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CONFORMATIONAL CHARACTERIZATION OF GLYCINE RESIDUES INCORPORATED INTO SOME HOMOPOLYPEPTIDES BY SOLID STATE ^{13}C NMR SPECTROSCOPY. II.

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ABSTRACT

A ^{13}C NMR study of some polypeptides containing ^{13}C enriched [2-^{13}C]glycine (Gly*) residues as a minor component (8%) in the solid state was made in order to clarify a conformational effect on the ^{13}C chemical shifts of the isotropic average value and the principal values of chemical shift tensors (σ_{11}, σ_{22} and σ_{33}) of glycine methylene carbons (Gly C_α) taking antiparallel β-sheet, 3_1-helix, α-helix and ω-helix forms The latter two conformations are achieved by incorporating Gly residues in the homopolypeptides of other amino acids taking the respective conformations By selective enrichment with ^{13}C, it was found that the isotropic chemical shifts and principal values of the chemical shift tensor of Gly C_α incorporated in some homopolypeptides are displaced depending on their conformations

INTRODUCTION

It has been demonstrated that ^{13}C NMR chemical shifts of a number of polypeptides and proteins in the solid state as determined by the cross polarization–magic angle spinning (CP-MAS) method are significantly displaced depending on their particular conformations, such as α-helix, β-sheet, 3_1-helix and ω-helix [1–10]. In particular, the ^{13}C chemical shifts of individual amino acid residues of peptides and proteins are not so influenced by a specific amino acid sequence but by the local conformation and a nature of hydrogen bonding of the residue under consideration.

In a previous paper [11], we reported that the isotropic Gly carbonyl carbon (Gly CO) chemical shifts and chemical shift tensors are significantly displaced depending on their conformational state and that the principal values (σ_{11}, σ_{22}

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and σ_{33}) of the chemical shift tensors are displaced more than the isotropic chemical shift values. It is found that the direction of the most sensitive component, σ_{22}, is the same as that of the C=O bond. This suggests that the Gly CO signals could be mainly accounted for by the nature of the hydrogen bond.

In the attempt to obtain further information on the relationship between the conformation and ^{13}C chemical shift, a study of the C_α carbon in Gly residue (Gly C_α) is expected to yield important results complementing the previous work [11] on the carbonyl carbon. In this paper, we attempt to analyze the isotropic ^{13}C chemical shifts and individual components of ^{13}C chemical shift tensors of the glycine residue methylene carbons of polypeptides taking various conformations, such as β-sheet, 3_1-helix, α-helix and ω-helix forms, and discuss the conformational dependence of Gly C_α carbon chemical shifts. In general, the experimental error of the chemical shift values estimated by ^{13}C CP-MAS method is still rather large (>0.2 ppm) compared with solution state ^{13}C NMR. We have already observed a slight change in Gly C_α chemical shifts for a series of silk fibroin samples and sequential polypeptides [5], but the chemical shifts were sometimes the same within the experimental error. Thus, increase of the signal-to-noise ratio and enhancement of the resolution for ^{13}C spectra are required for analysing the ^{13}C chemical shift of the C_α carbon in the Gly residue. Such a situation may be accomplished by the incorporation of a small amount of [2-^{13}C]Gly residue (methylene carbon is enriched by ^{13}C) into homopolypeptides, where the respective conformations remain unchanged. For this purpose, we adopted the incorporation of ^{13}C enriched [2-^{13}C]glycine residues into poly(β-benzyl-L-aspartate) ((Asp(OBzl))$_n$).

EXPERIMENTAL SECTION

Materials

Poly[2-^{13}C]glycine: Poly[2-^{13}C]glycine with a ^{13}C purity of 8 at.% was prepared by heterogeneous polymerization of [2-^{13}C]glycine *N*-carboxy anhydride (NCA) initiated by n-butylamine in acetonitrile at room temperature, where the NCA was synthesized from a mixture of [2-^{13}C]glycine (Merck, Lot no. 1017J, isotope purity: 91.6 at.%) and glycine (Nihon Rika Co.). The mole ratio of NCA to initiator ([A]/[I] ratio) was chosen as 100. The polymer yield was about 90%. The as-polymerized crystals took the antiparallel-β-sheet form ((Gly)$_n$ form I). The conformation of this sample was converted to 3_1-helix ((Gly)$_n$ form II) by precipitation from the saturated aqueous lithium bromide solution [12].

The conformational characterization of the host polypeptides was made on the basis of the conformation-dependent ^{13}C NMR chemical shifts [3] determined by the ^{13}C CP-MAS method and partly by IR spectroscopy. Each sample thus prepared gave IR and CP-MAS spectra almost identical to the respective

TABLE 1

Synthesis and characterization of poly([2-^{13}C]glycine) and [2-^{13}C]glycine-containing polypeptides

Sample[a]	Gly content (%)	Initiator	Solvent	A/I ratio[b]	Conformation[c]
$(Gly^*)_n$ I	100	n-$BuNH_2$	CH_3CN	100	β-sheet
$(Gly^*)_n$ II	100	n-$BuNH_2$	CH_3CN	100	3_1-helix
$(Asp(OBzl),Gly^*)_n$	8	Et_3N	$ClCH_2CH_2Cl$	100	α-helix
$(Asp(OBzl),Gly^*)_n$	8	Et_3N	$ClCH_2CH_2Cl$	100	ω-helix

[a]Symbol * indicates methylene carbon enriched by ^{13}C
[b]Theoretical number of degree of polymerization
[c]Determined by the conformation-dependent ^{13}C NMR chemical shifts of host residues and partly by the characteristic bands in IR spectra.

reference data of $(Asp(OBzl))_n$ reported previously [5]. (According to this characterization, it is apparent that the molecular weights of the samples are high enough for the conformational study [2].)

Copolypeptides: Copolypeptide, $(Asp(OBzl),Gly^*)_n$, was prepared by polymerization of [2-^{13}C]Gly NCA (90 at.%) with β-benzyl-L-aspartate NCA initiated by triethylamine in dichloroethane. A [2-^{13}C]glycine NCA content of about 8% and the [A]/[I] ratio of 100 were chosen. The α-helical form of $(Asp(OBzl),Gly^*)_n$ was converted to the ω-helical form by annealing at 123 °C for 10 min. The synthetic conditions and characterization of the samples used here are summarized in Table 1.

^{13}C NMR measurement

^{13}C CP-MAS NMR spectra were recorded at 67.80 MHz with a JEOL GX-270 spectrometer equipped with a CP-MAS accessory at room temperature. Field strength of the ^{1}H decoupling was 1.2 mT The contact time was 2 ms and repetition time was 5 s. Spectral width and data points were 40 kHz and 8 K, respectively. Samples were placed in a bullet-type rotor and spun as fast as 3–4 kHz. Powder pattern spectra were recorded with the same instrument without spinning. Spectra were usually accumulated 100–2000 times to achieve a reasonable signal to noise ratio. ^{13}C chemical shifts were calibrated indirectly through external adamantane (29.5 ppm relative to tetramethylsilane ($(CH_3)_4Si$)).

To obtain the three principal components of the shielding tensors (σ_{11}, σ_{22} and σ_{33}, from the downfield to upfield), a fitting was done by superimposing the theoretical powder pattern line shape convoluted with Lorentzian function (symmetrical broadening function) to the observed powder patterns. The theoretical line shape function expressed by Bloembergen and Rowland [13] was applied to the present case. This procedure has been detailed elsewhere [11].

RESULTS AND DISCUSSION

Isotropic chemical shifts

Figures 1 a–d show 67.8 MHz ^{13}C CP-MAS NMR spectra of $(Gly)_n$ I (antiparallel β-sheet), $(Gly)_n$ II (3_1-helix), α-helical $(Asp(OBzl),Gly^*)_n$ and ω-helical $(Asp(OBzl),Gly^*)_n$, respectively. All the isotropic chemical shifts are listed in Table 2. The C_α chemical shift of $(Gly)_n$ II is slightly displaced upfield by 0.6 ppm with respect to that of $(Gly)_n$ I. These chemical shifts are at the same positions as obtained in a previous study [11], where the methylene carbons were in natural abundance and the signal intensities were insufficient for a detailed examination. The value of 0.6 ppm is a significant change compared with the experimental error (± 0.2 ppm). Kricheldorf and Müller [9] reported an additional two peaks in the C_α region of $(Gly)_n$ II sample, of which the

TABLE 2

^{13}C Chemical shifts of polypeptides containing glycine residues characteristic of the β-sheet, 3_1-helix, α-helix, and ω-helix forms (± 0.2 ppm from TMS)

Sample[a]	Conformation[b]	C_α[c]	^{13}C chemical shift				Ref
			Carbonyl	C_β	Phenyl	Benzyl	
$(Gly^*)_n$ I	β-sheet	44.3$^+$	169.7				this work
$(Gly^*)_n$ II	3_1-helix	43.7$^+$	172.8				this work
$(Gly^{\#})_n$ II	β-sheet	44.2$^+$	169.5				11
$(Gly)_n$ II	β-sheet	44.2$^+$	169.4				4
$(Gly^{\#})_n$II	3_1-helix	45.3$^+$	170.2				9
$(Gly)_n$ II	3_1-helix	44.2$^+$	172.8				11
		44.2$^+$	173.3				4
		43.0$^+$	173.1				9
Glycine(amino acid)		46.0$^+$					13
$(Asp(OBzl),Gly^*)_n$	α-helix	53.9	175.0	35.2	129.0	66.7	this work
		44.8$^+$					
$(Asp(OBzl),Gly^*)_n$	ω-helix	51.0	171.8	34.8	130.0	66.5	this work
		45.1$^+$					
$(Asp(OBzl))_n$	α-helix	54.4	175.9	34.8	130.8	66.7	3
		54.6	175.9	35.2	130.2	67.1	9
	α_L-helix	51.9	172.1	34.8	130.2	67.1	3
	ω-helix	51.5	172.3	33.9	130.0	66.3	3
	β-sheet	50.2	170.6	36.1	130.0	66.9	3

[a]Symbol * indicates methylene carbon enriched by ^{13}C, and symbol # indicates carbonyl carbon enriched by ^{13}C

[b]α-Helical form is right-handed unless otherwise specified

[c]Symbol $^+$ indicates chemical shift for glycine residue

upfield peak was assigned to that of $(Gly)_n$ II. This result coincides with our observation.

For the samples which take the α-helix and ω-helix form, small peaks for the CO, C_α, C_β, benzyl and phenyl carbons of Asp(OBzl) residue are also seen in Fig. 1, in addition to the sharp intense Gly C_α peak. A comparison of the ^{13}C chemical shifts of these carbons with reference data for $(Asp(OBzl))_n$ reported previously leads to the result that the two $(Asp(OBzl), Gly^*)_n$ samples take the α- and ω-helical forms, respectively. Thus, it is suggested that the Gly residue in the copolymer also takes the respective helical form. The Gly C_α carbon signal for the α-helix form resonates downfield by 0.5 ppm relative to $(Gly)_n$ I. Thus, one can discriminate the α-helix form from the 3_1-helix form by means of Gly C_α chemical shift, although there is no significant difference between them in the isotropic Gly CO chemical shifts.

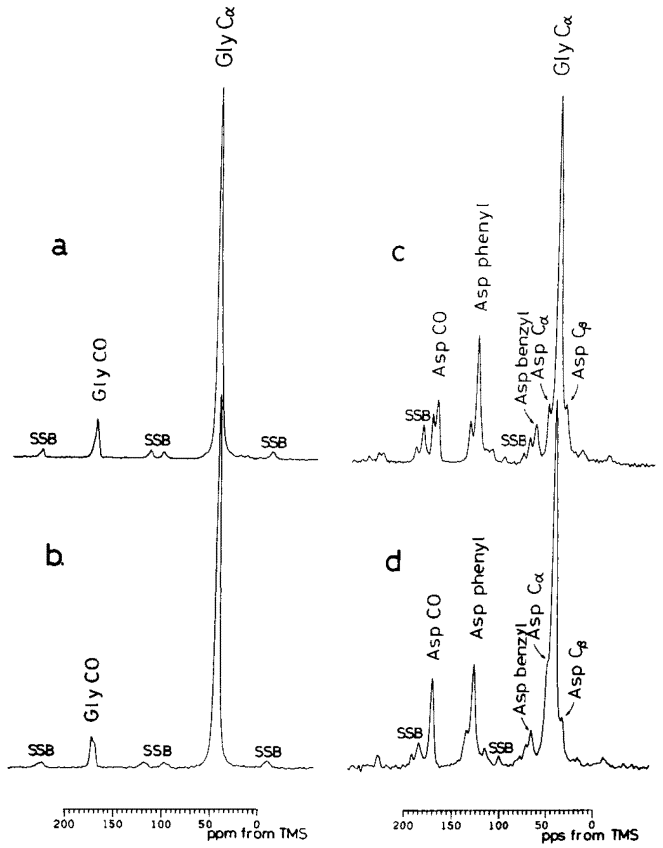

Fig 1 68 50 MHz ^{13}C CP-MAS NMR spectra of poly[2-^{13}C]glycine and [2-^{13}C]glycine-containing polypeptides (a) $(Gly^*)_n$ I, (b) $(Gly^*)_n$ II, (c) $(Asp(OBzl),Gly^*)_n$ (α-helix), (d) $(Asp(OBzl),Gly^*)_n$ (ω-helix)

However, it is also found that the difference in Gly C_α chemical shift is very small between the α-helix and the β-sheet form. In general, the C_α signals for the α-helix form resonate further downfield by 4–6 ppm compared with the β-sheet form. Such a small chemical shift change may come from the fact that glycine is the only amino acid with no side chain.

The Gly C_α signal of the α-helical sample is displaced slightly upfield by 0.3 ppm compared with the ω-helical one. The order in these C_α chemical shifts is opposite to that for $(Asp(OBzl))_n$, where the Asp(OBzl) C_α signal for the α-helix form appears 2.9 ppm downfield compared with the ω-helix form. In the previous study [11], the Gly CO chemical shifts for the α-helix, ω-helix and β-sheet forms showed the same order as in the case of $(Asp(OBzl))_n$ CO chemical shifts, where the conformation dependence was explained in terms of hydrogen bonding. In the case of the Gly C_α chemical shift, however, the hydrogen bonding effect would not be significant. Thus, the conformational change of the Gly C_α chemical shift can be ascribed to a change of electronic structure containing many small contributions. In spite of its relatively small chemical shift variation, we can distinguish all the four conformations in terms of the Gly C_α isotropic chemical shift

Chemical shift tensors

Figure 2 a–d shows 67.8 MHz ^{13}C powder spectra of $(Gly)_n$ I, $(Gly)_n$ II, and α- and ω-helical $(Asp(OBzl),Gly^*)_n$, respectively. These powder spectra were expanded when we fitted the theoretical powder pattern line shape to the observed spectra. The fitting was done by taking into account the isotropic ^{13}C chemical shift determined by ^{13}C CP/MAS NMR and the value of σ_{22} which was directly read from the observed powder pattern spectra. As shown in Fig. 3, a significant difference in the three principal components can be identified from this fitting. For this, the error limit of σ_{22} is surely smaller than ± 1 ppm. However, the error limit of σ_{11} nd σ_{33} is larger than that of σ_{22}. Therefore, the error limit of the fitting for the observed powder pattern spectra would be ± 1 ppm. All the tensor components of the Gly C_α carbon determined from these spectra and those of glycine amino acid [13] are listed in Table 3 (Fig. 4). The chemical shift anisotropy $(\sigma_{11}-\sigma_{33})$ of the Gly C_α carbon is much smaller than that of the Gly CO (about 150 ppm). This is because the glycine residue has no side chain and has a C_{2v} symmetry axis.

The chemical shift anisotropy of $(Asp(OBzl), Gly^*)_n$ was estimated to be larger than that of $[Gly]_n$. This could be attributed to the overlap of the Gly C_α signal with the Asp(OBzl) C_α and Asp(OBzl) C_β signals. In fact, the values of the tensor components σ_{11} and σ_{33} determined from these spectra are somewhat ambiguous (uncertainty ± 2 ppm). Nevertheless, it can be said that the downfield shift of the isotropic Gly C_α chemical shifts for the α- and ω-helix forms relative to $(Gly)_n$ I is caused by the significant downfield shift of the σ_{11}

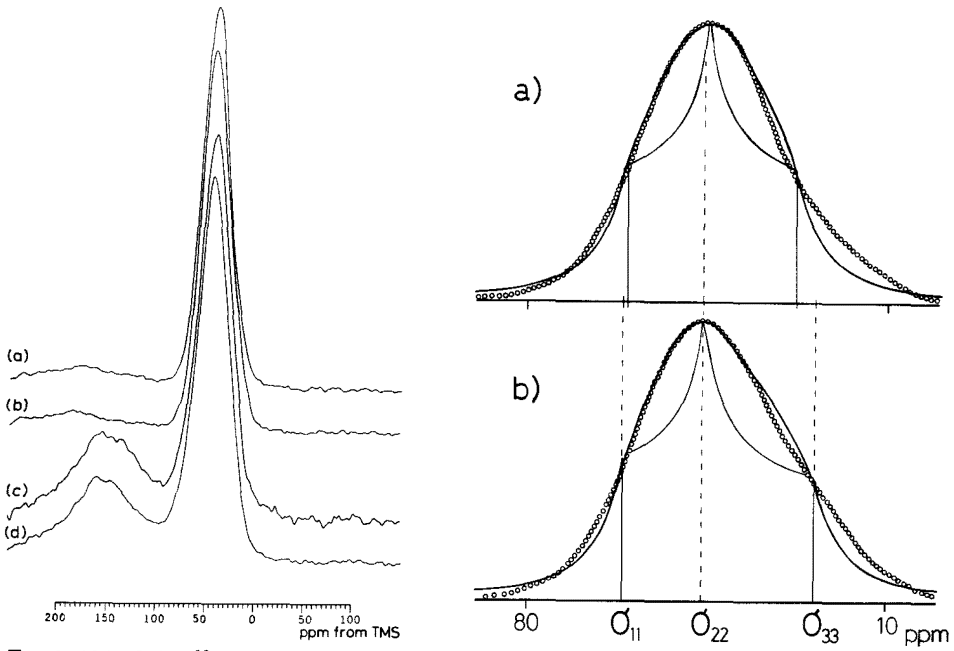

Fig 2. 68.50 MHz ^{13}C NMR powder pattern spectra of poly[2-^{13}C]glycine and [2-^{13}C]glycine-containing polypeptides (a) $(Gly^*)_n$ I, (b) $(Gly^*)_n$ II, (c) $(Asp(OBzl),Gly^*)_n$ (α-helix), (d) $(Asp(OBzl),Gly^*)_n$ (ω-helix)

Fig. 3 Schematic representations of theoretical powder pattern, theoretical powder pattern convoluted with the Lorentzian function, and experimental powder pattern (circles are experimental points) for (a) $(Gly^*)_n$ I and (b) $(Gly^*)_n$ II

component, which compensates the slight upfield shift of the σ_{33} component. Similarly, the upfield shift of the σ_{33} component also leads to a slight variation of the isotropic chemical shift of $(Gly)_n$ II relative to $(Gly)_n$ I.

The principal values and their directions for C_α carbon chemical shift tensors of glycine amino acid were determined by Haberkorn et al. [14]. They found that the orientation of each component is fairly typical and indicates that the methylene group of the glycine residue has approximately C_{2v} symmetry, similar to the case of the CH_2 groups in n-paraffins. The σ_{11} component is almost in the same direction as the H···H vector connecting the two protons of the CH_2 moiety, and σ_{22} nearly bisects the H–C–H angle. The σ_{33} component is found to be normal to the H–C–H plane. It is assumed that the orientation of Gly C_α chemical shift tensors of polyglycine and polypeptides containing glycine residues are not very different from that of free glycine. Although the σ_{22} component can be directly estimated from the powder spectra so that the error limit is small (uncertainty ± 0.5 ppm), it is difficult to obtain a clear relationship between σ_{22} and the isotropic chemical shift change. This may be because σ_{22} is aligned in the direction representing the C_{2v} symmetry axis bi-

TABLE 3

^{13}C chemical shift tensor components of glycine methylene carbons (ppm from TMS)

Sample [a]	Conformation	Tensor component [b]				Anisotropy breadth		Ref.
		σ_{iso}	σ_{11}	σ_{22}	σ_{33}	$\Delta\sigma$ [c]	$\sigma_{11}-\sigma_{33}$	
$(Gly^*)_n$ I	β-sheet	44 3	60	45 0	28	−25	32	this work
$(Gly^*)_n$ II	3_1-helix	43.7	61	45 7	25	−28	36	this work
$(Asp(OBzl),Gly^*)_n$	α-helix	44 8	65	44 1	25	−30	40	this work
$(Asp(OBzl),Gly^*)_n$	ω-helix	45 1	65	46 5	24	−32	41	this work
Glycine (amino acid)		46 0	65 3	46 2	26 6	−29	39	13
			66 8	44 7	26 7	−29	40	

[a] Symbol * indicates methylene carbon enriched by ^{13}C
[b] Uncertainty ± 2 ppm
[c] $\Delta\sigma = \sigma_{33} - \frac{1}{2}(\sigma_{11} + \sigma_{22})$

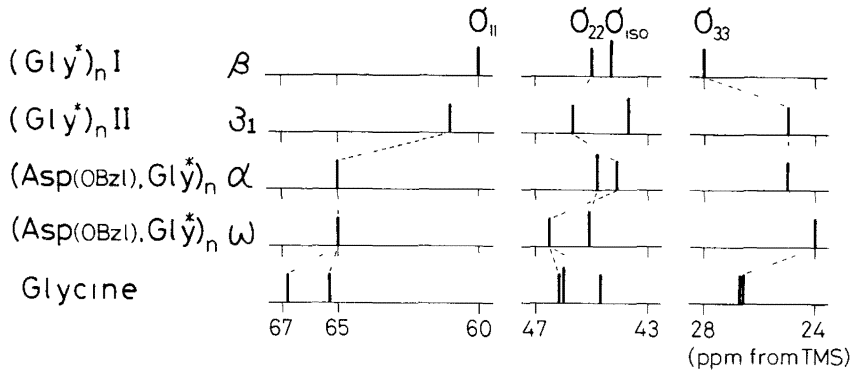

Fig 4 Observed stick spectra of ^{13}C NMR isotropic chemical shifts and tensor components of glycine methylene carbons of poly[2-^{13}C]glycine, [2-^{13}C]glycine-containing polypeptides, and glycine (amino acid).

secting the H–C–H plane. Facelli et al. [15] showed that the shielding components σ_{22} and σ_{33} showed a range of variation narrower than σ_{11} for methylene carbon for a series of cycloalkanes, cycloalkenes and heterocyclic compounds. Further, Veeman [16] has pointed out that the standard deviations of each component of methylene carbons are 15 ppm for σ_{11}, 10 ppm for σ_{22}, and 17 ppm for σ_{33}. These facts accord with the relative insensitivity of the σ_{22} component.

Contrary to the case of Gly CO, where the σ_{22} component plays an important role in determining the isotropic chemical shift change, there is no corresponding component in Gly C_α chemical shift tensors.

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