# Determination of the Solid-State Conformations of Polyalanine Using Magic-Angle Spinning NMR Spectroscopy

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Conformations of the powder samples of poly-L-alanine with molecular weights of 356 Da (tetraalanine), 15 000 Da (PLA-200), and 23 600 Da (PLA-333) were characterized by $^{13}C$ cross-polarization magic-angle spinning (CPMAS) and $^{1}H$ combined rotation and multiple pulse (CRAMPS) solid-state NMR spectroscopy. From the $^{13}C$ and $^{1}H$ isotropic chemical shift values, it is predicted that the main chain conformations of tetraalanine and PLA-200 are mainly $\beta$-sheet while the conformation of PLA-333 is mainly an $\alpha$-helix. It is unusual and interesting that a high molecular weight homopolypeptide, PLA-200, has a $\beta$-sheet conformation rather than an $\alpha$-helix conformation. The effect of dichloroacetic acid (DCA) solvent on the backbone conformation of these peptides was also studied. It is inferred from solid-state NMR results that conformations of tetraalanine and PLA-333 are similar before and after crystallization from DCA. On the other hand, the backbone conformation of PLA-200 is 60% $\alpha$-helix and 40% $\beta$-sheet after crystallization from the DCA solvent. Variable temperature studies are also reported.

## Introduction

Structural studies of homopolypeptides are important to understand the relationship between the structure and the function of biopolymers. In the recent past, solid-state NMR spectroscopy has been successfully applied to the structural investigation of homopolypeptides.[1−3] In particular, magic-angle spinning (MAS) spectroscopy is well recognized as a powerful method complementary to X-ray crystallography for obtaining different types of conformations adopted by polypeptides. This approach is a powerful utilization of the conformation-dependent chemical shift as an intrinsic probe to elucidate the conformational features in the solid-state as viewed from the individual amino acid residues under consideration. It has been demonstrated that the isotropic chemical shifts of $^{13}C$ nuclei present in the backbone of polypeptides, determined by the MAS method, are significantly displaced depending on their particular conformations such as right- and left-handed $\alpha$-helices, $\omega$-helix, $3_1$-helix, collagen-like triple helix, $\beta$-sheet, silk I form, and random coil.[4−14] A recent study demonstrated the relationship between the isotropic chemical shifts of $\alpha$-protons and the backbone conformation of homopolypeptides using combined rotation and multiple pulse (CRAMPS) spectroscopy.[15] Thus, it is feasible to carry out the conformational characterization of a number of polypeptides if the chemical shifts of these nuclei from suitable reference polypeptides are available.

We report here the backbone conformations of polyalanine powder samples with three different molecular weights determined by $^{13}C$ cross-polarization magic-angle spinning (CPMAS) and $^{1}H$ CRAMPS spectroscopy. The effect of DCA solvent and temperature on the backbone conformation of PLA samples is also reported.

## Experimental Section

**Polyalanine Samples.** All the polyalanine samples were obtained from Sigma chemical company. Tetraalanine, and polyalanine samples, with molecular weights of 15 000 (PLA-200) and 23 600 Da (PLA-333) were used without any further purification. The parameter *n* in PLA-*n* represents the degree of polymerization (Dpn) of a polyalanine sample. To study the effect of DCA, these samples were crystallized from dichloroacetic acid (DCA) solvent. The solubility of polyalanines in DCA solvent was poor, so a small amount of chloroform was added to obtain a clear solution of polyalanine samples in DCA. After that, the clear polyalanine solutions were added to ether for crystallization. The crystallized samples were filtered and the solvents were dried in the vacuum.

**NMR Methods.** All solid-state $^{13}C$ CPMAS and $^{1}H$ CRAMPS experiments were performed on a Chemagnetics Infinity 400 spectrometer equipped with a 9.4 T wide-bore JMT magnet. Resonance frequencies of $^{13}C$ and $^{1}H$ nuclei were 100.65 and 400.14 MHz, respectively. A 5 mm $^{1}H$ CRAMPS and a 5 mm double resonance probe purchased from Chemagnetics were used. A 5 mm glass rotor was used for $^{1}H$ CRAMPS experiments, while a 5 mm Zirconia rotor was used for $^{13}C$ CPMAS experiments. The spinning frequencies were $1.2 \pm 0.001$ kHz and $(5.5-8.5) \pm 0.001$ kHz for $^{1}H$ CRAMPS and $^{13}C$ CPMAS experiments, respectively. For $^{1}H$ CRAMPS experiments,[16−19] a BR-24 pulse sequence[20] was used with a 90° pulse width of 1.3 $\mu$s, a cycle time of 115.2 $\mu$s, a 16 ms acquisition time, and a recycle time of 10 s. The $^{1}H$ chemical shift was calculated using an experimentally determined scaling factor of 0.4 and was externally referenced using the $^{1}H$ CRAMPS spectrum of adipic acid relative to tetramethylsilane (TMS) $(CH_3)_3Si$ ($\delta$ = 0 ppm). Acetone and adipic acid were used as test samples to set up the experimental conditions to perform $^{1}H$ CRAMPS experiments on polyalanine samples. The $^{1}H$ chemical shifts of polyalanine are estimated within $\pm 0.1$ ppm accuracy. The shim parameters obtained for the water sample were kept constant throughout the measurement session to avoid shim-induced field change. $^{13}C$ spectra were obtained using a single-contact CP pulse sequence with a 2 ms contact time, a 20 ms acquisition time, and a 5 s recycle delay time. An rf field strength of 70 kHz was used for the cross-polarization, while the protons were

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**TABLE 1. Carbon-13 Chemical Shifts Characteristic of the Backbone Conformation of Polyalanine Powder Samples Obtained from Previous Studies Reported in the Literature (±0.5 ppm from TMS)**

| nuclei | $C_\alpha$ | | | | $C_\beta$ | | | | $C_o$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| conformation | α-helix | β-sheet | ref | random coil[a] | α-helix | β-sheet | ref | random coil[a] | α-helix | β-sheet | ref | random coil[a] |
| chemical shift | 52.4 | 48.2 | 7 | | 14.9 | 19.9 | 7 | | 176.4 | 171.8 | 7 | |
| | 52.3 | 48.7 | 8 | 51.1 | 14.8 | 20.0 | 8 | 15.7 | 176.2 | 171.6 | 8 | 176.1 |
| | 52.8 | 49.3 | 9 | | 15.5 | 20.3 | 9 | | 176.8 | 172.2 | 9 | |

[a] Solution data taken from ref 7.

decoupled with a 100 kHz rf field. The $^{13}C$ free induction decays were obtained in the presence of proton decoupling using the two-pulse phase modulation (TPPM) sequence.[21] The $^{13}C$ chemical shift frequencies were referenced to TMS by setting the observed $^{13}C$ signals of solid adamantane to 29.5 and 38.56 ppm. The line width measured from the $^{13}C$ CPMAS spectrum of adamantane was less than 8 Hz. Experimental data were processed with a Sun Sparc computer using the Spinsight software from Chemagnetics. For FID workup, 2 K data points were zero-filled to 8 K points after apodization using a 20 Hz exponential line broadening.

## Results and Discussion

**Conformational Analysis Using $^{13}C$ CPMAS Experiments.** It is well-known that polyalanine samples with a high molecular weight form an α-helical conformation and those with a low molecular weight form a β-sheet conformation.[7,22−25] This has been well predicted by experimental studies on homopolypeptides using X-ray diffraction[22−24] and IR spectroscopy.[22,23,26] Characteristic bands at 1656 and 370 $cm^{-1}$ in the infrared spectrum and at $2\theta = 11.5°$ (the (100) reflection), 20.8° (the (110) reflection), and 24° (the (200) reflection) from the hexagonal crystal with $a = b = 8.55$ Å and $c = 70.3$ Å in X-ray diffraction predict an α-helix conformation. A β-sheet conformation is characterized by the peaks at 1630 and 430−445 $cm^{-1}$ in the infrared spectrum and by the diffraction patterns at $2\theta = 16.7°$ (the (020) reflection) and 20.2° (the (110) reflection) arising from the orthorhombic crystal with $a = 4.79$, $b = 10.7$, and $c = 6.88$ Å. This has also been confirmed in solid-state NMR studies using the chemical shifts of $^{13}C$, $^{15}N$, and $^{1}H$ nuclei from the backbone of polyalanine powder samples.[1−3,15] In fact, the relationship between the $^{13}C$ isotropic chemical shift values and the secondary structure of homopolypeptides has been very well established (see Table 1). It is also clear from solid-state NMR studies that polyalanines with a degree of polymerization (Dpn) less than 16 form a β-sheet and those with Dpn > 16 form an α-helix conformation.[7] For example, it has been predicted that poly-L-alanines with molecular weights of 1200 Da, 4700 Da, 50 000 Da and 200 000 Da form an α-helix conformation while an octapeptide, H-$[Ala]_8$−NHBu, forms a β-sheet conformation.[7] Therefore, it is straightforward to determine the backbone conformation directly from the $^{13}C$ isotropic chemical shifts of α-, β-, and carbonyl carbons of polyalanine powder samples. In this paper, a complete conformational study of three different polyalanine powder samples is reported.

Carbon-13 CPMAS spectra of tetraalanine, PLA-200, and PLA-333 obtained at room temperature are given in Figure 1. The $^{13}C$ spectra of polyalanine samples have all three sites well resolved and easily assigned with the resonances from carboxyl and carbonyl carbons in the low-field region at 172−182 ppm, the α carbon region at 48−54 ppm, and the methyl carbon in the high field region at 15−25 ppm. Line broadening due to $^{14}N$ nuclei is negligible due to the use of a relatively high magnetic field (9.4 T). Further narrowing in the $^{13}C$ spectral lines is because of the high MAS speed and TPPM proton decoupling. For example, the full line width at half-height of the $^{13}C_\alpha$ peak in PLA-333 is 1 ppm. Carbon-13 isotropic chemical shift values measured from the CPMAS spectra along with the predicted backbone conformation of the peptides are summarized in Table 2. The dominant resonance peaks at 21.8, 51.2, and 172.6 ppm from the $^{13}C$ spectrum of tetraalanine, shown in Figure 1A, predict a β-sheet conformation for the tetraalanine molecule in the solid state. The resonance peak at

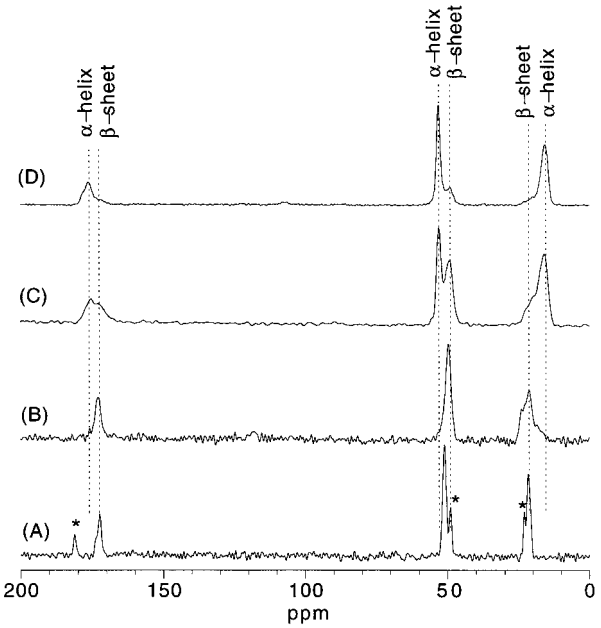


**Figure 1.** Carbon-13 CPMAS spectra of solid polyalanine samples: (A) tetraalanine (β-sheet); (B) polyalanine with a molecular weight of 15 000 Da (~92% β-sheet and ~8% α-helix); (C) polyalanine with a molecular weight of 15 000 Da crystallized in dichloroacetic acid solvent (~40% β-sheet and ~60% α-helix); (D) polyalanine with a molecular weight of 23 600 Da (~85% α-helix and ~15% β-sheet). Peaks marked by an asterisk arise from $^{13}C$ nuclei in C- and N-terminals of the polypeptides.

**TABLE 2. Carbon-13 Chemical Shifts Characteristic of the Backbone Conformation of Polyalanine Powder Samples Obtained from the Present Work Using CPMAS Experiments at Room Temperature (±0.5 ppm from TMS)**

| nuclei | PLA-4[a] | PLA-200[a] | PLA-200[b] | PLA-333[a] | conformation |
|---|---|---|---|---|---|
| $C_\beta$ | | 16.1 | 16.1 | 15.8 | α-helix |
| | 21.8 | 21.6 | 20.3 | 20.0 | β-sheet |
| | 23.2 | | | | *c* |
| $C_\alpha$ | 49.2 | | | | *c* |
| | 51.2 | 49.9 | 49.4 | 49.1 | β-sheet |
| | | 53.5 | 53.1 | 53.2 | α-helix |
| C═O | 172.6 | 173.1 | 173.1 | 172.1 | β-sheet |
| | 174.1 | | | | *c* |
| | | 176.2 | 175.7 | 176.3 | α-helix |
| | 181.4 | | | | *c* |

[a] Data obtained from commercial sample without further purification. [b] Data obtained from samples recrystallized in dichloroacetic acid solvent. [c] Resonance peaks from $^{13}C$ nuclei in the C- and N-terminals of the peptides.

181.4 ppm can be assigned to the carboxyl carbon of the peptide. Other resonance peaks at 23.2, 49.2, and 174.1 ppm in Figure 1A could be attributed to the terminal carbon nuclei that are in a different chemical environment (or near the C- and N-terminals of the peptide), as compared to carbon nuclei in the peptide bonds. This spectrum, shown in Figure 1A, does not have any resonance peaks that represent an α-helical conformation of tetraalanine peptide. This prediction completely agrees with the reported studies in the literature.[7,8]

Carbon-13 CPMAS spectrum of PLA-200 is shown in Figure 1B. There are two sets of resonances in the spectrum. One set of resonance peaks at 21.4, 50.0, and 173.1 ppm predicts a *β*-sheet (∼87%) conformation, while the second set of peaks at 16.3, 53.5, and 176.2 ppm predicts an α-helix (∼13%) conformation for PLA-200. It is important to note that the α-helix conformation of the PLA-200 peptide is only about 13%. This result contradicts the general prediction of the secondary structure of homopolypeptides reported in the literature.[7] Since PLA-200 is a high molecular weight molecule, it is expected to form mainly an α-helical conformation rather than a *β*-sheet conformation. The origin of other resonance peaks at 18.8 and 24.2 ppm in Figure 1B is not known.

Resonance peaks at 15.8, 53.2, and 176.3 ppm from the $^{13}C$ spectrum of PLA-333 as shown in Figure 1D predict that the main chain conformation of PLA-333 is α-helix (∼85%). Further, additional peaks at 21.8, 49.1, and 172 ppm confirm the presence of a *β*-sheet conformation (∼15%) of PLA-333. This result is in complete agreement with the published studies on polyalanine samples in the literature.[7]

The commercial polyalanine samples were crystallized from DCA solvent to investigate the conformation dependency on the DCA treatment. It is known that DCA induces helical structure for hydrophobic homopoly(amino acids) (i.e., poly-L-alanine) and poly-L-leucine).[27] Carbon-13 spectra were collected for all three polyalanine samples after crystallization from DCA solvent. Tetraalanine shows a small amount of α-helix conformation (∼2%) when the DCA solvent was not completely removed. The presence of DCA solvent was judged from a $^{13}C$ resonance peak at 65 ppm (spectrum is not shown). The percentage of α-helix conformation goes to 0 when DCA was completely removed. In the case of PLA-333, DCA decreases the *β*-sheet conformation from 16% to 14% (spectrum is not shown). On the other hand, the spectrum of PLA-200 as shown in Figure 1C was significantly different after crystallization from DCA. Isotropic $^{13}C$ chemical shift values of PLA-200 are given in Table 2. It is clear that the resonance peaks at 16.1, 53.1, and 175.7 ppm confirm the presence of an α-helical (60%) conformation in addition to the presence of a *β*-sheet (40%) conformation. Thus, the DCA solvent treatment has increased the α-helix conformation by a factor of 5, while it decreased the *β*-sheet conformation by a factor of 2.2.

Variable temperature experiments were conducted on polyalanine samples over the temperature range from 18 to 180 °C. Carbon-13 CPMAS spectra of all the polyalanine samples collected at various temperatures were found to be similar, except for the PLA-200 sample crystallized from the DCA solvent. This confirms the stability of the backbone conformations of polyalanine samples. Carbon-13 CPMAS spectra of PLA-200 powder sample crystallized from the DCA solvent at various temperatures are shown in Figure 2. Increase in temperature increases the percentage of the *β*-sheet conformation as shown in Figures 2 and 3. Here, we used the signal intensity of $^{13}C_\alpha$ to determine the conformation of PLA-200 as the peaks are well resolved for accurate measurements. At 160 °C, the

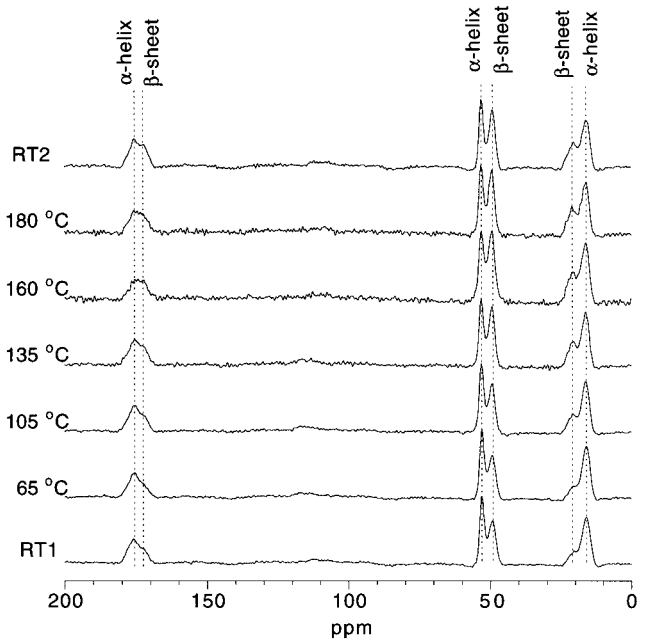


**Figure 2.** Carbon-13 CPMAS spectra of polyalanine with a molecular weight of 15 000 Da crystallized in dichloroacetic acid solvent obtained at various temperatures. The room temperature $^{13}C$ spectra, RT1 and RT2, were obtained before and after performing variable temperature studies, respectively.

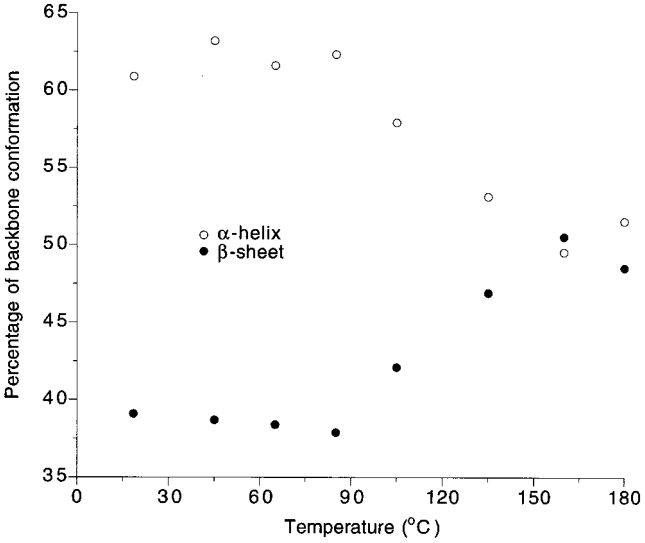


**Figure 3.** Change of the backbone conformation of polyalanine with a molecular weight of 15 000 Da crystallized in dichloroacetic acid solvent as a function of temperature obtained from the $^{13}C$ CPMAS spectroscopy.

percentage of α-helix and *β*-sheet conformations is almost equal, but at 180 °C, the percentage of *β*-sheet conformation decreases to 48.5% as shown in Figure 3. Interestingly, after performing the experiments at high temperature, the percentage of conformations at room temperature was 45% *β*-sheet and 55% α-helix as shown in Figure 3. This means that the major fraction of the conformational change from α-helix to *β*-sheet due to high temperature is permanent. The reason for this observation may be related to the conformational transition (from α-helix to *β*-sheet) of polyalanine samples due to mechanical stress applied to polyalanine films or fibers in contact with hot water.[8,28]

Fourier transform infrared (FTIR) spectra (4000−400 $cm^{-1}$) of KBr disks containing PLA-200 samples (commercial PLA-200 and a PLA-200 sample that contains equal amounts of

**TABLE 3. Distribution of Isotropic Chemical Shift (Δ) of $^{13}C$ and $^{1}H$ Nuclei**

| | α-helix (ppm) | | | β-sheet (ppm) | | |
|---|---|---|---|---|---|---|
| nuclei | min | max | Δ | min | max | Δ |
| $C_\beta$ | 14.8 | 16.1 | 1.3 | 19.9 | 21.8 | 1.9 |
| $C_\alpha$ | 52.3 | 53.5 | 1.2 | 48.2 | 51.2 | 3.0 |
| C═O | 175.7 | 176.8 | 1.1 | 171.6 | 173.1 | 1.5 |
| $H_\alpha$ | 3.5 | 3.9 | 1.1 | 5.0 | 5.1 | 0.1 |

α-helix and β-sheet conformations) were obtained on a Nicolet 5-DX FTIR spectrometer in order to confirm the results from solid-state NMR experiments. A major peak at 1630 $cm^{-1}$ in the FTIR spectrum, characteristic of a β-sheet conformation, was observed in both the PLA-200 samples. This is in good agreement with the NMR data. However, because of the overlap of bands at 1630 and 1656 $cm^{-1}$, it was not possible to estimate the percentage of α-helix and β-sheet conformations from the FTIR spectra of PLA-200 samples.

Solid-state NMR experiments were also performed on a trialanine powder sample. Carbon-13 chemical shifts measured from the CPMAS experiments were in complete agreement with the results reported in the literature.[8] The main chain β-sheet conformation was the same after crystallization from the DCA solvent and was also stable against the variation of temperature.

Experimental results predict a wide range of $^{13}C$ chemical shifts (Δ) for each conformation of polyalanine as summarized in Table 3. As shown in Tables 2 and 3, a β-sheet conformation could have the chemical shifts in the following ranges: 19.9−21.8 (Δ = 1.9), 48.2−51.2 (Δ = 3), and 171.6−173.1(Δ = 1.5) ppm. Similarly, α-helical conformation could result in $^{13}C$ peaks in the following ranges: 14.8−16.1 (Δ = 1.3), 52.3−53.5 (Δ = 1.2) and 175.7−176.8 (Δ = 1.1) ppm. Since the differences in $^{13}C$ chemical shifts of different conformations are so narrow, as evident from Table 1, care must be taken in predicting the backbone conformation of polypeptides based on the isotropic $^{13}C$ chemical shifts. For example, the $^{13}C$ isotropic chemical shifts display a good deal of overlap between α-helical and random coil conformations as evident from Table 1. Therefore, establishing an index of $^{13}C$ chemical shifts for each conformation will be useful to determine the backbone conformation of polypeptides correctly.

**Conformational Analysis Using $^{1}H$ CRAMPS Experiments.** Recent progress in $^{1}H$ CRAMPS spectroscopy has shown a dramatic improvement in resolution and sensitivity.[16−20,29−33] It has been successfully applied to a variety of studies in the solid state.[17,30,31,33] In the present work, $^{1}H$ CRAMPS experiments were performed on all polyalanine samples to examine the use of the chemical shifts of protons attached to the α-carbon ($H_\alpha$) to determine the backbone conformation of homopolypeptides. Proton CRAMPS spectra of polyalanine samples are shown in Figure 4. Proton chemical shift values measured from the CRAMPS spectra are summarized in Table 4 along with the predicted secondary structure. There are three different sets of resonance peaks in Figure 4 corresponding to $H_\beta$, $H_\alpha$, and NH protons of polyalanine molecules. From Figure 4, it is clear that the chemical shift of $H_\alpha$ is conformation dependent. The $H_\alpha$ chemical shifts of tetraalanine (Figure 4A), PLA-200 (Figure 4B), and PLA-333 (Figure 4D) are 5.0, 5.0, and 3.5 ppm, respectively, from TMS. This predicts a β-sheet conformation for tetraalanine and PLA-200 but an α-helix conformation for PLA-333. On the other hand, the $H_\alpha$ chemical shift values of PLA-200 crystallized from the DCA solvent are 3.5 and 4.7 ppm (refer to Figure 4C) and clearly predict a combination of α-helix and β-sheet conformations. From the intensity of $H_\alpha$ peaks of PLA-200, it may be estimated that the conformations

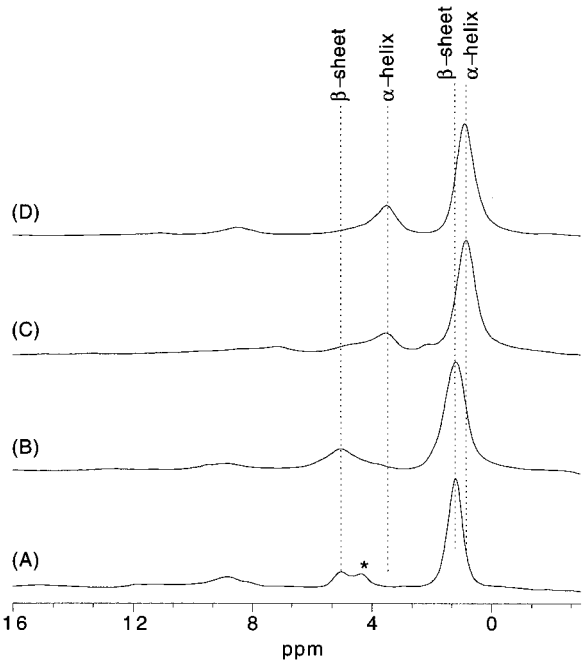


**Figure 4.** Proton CRAMPS spectra of polyalanine powder samples: (A) tetraalanine (β-sheet); (B) polyalanine with a molecular weight of 15 000 Da (β-sheet); (C) polyalanine with a molecular weight of 15 000 Da after crystallization from dichloroacetic acid solvent (60% α-helix and 40% β-sheet); (D) polyalanine with a molecular weight of 23 600 Da (α-helix). The peak indicated by an asterisk could be assigned to methyl proton in the N-terminal of the tetraalanine peptide.

**TABLE 4. Proton Chemical Shifts Characteristic of the Backbone Conformation of Polyalanine Powder Samples Obtained Using CRAMPS Experiments at Room Temperature (±0.1 ppm from TMS)**

| nuclei | PLA-4[a] | PLA-200 | PLA-200[b] | PLA-333[a] | conformation |
|---|---|---|---|---|---|
| $H_\alpha$ | | | 3.5 | 3.5 | α-helix |
| | 4.4 | | | | *c* |
| | 5.0 | 5.0 | 5.0 | | β-sheet |
| $H_\beta$ | | | 0.9 | 0.9 | α-helix |
| | 1.2 | 1.2 | | | β-sheet |

[a] Data obtained from commercial sample without further purification. [b] Data obtained from samples recrystallized in dichloroacetic acid solvent. [c] Resonance of a methyl proton in the N-terminal of the tetraalanine.

are 60% α-helix and 40% β-sheet. It is gratifying that the conformations of PLA samples predicted from the $^{1}H$ chemical shift values (refer to Table 4) completely agree with the results from the $^{13}C$ chemical shifts (refer to Table 2). The $H_\alpha$ chemical shift value of 3.5 ppm measured for the α-helical conformation of PLA-200 sample crystallized from the DCA solvent (refer Figure 4C) is close to those values in solution (3.94−3.95 ppm from DSS),[34−36] while 5.0 ppm for the β-sheet conformation of PLA-333 is identical to the previously reported $^{1}H$ CRAMPS results.[15] Proton CRAMPS experiments were repeated several times to confirm the chemical shift values, and the results were identical each time. The $H_\alpha$ isotropic chemical shifts of polyalanine studied thus far are summarized in Tables 3 and 4. It will certainly be useful to determine the range of $H_\alpha$ chemical shifts for different conformations from $^{1}H$ CRAMPS experiments on various homopolypeptides.

While the conformational sensitivity of α-proton resonances is well demonstrated, it is worth while to find out the sensitivity of other proton resonances that might display a conformational dependency. It is important to mention here that $H_\beta$ chemical shifts are also conformation dependent as shown in Figure 4. The $H_\beta$ chemical shift is 0.9 ppm for α-helix and 1.2 ppm for

$\beta$-sheet conformations. On the other hand, solution NMR results predict that the $H_\beta$ chemical shift is 1.4 ppm for α-helix and 1.0 ppm for $\beta$-sheet conformations. The reason for the difference in $H_\beta$ chemical shifts predicted from solution and solid-state NMR experiments is not clear now.

Solution NMR studies[34−36] have proven most obviously that the amide protons display an upfield shift for helices and a downfield shift for $\beta$-strands. But, unfortunately, the amide proton resonance peaks in the CRAMPS spectra are broad due to the unaveraged $^1H-^{14}N$ dipolar interaction. However, it is worthwhile to investigate the conformation dependency of the chemical shifts of amide protons by completely decoupling the N−H dipolar couplings. This could be made possible by $^1H$ CRAMPS experiments or through two-dimensional $^1H-^{15}N$ heteronuclear chemical shift correlation experiments on PLA samples uniformly labeled with the $^{15}N$ isotope. Such a study would be more powerful as the resonance frequency of amide protons can not only be used to determine the conformation of the polypeptide but also to characterize the backbone dynamics of the polypeptide. Proton CRAMPS experiments performed at higher magnetic fields[37] with the use of higher magic-angle sample spinning frequency[37,38] would greatly advance the study of conformation of homopolypeptides based on the $^1H$ chemical shift values. The inclusion of substantially more proton data in the coming years may eventually lead to routine structural studies of polypeptides using solid-state NMR spectroscopy.

## Conclusion

We have successfully used the chemical shift values of $^{13}C$ and $^1H$ nuclei to determine the backbone conformation of polyalanine samples in the solid state. Interestingly, a polyalanine sample with a molecular weight of 15 000 Da (PLA-200) is a stable $\beta$-sheet conformation, which is unusual as it is expected to be an α-helical conformation due to its high molecular weight. Changes in the conformation of PLA samples due to treatment with DCA solvent were also investigated using solid-state NMR experiments. Our results predict that PLA-200 changes its conformation from $\beta$-sheet to a combination of $\beta$-sheet and α-helix whereas tetraalanine and PLA-333 do not change their conformations upon crystallization from the DCA solvent. Results from $^{13}C$ CPMAS and $^1H$ CRAMPS experiments infer that the chemical shift values of either $^{13}C$ or $^1H_\alpha$ alone are sufficient to predict the conformation as well as to show conformational changes of polyalanine samples due to solvents. Variable-temperature studies reveal that the main chain conformation of PLA-200 crystallized from DCA changes from 40% $\beta$-sheet and 60% α-helix at room temperature to 50% α-helix and 50% $\beta$-sheet at 160 °C. It is noteworthy that the conformational transition from α-helix to $\beta$-sheet is not completely reversible when the temperature is decreased to room temperature. From the results presented in this paper, we strongly believe that the measurement of a range of isotropic chemical shift values from $^{13}C$ CPMAS and $^1H$ CRAMPS experiments will be useful as an index to determine the backbone conformation of polypeptides. Thus, it is obvious that high-resolution solid-state NMR spectroscopy, at higher external magnetic fields with higher spinning speeds,[37] would be fruitful to the structure determination of homopolypeptides.

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## References and Notes

(1) Saito, H.; Ando, I. In *Annual Reports on NMR Spectroscopy*; Webb, G. A., Ed.; Academic Press: London, 1989; Vol. 22, pp 209−290.

(2) Ando, I.; Yamanobe, T.; Asakura, T. *Prog. NMR Spectrosc.* **1990**, *22*, 349−400.

(3) 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. 24, pp 55−98.

(4) Taki, T.; Yamashita, S.; Satoh, M.; Shibata, A.; Yamashita, T.; Tabeta, R.; Saito, H. *Chem. Lett.* **1981**, 1803−1806.

(5) Muller, D.; Krichelderf, H. R. *Polym. Bull.* **1981**, *6*, 101−108.

(6) Opella, S. J.; Hexem, J. G.; Frey, M. H.; Cross, T. A. *Philos. Trans. R. Soc. London* **1981**, *A299*, 665−683.

(7) Saito, H.; Tabeta, R.; Shoji, A.; Ozaki, T.; Ando, I. *Macromolecules* **1983**, *16*, 1050−1057.

(8) Kricheldorf, H. R.; Mutter, M.; Maser, F.; Muller, D.; Forster, H. *Biopolymers* **1983**, *22*, 1357−1372.

(9) Kricheldorf, H. R.; Muller, D. *Macromolecules* **1983**, *16*, 615−623.

(10) Saito, H.; Tabeta, R.; Asakura, T.; Iwanaga, Y.; Shoji, A.; Ozaki, T.; Ando, I. *Macromolecules* **1984**, *17*, 1405−1412.

(11) Shoji, A.; Ozaki, T.; Saito, H.; Tabeta, R.; Ando, I. *Macromolecules* **1984**, *17*, 1472−1479.

(12) Yamanobe, T.; Ando, I. *J. Chem. Phys.* **1985**, *83*, 3154−3160.

(13) Ando, S.; Yamanobe, T.; Ando, I.; Shoji, A.; Ozaki, T.; Tabeta, R.; Saito, H. *J. Am. Chem. Soc.* **1985**, *107*, 7648−7652.

(14) Ando, S.; Ando, I.; Shoji, A.; Ozaki, T. *J. Mol. Struct.* **1989**, *192*, 153−161.

(15) Shoji, A.; Kimura, H.; Ozaki, T.; Sugisawa, H.; Deguchi, H. *J. Am. Chem. Soc.* **1996**, *118*, 7604−7607.

(16) Gerstein, B. C.; Pembleton, R. G.; Wilson, R. C.; Ryan, L. M. *J. Chem. Phys.* **1977**, *66*, 361−362.

(17) Bronnimann, C. E.; Hawkins, B. L.; Zhang, M.; Maciel, G. E. *Anal. Chem.* **1988**, *60*, 1743−1750.

(18) Maciel, G. E.; Bronnimann, C. E.; Hawkins, B. L. In *Advanced Magnetic Resonance*, Warren, W. S., Ed.; Academic Press: New York, 1990; Vol. 14, pp 125−150.

(19) Gerstein, B. C.; Dybowski, C. R. *Transient Techniques in NMR of Solids*; Academic Press: New York, 1985; Chapter 5. Gerstein, B. C. CRAMPS. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D. M., Harris, R. K., Eds.; John Wiley & Sons: Chichester, U.K., 1996; Vol. 3, pp 1501−1509.

(20) Burum, D. P.; Rhim, W. K. *J. Chem. Phys.* **1979**, *71*, 944−956.

(21) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, *103*, 6951−6958.

(22) Itoh, K.; Nakahara, T.; Shimanouchi, T.; Oya, M.; Uno, K.; Iwakura, Y. *Biopolymers* **1968**, *6*, 1759−1766.

(23) Fujie, A.; Komoto, T.; Oya, M.; Kawai, T. *Makromol. Chem.* **1973**, *169*, 301−321.

(24) Brown, D. A.; Trotter, I. F. *Trans. Faraday Soc.* **1956**, *52*, 537−548.

(25) Arnott, S.; Dover, S. D.; Elliot, A. *J. Mol. Biol.* **1967**, *30*, 201−208.

(26) Miyazawa, T. *Poly-α-Amino Acids*; Fasman, G. D., Ed.; Marcel Dekker: New York, 1967; Chapter 2.

(27) Shoji, A.; Kawai, T.; Nishioka, A. *Macromolecules* **1977**, *10*, 1292−1298.

(28) Noguchi, J.; Tokura, S.; Nishi, N. *Angew. Makromol. Chem.* **1972**, *22*, 107−131.

(29) Chu, P. J.; Potrzebowski, M. J.; Gao, Y.; Scott, A. I. *J. Am. Chem. Soc.* **1990**, *112*, 881−883.

(30) Naito, A.; Root, A.; McDowell, C. A. *J. Phys. Chem.* **1991**, *95*, 3578−3581.

(31) Buszko, M. L.; Bronnimann, C. E.; Maciel, G. E. *J. Magn. Reson.* **1993**, *103A*, 183−187.

(32) Liu, C. H. C.; Maciel, G. E. *Anal. Chem.* **1996**, *68*, 1401−1407.

(33) Gu, Z.; Ridenour, C. F.; Bronnimann, C. E.; Iwashita, T.; McDermott, A. *J. Am. Chem. Soc.* **1996**, *118*, 822−829.

(34) Wishart, D. S.; Sykes, B. D. In *Methods in Enzymology*; James, T. L., Oppenheimer, N. J., Eds.; Academic Press: San Diego, CA, 1994; Vol. 239, pp 363−392.

(35) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *FEBS Lett.* **1991**, *293*, 72−80.

(36) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *J. Mol. Biol.* **1991**, *222*, 311−333.

(37) van Rossum, B. J.; Forster, H.; deGroot, H. J. M. *J. Magn. Reson.* **1997**, *124*, 516−519.

(38) Ernst, M.; Verhoeven, A.; Meier, B. H. *J. Magn. Reson.* **1998**, *130*, 176−185.