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REVIEW

Some aspects of the NMR chemical shift/structure correlation in the structural characterization of polymers and biopolymers

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Studies on the NMR methodology for characterizing the structure of polymers and biopolymers based on the understanding of the NMR chemical shift/structure correlation using solution-state and solid-state NMR experiments, the development of the NMR chemical shift theory and their combination have been reviewed.
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The first observation, according to the best of knowledge, of a high-resolution 17.735-MHz ^{1}H NMR spectrum of uncured Heva rubber in a CS_2 solution was made in 1957 by Gutowsky *et al.*,[1] who observed the CH_3, CH_2 and CH signals to appear separately at different chemical shift positions. It was shown that the NMR chemical shift provides information on the chemical structure of polymers and can be used effectively in the chemical structure analysis and in the analysis of organic compounds.[1,2] Furthermore, in 1960, Bovey and Tiers,[3] Nishioka *et al.*[4] and Johnsen and Tessmar[5] independently reported a very important discovery for polymer science: the α-CH_3 signals in the ^{1}H NMR spectrum of poly(methyl methacrylate)s with different tacticities in chloroform solution appear at different chemical shift positions, in which the three splitting signals were assigned to the *rr*, *mr* and *mm* triads from upfield on the basis of sophisticated and reasonable experiments, where *m* and *r* are the meso and racemic dyads, respectively. Since that time, NMR spectroscopy has been well recognized to be the most powerful method available for characterizing the structures of polymers. This means that the chemical shift (chemical shielding), as one of the important NMR parameters,[6] can be meaningfully used for the structural characterization of polymers.

With such a foundation, the sophisticated understanding and development of the NMR chemical shift/structure correlation for the structural characterization of polymers and biopolymers have been implemented as one of our research programs. In this review, it is shown by the author and his colleagues that understanding the NMR chemical shift/structure correlation provides a powerful methodology for the structural characterization of polymers and biopolymers based on the sophisticated development of solution-state and solid-state NMR experiments, the development of NMR chemical shift theory, and their combination.[7,8]

THE CONCEPT OF THE NMR CHEMICAL SHIFT/STRUCTURE CORRELATION

A polymer chain has an enormous number of chemical bonds. In the solution state, the NMR chemical shifts of polymers are generally the averaged values over all of the possible conformations because of the rapid interconversions by rotation around the chemical bonds. However, in solids, the chemical shifts are often characteristic of specific conformations because of strongly restricted rotation around the bonds.[7,8] The NMR chemical shift is affected by a change in the electronic structure caused by the conformational change. Solid-state NMR chemical shifts, therefore, provide useful information about the electronic structure of a polymer chain and multiple polymer chains with a fixed structure. Furthermore, the complete chemical shift tensor components can often be determined because the chemical shift is, in principle, the second-rank tensor quantity. The complete chemical shift tensor components (the chemical shift anisotropy) of polymers and biopolymers provide information about the local symmetry of the electron cloud around the nucleus, and therefore, provide much more detailed knowledge about the conformation associated with electronic structure compared with the averaged chemical shift. To completely understand the NMR chemical shift/structure correlation of polymers and biopolymers, a sophisticated approach that combines solution-state and solid-state NMR experiments and NMR chemical shift theory has been implemented.

A brief account of the NMR chemical shift

The NMR chemical shifts of atoms in a polymer chain or multiple polymer chains, including biopolymers, depend on the electronic and molecular environments of the nuclei. The chemical shift for atom A

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(σ_A) can be precisely estimated as a sum of the following terms according to the theory of the chemical shift.[9–13]

$$\sigma_A = \sigma^d + \sigma^p, \tag{1}$$

where σ^d and σ^p are the diamagnetic term and the paramagnetic term, respectively. For nuclei with $2p$ electrons, such as ^{13}C, ^{15}N, ^{17}O, and so on, considered here, the relative chemical shift is predominantly governed by the paramagnetic term.[6–8] In the case of the ^1H chemical shift, another term, σ', is added to Equation (1), which can result from significant contributions to the chemical shift from the magnetic anisotropy effect, polar effect and ring current effect. Here, it is noted that the symbol σ is used for the calculated chemical shift, the symbol δ is used for the experimental chemical shift, and that a positive sign for σ signifies shielding and a positive sign for δ signifies deshielding.

Before describing details of the protocol for evaluation of the diamagnetic and paramagnetic contributions, it is noted that in an isotropic liquid or solution sample, the NMR chemical shift σ is observed as a scalar quantity σ_{iso}, the isotropic chemical shift, due to the fast tumbling of molecules and polymers. However, in a solid or oriented molecules and polymers (such as a liquid crystalline or single crystalline samples), the chemical shift is not an isotropic parameter but a second-rank tensor. The components of the chemical shift tensor in these samples can be specified by σ_{ij}, where i, j = x, y or z in a reference frame fixed on the nucleus in a molecule and a polymer. Thus, the observation of the chemical shift tensor components, in principle, leads to more fruitful information about the structure compared with the observation of the σ_{iso}. The magic angle spinning (MAS) experiment of a solid provides the σ_{iso} as the isotropic average of the three principal components of the tensor as given by $(\sigma_{11}+\sigma_{22}+\sigma_{33})/3$. Therefore, the line width of the NMR signal becomes very narrow.

Here, we are concerned with the protocol for evaluating the diamagnetic term $\sigma^d{}_{\alpha\beta}$ and the paramagnetic term $\sigma^p{}_{\alpha\beta}$ for small-size or medium-size model molecules of polymers and biopolymers. The wave function ψ_m for an electron m is expressed by

$$\sigma^d_{\alpha\beta} = \frac{\mu_0}{4\pi}\frac{e^2}{m_e^2}\sum_m^{occ}\langle\psi_m|r^{-3}(r^2\delta_{\alpha\beta} - r_\alpha r_\beta)|\psi_m\rangle \tag{2}$$

and

$$\sigma^p_{\alpha\beta} = -\frac{\mu_0}{4\pi}\frac{e^2}{m_e^2}\sum_m^{occ}\sum_n^{unocc}({}^1E^n_m - {}^1E_0)^{-1} \times\left\{\langle\psi_m|r^{-3}L_\alpha|\psi_n\rangle\langle\psi_n|L_\beta|\psi_m\rangle + \langle\psi_m|L_\beta|\psi_n\rangle\langle\psi_n|r^{-3}L_\alpha|\psi_m\rangle\right\}, \tag{3}$$

using the sum-over-states (SOS) method,[6] where the subscripts α and β denote the Cartesian components (x,y,z), μ_0 is the permeability of free space, m_e is the mass of the electron, and L_α is the orbital angular momentum operator. $\sum_n^{occ}$ and $\sum_n^{unocc}$ are the summation over the occupied and unoccupied molecular orbitals, respectively. The term $({}^1E^n{}_m - {}^1E_0)$ is the electronic singlet-singlet excitation energy from the mth to the nth orbital.

Two approaches for NMR chemical shift calculations of polymers and biopolymers

Two sophisticated approaches for calculating the NMR chemical shifts of polymers and biopolymers have been developed to understand the NMR chemical shift/structure correlation. The first approach (1) is for polymers and biopolymers in solution. A single polymer chain in solution can assume an enormous number of conformational configurations because of the rapid rotation around the chemical bonds. Thus, if the rotation around the bonds is very fast on the NMR timescale, the chemical shift for any specified atom A is observed as that averaged over the possible preferred conformations ($\langle\sigma_A\rangle$). $\langle\sigma_A\rangle$ is given as $\langle\sigma_A\rangle = \sum_{i=1}^n P_i\sigma_i$, where the numerical indices refer to the preferred conformations and P_i and σ_i are the probability of occurrence and the chemical shift of preferred conformation i, respectively. In approach (1), model molecules such as dimers, trimers, and so on. are used as the local structures of the polymer chains, and information about the conformational configuration of a polymer chain in solution has been obtained using a combination of chemical shift theory and polymer statistical mechanics. Such an approach has been carefully applied to characterize the stereochemical structures of some polymer systems in the solution state.[7,8] For example, stereochemical structure (such as dyad, triad and tetrad tacticities) of poly(vinyl chloride) were successfully characterized through the observation of ^1H and ^{13}C NMR spectra in solution and calculating the chemical shift using approach (1).[14–16] Furthermore, this approach led to establishment of using the chemical shift (contour) map for characterizing the second-order structure of polypeptides and using the hydrogen-bonded structure to analyze various types of amino acid residues of peptides and polypeptides. It should be recognized that the chemical shift calculations on model molecules are not always readily applicable to polymers in the crystalline state, because of the existence of long-range intrachain and interchain interactions. Electrons are constrained to a finite region of space in small molecules, whereas this is not necessarily the case for polymers, and thus another approach is required. The second approach (2) is to employ the tight-binding molecular orbital (TB MO) theory, which is well known in the field of solid-state physics, to describe the electronic structures of linear polymers with periodic structure within the framework of the linear combination of atomic orbitals approximation for the electronic eigenfunctions.[17–19] Thus, the chemical shift theory has been combined with the TB MO theory, which can take into account long-range intrachain and interchain interactions in polymer systems. Such an approach is useful for obtaining an appropriate knowledge of the relationship between the electronic structure and the ^{13}C NMR chemical shifts of solid polymers.[20–33]

In the TB approximation, the wave function ψ (**k**) for an electron at position **r**, which belongs to the nth crystal orbital (CO), is expressed with Bloch's theory as follows:

$$\psi_n(\mathbf{k}) = N^{-1/2}\sum_v\sum_{\mathbf{R}} C_{vn}(\mathbf{k})\varphi_v(\mathbf{r}-\mathbf{R})\exp(i\mathbf{k}\bullet\mathbf{R}), \tag{4}$$

where v is the index of the atomic orbital, N is the total number of unit cells, **k** is the wave vector expressed by $\mathbf{k}_x+\mathbf{k}_y+\mathbf{k}_z$ and **R** is the lattice vector. The symbol i denotes the imaginary number, and $C_{vv}(\mathbf{k})$ is the expansion coefficient for the atomic orbital $\phi_v(\mathbf{r}-\mathbf{R})$. Using the obtained expansion coefficients $C_{vv}(\mathbf{k})$, the ^{13}C NMR chemical shift can be expressed by σ_A (**k**) as a function of **k**.[22,23]

The protocol for calculating the NMR chemical shift by the SOS method in the framework of the linear combination of atomic orbital approximation with the neglect of integrals involving more than two centers is derived. If the integrals involve the product of atomic orbitals on different atoms, the diamagnetic term $\sigma^d{}_A(\mathbf{k})$ can be

expressed by

$$\sigma^{d}_{\alpha\beta}(\mathbf{k}) = \frac{\mu_0}{4\pi}\frac{e^2}{m_e^2}\sum_{m}^{occ}\langle\psi_m(\mathbf{k})|r^{-3}(r^2\delta_{\alpha\beta}-r_\alpha r_\beta)|\psi_m(\mathbf{k})\rangle$$
$$= \frac{\mu_0}{4\pi}\frac{e^2}{m_e^2}\frac{1}{N}\sum_{m}^{occ}\sum_{\nu\nu'}C^*_{\nu m}C_{\nu' m}\left\langle\sum_{\mathbf{R}}\exp(i\mathbf{k}\cdot\mathbf{R})\nu^{\mathbf{R}}\right|$$
$$\times r^{-3}(r^2\delta_{\alpha\beta}-r_\alpha r_\beta)\left|\sum_{\mathbf{R}'}\exp(i\mathbf{k}\cdot\mathbf{R}')\nu'^{\mathbf{R}'}\right\rangle \quad (5)$$
$$= \frac{\mu_0}{4\pi}\frac{e^2}{m_e^2}\sum_{m}^{occ}\sum_{\nu\nu'}C^*_{\nu m}C_{\nu' m}\sum_{\mathbf{R}}\exp(i\mathbf{k}\cdot\mathbf{R})$$
$$\times\left\langle\nu^{\mathbf{R}}\left|r^{-3}(r^2\delta_{\alpha\beta}-r_\alpha r_\beta)\right|\nu'^{\mathbf{R}'}\right\rangle,$$

where the asterisk denotes the complex conjugate and $<\nu^{\mathbf{R}}|$ is the νth atomic orbital in the **R**th unit cell. The paramagnetic term $\sigma^{p}{}_{A}(k)$ can be expressed by

$$\sigma^{p}_{\alpha\beta}(\mathbf{k}) = -\frac{\mu_0}{4\pi}\frac{e^2}{m_e^2}\sum_{m}^{occ}\sum_{n}^{unocc}({}^1E_m-{}^1E_0)^{-1}$$
$$\times\{\langle\psi_m(\mathbf{k})|r^{-3}L_\alpha|\psi_n(\mathbf{k})\rangle\langle\psi_n(\mathbf{k})|L_\beta|\psi_m(\mathbf{k})\rangle$$
$$+\langle\psi_m(\mathbf{k})|L_\beta|\psi_n(\mathbf{k})\rangle\langle\psi_n(\mathbf{k})|r^{-3}L_\alpha|\psi_m(\mathbf{k})\rangle\}$$
$$= -\frac{\mu_0}{4\pi}\frac{e^2}{m_e^2}\sum_{m}^{occ}\sum_{n}^{unocc}({}^1E^n_m-{}^1E_0)^{-1}\times\left[\frac{1}{N}\sum_{\nu\nu'}C^*_{\nu m}C_{\nu' n}\right.$$
$$\left\langle\sum_{\mathbf{R}}\exp(i\mathbf{k}\cdot\mathbf{R})\nu^{\mathbf{R}}\right|r^{-3}L_\alpha\left|\sum_{\mathbf{R}'}\exp(i\mathbf{k}\cdot\mathbf{R}')\nu'^{\mathbf{R}'}\right\rangle$$
$$\times\frac{1}{N}\sum_{\nu\nu'}C^*_{\nu' n}C_{\nu m}\left\langle\sum_{\mathbf{R}'}\exp(i\mathbf{k}\cdot\mathbf{R}')\nu'^{\mathbf{R}'}\right|L_\beta\left|\sum_{\mathbf{R}'}\exp(i\mathbf{k}\cdot\mathbf{R})\nu^{\mathbf{R}}\right\rangle$$
$$+\frac{1}{N}\sum_{\nu\nu'}C^*_{\nu m}C_{\nu' n}\left\langle\sum_{\mathbf{R}}\exp(i\mathbf{k}\cdot\mathbf{R})\nu^{\mathbf{R}}\right|L_\beta\left|\sum_{\mathbf{R}'}\exp(i\mathbf{k}\cdot\mathbf{R}')\nu'^{\mathbf{R}'}\right\rangle$$
$$\left.\times\frac{1}{N}\sum_{\nu\nu'}C^*_{\nu' n}C_{\nu m}\left\langle\sum_{\mathbf{R}'}\exp(i\mathbf{k}\cdot\mathbf{R}')\nu'^{\mathbf{R}'}\right|r^{-3}L_\alpha\left|\sum_{\mathbf{R}'}\exp(i\mathbf{k}\cdot\mathbf{R})\nu^{\mathbf{R}}\right\rangle\right], \quad (6)$$

where the angular momentum integral has a non-zero value only between orbitals with the same angular momentum quantum number because of the orbital orthogonality. The detailed derivation of these equations for evaluating $\sigma_A(\mathbf{k}) = \sigma^{d}{}_{A}(\mathbf{k}) + \sigma^{p}{}_{A}(\mathbf{k})$ has been previously reported.[22,23]

To compare the calculated chemical shift σ_A with the experimental chemical shift, one needs to integrate over the first Brillouin zone as expressed by

$$\sigma_A = \frac{\Omega}{8\pi^3}\int_{BZ}\{\sigma^{d}_{A}(\mathbf{k})+\sigma^{p}_{A}(\mathbf{k})\}d\mathbf{k}, \quad (7)$$

where Ω is the volume of the primitive cell. Thus, by such a treatment of $\sigma_A(\mathbf{k})$ over **k**, the NMR chemical shift σ_A can be compared with the experimental chemical shift.

Single polymer chain and multi polymer chains approaches

Conformation-dependent ^{13}C chemical shifts of various types have been systematically studied for a single polymer chain such as polyethylene, *cis*- and *trans*-polyacetylenes, polyoxymethylene, polypyrrole, polyoxyethylene and polypeptides such as polyglycine, poly(L-alanine), poly(β-benzyl L-aspartate) and poly(L-proline).[20–31] It has been shown that the concept of the ^{13}C chemical shift/structure correlation that has been clarified by the experimental and calculated results is a very useful means for the structural characterization of polymer and biopolymer systems. For example, the experimental ^{13}C chemical shifts for the α-helix, ω-helix and β-sheet forms of polypeptides in the solid state have been verified to be conformation-dependent.[24,25]

Next, we are concerned with the crystallographic effects on the ^{13}C chemical shifts of polyethylene, which is in the orthorhombic form when obtained under ordinary state conditions and in the monoclinic form when obtained under special state conditions. It is well known that the orthorhombic form is more stable than the monoclinic form. In the former, the all-*trans* zigzag planes are perpendicular to each other but are parallel to each other in the latter. The ^{13}C chemical shift calculations of polyethylene have been carried out with the TB CNDO/2 SOS method by using three infinite polyethylene chains in the orthorhombic and monoclinic forms by changing the interchain distance R.[26] The dependence of the calculated ^{13}C chemical shift (σ) of the CH_2 carbons of the central polyethylene chain in orthorhombic and monoclinic polyethylenes on the interchain distance R has been employed. The calculations have been carried out by varying R from 3 to 5 Å. For the orthorhombic form, the ^{13}C chemical shift (σ_{orth}) moves upfield as R increases from 3 to 4.5 Å and downfield as R increases beyond 4.5 Å. For the monoclinic form, the overall tendency of the R dependence on the ^{13}C chemical shift (σ_{mono}) is close to that for the orthorhombic form except that the value of R at which the downfield shift occurs is approximately 4.0 Å. It was determined from X-ray diffraction that the R value for the orthorhombic form is 4.0 Å and that for the monoclinic form is 4.08 Å. At these R values, the ^{13}C chemical shift values (σ) for the orthorhombic and monoclinic forms are −58.6 and −56.7 p.p.m., respectively, where the negative sign denotes deshielding. Therefore, as the experimental chemical shift (δ) is deshielding, the relative difference in the calculated chemical shift (σ) should be compared with the observed δ. This means that the expective ^{13}C chemical shift of orthorhombic polyethylene appears at a higher field by 1.9 p.p.m. than that of monoclinic polyethylene. As the experimental ^{13}C chemical shift of orthorhombic polyethylene appears at a higher field by 1.2 p.p.m. than that of monoclinic polyethylene, the calculations reasonably explain the experimental results. From this result, it can be seen that such a chemical shift displacement is caused by a local change in the intermolecular interactions when going from the orthorhombic form to the monoclinic form. Furthermore, the ^{13}C chemical shifts and the electronic structure for seven polyethylene chains with the orthorhombic and monoclinic forms have been studied by using the TB INDO/S SOS method.[30] The ^{13}C chemical shift calculations reasonably explain the experimental results. From these results, the angle φ between the b axis in the orthorhombic lattice system and the *all-trans zigzag* plane was determined to be 25°–42°. The total energy for the unit cell was determined to be −95.1997 eV for orthorhombic polyethylene and −95.1989 eV for monoclinic polyethylene. These calculated results agree with the experimental results that the orthorhombic form is more stable than the monoclinic form.

Three-dimensional (3D) polymer crystals

The protocol for calculating the NMR chemical shift of a 3D polymer crystal by a combination of *ab initio* TB MO theory and the SOS method of the chemical shift theory has been derived.[32] This has the potential for understanding and developing NMR chemical shift/

structure correlations for the structural characterization of 3D polymer crystals, organic crystals, and so on.

This protocol has been applied to the calculation of the ^{13}C NMR chemical shifts of 3D polyethylene crystals with the orthorhombic and the monoclinic forms by using the STO-3G minimal basis set. It is noted that the ^{13}C chemical shift position of polyethylene with the orthorhombic form appears at a higher field by approximately 1 p.p.m. than that of the monoclinic form. It is very significant to clarify whether or not such a chemical shift difference comes from the difference in the electronic structure between the crystallographic forms. By using three and seven infinite polyethylene chains as the orthorhombic and monoclinic models in a semi-empirical MO framework, it was approximated that the chemical shift difference comes from the packing effect arising from different crystallographic forms. For this analysis, the effects of the interchain and intrachain interactions on the ^{13}C NMR chemical shift and the band structure have been sophisticatedly evaluated by changing the *a*, *b* and *c* axis lattice constants in a polymer crystal.

Furthermore, this *ab initio* MO approach for a polymer crystal has been applied to elucidate the effect of the intrachain and interchain interactions on the ^{13}C chemical shifts of *cis*- and *trans*-polyacetylenes in the solid state.[33] Before expanding the approach to a 3D polyacetylene crystal, it is useful to examine a single polyacetylene chain with the *trans*- and *cis*-forms to clarify the intermolecular interaction effect of the electronic structure on the NMR chemical shift behavior. The total energy per monomer unit and the NMR chemical shielding for a single polyacetylene chain with the *cis*- and *trans*-forms are obtained by using the *ab initio* TB MO method within the framework of the STO-3G minimal basis set. It was shown that the total energy per monomer unit for *trans*-polyacetylene is lower than that for *cis*-polyacetylene by 0.0069 a.u. This means that the *trans*-form is more stable than the *cis*-form. The trends in the calculated results qualitatively explain the experimental finding that undoped *trans*-polyacetylene is thermally more stable than *cis*-polyacetylene. Next, we are concerned with the calculated chemical shieldings for *cis*-and *trans*-polyacetylenes. The experimental ^{13}C chemical shift of the *cis*-form appears at a higher field by 10 p.p.m. than that of the *trans*-form. However, the calculation shows that the ^{13}C chemical shift of the *cis*-form is at slightly a higher field by 0.1 p.p.m. than that of the *trans*-form. When compared with the experimental results, the chemical shift difference between the *cis*- and *trans*-forms is very small. It has been shown that the single polymer chain model is insufficient to reasonably explain the experimental results and that the interchain interactions must be taken into account in a 3D polymer crystal.

The electronic structures and ^{13}C chemical shifts of 3D infinite *cis*- and *trans*-polyacetylene crystals with the orthorhombic crystallographic form have been calculated by using the *ab initio* TB MO method within the framework of the STO-3G minimal basis set. The total energies per monomer unit and the chemical shifts have been obtained. The total energy per monomer unit for the *trans*-form is lower than that of the *cis*-form by 0.0024 a.u. The *trans*-form is reasonably predicted to be more stable than the *cis*-form. The ^{13}C chemical shift of the *cis*-form in polyacetylene crystal appears at a higher field by 1.0 p.p.m. than that of the *trans*-form; this was calculated by using the experimental lattice parameters. The calculated results more closely approach the experimental values compared with the results from the single polymer chain model. This shows that the intermolecular interactions have an important role for the ^{13}C chemical shift behavior. When the lattice parameter *a* is reduced to 5 Å (signifying an increase in the intermolecular interactions), the ^{13}C chemical shift difference between the *cis*- and *trans*-forms becomes 4.5 p.p.m. Then, when the length of the lattice parameter *a* is approximately 4.7 Å, the calculated ^{13}C chemical shift difference becomes 10 p.p.m. and then agrees with the experimental value. This result indicates that the ^{13}C chemical shift is a very sensitive parameter for investigating the intermolecular interactions in a polymer crystal. From the above results, it has been shown that structural elucidation of polymer crystals and organic crystals can be carried out by a combination of the NMR ^{13}C chemical shift experiments and the 3D polymer crystal MO/chemical shift method developed by us.

CONFORMATION-DEPENDENT NMR CHEMICAL SHIFTS OF PEPTIDES AND POLYPEPTIDES IN THE SOLID STATE

The primary structures of synthetic polypeptides consisted of repeating sequences of certain amino acid residues are not as complicated as those in proteins. Synthetic polypeptides are very important polymers in polymer science and protein science because the characteristic properties related to the structure lead to the expansion of research fields in polymer science and are very different from conventional synthetic polymers. Furthermore, synthetic polypeptides are sometimes used as model biomolecules for proteins because they form the α-helix, β-sheet, ω-helix, and so on. under appropriate conditions. From such situations, synthetic polypeptides can be considered to be 'interdisciplinary' macromolecules that are very important for research works in both polymer science and protein science.

As is well known, most of the peptides, polypeptides and proteins considered here consist of repeating sequences of peptide bonds with 20 different types of substituents at the C_α carbon. The limited conformations such as the α-helix, ω-helix, β-sheet, and so on. are taken into consideration by using a set of the possible dihedral angles (Φ, Ψ) around the N-C_α and C_α-C(= O) bonds. In the solution state, the NMR chemical shift of these biomolecules with the possible rotation around the bonds sometimes becomes the averaged value for the possible rotations around the peptide bonds in the NMR timescale. In the solid state, however, the chemical shift is characteristic of specific conformations because the internal rotation around the peptide bonds is fixed. This shows that the NMR chemical shift can be used to elucidate the conformation of polypeptides and proteins in the solid state. It has been experimentally and theoretically shown that the NMR chemical shift of polypeptides and proteins is a very important NMR parameter for determining the main-chain conformation, and some studies on the structural characterization of polypeptides and proteins by using such a methodology will be discussed below.

Conformation-dependent ^{13}C chemical shifts

For example, it has been shown that the ^{13}C chemical shifts of the C_α, C_β and amide C = O carbons of poly(L-alanine) ($(Ala)_n$) are closely related to its particular conformations.[34] The observed ^{13}C CP-MAS (cross polarization—MAS) NMR spectra of solid poly(L-alanine) show the C_α, C_β and amide C = O carbon signals to be well resolved between the α-helix and β-sheet forms. It has also been shown from the plots of the observed ^{13}C chemical shifts against the number-average degree of polymerization that the ^{13}C chemical shifts of $(Ala)_n$ with the α-helix form ($n > 16$) and the β-sheet form ($n < 16$) are unchanged for the peptides of various molecular weight within the experimental error, and thus can serve to characterize the α-helix form and the β-sheet form. The chemical shifts of the C_α and carbonyl carbons of the α-helix form are significantly displaced downfield by 4.2 and 4.6 p.p.m., respectively, relative to those of the

β-sheet form, whereas the chemical shift of the C_β carbon of the α-helix form is displaced upfield by approximately 5 p.p.m. with respect to that of the β-sheet form. For this reason, the ^{13}C chemical shift values can be used to describe the local conformation. In addition, the ^{13}C chemical shift of randomly coiled $(Ala)_n$ in trifluoroacetic acid solution has a value between those of the α-helix form and β-sheet form.

The existence of such characteristic displacements of ^{13}C chemical shifts is not limited to the Ala residue. Table 1 summarizes the ^{13}C chemical shift values of various amino acid residues in the α-helix and β-sheet forms relative to tetramethylsilane.[35–38,40] Here, it is seen that the C_α and C=O peaks of the α-helix form are all displaced downfield with respect to those of the β-sheet form, consistent with the experimental data of $(Ala)_n$. Furthermore, the ^{13}C chemical shift values are significant for the various conformations of poly(β-benzyl L-aspartate) (PBLA; $(Asp(OBzl))_n$), such as the right-handed $\alpha(\alpha_R)$-helix, left-handed $\alpha(\alpha_L)$-helix, left-handed $\omega(\omega_L)$-helix and β-sheet forms, which are achieved by appropriate treatments to the polypeptide chain.[41] It is shown that the absolute ^{13}C chemical shifts of the C_α and C_β carbons are affected by the chemical structure of the individual amino-acid residues and can be used effectively for conformational studies of the particular amino acid residues in polypeptides and proteins. However, the C=O chemical shifts do not seem to be affected by the residue structure and thus can be used to determine the main-chain conformation. This method has been applied for the structural characterization of collagen proteins,[42] wool keratin,[43,44] silk protein,[45] polypeptide liquid crystals,[46,47] polypeptide gels,[48,49] and polypeptide blends.[50,51] Other peptide systems analyzed by this method are reviewed in Saito *et al.*[40,52]

It is known that changes in the helix sense of polypeptides in the solid state often occur because of changes in external conditions, such as temperature and so on, and changes in the side-chain conformation also often induce changes in the helix sense of the main-chain or the other conformations. The change in helix sense subsequently changes the nature of the polypeptides. PBLA samples with various secondary structures can be obtained by heat treatment with different temperatures.[41] This has been demonstrated by real-time temperature-variable ^{13}C CP-MAS NMR measurements.[53] It was seen that PBLA takes the α_R-helix form at room temperature, and by elevating the temperature, the α_R-helix form is gradually changed to the ω_L-helix form and, at the same time, slightly to the β-sheet form. Then, at approximately 150 °C, the α_R-helix form completely disappears and the ω_L-helix form becomes the major component, and at 173 °C, the ω_L-helix form is completely changed to the β-sheet form. Furthermore, real-time temperature-variable ^{13}C CP-MAS NMR measurements have shown[54,55] that poly(γ-*n*-alkyl L-glutamate) with long *n*-octadecyl side chains forms the thermotropic liquid-crystalline state by the melting of the side chains at approximately 50 °C because the main chain only takes the rigid α-helix form at temperatures higher than approximately 50 °C, but poly(β-*n*-alkyl L-aspartate) with long *n*-octadecyl side chains does not form the thermotropic liquid-crystalline state by the melting of the side chains at approximately 50 °C because the main-chain changes from the α_R-helix form to the α_L-helix form at temperatures higher than approximately 50 °C and thus is not rigid. Such a transitional change in the helix sense from the right-handed helix form to the left-handed helix form induces changes in the nature of the polypeptides.

Conformation-dependent ^{15}N chemical shifts

Conformation-dependent ^{15}N chemical shifts of solid homopolypeptides, such as ^{15}N-labeled poly(L-alanine) with the α-helix and β-sheet forms, poly(L-leucine) with the α-helix and β-sheet forms, and so on,[56,57] have been studied by solid state ^{15}N CP-MAS NMR as for the conformation-dependent ^{13}C chemical shift case. It has been confirmed from these experimental results that the isotropic ^{15}N chemical shifts caused by the peptide backbone of homopolypeptides in the solid state exhibit a significant conformation-dependent change from observations and theoretical calculations. It has been shown that the δ_{iso}s for the α-helix form of the homopolypeptides are 97.0–99.2 p.p.m. relative to Gly-^{15}N and those for the β-sheet form are 99.0–107.0 p.p.m., and thus the α-helix form appears upfield by approximately 1.2–10.0 p.p.m. with respect to the β-sheet form. The conformation-dependent ^{15}N chemical shift obviously depends on the structure of the individual amino-acid residues. Some ^{15}N chemical shift differences are rather small, but they should be within acceptable limits for homopolypeptides. The variations of the δ_{iso} values for various types of homopolypeptides are approximately 2.5 p.p.m. in the α-helix form and approximately 7.5 p.p.m. in the β-sheet form. Additionally, the δ_{iso} values of the β-sheet form of the L-Leu, L-Val and L-Ile residues with alkyl side chains appear downfield with respect to that of the L-Ala residue. In contrast, the δ_{iso} values for the β-sheet form of L-Asp(OBzl), L-Glu(OBzl)(γ-benzyl L-glutamate) and L-Glu(OMe)(γ-methyl L-glutamate) residues with a side-chain ester appear upfield with respect to the δ_{iso} value of the L-Ala residue. This means that the ^{15}N chemical shift difference between the α-helix and β-sheet forms depends on the side-chain structure of the individual amino-acid residues. Another result is that the δ_{iso} values of the right-handed $\alpha(\alpha_R)$-helix and left-handed $\alpha(\alpha_L)$-helix forms of PBLA in the solid state are 99.2 and 97.0 p.p.m., respectively, and thus the former appears at a lower field by 2.2 p.p.m. than the latter. This means that the ^{15}N δ_{iso} results provide information about the helix sense as for the ^{13}C δ_{iso} results.

Isotropic ^{15}N chemical shifts and tensor components have been determined by solid-state ^{15}N CP-MAS and CP NMR, respectively, for a series of polypeptides $[Ala^*, X]_n$ containing ^{15}N-labeled L-alanine (Ala*) and other amino acids (X: residues with a natural abundance of ^{15}N), such as glycine, L-alanine, D-alanine, L-valine, β-benzyl-L-aspartate, and so on. The conformations for these polypeptides are characterized on the basis of their conformation-dependent ^{13}C chemical shifts.[58] It has been demonstrated that the δ_{iso} is useful for the conformational study of copolypeptides with identical primary structures (amino-acid sequences), and in addition, the δ_{22} value of the Ala* residue in a copolypeptide is closely related to the main-chain conformations (such as the right-handed and left-handed α-helices and β-sheet forms) rather than the amino-acid sequence. Thus, the ^{15}N chemical shifts of solid polypeptides is conformation-dependent.[59]

Conformation-dependent ^{17}O chemical shifts

Conformation-dependent ^{17}O NMR chemical shifts of the amide carbonyl carbons of the Gly residue of solid polyglycine with the 3_1-helix and β-sheet forms and of the Ala residue of solid poly(L-alanine) with the α-helix and β-sheet forms have been studied to develop a methodology for structural characterization in addition to the analysis of the ^{13}C and ^{15}N nuclei of the amino-acid residues of polypeptides.[60] To accomplish this, we must overcome two difficult problems. The first problem is that the ^{17}O nucleus has a very low natural abundance of 0.037%. The second problem is that because the ^{17}O nuclear spin number is 5/2, the quadrupolar splitting of the carbonyl oxygen of the amino-acid residue in solids is very large and thus the amide ^{17}O signal is extremely broad. This implies that it is not easy to determine reliable ^{17}O chemical shift in solids. For this

Table 1 Conformation-dependent ^{13}C chemical shifts of amino-acid residues in solid polypeptides (p.p.m. from tetramethylsilane)[a,b]

Amino-acid residues	C_α				C_β				*amide C=O*			
	α-helix	*β-sheet*	*random coil*[c]	Δ^d	*α-helix*	*β-sheet*	*random coil*[c]	Δ^d	*α-helix*	*β-sheet*	*random coil*[c]	Δ^d
Ala												
	52.4	48.7	51.1	4.2	14.9	19.9	15.7	−5.0	176.4	171.8	176.1	4.6
	52.3	48.7		3.6	14.8	20.0		−5.2	176.2	171.6		4.6
	52.8	49.3		3.5	15.5	20.3		−4.8	176.8	172.2		4.6
Leu												
	55.7	50.5		5.2	39.5	43.3		−3.8	175.7	170.5	175.7	5.2
	55.8	51.2		4.6	43.7[e]	39.6[e]		(4.1)	175.8	171.2		4.5
Val												
	65.5	58.4	61.2	7.1	28.7	32.4	31.7	−3.7	174.9	171.8	174.4	3.1
	58.2				32.4				171.5			
Ile												
	63.9	57.8	61.1	6.1	34.8	39.4	37.1	−4.6	174.9	172.7	175.8	2.2
		57.1				33.1			171.0			
Glu(OBzl)												
	56.4	51.2		5.2	25.6	29.0		−3.4	175.6	170.4		5.3
	56.8	51.1		5.7	25.9	29.7		−3.8	175.4	172.2		3.2
Asp(OBzl)												
	53.4	49.2		4.2	33.8	38.1		−4.3	174.9	169.8		5.1
	53.6[f]				34.2[f]				174.9			
Lys[e]												
	57.4				29.9				176.5			
Lys(Z)												
	57.6	51.4		6.2	29.3	28.5		−0.8	175.7	170.4		5.3
Arg[e]												
	57.1				28.9				176.8			
Phe												
	61.3	53.2		8.1	35.0	39.3		−4.3	175.2	169.0		6.2
Met												
	57.2	52.2		5.0	30.2	34.8		−4.6	175.1	170.6		4.5
Gly												
	52.2[b]	43.2		9.0					176.7[b]	168.5		8.2

[a]Saito *et al.*,[35] Saito and Ando,[36] Ando *et al.*[37] and Saito.[38]
[b]Chemical shift values of Gly residue with the α-helix incorporated into α-helical poly(L-alanine). Ando *et al.*[39]
[c]In CF_3COOH solution, a few drops of H_2SO_4 were added in the case of $(Ile)_n$ and $(Leu)_n$.
[d]Difference in the ^{13}C chemical shifts of the α-helix form relative to those of the β-sheet form.
[e]This assignment should be reversed.
[f]Erroneously assigned the left-handed α-helix.

reason, we have developed an ^{17}O-labelling method for preparing polyglycine and poly(L-alanine) with 6% and 10% ^{17}O-labeled amide carbonyl oxygens of the Gly and Ala residues, respectively,[60–62] and have performed solid-state ^{17}O NMR measurements with different NMR frequencies[61,63,64] and with high NMR frequency and high-speed MAS[64,65] and performed ^{17}O spectral analysis.[66]

Solid-state ^{17}O NMR spectra of Gly- and Ala-containing peptides and polypeptides have been measured by using the static CP method at certain NMR frequencies (270, 400 and 500 MHz for ^1H).[61] The chemical shift (δ), quadrupolar coupling constant (e^2qQ/h) and asymmetric parameter of the electric field gradient (η) have been determined by computer simulations of the obtained spectra. First,

the isotropic chemical shifts and tensor components are employed. From the static CP experiments, it was determined that the δ_{iso} values for the 3_1-helix and β-sheet forms in polyglycine[61] are 288 and 299 p.p.m., respectively, relative to external liquid water ($\delta = 0$ p.p.m.), and thus the 3_1-helix form appears at a higher field by 11 p.p.m. than the β-sheet form. The static CP experiments also determined that the δ_{iso} values for the α-helix and β-sheet forms in poly(L-alanine)[63] are 303 and 265 p.p.m., respectively, and thus the α-helix form appears at a lower field by 38 p.p.m. than the β-sheet form. This indicates that the ^{17}O δ_{iso} is very sensitive to the secondary structure and thus is conformation dependent. Furthermore, we are concerned with the tensor components (δ_{11}, δ_{22} and δ_{33}, from downfield to upfield), although the experimental errors are not so small due to the broad spectra. It has been determined that the tensor components (δ_{11}, δ_{22} and δ_{33}) for the β-sheet and 3_1-helix forms in polyglycine[61] are (574, 425 and -101 p.p.m.) and (562, 410 and -108 p.p.m.), respectively, and that the tensor components (δ_{11}, δ_{22} and δ_{33}) for the α-helix and β-sheet forms in poly(L-alanine)[63] are (595, 435 and -121 p.p.m.) and (514, 390 and -110 p.p.m.), respectively. From these experimental results, it is seen that all of the tensor components for the β-sheet form in polyglycine appear at a lower field than the 3_1-helix form in polyglycine, and in poly(L-alanine), the δ_{11} and δ_{22} for the α-helix form appear at a lower field than the β-sheet form, and the δ_{33} for the α-helix form appears at a higher field than for the β-sheet form.

Furthermore, to determine the exact isotropic ^{17}O chemical shift value, a combination of high-field magnetic and high-speed MAS methods has been used. As one example, the high-resolution solid-state NMR spectra of solid poly(L-alanine)s with the α-helix and β-sheet forms have been measured by using a high-field magnet (800 MHz for ^1H) and a high-speed MAS (25 kHz) as shown in Figure 1.[64] The δ_{iso} values for the α-helix and β-sheet forms have been determined to be 319 and 286 p.p.m., respectively. It is shown that the α-helix form appears at a lower field by 33 p.p.m. than the β-sheet form. These values are approximately those determined by the static CP method. It is seen from these results that the conformation-dependent ^{17}O chemical shift is remarkably larger than the corresponding conformation-dependent ^{13}C and ^{15}N chemical shifts. However, the δ_{iso} values for the 3_1-helix and β-sheet forms in solid polyglycine are determined to be 293 and 304 p.p.m., respectively. It is shown that the 3_1-helix form appears at a higher field by 11 p.p.m. than the β-sheet form. From these results, it is seen that these values are approximately those determined by static CP method. Furthermore, we can obtain the e^2qQ/h value from the observed ^{17}O NMR spectral analysis. It has been determined that the e^2qQ/h values for the α-helix and β-sheet forms in poly(L-alanine) are 8.59 and 8.04 MHz, respectively, and those for the 3_1-helix and the β-sheet forms in polyglycine are 8.21 and 8.36 MHz, respectively. The e^2qQ/h will be discussed as related to the length of the amide hydrogen bond $>C=O\cdots H\text{-}N<$[67–69] and the experimental results[64,65] and theoretical calculations[66] will be compared under the section head Isotropic ^1H chemical shifts.

Lemaitre *et al.*[70] measured the solid-state MAS ^{17}O NMR spectra of a selectively labeled transmembrane peptide, ^{17}O-[L-Ala12]-WALP23, that was single-labeled at Ala-12 as a lyophilized powder and incorporated in hydrated vesicles at 81.345 and 108.419 MHz. The spectral pattern is very similar to that of $(Ala)_n$ with the α-helix form.[64] The isotropic chemical shift for the labeled peptide at 317.5 p.p.m. is very close to that of $(Ala)_n$ with the α-helix form at 319 p.p.m., therefore, the peptide takes the α-helix form. The MAS ^{17}O spectrum was measured at 81.345 MHz for ^{17}O-[L-Ala12]-WALP23 in hydrated DSPC vesicles (1:10 peptide/lipid molar ratio) with a single resonance from the label inserted in alanine-12 centered at approximately 280 p.p.m. The lyophilized MLV sample was hydrated with one weight equivalent of water, and the spectrum was acquired at room temperature with the lipids in the liquid crystalline phase. Recently, the number of solid-state ^{17}O NMR studies on the structures of various types of peptides and biomolecules has been increasingly reported.

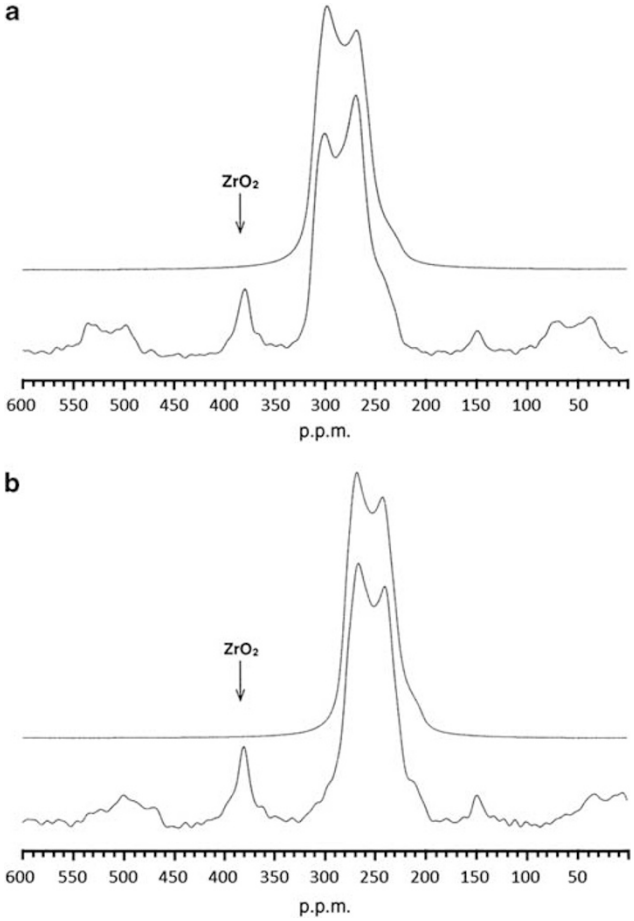

Figure 1 The observed and theoretically simulated MAS ^{17}O (**a**) NMR spectra spun at 25 kHz for solid $(L\text{-Ala})_n$, (**b**) $n = 100$, with an α-helix form and solid $(L\text{-Ala})_n$, $n = 5$, with a β-sheet form at 108.6 MHz (800 MHz for ^1H) at room temperature. From Yamauchi *et al.*[64]

Conformation-dependent ^1H chemical shifts

Shoji *et al.*[71,72] showed using ^1H CRAMPS (combined rotation and multiple pulse spectroscopy) measurements on various homopolypeptides in the solid state that the ^1H chemical shifts of the H_α signal of homopolypeptides with the α-helix and β-sheet forms are 3.9–4.0 p.p.m. and 5.1–5.5 p.p.m., respectively, relative to tetramethylsilane. Thus, the solid-state ^1H chemical shift is as conformation-dependent as the ^{13}C, ^{15}N and ^{17}O chemical shifts, which is very useful for conformational analysis of polypeptides in the solid state.

Yamauchi *et al.*[73,74] developed a new NMR technique for measuring high-resolution solid-state ^1H NMR by using the FSLG-2 (frequency-switched Lee-Goldburg) homonuclear dipolar decoupling method combined with high-speed MAS and have carried out the structural characterization of polypeptides in the solid state, as described below.

By using this technique, the structural characterization of Nylon 6, a polyamide, in the solid state has been performed by measuring solid-state ^{1}H NMR spectra over a wide range of temperatures.[75]

CONCEPT OF NMR CHEMICAL SHIFT MAP

In the crystalline state, structural information obtained from the chemical shift of polypeptides corresponds to the fixed conformation as mentioned above. The calculation of the ^{13}C chemical shifts for the dipeptide fragments (*N*-acetyl-*N*′-methyl-L-alanineamide) [Ac-L-Ala-NHMe] of poly(L-alanine) and L-alanine residues containing proteins has been attempted using the FPT (finite perturbation theory) INDO method as functions of the dihedral angles (Φ, Ψ), varied in 15° intervals. A contour map has been made as a function of Φ and Ψ in the transversal and longitudinal axes, respectively, for the ^{13}C chemical shift using the obtained chemical shift values to understand and predict the ^{13}C chemical shift behavior of polypeptides associated with the secondary structure elements, such as the α-helix, β-sheet, and so on.[76] This chemical shift map is a very useful representation of the chemical shift behavior resulting from changing the dihedral angles as in a Ramachandran energy map. By comparing the experimental data and the predicted values given by this chemical shift map, the ^{13}C chemical shifts of Ala residues in polypeptides and proteins can be successfully predicted. This has been used to understand the ^{13}C chemical shift behavior of collagen protein[42] and silk proteins[45] in the solid state.

Furthermore, *ab initio* calculations for the NMR chemical shifts have become available for medium-size molecules as a consequence of remarkable advances in the performance of workstations, personal computers and supercomputers. This allows for a quantitative discussion on the chemical shift behavior. As an example, the *ab initio* MO calculation with the 4–31G basis set using the GIAO-CHF (gauge-independent atomic orbital-coupled Hartree–Fock) method was carried out on *N*-acetyl-*N*′-methyl-L-alanineamide, the same model molecules as the case of the above FPT INDO calculation.[77] The isotropic ^{13}C chemical shift map of the C_β carbon as a function of the dihedral angles (Φ, Ψ) was calculated as shown in Figure 2, where the positive sign indicates shielding. The overall trend of this map is similar to that obtained by the FPT INDO method. The calculated isotropic chemical shift (σ) for the C_β carbon are 186.4 p.p.m. for the dihedral angles (Φ, Ψ) corresponding to the anti-parallel $\beta(\beta_A)$-sheet form, 189.4 p.p.m. for the right-handed $\alpha(\alpha_R)$-helix form and 189.6 p.p.m. for the left-handed $\alpha(\alpha_L)$-helix form, and the observed isotropic chemical shifts (δ) are 21.0 p.p.m. for the β_A-sheet form, 15.5 p.p.m. for the α_R-helix form and 15.9 p.p.m. for the α_L-helix form. Such experimental chemical shift behavior is well explained by the calculated chemical shift behavior. It is found that the changes in the dihedral angles (Φ, Ψ) dominate the isotropic chemical shift behavior of the C_β carbon of the L-alanine residue.

As mentioned above, the principal values of the chemical shift tensor provide information about the 3D electronic state of a molecule. However, to understand the behavior of the principal values, one should obtain information about the orientation of the principal axis system of the chemical shift tensor with respect to the molecular fixed frame. The orientations of the principal axis systems of the chemical shift tensors of the C_β-carbons in Ala have been calculated for some peptides whose Ala moieties have different main-chain dihedral-angles: (Φ, Ψ) = ($-57.4°$, $-47.5°$) (α_R-helix form), ($-138.8°$, $134.7°$) (β_A-sheet form), ($-66.3°$, $-24.1°$) (3_{10}^R-helix form) and ($-84.3°$, $159.0°$) (3_1-helix form). The σ_{33} component nearly lies along the C_α-C_β bond for all the peptides considered here,

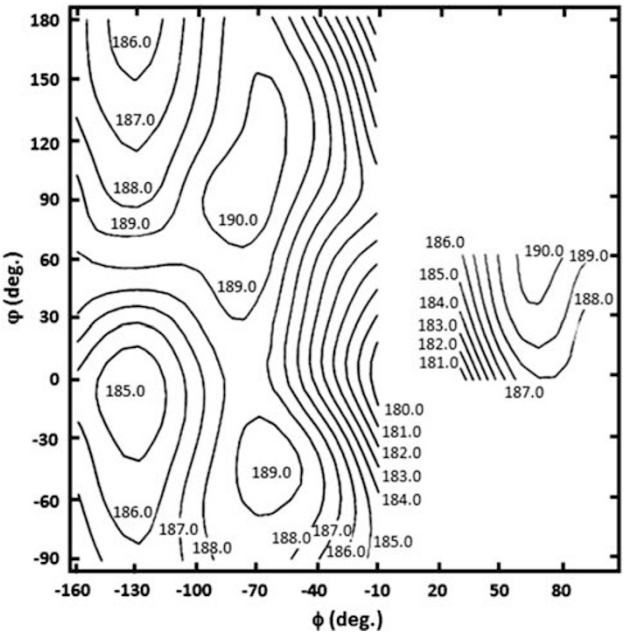

Figure 2 The isotropic ^{13}C chemical shift contour map for the C_β carbon of the L-alanine residue with the dihedral angles (Φ, Ψ) calculated by using the GIAO-CHF method with the 4–31 G *ab initio* basis set according to the concept of the NMR chemical shift map,[76] in p.p.m. The 4–31 G basis set optimized the geometries for the model molecules, *N*′-acetyl-*N*′-methyl-L-alanine amide. From Asakawa *et al.*[77]

and the σ_{11} is nearly also perpendicular to the plane defined by the C_β, C_α, and N atoms in the Ala residue.[78] The σ_{22} component is parallel to the plane. These results agree with the experimentally determined direction of the σ_{33} for the C_β-carbon in the L-Ala amino-acid residue by Naito *et al.*[79] The σ_{11} component for the dihedral angles corresponding to the β_A-sheet form is 37.06 p.p.m. This shows a downfield shift of approximately 9 p.p.m. with respect to that for the α_R-helix form. This means that the σ_{11} dominates the downfield shift on the isotropic chemical shift of the C_β carbon for the β_A-sheet form. Because the σ_{11} is not oriented along a specified chemical bond, it is not easy to intuitively comprehend 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 might be important for understanding the σ_{11} behavior.

For all of the dihedral angles employed in the calculations, the σ_{33} component of the chemical shift tensor of L-alanine C_α-carbons lies approximately along the C_α-$C'(=O)$ bond. However, for the dihedral angles (Φ, Ψ) = ($-57.4°$, $-47.5°$) (α_R-helix form), ($-66.3°$, $-24.1°$) (3_{10}^R-helix form) and ($-84.3°$, $159.0°$) (3_1-helix form), the σ_{22} component diverges slightly from the C_α-C_β bond and for (Φ, Ψ) = ($-138.8°$, $134.7°$) (β_A-sheet form), the σ_{11} component is along this direction. The tensor component that is nearly along the C_α-C_β bond is 47.53 p.p.m. for the β_A-sheet form, 61.93 p.p.m. for the α_R-helix form, 64.74 p.p.m. for the 3_{10}^R-helix form and 65.79 p.p.m. for the 3_1-helix form. Changes in the dihedral angles cause a large deviation of the chemical shift tensor component that is along the C_α-C_β bond. Moreover, because the σ_{33} depends on changes from one dihedral angle to another, it is obvious that the dihedral angle is explicitly dependent on the σ_{33}. It is thought that if the carbonyl group in the Ala residue forms a hydrogen bond, the σ_{33} will be most likely affected. de Dios *et al.*[80] studied the ^{13}C chemical shift behavior

of polypeptides and proteins by using *ab initio* MO calculation of the ^{13}C chemical shifts of several types of oligo-peptides such as in the representation of the FPT INDO ^{13}C chemical shift map as a function of the dihedral angles.

Another significant and empirical correlation of the ^{13}C chemical shift map has been made by Spera and Bax[81] on the basis of a database of the experimental ^{13}C chemical shift values of 442 residues of various globular proteins in solution for which the dihedral angles (Φ, Ψ) are known with good precision by X-ray work, resolved at 1.0–2.2 Å. Their ^{13}C chemical shift maps of the C_α and C_β carbons show the distribution of the secondary shifts in the α-helix and β-sheet forms. These are consistent with the solid state ^{13}C chemical shifts of homopolypeptides. For the C_α carbon, the average secondary shift for the α-helix form (119 residues) appears at a lower field by approximately 4.6 p.p.m. compared with the β-sheet form (126 residues), and for the C_β carbon, the former appears at a higher field by approximately 2.6 p.p.m. The chemical shift dispersion collected from globular proteins in solution is much larger than that from solid polypeptides. In addition, the database, containing 3796 $^{13}C_\alpha$ chemical shifts and 2794 $^{13}C_\beta$ chemical shifts as obtained from 40 different proteins, was used for the preparation of the chemical shift maps by Asakura *et al.*[82,83] It is shown that the chemical shift map is useful for the structural characterization of proteins, and the solution conformation of the protein is essentially similar to that in the solid state.

NMR CHEMICAL SHIFTS AND HYDROGEN-BONDED STRUCTURES ASSOCIATED WITH THE SECONDARY STRUCTURE OF SOLID PEPTIDES AND POLYPEPTIDES

It is well known that hydrogen bonds have an important role in forming the secondary structures of peptides and polypeptides, including proteins. Thus, the nature of the hydrogen bond has been widely studied by various spectroscopic methods.[84] High-resolution NMR spectroscopy has also been used as one of the most powerful means for obtaining useful information about details of the hydrogen-bonded structure as associated with the secondary structure. Therefore, the chemical shifts of the amide carbonyl carbon, amide nitrogen, amide carbonyl oxygen and amide hydrogen of the amino-acid residues associated with hydrogen bonding have provided useful information about the secondary structure of polypeptides.[85]

The NMR chemical shift is one of the most important parameters for providing information about molecular structures including hydrogen bonds. Because the electronic structure around the amide carbonyl-carbon and nitrogen in peptides and polypeptides is greatly affected by the nature of the hydrogen bond, the NMR chemical shifts for the nuclei involved are sensitive to the spatial arrangement of the nuclei comprising the hydrogen bond.

However, it is difficult to exactly determine the effect of hydrogen bonding on the chemical shifts in solution because the observed chemical shifts of peptides are often the averaged value over rotational isomers resulting from the interconversion caused by rapid rotations about the bonds. In contrast, the chemical shifts in the solid state provide direct information about the hydrogen bond of the peptides and polypeptides with a fixed conformation. Taking advantage of this, systematic studies have been performed on the effect of hydrogen bonding on the chemical shifts of the amide carbonyl carbon, amide nitrogen, amide oxygen and amide hydrogen of the amino acid residues for peptides and polypeptides in the solid state to obtain detailed information about the hydrogen-bonded structure.[85] In this section, some of the studies on the hydrogen-bonded structure of peptides and polypeptides in the solid state based on measurements and theoretical calculations of NMR chemical shifts will be discussed.[59,85–87]

Isotropic ^{13}C chemical shifts and chemical shift tensor components

The effect of hydrogen bonding on the ^{13}C chemical shift of the carbonyl carbon has been studied in several amino-acid residues.[88–92] The observed isotropic chemical shifts (δ_{iso}) of the amide C=O carbons of the Gly, L-Ala, L-Val, L-Leu and L-Asp residues of peptides and polypeptides in the solid state were plotted against the N···O hydrogen bond length ($R_{N\cdots O}$), as shown in Figure 3. This figure shows that a decrease in $R_{N\cdots O}$ leads to a downfield shift. There is an approximately linear relationship between δ_{iso} and $R_{N\cdots O}$ for the peptides considered here, expressed as $\delta_{iso} = a - b\ R_{N\cdots O}$, where δ_{iso} is expressed in p.p.m., $R_{N\cdots O}$ is in Å, and a (in p.p.m.) and b (in p.p.m./Å) are constant. The values of a and b were calculated to be 206.0 and 12.4 for the Gly residue, 237.5 and 21.7 for the Ala residues, 202.2 and 10.0 for the Leu residue, 215.4 and 14.2 for the Val residue and 199.0 and 9.6 for the Asp residues, respectively, by using the least squares method. There is also an approximately linear relationship between the δ_{22} and $R_{N\cdots O}$ for the Gly and Ala residues, and the values of a and b were calculated to be 262.9 and 30.2 for the Gly residue and 344.7 and 54.5 for the Ala residue, respectively.

These relationships indicate that the hydrogen bond length can be determined through the observation of the ^{13}C chemical shift of the carbonyl carbon in the amino acid residues in peptides and polypeptides. The slope b of the variation of δ_{iso} against the hydrogen bond length for these amino acid residues decreases in the order Ala > Val > Gly > Leu ≈ Asp. The magnitude of the intercept decreases in the order Ala > Val > Gly > Leu ≈ Asp. The magnitude of the slope b decreases in the same order as the intercept a. These results show that the values of a and b are characteristic of individual amino-acid residues. It is noted that the amide carbonyl carbon chemical shifts

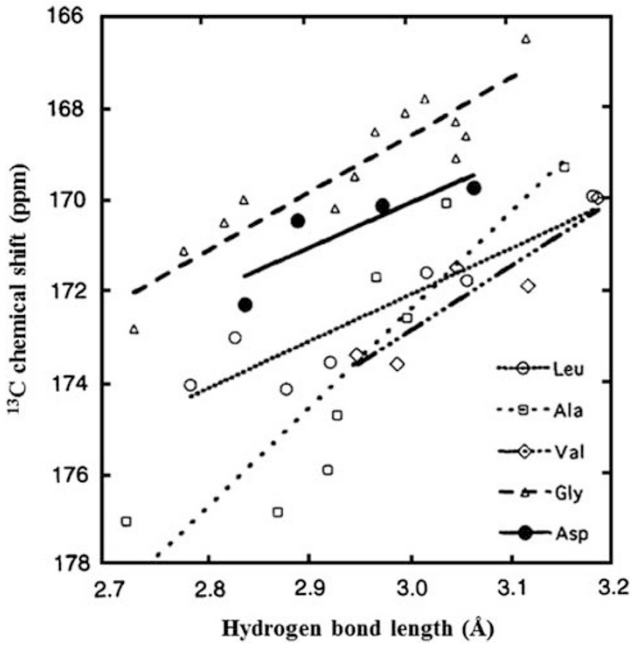

Figure 3 The correlation between the ^{13}C chemical shifts of the amide carbonyl carbons in Gly, L-Ala, L-Val, L-Leu and L-Asp residues of oligopeptides and polypeptides in the solid state and the hydrogen bond length between the amide nitrogen and the amide carbonyl oxygen ($R_{N\cdots O}$). ^{13}C chemical shifts are in p.p.m. relative to tetramethylsilane. From Tsuchiya *et al.*[90]

show this hydrogen bond length dependence not only in oligopeptides but also in polypeptides, such as polyglycine and poly(L-alanine). This suggests that the ^{13}C chemical shift of any amino acid carbonyl-carbon that accepts the hydrogen bond which is formed between the amide $>C=O$ and amide $>N$-H is strongly influenced by the hydrogen bond length.

Some Gly-containing peptides in the crystalline state have a hydrogen bond between the $>C=O$ and $-NH_3^+$, where the N-terminus is protonated as NH_3^+ and the C-terminus is unprotonated.[88] The carbonyl ^{13}C chemical shifts are linearly displaced upfield with decreasing $R_{N\cdots O}$, in contrast to the $>C=O\cdots H-N<$ hydrogen bond. Next, we consider the ^{13}C chemical shift calculations for the amide carbonyl carbons of some amino-acid residues by using the corresponding dipeptide hydrogen bonded with two formamide molecules in the calculations utilizing the FPT INDO method as a function of $R_{N\cdots O}$.[88] The theoretical calculations show that the isotropic ^{13}C chemical shifts for all of the amino-acid residues exhibit approximately linear downfield shifts for short $R_{N\cdots O}$ values. This experimental finding should be reasonably explained by the calculated results in the $R_{N\cdots O}$ region. However, the ^{13}C chemical shifts of the carbonyl carbon of the *N*-acetyl-*N*′-methylglycineamide hydrogen bond between the $>C=O$ and $-NH_3^+$ are calculated as a function of the hydrogen bond length. The ^{13}C chemical shift moves upfield for decreasing values of $R_{N\cdots O}$, agreeing with the experimental results. This trend is opposite to that for found peptides that have hydrogen bonds between the amide $>C=O$ and H-N$<$ groups.

Let us consider the ^{13}C chemical shift tensor behavior of the amide carbonyl carbon of peptides with a hydrogen-bonded structure. It has been reported that the δ_{11} component is in the amide sp^2 plane and lies along a direction normal to the C=O bond, the δ_{22} component lies almost along the amide C=O bond, and the δ_{33} component is aligned perpendicular to the amide sp^2 plane.[88,89] It is expected that the principal values of the ^{13}C chemical shift tensors (δ_{11}, δ_{22} and δ_{33}) are, in principle, more valuable as parameters for obtaining the detailed information about the hydrogen bonding that can be used to determine the electronic structure compared with the isotropic ^{13}C chemical shift ($\delta_{iso}=(\delta_{11}+\delta_{22}+\delta_{33})/3$).

The exact tensor components were determined from the Herzfeld–Berger analysis[93] of the spinning side bands of the ^{13}C CP—slow MAS NMR spectra of carbonyl ^{13}C-labeled Ala-containing peptides, such as AcAlaNHMe, AcAlaAibOMe, AlaGlyGly·H_2O, AlaSer and AlaProGly·H_2O, and poly(L-alanine) with the α-helix form.[89] The experimental δ_{22} values are the most sensitive to $R_{N\cdots O}$, and the δ_{22} values move linearly downfield with decreasing $R_{N\cdots O}$, except for the δ_{22} of AlaProGly·H_2O; the covalent bond between the Ala and Pro residues of AlaProGly·H_2O does not form a peptide bond but an imide bond. For this, the electronic structure of the Ala carbonyl-carbon in AlaProGly·H_2O is thought to be different from that of the Ala carbonyl-carbon forming the peptide bond, and hence, the chemical shift for the Ala carbonyl-carbon might be sensitive to both the $R_{N\cdots O}$ and the nature of the bonds. A decrease in $R_{N\cdots O}$ leads to a slight upfield shift in the δ_{11}, except for AlaProGly·H_2O. The experimental δ_{33} values are almost independent of $R_{N\cdots O}$, with some scatter on the data. With a decrease in $R_{N\cdots O}$, there is a large downfield shift of the isotropic ^{13}C chemical shifts, δ_{iso}, resulting from the behavior of the δ_{22} in overcoming that of the δ_{11}. McDermott *et al.*[94] obtained similar results on the amide carbonyl carbons of various types of amino acids.

To understand the relationships between the ^{13}C chemical shift and the hydrogen bond length, the FPT INDO calculations on the ^{13}C chemical shift tensor components of some model peptides have been performed.[88,89] The calculations show that the σ_{22} is the most sensitive to a change of $R_{N\cdots O}$ and moves linearly downfield with a decrease in $R_{N\cdots O}$. Correspondingly, the σ_{11} increases with a decrease in $R_{N\cdots O}$, whereas the σ_{11} is insensitive to changes in $R_{N\cdots O}$. The results of the theoretical calculations are in agreement with the experimental results. Such an agreement indicates that the changes in the ^{13}C chemical shift originate predominately from a change in the electronic state of the amino carbonyl groups caused by the variation in the hydrogen bond length.

Next, we are concerned with the relationship between the carbonyl ^{13}C chemical shifts and the $R_{N\cdots O}$ values for a Gly residue incorporated into $(Ala)_n$, $(Leu)_n$ and $(Val)_n$ to obtain structural information regarding how the corresponding Gly residue is incorporated with the same secondary structure into the host polypeptides.[95] The $R_{N\cdots O}$ value for the α_R-helix form is in the range of approximately 2.7–2.8 Å and that for the β-sheet form is approximately 3.0 Å, as determined from X-ray diffraction. The $R_{N\cdots O}$ values for the guest Gly residue incorporated into host copolypeptides can be determined by using the relation $\delta=a-bR_{N\cdots O}$ and observing the amide carbonyl ^{13}C chemical shifts, δ, of the guest Gly residue. The analysis of the ^{13}C chemical shift of the Gly residue revealed that the hydrogen bond lengths of the guest Gly residue ($R_{N\cdots O}$) are both 2.7 Å in $(Leu, Gly^*)_n$ and $(Ala, Gly^*)_n$, where the host Leu and Ala residues take the α_R-helix form and Gly* indicates the ^{13}C-labeled glycine residue. This is in agreement with the values of 2.7 and 2.8 Å for the host Leu and Ala residues. Therefore, the guest Gly residue is completely incorporated into host polypeptides with the α_R-helix form. Similar results are obtained for $(Val, Gly^*)_n$ and $(Ala, Gly^*)_n$ in the β-sheet form.

Isotropic ^{15}N chemical shifts and chemical shift tensor components

The isotropic ^{15}N chemical shifts and the tensor components of the glycine residue in a variety of peptides with a terminal Boc group have been measured to clarify the relationship between the ^{15}N chemical shift (relative to a saturated $^{15}NH_4NO_3$ solution in water) and $R_{N\cdots O}$.[96,97] It has been shown that there is a clear relationship between the observed isotropic ^{15}N chemical shifts (δ_{iso}) of GlyNH in BocGly, BocGlyAla, BocGlyPhe, BocGlyAib and BocGlyProOBzl in the solid state and their $R_{N\cdots O}$ values determined by X-ray diffraction. The $R_{N\cdots O}$ values of the peptides used here are in the 2.95–3.08 Å range. However, the hydrogen bond angles ($\angle C=O\cdots N$) are somewhat variable and are in the 113–155° range. The chemical shifts move downfield with a decrease in $R_{N\cdots O}$. The δ_{11} and δ_{33} components are more sensitive to a change in $R_{N\cdots O}$ than the δ_{22} component. A change of 0.2 Å in $R_{N\cdots O}$ results in a change of 20 p.p.m. in the δ_{11} and δ_{33} components but a change of 5 p.p.m. in the δ_{22} component. However, only the δ_{33} component moves linearly downfield with a decrease in $R_{N\cdots O}$; in the δ_{11} and δ_{22} components, there is no clear relationship with $R_{N\cdots O}$. Such a behavior is governed not only by the hydrogen bond length but also by the hydrogen bond angle. The ^{15}N chemical shift (σ_{iso}) and the chemical shift tensor components (σ_{11}, σ_{22} and σ_{33}) calculated by the FPT INDO method for *N*-acetyl-*N*′-methylglycine amide hydrogen bonded with a formamide molecule have shown that the isotropic ^{15}N chemical shifts move downfield with a decrease in $R_{N\cdots O}$. This explains the experimental results well. Further details of studies of solid-state ^{15}N NMR chemical shifts associated with hydrogen bonding in peptides and polypeptides are reviewed elsewhere.[40,59]

The directions of the GlyNH ^{15}N chemical shift tensor components have been determined using a BocGlyGlyGlyOBzl single crystal.[98] It

was shown experimentally that the σ_{11} component lies approximately along the N-H bond, the σ_{22} component is aligned in the direction perpendicular to the peptide plane, and the σ_{33} component lies approximately along the N-C_α bond. The FPT INDO calculation[96,97] shows that the σ_{11} component lies approximately along the N-H bond, in agreement with the experimental results, but the σ_{22} component lies approximately along the N-C_α bond and the σ_{33} component is aligned in the direction perpendicular to the peptide plane, which are different from the experimental results. It seems that it is not easy to determine the directions of the σ_{22} and σ_{33} components with significant experimental accuracy because their values are very close to each other. The calculated assignment of the σ_{22} and σ_{33} components seems to be acceptable, although it is difficult to differentiate between them. The direction of the σ_{11} component can be easily determined because its magnitude is significantly different from the others. In the theoretical calculation, the most shielded component σ_{33} is aligned in the direction perpendicular to the peptide plane because the orbitals of the nitrogen lone-pair electrons are in this direction. It has been shown from the experimental results that the σ_{33} component moves linearly downfield with a decrease in the hydrogen bond length; therefore, the σ_{33} component is not related to the hydrogen bond angle and the σ_{11} and σ_{22} components are related to the hydrogen bond length and the hydrogen bond angle. These have been reasonably explained by the calculation.

Isotropic ^{17}O chemical shifts and chemical shift tensor components

In addition to the studies on the conformation-dependent ^{17}O chemical shifts of polyglycine and poly(L-alanine), the association of the hydrogen bond structure with the ^{17}O NMR parameters such as the chemical shifts δ_{iso} and (δ_{11}, δ_{22} and δ_{33}), e^2qQ/h and η of Gly-containing peptides and polyglycine and poly(L-alanine) in the solid state have been studied.[61,63–65] Furthermore, these experimental data have been analyzed with the theoretical calculations of the association of the hydrogen-bonded structure with the NMR parameters for *N*-acetyl-*N*′-methylglycineamide hydrogen bonded with two formamide molecules by the FPT-MNDO-PM3 method[66] and the *ab initio* MO method[99] as a function of the hydrogen bond length $R_{N\cdots O}$. The hydrogen bond length is closely related to the higher-order structures, such as the α-helix, 3_1-helix, β-sheet and other forms. The above theoretical calculations predict that the δ_{iso} values moves upfield with a decrease in the $R_{N\cdots O}$. It is known from X-ray diffraction studies that the $R_{N\cdots O}$ values for the β-sheet form[100] and the 3_1-helix form[67] in polyglycine are 2.95 and 2.73 Å, respectively, and the $R_{N\cdots O}$ values for the α-helix and β-sheet forms in poly(L-alanine)[68,69] are 2.87 and 2.83 Å, respectively. Therefore, the experimental finding that the β-sheet form ($\delta_{iso} = 304$ p.p.m.) in polyglycine appears at a lower field by 11 p.p.m. than the 3_1-helix form ($\delta_{iso} = 293$ p.p.m.) is reasonably explained by the chemical shift calculations. The experimental finding that the α-helix form ($\delta_{iso} = 319$ p.p.m.) in poly(L-alanine) appears at a lower field by 33 p.p.m. than the β-sheet form ($\delta_{iso} = 286$ p.p.m.) also agrees with the chemical shift calculations on *N*-acetyl-*N*′-methylglycineamide.

Furthermore, we are concerned with the experimental ^{17}O chemical shift tensor components (δ_{11}, δ_{22} and δ_{33}) for the Gly residues of the Gly-containing peptides (GlyGly and GlyGly·HNO_3) and polyglycine. It has been shown that all of the tensor components move to a high field with a decrease in $R_{N\cdots O}$.[60] However, the hydrogen bond length dependence of the calculated chemical shift tensor components of the Gly carbonyl oxygen for the model molecule system of *N*-acetyl-*N*′-methylglycine amide hydrogen bonded with two formamide molecules shows that all of the tensor components move largely downfield with an increase in $R_{N\cdots O}$. This qualitatively explains the experimental trend.

The plots of the experimental e^2qQ/h values for the Gly residues of the Gly-containing peptides (GlyGly and GlyGly·HNO_3) and polyglycine against the hydrogen bond length show that the e^2qQ/h values decrease linearly with a decrease in $R_{N\cdots O}$. This relationship can be expressed by e^2qQ/h (MHz) $= 5.15 + 1.16\,R_{N\cdots O}$ (Å).[61] Such a change results from a change in the q values, which are the largest component of the electric gradient tensor (V_{33}). This result shows that the decrease in the hydrogen bond length leads to the decrease of electric gradient. The q value seems to be very sensitive to changes in the hydrogen bonding length.

As mentioned above, it has been shown that the significance of solid-state ^{17}O NMR studies has been revealed in the deeper understanding of the hydrogen-bonded structure of solid biopolymers.

Isotropic ^{1}H chemical shifts

In addition to the ^{13}C, ^{15}N and ^{17}O NMR studies on the hydrogen-bonded structure, it may be significant to clarify whether there is a relationship between the amide ^{1}H NMR chemical shift and the hydrogen bond length $R_{N\cdots O}$ for peptides and polypeptides in the solid state, and also whether this is supported by theoretical calculations because this information could lead to further elucidation of the hydrogen-bonded structure. However, before doing this experiment, we must overcome some technical NMR problems. For example, it is necessary to reduce the large dipolar interaction between the proton nuclei to obtain a small linewidth. The CRAMPS method is one of the solutions to obtain a ^{1}H NMR spectrum with a reasonable resolution. To obtain a high-resolution ^{1}H NMR spectrum for peptides and proteins in the crystalline state, there are some reports in which a combination of MAS and CRAMPS techniques is used.[71,72] However, the CRAMPS method requires a slow MAS speed for sampling the data points during acquisition (for example, the BR24 multi-pulse sequence needs less than approximately 3 kHz for sampling). The spinning rate of 3 kHz is not enough to provide a high-resolution ^{1}H NMR spectrum to analyze the amide proton chemical shift in peptides and polypeptides because the amide proton is directly bonded to the quadrupolar ^{14}N nucleus. As reported by McDermott *et al.*,[101,102] amino acids take up NH_3^+ forms, so the quadrupolar effect on the proton linewidth by the ^{14}N becomes very small because of its high symmetry. Therefore, the NH_3^+ proton linewidth in the amino acids become much sharper than that of amide protons in the peptides and polypeptides considered here.

Let us consider the relationship between the $R_{N\cdots O}$ and the hydrogen-bonded amide ^{1}H chemical shift data obtained from high-resolution ^{1}H NMR spectra of hydrogen-bonded Gly-containing peptides and polypeptides in the solid state. The ^{1}H NMR spectra was obtained at a high MAS speed of 30 kHz and a high frequency of 800 MHz for the removal of the dipolar coupling and quadrupolar coupling with amide ^{14}N.[73] The ^{1}H MAS NMR spectra of Gly-containing peptides and polyglycine show that the amide proton, α-proton and side-chain protons are straightforwardly assigned because their peaks are clearly resolved from each other. The amide ^{1}H chemical shift must be carefully assigned. By using an NMR technique to measure the high-resolution solid-state ^{1}H NMR designed with the FSLG-2 homonuclear dipolar decoupling method[103–106] combined with high-speed MAS, the high-resolution solid-state ^{1}H NMR spectra of amino acids, peptides and polypeptides have been obtained with more reasonable resolution for the amide ^{1}H signals. The chemical shifts of the amide ^{1}H provide

information about the hydrogen-bonded structure, compared with other high-resolution solid-state ^{1}H NMR methods.[74] The determined Gly amide ^{1}H chemical shift values of peptides and polypeptides in the solid state were plotted against the $R_{N\cdots O}$ as determined from X-ray diffraction (not shown), and the amide ^{1}H chemical shifts moved downfield with a decrease in $R_{N\cdots O}$. The relation between the amide ^{1}H chemical shifts (δ_H) and the $R_{N\cdots O}$ was determined to be $\delta_H = 25.4–5.9\,R_{N\cdots O}$ (p.p.m.). Therefore, by observing the amide ^{1}H chemical shift value, the value of $R_{N\cdots O}$ can be determined. Furthermore, the *ab initio* MO calculation and the neutron diffraction data show that the reduction of $R_{N\cdots O}$ leads to a decrease in the hydrogen bond length ($R_{O\cdots H}$) between the amide hydrogen and the amide carbonyl oxygen.[96] Thus, the ^{1}H chemical shifts move downfield with a decrease in $R_{O\cdots H}$. The isotropic ^{1}H chemical shifts and the tensor components of hydrogen-bonded Gly amide protons of two hydrogen-bonded GlyGly molecules were also calculated by using the Gaussian 96 program with the *ab initio* 6–31 G** basis set by changing $R_{N\cdots O}$ from 3.5 to 2.6 Å according to its crystal structure as determined by X-ray diffraction.[107] The calculated chemical shifts move downfield by 2.5 p.p.m. from 6.9 to 9.4 p.p.m. as $R_{N\cdots O}$ is decreased from 3.30 to 2.72 Å, and the calculation qualitatively explains the experimental results. However, quantitative agreement was not obtained. This may be because, strictly speaking, the position of the amide proton in the >N-H···O=C< hydrogen bond depends on $R_{N\cdots O}$,[95] but in this calculation the N-H bond length was fixed at 1.0 Å.

Shoji *et al.*[108] have determined the hydrogen-bonded amide N-H bond lengths of poly(L-alanine)s with the α-helix and β-sheet forms by analyzing the ^{1}H CRAMPS NMR spectra of fully ^{15}N-labeled poly(L-alanine)s in the solid state. The N-H dipolar spinning sideband pattern of α-helical poly(L-alanine) is different from that of β-sheet poly(L-alanine). The sideband pattern is characteristic of the N-H bond length. From the sideband pattern analysis, the N-H bond lengths for the α-helix and β-sheet forms of poly(L-alanine) were determined to be 1.09 and 1.12 Å, respectively. It was shown that the N-H bond length of the former is shorter than that of the latter.

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