References and Notes

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Conformational Studies of Poly(alanine)s in Dichloroacetic Acid by Nuclear Magnetic Resonance

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ABSTRACT: Proton nuclear magnetic resonance spectra were studied for monodisperse L-alanine oligopeptides (the dimer, trimer, tetramer, and nonamer) containing an *n*-butylamide group at the C-terminal residue and for the random and block copolymers of D- and L-alanines having sharp molecular weight distributions in dichloroacetic acid (DCA). It was found that the NH signals of poly(L-alanine)s and some copoly(D,L-alanine)s were split into three peaks in DCA, suggesting that these NH peaks reflect the conformation of the polymers. These NH peaks were assigned to the terminal helix (helix–coil junction) and the random-coil and the inner helix, respectively, from the lowest field. On the basis of the above assignment, the microconformations of poly(alanine)s in DCA were examined quantitatively. Moreover, it was concluded from the specific nature of the solvent that both the effects of the hydrophobic side chains of the polymers and the acidity of the solvent are most important for the formation of the helical conformation of poly(L-alanine).

The proton nuclear magnetic resonance (^{1}H-NMR) study of poly(amino acid)s in solution was reported for the first time by Bovey et al.[2] in 1959. Later, the NMR spectra of synthetic polypeptides and biological peptides were extensively applied to a study of their conformation.[3]

Recently, the conformational transitions of many polypeptides were studied in solution by ^{1}H-NMR spectroscopy, and the mechanisms of the helix–coil and β-coil transitions have been clarified to a considerable extent. However, microconformational studies by ^{1}H NMR, such as the studies of the tacticities of the synthetic vinyl polymers (i.e., poly(methyl methacrylate) and poly(vinyl acetate)), have been very few.

More recently, Paolillo et al.[4] measured the ^{1}H-NMR spectra of the block copolymers of benzyl L-glutamate and benzyl L-aspartate and observed double peaks for the helical α-CH signals. They assigned one of the double peaks to the right-handed helix of the benzyl L-glutamate block and the other to the left-handed helix of the benzyl L-aspartate block. We found that the cis and trans conformations of the amide bond [–N(CH_3)-CO–] of copoly(L-alanine, *N*-methyl-L-alanine)s are closely related to the second structures of the copolymers.[5]

In this report, keeping these results in mind, we attempted to clarify the microconformation of polypeptides in solution by the NMR method, using monodisperse oligo-L-alanines (the dimer, trimer, tetramer and nonamer) containing an *n*-butylamide group at the C-terminal residue, poly(L-alanine)s having sharp molecular weight distributions, and a series of random and block copolymers of D- and L-alanines.

It is well known that the poly(L-alanine) takes partially helical conformations in dichloroacetic acid (DCA)[6] and that its helix–coil transition occurs gradually over a wide range of temperatures or solvent compositions. Therefore, poly(L-alanine) is an ideal polymer for investigation of microconformations of the helix and random-coil parts of partially helical polymers.

Further, it is of interest in relation to the mechanism of the helix–coil transition why DCA is a helix-supporting solvent for poly(L-alanine), whereas this is a coil-supporting solvent for various other poly(amino acid)s.

From the above standpoints, we have studied the microconformation of various poly(alanine)s in DCA using the ^{1}H-NMR technique.

Experimental Section

Materials. L-Alanine (commercial), oligo-L-alanines, poly(L-alanine)s (PLA), poly(D-alanine) (PDA), and the random copolymers (PDLA-R) and block copolymers (PDLA-B) of D- and L-alanines were used.

A series of oligo-L-alanines containing an *n*-butylamide group at the C-terminal residue were synthesized as described earlier.[7] They are shown as follows: H-[NH-CH(CH_3)-CO]$_n$-NHCH$_2$CH$_2$CH$_2$CH$_3$ where n = 2, 3, 4, and 9, respectively.

Poly(L-alanine)s, poly(D-alanine) and the D,L-copolymers having various molecular weights and sharp molecular weight distributions[8] were obtained by the heterogeneous polymerization[9] of L- or D-alanine *N*-carboxyanhydride (NCA) and copolymerization of the NCAs in acetonitrile using *n*-butylamine as initiator, respectively. Table I shows the samples used in this experiment, the degree of polymerization, and the compositions of the copolymers.

Measurement of ^{1}H-NMR Spectra. We obtained high-resolution ^{1}H-NMR measurements over the temperature range from 25 to 100 °C with a concentration of 5 w/v % in DCA. We used a 100-MHz NMR apparatus (JNM-PS100) manufactured by JEOL.

Chemical shift is denoted by the δ value (ppm) from tetramethylsilane (TMS), which is the external standard and is corrected with regard to bulk magnetic susceptibility. Spin decoupling was applied at 25 °C, and a du Pont 310 analyzer was used for peak separation of the NMR spectra by assuming Lorentzian peaks.

Correction with Regard to Bulk Magnetic Susceptibility. By

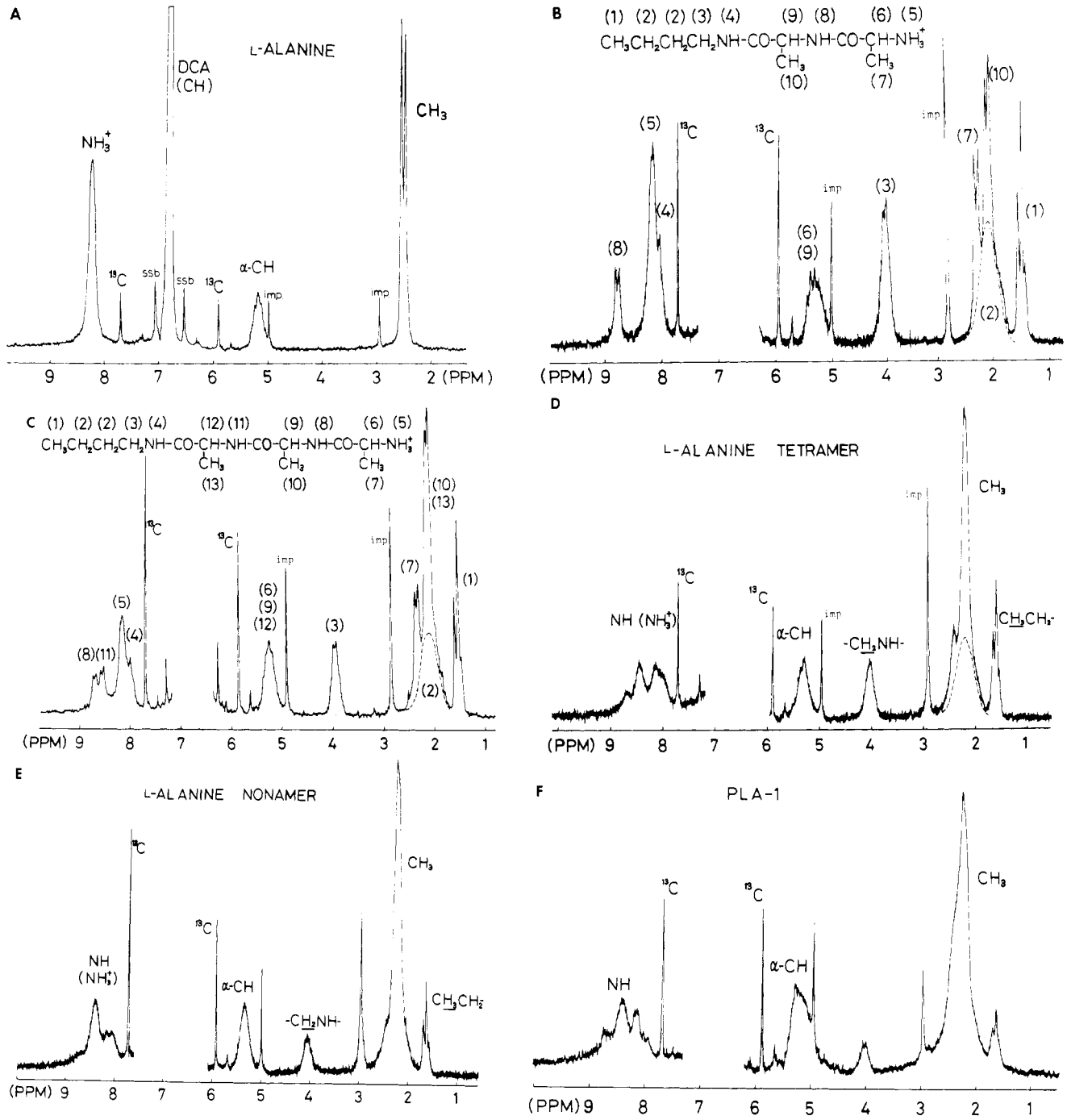

Figure 1. The NMR spectra and the assignments of the peaks for a series of oligo-L-alanines and poly-(L-alanine) in DCA at 28 °C: (A) L-alanine, (B) dimer, (C) trimer, (D) tetramer, (E) nonamer, and (F) PLA-1, respectively. The impurity peaks (imp) are due to the impurities of the solvent; those are confirmed to be $CHCl_2COOH$ (4.98 ppm) and CH_3COOH (2.92 ppm), respectively, which may not affect the results and discussion.

using the external method for the exact evaluation of the chemical shift values from TMS, we should correct with regard to the bulk magnetic susceptibility.[10]

The bulk magnetic susceptibility (X) of TMS at 30 °C is given by;[11]

$$X_{30\,°C}(\text{TMS}) = -0.537 \times 10^{-6}\ \text{Hz} \quad (1)$$

The correction with regard to the bulk magnetic susceptibility of DCA was possible by means of the NMR measurement using a double tube and the results gave;

$$X_{28\,°C}(\text{DCA}) = -0.773 \times 10^{-6}\ \text{Hz} \quad (2)$$

The position of the CH peak of the solvent (DCA) is 6.79 ppm (δ value at 28 °C), and it does not shift with temperature. Accordingly, it may be used as the standard peak for the chemical shift.

Measurement of Optical Rotatory Dispersion. Optical rotatory dispersion (ORD) measurements were carried out with a Jasco ORD/UV-5 spectropolarimeter equipped with a Haake thermostat using jacketed quartz cells (0.1, 0.01, and 0.001 dm) in the temperature range from 20 to 90 °C. Measurements were made over the range of wavelength 600–300 nm. The Moffitt–Yang parameters[12] (the a_0 and b_0 values) were calculated from the equation

$$[m']_\lambda = a_0\lambda_0^2/(\lambda^2 - \lambda_0^2) + b_0\lambda_0^4/(\lambda^2 - \lambda_0^2)^2 \quad (3)$$

where $[m']_\lambda$ is the reduced mean residue rotation at the wavelength λ (in nanometers) and the value of 212 nm was assumed for λ_0.[13] Refractive index correction was applied to all the calculations through the use of $n^{22}D(\text{DCA}) = 1.4659$.[14]

Results and Discussion

The NMR Spectra of L-Alanine Oligomers. Figure 1 shows the NMR spectra and the assignments of the peaks for

Table I
Characteristics of the Samples Used

Sample	$\overline{DP}_n$[c]	L composition,[d] %
L-Alanine	1	
Dimer (n = 2)	2	
Trimer (n = 3)	3	
Tetramer (n = 4)	4	
Nonamer (n = 9)	9	
PLA-1	16	
PLA-5	65	
PLA-A	120	
PDA-10	150	
PLA-10	160	
PLA-50	>160	
PDLA-RL90[a]	120	91
PDLA-RD75	120	22
PDLA-RL60	130	59
PDLA-RL50	120	50
PDLA-BL90[b]	150	82
PDLA-BL75	150	76
PDLA-BL60	150	61
PDLA-BL50	140	50

[a] PDLA-R denotes the random copolymers of D- and L-alanines. [b] PDLA-B denotes the block copolymers of D- and L-alanines. [c] The number average degree of polymerization ($\overline{DP}_n$) was calculated from the concentration of NH_3^+ end groups in TFA, as determined by NMR.[9] [d] D,L composition was calculated from $[\alpha]_{365}$ in TFA by the ORD method.[9]

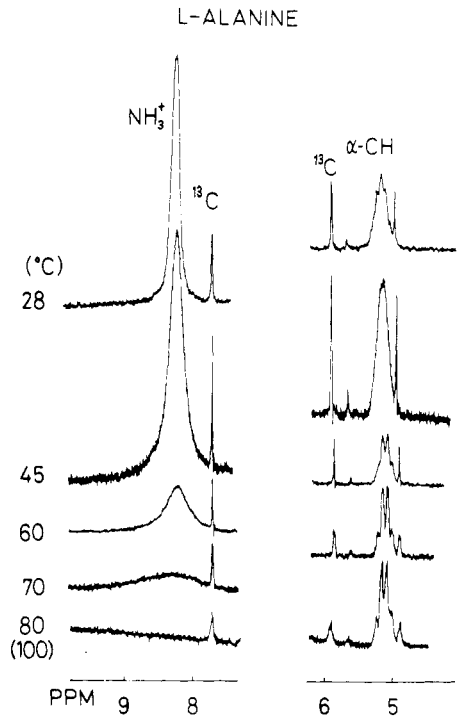

Figure 2. The NH_3^+ and α-CH proton spectra of L-alanine in DCA at various temperatures.

a series of oligo-L-alanines in DCA at 28 °C. The tentative assignments of the peaks involved were made on the basis of the direct inspection of the spectra, the integration of the peak intensities, the spin-decoupling method, and the separation of the peaks by changing temperature. For L-alanine (Figure 1A), the peaks are assigned to NH_3^+ (8.23 ppm), solvent CH (6.79 ppm), α-CH (5.16 ppm), and β-CH_3 (2.51 ppm), respectively. From the calculation of the intensities of these peaks, it was confirmed that the N-terminal group of L-alanine has been protonated as H_3^+N-CH(CH_3)-COOH in DCA. The chemical shift value of the individual peak was constant in the

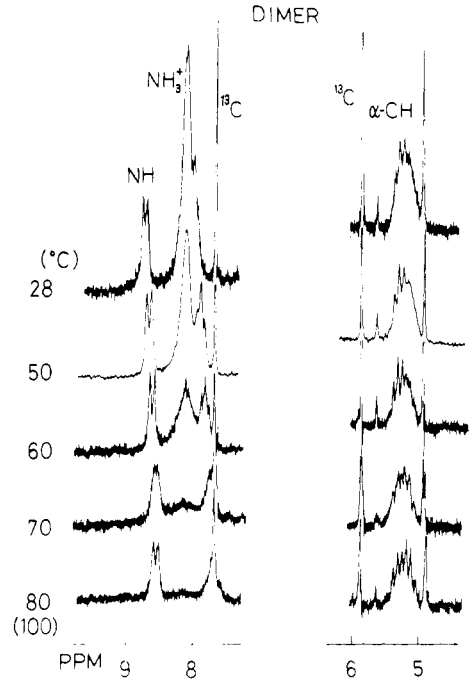

Figure 3. The NH, NH_3^+, and α-CH proton spectra of the dimer in DCA at various temperatures.

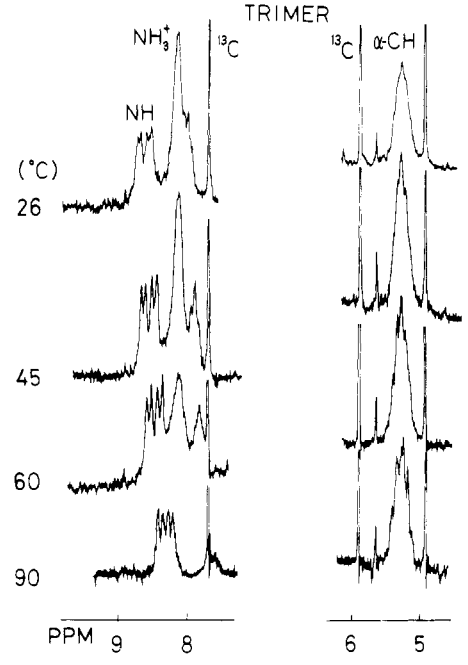

Figure 4. The NH, NH_3^+, and α-CH proton spectra of the trimer in DCA at various temperatures.

temperature range studied (20–100 °C).

Figure 2 shows the NH_3^+ and α-CH proton spectra of L-alanine in DCA at various temperatures. The NH_3^+ peak becomes broader and simultaneously its intensity decreases with increasing temperature up to 60 °C. This result is quite similar to that observed in trifluoroacetic acid (TFA).[10] Moreover, the splitting of the α-CH peak undergoes changes corresponding to the broadening of the NH_3^+ peak. Thus, the NMR spectra of L-alanine in DCA were similar to that in TFA.

For the dimer (Figure 1B), the peaks are assigned to NH (8.00–8.80 ppm), NH_3^+ (8.13 ppm), solvent CH (6.79 ppm), solvent CH (^{13}C satellites; 7.69 and 5.89 ppm), α-CH (5.33

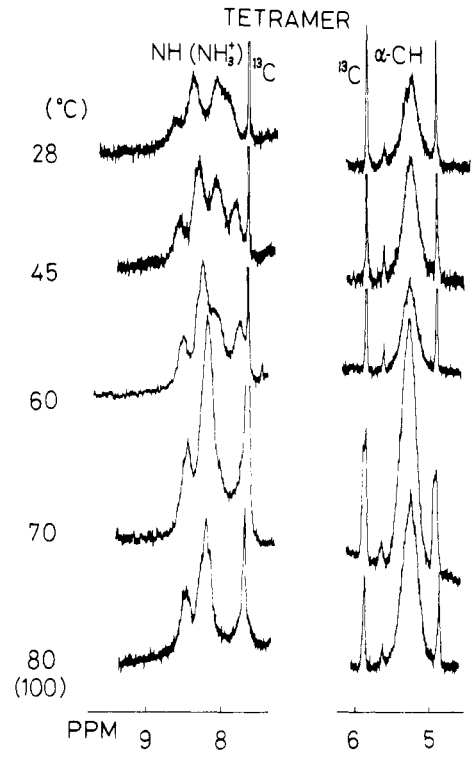

Figure 5. The NH, NH_3^+, and α-CH proton spectra of the tetramer in DCA at various temperatures.

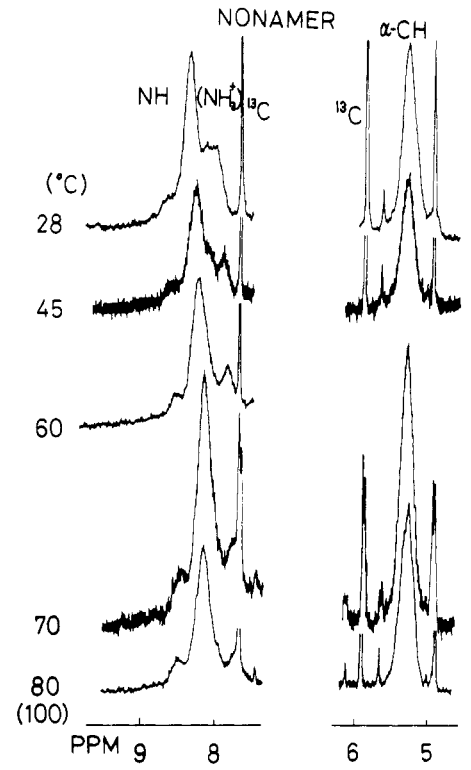

Figure 6. The NH, NH_3^+, and α-CH proton spectra of the nonamer in DCA at various temperatures.

ppm), $-NH-CH_2-$ (4.01 ppm), β-CH_3 (2.14–2.33 ppm), $CH_2CH_2CH_3$ (2.14 ppm), and CH_2CH_3 (1.50 ppm), respectively. The NMR spectra of the dimer in DCA were also similar to that in TFA.[10] The NH peak of the *n*-butylamide group, however, shifted to 0.11 ppm higher field than the NH_3^+ peak in DCA; this result was different from that observed in TFA. By taking into account this difference, it may be concluded that the NMR spectra of the dimer in DCA were rather similar to those in the TFA 50%–$CDCl_3$ 50% mixture (which is a helix-supporting solvent for PLA[10]). This may explain why poly(L-alanine) takes a helical conformation in DCA (strong acid) as will be discussed later.

Figure 3 shows the NH, (NH_3^+), and α-CH proton spectra of the dimer at various temperatures. The NH_3^+ peak becomes broader again, as the temperature rises, while its chemical shift does not change. On the other hand, the *n*-butylamide NH peak moves to a higher field with increasing temperature, eventually overlapping one of the ^{13}C satellites (7.69 ppm) of the solvent. The NH peak of the C-terminal residue of the dimer also moves to higher field as the temperature increases. Finally, only this NH peak is observed above 80 °C.

For the trimer (Figure 1C), the peaks are assigned to NH (8.53 and 8.69 ppm), NH_3^+ (8.13 ppm), $-NH-CH_2-$ (7.99 ppm), solvent CH (6.79 ppm), α-CH (5.29 ppm), $-NH-CH_2-$ (4.03 ppm), $H_3^+N-CH(CH_3)$ (2.41 ppm), $-NH-CH(CH_3)-CO-$ (2.17 ppm), $CH_2CH_2-CH_3$ (2.1–2.2 ppm), and CH_2-CH_3 (1.57 ppm), respectively. It was found that a new NH peak (8.53 ppm) due to the internal residue was observed for the trimer, although most of the other signals were very similar to the characteristic signals of the dimer. For the NH and β-CH_3 peaks, the chemical shift values of the N-terminal residue were different from those of the C-terminal residue. That is, they are assigned from the low-field side to the NH proton of the internal residue, C-terminal residue, N-terminal residue (NH_3^+), and that of the *n*-butylamide group, respectively. On the other hand, the β-CH_3 peak at lower field is assigned to the N-terminal residue, in contrast to the assignment of the NH peaks. This difference in the chemical shift between the NH and β-CH_3 peaks may be mainly due to the magnetic anisotropy and the electric effect induced by carbonyl groups.

Figure 4 shows the NH, (NH_3^+), and α-CH proton spectra of the trimer at various temperatures. The behavior of the individual peaks of the trimer was quite similar to those of the dimer as the temperature increased. Accordingly, the two NH peaks due to the C-terminal and internal residues are clearly observed above 90 °C for the trimer. The intensity ratio of these two peaks was 1:1, which is equal to the ratio of the numbers of the C-terminal and internal residues of the trimer.

Figures 1D and 5 show the NMR spectrum of the tetramer and the NH, (NH_3^+), and α-CH proton spectra at various temperatures, respectively. All of the signals of the tetramer were quite similar to the characteristic signals of the trimer. It is, however, worth noting that the NH and β-CH_3 peaks assigned to the internal residues are observed only above 80 °C and that their intensity ratio is 1:2, which is equal to the ratio of the numbers of the C-terminal and internal residues of the tetramer.

Figures 1E and 6 show the NMR spectrum of the nonamer and the NH, (NH_3^+), and α-CH proton spectra at various temperatures, respectively. The assignments of all the peaks are quite similar to the characteristic signals of the other oligomers mentioned above. For the nonamer, it was found that the intensities of the NH (8.34 ppm) and β-CH_3 peaks (2.18 ppm) due to the internal residues of the oligomers increased further in proportion to the numbers of the internal residues involved, as compared with those of the tetramer. Consequently, the two NH peaks due to the C-terminal and internal residues are observed only above 80 °C, and their intensity ratio is equal to the ratio of the numbers of the C-terminal and internal residues, that is 1:7. Therefore, we might assume that the intensity ratio of these two NH peaks for poly(L-alanine)s will become noticeably smaller with increasing the molecular weight; i.e., 1:14 for PLA-1 ($\overline{DP}_n$ = 16) and 1:158 ($\overline{DP}_n$ = 160).

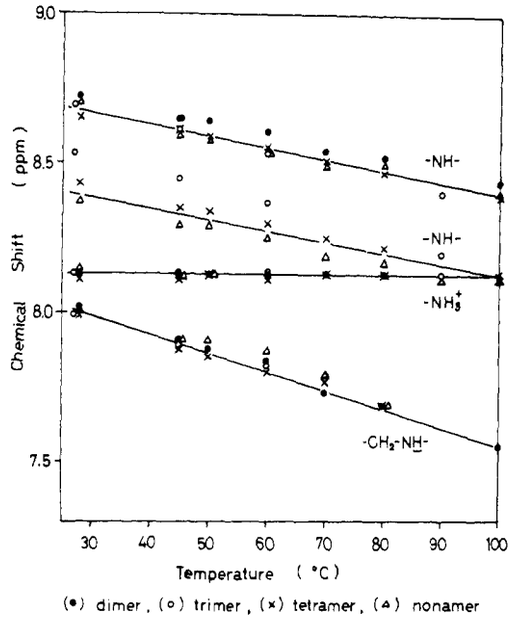

Figure 7. The dependence of the chemical shifts of the NH and NH_3^+ peaks of oligo-L-alanines in DCA upon temperature.

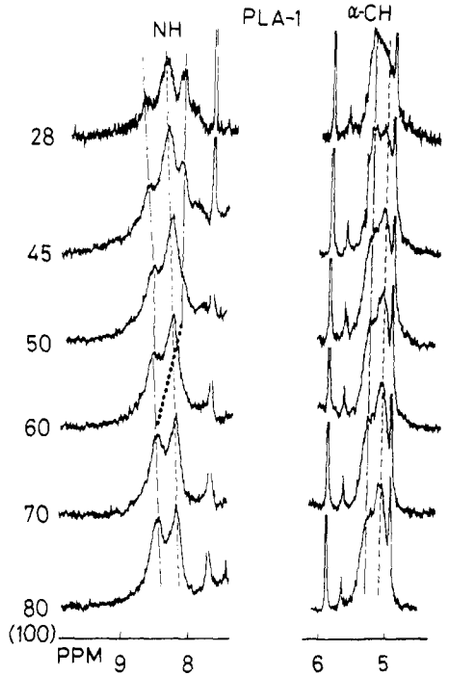

Figure 8. The NH, (NH_3^+), and α-CH proton spectra of PLA-1 in DCA at various temperatures.

On the contrary, no appreciable spectral changes in the α-CH peak could be observed for the oligo-L-alanines used, as is shown in Figures 3, 4, 5, and 6.

Figure 7 shows the dependence of the chemical shift values of the NH and NH_3^+ peaks of the oligo-L-alanines upon temperature. The NH peaks move to higher field approximately linearly with increasing temperature, whereas the chemical shift of the NH_3^+ peak is constant (8.10 ppm) in the temperature range studied. The NH peak intensity due to the internal residues of the oligomers increases in proportion to the molecular weight over the whole temperature range. These results seem to show reasonably that the oligo-L-alanines take nearly random-coil conformation in DCA, in good agreement

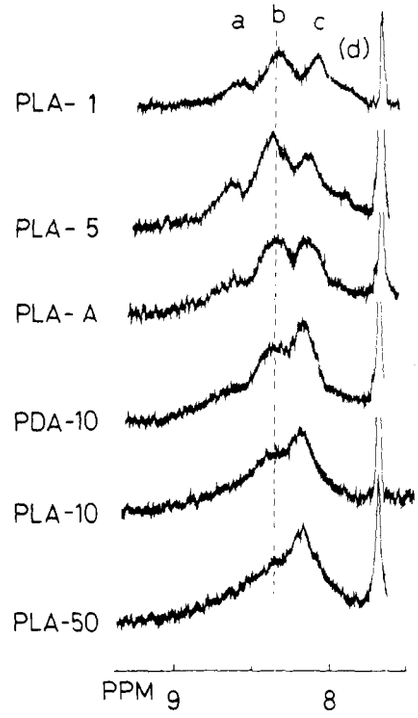

Figure 9. The NH, (NH_3^+), and α-CH proton spectra of poly-(L-alanine)s with various molecular weights and poly(D-alanine) in DCA at 28 °C.

with the results of the optical rotatory dispersion.[7] Thus, it may be said that the general features of the NMR spectra of oligo-L-alanines in DCA are quite similar to those in TFA.

The NMR Spectra of Poly(L-alanine)s. The NMR spectrum of the polymer PLA-1 and the NH, (NH_3^+), and α-CH proton spectra at various temperatures are shown in Figures 1F and 8, respectively. The peaks are assigned to NH and NH_3^+ (8.69, 8.41, 8.15, and 7.95 ppm), solvent CH (6.79 ppm), α-CH (5.21 ppm), and β-CH_3 (2.21 ppm), respectively. The NMR spectral pattern of PLA-1 was that to be expected from our data for the oligo-L-alanines in DCA. Further, the substantial change in the chemical shifts of all the peaks (except for the NH peaks) could not be observed as the molecular weight increased. On the other hand, the NH signals of PLA-1 were split into four peaks in DCA, and their chemical shifts moved to higher fields as the temperature increased (Figure 8). As a result, only two NH peaks were observed at 70–100 °C, which is different from the results obtained for oligo-L-alanines. This finding suggests that these NH peaks should reflect the conformation of the polymers.

In order to elucidate this point, the ^{1}H-NMR spectra of poly(L-alanine)s having various molecule weights were studied in DCA. Figure 9 shows the NH and NH_3^+ peaks of poly(L-alanine)s with different molecular weight and poly(D-alanine) in DCA at 28 °C. The NH peaks split into at most four (being denoted hereafter as the a, b, c, and d peaks from the lowest field, respectively) and became broader, and their peak intensities changed with the increasing molecular weight, whereas their chemical shift did not change. Specifically, the intensity of the c peak increased, while that of the a and b peaks decreased relatively. The chemical shift value of the b peak in poly(L-alanine)s agreed with the NH peak of the internal residues of oligo-L-alanines. Since the stability of the helix should increase as the molecular weight increased, it may be assumed that the b peak is due to random coil and the c peak is related to the helical conformation. On the other hand, the intensity of the d peak at the highest field, which may be assigned to the n-butylamide NH of the polymer, was very

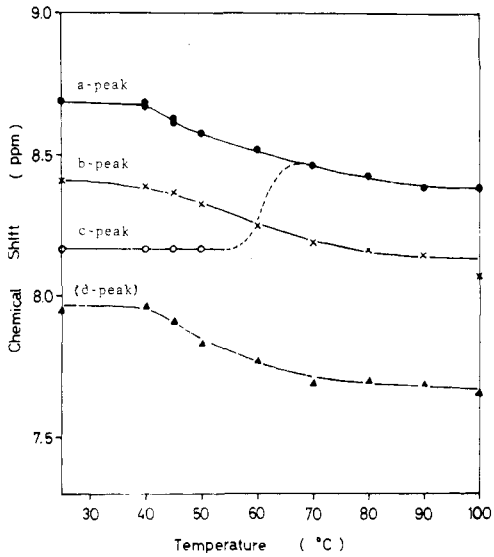

Figure 10. The dependence of the chemical shift values of the NH, (NH_3^+) peaks (the a, b, c, and d peaks) of PLA-1 in DCA upon temperature.

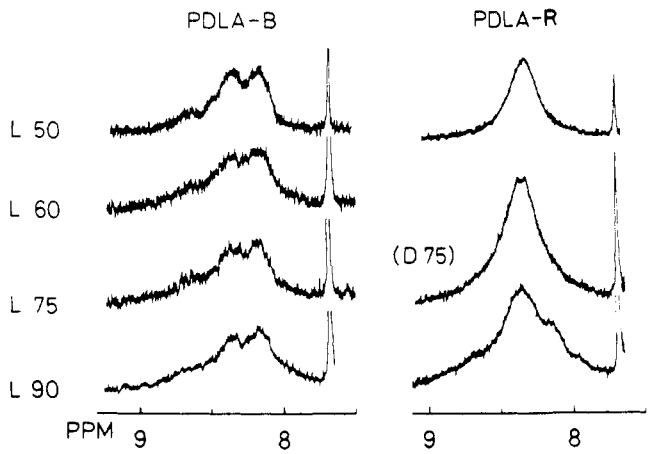

Figure 11. The NH proton spectra of random and block copoly(D,L-alanine)s in DCA at 28 °C.

weak, and it decreased with increasing molecular weight. This NH peak could not be observed for the high molecular weight PLAs. Consequently, the d peak will not be discussed further.

Figure 10 shows the dependence of the chemical shift of these three peaks upon temperature. The chemical shift of the c peak was constant in the range of 25 to 60 °C and both the a and b peaks shifted to higher fields. As was reported in the preceding paper,[10] the random-coil peak of the NH in the mixture of TFA 50% and $CDCl_3$ 50% shifted to lower fields by at most 0.16 ppm in the temperature range of 25–60 °C, whereas the chemical shift of the helical peak was constant. Also from this point the c peak may be assigned to the helical peak. As will be discussed later, the a peak is assigned to the helical peak but is of a different nature from the c peak, since it shifted considerably to higher fields with the increasing temperature. As for the α-CH peaks, the double peaks were observed for the PLAs, one in the lower field is assigned to the helical conformation and the other to the random coil (Figure 8). Such results have never been obtained for polypeptides in any organic solvent, and it may be very important to know why the PLA molecules take the helical conformation in DCA, as will be discussed later.

The NMR Spectra of Various Copoly(D,L-alanine)s. In order to clarify the difference in the chemical shift behavior

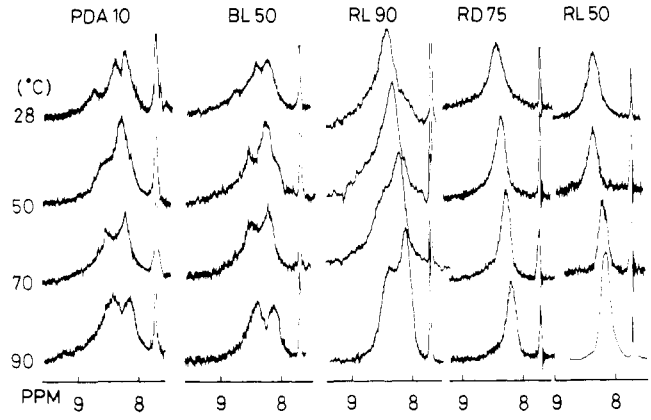

Figure 12. The dependence of the chemical shifts of the NH peaks of PDA-10 and random and block copolymers of D- and L-alanines in DCA upon temperature.

between the a and b peaks, we examined the NH spectra of the random copolymers (which give the model of the partially helical molecules) and the block copolymers (which give the model of the once-broken-rod molecules composed of the right-handed and left-handed helices) in DCA. The results are presented in Figure 11. For the D,L-block copolymers, any remarkable change did not occur in the NH spectra but the spectra approached to those of poly(L-alanine) with the increasing L component of the copolymer. This result shows that the position of the D–L junction of these A–B type D,L-block copolymers did not affect the NH signals. It may be said, moreover, that the peak splitting is independent of whether the helices are right handed or left handed.

For the D,L-random copolymers, the PDLA-RL50 and PDLA-RL60 gave a single NH peak, indicating the random-coil conformation. Also for PDLA-RL25 (or PDLA-RD75), a single NH peak was still observed, though its width became slightly greater as compared with the just-mentioned two copolymers. In addition, the chemical shift of the peak was identical with that of the b peak, and neither the a peak nor c peak were observed clearly. It is thus evident that this copolymer takes an almost random-coil conformation in DCA. On the other hand, the PDLA-RL90, which takes a partially helical conformation,[6] gave the three NH peaks corresponding to the a, b, and c, peaks, the spectra of which were similar to those of PLA. From the above results, it may be concluded that the three NH peaks observed for PLA-1 in DCA are assigned as follows; one (the b peak) is due to the random coil and the other two (the a and c peaks) due to the helices, respectively. The decrease in the intensity of the c peak from PLAs to PDLA-RL90 was much larger than the corresponding decrease in the a peak. This suggests that the c peak is due to the internal residues of the helices, whereas the a peak is due to the helical conformation near the junctions of the helix and the coil.

In order to confirm this assignment, we examined the dependence of the chemical shifts of the NH peaks and their intensities upon temperature. The results are shown in Figure 12. At the temperature studied, PDLA-RL50, RL60, and RL25 gave a single NH peak reflecting the random-coil conformation in DCA, while the others (PLAs, PDA, and PDLA-RL90) gave at most three NH peaks. The chemical shifts of the latter occurred toward higher fields and the intensity of the b peak increased as the temperature increased, whereas that of the c peak decreased. These changes correspond to the helix-to-coil transition with increasing temperatures.

It is of particular interest that as the temperature was increased the amount of terminal helix gradually decreased. At

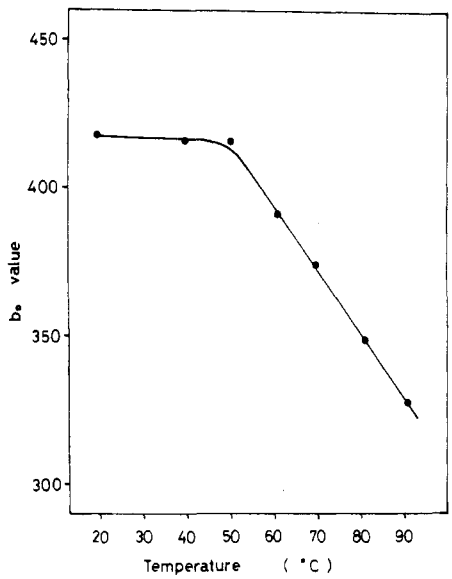

Figure 13. The dependence of the b_0 values of PDA-10 in DCA upon temperature (a polymer concentration = 5 w/v %).

higher temperatures, only the two peaks due to the terminal helix and the random coil remained. This suggests that the process of destruction of the helix occurs via the partial helices. It is also obvious that the helix-to-coil transitions of the D,L-random copolymers, which originally were partially helical, occurred at a lower temperature than for the homopolymers or block copolymers.

Conformations of Poly(alanine)s. On the basis of the data obtained in this study, we shall examine quantitatively the microconformation of poly(alanine)s in DCA.

For PLA-1 ($\overline{DP}_n$ = 16), the intensity ratios of the a, b, and c NH peaks were 27, 51, and 22%, respectively. Since the average degree of polymerization was around 16, we can calculate that the chain, on the average, consists of a helical part of 7.8 residues (including the terminal helix of about 4.3 residues) and a coil part of 8.2 residues. This implies that two or three residues of both ends of the helical segments give the a peak and four residues of both chain ends take nearly the coil conformation. As the molecular weight increases, the number of residues belonging to the inner helix increases, but they still seem to take partially helical conformations.

For PLA-10, we can calculate, assuming that the terminal helix consists on the average of 4.3 residues of L-alanine, as follows: On the average, one helical segment consists of ca. 13 residues, and the number of helical segments is ca. 6. For the PDLA-RL90, we can calculate that, on the average, one helical segment consists of 9–10 residues and the number of helical segments is 2–3. The results obtained in a similar manner from the NH spectra of various poly(alanine)s in DCA are summarized in Table II.

Figure 13 shows the dependence of the b_0 values of PDA-10 in DCA upon the temperature. This shows that the helical conformation became less stable with increasing temperature and the destruction of the helix commences at above 60 °C.

According to the NMR results (Figures 8 and 12), PLA-10 takes a partially helical conformation (helical content is about 50%) in DCA at high temperatures (90–100 °C), which is in good agreement with the just-mentioned ORD results (b_0 = −330, corresponding to a helical content of about 52%) at 92 °C. Moreover, the NMR results indicate that the length of the helical sequences in the chain becomes smaller and the number of helix–coil junctions increases with increasing temperature. In this manner, the microconformations of these polymers and copolymers are reflected in the NMR spectra

Table II
The Percentage Area of the NH Peaks (the a, b, and c peaks) of Various Poly(alanine)s in DCA and Their Microconformation

Sample	$\overline{DP}_n$	Peak area, % a	b	c	$\overline{m}^a$	$\overline{h}^b$ (residues)
PLA-1	16	27	51	22	0	7.8
PLA-10	160	19	42	39	7.0	13.3
PDLA-RL90	120	12.5	72.5	15	3.5	9.5
PDLA-RL25 (RD75)	120	5.5	92	2.5	1.5	6.3
PDLA-RL60	130	5.3	93	1.7	1.6	5.7
PDLA-BL90	150	16	59	25	5.6	11.1
PDLA-BL75	150	19	55	26	6.6	10.2
PDLA-BL60	150	19	50	31	6.6	11.4

[a] The number of helical segments in partially helical polymers ($\overline{m}$) was calculated by the equation $\overline{m} = a\overline{DP}_n/430$. [b] The average length of helical segments of partially helical polymers ($\overline{h}$) was calculated by the equation $\overline{h} = (a + c)\overline{DP}_n/100\overline{m}$.

in DCA. These results seem to be closely related to the specificity of this solvent. DCA works usually as a helix-supporting solvent for some poly(amino acids) (i.e., poly(L-alanine) and poly(L-leucine)), which possess strong intermolecular interaction (cohesive forces) due to their hydrophobic side chains, since DCA is a weaker acid than TFA (coil solvent for them). Therefore, when $CDCl_3$ (a helix-supporting solvent) was added to the DCA solution of poly(L-alanine), the amount of helix did not increase at all. On the other hand, DCA works generally as a coil-supporting solvent for other polymers because of its acidic nature. Thus, DCA has two opposite characters for polypeptides and they may be explained in terms of the strength of its acidity. We compared the dissociation constants (K) for DCA and TFA[15] at 25 °C as follows:

$$K_{DCA}^{25\,°C} = 5.534 \times 10^{-2} \quad (pK_a = 1.257) \tag{4}$$

$$K_{TFA}^{25\,°C} = 0.589 \quad (pK_a = 0.230) \tag{5}$$

Consequently, it may be said that the hydrophobic side chains play an important role in stabilizing the helical conformation of PLA. It may be concluded from the above results that both the effects of the hydrophobic side chains of polymers and the acidity of the solvent are most important for the formation of the helical conformation.

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