Conformational Study of Solid Polypeptides by ^{1}H Combined Rotation and Multiple Pulse Spectroscopy NMR

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Abstract: The relation between the ^{1}H chemical shift and the conformation of linear homopolypeptides and cyclic dipeptides in the solid state has been studied utilizing the ^{1}H combined rotation and multiple pulse spectroscopy (CRAMPS) NMR method. It was found that the ^{1}H chemical shift of the H_α signal of homopolypeptides depends on the secondary structure such as α-helix or β-sheet form, whereas those of the side-chain proton signals (H_β, H_γ, H_δ, etc.) are almost independent of the secondary structure. The ^{1}H chemical shifts of the H_α signal of homopolypeptides having the α-helix and the β-sheet forms were 3.9−4.0 ppm and 5.1−5.5 ppm, respectively. Accordingly, the ^{1}H chemical shift of the H_α is very useful for conformational analysis of polypeptides in the solid state. Furthermore, it is shown that the ^{1}H chemical shift of the H_α and the NH signals of cyclic dipeptides are sensitive to the ring conformation and the hydrogen bond length in the solid state.

Introduction

High-resolution and solid-state NMR spectroscopy, especially for the ^{13}C and ^{15}N nuclei, is very useful for structural analysis of polypeptides and proteins in the solid state.[1−10] In our previous papers,[3,4] it has been demonstrated that ^{13}C chemical shifts of solid polypeptides depend on conformation. In particular, the ^{13}C chemical shift of the backbone carbonyl carbon (C═O) depends mainly on main-chain conformation, and it is rather insensitive to the variety of side-chain effects of the amino-acid residues and the neighboring amino-acid sequence effects.[5] Thus, the ^{13}C chemical shift of the backbone C═O is very useful for the overall conformational analysis of polypeptides and proteins in the solid state. In contrast, the ^{13}C chemical shifts of the α-carbon (C_α) and β-carbon (C_β) are useful for the local conformational analysis of the specific amino acid residues,[3−5] since the ^{13}C chemical shifts of the C_α and C_β signals depend on both the main-chain conformation and the side-chain structure (but they do not depend on the neighboring amino-acid sequence).

Further, our recent papers[7−10] have shown that the ^{15}N chemical shift of solid polypeptides is strongly influenced not only by the local conformations but also by the neighboring amino-acid sequence effects and the side-chain effects of the individual amino-acid residues. Thus, the ^{15}N chemical shift is also very useful for the structural analysis such as the conformation, the neighboring amino-acid sequence effect, and the side-chain effects of the individual amino-acid residues in solid polypeptides, if these effects can be separated.

Since, however, ^{13}C and ^{15}N measurements are difficult and time consuming, we wanted to explore if the same information could be obtained more rapidly by high-resolution ^{1}H NMR of these solids.

The line width of ^{1}H CRAMPS (combined rotation and multiple pulse spectroscopy[11,12]) spectra is controlled by the nature of the samples and cannot be narrowed over a certain limit. Further, since chemical shifts are scaled in CRAMPS spectra and since the scaling factor may not be constant,[13] ^{1}H CRAMPS spectra, the technique of which was recently reviewed,[14] have not been used as a general structural analysis technique for organic compounds. It is possible, however, to obtain a reliable chemical shift scale by careful adjustment and use of internal standards. Such a calibration procedure has been carried out by us, and the offset dependence of the scaling factor is shown in Figure 1 for a range of 20 ppm. It can be seen that, in the important region (0−15 ppm), the deviation of the scaling factor is in the order of 0.1 ppm, which is very acceptable for solid-state spectra.

We have studied various kinds of homopolypeptides such as poly(L-alanine) (PLA), poly(L-leucine) (PLL), and poly(γ-benzyl-L-glutamate) (PBLG), which show characteristic differ-

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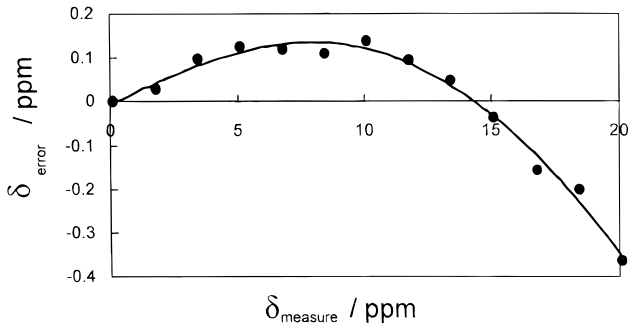

Figure 1. Plots of the ^{1}H chemical shift error (δ_{error}) against the observed value ($\delta_{measure}$) of the peak shift of silicon−rubber. Note: $\delta_{error} = \delta_{calcd} - \delta_{measd}$, where δ_{calcd} is the calculated chemical shift value using the scaling factor of 0.4. δ_{measd} is the chemical shift value measured from the offset frequency dependency of the peak shift.

Table 1. Synthetic Homopolypeptide Samples and Their Characteristics[g]

sample	DP$_n$[a]	synthetic method	conformation[b]
H-[Ala]$_8$-NHBu		activated ester	β-sheet
[Ala]$_n$-1	16	NCA	α-helix[e]
[Ala]$_n$-5	65	NCA	α-helix
[Leu]$_n$-1	A/l[c] = 5	NCA	β-sheet[f]
[Leu]$_n$-2	A/l[c] = 100	NCA	α-helix
H-[Glu(OBzl)]$_6$-OBzl		activated ester	β-sheet
[Glu(OBzl)]$_n$	h.m.w[d]	NCA	α-helix

[a] The number-averaged degree of polymerization (ref 15). [b] Conformations of these samples were determined by the ^{13}C and/or ^{15}N CP-MAS NMR, IR and far-IR spectroscopic methods. [c] The molar ratio of the monomer (A) to the initiator (I), which corresponds to the theoretical number-averaged degree of polymerization. [d] High-molecular-weight (fibrous) sample. [e] Containing small amounts of β-sheet. [f] Containing only a small amount of α-helix. [g] Abbreviations: Ala, L-alanine; Leu, L-leucine; Glu(OBzl), γ-benzyl-L-glutamate; NHBu, *n*-butyl amide; OBzl, benzyl ester; NCA, *N*-carboxy-α-amino acid anhydride; α-helix, right-handed α-helix; β-sheet, anti-parallel β-sheet.

ences in conformation such as the right-handed α-helix (α-helix) and the antiparallel β-sheet (β-sheet) forms, and some cyclic dipeptides such as cyclo(L-Ala-L-Ala) and cyclo(L-Ala-D-Ala), which show characteristic differences in ring conformation and crystal structure, in order to systematically test the power of ^{1}H CRAMPS NMR for the structural analysis of polypeptides in the solid state. Solution studies are not possible or difficult, since the structure of these materials will change by dissolution in many solvents.

Experimental Section

Materials. A variety of linear homopolypeptide and homo-oligopeptide samples used in this study were synthesized in our laboratory.[15,16] The physical state of these samples is a semicrystalline state. Synthetic conditions and characteristics of these samples are shown in Table 1.

Cyclic dipeptides, cyclo[L-Ala-L-Ala] and cyclo[L-Ala-D-Ala] (LL and LD, respectively), used in this study were also synthesized in our laboratory.[17] The physical state of these samples is a polycrystalline state. Synthetic conditions and characteristics of these samples are shown in Table 2. Conformational characterization of these samples were made on the basis of conformation-dependent ^{13}C chemical shifts determined from the CP-MAS NMR method,[3−5,18] from the characteristic bands in the IR and far-IR spectra,[19,20] and also by the X-ray diffraction data.[21,22]

Table 2. Cyclic Dipeptide Samples and Their Characteristics

	sample[a]	synthetic method	ring conformation[b]
LL	cyclo[Ala-Ala]	activated ester	boat
LD	cyclo[Ala-D-Ala]	activated ester	planar

[a] Abbreviations: Ala, L-alanine; D-Ala, D-alanine. [b] Ring conformations of these samples were determined by the X-ray crystallographic references[21,22] and the ^{13}C CP-MAS NMR method.[18]

^{1}H CRAMPS NMR Measurements. The solid-state ^{1}H CRAMPS NMR measurements were performed on a Chemagnetics CMX 300 spectrometer operating at 300 MHz, equipped with a 5 mm CRAMPS probe. The BR-24 pulse sequence[23] was used, and the $\pi/2$ pulse width was 1.3 μs. The rotational frequency was exactly controlled in the range 1.5−2.5 kHz, and the cycle time of BR-24 was 108 μs. The recycle delay was 10 s, and spectra were usually accumulated 32 times to achieve a reasonable signal-to-noise ratio for the samples. The ^{1}H chemical shift was calculated with scaling factor of 0.40 for all samples, which was determined experimentally. The ^{1}H CRAMPS spectra were recorded first without internal standard and calibrated afterward with internal silicon−rubber (δ 0.12 ppm) relative to tetramethylsilane $(CH_3)_4Si$ (δ 0). The 300 MHz ^{1}H CRAMPS NMR spectra of poly(L-alanine) in the solid state with silicon−rubber as an internal standard and without an internal standard are shown in Figure S1.[24] Thus, the experimental errors of the ^{1}H chemical shifts are estimated within ±0.1 ppm (0−15 ppm). The typical half-width was 30 Hz, and the total measurement time for one sample was usually 5 min.

Results and Discussion

Conformational Characterization of Solid Polypeptides. Table 1 shows the synthetic conditions and characteristics of the samples used in this study. Conformational characterization of these samples was made on the basis of conformation-dependent ^{13}C and ^{15}N chemical shifts determined using CP-MAS NMR[4,5,7] and also by the characteristic bands in the IR and far-IR spectra.[19,20] From the ^{13}C NMR and ^{15}N NMR data, it is clear that the main-chain amide C═O, C$_\alpha$, side-chain C$_\beta$, and main-chain amide ^{15}N signals are conformation-dependent. (1) C═O: 175.8 ± 0.8 ppm (α-helix) and 170.9 ± 1.2 ppm (β-sheet). (2) C$_\alpha$: 52.4 ppm (α-helix, PLA), 48.2 ppm (β-sheet, PLA); 55.7 ppm (α-helix, PLL), 50.5 ppm (β-sheet, PLL); 56.4 ppm (α-helix, PBLG), 51.2 ppm (β-sheet, PBLG). (3) C$_\beta$: 14.9 ppm (α-helix, PLA), 19.9 ppm (β-sheet, PLA); 39.5 ppm (α-helix, PLL), 43.3 ppm (β-sheet, PLL); 25.6 ppm (α-helix, PBLG), 29.0 ppm (β-sheet, PBLG) (±0.5 ppm from $(CH_3)_4$-Si). (4) ^{15}N: 98.6 ppm (α-helix, PLA), 101.8 ppm (β-sheet, PLA); 97.0 ppm (α-helix, PLL), 107.0 ppm (β-sheet, PLL); 97.6 ppm (α-helix, PBLG), 99.5 ppm (β-sheet, PBLG) (±0.5 ppm from $^{15}NH_4NO_3$). These results obtained for the conformational characterization of polypeptides on the basis of the chemical shift displacements are in good agreement with the IR and far-IR results.

Conformation-Dependent ^{1}H Chemical Shift of Homopolypeptides. Figure 2 shows the 300 MHz ^{1}H CRAMPS NMR spectra of PLAs: (A) H-[Ala]$_8$-NHBu (octapeptide, β-sheet form), (B) [Ala]$_n$-1 (averaged degree of polymerization (DP$_n$) = 16, α-helix + β-sheet form), and (C) [Ala]$_n$-5 (DP$_n$ = 65, α-helix) in the solid state. The ^{1}H CRAMPS NMR spectra showed high-resolution proton signals separated into three

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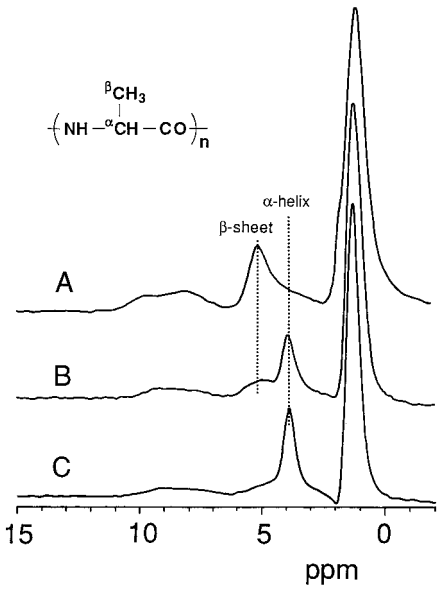

Figure 2. 300 MHz ^{1}H CRAMPS NMR spectra of poly(L-alanines): (A) H-[Ala]$_8$-NHBu (β-sheet form), (B) [Ala]$_n$-1 (α-helix + β-sheet form), and (C) [Ala]$_n$-5 (α-helix) in the solid state.

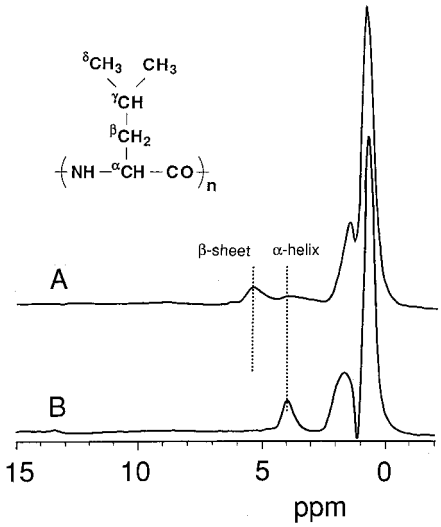

Figure 3. 300 MHz ^{1}H CRAMPS NMR spectra of poly(L-leucines): (A) [Leu]$_n$-1 (β-sheet) and (B) [Leu]$_n$-2 (α-helix) in the solid state.

regions (NH, H$_\alpha$, and H$_\beta$ peaks) for PLA. From Figure 2, it was induced that (1) the ^{1}H chemical shift of the H$_\alpha$ signal from the methyne protons is conformation-dependent [α-helix (3.9 ppm) and β-sheet (5.1 ppm)], (2) the ^{1}H chemical shift of the H$_\beta$ methyl protons is nearly conformation-independent [1.2−1.4 ppm], and (3) the NH signal is relatively broad due to residual dipolar coupling by the quadrupolar interaction between amide proton and ^{14}N nucleus. Therefore the chemical shift of the methyne proton in the α-helix is 1.2 ppm upfield from that in the β-sheet form, indicating that the ^{1}H chemical shift of the H$_\alpha$ signal is a useful fingerprint for conformational analysis of PLA in the solid state.

Figure 3 shows the 300 MHz ^{1}H CRAMPS NMR spectra of PLLs: (A) [Leu]$_n$-1 (β-sheet plus a small quantity of α-helix) and (B) [Leu]$_n$-2 (α-helix) in the solid state. The ^{1}H CRAMPS NMR spectra showed high-resolution proton signals separated into three regions (NH, H$_\alpha$, and other proton signals) for PLL, which is the same result as PLA. For PLL, it is found that (1) the ^{1}H chemical shift of the H$_\alpha$ signal is conformation-dependent [α-helix (4.0 ppm) and β-sheet (5.5 ppm)], (2) the ^{1}H chemical shifts of the H$_\beta$, H$_\gamma$, and H$_\delta$ signals are nearly conformation-independent [H$_\beta$ and H$_\gamma$ signal (1.5−1.6 ppm, overlapping), and H$_\delta$ signal (0.8−0.9 ppm)], and (3) the NH signal is again relatively so broad as to be almost nondetectable. The ^{1}H chemical shift of the H$_\alpha$ signal is conformation-dependent, and the chemical shift of the methyne proton in the α-helix is 1.5 ppm upfield from that in the β-sheet form. These results show that the ^{1}H chemical shift of the H$_\alpha$ signal is very useful for conformational analysis of PLL in the solid state, in spite of the fact that the L-leucine residue has a bulky side group.

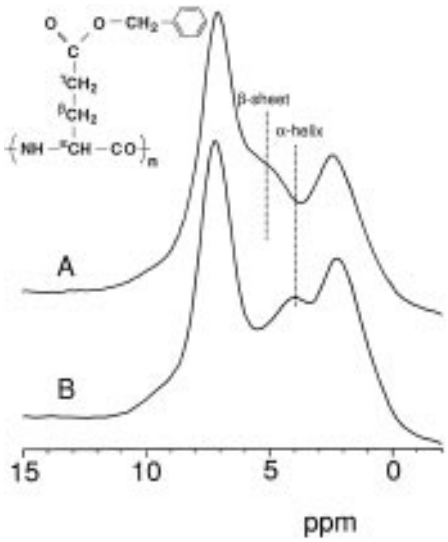

Figure 4. 300 MHz ^{1}H CRAMPS NMR spectra of poly(γ-benzyl-L-glutamates): (A) H-[Glu(OBzl)]$_6$-OBzl (β-sheet) and (B) [Glu(OBzl)]$_n$ (α-helix) in the solid state.

Figure 4 shows the 300 MHz ^{1}H CRAMPS NMR spectra of PBLGs: (A) H-[Glu(OBzl)]$_6$-OBzl (β-sheet) and (B) [Glu-(OBzl)]$_n$ (α-helix) in the solid state. The ^{1}H CRAMPS NMR spectra showed high-resolution proton signals separated into three regions (NH, phenyl H, benzyl H; H$_\alpha$; and H$_\beta$, H$_\gamma$ signals) for PBLG. The γ-benzyl-L-glutamate residue has a side-chain benzyl ester, and therefore, the ^{1}H NMR signals are broad over the wide range of chemical shifts. However, it was clear that (1) the ^{1}H chemical shift of the H$_\alpha$ signal is conformation-dependent [α-helix (4.0 ppm) and β-sheet (nearly 5.2 ppm)], (2) the ^{1}H chemical shift of the H$_\beta$ and H$_\gamma$ signals are nearly conformation-independent (2.2−2.4 ppm, overlapping), (3) the NH signal is overlapping with the phenyl protons and almost nondetectable, and (4) the ^{1}H chemical shift of the phenyl signal (7.1−7.2 ppm) is also overlapping with the benzyl protons. The ^{1}H chemical shift of the H$_\alpha$ signal is conformation-dependent, and the chemical shift of the methyne proton in the α-helix is nearly 1.2 ppm upfield from that in the β-sheet form. The chemical shift of the H$_\alpha$ proton in polypeptides appears to be useful for conformational analysis of PBLG in the solid state.

Next, we have compared our results in the solid state to solution-state NMR measurements[25−27] of the dependence of methyne proton chemical shifts on conformation. As a result, we found that (1) in the α-helical polypeptides used in this study, the α-proton chemical shifts in the solid state (3.9−4.0 ppm from TMS) are identical with those in solution[25] (3.94−3.95 ppm from DSS), and (ii) in the β-sheet polypeptides, on the contrary, the α-proton chemical shifts in the solid state (5.1−

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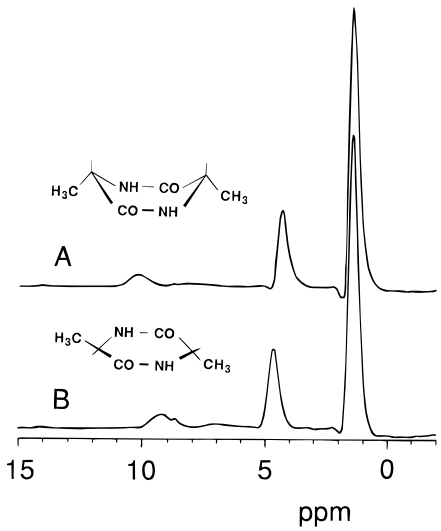

Figure 5. 300 MHz ^{1}H CRAMPS NMR spectra of the cyclic dipeptides cyclo[L-Ala-L-Ala] (LL) and cyclo[L-Ala-D-Ala] (LD), in the solid state.

5.5 ppm) are nearly 0.4−0.8 ppm downfield from those in solution[25] (4.70−4.71 ppm from DSS), the reason of which is not clear now.

In conclusion, the ^{1}H chemical shift of the H_α signal is important and useful for conformational analysis of these systems in the solid state: α-helix (3.9−4.0 ppm) and *β*-sheet (5.1−5.5 ppm).

Conformation-Dependent ^{1}H Chemical Shifts of Cyclic Dipeptides. Figure 5 shows the 300 MHz ^{1}H CRAMPS NMR spectra of the cyclic dipeptides cyclo[L-Ala-L-Ala] (LL) and cyclo[L-Ala-D-Ala] (LD) in the solid state. The ^{1}H CRAMPS NMR spectra showed a simple high-resolution proton signal pattern separated into three regions (NH, H_α, and H_β signals) for LL and LD. It is known that the ring conformation of LL is the boat form (C_β: equatorial), and that of LD is the planar form in the solid state by the X-ray crystal diffraction analysis.[21,22] From Figure 5, it was demonstrated that (1) the ^{1}H chemical shift of the H_α signal is different between LL (4.3 ppm) and LD (4.7 ppm), (2) the ^{1}H chemical shifts of the H_β signals are almost identical for LL and LD (1.4−1.5 ppm), and (3) the ^{1}H chemical shift of the NH signal is quite different between LL (10.3 ppm) and LD (9.3 ppm). The chemical shift difference of the H_α signal is attributable mainly to the differences in ring conformation.

According to the X-ray crystal diffraction studies by Sletten et al.,[21,22] the intermolecular hydrogen bond (O···H) lengths are 1.89 and 1.91 Å for LL and 1.94 Å (equivalent) for LD, respectively. The chemical shift difference of the NH signal may be explained by considering an intermolecular hydrogen-bonding effect of the cyclic dipeptides. In order to verify that there exists a relation between the ^{1}H (NH) chemical shift and the hydrogen bond length, further details of the relation should be clarified together with results on other cyclic dipeptides, which we are studying currently. In addition, it is important that in these systems, the NH signal is easily visible, and that the chemical shift difference between LL and LD is very large. Accordingly, it may be anticipated that obtaining the ^{1}H chemical shift of the NH signal by ^{1}H CRAMPS NMR opens the possibility for the analysis of ring conformation and for the study of hydrogen bonding of cyclic dipeptides in the solid state.

Conclusion

We have successfully measured the ^{1}H CRAMPS NMR spectra of some homopolypeptides and cyclic dipeptides in the solid state. We determined the ^{1}H chemical shift of these peptides within the error limit of ±0.1 ppm. As a result, it was found that (1) the ^{1}H chemical shift of the H_α signal of solid homopolypeptides such as PLA, PLL, and PBLG is conformation-dependent [α-helix (3.9−4.0 ppm) and *β*-sheet (5.1−5.5 ppm)], (2) the ^{1}H chemical shift of the H_β signal is conformation-independent and is strongly affected by the chemical structure of the individual amino acid residue [PLA (1.2−1.4 ppm), PLL (1.5−1.6 ppm), and PBLG (2.2−2.4 ppm)], and (3) the NH signal is very broad or almost nondetectable. Thus, the ^{1}H chemical shift of the H_α signal of solid homopolypeptide is very useful for the conformational analysis of polypeptides in the solid state. Further, from the results of cyclic dipeptides, it may be anticipated that the ^{1}H chemical shift of the NH peak may be useful for the analysis of ring conformation and for the study of hydrogen bonding of cyclic dipeptides in the solid state.

Supporting Information Available: Figure S1 of ^{1}H CRAMPS NMR spectra (A) with silicon−rubber as an internal standard and (B) without internal standard (1 page). See any current masthead page for ordering and Internet access instructions.

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