ACS SYMPOSIUM SERIES **732**

Modeling NMR Chemical Shifts

Gaining Insights into Structure and Environment

Julio C. Facelli, EDITOR
University of Utah

Angel C. de Dios, EDITOR
Georgetown University

American Chemical Society, Washington, DC

Chapter 9

^{17}O NMR Chemical Shifts in Peptides

S. Kuroki[1], K. Yamauchi[2], Hiromichi Kurosu[3], S. Ando[1], Isao Ando[1],
A. Shoji[4], and T. Ozaki[4]

[1]Department of Chemistry and Materials Science, Tokyo Institute
of Technology, Ookayama, Meguro-ku, Tokyo 152–8852, Japan
[2]Solid NMR Application, Bruker Japan Company Ltd., Ninomiya,
Tsukuba-shi, Ibaraki 305–0015, Japan
[3]Department of Human Life and Environment, Nara Women's University,
Kita-uoya, Nishimachi, Nara 630–8263, Japan
[4]Department of Biological Sciences, Gunma University, Tenjin-cho, Kiryu-shi,
Gunma 376–8515, Japan

The O-17 NMR spectra of L-alanine containing polypeptides in the solid state were measured chemical shift, quadrupolar coupling constant and asymmetric parameter information obtained. The relationship between the hydrogen-bonding structure and these NMR parameters was investigated. Theoretical calculations of the electric field gradients and chemical shielding values of hydrogen-bonded carbonyl oxygens in peptides were also carried out, and it was found that there exists a relationship between hydrogen-bonding structure and the NMR parameters such as quadrupolar coupling constants and chemical shieldings.

The oxygen atom is one of the more important atoms involved with hydrogen-bonding in polymers such as peptides and polypeptides. Nevertheless, few solid-state ^{17}O NMR studies of such polymers have been carried out. This is due to the very weak sensitivity of solid-state ^{17}O NMR measurements, due to the two following reasons. First, the ^{17}O nucleus has a very low natural abundance, 0.037 %. Second, the ^{17}O nuclear spin quantum number(I) is 5/2, which implies a quadrupolar nucleus, and so the ^{17}O signal is broadened by nuclear quadrupolar effects in the solid. On the other hand, solution-state ^{17}O NMR spectroscopy has been successfully employed to elucidate a number of structural problems in organic chemistry, because the ^{17}O signal becomes relatively sharp due to the removal of the quadrupolar interaction by fast isotropic reorientation in solution. For example, as the oxygen atom is directly associated with the formation of a hydrogen bond, hydrogen bonding for the carbonyl group in various compounds often results in large low frequency shifts of the carbonyl ^{17}O NMR signal. From these results, solution-state ^{17}O NMR has been established as a means for structural characterization.

In such situations, it can be expected that solid-state ^{17}O NMR will provide a deep insight into understanding the hydrogen-bonding structures of solid polymers, such as peptides and polypeptides(1). High-resolution ^{17}O NMR spectra of solid polyglycine(PGI(β-sheet) and PGII(3_1helix)), glycylglycine(GlyGly), glycylglycine nitrate (GlyGly•HNO_3), have been measured previously, in order to obtain three kinds of NMR parameters: the chemical shift(δ), quadrupolar coupling constant(e^2qQ/h) and asymmetry parameter(η), in order to understand the relationship between these NMR parameters and the hydrogen-bonding structure(2). From these observed ^{17}O carbonyl NMR spectra, it is found that e^2qQ/h values decrease linearly with a decrease in the hydrogen bond length. This indicates that it is possible to determine the hydrogen bond length through the observation of e^2qQ/h values. The chemical shift values in peptides and in polypeptides move to low frequency with a decrease in the hydrogen bond length. However, there is a difference in the chemical shift value between peptides and polypeptides, which may come from differences in molecular packing. From these experimental findings, it was demonstrated that ^{17}O NMR spectroscopy is a useful mean for elucidating the hydrogen-bonding structure in solid peptides and polypeptides.

In addution, it was also demonstrated that the quantum-chemical approach of calculating the quadrupolar coupling constants and the chemical shieldings will provide systematic information on the conformation and electronic state. The theoretical calculations of the electric field gradients of hydrogen-bonded carbonyl oxygen were successfully carried out by the FPT-MNDO-PM3 method. From these calculated results, it was found that the calculated e^2qQ/h value decreases with a decrease in the hydrogen bond length, and the chemical shielding values move to low frequency with a decrease in the hydrogen bond length. These calculations explain the experimental findings obtained in the NMR measurements. Also, the directions of the principal axes of the electric field gradient tensor and chemical shielding of the carbonyl oxygen were obtained with resonable accuracy(3).

In addition to the glycine-containing polypeptides and peptides, we show here new results on the ^{17}O NMR chemical shift and quadrupolar coupling constant of poly(L-alanine)s in both α-helix and β-sheet forms, in the solid state. There results help clarify the relationship between hydrogen-bond length and e^2qQ/h and chemical shift parameters.

Experimental Section

Sample Preparations. 10% ^{17}O-labeled the L-alanine methyl ester in $Na^{17}OH$/methanol solution, where $Na^{17}OH$ was prepared by reaction of 20% ^{17}O-labeled water with Na metal. Further, L-Ala N-carboxyanhydride(NCA) was prepared using 10% ^{17}O-L-alanine. 10% ^{17}O-labeled poly(L-alanine)[$(L\text{-}Ala)_n$] was prepared by heterogeneous polymerization of 10% ^{17}O-labeled NCA in acetonitrile by using n-butylamine as the initiator. The conformational characterizations of these polymers were made on the basis of ^{13}C CP/MAS(cross polarization/magic angle spinning) by using reference data of ^{13}C chemical shift values for various polypeptides, which are associated with the main-chain conformation. $[L\text{-}Ala]_n$ with A/I=100 takes α-helix form, and $[L\text{-}Ala]_n$ with A/I=5 takes β-sheet forms, respectively.

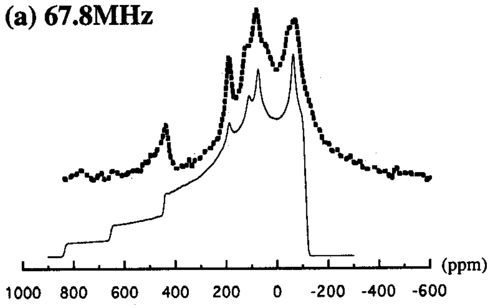
Solid state ^{17}O NMR Measurements. Static ^{17}O CP NMR spectra were recorded with a JEOL GSX-270(6.3T) spectrometer and a JEOL GSX-500(11.7T) spectrometer operating at 36.6 and 67.8 MHz, respectively, with a CP/MAS accessory at room temperature. In the CP static methods, Mg(^{17}OH)$_2$ was used for ^{1}H-^{17}O CP matching($\gamma_H B_H = 3\gamma_o B_o$). The ^{1}H $\pi/2$ pulse length was 5μs and the ^{17}O $\pi/2$ pulse length was 5μs for a solid sample(which corresponded to 15μs for a solution sample). The ^{1}H decoupling field strength was 50 kHz and repetition time was 5s. According to the previous work(2) on ^{17}O CP NMR experiments for polyglycines and its peptides, 9ms was used as the appropriate contact time. The ^{17}O chemical shifts were calibrated through external liquid water(δ= 0 ppm). Further, ^{17}O MAS NMR spectra were recorded with a Bruker AVANCE400(9.4T) spectrometer, a Bruker AVANCE 500(11.7T) spectrometer, and a Bruker AVANCE800(18.8T) spectrometer with a high-speed MAS accessory operating at 54.2, 67.8, and 108.6MHz. The sample spinning rate is 15kHz at 54.2 and 67.8MHz, and 25kHz at 108.6MHz, respectively. These spectra were obtained by single $\pi/2$ pulse with ^{1}H decoupling experiments.

Solid State ^{17}O NMR Spectral Analysis. The ^{17}O nucleus has a 5/2 spin and so has a quadrupolar moment. This means that static and MAS ^{17}O NMR spectrum contains information about quadrupolar interactions and the chemical shift. In order to obtain the quadrupolar coupling constant and chemical shift separately, we have carried out spectral analysis with theoretical calculation taking into account quadrupolar interactions and chemical shift interactions.

Results and Discussion

^{17}O NMR spectra of poly(L-alanine)s: Figures 1 and 2 shows the static ^{17}O CP NMR spectra of solid (L-Ala)$_n$[A/I=100] with an α-helix form, and solid (L-Ala)$_n$[A/I=5] with a β-sheet form at 67.8 MHz(a) and 36.6 MHz(b), respectively(4). The spectrum at 36.6 MHz consists of two major split signals, but that at 67.8 MHz has one major signal overlapping with two signals. Such large variation comes from the quadrupolar interaction because the appearance of the spectrum depends on the NMR frequency. If the NMR frequency is extremely high, the influence of quadrupolar interactions may be neglected in the spectrum.□From these static spectra we can obtain the NMR parameters such as chemical shift tensors(δ_{11}, δ_{22}, and δ_{33}), quadrupolar coupling constant(e^2qQ/h), asymmetry parameter of electric field gradient(η),and the Euler angle(α,β,γ) between the principal axes of the quadrupolar tensor and the chemical shift tensor by theoretical simulation, but it is difficult to determine these eight parameters exactly at the same time. For the case of MAS spectra, we can obtain only three NMR parameters such as isotopic chemical shift(δ_{iso}), e^2qQ/h , and η. It is not so difficult to determine these three NMR parameters from MAS spectra compared with the case of static spectra. But until now, the typical sample spinning rate is below 10kHz, it was not enough to separate the centerband signal and sideband signals of ^{17}O NMR spectrum so that identical quadrupolar MAS signal could not be observed. Very recently the high speed MAS technique has been developed and we can observed the centerband signal of ^{17}O NMR signal separated from the sideband signals easily. Figures 3 and 4 shows the

(a) 67.8MHz



(b) 36.6MHz

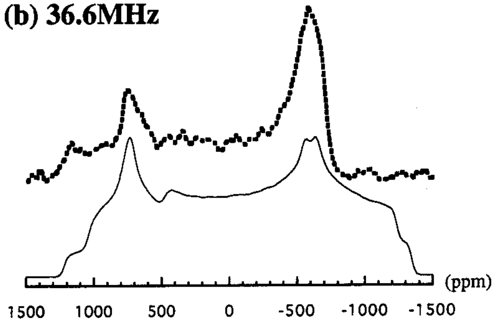
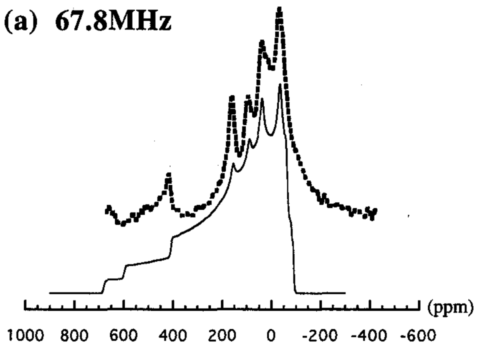


Figure 1. 67.8MHz (a) and 36.6MHz (b) ^{17}O CP static NMR spectra of (L-Ala)$_n$ with the α-helix form (A/I=100) in the solid state.

(a) 67.8MHz



(b) 36.6MHz

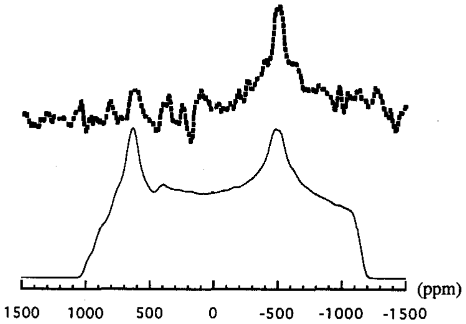


Figure 2. 67.8MHz (a) and 36.6MHz (b) ^{17}O CP static NMR spectra of (L-Ala)$_n$ with the β-sheet form (A/I=5) in solid state.

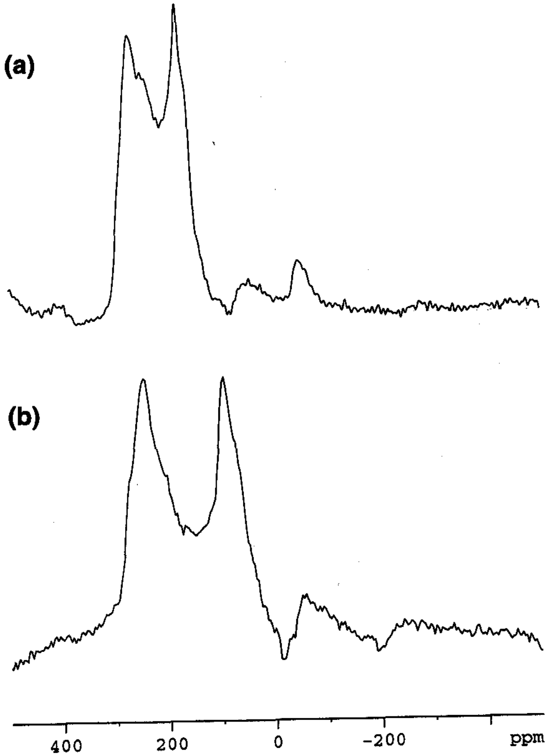


Figure 3. ^{17}O MAS NMR spectra of solid (L-Ala)$_n$[A/I=100] with an α-helix form at 67.8 MHz(a) and 54.2 MHz(b) spun at 15kHz.

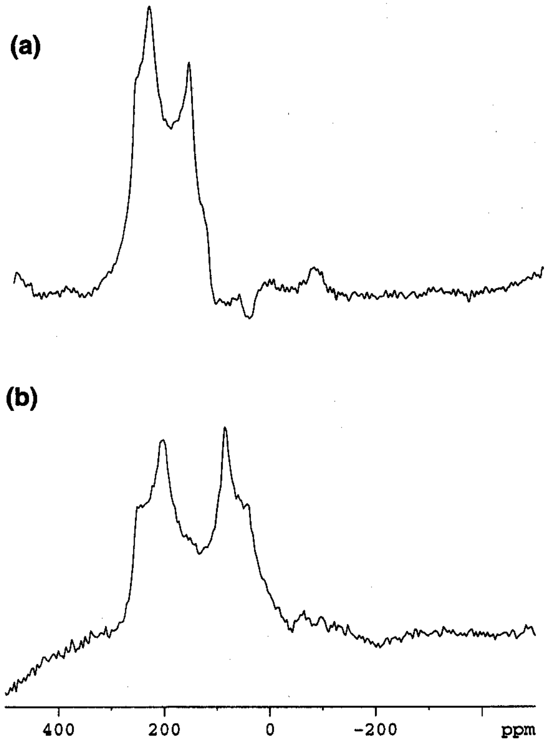


Figure 4. ^{17}O MAS NMR spectra of solid (L-Ala)$_n$[A/I=5] with a β-sheet form at 67.8 MHz(a) and 54.2 MHz(b) spun at 15kHz.

^{17}O MAS NMR spectra of solid (L-Ala)$_n$[A/I=100] with an α-helix form, and solid (L-Ala)$_n$[A/I=5] with a β-sheet form at 67.8 MHz(a) and 54.2 MHz(b) spun at 15kHz, respectively. At 54.2MHz, the centerband signal was not separated from the sideband signal both poly(L-alanine)s, though at 67.8 MHz the centerband signal was separated from the sideband signals. The centerband linewidth at 54.2MHz was broadened compared with that at 67.8MHz. The centerband linewidth is proportional to $\omega_Q{}^2/\omega_L$. ω_L is the Larmor frequency and ω_Q is proportional to e^2qQ/h. Therefor, it can be said that high-frequency measurement is needed for a ^{17}O MAS experiment simultaneously with high-speed sample spinning. In addition, the ^{17}O MAS NMR spectra spun at 25kHz of solid (L-Ala)$_n$[A/I=100] with an α-helix form, and solid (L-Ala)$_n$[A/I=5] with a β-sheet form at 108.6 MHz are shown in Figure 5. In these spectra, the centerband signal was completely separated from the sideband signals. The obtained NMR parameters for the α-helix form and the β-sheet form of poly(L-alanine)s are shown in Table I, together with the NMR parameters obtained from polyglycines I(β-sheet form) and II(3_1-helix form) as reported previously(2). Table I also shows the hydrogen-bond length N...O of poly(L-alanine)s and polyglycines obtained by X-ray analysis. It was found that e^2qQ/h value decreases with a decrease in the hydrogen bond length, and the chemical shift values move low frequency with a decrease in the hydrogen bond length. This results coincided with glycine residue containing peptides and polypeptides results(2).

Electric field gradient and oxygen nuclear shielding calculations of the carbonyl oxygen of the model hydrogen-bonding system; The theoretical approach of calculating the quadropular coupling constants and the chemical shieldings on the basis of quantum chemistry provides systematic information on the hydrogen-bonding structure and its electronic state. The theoretical calculations of the electric field gradients and of hydrogen-bonded carbonyl oxygen of glycine residue were successfully carried out by the FPT-MNDO-PM3 method(3). Figures 6 and 7 shows these calculated results, and it was found that the calculated e^2qQ/h value decreases with a decrease in the hydrogen bond length, and the chemical shielding values move low frequency with a decrease in the hydrogen bond length. These calculations explain the experimental finding obtained in the NMR measurement. Further, in this study we calculated the oxygen chemical shielding values by the ab-initio GIAO-CHF method using model compound. The ^{13}C chemical shielding calculated by the ab-initio GIAO-CHF method reasonably explained the experimental findings(6, 7). Figure 8 shows the calculated oxygen chemical shielding against the hydrogen bond length N...O. The chemical shielding values moved low frequency with a decrease in the hydrogen bond length. This result coincided with the results obtained by the FPT-MNDO-PM3 method and explain the experimental finding obtained in the NMR measurement.

Summary

We can successfully measure ^{17}O MAS NMR spectra of poly(L-Ala)s using high-field and high-speed magic-angle spinning technique. From the ^{17}O MAS spectra three kinds of NMR parameters such as chemical shift, quadrupolar coupling constant and asymmetric parameter were obtained exactly and understood the relationship

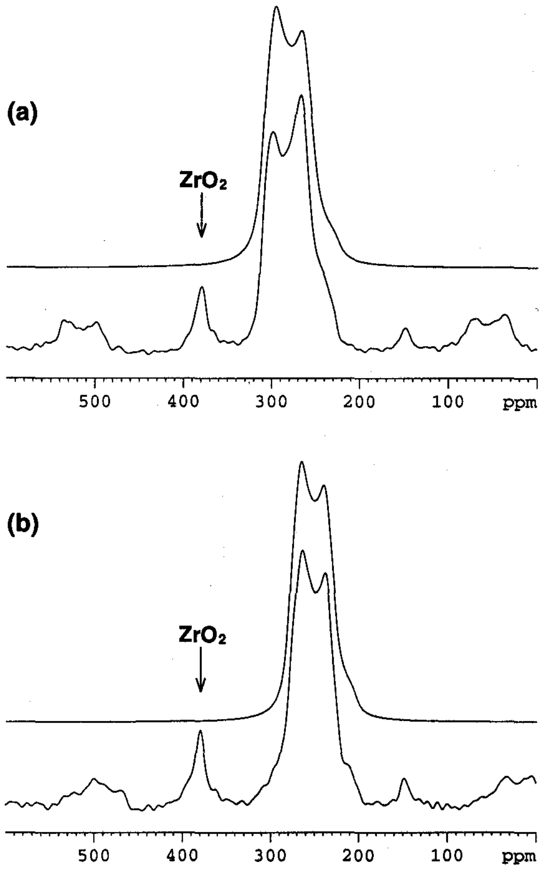


Figure 5. ^{17}O MAS NMR spectra spun at 25kHz of solid $(L\text{-}Ala)_n[A/I=100]$ with an α-helix form(a), and solid $(L\text{-}Ala)_n[A/I=5]$ with a β-sheet form(b) at 108.6 MHz with theoretically simulated spectra.

Table I. The determined NMR parameters of $(L\text{-}Ala)_n$ in this work together with those of $(Gly)_n$.

Sample		e^2qQ/h(MHz)	η	δ_{iso}(ppm)	$R_{N...O}$(Å)
$(L\text{-}Ala)_n$	α-helix	8.59	0.28	319	2.87
	β-sheet	8.04	0.28	286	2.83
$(Gly)_n$	PG II	8.30	0.29	288	2.73
	PG I	8.55	0.26	299	2.95

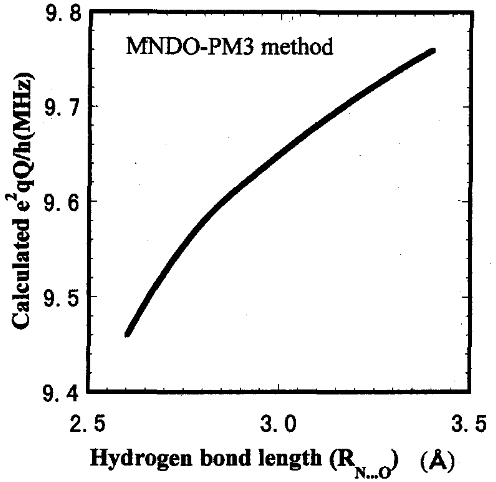


Figure 6. Plots of the calculated e^2qQ/h using the FPT-MNDO-PM3 method against the hydrogen bond length.

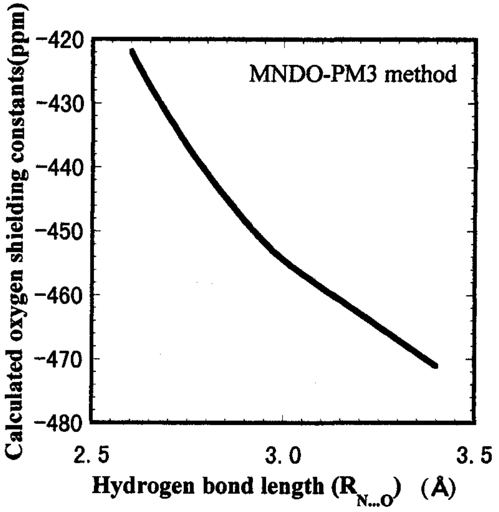


Figure 7. Plots of the calculated oxygen chemical shielding using the FPT-MNDO-PM3 method against the hydrogen bond length.

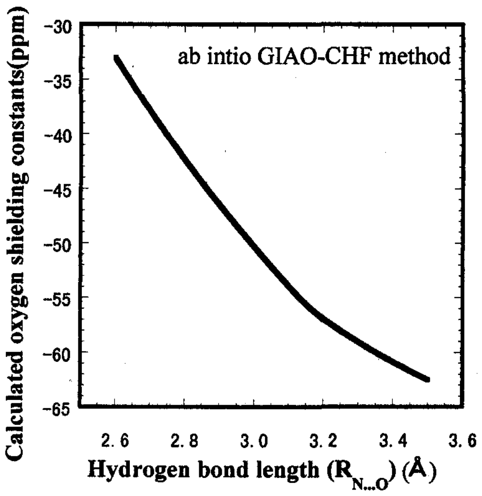


Figure 8. Plots of the calculated oxygen chemical shielding using the GIAO-CHF method against the hydrogen bond length.

between the hydrogen-bonding structure and these NMR parameters. Further, the theoretical calculations of the electric field gradients and the chemical shielding values of hydrogen-bonded carbonyl oxygens in peptides were carried out by the FPT-MNDO-PM3 and ab-inito GIAO-CHF methods, and it was found that there is a relationship between the hydrogen-bonding structures and the NMR parameters such as quadrupolar coupling constants and the chemical shielding values.

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