguchi, K.; Ando, I.; Magoshi, J. *J. Mol. Struct.* **1998**, *441*, 251−266.
(18) Kuroki, S.; Ando, S.; Ando, I.; Shoji, A.; Ozaki, T.; Webb, G. A. *J. Mol. Struct.* **1990**, *240*, 19−29.
(19) Kuroki, S.; Asakawa, N.; Ando, S.; Ando, I.; Shoji, A.; Ozaki, T. *J. Mol. Struct.* **1991**, *245*, 69−80.
(20) Hiyama, Y.; Niu, C. H.; Chien, H.; Silverton, J. V.; Bavoso, A.; Torchia, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 2378−2383.
(21) Oas, T. G.; Hartzell, C. J.; Dahlquist, F. W.; Drobny, G. P. *J. Am. Chem. Soc.* **1987**, *109*, 5962−5966.
(22) Hartzell, C. J.; Whitfield, M.; Oas, T. G.; Drobny, G. P. *J. Am. Chem. Soc.* **1987**, *109*, 5966−5969.
(23) Valentine, K. G.; Rockwell, A. L.; Gierasch, L. M.; Opella, S. J. *J. Magn. Reson.* **1987**, *73*, 519−523.
(24) Glushka, J.; Lee, M.; Coffin, S.; Cowburn, D. *J. Am. Chem. Soc.* **1989**, *111*, 7716−7722.
(25) Le, H.; Oldfield, E. *J. Biomol. NMR* **1994**, *4*, 341−348.
(26) Le, H.; Oldfield, E. *J. Phys. Chem.* **1996**, *100*, 16423−16428.
(27) Kyotani, H.; Kanetsuna, H. *J. Polym. Sci., Polym. Phys. Ed.* **1972**, *10*, 1931−1939.
(28) Saito, H.; Tabeta, R.; Ando, I.; Ozaki, T.; Shoji, A. *Chem. Lett.* **1983**, 1437−1440.
(29) Akieda, T.; Mimura, H.; Kuroki, S.; Kurosu, H.; Ando, I. *Macromolecules* **1992**, *25*, 5794−5797.
(30) Bradbury, E. M.; Carpenter, B. G.; Stephens, R. M. *Biopolymers* **1968**, *6*, 905−915.
(31) Bradbury, E. M.; Brown, L.; Downie, A. R.; Elliott, A.; Fraser, R. D. B.; Hanby, W. E. *J. Mol. Biol.* **1962**, *5*, 230−247.
(32) Baldwin, J. P.; Bradbury, E. M.; McLuckie, I. F.; Stephens, R. M. *Macromolecules* **1973**, *6*, 83−91.
(33) Arnott, S.; Wonacott, A. *J. Mol. Biol.* **1966**, *21*, 371−383.
(34) Arnott, S.; Dover, S. D. *J. Mol. Biol.* **1967**, *30*, 209−212.
(35) Elliott, A. In *Poly-α-Amino Acids*; Fasman, G. D., Ed.; Marcel Dekker: New York, 1967; Chapter 1.
(36) Kameda, T.; Takeda, N.; Kuroki, S.; Kurosu, H.; Ando, S.; Ando, I.; Shoji, A.; Ozaki, T. *J. Mol. Struct.* **1996**, *384*, 17−23.
(37) Oas, T. G.; Hartzell, C. J.; McMahon, T. J.; Drobny, G. P.; Dahlquist, F. W. *J. Am. Chem. Soc.* **1987**, *109*, 5956−5962.

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