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Communications to the Editor

Determination of N−H Bond Lengths of ^{15}N-Labeled Poly(L-alanines) by ^{1}H CRAMPS NMR

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An accurate determination of the N−H covalent bond length is important for elucidating polypeptide conformations because the N−H bond length directly mirrors the >N−H···O=C< hydrogen bond strength, and is responsible for the long-range order in the system. For this purpose, CRAMPS (combined rotation and multiple pulse spectroscopy)[1−6] of ^{1}H has great potential for conformational analysis of polypeptides in the solid state.

We have previously studied the relationship between the ^{1}H chemical shifts of the amide proton (NH) and the conformation of some solid polypeptides by ^{1}H CRAMPS.[7,8] In that study, the ^{1}H NMR signal of the NH group with ^{15}N in natural abundance is very broad and exhibits asymmetric doublet patterns due to the residual heteronuclear dipolar coupling between the quadrupolar ^{14}N nucleus and the amide proton.[7−9] To get a sharp ^{1}H NMR signal and a symmetric singlet pattern of the NH group, we have measured ^{1}H CRAMPS NMR spectra of fully ^{15}N-labeled poly(L-alanines) which have been prepared to eliminate the effects of the quadrupolar ^{14}N nuclei. In the ^{1}H CRAMPS NMR experiment of the ^{15}N-labeled poly(L-alanines) at 2.0 kHz MAS (magic angle spinning) speed, the ^{15}N−^{1}H dipolar sideband patterns of the NH signal were observed. The magnitude of the ^{15}N−^{1}H heteronuclear dipolar coupling is proportional to the inverse cube of the distance between ^{15}N and ^{1}H nuclei.[3,4,10] Thus, the ^{15}N−^{1}H bond lengths can be measured by observing the dipolar line width, which can be computed from the ^{15}N−^{1}H dipolar spinning sidebands. The ^{15}N−^{1}H dipolar interaction has been measured utilizing ^{15}N SLF (separated local field) spectroscopy.[10−12] In this method, the ^{15}N−^{1}H dipolar interaction is separated from the ^{15}N chemical shift anisotropy by using two-dimensional (2D) NMR technique. The ^{15}N−^{1}H dipolar interaction can be also estimated directly from the ^{1}H CRAMPS NMR, because under CRAMPS, the ^{1}H chemical shift anisotropy is averaged to zero. The N−H bond lengths of poly(L-alanines) are almost indeterminate by X-ray diffraction. It is also difficult to determine the N−H bond length from the neutron diffraction, because this method needs deuterium-labeled poly(L-alanine) in a single crystal state.

In this communication, we report that the ^{15}N−^{1}H bond lengths of fully ^{15}N-labeled poly(L-alanines) adopting the right-handed α-helix (α-helix) and antiparallel β-sheet (β-sheet) conformations can be determined from the ^{15}N−^{1}H dipolar sideband patterns observed in the ^{1}H NMR spectra. To observe the full spectral width of the dipolar sideband patterns, the quadrature-phase (QD) detection measurement was performed.[13] We demonstrate the first practical use of the QD-CRAMPS method in the present work.

In this study we used fully ^{15}N-labeled poly(L-alanines) (99 at. % of ^{15}N purity; MASSTRACE, Inc.), which were synthesized by the N-carboxy-α-amino acid anhydride (NCA) method in our laboratory.[8] Conformations of these samples were characterized on the basis of

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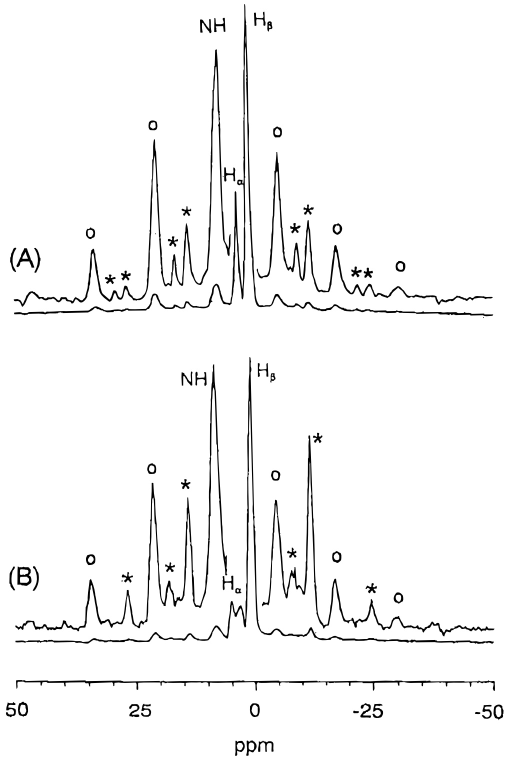

Figure 1. 300 MHz ^{1}H CRAMPS NMR spectra of fully ^{15}N-labeled poly(L-alanines) for (A) α-helix and (B) β-sheet conformations in the solid state. Peak assignment: NH, 10−8 ppm; H_α, 3.9−5.0; H_β, 1.4−1.2 ppm. Note: $-N-CH_2-$ peak (3.2−3.5 ppm) of *n*-butylamide group (initiator of polymerization) in spectrum B. The sign (○) indicates the spinning sidebands (SSBs) of NH signal and the sign (∗) indicates SSBs of H_α and H_β signals.

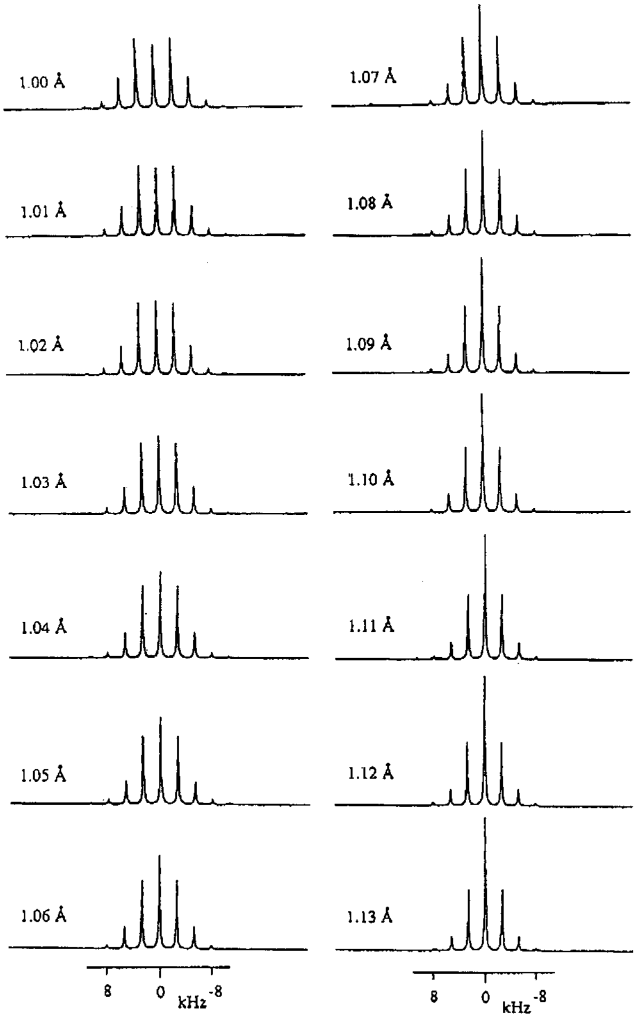

Figure 2. Simulated spectra of ^{1}H CRAMPS NMR for the ^{1}H dipolar sideband patterns in a series of different N−H bond lengths (scaling factor; 0.53, spinning frequency; 2 kHz, and line width; 160 Hz).

conformation-dependent ^{13}C and ^{15}N chemical shifts determined from CP-MAS NMR measurements.

The solid-state ^{1}H CRAMPS NMR measurements were performed on a Chemagnetics CMX 300 spectrometer equipped with a CRAMPS probe with 5 mm rotor.[7,8] Quadrature-phase detection was carried out according to the phase-cycling technique proposed by Burum et al.[13] Here, we used the MREV-8 pulse sequence[14] for homonuclear decoupling. The $\pi/2$ pulse width was 1.1 μs, and τ was 2.4 μs. The cycle time of the MREV-8 was 28.8 μs, and the rotational frequency was controlled to 2.0 kHz. Silicone rubber (δ = 0.12 ppm) relative to tetramethylsilane $(CH_3)_4Si$ (δ = 0 ppm) was used as an internal standard. The ^{1}H chemical shift was calculated with the scaling factor of 0.53 for all samples, which was determined experimentally in the same manner as described before.[7]

A spectral simulation was performed on an NEC 9801 personal computer equipped with a transputer (INMOS, U.K.) using the SLFDIP program, which was written in FORTRAN 77 language based on the theory described by Naito et al.[12]

Figure 1 shows the ^{1}H CRAMPS NMR spectra of fully ^{15}N-labeled poly(L-alanines) for (A) α-helix and (B) β-sheet conformations in the solid state. The spinning sidebands (SSBs) of the methine proton (H_α) and methyl proton (H_β) signals of poly(L-alanines) are indicated by asterisks (∗), and those of the amide proton (NH) signal are by open circles (○). The SSBs of the H_α and H_β signals fell off rapidly compared to those of the amide protons, whereas those of the NH signal gradually decreased due to the quite large ^{15}N−^{1}H heteronuclear dipolar interaction. Here, we have confirmed that the integral intensities of the SSBs at left side are almost the same as the corresponding one at right side, whereas their peak heights are different. The N−H dipolar SSB pattern of α-helical poly(L-alanine) was different from that of β-sheet poly(L-alanine).

Figure 2 shows the simulated spectra for a series of different N−H bond lengths at a rotor frequency of 2.0 kHz. The sideband pattern is sensitive to N−H bond length. The relative intensity of SSBs to the center peak decreases with longer N−H distance. It is therefore possible to determine the N−H bond length within an accuracy of 0.01 Å by a careful comparison of the integral ratio of the center peak to the sideband intensities of the dipolar spectrum obtained experimentally compared to that of the simulated spectra.

Figure 3 shows the plots of the intensity ratio of the first and second sideband peak to the center signal of the simulated and experimentally obtained ^{15}N−^{1}H dipolar spectra vs the order of the sidebands. We thus determined the N−H bond lengths for the poly(L-alanines) to be 1.09 and 1.12 Å for the α-helix and β-sheet conformations, respectively, with an accuracy of 0.01 Å. Therefore, the N−H distance in the β-sheet was 0.03 Å longer than that in the α-helix.

The above N−H bond length difference between the α-helix and β-sheet forms seems to be related to the

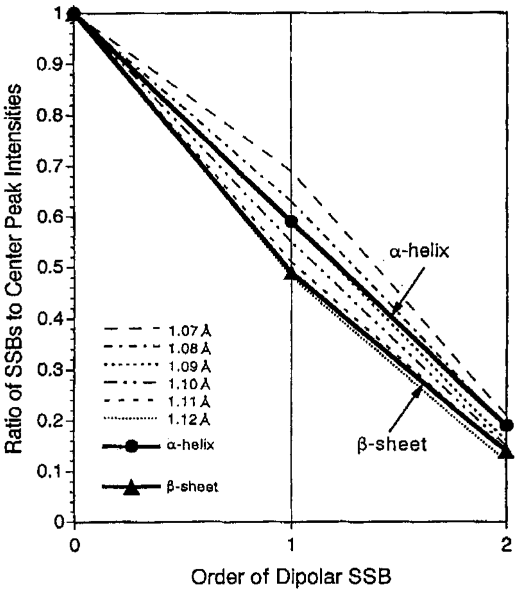

Figure 3. Plots of the intensity ratio of the first and second order sideband intensities to the center signal of the simulated and experimentally obtained $^{15}N-^{1}H$ dipolar spectra of poly(L-alanines) vs the order of the sidebands. The experimental results are indicated by the heavy lines.

hydrogen bond strengths and amide proton chemical shifts. According to the X-ray diffraction studies of poly(L-alanines) by Arnott et al.,[15,16] the distances between the nitrogen and oxygen atoms are 2.87 and 2.83 Å for the α-helix and β-sheet forms, respectively.

On the other hand, the ^{1}H chemical shift value of the NH group in the α-helix was 0.6 ppm upfield from that of the β-sheet form (α-helix, $\delta = 8.0$ ppm; β-sheet, $\delta = 8.6$ ppm).[8] Also, the above ^{1}H chemical shift displacement was consistent with that of the ^{15}N chemical shift data in poly(L-alanines).[17−19] These results suggest that the hydrogen bond strength in the β-sheet was stronger than that in the α-helix for poly(L-alanines). Accordingly, it is reasonable to conclude that the amide proton of the β-sheet poly(L-alanine) is more strongly attracted to the oxygen than that of the α-helical poly(L-alanine).

In conclusion, we have successfully determined the accurate N−H bond lengths for the fully ^{15}N-labeled poly(L-alanines) adopting the α-helix (1.09 Å) and β-sheet (1.12 Å) conformations from the ^{1}H CRAMPS NMR spectra. Thus, we found that the N−H distance in the β-sheet was 0.03 Å longer than that in the α-helix for poly(L-alanines). This result is consistent with the hydrogen bond distances between the nitrogen and oxygen atoms from the X-ray diffraction and with the ^{1}H chemical shifts of amide proton signals from ^{1}H CRAMPS method.

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