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HYDROGEN BOND LENGTH AND ^{15}N NMR CHEMICAL SHIFT OF THE GLYCINE RESIDUE OF SOME OLIGOPEPTIDES IN THE SOLID STATE

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ABSTRACT

CP–MAS and CP–static ^{15}N NMR spectra were measured for a variety of solid oligopeptides containing the glycine residue, the crystal structures of which had already been determined by X-ray diffraction From the results of the observed ^{15}N chemical shifts, it was found that the isotropic ^{15}N chemical shifts (σ_{iso}) of the glycine residues move downfield with a decrease of hydrogen bond length ($R_{N\ O}$) between the nitrogen and oxygen atoms in the amide groups, and that the principal value of σ_{33} moves linearly downfield with a decrease of $R_{N\ O}$ There is no relationship between the principal value of σ_{11} or σ_{22} and $R_{N\ O}$ This indicates that such a linear downfield shift of σ_{33} contributes predominantly to the downfield shift of σ_{iso} Quantum chemical calculations of the ^{15}N shielding constant for the model compounds were carried out by the FPT–INDO method, and the relationship between ^{15}N chemical shift and $R_{N\ O}$ discussed

INTRODUCTION

^{15}N NMR spectroscopy has been demonstrated to provide useful information about structure and dynamics of synthetic polypeptides and natural proteins in solution [1–6]. It has been reported that the ^{15}N nucleus is highly sensitive to structural and conformational changes of the molecules. Most recently, high-resolution solid-state ^{15}N NMR has been developed by means of the cross polarization–magic angle spinning (CP–MAS) method, and has been applied to the conformational characterization of polypeptides and oligopeptides [7]. It has been demonstrated that the ^{15}N chemical shifts in the peptide backbone of a variety of polypeptides exhibit a significant conformation-dependent change [8–10], the α_R-helix form (97.0–99.2 ppm) appearing more upfield than the β-sheet form (99.5–107.0 ppm).

However, if the exact principal values of ^{15}N chemical shift tensors (σ_{11}, σ_{22} and σ_{33}) are available, this provides more detailed information about the struc-

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ture of polypeptides and oligopeptides, which are closely associated with electronic structure, compared with the isotropic chemical shift value ($\sigma_{iso}=(\sigma_{11}+\sigma_{22}+\sigma_{33})/3$) which is determined by MAS. Because a nitrogen atom possesses lone-pair electrons, it is of interest to examine the effects of these electrons on the isotropic ^{15}N chemical shift (σ_{iso}) and the principal values of the ^{15}N chemical shift tensors (σ_{11}, σ_{22} and σ_{33}). Further, the direction of the principal axes of the glycine residue ^{15}N shielding tensor components has been experimentally determined by some investigators [11].

Our previous work [7] reported that in a variety of solid oligopeptides (X–GlyGly, where Gly is glycine residue and X is some other amino acid residue) the decrease of the N–H bond length in the $>C=O\cdots H-N<$ type hydrogen bond for the C-terminal Gly residue leads to a linear increase in ^{15}N shielding, but there is no clear relationship between the ^{15}N chemical shifts and $N\cdots O$ separation. The absence of any such relationship may be caused by some effect of the other amino acid (X). For this reason, when we study details of the hydrogen bond through the ^{15}N chemical shift, we must choose oligopeptides without any effect from X on the ^{15}N chemical shift of the Gly residues. If oligopeptides with a *tert*-butyloxycarbonyl (Boc) group at the terminus are chosen, we can expect that they will not have any effect from X on the ^{15}N chemical shift of Gly residue. (The Boc group is sometimes used as the terminal group in oligopeptides.)

As a continuation of our ^{15}N NMR study on the hydrogen bond in oligopeptides, in this work we attempt to measure isotropic ^{15}N chemical shifts and individual components of the ^{15}N chemical shift tensors of the glycine residue (Gly) in a variety of oligopeptides with a terminal Boc group, and to clarify the relationship between hydrogen bond length and isotropic chemical shifts and individual components of chemical shift tensors. The results obtained will be discussed in comparison with our previous NMR studies about hydrogen bonds. Further, to obtain a deep insight into the hydrogen bond, we attempt to calculate the ^{15}N chemical shift and tensor components of the glycine amide nitrogen atoms by employing quantum chemical methods.

EXPERIMENTAL

Materials

A series of oligopeptides containing the ^{15}N-labeled glycine residue (a ^{15}N purity of about 10%) was prepared by condensing of Boc-glycine *N*-hydroxysuccinimide-activated esters with amino acids [12]. The *N*-terminals of all the peptides considered here were protected by a *tert*-butyloxycarbonyl (Boc) group using Boc-S-(*tert*-butyl)-S-(4,6-dimethylpyrimidin-2-ylthiocarbonate). A mixture of ^{15}N labeled glycine (Merck Inc., isotope purity 99atom%) and glycine (Nihon-Rika Co.) was used to obtain remarkably intense signals in the

^{15}N NMR spectra. The samples obtained were slowly recrystallized from ethyl acetate solution according to the procedure used on X-ray diffraction studies [13–17]

^{15}N NMR measurements

^{15}N CP–MAS NMR spectra were recorded at room temperature with a JEOL GSX-270 spectrometer operating at 27.25 MHz equipped with a CP–MAS accessory. The field strength of ^{1}H decoupling was 1.2 mT. The contact time was 5 ms, and repetition time 10 s. Spectral width and data points were 10 kHz and 8 k, respectively. Samples were placed in a cylindrical rotor and spun as fast as 4–5 kHz. Powder pattern spectra were recorded with the same instrument but without magic angle spinning. Spectra were usually accumulated 100–2000 times to achieve a reasonable signal-to-noise ratio for the ^{15}N chemical shifts. ^{15}N chemical shifts were calibrated indirectly through external glycine-^{15}N (σ=11.59 ppm; line width=17 Hz), relative to saturated $^{15}NH_4NO_3$ solution (σ=0 ppm) in H_2O.

To obtain the three principal components of the shielding tensors (σ_{11}, σ_{22}, and σ_{33}, from downfield to upfield), a fitting was carried out by superimposing the theoretical powder pattern line shape with a Lorentzian function (sym-

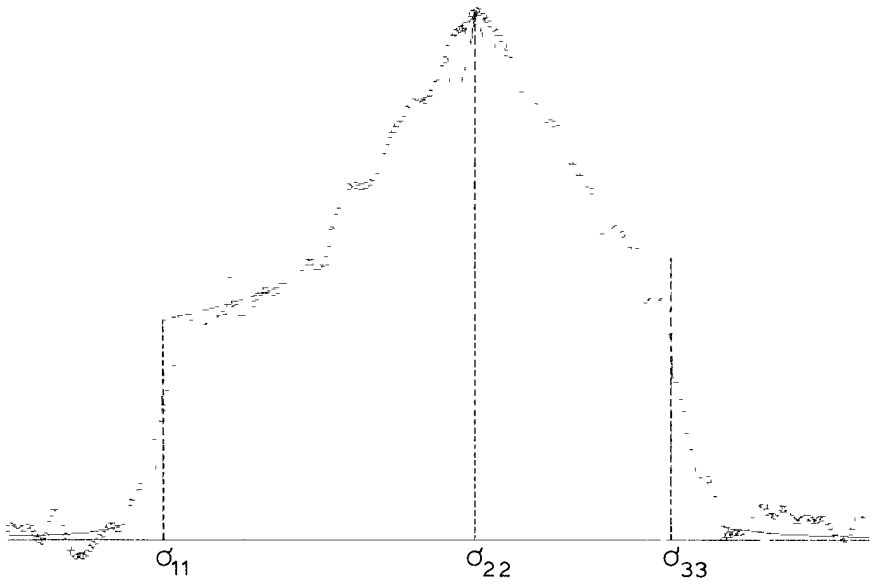

Fig 1 Schematic representations of experimental powder pattern and theoretical powder pattern convoluted with the Lorentzian function The circles are the experimental data for BocGlyAla, σ_{11}=121 5 ppm, σ_{22}=52 2 ppm and σ_{33}=11 1 ppm

metrical broadening function) to the observed powder pattern as shown in Fig. 1.

^{15}N Chemical shift calculations

In this work, we calculated relative ^{15}N NMR chemical shifts (magnetic shielding constants) of a dipeptide fragment, *N*-acetyl-glycine methylamide (forming hydrogen bonds with a formamide molecule), employing the finite perturbation theory (FPT)–INDO method, as shown in detail in a previous paper [18]. The bond lengths and bond angles proposed by Momany et al. [19] were used.

A HITAC M780H computer at the Computer Center of the Tokyo Institute of Technology and a HITAC S-820 computer at the Computer Center of the Institute for Molecular Science, Okazaki, were used for the calculations.

RESULTS AND DISCUSSION

^{15}N NMR chemical shifts of the glycine residue of BocGly peptides in the solid state

A 27.25 MHz ^{15}N CP–MAS NMR spectrum and a 27.25 MHz ^{15}N CP powder pattern spectrum of *tert*-butyloxycarbonyl-glycylalanine (BocGlyAla) in the solid state are shown as a typical example in Fig. 2. ^{15}N spectra of the other remaining samples were also obtained with similar resolutions. In the BocGly peptides, a nitrogen of the glycine residue forms a urethane bond. Thus, its ^{15}N signal appears more upfield compared with that of the amide nitrogens. Signals were easily assigned with reference to solution-state ^{15}N NMR data reported previously [20].

All the isotropic ^{15}N chemical shift values (σ_{iso}) and the principal values of the ^{15}N chemical shift tensors (σ_{11}, σ_{22} and σ_{33}; from downfield to upfield) are shown in Table 1. The geometrical parameters obtained by X-ray diffraction

TABLE 1

Observed ^{15}N isotropic chemical shift and chemical shift tensor components of glycine amide nitrogen of BocGly-containing peptides (ppm from $^{15}NH_4NO_3$)

Sample	σ_{iso}	σ_{11}	σ_{22}	σ_{33}
BocGly	59 75	127 0	51 2	1 1
BocGlyAla	61 61	121 5	52 2	11 1
BocGlyPhe	54 04	112 7	56 1	−6 7
BocGlyAib	57 70	127 0	49 1	−3 0
BocGlyProOBzl	53 06	117 2	50 6	−8 6

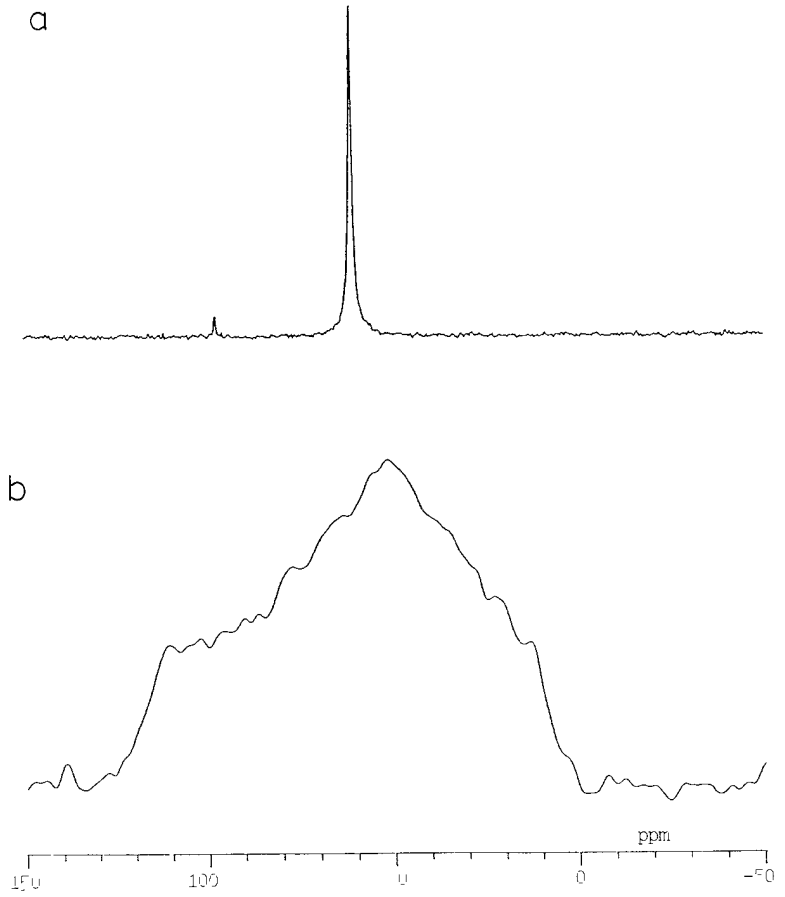

Fig 2 27 25 MHz ^{15}N CP-MAS NMR spectrum(a) and 27 25 MHz ^{15}N powder pattern spectrum(b) of BocGlyAla in the solid state

studies [13–17] are shown in Table 2, where some of the geometrical parameters were estimated by using unit cell parameters and fractional coordinates given in the literature. The hydrogen bond lengths ($R_{N\cdots O}$) of peptides used in this work are in the range 2.95–3.08 Å. On the other hand, the hydrogen bond angles ∠ C=O···N are somewhat variable and are in the range 113–155°.

Figure 3 shows the plot of the observed isotropic ^{15}N chemical shifts (σ_{iso}) of Gly NH against the hydrogen bond length $R_{N\cdots O}$. It is found that there is a clear relationship between σ_{iso} and $R_{N\cdots O}$, and a decrease of $R_{N\cdots O}$ leads to a decrease in shielding. This trend is similar to that in the relationship between carbonyl ^{13}C chemical shifts and $R_{N\cdots O}$ [20]. Figures 4(a)–(c) show the plot of the observed principal values (σ_{11}, σ_{22} and σ_{33}) of the ^{15}N chemical shift tensor of Gly NH against $R_{N\cdots O}$, respectively. It is found that σ_{11} and σ_{33} are

TABLE 2

Geometrical parameters of glycine residue amide nitrogens of BocGly-containing peptides

Sample	Dihedral angle (deg)			Hydrogen bond geometry				Ref
	ω	ϕ	ψ	N···O (Å)	H···O (Å)	∠N-H· ·O (deg)	∠N···O=C (deg)	
BocGly	174 0	−61 5	−15 1	3 01	-	-	113 1	13
BocGlyAla	178 8	−125 6	45 0	2 95	2 02	161 1	-	14
BocGlyPhe	−176 9	−88 0	−14 5	3 08	-	-	155 4	15
BocGlyAib	−159 4	89 5	165 1	3 08	-	-	116 4	16
BocGlyProOBzl	172 2	−109 5	164 4	3 10	2 24	163 4	136 7	17

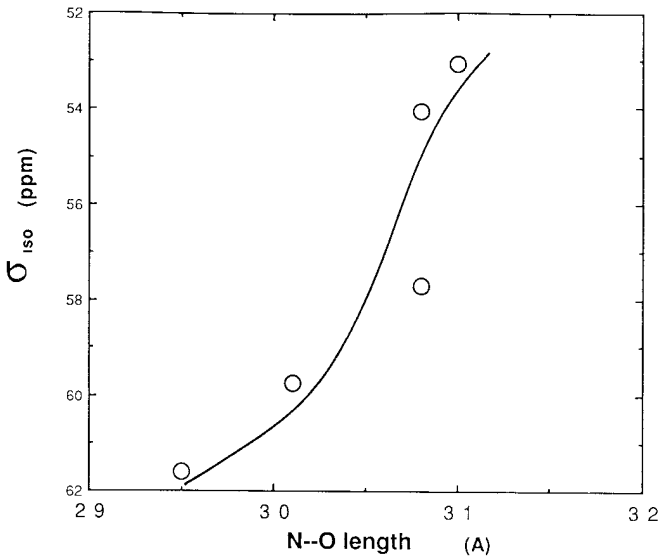

Fig 3 Plots of the observed isotropic ^{15}N chemical shifts (σ_{iso}) in the solid state against the N···O hydrogen bond length ($R_{N\ O}$)

more sensitive than σ_{22} to a change in $R_{N\ O}$. A change of 0.2 Å in $R_{N\ O}$ leads to a change of 20 ppm in σ_{11} and σ_{33}, but a change of only 5 ppm for σ_{22}. However, only σ_{33} is shifted linearly downfield with a decrease of $R_{N\ O}$, and in σ_{11} and σ_{22} there is no clear relationship with $R_{N\ O}$. These results show that such behavior is governed not only by the hydrogen bond length, but also by the hydrogen bond angle.

Theoretical calculation of ^{15}N shielding constant

In order to obtain a deep insight for the experimental finding that the isotropic ^{15}N chemical shift and the principal value of σ_{33} depend upon the hy-

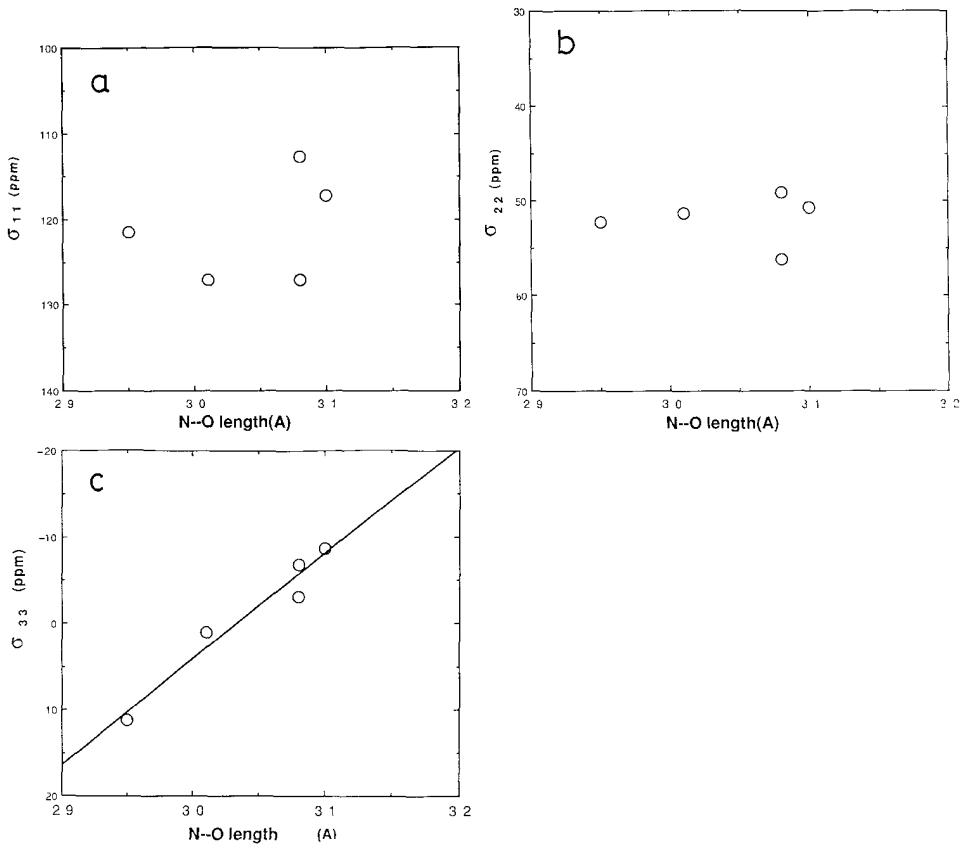

Fig 4 Plots of the observed principal values of ^{15}N chemical shift tensors (a) σ_{11}, (b) σ_{22}, and (c) σ_{33}, against the N···O length ($R_{N\ O}$)

drogen bond length, theoretical calculations of ^{15}N chemical shifts were carried out by the FPT–INDO method.

Figures 5(a)–(d) show the calculated isotropic ^{15}N shielding (σ_{iso}) and the paramagnetic terms of the shielding tensor components (σ_{11}, σ_{22} and σ_{33}) of Gly NH in the model compound. The calculated values are all expressed in ppm with an opposite sign to the experimental chemical shift values shown in Table 1. 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 diamagnetic and paramagnetic terms. However, the behavior of the shielding tensor can be predominantly explained by the paramagnetic term, since the diamagnetic term is isotropic. As shown in Fig. 5(a), a decrease of $R_{N\ .\ O}$ leads to a decrease of σ_{iso}. This agrees with the observed results. Therefore, such a relationship suggests that isotropic ^{15}N chemical shift values can

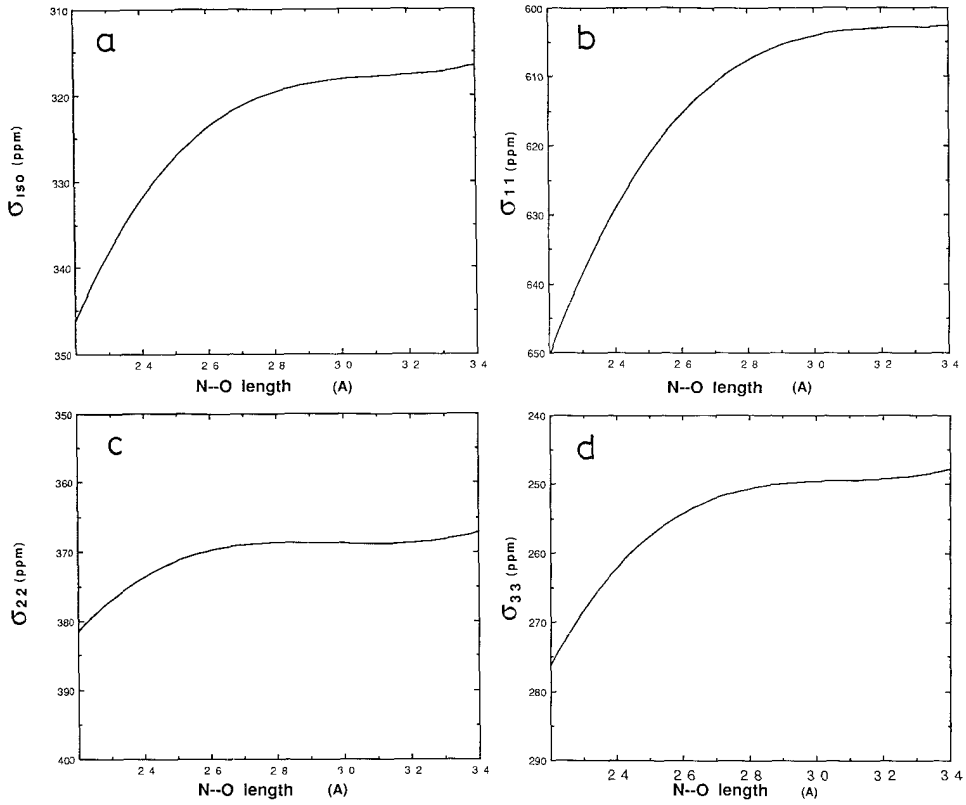

Fig 5 Variation of the calculated ^{15}N shielding constant and its tensor components with the N···O hydrogen bond length ($R_{N\ O}$) (a) isotropic shielding constant σ_{iso}, (b) σ_{11}, (c) σ_{22}, and (d) σ_{33}

be used in estimation of $R_{N\ O}$, just as in the case of carbonyl ^{13}C chemical shifts reported previously [21]. Figures 5(b)–(d) show the $R_{N\ O}$ dependence of the calculated principal values of the ^{15}N chemical shift tensor (σ_{11}, σ_{22} and σ_{33}). It is shown that a decrease in $R_{N\ O}$ leads to decreases in shielding of σ_{11}, σ_{22} and σ_{33}, with magnitudes in the order $\sigma_{11} > \sigma_{33} > \sigma_{22}$. This agrees with the observed order. Further, a linear decrease of σ_{33} with a decrease in $R_{N\ O}$ agrees with the observed result. However, in the experimental results there is no relationship between $R_{N\ O}$ and σ_{11} or σ_{22}, but in the calculation there is such a relationship. Why is there this discrepancy? In the above calculations, we are concerned with the hydrogen bond length only. To understand deeply hydrogen bonding, another important factor such as the hydrogen bond angle (i.e. the distortion of the hydrogen bond from linearity) should be taken into account.

For this reason, we calculated the ^{15}N chemical shift as a function of the

hydrogen bond angle ($\angle$ N–H···O) using the model compound (Fig. 6). The H···O length was fixed as 1.75 Å and then the hydrogen bond angle θ ($\angle$ N–H···O) was varied. Figures 7(a)–(d) show the plot of σ_{iso}, and σ_{11}, σ_{22} and σ_{33} against the angle θ, respectively.

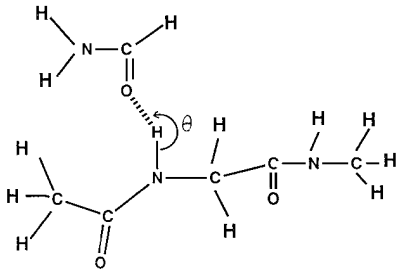

Fig 6 Molecular structure of *N*-acetyl-*N'*-methylglycine amide hydrogen-bonded with a formamide molecule

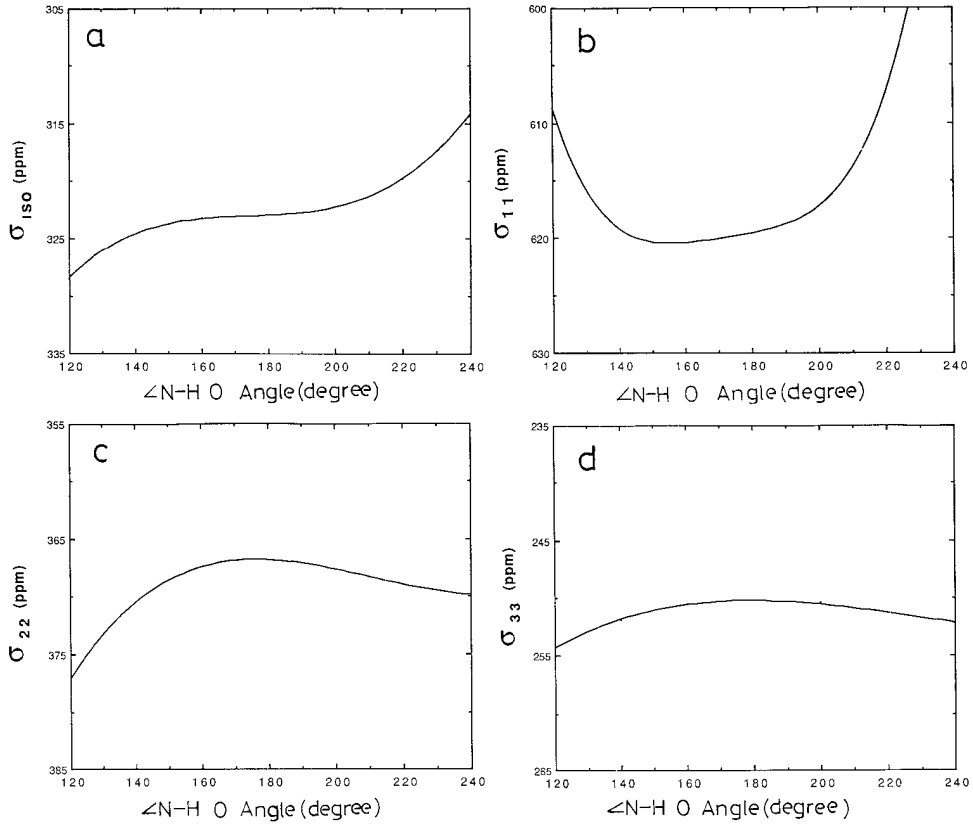

Fig 7 Variation of the calculated ^{15}N shielding constant and its tensor components with the hydrogen bond angle ($\angle$ N-H···O) (a) isotropic shielding constant σ_{iso}, (b) σ_{11}, (c) σ_{22}, and (d) σ_{33}

Figure 7(a) shows the ∠N–H···O dependence of the calculated isotropic ^{15}N shielding (σ_{iso}). A change in the angle θ from 140° to 220° leads to a change of 5 ppm in σ_{iso} compared with the value of about 20 ppm induced by a change of $R_{N\ O}$ from 2.4 to 3.2 Å. Figures 7(b)–(d) show the ∠N–H···O dependence of the calculated principal values of shielding tensors (σ_{11}, σ_{22} and σ_{33}) The magnitudes of changes in σ_{11} and σ_{22} are about 10 and 4 ppm, respectively, in going from θ=140° to 220°. In this range of θ, the magnitudes of changes in σ_{11} and σ_{22} are about 30 and 4 ppm, respectively, in going from 2.4 to 3.2 Å. On the other hand, the magnitude of change in σ_{33} is about 1 ppm in going from θ=140° to 220° and in this range of θ the magnitude of change in σ_{33} is about 15 ppm in going from $R_{N\ O}$=2.4 to 3.2 Å. Therefore, it can be said that σ_{11} and σ_{22} are relatively sensitive to change of θ, but σ_{33} is relatively insensitive, when compared with their $R_{N\ O}$ dependences. For these results, we can understand the above experimental finding that there is a relationship between hydrogen bond length and σ_{33} or σ_{iso}, in spite of the distribution in the hydrogen bond angles, but there is no clear relationship between $R_{N\ O}$ and σ_{11} or σ_{22}.

The direction of the principal axes of the Gly NH shielding tensor

The direction of the principal axes of the Gly NH ^{15}N shielding tensor components has been determined by an NMR study of a BocGlyGlyGlyOBzl single crystal [11]. It has been reported that the σ_{11} component lies approximately along the N–H bond, the σ_{33} component lies approximately along the N–C′ bond, and the σ_{22} component is aligned in the direction perpendicular to the peptide plane.

The results of the FPT–INDO calculations are shown in Fig 8. The σ_{11} component lies approximately along the N–H bond, in agreement with the experimental results. However, the σ_{22} component lies approximately along the N–C′ bond and the σ_{33} component is aligned in the direction perpendicular to the peptide plane, which is different from the experimental results. Experimentally it is not easy to determine the direction of σ_{22} and σ_{33}, because their magnitudes are very close to each other. The present assignments of σ_{22} and σ_{33} seem to be acceptable, although it is difficult to differentiate between them. The direction of σ_{11} can be easily determined, because its magnitude is very different from the others

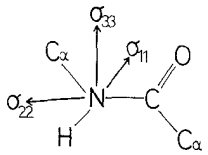

Fig 8 Orientation of the principal axes of the calculated ^{15}N chemical shift tensors of glycine residue amide nitrogen

The reason why in the theoretical calculation the most shielded component σ_{33} is aligned in the direction perpendicular to the peptide plane is due to the fact that the orbitals of the nitrogen atom lone-pair electrons are in this direction. The experimental results (that σ_{33} moves linearly downfield with a decrease of the hydrogen bond length, that σ_{33} is not related to hydrogen bond angle, and that σ_{11} and σ_{22} are related to hydrogen bond length and hydrogen bond angle) were reasonably explained by the theoretical calculations.

Finally, we can conclude the following. The observed isotropic ^{15}N chemical shift moves downfield with decrease in $R_{N \quad O}$ and the principal value of the σ_{33} component moves linearly downfield with such a decrease. This was justified by quantum chemical calculations. Further, quantum calculations show that the σ_{11} and σ_{22} components are related not only to the hydrogen bond length, but also to the hydrogen bond angle. This explains the experimental finding that there is no relationship between the hydrogen bond length and σ_{11} or σ_{22}. From this, it can be said that the ^{15}N chemical shift of amide nitrogen provides useful information about the hydrogen bond length and the hydrogen bond angle.

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