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^{13}C and ^{2}H NMR studies of *N*-acetylglycine oligomer ethyl esters (residue number, $n = 3$–9) and selectively C-deuteriated oligomer acid types ($n = 3$, 4): Intermolecular hydrogen-bonding distance and segmental mobility

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N-Acetylglycine oligomer ethyl esters (residue number, $n = 3$–9) and selectively C-deuteriated oligomer acid types ($n = 3$, 4) have been synthesized. Two crystalline modifications, the A- and B-series [which correspond to polyglycine (PG) II and I in structure, respectively] have been prepared. For the two series of oligomers, the intermolecular hydrogen-bond distance ($R_{N\cdots O}$) and segmental mobility were evaluated using ^{13}C CP MAS and ^{2}H NMR spectra. The results showed that the $R_{N\cdots O}$ values for the A- and B-series of oligomer ethyl esters closely correspond to those of PGII and PGI, respectively, and that for the two series of C-deuteriated oligomers segmental mobilities of the CH_2 groups of both the N- and C-terminal residues are more restricted than those of the methylenes sandwiched between the terminal residues.

In our previous study,[1] we demonstrated that two crystalline modifications, solid-A and -B are obtained for *N*-acetylglycine trimer, tetramer and pentamer acid types, and that these correspond to polyglycine (PG) II and I, respectively, in structure.[2–5] Furthermore, a solid-A $\rightleftharpoons$ solid-B conversion, which is similar to the PGII $\rightleftharpoons$ PGI conversion, is possible for these oligomers. However, the cause of such a conversion for these simple oligomers remains unresolved.

We might expect inter- or intra-molecular hydrogen bonds to play a critical role in the mechanism for the A $\rightleftharpoons$ B conversion.[1] Indeed, the significant influence of both the N- and C-terminal residues on a hydrogen-bonding system has already been pointed out for oligopeptides taking up a specific secondary structure.[6,7] Elucidation of the role of the hydrogen bonds in these oligomers is therefore essential for understanding further the mechanism of such an interconversion.

In the present study, *N*-acetylglycine oligomer ethyl esters (residue number, $n = 3$–9) and selectively C-deuteriated *N*-acetylglycine oligomer acid types ($n = 3$, 4) were prepared by a stepwise procedure. ^{13}C and ^{2}H NMR spectra and ^{2}H spin–lattice relaxation times have been measured for these oligomers in the solid state, and hydrogen-bond distances and segmental mobilities have been evaluated.

Experimental

Materials

N-Acetylglycine oligomer ethyl esters [$CH_3CO(NHCH_2CO)_n OC_2H_5$, $n = 3$–9; AcG_nEt] were synthesized by previously described stepwise procedures.[8]

Selectively C-deuteriated *N*-acetylglycine trimers [$AcG_1^*G_2G_3$, $AcG_1G_2^*G_3$ and $AcG_1G_2G_3^*$ (the asterisk denotes the CD_2-glycine residue): AcG_mD3, m (the numbering of the C-deuteriated glycine residue) = 1–3] and tetramers ($AcG_1^*G_2G_3G_4$, $AcG_1G_2^*G_3G_4$, $AcG_1G_2G_3^*G_4$ and $AcG_1G_2G_3G_4^*$; AcG_mD4, $m = 1$–4) were prepared by stepwise procedures as follows. The N- or C-terminus of 2,2-[$^{2}H_2$]glycine (G*; 98 atom% D, Aldrich Chem. Co.) was protected by a carbobenzoxy group (Z-G*)[9] or a *p*-nitrobenzyl ester group (G*-ONb),[10] respectively. Z-G* or G*-ONb was condensed with the C- or N-terminus-protected glycine (G-ONb or Z-G) to prepare Z-G*-G-ONb or Z-G-G*-ONb, respectively, using a method similar to that described by Anderson *et al.*[11] The Z-group of these compounds was removed in 25% HBr–acetic acid solution to obtain N-terminus-free diglycine *p*-nitrobenzyl esters. The diglycine *p*-nitrobenzyl esters thus obtained were further condensed with Z-G. This procedure was repeated until the N- and C-terminus-protected glycine oligomers with the required residue number ($n = 3$ or 4) were obtained. Other C-deuteriated glycine oligomers were prepared by introducing the Z-G* group in the required position. Then, the Z- and -ONb groups of the N- and C-terminus-protected glycine oligomer were removed by Pd-catalytic reduction to obtain the terminus-free oligoglycine. Selectively C-deuteriated *N*-acetylglycine oligomers were prepared by acetylation of the corresponding terminus-free C-deuteriated oligoglycines with acetic anhydride.[12] The undeuteriated trimer (AcG3H) and tetramer (AcG4H) acid types were prepared by hydrolysis of the corresponding esters.

These oligomers were treated with lithium bromide (LiBr) aqueous solution or dichloroacetic acid (DCA) in the manner described previously.[1] The samples treated with the LiBr aqueous solution and with DCA are termed the A- and B-series samples, respectively. The abbreviations for the two crystalline modifications of these oligomers are AcG_nEt-A and AcG_nEt-B for the A- and B-series of the AcG_nEt samples, respectively, AcG_mD3-A and AcG_mD4-A for the A-series of the trimer and the tetramer acid types, and AcG_mD3-B and AcG_mD4-B for the B-series of the trimer and tetramer acid types, respectively.

Samples were identified by elemental analysis and agreement between the calculated and observed values was within 0.5%. The isotopic purity of each C-deuteriated oligomer, determined by ^{1}H NMR, was 98%.

Methods

Solid-state high-resolution ^{13}C NMR measurements were recorded on a Varian UNITY-400 plus spectrometer operated at 100.6 MHz with cross-polarization magic-angle-spinning (CP MAS) at 25 °C. ^{13}C CP MAS spectra were obtained using the following parameters: acquisition time 0.102 s, contact time 10 ms, delay time 5 s, pulse width 4.5 μs and spectral width 40 241.4 kHz. NMR signals were obtained by the use of a cross-polarization sequence (XPOLAR) and accumulated 1024–8192 times for 8192 data points. The ^{13}C chemical shifts (in ppm) are given relative to an external adamantane standard (the chemical shift of the upper field peak was set to δ 29.5).

^{2}H NMR powder pattern spectra were also measured at 61.4 MHz using a Varian UNITY-400 plus spectrometer at 25 °C. A quadrupolar-echo pulse sequence (SSECHO) was used with an acquisition time of 0.008 s, pulse width of 2.9 μs and spectral width of 2.0 MHz. Two delay times (3 and 10 s) were used to measure a T_1 value by the saturation recovery method.[13] ^{2}H NMR signals were accumulated 512–4096 times for 32 768 data points. The ^{2}H chemical shifts (in ppm) are given relative to an external [$^{2}H_4$]malonic acid standard.

X-Ray powder diffraction patterns were obtained by use of an RAD-RC diffractometer with a countermonochromator (Cu-Kα X-ray source; 40 kV, 80 mA).

Results and Discussion

X-Ray powder diffraction patterns were measured for the A- and B-series samples and compared with those of PGII and PGI.[2,3] Very intense reflections at 4.16–4.19 Å and weak ones at 3.11–3.26 Å were observed in common for all the A-series samples containing acid types, and these reflections correspond closely to the 4.14 and 3.09 Å reflections, respectively, of PGII. The patterns for the samples of AcG$_n$Et-B (n = 6–9) and for the B-series of the trimer and tetramer acid types contained very intense reflections at 3.35–3.38 Å and medium ones at 4.35–4.42 Å, and these corresponded to the 3.42 and 4.36 Å reflections respectively, of PGI. These observations suggest that the A-series oligomers adopt a PGII-type helical structure while the B-series of ester samples (from hexamer to nonamer) and the trimer and tetramer acid types take up a β-sheet structure similar to that of PGI. Secondary structures of the A- and B-series samples were confirmed by X-ray powder diffraction patterns. However, for the AcG3Et-B, AcG4Et-B and AcG5Et-B samples, very intense reflections characteristic of PGII were observed, and therefore these three B-series samples can be regarded as PGII-type oligomers.

^{13}C Chemical shifts of peptide C=O carbons and intermolecular hydrogen-bond distances

^{13}C CP MAS NMR spectra of the A- and B-series samples in the solid state were measured in order to evaluate the hydrogen-bond distance between nitrogen and oxygen ($R_{N\cdots O}$) in these oligomers. Tentative assignment was then made by comparing the chemical shifts with those for the glycine residue and peptide C=O carbon atoms of other oligopeptides containing a glycine residue.

Fig. 1 shows the ^{13}C signals for the peptide C=O carbon atoms in these oligomers over the range of residue numbers. The observed ^{13}C chemical shifts (δ) for these signals (CO) are listed in Table 1 together with the corresponding data for PGII and PGI.[14] A close correspondence is seen between the CO ^{13}C δ values of the A- and B-series samples and those of PGII and PGI, respectively.

The ^{13}C resonance signals of the glycine C$^{\alpha}H_2$ carbon atoms in the A- and B-series differ. It seems that this difference is characteristic only of the A- and B-series oligomers, since there is no corresponding difference in the chemical shifts of PGII and PGI.

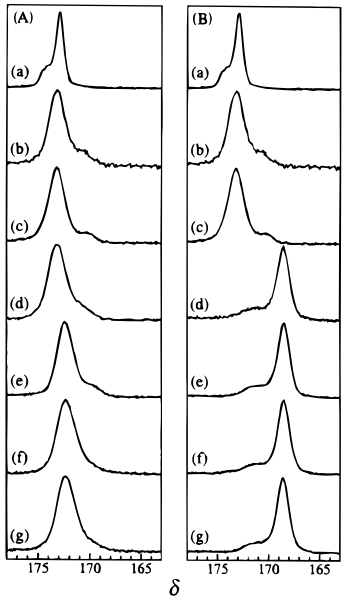

Fig. 1 ^{13}C NMR spectra of the C=O resonance in solid AcG$_n$Et samples: (A) A-series and (B) B-series; (a) n = 3, (b) n = 4, (c) n = 5, (d) n = 6, (e) n = 7, (f) n = 8 and (g) n = 9

Table 1 ^{13}C Chemical shifts δ (in ppm) and hydrogen-bond distances $R_{N\cdots O}$ (in Å) for the AcG$_n$Et-A, AcG$_n$Et-B samples (n = 3–9) and for the A- and B-series of AcG3H and AcG4H (n = 3*, 4*)

	A-series			B-series		
n	δ (C$^{\alpha}H_2$)	δ (C=O) (peptide)	$R_{N\cdots O}$ [a]	δ (C$^{\alpha}H_2$)	δ (C=O) (peptide)	$R_{N\cdots O}$ [a]
9	41.89	172.36	2.71	43.65	168.50	3.03
8	41.94	172.36	2.71	43.50	168.45	3.03
7	41.89	172.36	2.71	43.41	168.45	3.03
6	42.62	173.14	2.64	43.45	168.45	3.03
5	42.72	173.14	2.66	—	—	—
4	42.89	173.13	2.65	—	—	—
3	42.48	172.85	2.66	—	—	—
4*	42.53	172.75	2.68	44.68	169.33	2.95
3*	42.92	173.24	2.64	44.92	169.48	2.96
		PGII[b]			PGI[b]	
polyglycine	44.20	172.80	2.68	44.20	169.50	2.94

[a] Evaluated from eqn. (1). [b] Ref. 15.

The ^{13}C signals of the peptide C=O carbon atoms for the A- and B-series samples of the trimer and tetramer acid types are somewhat different in chemical shift but these differences are clearly attributable to PGII- and PGI-type structures, respectively.

Ando *et al.*[14] have reported that the ^{13}C chemical shifts of peptide-carbonyl carbons in glycine-containing peptides reflect the hydrogen-bond distances in peptides. They found that the ^{13}C chemical shifts of the carbonyl carbon atoms in a C=O···H—N type hydrogen-bonding system shift linearly downfield with a decrease in length of the hydrogen bond. Moreover, the ^{13}C chemical shift values of the carbonyl carbon atoms for PGI and PGII could be reasonably explained in terms of the difference in length of the hydrogen bond.

Asakawa *et al.*[15] have also found that there exists a linear relationship between CO ^{13}C δ and $R_{N\cdots O}$ [eqn. (1)], which can be used to estimate intra- or inter-molecular hydrogen-bond distances for glycine-containing peptides,

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

where δ_{iso} is the ^{13}C chemical shift of the peptide C=O carbon atom belonging to the glycine residue moiety.

$R_{N\cdots O}$ values were evaluated for the ethyl ester samples of the A- and B-series by using eqn. (1), and are also listed in Table 1. The $R_{N\cdots O}$ values, 2.64–2.71 Å for the A-series and 3.03 Å for the B-series, closely correspond to the N···O hydrogen bond lengths, 2.68 Å for PGII and 2.94 Å for PGI, respectively. This result indicates that the hydrogen-bond distances obtained for the A- and B-series of the oligomer ethyl esters are very similar to those for PGII and PGI. Similarly, the $R_{N\cdots O}$ values of the A- and B-series samples for the trimer and tetramer acid types correspond well to those of PGII and PGI, respectively.

^2H NMR spectra of selectively C-deuteriated oligomers and segmental mobilities

Wideline ^2H NMR spectra and ^2H spin–lattice relaxation times (T_1/s) were measured for the samples of selectively C-deuteriated A- and B-series in the solid state, in order to evaluate the mobility of each glycine residue in these oligomers.

Fig. 2 shows representative ^2H NMR spectra for the samples of selectively C-deuteriated A- and B-series, and the spectral features of all the C-deuteriated samples exhibit a typical quadrupolar splitting. The split separations of 116.24–117.22 kHz for the trimers and 114.29–118.68 kHz for the tetramers are almost independent of the position of the C-deuteriated glycine residue. Furthermore, it is evident that

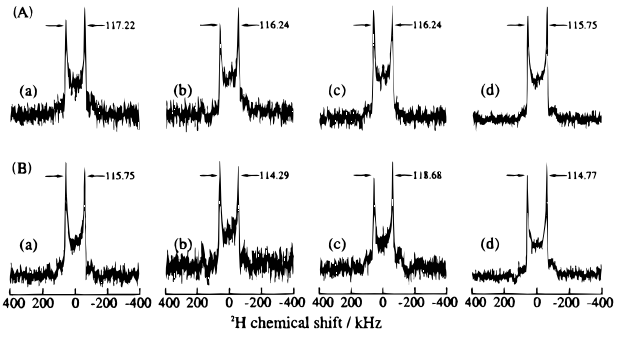

Fig. 2 ^2H NMR spectra of selectively C-deuteriated *N*-acetylglycine tetramer acid types in the solid state: (A) A-series [(a) AcG1D4-A, (b) AcG2D4-A, (c) AcG3D4-A, (d) AcG4D4-A] and (B) B-series [(a) AcG1D4-B, (b) AcG2D4-B, (c) AcG3D4-B (d) AcG4D4-B]

Table 2 ^2H T_1 values (in s) for selectively C-deuteriated *N*-acetylglycine oligomer acid types

	A-series	B-series
AcG1D3	4.2 ± 0.3	9.4 ± 1.7
AcG2D3	3.6 ± 0.2	4.6 ± 0.3
AcG3D3	2.4 ± 0.2	5.0 ± 0.3
AcG1D4	4.2 ± 0.3	6.8 ± 1.1
AcG2D4	5.9 ± 0.4	5.8 ± 0.4
AcG3D4	4.6 ± 0.3	6.2 ± 0.4
AcG4D4	2.6 ± 0.2	4.1 ± 0.3

there is not a marked difference in the split separations for the A- and B-series oligomers.

The observed T_1 values are listed in Table 2. For the A-series oligomers, the T_1 values of the C-terminal CD_2 groups tend to become smaller, compared with those of the CD_2 groups sandwiched between the C- and N-terminal residues. In particular, for AcG*m*D4-A the T_1 value of the N-terminal CD_2 group also exhibits a tendency to become small. The results show that both the N- and C-terminal methylene groups are restricted and that the segmental mobility of CD_2 groups sandwiched between these termini is larger than that of either of the terminal methylenes. For the B-series oligomers, the T_1 value of the CD_2 group decreases in the order $^1CD_2 > {}^2CD_2 > {}^3CD_2$ (or $^1CD_2 > {}^2CD_2 > {}^3CD_2 > {}^4CD_2$), indicating that the segmental mobility of the C-terminal methylene group is markedly restricted for samples in the B-series.

Thus, the dependence of the ^2H T_1 value on the location of C-deuteriated glycine residue is obviously different for the A- and B-series samples.

From the IR and Raman spectral data of the terminal CO_2H groups of these acid type oligomers, we may assume that the mode of the hydrogen-bonding system, in which the terminal CO_2H group participates, is different for the A- and B-series samples. In fact, in our previous study[1] we have shown that strongly hydrogen-bonded cyclic dimers and weakly hydrogen-bonded types of CO_2H groups coexist in the A-series samples of the C-undeuteriated acid type oligomers while only the weakly hydrogen-bonded type of CO_2H groups exists in the B-series oligomers.

Thus, the ^2H T_1 results obtained in this present study may signal a difference in the hydrogen-bonded types of terminal CO_2H groups between the A- and B-series. For the A-series of trimer and tetramer acid types, formation of strongly hydrogen-bonded CO_2H cyclic dimers probably leads to a restricted state of the C-terminal glycine and this restriction contributes to the smaller T_1 value of the C-terminal glycine residue.

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References

1 H. Okabayashi, K. Ohshima, H. Etori, K. Taga, T. Yoshida and E. Nishio, *J. Phys. Chem.*, 1989, **93**, 6638.
2 W. T. Astbury, C. E. Dalgliesh, S. E. Darmon and G. B. B. M. Sutherland, *Nature* (*London*), 1948, **162**, 596.
3 W. T. Astbury, *Nature* (*London*), 1949, **163**, 722.
4 F. H. C. Crick and A. Rich, *Nature* (*London*), 1955, **176**, 780.
5 S. Krimm, *Nature* (*London*), 1966, **212**, 1482.
6 T. Uehara, H. Okabayashi, K. Taga, T. Yoshida and H. Kojima, *J. Chem. Soc., Faraday Trans.*, 1993, **89**, 2743.
7 K. Ohshima, H. Okabayashi and T. Yoshida, *J. Chem. Soc., Faraday Trans.*, 1994, **90**, 3483.

8 H. Etori, K. Taga, K. Watanabe, T. Yoshida and H. Okabayashi, *J. Raman Spectrosc.*, submitted.
9 J. P. Greenstein and M. Winitz, in *Chemistry of the Amino Acids*, John Wiley and Sons, New York, 1961, vol. 2, p. 891.
10 Ref. 9, p. 942.
11 G. W. Anderson, J. E. Zimmerman and F. M. Callahan, *J. Am. Chem. Soc.*, 1967, **89**, 5012.
12 R. M. Herbst and D. Shemin, in *Organic Syntheses*, *Collect. Vol. 2*, ed. A. H. Blatt, John Wiley and Sons, New York, 1966, p. 11.
13 N. Bloembergen, E. M. Purcell and R. V. Pound, *Phys. Rev.*, 1948, **73**, 679.
14 S. Ando, I. Ando, A. Shoji and T. Ozaki, *J. Am. Chem. Soc.*, 1988, **110**, 3380.
15 N. Asakawa, H. Kurosu, I. Ando, A. Shoji and T. Ozaki, *J. Mol. Struct.*, 1994, **317**, 119.

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