Journal of Molecular Structure, 212 (1989) 123–135
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

A CONFORMATIONAL STUDY OF OLIGOPEPTIDES CONTAINING GLY–PRO SEQUENCE IN THE SOLID STATE BY ^{13}C CP-MAS NMR

SHINJI ANDO, KEISUKE MATSUMOTO and ISAO ANDO

Department of Polymer Chemistry, Tokyo Institute of Technology, Meguro-ku, Ookayama, Tokyo 152 (Japan)

AKIRA SHOJI and TAKUO OZAKI

Department of Industrial Chemistry, College of Technology, Gunma University, Tenjin-cho, Kiryu-shi, Gunma 376 (Japan)

(Received 24 October 1988)

ABSTRACT

In order to investigate the applicability and its usefulness of the conformation-dependent ^{13}C chemical shift to the molecular structures of oligopeptides in the solid state, we recorded high resolution ^{13}C CP-MAS NMR spectra of a series of oligopeptides having Gly–Pro sequence. Using the ^{13}C chemical shifts of polypeptides with particular conformations and the hydrogen bond dependence of Gly CO chemical shift, we obtained useful information about the local conformation of oligopeptides and examined the molecular structure of the peptides which had not been determined by X-ray diffraction. It was revealed that ^{13}C CP-MAS NMR is also effective in characterizing the conformation of oligopeptides in the solid state.

INTRODUCTION

As has been revealed in a series of ^{13}C CP-MAS NMR studies of synthetic and biological polypeptides [1], every amino acid residue in polypeptides has characteristic ^{13}C chemical shift values in the solid state. Since this shift varies depending on the conformation such as α-helix, β-sheet and 3_1-helix, we can regard it as an intrinsic probe of the secondary structure of peptides. It was also clear from the experimental [2] and theoretical studies [3] that such a chemical shift change is not caused by the specific peptide sequence, but by the local conformation and intermolecular interactions. This implies that the ^{13}C chemical shift in the solid state grossly depends on very confined electronic structure around the nucleus under consideration.

We have reported previously that the behavior of ^{13}C chemical shifts of the glycine residue carbonyl carbons (Gly CO) of oligo- and polypeptides are clearly explained in terms of the intermolecular hydrogen bond [4,5]. The experi-

0022-2860/89/$03.50

mental finding that ^{13}C chemical shifts of Gly CO of polyglycine with forms I and II were also satisfactorily interpreted by the difference of hydrogen bond lengths (N···O length in >N–H···O=C< type hydrogen bond) indicates the absence of polymer effect on the ^{13}C chemical shift in the solid state. This suggests the possibility of also utilizing the ^{13}C chemical shift as a conformational probe for rather short sequence peptides. Although several ^{13}C CP-MAS NMR spectra of oligopeptides have been reported, the conformational studies were limited to cyclic peptides having the specific turn-structures [6–8] with the exceptions of Pro–Leu–Gly–NH_2 [9] and Leu-enkephalin [10].

To begin with, in order to examine the possibility of expanding the applicability of the ^{13}C chemical shift measurements, we have applied this technique to the conformational analysis of a series of linear oligopeptides in the solid state whose crystal structures have already been determined. The conformations of the samples containing the sequence Z–Gly–Pro (Z = benzyloxycarbonyl) studied here are tabulated in Table 1. It is interesting to note that the conformation entirely changes upon increasing the peptide sequence. Dipeptide [11] and tripeptide [12] take the antiparallel β-sheet conformation and the 3_1-helix-like conformation, respectively, which are the particular conformations frequently found in polypeptides. It is well known that oligopeptides mostly take one of the conformations which are classified as the secondary structures of polypeptides or proteins [15]. On the other hand, tetra- [13] and pentapeptide [14] have the "β-turn" conformation. This is occasionally found in cyclic peptides and proteins, but is rarely observed in the synthetic polypeptides.

According to the result of X-ray study, the 3_1-helix-like conformation of Z–Gly–Pro–Leu is characterized by a *cis* arrangement at the Gly–Pro sequence. We can also obtain information about the *cis/trans* isomerism relating to the Pro residue by checking the chemical shift difference between Pro C_β and $C\gamma$ carbons [16].

TABLE 1

Oligopeptides containing the Gly-Pro sequence and their solid state conformations

Sample	Structure	Ref.
Z-Gly-Pro	β-Sheet	11
Z-Gly-Pro-Leu	3_1-Helix	12
Z-Gly-Pro-Leu-Gly	β-Turn	13
Z-Gly-Pro-Leu-Gly-Pro	β-turn	14
Z-Gly-Pro-Gly-Gly	Unknown	
Z-Gly-Pro-Ala-Gly	Unknown	
Z-Gly-Pro-Ala-Ala	Unknown	
Z-Gly-Pro-Val-Gly	Unknown	

In addition, the hydrogen bond plays an important role in forming stable conformations of peptides in the crystalline state. The molecular structures of linear peptides are generally constructed by intermolecular hydrogen bonds. However, the β-turn structure is stabilized by the intramolecular hydrogen bond. For this, we planned to investigate the nature of the hydrogen bond by incorporating ^{13}C-enriched [1-^{13}C]glycine residues as the first residue in the peptide sequence (the amino acid residue is numbered following the Z-group, for example Leu is the third residue in Z–Gly–Pro–Leu). From the chemical shift of Gly CO, we can obtain information about whether there is a hydrogen bond or not and can estimate the hydrogen bond length on the basis of our previous results [5].

Further, we made an attempt to assess the conformations of a series of peptides whose crystal structures have not yet been determined. By employing the knowledge and procedure deduced from the results of peptides with known structures, we can obtain useful information about the conformations of peptides with unknown structures. It should be noted that oligopeptides with several residues are sometimes very difficult to crystallize for X-ray studies because of the low energy barriers of the skeletal bonds. The other spectroscopic tools, such as IR, etc., do not give immediate information about the conformation in the solid state. The CP-MAS NMR technique can also be applied for the samples which are not crystallized or are composed of several conformers.

EXPERIMENTAL

Materials

A series of oligopeptides containing the [1-^{13}C]glycine residue (5%) were prepared according to the fragment condensation of *N*-hydroxysuccinimide esters and amino acids by the DCCI (*N,N'*-dicyclohexylcarbodiimide) coupling method [17]. The N-terminals of all the peptides were protected by Z-groups using Z-chloride. A mixture of [1-^{13}C]glycine (Merck Inc., isotope purity 90 atom%) and glycine (Nihon-Rika) were provided to get remarkably intense signals in the ^{13}C NMR spectra. The samples obtained were slowly recrystallized from ethyl acetate solution according to the procedure used in X-ray diffraction studies [11–14]. The samples whose crystal structures had not been reported were also recrystallized from ethyl acetate by the vapor diffusion method with n-hexane as precipitant. The polycrystalline samples obtained were ground with an agate mortar before the NMR measurement to eliminate the orientation anisotropy of crystals in the spinning rotor.

^{13}C CP-MAS NMR measurement

^{13}C CP-MAS NMR spectra were recorded at room temperature with a JEOL GX-270 spectrometer at 67.80 MHz equipped with a CP-MAS accessory. The

field strength of ^{1}H decoupling was 1.2 mT. Contact time was 2 ms, and repetition time was 5 s. Spectral width was 27 kHz and data points were 8 k. Samples were placed in a bullet-type rotor and spun at about 3 kHz. Spectra were accumulated 200–500 times to achieve a reasonable signal-to-noise ratio. ^{13}C chemical shifts were calibrated indirectly through the adamantane peak observed upperfield (29.5 ppm relative to tetramethylsilane ($(CH_3)_4Si$)).

RESULTS AND DISCUSSION

^{13}C Chemical shift of Gly CO

A 67.80 MHz ^{13}C CP-MAS NMR spectrum of Z–glycylprolylleucylglycine (Z–Gly–Pro–Leu–Gly) is shown as a typical example in Fig. 1. ^{13}C CP-MAS spectra of other remaining samples were also obtained with similar resolutions. The Gly CO signal can be straightforwardly assigned from its intensity. Other signals were also assigned with reference to the previous ^{13}C CP-MAS NMR studies and solution state ^{13}C NMR. The ^{13}C chemical shifts of the glycine

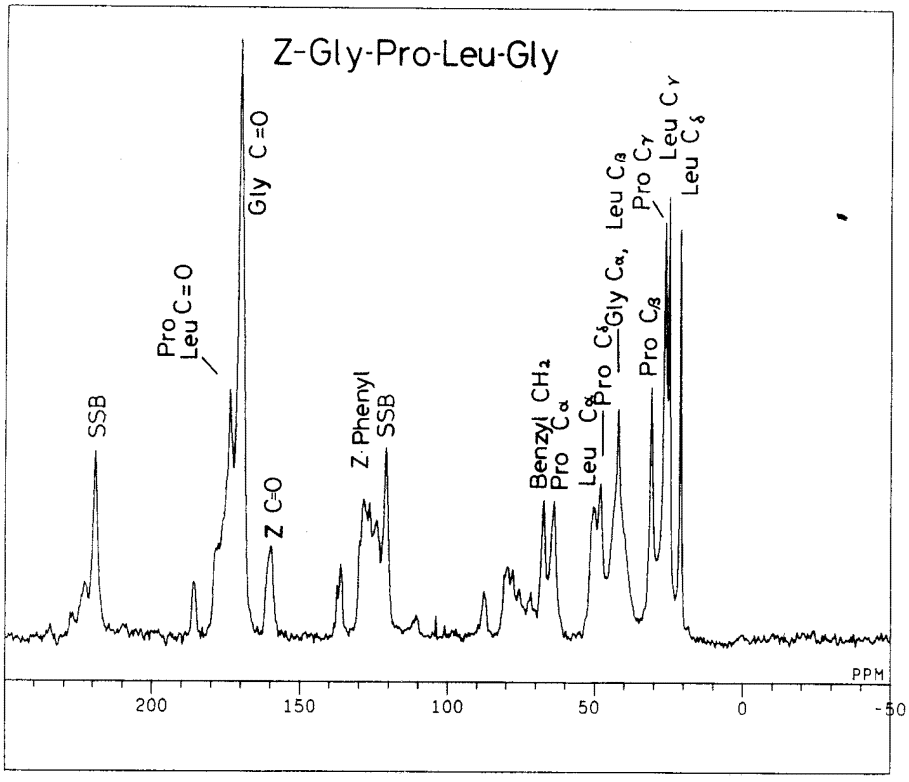


Fig. 1. ^{13}C CP-MAS (67.80 MHz) NMR Spectra of Z-glycylprolylleucylglycine.

residue, aliphatic carbons of Ala, Leu and Val residues and the $\Delta\beta\gamma$ parameters of the Pro residue (difference in ^{13}C chemical shift between Pro Cβ and Pro Cγ carbons) are listed in Table 2. On the whole, signal separation was very good, except for the region of carbonyl carbons other than the Gly CO signal (170–180 ppm).

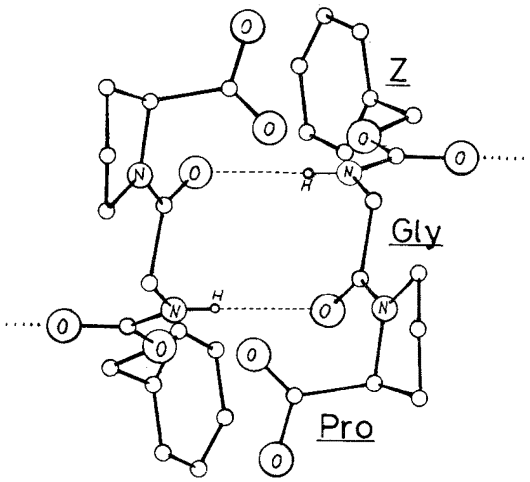
Figure 2 shows the schematic crystal structures of peptides examined in this study [11–13]. The conformations and hydrogen-bonding networks of Z–Gly–Pro and Z–Gly–Pro–Leu are very close to those of antiparallel β-sheet and 3_1-helix, respectively. As shown in Table 2, Gly COs of these peptides which are resonated at 168.5 and 172.2 ppm are consistent with polyglycine form I (antiparallel β-sheet, 169.5 ppm) and form II (3_1-helix, 172.8 ppm) conformations, respectively [4, 5]. In our previous study [5], it was revealed that there is a linear relationship between te Gly C ^{13}C chemical shift and its hydrogen bond length. Also in this case, the hydrogen bond length of Z–Gly–Pro–Leu (2.64 Å [11]; O···O length in Gly C=O and H–O–Leu) is much shorter than that of Z–Gly–Pro (2.91 Å [12]; O···N length in Gly C=O and H–N–Gly) as determined by X-ray studies. Figure 3 shows the plot of ^{13}C chemical shifts of Gly CO of the peptides considered here and those studied in the previous study against the hydrogen bond length. Thus, the chemical shift difference can be primarily attributed to the difference of hydrogen-bond length. From the other point of view, we can say that ^{13}C chemical shift of Gly CO is displaced depending on its conformation, because the hydrogen bond length generally reflects the conformation owing to its steric arrangement of the molecules in the crystal [18]. This might be the origin of "the conformation-dependent chemical shift" of Gly CO in the solid state.

On the other hand, Gly CO of the peptides taking the β-turn structure (Z–Gly–Pro–Leu–Gly and Z–Gly–Pro–Leu–Gly–Pro) are resonated in the midst of the above two samples. The chemical shifts of 170.1 and 170.3 ppm of Gly COs in β-turn peptides are slightly displaced downfield compared with the peptides forming intermolecular hydrogen bonds. As mentioned above, the Gly CO in β-turn forms the intramolecular hydrogen bonds with Gly N–H of the fourth residue. Although this bond stabilizes the turn structure, the local conformation around the Gly–Pro sequence is much constrained and the hydrogen bond deviates markedly from linearity. The fact that the hydrogen bond angle of C=O··N is about 120° in the β-turn structure is in contrast to that of the corresponding angle in non-cyclic di- and tripeptides which is about 150° as shown in the previous study [5]. This characteristic bending forces the N–H group to direct the π electrons on the C≡O bond and it caused the relative downfield shift. In addition, the β-turn conformation is known as an appreciable fixed structure. Ashida and co-workers [14,15,19,20] carried out systematic X-ray studies on a series of β-turn peptides and reported that all the skeletal structures of the turning portions containing Pro residue fell in the same structure with a difference of about 0.1 Å. Therefore the Gly CO chemical shift

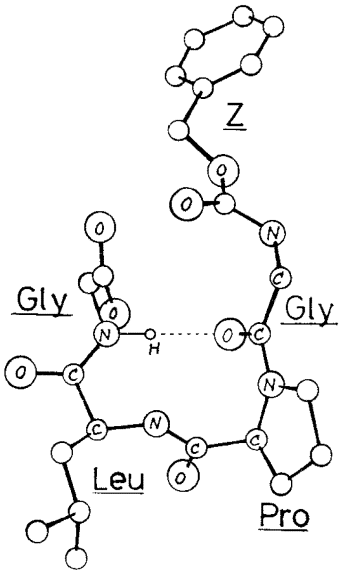
TABLE 2

^{13}C Chemical shifts of some peptides and reference polypeptides in the solid state (± 0.5 ppm from TMS)

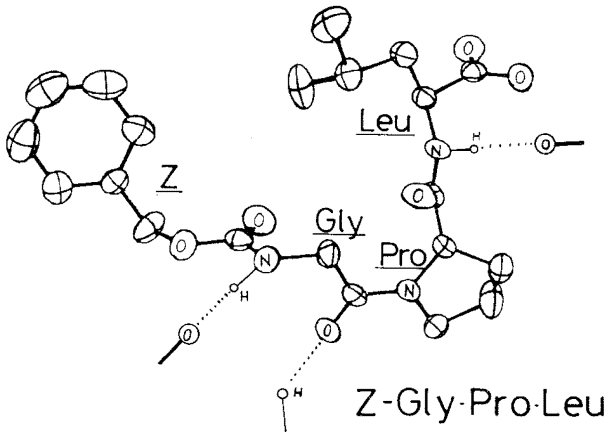
Sample	Conformation	Gly		Pro	Ala		Leu		Val	
		C=O	Cα	$\Delta\beta\gamma$	Cα	Cβ	Cα	Cβ	Cα	Cβ
Z-Gly-Pro	β-Sheet	168.5	45.1	3.3						
Z-Gly-Pro-Leu	3_1-Helix	172.2	43.2	9.1			53.3	38.5		
Z-Gly-Pro-Leu-Gly	β-Turn	170.1	42.0	4.7			50.4	41.6		
Z-Gly-Pro-Leu-Gly-Pro	β-Turn	170.3	43.0	4.7			51.1	41.6		
Z-Gly-Pro-Gly-Gly	(3_1-Helix)	172.7	43.6	4.0						
Z-Gly-Pro-Ala-Gly	(3_1-Helix)	172.1	43.3	7.6	48.7	16.7				
				9.9						
Z-Gly-Pro-Ala-Ala	(Triple)	167.6	43.1	4.9	47.5	16.4				
						19.3				
Z-Gly-Pro-Val-Gly	(β-Sheet)	169.7	42.4	4.9					59.1	33.5
				10.1						
[Gly]$_n$	β-Sheet	169.5	44.2							
	3_1-Helix	172.8	44.2							
[Pro]$_n$	10_3-Helix (*cis*)			9.3						
	3_1-Helix (*trans*)			2.4						
[Ala]$_n$	α-Helix				53.4	15.9				
	β-Sheet				49.2	20.9				
[Leu]$_n$	α-Helix						56.7	40.5		
	β-Sheet						51.5	44.3		
[Val]$_n$	α-Helix								66.5	29.7
	β-Sheet								59.4	33.4
[Ala,Gly]	α-Helix	172.7			53.2	15.6				
[Leu,Gly]	α-Helix	172.4					56.4	40.0		
[Val,Gly]	β-Sheet	169.5							59.0	33.0
[Ala-Gly-Gly]$_n$	3_1-Helix	172.1	43.0		48.7	17.4				
[Pro-Ala-Gly]$_n$	Triple	168.2	43.5	4.6	48.3	17.6				
[Pro-Gly-Pro]$_n$	Triple	167.2	43.0	4.0						



Z-Gly-Pro : β-sheet



Z-Gly-Pro-Leu-Gly : β-turn



Z-Gly-Pro-Leu : 3_1-helix

Fig. 2. Schematic crystal structures for the three types of conformation: antiparallel β-sheet, 3_1-helix-like conformation and β-turn.

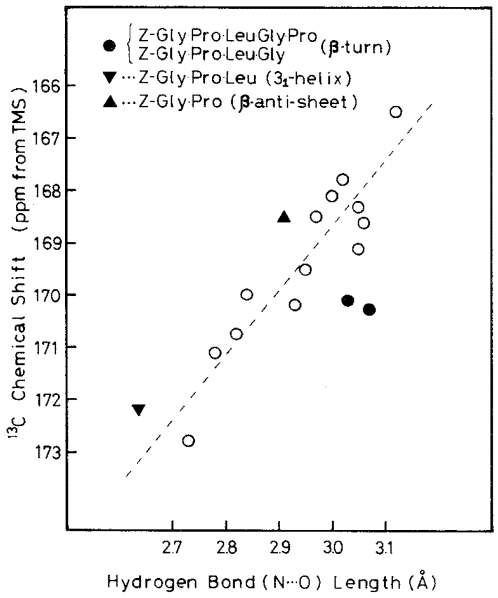


Fig. 3. Plots of the observed Gly CO ^{13}C chemical shifts in the solid state against the hydrogen bond length. Open circles are of the polypeptides and oligopeptides studied in the previous study [5]. All the Gly COs form C≡O···H–N type hydrogen bonds with the exception of Z-Gly-Pro-Leu, in which Gly CO forms the hydrogen bond with the O–H group of the Leu residue of the neighboring molecule.

around 170 ppm can be regarded as the characteristic value for the β-turn conformation.

$\Delta\beta\gamma$ Parameter in Pro Residue

As pointed out by some solution ^{13}C NMR [16] and ^{13}C CP-MAS NMR studies [6,7,9], the $\Delta\beta\gamma$ parameter is well correlated with the *cis/trans* isomerism of the peptide bond in the X–Pro sequence (X indicates another amino acid residue). Generally, a value of $\Delta\beta\gamma$ of more than 8 ppm is attributed to a *cis* conformation and a value less than 6 ppm to *trans*. In Table 3, we tabulate the observed $\Delta\beta\gamma$ values of peptides together with the observed ones by others, whose crystal structures have already been determined. The $\Delta\beta\gamma$ values in Z–Gly–Pro, Z–Gly–Pro–Leu, Z–Gly–Pro–Leu–Gly and Z–Gly–Pro–Leu–Gly–Pro are in agreement with the results of X-ray diffraction studies, where only Z–Gly–Pro–Leu has a *cis* arrangement at the Gly–Pro bond. In addition, this parameter is well correlated with the dihedral angle of Pro ψ as shown in Fig. 4, in which the dashed lines indicate the relationship introduced from the solution NMR studies by Siemion et al. [32]. There is no difference between the relationships obtained from the solution and solid state measurements. In par-

TABLE 3

Δβγ Parameters of the Pro residue in some peptides with their geometrical parameters

Sample	X-Pro bond	Pro (ϕ,ψ) (degrees)	Pro *Δβγ* (ppm)	Ref.	
				NMR	Structure
Z-Gly-Pro	*Trans*	(−70,150)	3.3	This work	11
Z-Gly-Pro-Leu	*Cis*	(−72,145)	9.1	This work	12
Z-Gly-Pro-Leu-Gly	*Trans*	(−63,−27)	4.7	This work	13
Z-Gly-Pro-Leu-Gly-Pro	*Trans*	(−63,−23)	4.7	This work	14
Boc-Gly-Pro	*Trans*	(−70,156)	4.9	This work	23
Boc-Pro-Val-Gly	*Cis*	(−81,163)	11.1	This work	24
Boc-Pro-Leu-Gly	*Trans*	(−65,−21)	3.6	This work	25
Polyproline I	*Cis*	(−83,158)	9.3	21	26
			9.9	22	
Polyproline II	*Trans*	(−78,149)	2.4	21	27
			2.9	22	
Cyclo(D-Phe-Pro-Gly-D-Ala-Pro)	*Trans*	(−82,59	0.7	7	28
	Trans	(−64,128)	4.3		
Cyclo(Gly-Pro-Gly)$_2$	*Trans*	(−53,126)	3.7	7	29
	Trans	(−66,−36)	5.5		
Cyclo(Phe-Pro-D-Ala)$_2$	*Cis*	(−83,157)	10.1	6	30
	Cis	(−70,165)			
Cyclo(Gly-Pro-D-Ala)$_2$	*Trans*	(−54,125)	3.5	6	31
	Trans	(−70,116)			
Pro-Leu-Gly-NH$_2$	*Trans*	(— ,153)	3.8	9	39

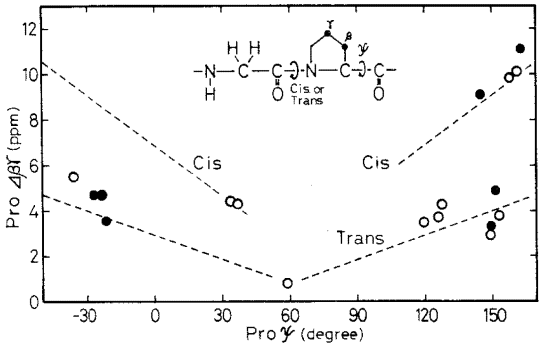


Fig. 4. Plots of the observed Pro *Δβγ* (difference in ^{13}C chemical shift between Pro Cβ and Cγ) against the dihedral angle ψ of the Pro residue for the peptides tabulated in Table 3.

ticular, the value of 0.7 ppm for $\Delta\beta\gamma$ observed in the γ-turn conformation by Opella and co-workers [7] plays an important role in justifying this relationship.

^{13}C Chemical shift of aliphatic carbons of the third residue

The conformation-dependent ^{13}C chemical shift of aliphatic carbons of homopolypeptides can be utilized as the reference data for particular conformations [1]. Hence, the most probable nucleus reflecting the molecular structure might be the aliphatic carbons of the third residue in the peptides considered here. For comparison we also tabulate in Table 2 the reference data of ^{13}C chemical shifts of polypeptides with various conformations. The aliphatic carbons of the Leu residue in Z–Gly–Pro–Leu–Gly and Z–Gly–Pro–Leu–Gly–Pro are resonated at a unique position, in which the Cα chemical shift is close to that of the polyleucine ($[Leu]_n$) β-sheet but Cβ is in the midst of those of $[Leu]_n$ α-helix and β-sheet and rather close to the α-helix. The dihedral angles (ϕ, ψ) of the third residue in the β-turn ($-107°$, $15°$) [15] are quite different from those of the α-helix ($-57°$, $-48°$) [33] and β-sheet ($-140°$, $135°$) [34] conformations. We might consider that these chemical shifts can be the reference data for the β-turn structure in addition to the Gly CO chemical shift.

We have reported that the calculated ^{13}C shielding constants of Ala Cα, Cβ and carbonyl carbons employing a dipeptide model by means of FPT-INDO theory exhibit conformation dependence comparable with the experimental data. This supports our view that conformation dependent ^{13}C chemical shifts can be applied for rather small peptides. As shown below, ^{13}C chemical shifts of aliphatic carbons of the third residues are informative in the course of determining the peptide conformations.

On the other hand, since the first Gly residue has no side chain, the chemical shift change of Gly Cα caused by the conformational change is much smaller than the other residues. We cannot obtain useful information about the conformation from the Gly Cα chemical shift despite its observability [35].

Conformational analysis of some peptides with unknown structures

We attempted to assess the conformation of related peptides whose structures have not been determined, on the basis of the knowledge deduced from the above results and the previous ^{13}C CP-MAS NMR studies.

Z-Gly-Pro-Gly-Gly

The relatively downfield signal of Gly CO (172.7 ppm) in Z–Gly–Pro–Gly–Gly comes from a shorter hydrogen bond length of around 2.7 Å. This chemical shift is close to that of polyglycine form II ($[Gly]_n$ II) taking the 3_1-helix form with a hydrogen bond length of 2.73 Å [18]. In addition, it is known that po-

lyproline form II ($[Pro]_n$ II) takes the left-handed 3_1-helix form in the solid state and the tripeptide unit of this sample, Pro–Gly–Gly–OH [36] has a similar structure to $[Gly]_n$ II. A Pro $\Delta\beta\gamma$ value of 4 ppm indicates that the Gly–Pro bond is *trans*. From these results, it can be said that this peptide may assume a 3_1-helix-like conformation which is similar to that of $[Gly]_n$ II or $[Pro]_n$ II with a *trans* X–Pro bond, but not to Z–Gly–Pro–Leu or $[Pro]_n$ I with a *cis* X–Pro bond.

Z-Gly-Pro-Ala-Gly

The Gly CO chemical shift of this peptide (172.1 ppm) is also relatively deshielded. This suggests that the hydrogen bond length is about 2.75 Å. Such results indicate that neither the β-sheet nor the β-turn structure exist. The appearance of the two signals for Pro Cγ suggests the existence of two different conformers, but the discrepancy in conformation between them is not very large, because their $\Delta\beta\gamma$ values are close together and are attributed to *cis* conformations. Comparing the ^{13}C chemical shifts of the Ala residue of this peptide with those of polyalanine ($[Ala]_n$ α-helix and β-sheet) and $[Ala–Gly–Gly]_n$ (3_1-helix), Cα and Cβ signals have values closer to a 3_1-helical copolypeptide. From the above results, it can be said that Z–Gly–Pro–Ala–Gly might have a similar structure to Z–Gly–Pro–Leu or $[Pro]_n$ I with a right handed 10_3-helix whose X–Pro bonds are in a *cis* arrangement.

Z-Gly-Pro-Ala-Ala

The most shielded chemical shift of Gly CO (167.6 ppm) apparently indicates the absence of hydrogen bond between Gly CO and the other proton donor group. The possibility of the β-sheet, β-turn or 3_1-helix structures being taken can then be excluded. A similar phenomenon has already been found by Saito et al. [2] in the collagen-like polypeptides which take triple helical conformations ($[Pro–Gly–Pro]_n$ and $[Pro–Ala–Gly]_n$). The Gly CO chemical shifts of those polypeptides are substantially displaced upfield by 4.1–5.1 ppm with respect to those of the 3_1-helical conformation. This was explained by assuming that Gly CO of $[Pro–Gly–Pro]_n$ is not involved in hydrogen bond. In addition, chemical shifts of Ala Cα and Cβ of Z–Gly–Pro–Ala–Ala coincide with those of triple helical $[Pro–Ala–Gly]_n$ and 3_1-helical $[Ala–Gly–Gly]_n$ within experimental error. Pro $\Delta\beta\gamma$ values are almost the same in Z–Gly–Pro–Ala–Ala and $[Pro–Ala–Gly]_n$ and both values indicate a *trans* arrangement at the Gly–Pro bond. These facts suggest that this peptide takes a similar structure to the triple helical polypeptides.

However, it is difficult to suppose that such a small peptide forms a triple helical structure. As Saito et al. [2] have pointed out, the difference in dihedral angle between the 3_1-helix and triple helical conformations in the Gly residue is about 20° in the ψ angle and less than 10° in the ϕ angle. Therefore, this peptide takes a conformation similar to triple helical and 3_1-helical polypep-

tides without a hydrogen bond which involves Gly CO groups. This view is also supported by the fact that chemical shifts of Ala residues in Z–Gly–Pro–Ala–Gly taking a 3_1-helix-like conformation are very close to those of this sample.

Z–Gly–Pro–Val–Gly

Two sets of the signals of Pro Cβ and Pro Cγ are observed in this peptide. This suggests that there exist two conformations with a *cis* and *trans* Gly–Pro bond in the solid state. According to the Gly CO chemical shift of 169.7 ppm, the Gly CO group is included in the hydrogen bond with a length of 3.0 Å. This value suggests the β-sheet or β-turn conformation. On the other hand, Val Cα and Cβ signals of this peptide are resonated at the same position as the β-sheet in polyvaline ($[Val]_n$) and $[Val\text{-}Gly]_n$ within experimental error. Therefore, this peptide possibly takes the β-sheet structure. The Val residue is known as the most probable residue found in the sheet region of proteins [37] and it locates very rarely at the third position of the β-turn [37]. This is also supported by the fact that two analogous peptides, Boc–Pro–Val–Gly [24] and Boc–Pro–Val–Gly–NH_2 [38], have typical β-sheet structures.

CONCLUDING REMARKS

We have demonstrated an ability to interpret the behavior of ^{13}C chemical shifts of oligopeptides as the representation of conformation and the manner of hydrogen bonding in the crystalline state and also shown the possibility of applying this method to obtain an insight into the unknown structures of peptides. The conformational features drawn from the investigations of ^{13}C chemical shifts are in good agreement with those of crystal structures obtained by X-ray diffraction. The ^{13}C chemical shifts consistently indicate one particular conformation for each peptide whose crystal structure has not been determined. This method is also applicable to samples which are difficult to crystallize or consist of the mixtures of more than two conformers. Accordingly, the CP-MAS NMR approach is a very useful tool for investigating the conformation not only of polypeptides, but also of oligopeptides in the solid state.

REFERENCES

1 H. Saitô and I. Ando, Annu. Rep. NMR Spectrosc., 22 (1989) 209.
2 H. Saitô, R. Tabeta, A. Shoji, T. Ozaki, I. Ando and T. Miyata, Biopolymers, 23 (1984) 2279.
3 I. Ando, H. Saitô, R. Tabeta, A. Shoji and T. Ozaki Macromolecules, 17 (1984) 457.
4 S. Ando, T. Yamanobe, I. Ando, A. Shoji, T. Ozaki, R. Tabeta and H. Saitô, J. Am. Chem. Soc., 107 (1985) 7648.
5 S. Ando, I. Ando, A. Shoji and T. Ozaki, J. Am. Chem. Soc., 110 (1988) 3380.

6 S.K. Sarkar, D.A. Torchia, K.D. Kopple and D.L. VanderHart, J. Am. Chem. Soc., 106 (1984) 3328.
7 M.H. Frey, S.J. Opella, A.L. Rockwell and L.M. Gierasch, J. Am. Chem. Soc., 107 (1985) 1946.
8 A. Shoji, T. Ozaki, H. Saitô, R. Tabeta and I. Ando, Makromol. Chem. Rapid Commun., 5 (1984) 799.
9 H. Saitô and R. Tabeta, Chem. Lett., (1985) 83.
10 H. Saitô and R. Tabeta, in T. Shibata and S. Sakakibara (Eds.) Proc. Jpn. Symp. Peptide Chem., Protein Research Foundation, Osaka, 1987, p. 53.
11 I. Tanaka, T. Kozima, T. Ashida, N. Tanaka and M. Kakudo, Acta Crystallogr., Sect. B, 33 (1977) 116.
12 T. Yamane, T. Ashida, K. Shimonishi, M. Kakudo and Y. Sasada, Acta Crystallogr., Sect. B, 32 (1977) 2071.
13 T. Ueki, T. Ashida, M. Kakudo, Y. Sasada and Y. Katsube, Acta Crystallogr., Sect. B, 25 (1969) 1840.
14 S. Bando, N. Tanaka, T. Ashida and M. Kakudo, Acta Crystallogr., Sect. B, 34 (1978) 3447.
15 I.L. Karle, in E. Gross and J. Meienhofer (Eds.), The Peptide, Vol. 4, Academic Press, New York, 1981.
16 V. Madison, M. Atreyi, C.M. Deber and E.R. Blout, J. Am. Chem. Soc., 96 (1974) 6725.
17 (a) L. Zervas, D. Borovas and E. Gazis, J. Am. Chem. Soc., 85 (1963) 3660.
(b) E. Wunsch, Synthese von Peptiden, Thieme Verlag, Stuttgart, 1974, Teil I and II.
(c) N. Izumiya, T. Kato, M. Ohno and H. Aoyagi, Peptide Synthesis, Maruzen, Tokyo, 1975.
18 (a) G.N. Ramachandran, V. Sasisekharan and C. Ramakrishnan, Biochim. Biophys. Acta, 112 (1966) 168.
(b) B. Lotz, J. Mol. Biol., 87 (1975) 169.
19 T. Ashida, I. Tanaka, Y. Shimonishi and M. Kakudo, Acta Crystallogr., Sect. B, 33 (1977) 3054.
20 T. Ueki, S. Bando, T. Ashida, Y. Sasada and Y. Katsube, Acta Crystallogr., Sect. B, 27 (1971) 2219.
21 H. Saitô, R. Tabeta, A. Shoji, T. Ozaki and I. Ando, Macromolecules, 16 (1983) 1050.
22 D. Müller, J. Stutz and H.R. Kricheldorf, Makromol. Chem., 185 (1984) 1739.
23 E. Benedetti, M. Palumbo, G.M. Borona and C. Toniolo, Macromolecules, 9 (1976) 417.
24 I. Tanaka and T. Ashida, Acta Crystallogr., Sect. B, 36 (1980) 2164.
25 T. Ashida and I. Tanaka, Acta Crystallogr., B, 33 (1977) 3054.
26 J.A. Schellman and C. Schellman, in H. Neurath (Ed.), The Proteins, Vol. 2, 2nd edn., Academic Press, New York, 1964.
27 W. Traub and U. Schmueli, Nature (London), 198 (1963) 1165.
28 I.L. Karle, in A. Eberle, R. Geiger and T. Wieland (Eds.), Perspectives in Peptide Chemistry, Karger, Basel, 1981, p. 261.
29 E.C. Kostansek, W.E. Thiessen, D. Schomburg and W.N. Lipscomb, J. Am. Chem.Soc., 101 (1979) 5811.
30 G. Kartha, K.K. Bhandary, K.D. Kopple, A. Go and P.P. Zhu, J. Am. Chem. Soc., 106 (1984) 3844.
31 E.C. Kostansek, W.N. Lipscomb and W.E. Thiessen, J. Am. Chem. Soc., 101 (1979) 834.
32 I.Z. Siemion, T. Wieland and K.H. Pook, Angew. Chem., 19 (1975) 712.
33 S. Arnott and A.J. Wonacott, J. Mol. Biol., 21 (1966) 371.
34 S. Arnott, S.D. Dover and A. Elliot, J. Mol. Biol., 30 (1967) 201.
35 S. Ando, I. Ando, A. Shoji and T. Ozaki, J. Mol. Struct., 153 (1989) 192.
36 V. Lalitha, E. Subramanian and R. Parthasarathy, Int. J. Peptide Protein Res., 27 (1986) 223.
37 P.Y. Chou and G.D. Fasman, Adv. Enzymol., 47 (1978) 45.
38 T. Ashida, T. Kojima, I. Tanaka and T. Yamane, Int. J. Peptide Protein Res., 27 (1986) 61.
39 L.L. Reed and P.L. Johnson, J. Am. Chem. Soc.,,95 (1973) 7523.