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The amide proton NMR chemical shift and hydrogen-bonded structure of peptides and polypeptides in the solid state as studied by high-frequency solid-state ^{1}H NMR

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Abstract

High-resolution ^{1}H NMR spectra of glycine (Gly)-containing peptides and polypeptides in the solid state were measured at 800 MHz and at high-speed magic-angle-spinning (MAS) of 30 kHz to elucidate the relationship between the hydrogen-bond length and ^{1}H NMR chemical shift to add to our previous experimental and theoretical findings that there is a relationship between the hydrogen-bond length and ^{13}C, ^{15}N and ^{17}O chemical shifts of various kinds of amino acid residues of peptides and polypeptides in the solid state. From these experimental results, it is found that the ^{1}H chemical shifts of Gly amide protons of Gly-containing peptides and polypeptides, for which the hydrogen-bond length between the nitrogen and oxygen atoms ($R_{N\ldots O}$) have already been determined by X-ray diffraction, move downfield with a decrease in $R_{N\ldots O}$. Theoretical calculations qualitatively explain these experimental results. © 2000 Published by Elsevier Science B.V.

1. Introduction

It has been known that the NMR chemical shifts of peptides and proteins sometimes offer structural information about their backbone and side chains [1–4]. The chemical shifts in peptides and polypeptides in the solid state have been successfully applied to studies of three-dimensional structural analyses of peptides and proteins with the intramolecular and/or intermolecular hydrogen bonds.

In a series of our previous papers [5–17], it has been experimentally and theoretically demonstrated that there is a linear relationship between the hydrogen-bond length and the ^{13}C [5–10], ^{15}N [11–13] and ^{17}O [14–17] NMR chemical shifts and their tensor components for the amide carbonyl carbon, amide nitrogen, and amide carbonyl oxygen of glycine (Gly), L-alanine (Ala), L-valine (Val), L-leucine (Leu), aspargine (Asn), and L-phenylalanine (Phe) amino acid residues in peptides and polypeptides in the solid state. This means that the NMR chemical shifts provide useful information about details of the hydrogen-bonded structure. In addition to these works, it may be significant to clarify whether there is a link between the relationship between the hydrogen-bond length between amide nitrogen and oxygen atoms ($R_{N\ldots O}$) and the solid state ^{1}H NMR

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chemical shift of hydrogen-bonded Gly-containing peptides and polypeptides in the solid state, and also by theoretical calculations because this leads to further elucidation of hydrogen-bonded structure.

However, before doing this experiment, we have some NMR technical problems. For example, we need to reduce the large dipolar interaction between proton nuclei, which leads to a large linewidth. The CRAMPS (combined rotational and multipulse spectroscopy) method is one method to get ^{1}H NMR spectra with reasonable resolution. For obtaining high-resolution ^{1}H NMR spectra for peptides and proteins in the crystalline state, there are some reports in which a combination of magic angle spinning (MAS) and CRAMPS techniques is used [18–20]. However, CRAMPS needs slow speed MAS for sampling the data point during acquisition (e.g., BR24 multi-pulse sequence needs less than about 3 kHz for sampling). The spinning rate of 3 kHz is not enough to get high-resolution ^1H NMR spectra for analyzing the amide proton chemical shift in peptides and polypeptides because the amide proton is directly bonded with a quadrupolar nitrogen-14 (^{14}N) nucleus. As reported by McDermott [18,19], amino acids take $^{+}NH_3$ forms, and so the quadrupolar effect on the proton linewidth through ^{14}N becomes small because of the high symmetry. Therefore, the NH_3 proton linewidth in the amino acids become much sharper than that of amide protons in peptides and polypeptides considered here.

In this Letter we aim to clarify the relationship between the hydrogen-bond length between amide nitrogen and oxygen atoms ($R_{N\ldots O}$) of hydrogen-bonded Gly-containing peptides and polypeptides in the solid state through the observation of high-resolution ^{1}H NMR spectrum at high-speed MAS of 30 kHz and high frequency of 800 MHz for removal of the dipolar coupling and quadrupole coupling with amide ^{14}N.

2. Experimental

2.1. Peptides and polypeptides samples

All the Gly-containing peptides and polypeptides prepared in our previous works were used in this work as listed in Table 1 [6,10,15], of which the hydrogen-bond length between the amide nitrogen and amide carbonyl oxygen atoms ($R_{N\ldots O}$) of Gly amino acid residues were already determined by X-ray diffraction.

2.2. NMR measurements

The solid-state high-resolution ^{1}H NMR experiments were preformed with a Bruker Avance 800 spectrometer operating at 800.13 MHz equipped with high-power amplifier and MAS accessory. The MAS probe was used and the sample of 12 μl was packed

Table 1
^{1}H NMR chemical shifts of hydrogen-bonded glycine residue amide proton for peptides as determined by high-speed MAS NMR and their geometrical parameters

Sample	Hydrogen-bonded glycine amide proton chemical shift δ (ppm)	Hydrogen-bond length $R_{N\ldots O}$ (Å)	Refs.
Poly glycine (form II)	9.04	2.73	[25]
Tyr–Gly–Gly	9.03	2.88	[30]
Pro–Gly–Gly	8.99	2.89	[21]
Val–Gly–Gly	8.80	3.05	[22]
Gly–Gly	8.59	2.94	[23]
Sar–Gly–Gly	8.57	3.06	[24]
Poly glycine (form I)	8.40	2.95	[29]
Ala–Gly–Gly	8.12	3.00	[26]
Gly–Gly · HCl	7.95	3.30	[28]
Gly–Gly · HNO_3	7.76	3.12	[27]

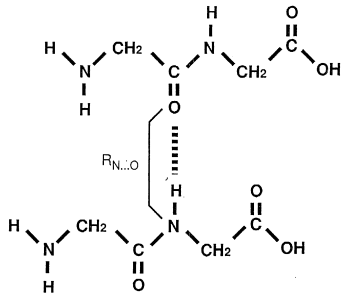

Fig. 1. A GlyGly supermolecule used in ^{1}H chemical shielding calculation.

in an NMR rotor with an outer diameter of 2.5 mm. The pulse excitation field was 150 kHz and applied 45° pulse. The typical measurement conditions were as follows. The MAS was 30 kHz, the recycle delay 10 s and the number of accumulation 8 times. The ^{1}H chemical shifts were calibrated relative to external DSS(3-trimethylsilyl-1-propanesulfonic acid sodium salt).

2.3. Theoretical calculations

The ^{1}H chemical shielding calculations were done with the GAUSSIAN 96 program with ab initio 6-31G** basis set using the optimized geometries of a GlyGly supermolecule, where two hydrogen-bonded GlyGly molecules (Fig. 1) were used by changing the hydrogen-bond length between amide nitrogen and oxygen atoms ($R_{N\ldots O}$) from 2.3 to 3.0 Å as referred to its crystal structure determined by X-ray diffraction. The calculated chemical shieldings are in ppm relative to tetramethylsilane (TMS).

3. Results and discussions

Fig. 2 shows ^{1}H NMR spectra of Gly-containing peptides obtained by single pulse method with a MAS rate of 30 kHz. The amide proton, α-proton, and the side-chain protons are straightforwardly assigned because their peaks clearly resolve with each other. The chemical shifts of the other functional groups are approximately the same as corresponding amino acids in aqueous solution. In this work, we are concerned with the amide proton chemical shift behavior for the individual Gly residues because the amide NH proton forms forming the hydrogen bond. The amide proton chemical shift must be assigned carefully. Thus, if the corresponding peak overlapped with the other peaks, it was decomposed by using computer-fitting and so the chemical shift was

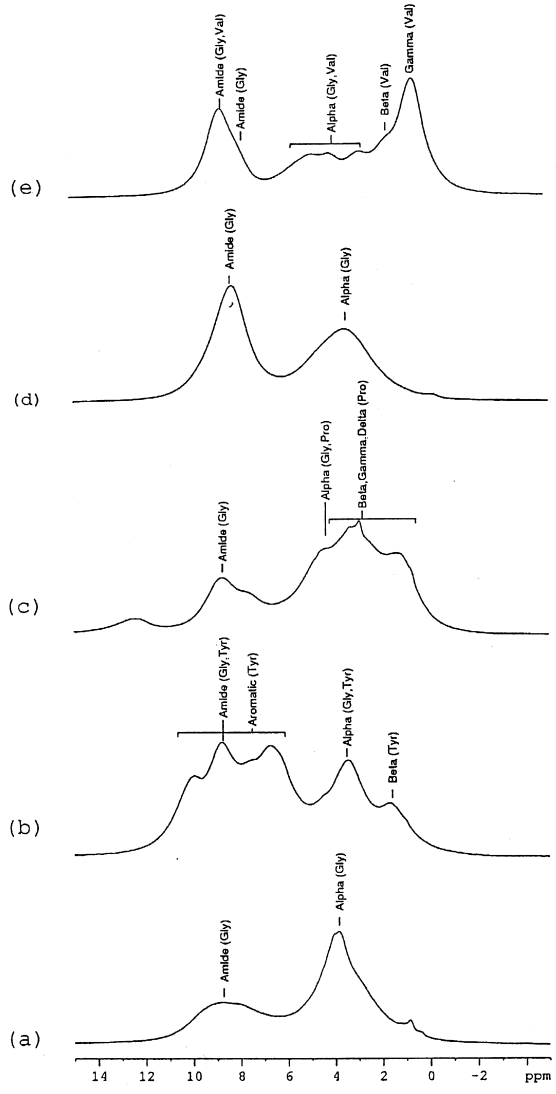

Fig. 2. The ^{1}H MAS NMR spectra of Gly-containing peptides and polyglycines by single pulse method at a MAS rate of 30 kHz at 800 Mhz. (a) Polyglycine (form II), (b) Tyr–Gly–Gly, (c) Pro–Gly–Gly, (d) Gly–Gly, (e) Val–Gly–Gly, (f) Sar–Gly–Gly, (g) polyglycine (form I), (h) Ala–Gly–Gly, and (i) Gly–Gly·HNO_3.

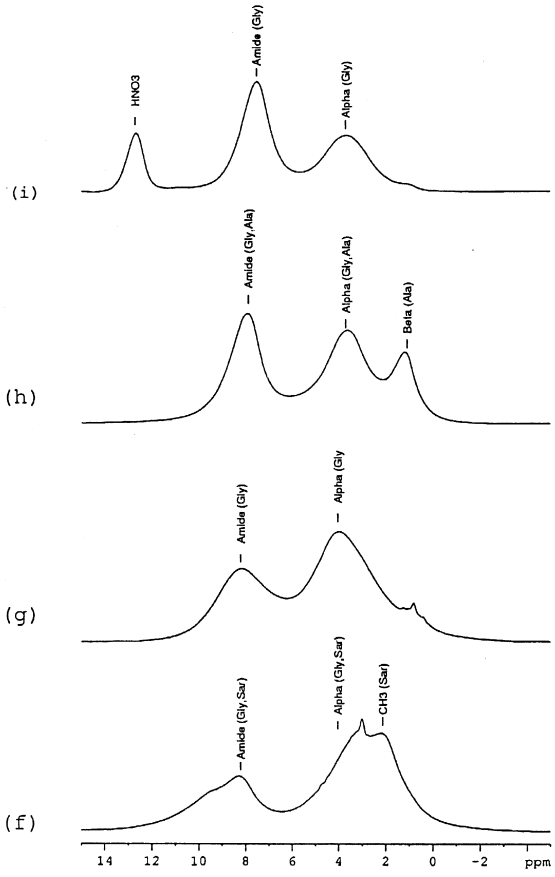

Fig. 2 (continued).

determined. Table 1 shows the determined Gly amide proton chemical shift values of peptides and polypeptides in the solid state together with the hydrogen-bond length between amide nitrogen and oxygen atoms ($R_{N\ldots O}$) as determined from X-ray diffraction. Here, it can be said that the reduction of $R_{N\ldots O}$ may lead to a decrease in the hydrogen-bond length between the amide proton and oxygen atom.

Fig. 3 shows the plots of the determined ^{1}H chemical shift values (δ) of hydrogen-bonded Gly amide protons of Gly-containing peptides and polyglycines in the solid state against the hydrogen-bond lengths between amide nitrogen and oxygen atoms ($R_{N\ldots O}$) as determined from X-ray diffraction. The bars indicate the experimental errors in the spectra. It is found that as $R_{N\ldots O}$ is decreased from 3.12 to 2.72 Å, amide ^{1}H chemical shift moves to downfield

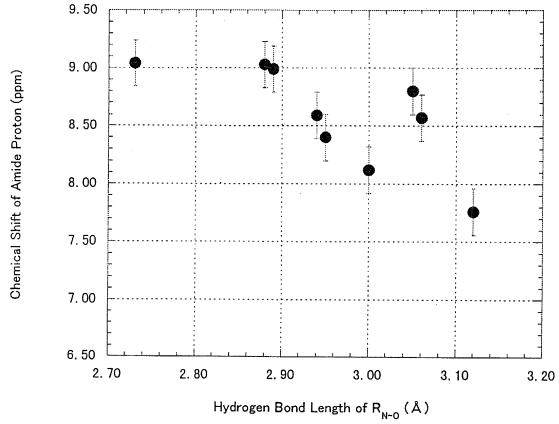

Fig. 3. Plots of the determined ^{1}H chemical shift values (δ) of hydrogen-bonded Gly amide protons of Gly-containing peptides and polyglycines in the solid state against their hydrogen-bond lengths between amide nitrogen and oxygen atoms ($R_{N\ldots O}$) as determined from X-ray diffraction. The bars indicate the experimental errors in the spectra.

by 1.28 ppm from 7.76 to 9.04 ppm. Thus, it can be said that the amide ^{1}H chemical shift moves to downfield with a decrease in hydrogen-bond lengths between amide nitrogen and oxygen atoms ($R_{N\ldots O}$). This means that the hydrogen-bond length $R_{N\ldots O}$ can be estimated through the observation of the amide ^{1}H chemical shift.

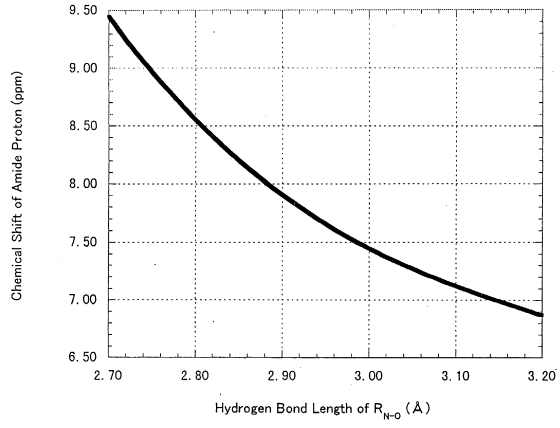

Fig. 4. Plots of the calculated ^{1}H chemical shieldings (σ) of hydrogen-bonded Gly amide protons of two hydrogen-bonded GlyGly supermolecules (as shown in Fig. 1) by using the GAUSSIAN 96 program with ab initio 6-31G** basis set against $R_{N\ldots O}$.

In order to elucidate such a downfield shift with a decrease in $R_{N\cdots O}$, the ^1H chemical shieldings (σ) of hydrogen-bonded Gly amide protons of two hydrogen-bonded GlyGly molecules were calculated by using the GAUSSIAN 96 program with ab initio 6-31G** basis set by changes of $R_{N\cdots O}$ from 3.5 to 2.6 Å. The calculated ^1H chemical shieldings (σ) were plotted against the $R_{N\cdots O}$ in Fig. 4. It is found that the calculated chemical shifts move to downfield by 2.5 ppm from 6.9 to 9.4 ppm as $R_{N\cdots O}$ is decreased from 3.20 to 2.72 Å. This shows that the calculation explains qualitatively the experimental results. However, quantitative agreement is not obtained. This may come form the fact that strictly the position of the amide proton in the $>N–H\cdots O=C<$ hydrogen bond depends on $R_{N\cdots O}$ [12], but in this calculation the N–H bond length is fixed to be 1.0 Å.

4. Conclusion

The proton MAS spectra at high frequency and at high-speed MAS have been successfully measured. The spectra obtained have better resolution than those by low-frequency MAS NMR with low-speed MAS. From these experimental results, it was found that the amide ^1H chemical shift moves downfield with a decrease in hydrogen-bond lengths between amide nitrogen and oxygen atoms ($R_{N\cdots O}$). Further, it was found that the ab initio calculations explain qualitatively the experimental results.

References

[1] H. Saito, T. Tabeta, A. Shoji, I. Ando, T. Asakura, in: G. Govil, C.L. Khetrapal, A. Saran (Eds.), Magnetic Resonance in Biology and Medicine, Tata McGraw-Hill, New Dehli, 1985, p. 195.

[2] H. Saito, I. Ando, Ann. Rept. NMR Spectrosc. 21 (1989) 210.

[3] I. Ando, T. Yamanobe, T. Asakawa, Prog. NMR Spectrosc. 22 (1990) 349.

[4] A. Shoji, S. Ando, S. Kuroki, I. Ando, G.A. Webb, Ann. Rept. NMR Spectrosc. 26 (1993) 55.

[5] S. Ando, T. Yamanobe, I. Ando, A. Shoji, T. Ozaki, R. Tabeta, H. Saito, J. Am. Chem. Soc. 110 (1988) 3380.

[6] N. Asakawa, S. Kuroki, H. Kurosu, I. Ando, A. Shoji, T. Ozaki, J. Am. Chem. Soc. 114 (1992) 3216.

[7] N. Asakawa, H. Kurosu, I. Ando, J. Mol. Struct. 323 (1994) 279.

[8] T. Kameda, I. Ando, J. Mol. Struct. 412 (1997) 197.

[9] N. Asakawa, T. Kameda, S. Kuroki, H. Kurosu, S. Ando, I. Ando, A. Shoji, Ann. Rept. NMR Spectrosc. 35 (1995) 233.

[10] K. Tsuchiya, A. Takahashi, N. Takeda, N. Asakawa, S. Kuroki, I. Ando, A. Shoji, T. Ozaki, J. Mol. Struct. 350 (1995) 233.

[11] S. Kuroki, N. Asakawa, S. Ando, I. Ando, A. Shoji, T. Ozaki, J. Mol. Struct. 245 (1991) 69.

[12] S. Kuroki, S. Ando, I. Ando, J. Mol. Struct. 240 (1990) 19.

[13] A. Shoji, T. Ozaki, T. Fujito, K. Deguchi, S. Ando, I. Ando, Macromolecules 22 (1989) 2861.

[14] S. Kuroki, A. Takahashi, I. Ando, A. Shoji, T. Ozaki, J. Mol. Struct. 323 (1994) 197.

[15] A. Takahashi, S. Kuroki, I. Ando, T. Ozaki, A. Shoji, J. Mol. Struct. 442 (1998) 195.

[16] S. Kuroki, K. Yamauchi, H. Kurosu, S. Ando, I. Ando, A. Shoji, T. Ozaki, ACS Ser. 732 (1999) 126.

[17] K. Yamauchi, S. Kuroki, I. Ando, A. Shoji, T. Ozaki, Chem. Phys. Lett. 302 (1999) 331.

[18] A. McDermott, C.F. Ridenour, in: R.K. Harris, D.M. Grant (Eds.), Encyclopedia of Nuclear Magnetic Resonance, John Wiley, New York, 1996, p. 3820.

[19] Y. Wei, A.E. McDermotte, ACS Symposium Series 732: Modeling NMR Chemical Shifts, 1999, p. 177.

[20] A. Shoji, H. Kimura, T. Ozaki, H. Sugisawa, K. Deguchi, J. Am. Chem. Soc. 118 (1996) 7604.

[21] V. Lalitha, E. Subramanian, R. Parthasarathy, Int. J. Pept. Protein Res. 27 (1986) 223.

[22] V. Lalitha, R. Murali, E. Subramanian, Int. J. Pept. Protein Res. 27 (1986) 472.

[23] A.B. Biswas, E.D. Hughes, B.D. Sharma, J.N. Wilson, Acta Crystallogr. B 24 (1968) 40.

[24] J.P. Glusker, H.L. Carrel, M. Berman, B. Gallen, R.M. Peck, J. Am. Chem. Soc. 99 (1977) 595.

[25] F.H.C. Crich, A. Rich, Nature (London) 176 (1955) 780.

[26] V. Lalitha, E. Subramanian, Indian J. Pure Appl. Phys. 23 (1985) 506.

[27] S.N. Rao, R. Parthasarathy, Acta Crystallogr. B 29 (1973) 2379.

[28] T.F. Koetzle, W.C. Hamilton, R. Parthasarathy, Acta Crystallogr. B 28 (1972) 2083.

[29] W.T. Astbury, C.H. Dalgleish, S.E. Darmon, G.B.B.M. Sutherland, Nature (London) 69 (1948) 596.

[30] W.M. Carson, M.L. Hackert, Acta Crystallogr. B 34 (1978) 1275.