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^{15}N Chemical Shift Tensors and Conformation of Solid Polypeptides Containing ^{15}N-Labeled L-Alanine Residue by ^{15}N NMR. 2. Secondary Structure Reflected in σ_{22}

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Abstract: A series of polypeptides $[Ala^*,X]_n$ containing ^{15}N-labeled L-alanine (Ala*) and other amino acids (X; natural abundance of ^{15}N) such as glycine, L-alanine, D-alanine, L-leucine, β-benzyl L-aspartate, γ-benzyl L-glutamate, γ-methyl L-glutamate, L-valine, L-isoleucine, and sarcosine were synthesized by the α-amino acid *N*-carboxy anhydride (NCA) method. Conformations of these polypeptides in the solid state were characterized on the basis of conformation-dependent ^{13}C chemical shifts in the ^{13}C cross-polarization-magic-angle spinning (CP-MAS) NMR spectra and of the characteristic bands in the infrared (IR) and far-IR spectra. Further, isotropic ^{15}N chemical shift (σ_{iso}) and chemical shift tensors (σ_{11}, σ_{22}, and σ_{33}) of the polypeptides were measured by the ^{15}N CP-MAS and the ^{15}N CP-static (powder pattern) methods, respectively. It was found that σ_{iso} is useful for the conformational study of homopolypeptides and copolypeptides with identical primary structures (amino acid sequences). In addition, it was demonstrated that the σ_{22} value of the Ala* residue in copolypeptide is closely related to the main-chain conformations (such as the right-handed and left-handed α-helices, and the β-sheet forms) rather than the amino acid sequence. Consequently, the σ_{22} value is very useful for conformational analysis of solid copolypeptides.

Recently, high-resolution and solid-state ^{15}N NMR has been increasingly applied to the investigation of polypeptides, proteins, and biopolymers.[1-12] This is primarily due to rapid progress in the development of both methodology and instrumentation. For example, sensitivity of the ^{15}N resonance has been greatly improved by introduction of the cross-polarization magic-angle spinning (CP-MAS) methods. However, little attempt has yet been made to relate the ^{15}N chemical shifts and chemical shift tensors to the structural parameters or primary, secondary, and higher ordered structures of synthetic polypeptides or natural proteins in the solid state. Furthermore, since a nitrogen atom possesses lone-pair electrons, it is of interest to examine the effects of the lone-pair electrons on the isotropic ^{15}N chemical shift (σ_{iso}) and the principal values of the ^{15}N chemical shift tensors (σ_{11}, σ_{22}, and σ_{33} from the downfield side), which are a measure of the electronic structure. Thus, it is now expected that ^{15}N NMR spectroscopy will contribute to and become increasingly important in the study on the structures and dynamics of synthetic polypeptides and natural proteins in the solid state.

In our previous papers,[13,14] we have demonstrated that (1) the σ_{iso} value determined by the ^{15}N CP-MAS NMR method is very sensitive to the primary structure such as the variety of amino acid residue and the amino acid sequence, as well as the secondary structure (particular conformations) such as the α-helix and β-sheet forms of polypeptides in the solid state and (2) the σ_{11}, σ_{22}, and σ_{33} values determined by the CP-static (powder pattern) spectra for ^{15}N-labeled copolypeptides are strongly influenced not only by the local conformations but also by the chemical nature of individual amino acid residues. In particular, it is noteworthy that one of the principal values, σ_{22}, may be useful for determination of the backbone conformation of polypeptides in the solid state.

In this paper, therefore, we attempt to elucidate the origin of the individual components of the ^{15}N chemical shift tensors of polypeptides in connection mainly with the primary structure such as the variety of amino acid residue and amino acid sequence and the secondary structure such as right-handed α-helix (α-helix), left-handed α-helix (α_L-helix[15]), and β-sheet conformations. For this purpose, we have prepared a series of ^{15}N-labeled polypeptides, $[Ala^*,X]_n$, consisting of ^{15}N-labeled L-alanine (Ala*) and other amino acids (X; natural abundance of ^{15}N). Here, the following amino acids were selected for the X residue: (1) L-alanine (Ala), D-alanine (D-Ala), and L-leucine (Leu), which contain nonpolar hydrocarbon side chains and stabilize an α-helix; (2) β-benzyl L-aspartate (Asp(OBzl)), γ-benzyl L-glutamate (Glu(OBzl)), and γ-methyl L-glutamate (Glu(OMe)), which contain polar side-chain esters and stabilize an α-helix; (3) glycine (Gly), which is optically inactive and stabilizes a β-sheet; (4) L-valine (Val) and L-isoleucine (Ile), which contain nonpolar hydrocarbon side chains and stabilizes a β-sheet; (5) sarcosine (Sar) = *N*-methylglycine, which destabilizes an α-helix and a β-sheet. Further, we have also investigated the dependence of the isotropic ^{15}N chemical shift and chemical shift tensors for the L-alanine content in $[Ala^*,X]_n$.

Experimental Section

Materials. A variety of ^{15}N-labeled polypeptides, $[Ala^*,X]_n$, were prepared by polymerization of Ala*-*N*-carboxy anhydride (Ala*-NCA)

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Table I. Isotropic ^{15}N Chemical Shift (σ_{iso}), ^{15}N Chemical Shift Tensors (σ_{11}, σ_{22}, σ_{33}), Anisotropy ($\Delta\sigma$), and Asymmetry Parameter (η) of Solid Polypeptides $[Ala^*,X]_n$ Containing ^{15}N-labeled L-Alanine Residue in the α-Helix, α_L-Helix, and β-Sheet Forms

sample	composition[a] (%)			conformn[b]	^{15}N chemical shift[c] (ppm)					
	Ala*	Ala	X		σ_{iso}	σ_{11}	σ_{22}	σ_{33}	$\Delta\sigma$[d]	η[e]
A1 $[Ala^*]_n$	20	80	0	α-helix	98.8	204	54.4	38	158	0.16
A2 $[Ala^*]_n$ - 5	20	80	0	β-sheet	102.2	201	61.7	44	148	0.18
A3-1 $[Ala^*,D\text{-}Ala]_n$	5	0	95	α_L-helix	96.7	197	57.1	36	151	0.21
A3 $[Ala^*,D\text{-}Ala]_n$	20	0	80	α_L-helix	96.5	198	55.1	36	153	0.19
A4 $[Ala^*,Gly]_n$	20	0	80	β-sheet	98.8	200	59.6	37	152	0.22
A5 $[Ala^*,Gly]_n$	20	60	20	α-helix	98.6	202	57.4	36	155	0.21
A6-1 $[Ala^*,Leu]_n$	5	0	95	α-helix	98.6	205	56.0	35	160	0.20
A6 $[Ala^*,Leu]_n$	20	0	80	α-helix	98.6	204	56.9	35	158	0.21
A6-2 $[Ala^*,Leu]_n$	5	45	50	α-helix	98.3	207	54.2	34	163	0.19
A6-3 $[Ala^*,Leu]_n$	5	75	20	α-helix	98.1	203	57.1	34	158	0.22
A7-1 $[Ala^*,Val]_n$	5	0	95	β-sheet	107.0	210	63.5	47	155	0.16
A7 $[Ala^*,Val]_n$	20	0	80	β-sheet[f]	99.7	202	62.4	35	153	0.27
A7-2 $[Ala^*,Val]_n$	5	25	70	α-helix[g]	98.6	201	53.1	42	154	0.11
A8 $[Ala^*,Ile]_n$	20	0	80	β-sheet	101.0	200	63.0	40	149	0.23
A9-1 $[Ala^*,Asp(OBzl)]_n$	5	0	95	α-helix	101.3	210	54.7	39	163	0.14
A9-2 $[Ala^*,Asp(OBzl)]_n$	10	0	90	α-helix	101.1	210	56.0	37	164	0.17
A9 $[Ala^*,Asp(OBzl)]_n$	20	0	80	α-helix	101.5	208	58.7	38	160	0.19
A10 $[Ala^*,Glu(OBzl)]_n$	20	0	80	α-helix	100.4	206	56.7	39	158	0.17
A11 $[Ala^*,Glu(OMe)]_n$	20	0	80	α-helix[h]	99.9	205	58.1	37	157	0.20
A12 $[Ala^*,Sar]_n$	20	0	80	?	99.0	198	62.2	37	148	0.26

[a] Copolymer composition (%). Abbreviations: Ala* = ^{15}N-labeled L-alanine (99 atom % of ^{15}N purity), Ala = L-alanine (natural abundance of ^{15}N), X = other amino acids (natural abundance of ^{15}N). [b] Abbreviations: α-helix = right-handed α-helix, α_L-helix = left-handed α-helix, β-sheet = antiparallel β-sheet. [c] ^{15}N chemical shifts of Ala* of polypeptides: ±0.5 ppm for σ_{iso} and σ_{22} and ±2 ppm for σ_{11} and σ_{33}, from $^{15}NH_4NO_3$. [d] Anisotropy: $\Delta\sigma = \sigma_{11} - (\sigma_{22} + \sigma_{33})/2$. [e] Asymmetry parameter: $\eta = (\sigma_{22} - \sigma_{33})/(\sigma_{11} - \sigma_{iso})$. [f] Major conformation of $[Ala^*,Val]_n$ (A7) is the β-sheet form containing small amounts (assumed below 10–20%) of the α-helix form. [g] Major conformation of $[Ala^*,Val]_n$ (A7-2) is the α-helix form containing small amounts (assumed below 10%) of the β-sheet form. [h] Major conformation of $[Ala^*,Glu(OMe)]_n$ is the α-helix form containing small amounts (assumed below 20–30%) of the β-sheet form.

and the corresponding amino acid-NCA (X-NCA) in acetonitrile (ACN) or 1,2-dichloroethane (DCE) at 30 °C by using *n*-butylamine as the initiator. Contents of the ^{15}N-labeled L-analine (99 atom % of ^{15}N purity; MSD Isotopes) in the polypeptides are about 5–20 mol % (Table I (supplementary material); see paragraph at end of paper regarding supplementary material). Conformational characterization of these samples was made on the basis of conformation-dependent ^{13}C chemical shifts determined from the CP–MAS NMR method[16-21] and also by the characteristic bands in the IR and far-IR spectra[22,23] (Table II (supplementary material); see paragraph at end of paper regarding supplementary material).

^{15}N and ^{13}C NMR Measurements. The solid-state ^{15}N and ^{13}C NMR measurements were performed on a JEOL GX-270 spectrometer operating at 27.4 and 67.80 MHz, respectively, equipped with a CP–MAS accessory. The contact time was 2 ms (for ^{15}N) and 4 ms (for ^{13}C), and the repetition time was 5 s (for ^{15}N) and 4 s (for ^{13}C). A 90° pulse width was typically 5.7–5.8 μs for both ^{15}N and ^{1}H under CP conditions and 5.3–5.4 μs for both ^{13}C and ^{1}H. Spectral width was 20 kHz (for ^{15}N) and 27 kHz (for ^{13}C), and data points were 8K points. Spectra were usually accumulated ca. 50–200 (for ^{15}N CP–MAS), 60–13 850 (for ^{15}N CP-static), and 110–4450 times (for ^{13}C CP–MAS) to achieve a reasonable signal to noise ratio for samples. The ^{15}N chemical shifts were calibrated indirectly by external [^{15}N]glycine (11.59 ppm, line width 17 Hz) relative to saturated $^{15}NH_4NO_3$ (0 ppm) solution in H_2O. The ^{13}C chemical shifts were calibrated indirectly by external adamantane (29.50 ppm relative to tetramethylsilane $(CH_3)_4Si$). The experimental errors of the isotropic ^{15}N and ^{13}C chemical shift values are estimated to be ≤ ±0.5 and ≤ ±0.2 ppm, respectively. The chemical shift tensor value of σ_{22} can be read directly from the observed powder pattern (CP-static) spectra with the accuracy being ≤±0.5 ppm.[14] The remaining two components of the tensors (σ_{11} and σ_{33}) were obtained by fitting the observed powder pattern with convoluted Lorentzian curves,[14,24,25] the error limits of σ_{11} and σ_{33} (≤±2 ppm) are larger than that of σ_{22}.

IR and Far-IR Measurements. IR and far-IR spectra were obtained for KBr disks with JEOL JIR-FX6160 Fourier transform IR (wavenumber range of 4000–200 cm^{-1}) and Jasco A-702 IR (4000–200 cm^{-1}) spectrophotometers.

Results and Discussion

Conformational Analysis of Solid Polypeptide. Conformational analysis of the samples used in this study was made on the basis of conformation-dependent ^{13}C chemical shifts determined from the CP–MAS NMR method[16-21] and also by the characteristic bands in the IR and far-IR spectra[22,23] (Table II (supplementary material); see paragraph at end of paper regarding supplementary material). In the previous papers,[17-21,24-27] we have shown that the ^{13}C NMR chemical shifts of solid polypeptides and proteins determined by the ^{13}C CP–MAS method depend significantly on their conformations such as α-helix, β-sheet, α_L-helix, 3_1-helix, collagen-like triple helix, ω_L-helix forms, and so on. In particular, the ^{13}C chemical shifts of the individual amino acid residue in polypeptides and proteins were found to be affected mainly by the local conformation, as defined by the torsional angles of the skeletal bonds, but not so strongly influenced by the amino acid sequence.[16] This view was further supported by our theoretical calculations of the contour map of the ^{13}C chemical shifts utilizing the finite perturbation (FPT) INDO theory[21,28] and the sum-over-states tight-binding MO theory.[29,30] It has also been established that the IR and far-IR spectroscopic methods are useful in conformational analysis of polypeptides and proteins. We have thus determined the conformations of the polypeptides in the solid state on the basis of these methods. From the ^{13}C NMR data (Table II (supplementary material)), it is obvious that the ^{13}C

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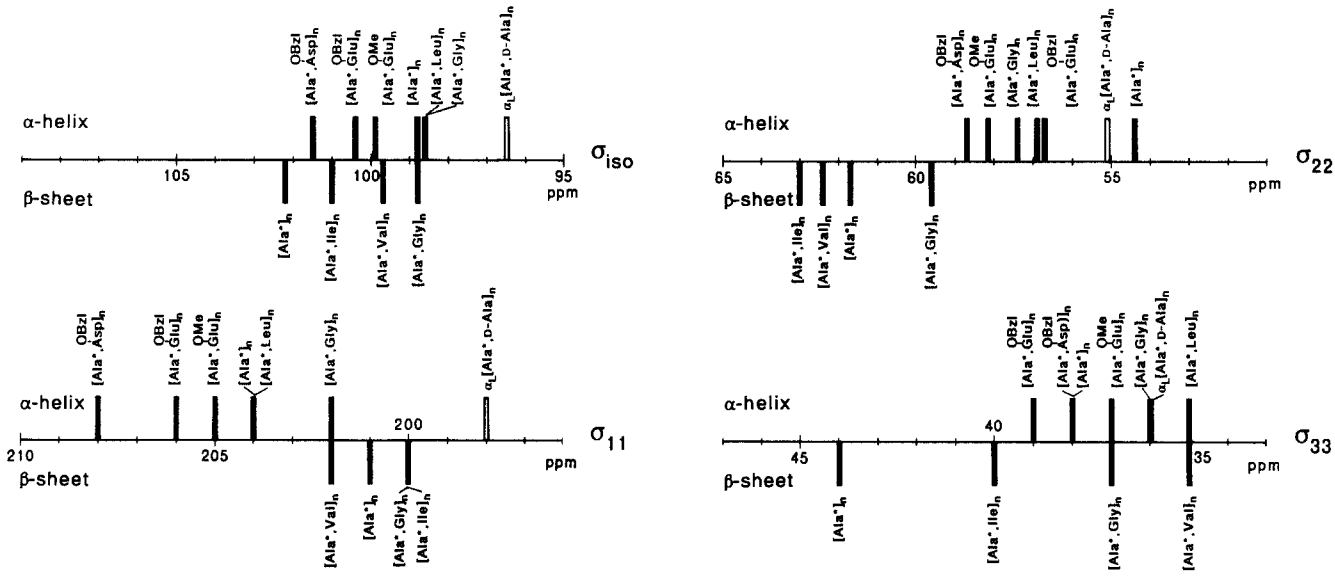

Figure 1. Diagram of the observed isotropic ^{15}N chemical shift (σ_{iso}) and ^{15}N chemical shift tensors (σ_{11}, σ_{22}, and σ_{33}) of the Ala* residue of some polypeptides $[Ala^*,X]_n$ (Ala* content is nearly 20%) in the solid state. The symbol α_L denotes the left-handed α-helix conformation of the L-alanine residues in the polypeptides.

chemical shifts of the main-chain amide carbonyl (C=O), C_α, and side-chain C_β signals of L-Ala residue are conformation-dependent. C=O: α-helix, 175.0–176.9 ppm; α_L-helix, 173.5–173.8 ppm; β-sheet, 172.0–172.7 ppm. C_α: α-helix, 53.2–54.2 ppm; α_L-helix, 50.2 ppm; β-sheet, 48.7–49.8 ppm. C_β: α-helix, 15.6–16.6 ppm; α_L-helix, 15.8 ppm; β-sheet, 20.3–20.8 ppm. The ^{13}C chemical shift displacements of the X residue among these conformations are consistent with those of the L-alanine residue as mentioned above. Further, all the NMR data are in good agreement with the IR and far-IR results (Table II (supplementary material)).

Most of the polypeptide samples commonly prefer only one stable conformation. A few exceptional cases are $[Ala^*,Val]_n$ (A7), $[Ala^*,Val]_n$ (A7-2), and $[Ala^*,Glu(OMe)]_n$ (A11), in which two components of conformations (α-helix and β-sheet) may coexist (see Table I). However, it must be kept in mind that an amount of one component is evidently dominant compared with the other. In addition, the conformation of $[Ala^*,Sar]_n$ (A12) is apparently different from the α-helix and β-sheet forms, although it is not defined at present. On the whole, according to this characterization, the molecular weights of the samples appear high enough to obtain desirable conformations and these polypeptides thus provide suitable systems for examining relations between conformations and ^{15}N NMR parameters.

Isotropic ^{15}N Chemical Shifts of the Ala* Residue of Polypeptides. Table I shows the isotropic ^{15}N chemical shift (σ_{iso}), chemical shift tensors (σ_{11}, σ_{22}, σ_{33}), anisotropy ($\Delta\sigma = \sigma_{11} - (\sigma_{22} + \sigma_{33})/2$), and asymmetry parameter ($\eta = (\sigma_{22} - \sigma_{33})/(\sigma_{11} - \sigma_{iso})$) of solid polypeptides containing Ala* residue in the α-helix, α_L-helix, and β-sheet forms.

For poly(L-alanines) ($[Ala^*]_n$, homopolypeptide), σ_{iso} of the Ala* residue is clearly conformation-dependent (α-helix, 98.8 ppm; β-sheet, 102.2 ppm), which is consistent with our previous results;[13] σ_{iso} of the α-helix form appears at higher field by 3.4 ppm than that of the β-sheet form. Experimental data were reproduced by our theoretical calculations of the ^{15}N chemical shifts (shielding constant) of poly(L-alanine) utilizing the FPT-INDO theory.[13]

For the copolypeptides $[Ala^*,X]_n$ on the contrary, the σ_{iso} values of the Ala* residue of the α-helix and β-sheet forms are observed in the ranges 98.1–101.5 and 98.8–107.0 ppm, respectively, as shown in Table I and Figure 1. This suggests that σ_{iso} depends not only on the secondary structure but also on the primary structure or probably on the higher ordered structure (although such effects must be small). Thus, the origin of ^{15}N chemical shifts is rather complex as compared with that of the ^{13}C chemical shifts, and it may be difficult to estimate the secondary structure of copolypeptides from the σ_{iso} value. However, it is emphasized that σ_{iso} of the α-helix always appears at higher field than that of the β-sheet form for the same kind of polypeptide. Thus, it is expected that σ_{iso} may be useful for the study on such a conformational change of homopolypeptides and copolypeptides (or natural proteins) with identical primary structure (amino acid sequence).

A further important result is that σ_{iso} gives information about the helix sense (right-handed or left-handed) of polypeptides by the ^{15}N CP–MAS NMR method. It has been already established by far-IR[31] and ^{13}C CP–MAS NMR[21] methods that the stable conformation of $[Ala^*, D\text{-}Ala]_n$ (A3-1 and A3) is the left-handed α-helix; the Ala* residues (minor component, 5–20 mol %) are incorporated into the left-handed α-helix of the major D-Ala residues. The σ_{iso} values (α_L-helix, 96.5–96.7 ppm) of the Ala* residues of $[Ala^*, D\text{-}Ala]_n$ (A3-1 and A3) are displaced upfield by ca. 2 ppm as compared with that of $[L\text{-}Ala]_n$ (α-helix, 98.6–98.8 ppm, which is exactly equal to the σ_{iso} of $[D\text{-}Ala]_n$). Similar ^{15}N chemical shift displacements of σ_{iso} were obtained for homopolypeptide $[L\text{-}Asp(OBzl)]_n$ (α-helix,[13] 99.2 ppm; α_L-helix, 97.0 ppm; ω_L-helix, 96.8 ppm; β-sheet,[13] 100.4 ppm), which will be published elsewhere.[32] This result supports that σ_{iso} is sensitive to the helix sense of polypeptides in the solid state.

^{15}N Chemical Shift Tensors of the Ala* Residue of Polypeptides. We now consider the relation between the ^{15}N chemical shift tensors and the conformation of polypeptides. Figure 1 shows the diagram of the observed ^{15}N NMR chemical shifts (σ_{iso}, σ_{11}, σ_{22}, and σ_{33}) of the Ala* residue of some polypeptides $[Ala^*,X]_n$ (Ala* content is nearly 20%). As Figure 1 shows, it is noteworthy for poly(L-alanine) that the σ_{22} and σ_{33} of the α-helix form is shifted upfield as compared with those of the β-sheet form, whereas the σ_{11} of the α-helix form is shifted downfield as compared with that of the β-sheet form. In particular, the chemical shift displacement of σ_{22}, the alignment of which is perpendicular to the peptide bond,[33,34] is very sensitive to the conformational changes of ^{15}N-labeled polypeptides, if ^{15}N CP–static spectra are available. A detailed discussion on σ_{22} of poly(L-alanine) was given in the previous paper.[14]

It has been reported that the alignment of the downfield tensor element σ_{11} is nearly parallel to the hydrogen-bonding (N–H···O) direction.[33-35] Therefore, it is anticipated that σ_{11} may offer

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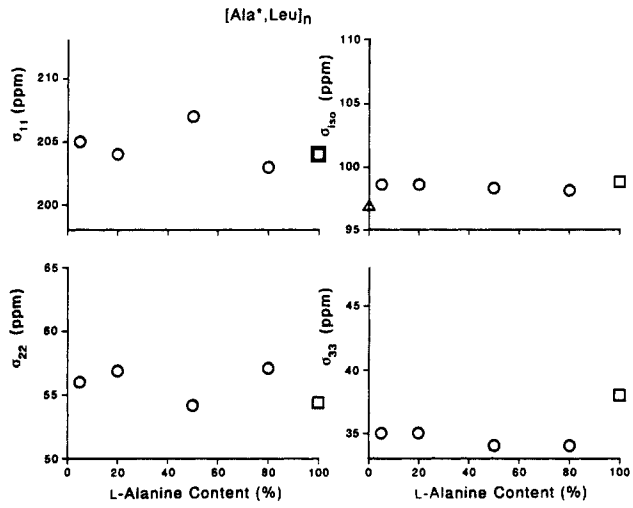

Figure 2. Plots of the isotropic ^{15}N chemical shift (σ_{iso}) and ^{15}N chemical shift tensors (σ_{11}, σ_{22}, σ_{33}) of the Ala* residue in $[Ala^*,Leu]_n$ against the L-alanine content (%): (○) α-helix form; (□) poly(L-alanine) (α-helix form); (△) poly(L-leucine) (α-helix form[13]).

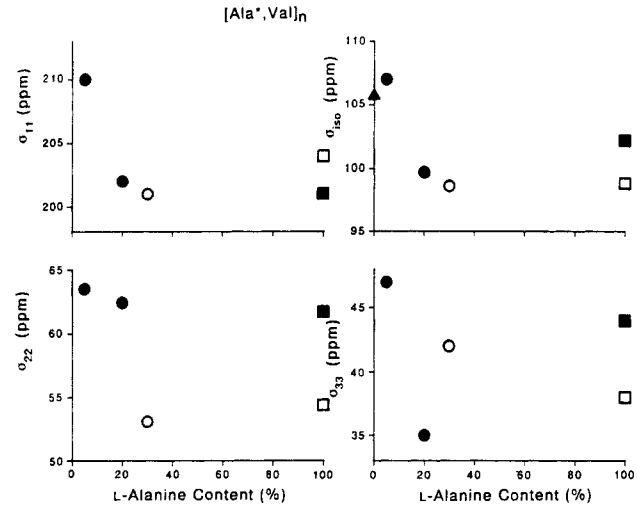

Figure 3. Plots of the isotropic ^{15}N chemical shift (σ_{iso}) and ^{15}N chemical shift tensors (σ_{11}, σ_{22}, σ_{33}) of the Ala* residue in $[Ala^*,Val]_n$ against the L-alanine content (%): (○) α-helix form; (●) β-sheet form; (□) poly(L-alanine) (α-helix form); (■) poly(L-alanine) (β-sheet form); (▲) poly(L-valine) (β-sheet form[13]).

certain information about the manner of the hydrogen bonding of polypeptides and proteins. In addition, it was clarified, in the present work, that σ_{11} of the Ala* residues of $[Ala^*, D\text{-}Ala]_n$ (A3, left-handed α-helix) is significantly shifted upfield by 6 ppm as compared with that of $[Ala^*]_n$ (A1, right-handed α-helix), whereas the difference between the right-handed and left-handed α-helices is not so large for σ_{22} (0.7 ppm) and σ_{33} (2 ppm). This indicates that σ_{11} is sensitive to the helix sense (such as the right-handed and left-handed α-helices) as well as the manner of the hydrogen bonding. The chemical shift variation of σ_{11} among the α-helical $[Ala^*,X]_n$ is larger than that of the β-sheet form; the displacement of the chemical shift tensors of the Ala* residue among various kinds of polypeptides $[Ala^*,X]_n$ (Ala* content of 20%) with the α-helix form are 202–208 ppm (variation ≤6 ppm) for σ_{11}, 54.4–58.7 (≤4.3 ppm) for σ_{22}, and 35–39 ppm (≤4 ppm) for σ_{33}, and those with the β-sheet form are 200–202 (≤2 ppm) for σ_{11}, 59.6–63.0 (≤3.4 ppm) for σ_{22}, and 35–44 ppm (≤9 ppm) for σ_{33}. The variation of the upfield tensor element σ_{33} (parallel to the C′–N bond direction[33,34]) among the α-helix forms is, in contrast, smaller than that of the β-sheet form. This result suggests that σ_{33} is related to the side-chain structures of solid polypeptides, although further study is needed to clarify the correlation between the specific structure (amino acid sequence, secondary structure, and higher ordered structure) and the ^{15}N chemical shift tensors.

Correlation between the ^{15}N Chemical Shifts (σ_{iso}, σ_{11}, σ_{22}, σ_{33}) and the Copolymer Composition. We discuss here five series of copolypeptides: $[Ala^*,Leu]_n$, $[Ala^*,Asp(OBzl)]_n$, $[Ala^*,Val]_n$, $[Ala^*,D\text{-}Ala]_n$, $[Ala^*,Gly]_n$.

For a series of $[Ala^*,Leu]_n$ where Leu has a hydrophobic alkyl side chain and stabilizes an α-helix conformation, σ_{11} and σ_{22} seem to depend slightly on the L-alanine content, as shown in Figure 2. It is noteworthy that the σ_{22} value at 50% L-alanine content is somewhat specific and smaller (upfield) than that of the other contents. It is interesting that the chemical shift displacements of σ_{11} and σ_{22} are similar (but inverse tendency), whereas the changes of σ_{11} are smaller than the error limits. The anisotropy ($\Delta\sigma$) of $[Ala^*,Leu]_n$ (A6-2) was maximum, and the asymmetry parameter (η) was minimum in this series, probably reflecting the difference of the amino acid sequence in copolypeptides. In contrast, the changes of σ_{iso} and σ_{33} are negligibly small, indicating that they are independent of the wide range of L-alanine content (5–80%).

For a series of $[Ala^*,Asp(OBzl)]_n$, where Asp(OBzl) residue has a carboxylic side-chain ester and stabilizes an α-helix form, only σ_{22} changes linearly with the L-alanine content, as shown in Table I. The σ_{22} is shifted downfield as the L-alanine content increases. The extent of the chemical shift changes is appreciably large (ca. 4 ppm) in spite of a quite narrow range of the L-alanine content (5–20%). The change of σ_{22} is probably due to the amino acid sequence in copolypeptides, but not due to the main-chain conformation of polypeptides. The σ_{iso}, σ_{11}, and σ_{33} values do not change over 5–20% L-alanine content, suggesting they are independent of the L-alanine content.

For a series of $[Ala^*,Val]_n$, where the Val residue has a hydrophobic side chain and stabilizes a β-sheet form, the stable conformation was found to be the β-sheet form at ≤20% L-alanine content (A7-1, A7) and α-helix form at ≥30% L-alanine content (A7-2), as shown in Table I. In this narrow range of L-alanine content (between 20 and 30%), only σ_{22} exhibits a drastic upfield shift (ca. 9 ppm) as the L-alanine content increases, as shown in Figure 3. On the other hand, very few chemical shift changes were observed for σ_{22} at 5–20% L-alanine content, where no conformational change occurred (β-sheet form). The above results indicate that σ_{22} may be governed mainly by the conformational factor of copolypeptides in the solid state, which is consistent with our assumption that σ_{22} is conformation-dependent, as described above. In contrast, the large chemical shift changes were seen for σ_{iso}, σ_{11}, and σ_{33} (other than σ_{22}) at 5–20% L-alanine content. The changes of these chemical shifts may be mainly due to the nature of L-valine residue or amino acid sequences, but not due to the main-chain conformation of copolypeptides.

For a series of $[Ala^*,D\text{-}Ala]_n$, where the D-Ala residue stabilizes the left-handed α-helix form as shown in Table I, most of the Ala* residues are incorporated into the left-handed α-helix of the D-alanine residue. This left-handed α-helix of the D-alanine residue is fully equivalent to the right-handed α-helix of the L-alanine residue. In this case, no chemical shift displacements of σ_{iso}, σ_{11}, and σ_{33} were detected over 5–20% L-alanine content and therefore, they are almost independent of L-alanine content, which is similar to the cases of $[Ala^*,Leu]_n$ or $[Ala^*,Asp(OBzl)]_n$ described above. The σ_{22} is shifted upfield as the L-alanine content increases. Therefore, the change of σ_{22} should not be ascribed to the main-chain conformation but to the nature of the D-alanine residue or the amino acid sequences in the copolypeptides.

At last, for a series of $[Ala^*,Gly]_n$, where the Gly residue has no asymmetric α-carbon and destabilizes an α-helix form, the differences in chemical shifts between A5 (α-helix, 80% L-alanine content) and A4 (β-sheet, 20% L-alanine content) are generally small, as shown in Table I. However, the difference of σ_{22} may be ascribed mainly to the secondary structure. In contrast, the difference (3.0 ppm) of σ_{22} between A5 and poly(L-alanine) (A1) is close to that in $[Ala^*,Leu]_n$ (2.7 ppm). Therefore, this difference may be ascribed to the amino acid sequences or to the side-chain

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interactions, but not to the secondary structure. It is important to investigate the displacements of the ^{15}N chemical shift tensors of the ^{15}N-labeled glycine residue (Gly*) in a series of copolypeptides $[Ala,Gly^*]_n$ as compared with those of Ala* residue in $[Ala^*,Gly]_n$, which will be published in the following paper.[36]

Conclusion

It was demonstrated that the isotropic ^{15}N chemical shift (σ_{iso}) is sensitive to the primary, secondary, and higher ordered structures of polypeptides in the solid state. It was found to be profitable to determine the main-chain conformation of homopolypeptides and copolypeptides with identical primary structure (amino acid sequence) on the basis of the conformation-dependent σ_{iso}. Thus, this method may be applicable to investigation of conformational changes in natural proteins with identical primary structures in the solid state. However, it may generally be difficult to estimate the main-chain conformation of a variety of copolypeptides and natural proteins on the basis of σ_{iso}. For such polypeptides, on the other hand, we showed that the ^{15}N chemical shift tensors (σ_{11}, σ_{22}, σ_{33}) give information on the structures of polypeptides rather than σ_{iso}, using a series of ^{15}N-labeled copolypeptides $[Ala^*,X]_n$. In particular, we have confirmed that σ_{22} is governed mainly by secondary structures (the right-handed or left-handed α-helix and the β-sheet form) rather than by the amino acid sequences of polypeptides. The σ_{22} of the Ala* residues in the copolypeptide with the α-helix form was separated from that of the β-sheet form. Therefore, it is now possible to determine the main-chain conformation of copolypeptides (or probably of natural proteins) on the basis of σ_{22}, if the ^{15}N-labeled copolypeptide or natural protein can be provided. Furthermore, the chemical shift tensors other than σ_{22} of the Ala* residue seem to be closely related not only to the secondary structure but also to the primary and higher ordered structures of copolypeptides. However, the correlation between these structures and the ^{15}N chemical shift tensors is not clarified at present, and therefore, a further study is needed.

Supplementary Material Available: Synthetic conditions of some ^{15}N-labeled polypeptides $[Ala^*,X]_n$ (Table I) and conformational characterization of solid polypeptides determined by the ^{13}C CP-MAS NMR, IR, and far-IR methods (Table II) (2 pages). Ordering information is given on any current masthead page.

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Low-Temperature NMR Study of Conformational Equilibration and Reversible Covalent Association in Dithioacetic Acid

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Abstract: The proton NMR spectrum of a dilute solution (0.2%) of dithioacetic acid at −106 °C in CD_2Cl_2 shows separate methyl and SH signals for the *E* and *Z* conformations, with the *Z* isomer favored by 0.12 kcal/mol. The free energy barriers at −89 °C are 8.8_5 and 8.9_7 kcal/mol. For the neat liquid or more concentrated solutions in several solvents, the compound is appreciably associated, with the amount of monomer depending upon concentration, temperature, and solvent. At 19.5 °C, the carbon peaks of neat dithioacetic acid are accounted for by monomer (61%), dimer (38%), and a small amount of cyclic trimer (ca. 1%). Spectra taken at lower temperatures in suitable solvents show that the dimer exists in two forms; the free-energy barriers for interconversion of the two forms are 8.7_6 and 8.7_7 kcal/mol.

Dithiocarboxylic acids have been known for over 100 years,[1,2] and several physical studies of members of this class have been reported.[2] Dithioformic acid is monomeric in the gas phase[3,4,5] and was found in a microwave study[3,4] to exist as a mixture of *E* and *Z* conformations, with the *Z* favored by 1.0 kcal/mol. An IR study[5] and molecular orbital calculations[5,6,7] also show that the *Z* conformation is lower in energy. The compound has been reported[8,9] to exist in the condensed phase as a cyclic trimer or polymer.[10]

The higher dithio acids have generally been assumed to be monomeric, except for possible association through hydrogen bonding.[2] The IR spectrum[11] of dithioisobutyric acid showed absorption at 2502 cm^{-1} as the neat liquid and at 2566 cm^{-1} in dilute (1.5%) solution in CCl_4; both peaks were observed at intermediate concentrations and were attributed to S–H stretching in hydrogen-bonded dimer and monomer, respectively. Because the thiocarbonyl absorption remained constant at about 1220 cm^{-1} for several concentrations in carbon disulfide, the dimerization was assumed to be of the type S–H···S(–H) and not S–H···S(=C). The position of the SH peak in the NMR spectrum shifts slightly upfield upon dilution in CCl_4 (δ 6.40 for the neat liquid to 6.02 for a 1.5% solution), and this shift also was taken[11] to be evidence for a monomer–dimer equilibrium of this type. The dipole moment for dithioisobutyric acid (2.13 D in benzene at 20 °C) was interpreted[11] as evidence for the *Z* conformation in this solution.

The room temperature proton NMR spectrum of trifluorodithioacetic acid was reported[12] to show a complex group of signals consisting of five peaks centered at δ 2.65, whose intensities after deuteration greatly decreased. The authors did not discuss possible reasons for the large number of peaks and interpreted the NMR spectrum,[12] mass spectrum,[12] and IR spectrum[12,13] in terms of the monomer, although later studies[11,14] give chemical shifts of δ 6.0

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