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A structural study of peptides and proteins containing L-alanine residues by ^{13}C NMR spectroscopy combined with ab initio chemical shift calculations

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Abstract

From the observation of solid-state ^{13}C NMR chemical shifts of L-alanine residues which are contained in some peptides, and the ^{13}C chemical shift calculations employing the coupled-Hartree–Fock method with gauge-invariant-atomic-orbital, it was found that the ^{13}C chemical shift of the C_β-carbon in the L-alanine residue is related to the main-chain dihedral angles, ϕ, ψ, but that of the C_α-carbon is affected not only by dihedral angles but also by hydrogen-bonding structure. These results were successfully applied to the structural study of proteins in solution, ribonuclease H from *Escherichia coli* and basic pancreatic trypsin inhibitor.

Introduction

Solid-state NMR spectroscopy offers unique structural information about the backbone and side chains of peptides and proteins [1–4]. It is well-known that peptides and proteins are found in almost every biological system and that they play an important role in biological processes. An understanding of how the conformation of peptides relates to their biological function is very important in unraveling the many biological systems in nature. The study of model peptides is of particular interest because they serve as partial model systems for proteins which are too large to crystallize easily for X-ray studies and are too large to study with conventional solution NMR. We have shown that the ^{13}C chemical shift behavior of poly(L-alanine) can be interpreted in terms of the change of the electronic structure which occurs not only from the change in the main-chain dihedral angles of the polypeptide but also from the intra- or interchain hydrogen bonds [5]. Furthermore, we have demonstrated that the solid-state isotropic chemical shifts of the carbonyl carbons in glycine [6] and L-alanine [7] residues which are contained in a series of peptides move linearly downfield with a decrease of the hydrogen-bond length between nitrogen and oxygen ($R_{N\cdots O}$) determined by X-ray or neutron diffraction studies. From these investigations, we can see that the correlation between the $R_{N\cdots O}$ and the chemical shift of the carbonyl carbon in the L-alanine residue in the solid state is different from that in the glycine residue. The correlations for glycine and L-alanine residues are as follows. For Gly

$$\delta_{iso} = 206.0 - 12.4R_{N\cdots O}(\text{Å}) \quad (1)$$

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For L-Ala

$$\delta_{iso} = 237.5 - 21.7R_{N\cdots O}(\text{Å}) \quad (2)$$

It can be said that a correlation for a specific amino acid residue will be unique. The implication of these correlation equations is that the hydrogen-bond length ($R_{N\cdots O}$) can be determined through the observation of ^{13}C chemical shift values of the L-alanine residue carbonyl carbon.

^{13}C chemical shifts of peptides in the solid state give us information about conformations, i.e. the main-chain dihedral angles (ϕ, ψ) of a peptide and the N···O hydrogen-bonded structure. In this article, we will propose a methodology for structural elucidation of peptides and proteins through the ^{13}C chemical shift. For this purpose, a combination of ^{13}C solid-state NMR experiments of peptides and the ^{13}C chemical shift calculations by the gauge-invariant-atomic-orbital and coupled-Hartree–Fock (GIAO–CHF) method was used.

As is well known, both the X-ray diffraction method and a distance-geometry method with solution NMR techniques are applied to the structural elucidation of proteins. In X-ray diffraction, a single crystal sample of a protein must be prepared. Since it is not generally easy to prepare a single crystal of a large protein, the X-ray diffraction method is sometimes not a powerful methodology for the structural elucidation of larger proteins. The distance-geometry method gives structural information on proteins in solution. This method employs the nuclear Overhauser effect (NOE) and *J*-coupling obtained by solution NMR experiments. However, as signal enhancement by NOE is proportional to r^{-6} (where r is the interproton distance), the larger the interproton distance, the larger the experimental error it inevitably contains. The main problem of these two methods is the lack of experimental data compared with the required number of parameters for determining the structure of a protein (over 1000 parameters of bond lengths, bond angles, and dihedral angles are needed). Hence, in addition to these methods, a further method is needed for structural elucidation. Therefore, this work intends to propose a methodology for the structural elucidation of larger proteins such as ribonuclease H (RNaseH) and basic pancreatic trypsin inhibitor (BPTI).

Experimental

Materials

A series of peptides containing L-alanine residues were synthesized according to a fragment condensation between the *N*-hydroxysuccinimide ester of an *N*-terminal protected amino acid and an amino acid [8]. An *o*-nitrophenylsulfenyl group (Nps) or a *t*-butyloxycarbonyl group (Boc) was used as the *N*-terminal protector group. A mixture of ^{13}C labeled L-[1-^{13}C]alanine (Merck, isotope purity 99 atom %) and ^{13}C unlabeled L-alanine (Nihon-Rika) was used to observe the accurate ^{13}C chemical shift value of the L-alanine carbonyl carbon in the ^{13}C NMR spectrum. Boc-L-alanyl-α-aminoisobutyric acid (Boc-Ala-Aib-OH), and Boc-L-alanyl-L-proline (Boc-Ala-Pro-OH) were synthesized by this procedure. Nps-Ala-OH and methylamine were used for the synthesis of Nps-*N'*-methyl-L-alanine amide by the dicyclohexylcarbodiimide method. HCl·Ala-NHMe was de-protected by HCl/dioxane solution. *N*-Acetyl-Ala-NHMe (Ac-Ala-NHMe) was prepared in pyridine by reacting anhydrous acetate with HCl·Ala-NHMe. Poly(L-[1-^{13}C]alanine) was prepared by polymerization of L-[1-^{13}C]alanine-*N*-carboxy anhydride. The synthesized peptides were recrystallized according to the same procedures as those used in the X-ray diffraction studies [9–13].

^{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 [14] accessory. The field strength of the ^{1}H decoupling was 1.2 mT, contact time was 2 ms, repetition time was 5 s, and spectral width was 27.0 kHz. 8 K data points were used. Samples were placed in a cylindrical rotor and spun at 3.9–4.5 kHz. Spectra

were accumulated 200–1000 times to achieve a reasonable signal-to-noise ratio. The ^{13}C chemical shifts were calibrated indirectly through the adamantane peak observed upfield (29.5 ppm relative to tetramethylsilane, TMS, $(CH_3)_4Si$).

Theoretical calculations

It is known that ^{13}C chemical shifts obtained from solid-state NMR are closely related to the electronic structure of a molecule, and so give information about the three-dimensional structure of the molecule. In order to reveal the correlation between the structure of peptides and the ^{13}C chemical shifts, we carried out the ^{13}C shielding calculations by GIAO–CHF [15,16]. In the GIAO–CHF method, the total shielding constant is estimated as the sum of the diamagnetic and paramagnetic contributions.

The diamagnetic contribution is largely due to the geometrical parameters of a molecule and an unperturbed bond order when the external magnetic field is not applied. The paramagnetic contribution, however, is largely due to not only its geometrical parameters and the unperturbed bond order but also a perturbed bond order when the molecule is in the magnetic field. In this work, we adopted a model molecule, *N*-acetyl-*N'*-methyl-L-alanine amide (Ac-L-Ala-NHMe) as shown in Fig. 1 [5]. All the bond lengths in this model molecule were optimized, except for the C–H bond lengths in the methyl groups, by the ab initio 4-31G molecular orbital method, and all the bond angles except for the methyl groups were also optimized by the same method. Calculated chemical shifts are given in ppm relative to methane (the calculated ^{13}C shielding of methane by the 4-31G/4-31G basis set [17] is 207.2 ppm and the observed ^{13}C chemical shift is −2.1 ppm relative to TMS). Finally, the calculated chemical shifts are given relative to TMS.

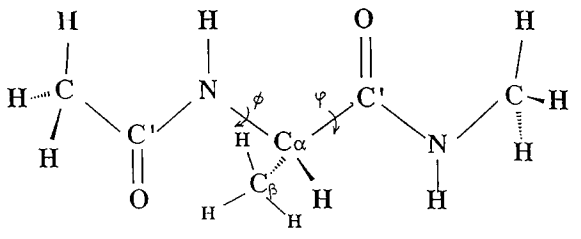

Fig. 1. Molecular structure of *N*-acetyl-*N'*-methyl-[1-^{13}C]L-alanine amide which was employed in the 4-31G GIAO–CHF calculation.

Sun 4 Spark Station 2 was used for the calculations. For the optimization of the geometrical parameters and the nuclear shielding constant calculation, it took about 26 h (the initial guess used here was Momany's standard values [18]) and 6 h, respectively. The nuclear shielding calculation also offers information about the relative orientation of the principal axis system of a chemical shielding tensor with respect to the molecular frame. Each of the tensor elements has information about the three-dimensional electronic structure around a nucleus. Thus, in this work, we also calculated the relative orientation of principal axes of the shielding tensors for the carbonyl, C_α-, and C_β-carbons in the L-alanine residue.

Results and discussion

^{13}C chemical shifts of the L-alanine C_α- and C_β-carbons in peptides in the solid state

Figure 2 shows a 67.8 MHz CP-MAS spectrum of *N*-acetyl-*N'*-methyl-[1-^{13}C]L-alanine amide (Ac-[1-^{13}C]L-Ala-NHMe) as a typical example. It was found that the unit cell of crystals of Ac-L-Ala-NHMe contains two molecules, the conformations of which are not identical. In Fig. 2, for the carbonyl, C_α- and C_β-carbons, the two signals which come from two different conformations were observed. ^{13}C CP-MAS spectra of the other remaining samples were also obtained with similar resolution. Table 1 shows the ^{13}C chemical shifts of the L-alanine C_α- and C_β-carbons in some peptides together with their geometrical parameters as determined by X-ray diffraction studies [9–13]. Some of the geometrical parameters were calculated by using the unit cell parameters and the fractional coordinates. Table 1 shows that the

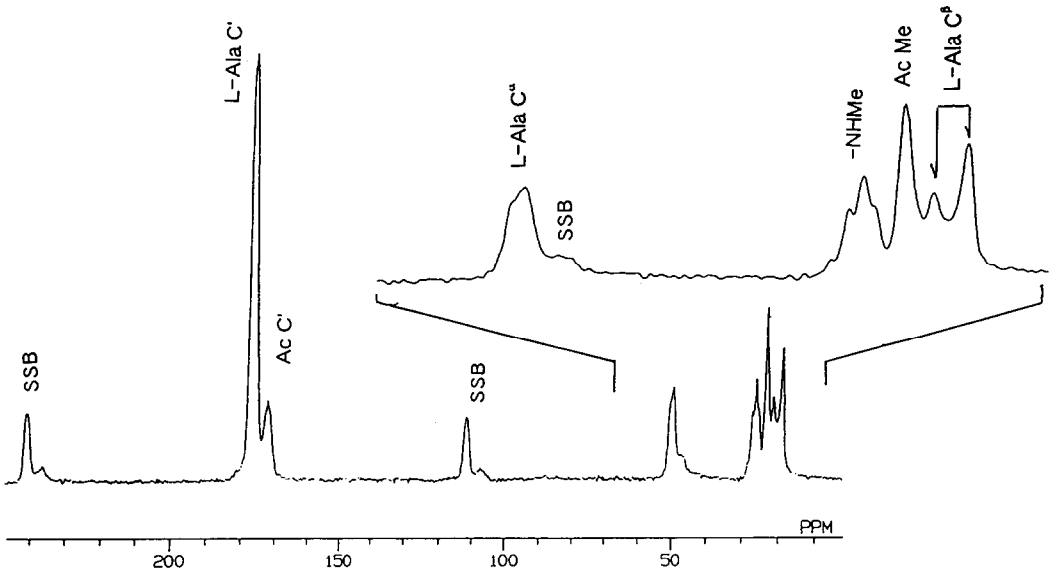

Fig. 2. A typical 67.8 MHz ^{13}C CP-MAS NMR spectrum of *N*-acetyl-*N'*-methyl-[1-^{13}C]L-alanine amide in the solid state.

L-alanine residues in the peptides have dihedral angles (ϕ, ψ) corresponding to several conformations such as right-handed $\alpha(\alpha_R)$-helix, antiparallel $\beta(\beta_A)$-sheet, right-handed 3_{10} $(3_{10}{}^R)$-helix, etc. The ^{13}C signal of the L-alanine C_α-carbon in poly(L-alanine) which forms the α_R-helix conformation appears upfield, by several ppm, of the other conformations; however, the ^{13}C chemical shift of the L-alanine C_β-carbon in the β_A-sheet conformation is 21.0 ppm, which is downfield of the other conformations. The ^{13}C chemical shift of the L-alanine C_α-carbon for the β_A-sheet conformation is 48.7 ppm, which appears upfield of the other conformations; however, the ^{13}C chemical shift for poly(L-alanine) which forms the α_R-helix conformation is 53.0 ppm, which is upfield, by several ppm, of the other conformations. The ^{13}C chemical shifts of the L-alanine C_α- and C_β-carbons for the other specified conformations were almost intermediate values between those for the α_R-helix and the β_A-sheet conformations. We can recognize from these results that ^{13}C chemical shifts of the C_α- and C_β-carbons in the L-alanine residue in peptides closely relates with the main-

Table 1
Observed ^{13}C chemical shifts of L-alanine residue C_α- and C_β-carbons for peptides including L-alanine residues in the solid state, as determined by ^{13}C CP-MAS NMR, and their geometrical parameters

Sample	^{13}C chemical shift (ppm)		Dihedral angle (deg)		
	C_α	C_β	ϕ	ψ	ω
Ac-Ala-NHMe	49.3, 50.4	18.8, 21.1	−84.3	159.0	173.3
			−87.6	154.8	171.9
Boc-Ala-Aib-OH	52.3	17.4	−66.3	−24.1	171.8
Boc-Ala-Pro-OH	49.2	17.2	−95.4	153.6	179.9
Poly(Ala)[a]	53.0	15.5	−57.4	−47.5	−179.8
Poly(Ala)[b]	48.7	21.0	−138.8	134.7	−178.5

[a]With the α^R-helix conformation.
[b]With the β_A-sheet conformation.

Table 2
Calculated ^{13}C chemical shifts (ppm) of L-alanine residue C_α- and C_β-carbons by the 4-31G -GIAO-CHF method

Sample	C_α				C_β			
	σ_{iso}	σ_{11}	σ_{22}	σ_{33}	σ_{iso}	σ_{11}	σ_{22}	σ_{33}
Ac-Ala-NHMe	43.62	65.79	46.46	18.91	15.94	33.80	17.97	−3.49
	–	–	–	–	–	–	–	–[a]
Boc-Ala-Aib-OH	45.71	64.74	55.04	26.37	15.84	32.47	19.03	−4.00
Boc-Ala-Pro-OH	–	–	–	–	–	–	–	–[a]
Poly(Ala)[b]	45.52	61.93	43.69	30.93	15.72	28.16	22.14	−3.16
Poly(Ala)[c]	44.73	62.02	47.53	24.64	18.74	37.06	21.70	−2.53

[a]The chemical shifts could not be calculated because of SCF failures.
[b]With the α^R-helix conformation.
[c]With the β_A-sheet conformation.

chain conformation. However, since the term "main-chain conformation" of the peptide implies not only the main-chain dihedral angles (ϕ, ψ) but also the hydrogen-bonding structure (hydrogen-bond length and angles), it is not apparent yet whether ^{13}C chemical shifts of the L-alanine C_α- and C_β-carbons should be governed by the dihedral angles for the L-alanine residue moiety. In order to estimate the contribution of changes of the dihedral angles to the ^{13}C chemical shifts of the L-alanine residue C_α- and C_β-carbons, we calculated the ^{13}C chemical shifts of L-alanine residue C_α- and C_β-carbons by the GIAO–CHF method with the 4-31G basis set. Ac-L-Ala-NHMe was used for the chemical shielding constant calculations as shown in Fig. 1. Table 2 summarizes the calculated isotropic chemical shifts (σ_{iso}) and the principal values of chemical shift tensor (σ_{11}, σ_{22}, and σ_{33}; $\sigma_{11} > \sigma_{22} > \sigma_{33}$) of L-alanine residue C_α- and C_β-carbons with the dihedral angles of the L-alanine moiety. The calculated isotropic chemical shift of the L-alanine residue C_β-carbon is 18.74 ppm when the dihedral angles (ϕ, ψ) correspond to the β_A-sheet conformation. This calculated chemical shift is displaced downfield relative to the other conformations; however, the calculated chemical shift for the α_R-helix conformation is displaced upfield relative to the others. The experimental results can be reasonably understood from these calculations. Moreover, the behavior of the principal values of the calculated chemical shift tensor offers information about the three-dimensional electronic structure of a molecule. Then, as seen from Table 2, the difference between the calculated isotropic ^{13}C chemical shifts of the C_β-carbon for the dihedral angles, $\phi, \psi = -138.8°, 134.7°$, which corresponds to the β_A-sheet conformation, and for the dihedral angles $\phi, \psi = -57.4°, -47.5°$, which corresponds to the α_R-helix conformation, is dominated by α_{11}. However, the difference between the chemical shifts for the β_A-sheet conformation and for the $3_{10}{}^R$-helix conformation is dominated by not only σ_{11} but also σ_{22}. In order to understand the correlation between the principal values and molecular structure, information is needed about the orientation of the principal axis system of a chemical shift tensor with respect to the molecular fixed frame. The chemical shift tensor orientation will be discussed below.

The calculated isotropic ^{13}C chemical shifts of the L-alanine residue C_α-carbon in some peptides are shown in Table 2. First, the calculated isotropic chemical shift of the L-alanine residue C_α-carbon is not sensitive to the difference in the main-chain dihedral angles of the L-alanine moiety. However, the experimental isotropic chemical shift indicates the explicit "conformational" dependency. It is thought that this discrepancy between the experimental and calculated results may be caused by hydrogen-bonding effects on the chemical shift of the L-alanine C_α-carbon because, in the

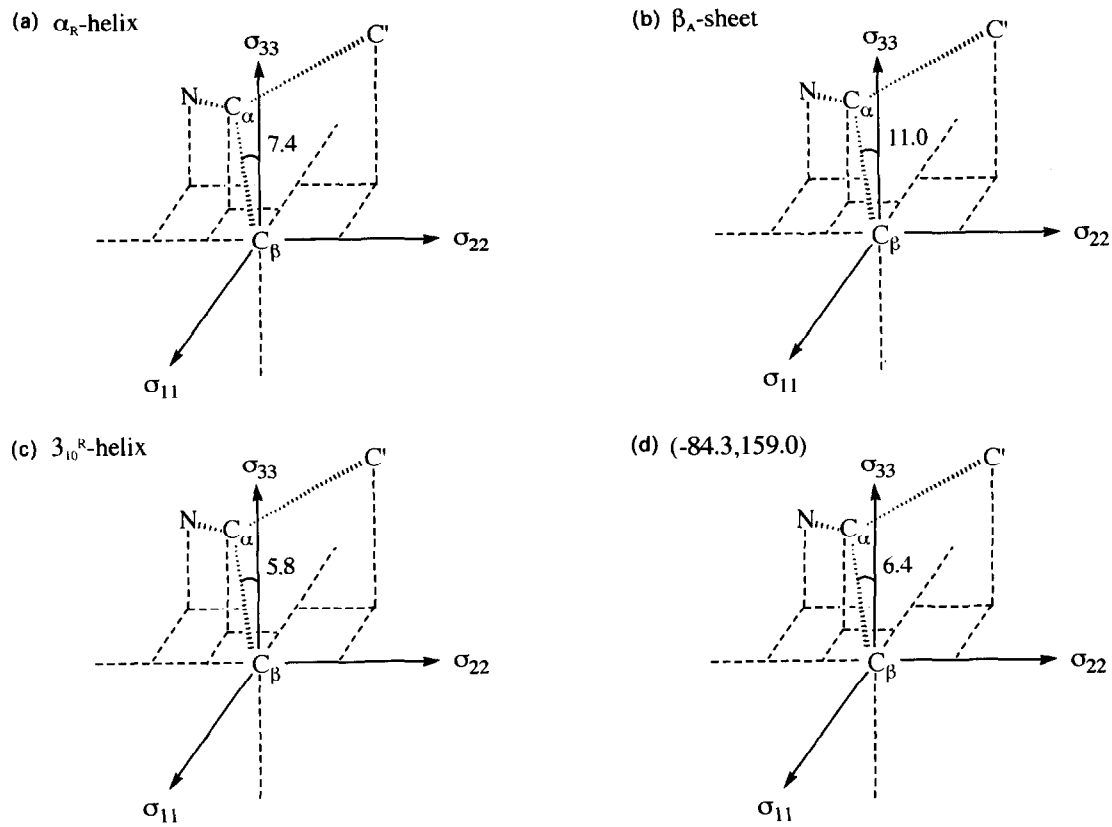

Fig. 3. Orientation of the principal axes of the calculated ^{13}C chemical shift of the L-alanine residue C_β-carbon: (a) $(\phi,\psi) = -57.4, 47.5°(\alpha^R$-helix); (b) $-138.8, 134.7$ (β_A-sheet); (c) $-66.3, -24.1°(3_{10}{}^R$-helix); (d) $-84.3, 159.0°$.

calculation, hydrogen bonding is not taken into account, but in the crystalline state hydrogen bonds are formed. The calculated principal values of the ^{13}C chemical shift tensor of the L-alanine C_α-carbon are also listed in Table 2. The calculated principal values for several main-chain dihedral angles are considerably different from each other. Moreover, since the orientation of the principal axes of the chemical shift tensor with respect to the molecular frame varies, the behavior of the principal values are complicated. This will be discussed below.

Orientation of the principal axis system of the calculated chemical shift tensor with respect to the molecular fixed frame

As mentioned above, the principal values of the chemical shift tensor give information about the three-dimensional electronic structure of a molecule. However, in order to understand the behavior of the principal values, information is needed about the orientation of the principal axis system of a chemical shift tensor with respect to the molecular fixed frame. Figures 3(a)–3(d) show the calculated orientations of the principal axis systems of the chemical shift tensors of the L-alanine C_β-carbons in some peptides whose L-alanine moieties have different main-chain dihedral angles: $(\phi,\psi) = -57.4, -47.5°$ (α^R-helix); $-138.8, 134.7°$ (β_A-sheet); $-66.3, -24.1°(3_{10}{}^R$-helix); $-84.3, 159.0°$, (the helix near the 3_1-helix). Figures 3(a)–3(d) show that the σ_{33} component nearly lies along the C_α–C_β bond for all peptides considered here, and they also show that the α_{11} is nearly perpendicular to the plane which is defined by the C_β, C_α, and N atoms in the L-alanine residue; σ_{22}, however, is parallel with

respect to the plane. These results agree with the experimentally determined direction of σ_{33} of the C_β-carbon in L-alanine amino acid by Naito et al. [19]. As seen from Table 2, the σ_{11} component for the dihedral angles corresponding to the β_A-sheet conformation is 37.06 ppm. This shows a downfield shift of about 9 ppm with respect to that for the α^R-helix conformation. This result means that the σ_{11} dominates the downfield shift on the isotropic chemical shift of the C_β-carbon for the β_A-sheet conformation. Since the σ_{11} does not orient to a specified chemical bond, it is not easy to comprehend intuitively the chemical shift tensor behavior of the C_β-carbon. However, it is obvious that the through-space interaction between the C_β-methyl group and its surroundings is important in understanding the σ_{11} behavior.

Figures 4(a)–4(d) show the calculated orientations of the principal axis systems of the chemical shift tensors of the L-alanine C_α-carbons in the peptides with respect to the molecular frames. As seen from these figures, the calculated oricntation of the principal axis system for the C_α-carbon is quite different from sample to sample. For all the dihedral angles employed in the calculations, the σ_{33} component of the ^{13}C chemical shift tensor in the L-alanine C_α-carbon always lies along the C_α–C′ bond. However, for the dihedral angles $(\phi, \psi) = -57.4, -47.5°$ (α^R-helix), $-66.3, -24.1°$ (3_{10}^R-helix), and $-84.3, 159.0°$, the σ_{11} component lies along the slightly deviated direction from the C_α–C_β bond; and for $(\phi, \psi) = -138.8, 134.7°$, β_A-sheet, the σ_{22} component is along this direction. As shown in Table 2, the principal value which is nearly along the C_α–C_β bond is 47.53 ppm for the β_A-sheet form, 61.93 ppm for the α_R-helix form, 64.74 ppm for the 3_{10}^R-helix form, and 65.79 ppm for $(\phi, \psi) = -84.3, 159.0°$, (the helix near 3_1-helix). The change of the dihedral angles causes the large deviation of the chemical shift tensor element which is along the C_α–C_β bond. Moreover, since σ_{33} depends on changes from one dihedral angle to another, it is obvious that

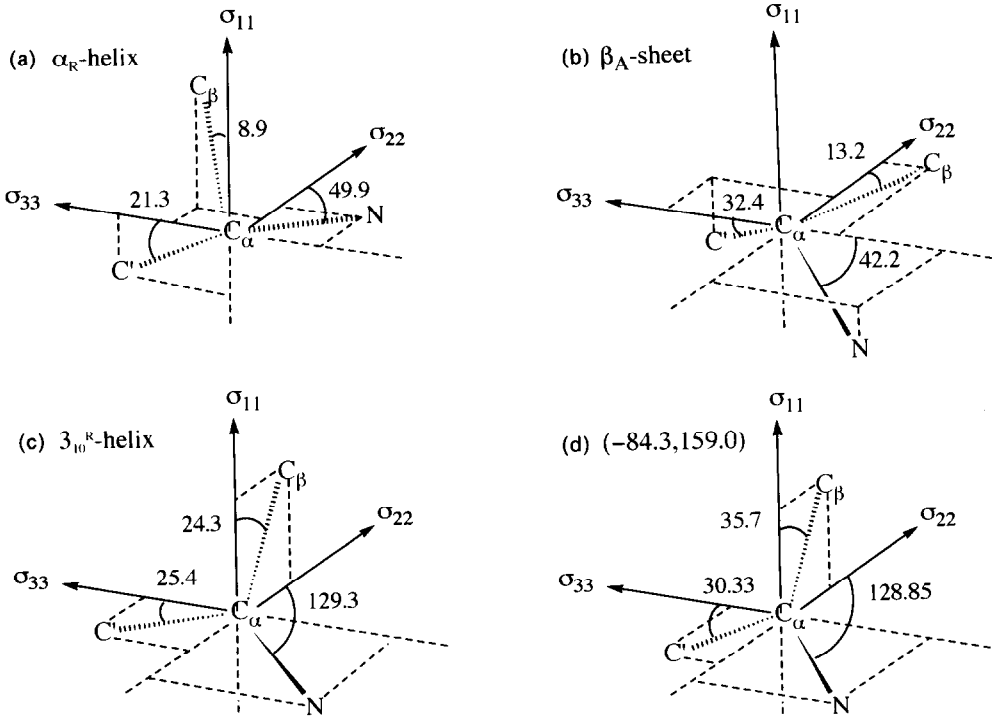

Fig. 4. Orientation of the principal axes of the calculated ^{13}C chemical shift of the L-alanine residue C_α-carbon: (a) $-57.4, 47.5°$ (α^R-helix); (b) $-138.8, 134.7°$, (β_A-sheet); (c) $-66.3, -24.1°$, (3_{10}^R-helix); (d) $-84.3, 159.0°$.

there exists the explicit dihedral angle dependency on σ_{33}. Then, it is thought that if the carbonyl group in the L-alanine residue forms a hydrogen bond, σ_{33} will probably be affected. The principal values of the chemical shift tensor of the L-alanine C_α-carbon in peptides have not been measured yet, because the chemical shift anisotropy is not large enough to accurately evaluate. However, since some experimental methods for the detection of a small degree of chemical shift anisotropy have been developed recently [20], we are trying to measure the principal values of the chemical shift tensor of the L-alanine C_α-carbon. We are also trying to determine the mutual orientation of the principal axis systems of the chemical shift tensor for the L-alanine carbonyl and C_α-carbons by using the $n = 3$ rotational resonance method [21].

Application to the structural study of proteins

Using two- and three-dimensional NMR and isotopic labeling techniques, Yamazaki and Nagayama carried out the assignments of backbone carbons of a large protein, RNase H, extracted from *Escherichia coli*, which consists of 155 amino acid residues and has a molecular mass of 17.6 kDa (see, for example, ref. 22). The samples were dissolved in 0.1 M deuterated acetate buffer of 80% H_2O/20% D_2O, pH 5.5. It was also found that the structure of RNase H, determined by NMR, agreed with that obtained from the X-ray diffraction study [23,24]. From these results, it is obvious that the main-chain conformation of RNase H in the solution state is quite similar to that in the crystalline state.

^{13}C chemical shifts of the C_β-carbons of the L-alanine residues in RNase H

The correlation between the ^{13}C chemical shifts of a peptide and its backbone conformation is of great interest for structural elucidation. We carried out calculations of the isotropic ^{13}C chemical shift

Table 3
Geometrical parameters of L-alanine residues in RNase H and BPTI determined by X-ray diffraction studies and ^{13}C NMR chemical shifts of the carbonyl C_α-, C_β-carbons in the corresponding residues

Amino acid residue	Dihedral angle (deg)			Hydrogen-bond length (Å)	Observed chemical shift (ppm)			Calculated chemical shift (ppm)	
	ϕ	ψ	ω	($R_{N\cdots O}$)	C′	C_α	C_β	C_α	C_β
A24	−144.6	134.7	178.7	2.83	173.7	49.2	21.3	45.35	18.54
A37	−160.5	150.4	179.9	3.27	172.1	50.2	19.7	45.93	17.41
A51	−55.2	−46.1	−179.1	3.20	175.5	53.3	19.3	45.56	15.68
A52	−71.3	−35.4	−178.6	3.24	176.1	52.7	15.3	45.32	15.70
A55	−65.8	−49.2	178.1	3.30	175.6	52.7	17.2	45.07	15.63
A58	−81.0	−11.5	176.8	–	176.1	51.2	17.2	45.14	16.73
A93	−62.4	−22.9	−179.0	–	175.8	53.1	16.0	45.87	15.94
A109	−53.5	−49.9	−177.5	3.22	178.4	52.9	15.8	45.46	15.80
A110	−72.2	−25.6	180.0	3.30	178.1	52.7	16.5	45.46	15.91
A125	−66.5	123.7	−179.8	–	176.1	50.8	16.4	45.54	14.86
A137	−53.7	−57.3	179.8	3.25	177.3	53.5	16.6	45.09	15.97
A139	−67.4	−38.4	177.7	–	178.9	52.9	15.7	45.40	15.57
A140	−67.6	−41.7	−179.0	3.06	177.7	52.7	15.3	45.31	15.56
A141	−60.5	−22.9	−178.9	3.31	175.8	52.7	16.0	45.94	16.00
A16	−76.2	172.0	170.6	–	–	50.5	18.5		
A25	−62.3	−28.0	177.0	–	–	53.0	17.5		
A27	−82.9	−23.1	−178.7	–	176.3	50.5	19.0		
A40	−62.5	151.1	170.6	–	176.9	52.5	18.5		
A48	−61.7	−35.9	−179.4	2.80	177.9	53.5	16.0		

of the C_β-carbons in the L-alanine residue by applying the GIAO–CHF method. The calculated shifts are shown in Table 3. A comparison of the calculated and experimental ^{13}C chemical shifts are shown in Fig. 5. From this, it is clear that the isotropic ^{13}C chemical shift of the L-alanine C_β-carbon is related to the main-chain dihedral angles of the L-alanine residue moiety of RNase H in aqueous solution. In this Figure, the observed chemical shift behavior of the L-alanine residue C_β-carbon is generally similar to that of the RNase H C_β-carbon in aqueous solution. Moreover, by using this comparison between the experimental and the calculated chemical shifts, an interaction between the L-alanine C_β-carbon and its surroundings, solvent molecules (H_2O) or side chains of the adjacent amino acid residues can be elucidated. For example, the data for A58 and A125, as shown in Fig. 5, deviate from the correlation curve. These L-alanine residues do not form any hydrogen bonds in the protein; that is, the solvent molecules or side chains of adjacent residues may be proximate to the C_β-methyl group. From such a situation, it is thought that the isotropic chemical shifts for the A58 and A125 C_β-carbons are affected by not only their main-chain

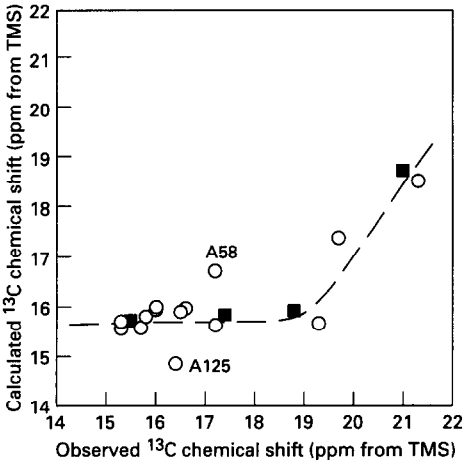

Fig. 5. Comparison of the calculated chemical shifts with the observed shifts of the L-alanine residue C_β-carbons in some peptides in the solid state (■) and in RNaseH in solution (○).

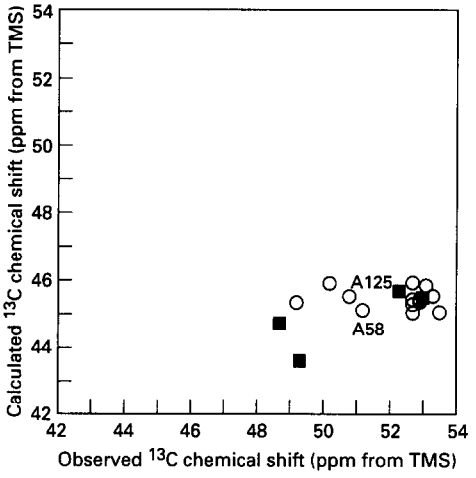

Fig. 6. Comparison of the calculated chemical shifts with the observed shifts of the L-alanine residue C_α-carbons in some peptides in the solid state (■) and in RNaseH in solution (○).

dihedral angles but also by the above mentioned interactions. It is important to detect these interactions when studying the functional aspects of a protein. The detection of such interactions in solution can only be done by solid-state NMR measurements and chemical shift calculations, as well as solution NMR and X-ray diffraction studies.

^{13}C chemical shifts of the C_α-carbons of the L-alanine residues in RNase H

We also carried out chemical shift calculations of the L-alanine residue C_α-carbons by the GIAO–CHF method with a 4-31G basis set. In this calculation, Ac-L-Ala-NHMe with the dihedral angles (ϕ, ψ) was employed. The experimental and calculated isotropic chemical shifts are shown in Table 3. A comparison between the experimental and the calculated ^{13}C chemical shifts is shown in Fig. 6. From this Figure, it was found that there is no clear relationship between the experimental and calculated chemical shifts. This is because we carried out the calculation without taking account of the hydrogen-bonding effect.

^{13}C chemical shifts of the carbonyl carbons of the L-alanine residues in RNase H

Figure 7 shows the plots for the ^{13}C chemical shifts of the L-alanine residue carbonyl carbons in RNase H against the $R_{N\cdots O}$ values obtained from the X-ray diffraction study. A solid, straight line in Fig. 7 was obtained from Eq. (2). In this Figure, there are many data points which have large deviations from the solid, straight line from Eq. (2). It is thought that this is not caused by the difference between the secondary structure in the solid state and that in aqueous solution, but by the lack of accuracy of the $R_{N\cdots O}$ values as determined by X-ray diffraction. Since structural analysis of proteins by X-ray diffraction gives covalent-bond lengths and bond angles with an accuracy of 10^{-2} Å, then the main-chain dihedral angles can be determined with sufficient accuracy [24]. In the case of a small protein such as BPTI, the hydrogen-bond length can be accurately determined [25]. Therefore, the datum for the carbonyl carbon of A48 in BPTI lies on the solid, straight line from Eq. (2) as shown in Fig. 7 [26]. However, through-space internuclear distances such as hydrogen-bond length in larger proteins (such as RNase H) determined by the X-ray diffraction study may include a large experimental error (about 0.3 Å) which results in accumulated errors of the main-chain dihedral angles [24]. This means that for the structural elucidation of larger proteins, it is useful to utilize chemical shifts of the carbonyl carbon as an indication of hydrogen-bond lengths.

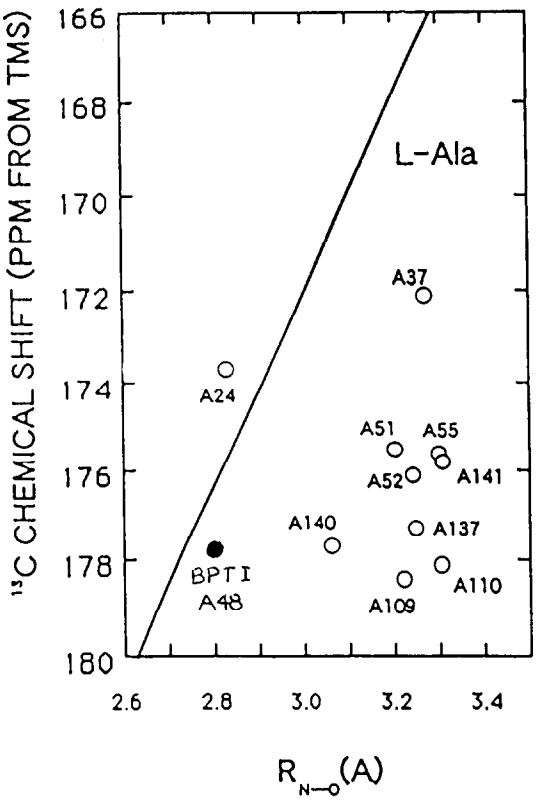

Fig. 7. Plots of the observed chemical shifts of the L-alanine residue carbonyl carbons in RNase H and BPTI in the aqueous solution against the hydrogen-bond lengths ($R_{N\cdots O}$). The straight line is the correlation between the observed ^{13}C chemical shifts of the L-alanine residue carbonyl carbons in some peptides in the solid state and their $R_{N\cdots O}$ values reported previously [7].

Conclusion

It is shown that ^{13}C chemical shifts of the L-alanine residue C_β-carbons in peptides in the solid state and in proteins in aqueous solution give information about the dihedral angle of the main-chain of the L-alanine moiety. However, the hydrogen-bonded structure as well as the dihedral angles affects the ^{13}C chemical shifts of the C_α-carbon in a complicated way. These results were obtained by the 4-31G GIAO–CHF calculation on the ^{13}C chemical shifts. Although there exists the experimental relation that the ^{13}C chemical shift of the L-alanine residue carbonyl carbon in solid peptides moves downfield linearly with a decrease of $R_{N\cdots O}$, we could not find such a relation with the ^{13}C chemical shift of the L-alanine carbonyl carbon in ribonuclease H in aqueous solution. This is because $R_{N\cdots O}$ values, determined by X-ray diffraction, inevitably include large experimental errors. Therefore, the chemical shifts of the carbonyl carbons in peptides and larger proteins can be used as an indication of hydrogen-bond length.

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