Hydrogen-Bonding Effect on ^{13}C NMR Chemical Shifts of L-Alanine Residue Carbonyl Carbons of Peptides in the Solid State

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Abstract: In order to investigate the relationship between hydrogen-bond length and ^{13}C NMR chemical shifts of L-alanine carbonyl carbons in peptides in the solid state, ^{13}C CP-MAS NMR spectra were measured for a series of peptides containing L-alanine residues, for which the crystal structures were already determined by X-ray diffractions. From the results of the observed ^{13}C chemical shifts, it was found that the isotropic ^{13}C chemical shifts (δ_{iso}) of L-alanine residues move linearly downfield with a decrease of hydrogen-bond length ($R_{N\cdots O}$) as expressed by $\delta_{iso} = 237.5-21.7R_{N\cdots O}$ ppm. Such a downfield shift of δ_{iso} predominantly arises from the large downfield shift of δ_{22}, one of the chemical shift tensor components (δ_{11}, δ_{22}, and δ_{33}) with decreasing $R_{N\cdots O}$. δ_{11} moves somewhat upfield with $R_{N\cdots O}$ and δ_{33} is not sensitive to a change of $R_{N\cdots O}$. The quantum-chemical calculation of the ^{13}C shielding constant for the model compound was carried out by the FPT-INDO method, and the hydrogen-bonding effect on the ^{13}C chemical shift in the)C=O···H—N(type hydrogen bond and the nature of the hydrogen bond are discussed.

Introduction

It is well-known that the hydrogen bond plays an important role in forming stable conformations of oligopeptides, polypeptides, and proteins.[1] Thus, the nature of the hydrogen bond has been widely studied by various spectroscopic methods. High resolution NMR spectroscopy has also been used as one of the most powerful tools for obtaining useful information about details of the hydrogen bond. The ^{13}C NMR chemical shift of the carbonyl carbon is thought to be the most sensitive to the spatial arrangement of the nuclei comprising the hydrogen bond, since the electronic structure of the carbonyl carbon is greatly affected by the nature of the hydrogen bond. In fact, it was reported that the formation of the hydrogen bond causes a downfield shift on the carbonyl carbon of peptides in solution.[2-6] It is difficult to estimate exactly the hydrogen-bond effect on the ^{13}C chemical shift because the observed chemical shifts of peptides are often the averaged value for all the rotational isomers owing to an interconversion by rapid rotation about the bonds in solution.

On the other hand, chemical shifts in the solid state provide useful information about the hydrogen bond of peptides with a fixed conformation. From such a point of view, most recently, Ando et al. have studied the hydrogen-bonding effect on the ^{13}C chemical shifts of carbonyl carbons for peptides containing glycine (Gly) residues in the solid state.[7] They have reported that ^{13}C chemical shifts were determined for a series of oligopeptides containing glycine residues, for which the crystal structures were already available from X-ray diffraction studies. They found that the ^{13}C chemical shifts of the carbonyl carbons in the)C=O··H—N(type hydrogen bond move linearly downfield with a decrease of the hydrogen-bond length. Furthermore, the ^{13}C chemical shift behavior of the carbonyl carbon of polyglycine with form I (β-sheet) for form II (3_1-helix) could be reasonably explained in terms of the difference in their hydrogen-bond lengths. These experimental results were reasonably explained by using ^{13}C chemical shift calculations based on the quantum-chemical method.

It is quite sure that any peptides containing Gly resides are excellent models for any proteins. However, since proteins have other amino acid residues besides the glycine residue, of which the α-carbon is asymmetric, it is necessary to employ such amino acid residues to clarify generally the hydrogen-bonding effect on the ^{13}C chemical shift. According to such a situation, the main purpose of the present work is to measure the ^{13}C chemical shifts of L-alanine carbonyl carbons (L-Ala CO) in peptides containing L-Ala residues in the solid state. On the basis of the observed results, to discuss the relationship between the ^{13}C chemical shift and the hydrogen-bond length, we use some peptides containing ^{13}C labeled L-alanine residues of which the hydrogen-bond length and the conformation are determined from X-ray studies. These results will be compared with those of peptides containing Gly residues as reported previously. We employ peptides whose L-Ala CO in the amide group is involved in the)C=O···H-N(type hydrogen bonds (L-Ala CO carbon is not in the terminal carboxyl group). Further, in order to understand the origin of the relationship between the ^{13}C chemical shift and the strength of the hydrogen bond, a much more detailed knowledge of peptides is required. For this purpose, we attempt to calculate the ^{13}C shielding constants and tensor components of the L-alanine carbonyl carbons by employing the quantum chemical method.

Experimental Section

Materials. A series of oligopeptides containing ^{13}C labeled L-[1-^{13}C]Ala residues (isotopic purity ≅ 5%), except L-alanyl-L-prolylglycine monohydrate (Ala-Pro-Gly·H_2O) (purchased from Sigma), were synthesized and recrystallized according to the same procedures as those in the X-ray diffraction studies on them. A mixture of ^{13}C labeled L-[1-^{13}C]alanine (Merck Inc., isotope purity 99 atom %) and ^{13}C unlabeled L-alanine (Nihon-Rika Co.) was used to observe the accurate ^{13}C chemical shift of the L-Ala CO carbon in the ^{13}C NMR spectra.

L-[1-^{13}C]Alanylglycylglycine (Ala*-Gly-Gly·H_2O) and L-[1-^{13}C]alanyl-L-serine (Ala*-Ser) were synthesized according to a fragment condensation of *N*-hydroxysuccinimide esters and acids.[8] The N-terminal of the active ester was protected by the *o*-nitrophenylsulfenyl (Nps) group

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Table I. Observed ^{13}C Chemical Shifts of L-Alanine-Residue Carbonyl-Carbons for Oligopeptides Containing L-Alanine Residue in the Solid State As Determined by ^{13}C CP-MAS NMR and Their Geometrical Parameters

	^{13}C chemical shift (ppm)				geometrical parameters					
					hydrogen-bond length (Å)	hydrogen-bond angle (deg)	dihedral angle (deg)			
sample	δ_{iso}	δ_{11}	δ_{22}	δ_{33}	N···O	C=O···N	ϕ	ψ	ω	ref
Ac-Ala-NHMe	175.9	245	186	96	2.92	138.3	-84.3	159.0	173.3	16
	177.0	241	196	94	2.72	132.4	-87.6	154.8	171.9	
Ac-Ala-Aib-OMe	174.7				2.93	154.5	-134.0	122.2	-174.1	17
	171.7				2.97	152.2	-129.8	121.5	-179.5	
Ala-Gly-Gly·H_2O	172.6	245	179	93	3.00	150.2		160.0	172.4	18
Ala-Ser	170.1	249	172	89	3.04	156.3		124.8	-178.0	19
Ala-Pro-Gly·H_2O	169.3	235	178	95	3.157	155.2		153.2	177.0	20
poly(Ala)[a]	176.8	243	194	94	2.87		-57.4	-47.5	-179.8	21

[a] With the α-helix.

for avoiding the racemization in the course of the coupling with amino acids. *N*-Acetyl-*N'*-methyl-L-[1-^{13}C]alanine amide (Ac-Ala*-NHMe) and *N*-acetyl-L-alanyl-α-aminoisobutyric acid methyl ester (Ac-Ala-Aib-OMe) (not labeled) were prepared in pyridine by reacting anhydrous acetate with HCl·Ala*-NHMe and HCl·Ala-Aib-OMe, respectively. Poly(L-[1-^{13}C]alanine) ((L-Ala*)$_n$) was prepared by polymerization of [1-^{13}C]alanine-*N*-carboxy anhydride (Ala*-NCA). All the samples prepared in this study form)C=O···H—N(type hydrogen bonds in the crystalline state.

The obtained polycrystalline samples were ground by agate mortar before the NMR measurements were carried out to eliminate the orientation anisotropy of crystals in the spinning rotor.

^{13}C NMR Measurements. Solid state ^{13}C NMR measurements were performed on a JEOL GSX-270 spectrometer operating at 67.80 MHz equipped with a CP–MAS accessory. The field strength of the ^1H decoupling was 1.2 mT, contact time was 2 ms, repetition time was 5 s, and spectral width was 27.0 kHz. Data points were 8K. Samples were placed in a cylindrical rotor and spun as fast as 1.5–4.5 kHz. ^{13}C powder pattern spectra are often recorded in order to determine principal values of a ^{13}C chemical shift tensor, but we adopted the spinning-sideband analysis,[23,24] which allows a determination of these parameters from MAS NMR spectra in a more direct way and with a higher accuracy by using simultaneously all the information contained in the spinning sidebands. Spectra were usually accumulated 200–800 times to achieve a reasonable signal-to-noise ratio. The ^{13}C chemical shifts were calibrated indirectly through the adamantane peak observed at upperfield (29.5 ppm relative to tetrametylsilane ($(CH_3)_4Si$)).

Theoretical Calculation. We employed the finite perturbation theory (FPT)[9-12] within the INDO (intermediate neglect of differential overlap) framework for calculating the ^{13}C shielding constant. The FPT INDO theory has an advantage of permitting the calculation of the paramagnetic term without the explicit wave functions of excited states and the one-electron excitation energies, which are rarely obtained in high accuracy by the usual semiempirical MO approximations. This approach reproduces reasonably well the experimental ^{13}C chemical shifts of the L-alanine residue in peptides.[13] According to the FPT-INDO framework,[9-12] the ^{13}C shielding constant can be estimated by the sum of $\sigma^d_{\alpha\beta}(A)$ (diamagnetic term) and $\sigma^p_{\alpha\beta}(A)$ (paramagnetic term).

The ^{13}C shielding constant calculation was carried out by a similar procedure as reported previously.[13] However, the contributions from AOs centered on two different nuclei are not involved. In the calculation, we adopted *N*-acetyl-*N'*-methyl-L-alanine amide (having the same skeletal bonds as peptide) forming hydrogen bonds with two formamide molecules as a model supermolecule as will be shown below. The bond lengths and bond angles proposed by Momany et al.[14] were used. The calculation was performed as a function of hydrogen-bond length (the distance between nitrogen and oxygen atoms, abbreviated as $R_{N\cdots O}$) for the right-handed form α-helix, β-sheet, and any specified helix form (which is taken in Ac-Ala-NHMe) with the three dihedral angles, ($\phi = -57°$, $\psi = -47°$), ($\phi = -139°$, $\psi = 135°$), and ($\phi = -88°$, $\psi = 155°$), respectively.

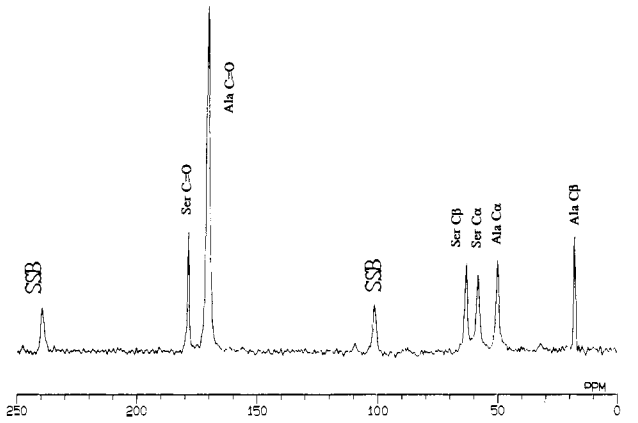

Figure 1. A typical 67.80 MHz ^{13}C CP–MAS NMR spectrum of L-[1-^{13}C]alanyl-L-serine in the solid state.

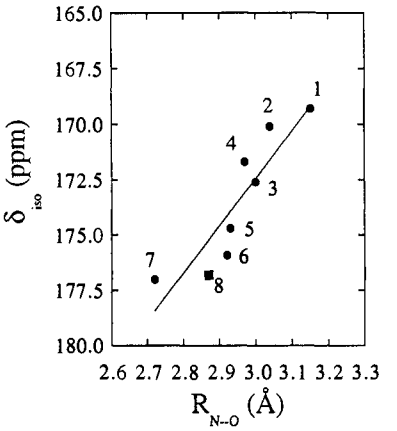

Figure 2. Plots of the observed ^{13}C chemical shifts in the solid state against the N···O hydrogen-bond length ($R_{N\cdots O}$): (1) Ala-Pro-Gly·H_2O, (2) Ala-Ser, (3) Ala-Gly-Gly·H_2O, (4, 5) Ac-Ala-Aib-OMe, (6, 7) Ac-Ala-NHMe, and (8) $(Ala)_n$.

A HITAC M660 computer at the Computer Center of the Tokyo Institute of Technology and a HITAC 280H computer at the Computer Center of the Institute for Molecular Science, Okazaki, were used for the calculation.

Results and Discussion

Isotropic ^{13}C Chemical Shifts of the L-Alanine Carbonyl Carbons in Peptides. A 67.80 MHz ^{13}C CP-MAS NMR spectrum of L-[1-^{13}C]alanylserine (Ala*-Ser) is shown as a typical example in Figure 1. ^{13}C CP–MAS spectra of the other remaining samples were also obtained with similar resolutions (figure not shown). The carbonyl carbon of the C-terminal carboxylic group appears downfield by several parts per million as a rather sharp peak relative to the internal amide carbonyl carbon.[15] The isotropic

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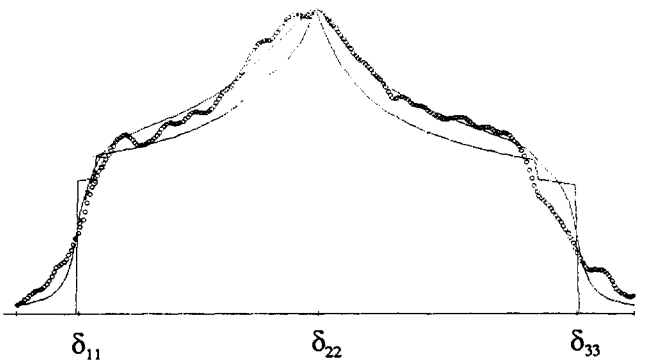

Figure 3. Schematic representation of experimental powder pattern and theoretical powder pattern convoluted with the Lorentzian function. The circles indicates the experimental data for Ala-Ser.

^{13}C chemical shifts of L-Ala CO carbons determined for all the peptides measured from ^{13}C CP-MAS are listed in Table I, together with the geometrical parameters obtained by X-ray diffraction studies. Some of the geometrical parameters were calculated by using the unit-cell parameters and fractional coordinates in the literature.

Figure 2 shows the plots of the observed isotropic ^{13}C chemical shifts (δ_{iso}) of L-Ala CO carbon against the N···O hydrogen-bond length ($R_{N\cdots O}$). It is found that a decrease of $R_{N\cdots O}$ leads to a downfield shift, and there exists approximately a linear relationship between the ^{13}C chemical shift and $R_{N\cdots O}$. It is noted that not only in oligopeptides (dimer or trimer) but also in polypeptides ((L-Ala)$_n$ with the α-helix form) the L-Ala CO carbon chemical shifts give a similar hydrogen-bond dependence. This suggests that the ^{13}C chemical shifts of any L-Ala CO taking the hydrogen bond which is formed between the amide >C=O and amide >N—H are predominantly determined by the length of the hydrogen bond. The expression for this relationship is

$$\delta_{iso} = 237.5 - 21.7R_{N\cdots O} \text{ (ppm)} \quad (1)$$

This relationship indicates that the hydrogen-bond length can be determined through the observation of ^{13}C chemical shift of the L-Ala CO carbon in peptides and polypeptides.

On the other hand, the relationship between the observed ^{13}C chemical shifts in the solid state of Gly CO and $R_{N\cdots O}$ has already determined by Ando et al.[7] as expressed by

$$\delta_{iso} = 210.0 - 12.4R_{N\cdots O} \text{ (ppm)} \quad (2)$$

As seen from these two relationships, the slope of the variation of the ^{13}C chemical shift of L-Ala CO carbon against the hydrogen-bond length is 1.7 times larger than that of Gly CO carbon. This shows the $R_{N\cdots O}$ dependence of the former is much larger than that of the latter.

Principal Values of ^{13}C Chemical Shift Tensor of the L-Alanine Carbonyl Carbon in Peptides. It is expected that the principal values of ^{13}C chemical shift tensors (δ_{11}, δ_{22}, and δ_{33}, from the downfield to upfield) are, in principle, more valuable as parameters for obtaining detailed information of hydrogen bond to be related with electronic structure compared with the isotropic ^{13}C chemical shift ($\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3$). A 67.80 MHz ^{13}C NMR powder pattern spectrum of L-alanyl-L-serine ([1-^{13}C, 5%]Ala-Ser) using the dipolar dephasing (DDph) pulse technique[22] is shown as a typical example in Figure 3. ^{13}C powder pattern spectra of the other remaining samples were also obtained with similar resolutions (figure not shown). As seen from these spectra, it is not usually easy to demonstrate exactly the principal values of the ^{13}C chemical shift tensor because of the overlap of powder patterns of several amino acid residues, which comes from the low concentration of ^{13}C-labeled L-Ala residue. For this, the spinning-sideband analysis[23,24] was adopted to determine the exact principal values of ^{13}C labeled L-Ala CO carbon.

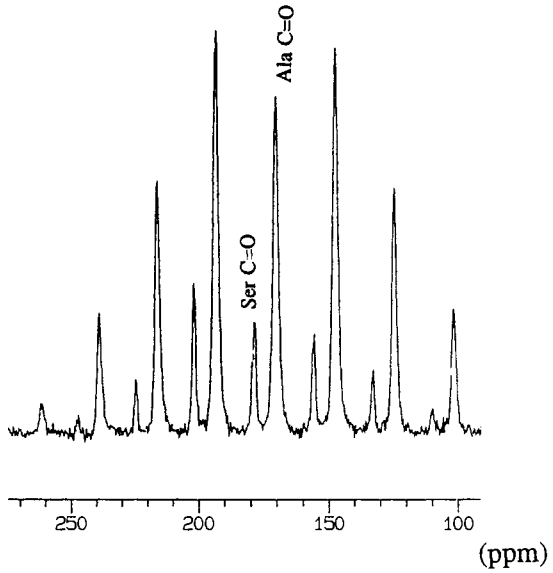

Figure 4. The 67.80 MHz ^{13}C CP-MAS NMR spectra of Ala-Ser in the solid state. The magic angle spinning rate is 1.55 kHz.

A 67.80 MHz ^{13}C CP-MAS spectrum of Ala-Ser with the spinning frequency at 1.55 kHz is shown in Figure 4. The above-mentioned spinning-sideband analysis was carried out to determine the principal values of the ^{13}C chemical shift tensor. The experimental errors of δ_{11}, δ_{22}, and δ_{33} are $<\pm 1.5$ ppm, $<\pm 0.7$ ppm, and $<\pm 1.5$ ppm, respectively.

The determined principal values of ^{13}C chemical shift tensors of peptides considered here are listed in Table I. The plots of δ_{11}, δ_{22}, and δ_{33} against the N···O hydrogen-bond length ($R_{N\cdots O}$) are shown in Figure 5, a–c, respectively. From this plot, it is found that the experimental δ_{22}s are the most sensitive to $R_{N\cdots O}$, and the δ_{22}s shift linearly downfield with a decrease of $R_{N\cdots O}$ except for the δ_{22} of Ala-Pro-Gly·H_2O. (As for Ala-Pro-Gly·H_2O, the covalent bond between the Ala and Pro residues does not form the peptide bond but the imide bond. For this, it is thought that the electronic structure of the L-Ala CO in Ala-Pro-Gly·H_2O is different from that of the L-Ala CO forming peptide bond, and, therefore, the L-Ala CO chemical shift is sensitive to both $R_{N\cdots O}$ and the nature of the bonds.) A decrease of $R_{N\cdots O}$ leads to a slight upfield shift on δ_{11} except for Ala-Pro-Gly·H_2O. The experimental δ_{33}s are almost independent of $R_{N\cdots O}$ with some scatter of the data. From the above results, it is demonstrated that the large downfield shift of the isotropic ^{13}C chemical shifts δ_{iso} with a decrease of $R_{N\cdots O}$ comes from the behavior of the δ_{22} in overcoming that of the δ_{11}.

^{13}C Shielding Constant Calculation. In Figure 7 (a–d) are shown the calculated isotropic shield constants (σ_{iso}) and their paramagnetic terms of tensor components (σ_{11}, σ_{22} and σ_{33}) of the L-Ala CO carbon of the model compounds (Figure 6) as a function of $R_{N\cdots O}$. The calculated values are all expressed in parts per million (ppm) with an opposite sign to that of the experimental. Note that the negative sign for the calculated shielding constant denotes deshielding, in contrast to the positive sign of the experimental chemical shift values. A shielding constant or tensor component is usually represented as a sum of the diamagnetic and the

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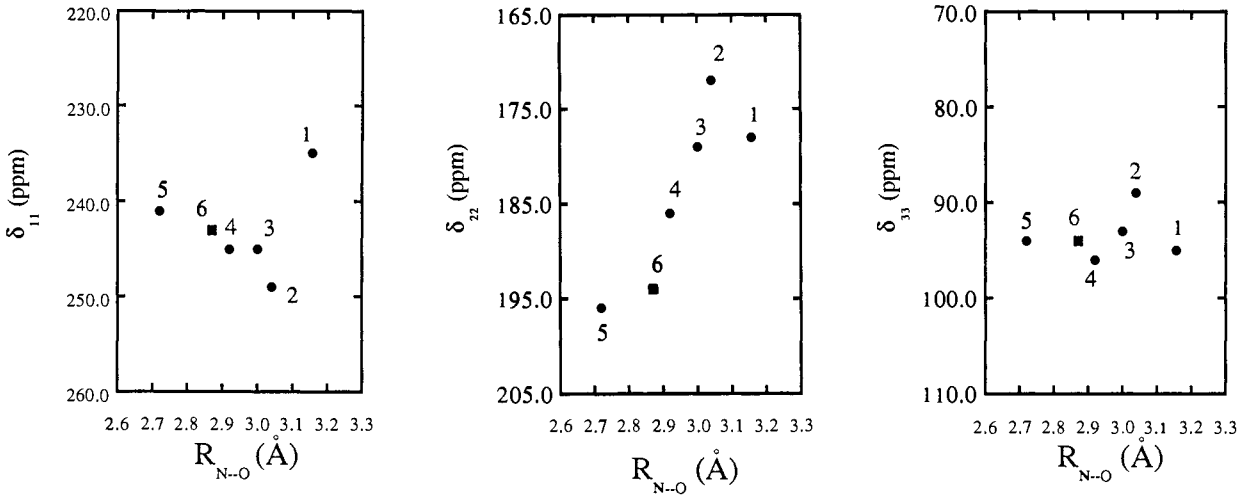

Figure 5. Plots of the observed principal values of ^{13}C chemical shift tensors for (a) δ_{11}, (b) δ_{22}, and (c) δ_{33} against the N···O hydrogen-bond length R_{N-O}: (1) Ala-Pro-Gly·H_2O, (2) Ala-Ser, (3) Ala-Gly-Gly·H_2O, (4, 5) Ac-Ala-NHMe, (6) $(Ala)_n$.

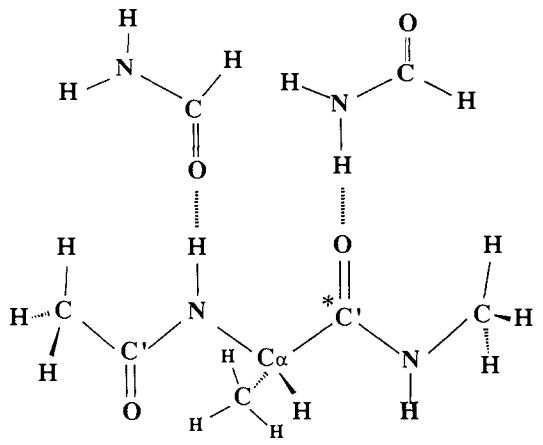

Figure 6. Molecular structure of *N*-acetyl-*N'*-methyl-L-alanine amide hydrogen-bonded with two formamide molecules. The calculation was made for the carbon marked by an asterisk.

paramagnetic terms. However, the relative change for the ^{13}C shielding tensors is predominantly governed by the paramagnetic term.

Figure 7a shows the R_{N-O} dependence of the calculated isotropic ^{13}C shielding constant (σ_{iso}) of the central L-Ala CO carbon of the model compound with the helix or β-sheet form. In the large R_{N-O} region, the values of σ_{iso} significantly depend on the conformational changes. On the other hand, in the short R_{N-O} region, R_{N-O} dominates the change in σ_{iso}, but the conformational differences are still there. Therefore, the experimental finding that the isotropic ^{13}C chemical shift moves linearly downfield with a decrease of R_{N-O} should be reasonably explained by the calculated results in the short R_{N-O} region. As the adopted semiempirical INDO MO approximation neglects some two-center electron integrals, the intermolecular interactions are considered to be reproduced reasonably in the short R_{N-O} region.[25] The variation of the total energy also supports this view as shown in Figure 8, where the total energy minimum appears around the R_{N-O} value of 2.3–2.5 Å.

As shown in Figure 7a, a decrease of R_{N-O} leads to a decrease of σ_{iso} in the short R_{N-O} region (R_{N-O} = 2.3–2.5 Å). In this region, the "hydrogen-bond length effect" on the ^{13}C chemical shift is much larger than the "conformation effect". These reasonably agree with the experimental results.

Parts b–d in Figure 7 show the R_{N-O} dependence of the calculated principal values of ^{13}C shielding tensor (σ_{111}, σ_{22}, and σ_{33}).

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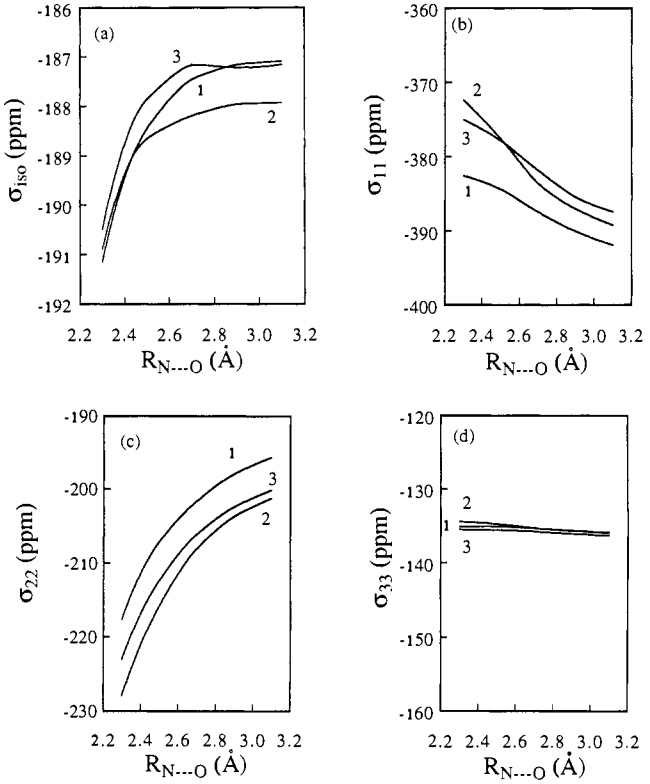

Figure 7. Variation of the calculated ^{13}C shielding constant and its tensor components with the N···O hydrogen-bond length R_{N-O} for (a) isotropic shielding constant σ_{iso}, (b) σ_{11}, (c) σ_{22}, and (d) σ_{33}: (1) α-helix, (2) β-sheet, and (3) helix (ϕ = −88°, ψ = 155°).

It is shown that a decrease of R_{N-O} leads to a decrease in the shielding of σ_{22} and an increase in that of σ_{11}. σ_{33} is not sensitive to a change of R_{N-O}. The magnitude of change for σ_{22} is much larger than that for σ_{11} and σ_{33}.

These results agree with the experimental ones. If we look at the calculation and experiments carefully, it can be said that the conformation effect on σ_{11} and σ_{22} in the calculation is relatively larger than the experimental results. The difference between the experiment and the calculation is probably due to the fact that the calculation was carried out under the assumption that the hydrogen-bonding angle (∠N···O═C) is 180°. In order to explain completely the experimental behavior, it seems that is is necessary to calculate the magnetic shielding by using a more accurate model which takes account of not only the conformation and the hydrogen-bond length, but also the hydrogen-bond angle.

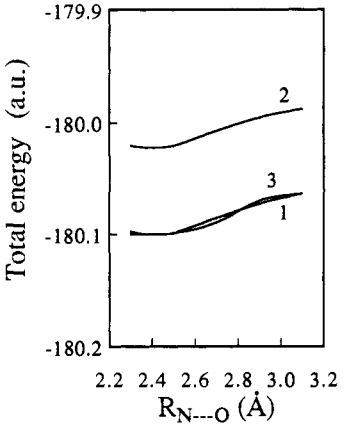

Figure 8. Variation of the calculated total energy against the N···O hydrogen-bond length $R_{N\cdots O}$ for *N*-acetyl-*N'*-methyl-L-alanine amide taking hydrogen bonds with two formamide molecules: (1) α-helix, (2) β-sheet, and (3) any specified helix ($\phi = -88°$, $\psi = 155°$).

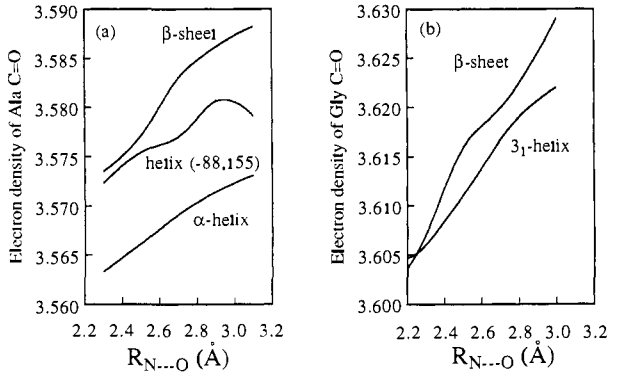

Figure 9. Variation of the calculated electron density against $R_{N\cdots O}$ for (a) L-Ala CO and (b) Gly CO carbon, respectively.

$R_{N\cdots O}$ Dependence of the Calculated Electron Densities on the Carbonyl Carbons of L-Ala and Gly. Figures 9, a and b, shows the $R_{N\cdots O}$ dependence of the calculated electron densities on the carbonyl carbons of L-Ala and Gly residues, respectively. The ^{13}C chemical shift is closely related to the electron density on the carbon atom. The smaller electron density on the carbon atom leads to the lower field shift. As shown in this figure, a decrease in $R_{N\cdots O}$ causes a decrease in the electron density. It, however, appears that the variation in the electron density on the L-Ala CO carbon is smaller than that on the Gly, and, on the other hand, the change of the experimental ^{13}C chemical shifts of L-Ala CO carbons is larger than that of the Gly. Thus, the above-mentioned discussion must be done by direct calculation of ^{13}C chemical shift.

Direction of the Principal Axes of the L-Ala CO Shielding Tensor. Figure 10, a–c, shows the direction of the principal axes of L-Ala CO ^{13}C shielding tensor components determined by theoretical calculations in this work, where the value of $R_{N\cdots O}$ of the model compounds considered here is 2.3 Å and the conformations are the α-helix ($\phi = -57°$, $\psi = -48°$), β-sheet ($\phi = -139°$, $\psi = 135°$) and any specified helix ($\phi = -88°$, $\psi = 155°$) forms, respectively. Figure 10d shows the direction of principal axes determined by the NMR study of [1-^{13}C]Ala[^{15}N]Ala powder sample.[26] The σ_{22} component nearly lies long the amide CO bond, and the σ_{11} is in the amide sp^2 plane and lies along the direction normal to the C=O bond. The σ_{33} component is aligned in the direction perpendicular to the amide sp^2 plane. This calculated direction of the principal axes of the L-Ala CO ^{13}C shielding tensor components does not conflict with previous experimental results.[26] Therefore, it is found that the σ_{22} value for the principal axis parallel to the C=O bond is the most sensitive to changes of $R_{N\cdots O}$.

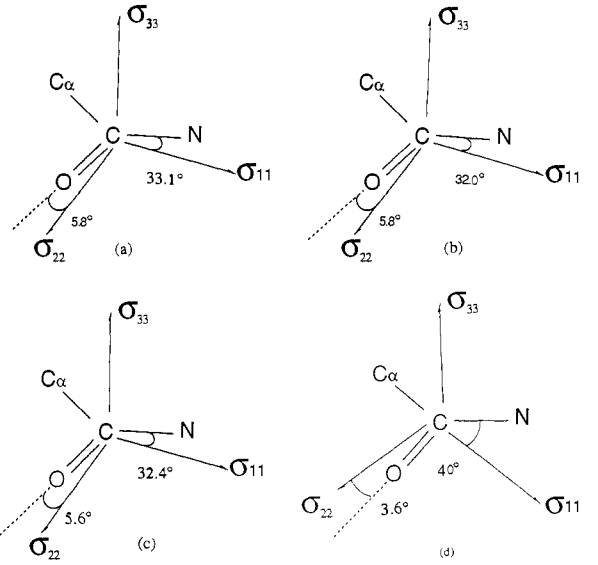

Figure 10. Orientation of the principal axes of the calculated and observed ^{13}C chemical shift tensors of L-alanine residue carbonyl carbon: (a) α-helix, (b) β-sheet, (c) any specified helix ($\phi = -88°$, $\psi = 155°$), and (d) [^{13}C]Ala[^{15}N]Ala (ref 26).

Conclusion

The observed isotropic ^{13}C chemical shift (δ_{iso}) of L-Ala CO in the amide-type hydrogen bond moves linearly downfield with a decrease of hydrogen-bond length. Such a downfield shift is predominantly governed by δ_{22}. δ_{11} moves somewhat upfield, and δ_{33} is not sensitive to changes in $R_{N\cdots O}$. These results could be justified by quantum chemical calculations. Further, calculations show that σ_{22} (corresponding to the observed δ_{22}) lies along the amide CO bond and is the most sensitive principal value of the ^{13}C carbonyl chemical shift tensor. The above results are of use in analyzing ^{13}C solid state NMR studies of polypeptides and proteins.

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