Hydrogen-bonded structure and \(^{13}\)C NMR chemical shift tensor of amino acid residue carbonyl carbons of peptides and polypeptides in the crystalline state. Part I

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Abstract

\(^{13}\)C chemical shift tensor components (\(\delta_{11}\), \(\delta_{22}\), and \(\delta_{33}\)) of glycine (Gly), L-valine (Val), L-leucine (Leu), and L-asparagine (Asp) residue carbonyl carbons (C=O) of peptides and polypeptides covering a wide range of hydrogen-bond lengths (\(R_{N\cdot O}\)) in the crystalline state have been measured by slow magic-angle-spinning solid-state \(^{13}\)C NMR. From these experiments, it is found that \(\delta_{22}\), which lies approximately along the amide C=O bond, moves linearly downfield with a decrease in \(R_{N\cdot O}\) and the slope and intercept of the variation of \(\delta_{22}\) against \(R_{N\cdot O}\) depend on the amino acid residue. Using this relationship, the \(R_{N\cdot O}\) values for polypeptides were determined by observation of the \(\delta_{22}\) of the guest Gly residue incorporated into host polypeptides. \(\delta_{11}\) and \(\delta_{33}\) are found to be insensitive to the change in \(R_{N\cdot O}\) and amino acid residues. Moreover, it is found that the sum of \(\delta_{11}\) and \(\delta_{33}\) is almost constant (357.5 \pm 3.5 ppm) and is independent of the amino acid residue. The quantum-chemical calculation on the \(^{13}\)C shielding constant for a peptide model compound was carried out by the finite perturbation theory within the INDO framework. This calculation acceptably explains the experimental results.

Keywords Hydrogen bonding, Solid state NMR spectroscopy, Chemical shift tensor, Peptides, Polypeptides

1. Introduction

As the > C = O H–N < (amide–amide) type of hydrogen bond plays an important role in forming the secondary structure for solid polypeptides and proteins [1], many extensive investigations have been carried out using various spectroscopic methods such as IR, UV, X-ray diffraction, etc [1–3]. These approaches have elucidated the nature of hydrogen bonds in polypeptides and proteins.

In our previous works on the intermolecular ‘‘amide–amide’’ type of hydrogen-bonding effect on the \(^{13}\)C NMR chemical shifts of the carbonyl carbons (C=O) of glycine (Gly) [4], L-alanine (Ala) [5], L-valine (Val) [6], L-leucine (Leu) [6], and L-asparagine (Asp) [6] peptide residues in the crystalline state, we have demonstrated that the isotropic \(^{13}\)C chemical shifts (\(\delta_{N\cdot O}\)) of Gly, Ala, Val, Leu, and Asp residues move linearly downfield with a decrease in hydrogen-bond length (\(R_{N\cdot O}\)) between nitrogen and
oxygen atoms in an amide-type hydrogen bond as expressed in ppm by

\[ \delta_{\text{i,e}}(\text{Gly}) = 206.0 - 12.4 \times R_{N\text{O}} \]  
\[ \delta_{\text{i,e}}(\text{Ala}) = 237.5 - 21.7 \times R_{N\text{O}} \]  
\[ \delta_{\text{i,e}}(\text{Leu}) = 202.2 - 10.0 \times R_{N\text{O}} \]  
\[ \delta_{\text{i,e}}(\text{Val}) = 215.4 - 14.2 \times R_{N\text{O}} \]  
\[ \delta_{\text{i,e}}(\text{Asp}) = 199.0 - 9.6 \times R_{N\text{O}} \]

where \( R_{N\text{O}} \) is expressed in Å. Using these expressions, we can determine the \( R_{N\text{O}} \) of some polypeptides in the crystalline state through the observed amide C = O chemical shifts. This approach provides a much simpler method to determine the \( R_{N\text{O}} \) compared with others such as X-ray diffraction and distance-geometry methods using solution NMR, and may be used to determine the hydrogen-bond lengths in both crystalline and non-crystalline regions.

In principle, the chemical shift for static samples is expressed as a second rank tensor. Thus, the chemical shift tensor components \( (\delta_{11}, \delta_{22}, \text{and} \delta_{33}) \) have potentially more detailed structural information compared with the isotropic chemical shift which is the average of the tensor components [7]. This is due to tensor components being closely associated with the electronic state. In our previous work, it has been reported that the principal values of the \( ^{13}\text{C} \) chemical shift tensor of \([L,^{13}\text{C}]\text{glycine-containing polypeptides depend on the secondary structure [8]}\) and that the \( \delta_{22} \) of the amide C = O tensor components for Ala

<table>
<thead>
<tr>
<th>Sample a</th>
<th>Carbonyl (^{13}\text{C} ) chemical shift (ppm)</th>
<th>Hydrogen-bond length (Å) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly residue</td>
<td>( \delta_{\text{iso}} \ \delta_{11} \ \delta_{22} \ \delta_{33} \ \delta_{11+33} )</td>
<td>( R_{N\text{O}} ) c</td>
</tr>
<tr>
<td>Gly'-Gly</td>
<td>168.1 242 174 88 331</td>
<td>2.97 [16]</td>
</tr>
<tr>
<td>Cl Ac-Gly'-Gly</td>
<td>170.1 244 176 91 335</td>
<td>2.82 [17]</td>
</tr>
<tr>
<td>Ala-Gly'-Gly</td>
<td>170.6 240 177 94 334</td>
<td>2.93 [18]</td>
</tr>
<tr>
<td>Val-Gly'-Gly</td>
<td>169.2 245 170 93 338</td>
<td>3.05 [19]</td>
</tr>
<tr>
<td>Gly'-Gly HNO3</td>
<td>168.3 248 168 89 337</td>
<td>3.12 [20]</td>
</tr>
<tr>
<td>Poly(Gly) β-sheet</td>
<td>169.6 245 173 91 336</td>
<td>2.91 [21]</td>
</tr>
<tr>
<td>Poly(Gly) α-helix</td>
<td>173.2 247 182 91 338</td>
<td>2.73 [22]</td>
</tr>
<tr>
<td>Poly(Ala,Gly') α-helix</td>
<td>173.0 243 181 95 338</td>
<td>– –</td>
</tr>
<tr>
<td>Poly(Ala,Gly') β-sheet</td>
<td>168.8 241 171 95 336</td>
<td>– –</td>
</tr>
<tr>
<td>Poly(Leu,Gly') α-helix</td>
<td>172.8 241 180 97 338</td>
<td>– –</td>
</tr>
<tr>
<td>Poly(Val,Gly') β-sheet</td>
<td>169.6 240 169 99 340</td>
<td>– –</td>
</tr>
<tr>
<td>Val residue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val'-Gly-Gly</td>
<td>169.2 245 170 93 338</td>
<td>3.05 [19]</td>
</tr>
<tr>
<td>Leu residue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boc-Pro-Ile'-Gly</td>
<td>173.0 249 183 88 336</td>
<td>2.83 [23]</td>
</tr>
<tr>
<td>DL-Leu'-Gly-Gly</td>
<td>172.0 246 178 92 338</td>
<td>3.06 [24]</td>
</tr>
<tr>
<td>Asp residue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp-Gly</td>
<td>170.3 242 175 93 336</td>
<td>2.98 [25]</td>
</tr>
<tr>
<td>Ala residue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ac-Ala'-NHMe</td>
<td>177.0 241 196 94 335</td>
<td>2.72 [26]</td>
</tr>
<tr>
<td>Poly(Ala) α-helix</td>
<td>175.9 245 189 96 341</td>
<td>2.92</td>
</tr>
<tr>
<td>Ala'-Gly-Gly</td>
<td>172.6 245 179 93 338</td>
<td>3.00 [18]</td>
</tr>
<tr>
<td>Ala'-Ser</td>
<td>170.1 249 172 89 338</td>
<td>3.04 [28]</td>
</tr>
</tbody>
</table>

a Amide carbonyl \(^{13}\text{C} \) chemical shifts for the asterisk-marked amino acid residues were measured.
b Determined by X-ray diffraction.
c Amide-amide hydrogen-bond length (Å) between nitrogen and oxygen atoms in a hydrogen bond.
residues in oligopeptides and polypeptides, which lies along to the C=O bond, is very sensitive to change in $R_{N_0}$ [5]. In addition to such investigations, in the present work we aim to study systematically the hydrogen-bonding effects on the principal values of the $^{13}$C chemical shift tensor for Gly, Val, Leu, and Asp residue carbonyl carbons of peptides and polypeptides in the crystalline state and to elucidate the relationship between the tensor components and hydrogen bond length. By further manipulation of the experimental results, the $^{13}$C shielding constant of the amino acid residue carbonyl carbon of a peptide model compound was calculated by the finite perturbation theory (FPT) [9–12] within the INDO framework.

2. Experimental section

2.1 Materials

A series of peptides containing Gly, Val, Leu, and Asp residues, for which the crystal structures had previously been determined by X-ray diffraction, were used as shown in Table 1: Glycine, alanine, valine, leucine, and asparagine residues in oligopeptides are abbreviated as Gly, l-Ala, l-Val, l-Leu, and l-Asp, respectively. Polyglycine, poly(l-alanine), poly(l-valine), poly(l-leucine), and poly(l-asparagine) are abbreviated as poly(Gly), poly(Ala), poly(Val), poly(Leu), and poly(Asp), respectively. Poly(Ala, Gly*) indicates poly(Ala) containing glycine residues with 90% $^{13}$C-labeled carbonyl carbons as a minor component (5%). Poly(Val, Gly*) and poly(Leu, Gly*) are poly(Val) and poly(Leu) containing $^{13}$C-labeled glycine residue with 90% $^{13}$C-labeled carbonyl carbon as a minor component (8%), respectively. These samples were prepared in a previous work [8]. For converting poly(Ala) from α-helix form to β-sheet form, a poly(Ala, Gly*) film cast from dichloroacetic acid solution was uniaxially drawn.

2.2 NMR measurement

$^{13}$C cross polarization–magic angle spinning (CP–MAS) NMR spectra were recorded at room temperature using a JEOL GSX-270 NMR spectrometer at 67.8 MHz with a CP–MAS accessory. Samples were contained in a cylindrical rotor and spun at 1530–1750 Hz. $^{13}$C chemical shifts were calibrated indirectly through the adamantane peak observed at the upper field (29.5 ppm relative to tetramethysilane [(CH$_3$)$_4$Si]). The $^{13}$C chemical shift tensor components were determined from the slow MAS sideband intensities by the Herzfeld and Berger method [13].

2.3 Theoretical calculation

The FPT within the INDO framework was used for calculating the $^{13}$C shielding constants. This approach reproduces reasonably the experimental $^{13}$C chemical shifts of some amino acid residues in peptides [4–6,14]. A shielding constant or tensor component is usually represented as the sum of the diamagnetic and paramagnetic terms. However, the $R_{N_0}$ dependence of the shielding tensor can be explained by the paramagnetic term, since the diamagnetic term is almost isotropic. In the $^{13}$C shielding constant calculation, we adopted N-acetyl-N'-methyl-$R$ amide (R = Gly, Ala, Val, Leu or Asp) as a peptide model, hydrogen-bonded with two formamide molecules as shown in Fig. 1. The bond lengths and bond angles proposed by Momany et al [15] were used. This peptide model is the same as reported previously [4–6]. The calculations were performed as a function of hydrogen-bond length for a typical β-sheet form.
poly(Gly) with a 3_1-helix form at the spinning rate of 1750 Hz is shown in Fig. 2. The other remaining samples gave similar sideband spectra. The determined \( \delta_{11}, \delta_{22}, \) and \( \delta_{33} \) components of the poly(Gly) with the 3_1-helix form are 247, 182, and 91 ppm, respectively. The experimental errors of \( \delta_{11}, \delta_{22}, \) and \( \delta_{33} \) are \( < \pm 1.7 \) ppm, \( < \pm 0.7 \) ppm, and \( < \pm 1.7 \) ppm, respectively. The tensor components of the other samples were determined in the same manner.

The determined isotropic \( ^{13} \text{C} \) chemical shifts and tensor components for Gly, Val, Leu, and Asp residue carbonyl carbons in oligopeptides are listed in Table 1, together with the \( R_{NO} \) values determined by X-ray diffraction, in addition to those of the Ala residue as reported previously by Asakawa et al. [5]. It has been reported that the \( \delta_{11} \) is in the amide sp\(^2\) plane and lies along the direction normal to the C = O bond, the \( \delta_{22} \) component lies almost along the amide C = O bond, and the \( \delta_{33} \) component is aligned in the direction perpendicular to the amide sp\(^2\) plane [29].

Fig. 3 shows the plots of the \( ^{13} \text{C} \) chemical shift tensor components (\( \delta_{11}, \delta_{22}, \) and \( \delta_{33} \)) against \( R_{NO} \) for Gly, Ala, Val, Leu, and Asp residues. The \( \delta_{22} \) for Gly, Leu, and Ala residues moves linearly downfield with a decrease in \( R_{NO} \) [5]. The slope and intercept of the variation of the \( \delta_{22} \) against \( R_{NO} \) vary depending on the amino acid residue. The slope of the variation of \( \delta_{22} \) against the \( R_{NO} \) for Ala residues in peptides is larger compared with the other amino acid residues. This shows that the \( \delta_{22} \) for Ala residue is the most

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Fig 2: A 67.8 MHz \( ^{13} \text{C} \) CP–MAS NMR spectrum of polyglycine with a 3_1-helix form in the solid state. The magic angle spinning rate is 1.75 kHz with the dihedral angle (\( \phi = -149.9^\circ, \psi = 146.5^\circ \)). A Sun SPARC station 2 was used for the calculation.

3. Results and discussion

3.1 Correlation between the hydrogen-bond length and \( ^{13} \text{C} \) chemical shift tensor components of Gly, Val, Leu, and Asp residue carbonyl carbons

A typical 67.8 MHz \( ^{13} \text{C} \) CP–MAS spectrum of polyglycine with a 3_1-helix form at the spinning rate of 1750 Hz is shown in Fig. 2. The other remaining samples gave similar sideband spectra. The determined \( \delta_{11}, \delta_{22}, \) and \( \delta_{33} \) components of the poly(Gly) with the 3_1-helix form are 247, 182, and 91 ppm, respectively. The experimental errors of \( \delta_{11}, \delta_{22}, \) and \( \delta_{33} \) are \( < \pm 1.7 \) ppm, \( < \pm 0.7 \) ppm, and \( < \pm 1.7 \) ppm, respectively. The tensor components of the other samples were determined in the same manner.

The determined isotropic \( ^{13} \text{C} \) chemical shifts and tensor components for Gly, Val, Leu, and Asp residue carbonyl carbons in oligopeptides are listed in Table 1, together with the \( R_{NO} \) values determined by X-ray diffraction, in addition to those of the Ala residue as reported previously by Asakawa et al. [5]. It has been reported that the \( \delta_{11} \) is in the amide sp\(^2\) plane and lies along the direction normal to the C = O bond, the \( \delta_{22} \) component lies almost along the amide C = O bond, and the \( \delta_{33} \) component is aligned in the direction perpendicular to the amide sp\(^2\) plane [29].

Fig. 3 shows the plots of the \( ^{13} \text{C} \) chemical shift tensor components (\( \delta_{11}, \delta_{22}, \) and \( \delta_{33} \)) against \( R_{NO} \) for Gly, Ala, Val, Leu, and Asp residues. The \( \delta_{22} \) for Gly, Leu, and Ala residues moves linearly downfield with a decrease in \( R_{NO} \) [5]. The slope and intercept of the variation of the \( \delta_{22} \) against \( R_{NO} \) vary depending on the amino acid residue. The slope of the variation of \( \delta_{22} \) against the \( R_{NO} \) for Ala residues in peptides is larger compared with the other amino acid residues. This shows that the \( \delta_{22} \) for Ala residue is the most

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Fig 3: Plots of the observed \( ^{13} \text{C} \) chemical shift tensor components for \( \delta_{11} \) (a), \( \delta_{22} \) (b) and \( \delta_{33} \) (c) for the amide carbonyl carbon in Gly (●), Ala (■), Val (○), Leu (△) and Asp (□) residues in peptides against the N–O hydrogen-bond length (\( R_{NO} \)). The experimental errors of \( \delta_{11} \) and \( \delta_{33} \) are indicated by an error bar.
sensitive to $R_{N\ O}$ compared with the other amino acid residues. The expression for the relationship as determined by the least-squares method for the oligopeptides containing Gly residue is expressed as follows:

$$\delta_{22} = 262.9 - 30.2R_{N\ O} \quad \text{(correlation coefficient} = 0.89)$$

(2)

where $\delta_{22}$ and $R_{N\ O}$ are in ppm and Å, respectively. This relationship indicates that $R_{N\ O}$ can be determined through the observation of the $^{13}$C chemical shift tensor of $\delta_{22}$ for the carbonyl carbon of the Gly residue in oligopeptides within an error of $\pm 0.7$ ppm. It should be noted that the Gly C = O carbon chemical shifts for poly(Gly) with β-sheet form and $3_1$-helix form are located on a straight line as expressed by eqn (2). This shows that the relationship can be applied to oligopeptides.

Next, we are concerned with $\delta_{11}$ and $\delta_{33}$. As seen from Fig. 3, the experimental data for $\delta_{11}$ and $\delta_{33}$ of Gly, Ala, and Leu residues scatter largely. The $\delta_{11}$ and $\delta_{33}$ are insensitive to changes in $R_{N\ O}$, but it seems that $\delta_{11}$ and $\delta_{33}$ slightly move upfield and downfield, respectively, with a decrease in $R_{N\ O}$. In order to clarify the relation between $\delta_{11}$ and $\delta_{33}$, $\delta_{11}$ was plotted against $\delta_{33}$ as shown in Fig. 4. The slope of the linear relationship between $\delta_{11}$ and $\delta_{33}$ values is about $-1$. When $\delta_{11}$ moves downfield, $\delta_{33}$ moves upfield by the same magnitude. Therefore, the sum of $\delta_{11}$ and $\delta_{33}$ is almost constant (337.5 ± 3.5 ppm) and is independent of the amino acid residue, except for GlyGly (the reason is unclear). This leads to the experimental finding that there is a linear relationship between $\delta_{330}$ and $\delta_{22}$ as expressed by

$$\delta_{330} = (1/3)\delta_{22} + 112.5 \text{ ppm (relative to TMS)} \quad \text{(3)}$$

Therefore, it can be said that the large downfield shift in $\delta_{330}$ with a decrease in $R_{N\ O}$ is predominantly governed by the decrease in $\delta_{22}$. Similar relationships have been reported by Oas et al. for peptides containing Gly amino acid residue [30].

3.2 Application of Eqs (1) and (2) to the determination of the hydrogen-bond length in Gly-containing oligopeptides

Here, we are concerned with the application of Eqs (1a)–(1e) and Eq (2) to the determination of the $R_{N\ O}$ value for the guest Gly residue incorporated into host copolypeptides. As reported previously, it was successfully identified from the reference data of poly(Ala) with the $\alpha$-helix form that the Ala residue in poly(Ala, Gly) takes the $\alpha$-helix form [31–33]. However, we still have a problem of whether the guest Gly residue incorporated into host polypeptides takes the same conformation as the host amino acid residues or not. The $R_{N\ O}$ values of the guest Gly residue in some host polypeptides determined using Eq (2) by the observation of the $\delta_{22}$ of Gly residue are listed in Table 2, together with the $R_{N\ O}$ values determined using Eqs (1a)–(1e) through the observation of $\delta_{330}$ as reported by Tsuchiya et al. [6].

The $^{13}$C chemical shift tensor component $\delta_{22}$ for Gly residue leads to the result that the hydrogen-bond lengths of the guest Gly residue ($R_{N\ O}$) in poly(Leu, Gly') and poly(Ala, Gly') of which the host Leu and Ala residues take the $\alpha$-helix form are 2.7 Å as estimated by using Eq (2). This value is in agreement with the hydrogen-bond lengths of 2.7 and 2.8 Å for the host Leu and Ala residues ($R_{N\ O}$ and $R_{N'}\ O$) respectively, determined using Eqs (1c) and (1b) through the observation of the $\delta_{330}$ for Leu and Ala residues in homo-poly(Leu) and poly(Ala) with an $\alpha$-helix form, respectively. Moreover, the $R_{N\ O}$ values of the guest Gly residue in poly(Leu, Gly') and poly(Ala, Gly') are very close to the hydrogen-bond length of 2.8 Å for the guest Gly residue ($R_{N\ O}$) determined using Eq (1a) through the observation of the $\delta_{330}$ for Gly residue in
Table 2
Hydrogen-bond lengths for some polypeptides and copolypeptides incorporated with Gly residues determined using Eqs (1a)–(1e) and Eq (2) through the observation of the $^{13}$C chemical shift tensor component $\delta_{22}$ and isotropic chemical shift $\sigma_{0}$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conformation</th>
<th>Hydrogen bond length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (Leu)</td>
<td>$\alpha$-helix</td>
<td>–</td>
</tr>
<tr>
<td>Poly (Leu, Gly')</td>
<td>$\alpha$-helix</td>
<td>2.7, 2.8</td>
</tr>
<tr>
<td>Poly (Val)</td>
<td>$\beta$-sheet</td>
<td>–</td>
</tr>
<tr>
<td>Poly (Val, Gly')</td>
<td>$\beta$-sheet</td>
<td>3.0, 3.0</td>
</tr>
<tr>
<td>Poly (Ala)</td>
<td>$\alpha$-helix</td>
<td>–</td>
</tr>
<tr>
<td>Poly (Ala, Gly')</td>
<td>$\alpha$-helix</td>
<td>2.7, 2.8</td>
</tr>
<tr>
<td>Poly (Ala)</td>
<td>$\beta$-sheet</td>
<td>–</td>
</tr>
<tr>
<td>Poly (Ala, Gly')</td>
<td>$\beta$-sheet</td>
<td>3.0, 3.1</td>
</tr>
</tbody>
</table>

- $^a$ Determined using Eq (2) and the $^{13}$C chemical shift tensor component $\delta_{22}$ for the Gly residue in copolypeptides
- $^b$ Determined using Eq (1a) and the isotropic $^{13}$C chemical shift for the Gly residue
- $^c$ Determined using Eq (1c) and the isotropic $^{13}$C chemical shift for the Leu residue
- $^d$ Determined using Eq (1d) and the isotropic $^{13}$C chemical shift for the Val residue
- $^e$ Determined using Eq (1b) and the isotropic $^{13}$C chemical shift for the Ala residue

4. Shielding constant calculations on a hydrogen-bonded peptide model compound

We have carried out FPT–INDO calculations on $^{13}$C shielding tensors of some model peptides in order to understand the obtained relationships between the tensor components and hydrogen-bonded length. Fig 5 shows the $R_{N\ O}$ dependence of the calculated nuclear shielding constants for $\sigma_{11}$, $\sigma_{22}$, and $\sigma_{33}$ of Gly, Ala, Val, Leu, and Asp C = O carbons. The negative sign for the calculated shielding constant indicates that the guest Gly residue is completely incorporated into host polypeptides with an $\alpha$-helix form. In the case of poly(Val, Gly') and poly(Ala, Gly') with the $\beta$-sheet form, similar results were obtained. Therefore, it can be concluded that the guest Gly residue is completely incorporated into the host polypeptides with a $\beta$-sheet form. These results indicate that the hydrogen-bond length for host residue in copolypeptides and proteins can be determined through the observation of $\delta_{22}$ of Gly residue in addition to the $\delta_{iso}$.
denotes deshielding and the positive sign of the observed chemical shift value deshielding. It is known that with the adopted semiempirical INDO MO approximation, the intermolecular interactions are reproduced in the short $R_{N_0}$ region [34]. In the calculation, the total energy minimum appears around the $R_{N_0}$ value of 2.3–2.5 Å. For this reason, the experiment was compared with the calculation in the short $R_{N_0}$ region $\sigma_{22}$ is the most sensitive to the change of $R_{N_0}$ and moves linearly downfield with a decrease in $R_{N_0}$. $\sigma_{33}$ moves somewhat upfield with a decrease of $R_{N_0}$. whereas $\sigma_{35}$ is insensitive to change of $R_{N_0}$. The results of the theoretical calculation agree well with the experimental results. Such an agreement between the calculated and the experimental results indicates that the $^{13}$C chemical shift changes predominately originate from the change of the electronic state of amino carbonyl groups caused by the hydrogen-bond length variation. Further, it can be said that the amino-acid residue dependence of the calculated tensor components is similar to the experimental one.

In conclusion, the experimental $^{13}$C chemical shift tensor component $\delta_{22}$ of Gly, Ala, Val, Leu, and Asp residues carbonyl carbons in the amide-type hydrogen bond move linearly downfield with a decrease of hydrogen-bond length and the slope and intercept of the variation of $\delta_{22}$ against $R_{N_0}$ depend on the amino acid residue. The sum of $\delta_{11}$ and $\delta_{33}$ is almost constant (337.5 ± 3.5 ppm) irrespective of the amino acid residues. The $R_{N_0}$ for a guest Gly residue in host polypeptides can be determined by the observation of $\delta_{22}$ of the guest Gly residue incorporated into host polypeptides. The theoretical calculation qualitatively explains the experimental results.

References

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