Conformational Study of Sequential Polypeptides of L-Alanine and D-Alanine

Takuo OZAKI, Masanao ŌYA, and Koichi ITOH*

*Department of Industrial Chemistry,
College of Technology, Gunma University,
1-5-1, Tenjin-cho, Kiryu, Gunma 376, Japan.*

**Department of Chemistry, School of Science and Engineering,
Waseda University, 3-4-1, Ohkubo, Shinjuku-ku, Tokyo 160, Japan.*

(Received June 26, 1980)

ABSTRACT: Infrared spectra in the 700—200 cm^{-1} and in the amide I and II regions were measured for poly(L-Ala-L-Ala-D-Ala), poly(L-Ala-L-Ala-D-Ala-D-Ala), poly(L-Ala-L-Ala-L-Ala-D-Ala), and poly(L-Ala-D-Ala-L-Ala-D-Ala) with different degrees of polymerization. The spectra showed that the films cast from aqueous solutions of poly(L-Ala-L-Ala-D-Ala), poly(L-Ala-L-Ala-D-Ala-D-Ala), and poly(L-Ala-L-Ala-L-Ala-D-Ala) take disordered conformations containing appreciable amounts of L-alanine residues with local right-handed α -helix-like structures and the D-alanine residues with local left-handed α -helix-like structures. The disordered conformations were converted to the extended β -form when treated with ethanol. The latter conformation is also found in water-insoluble portions of poly(L-Ala-L-Ala-D-Ala-D-Ala) and poly(L-Ala-L-Ala-L-Ala-D-Ala). On the other hand, poly(L-Ala-D-Ala-L-Ala-D-Ala) has a conformation different from those taken on other sequential polypeptides studied in this paper. The above findings were confirmed also by the measurement of the Raman spectra of poly(L-Ala-L-Ala-D-Ala) and poly(L-Ala-D-Ala-L-Ala-D-Ala).

KEY WORDS Polypeptide / Sequential Polypeptide / Poly(L-Ala-D-Ala) / Infrared Spectra / Raman Spectra / α -Helix Formation / Conformation of Polypeptide / Fraction of Polypeptide / Gel Chromatography / Sephadex / Peptide Synthesis /

The structure of polypeptides consisting of a known sequence of L- and D-amino acid residues has been studied to understand the factors determining polypeptide backbone conformations. Studies have been carried out both experimentally and theoretically,¹⁻⁷ but no decisive method has been found for determining the local conformations of the L- and D-amino acid residues of the copolymers. The circular dichroism and the infrared spectra in the amide I and II region, for example, can at most indicate the overall content of the ordered structures (the α -helical and β -form structures) and the random coil structure. Knowledge about the values of the (ϕ , ψ) angles⁸ taken by the constituent amino-acid residues of a given polypeptide chain is necessary to compare precisely the experimental and theoretical results.

In previous papers,⁹⁻¹¹ we demonstrated that the vibrational spectroscopy in the low frequency

region offers an especially useful means for clarifying the local as well as over-all structures of poly(α -amino acids) and copoly(α -amino acids). We also established a correlation between the vibrational bands in the low frequency region and the local geometry of the amino-acid residues.

In this paper, this correlation is used to analyze the infrared and Raman spectra of a series of sequential polypeptides of the L- and D-alanine residues with different degrees of polymerization. The results give detailed information about not only the local geometry of the constituent amino-acid residues but also the dependence of the conformation on the degree of polymerization.

Lontan *et al.*⁴ have studied the conformation of poly(L-alanyl-D-alanyl-L-alanyl-L-alanine) in solution by circular dichroism spectra. We have compared their results with those obtained from the

vibrational spectra in this paper.

EXPERIMENTAL

Preparation of Poly(L-alanyl-L-alanyl-D-alanine)

Poly(L-alanyl-L-alanyl-D-alanine) (abbreviated (Ala-Ala-D-Ala)_n* where *n* denotes the degree of polymerization.) was obtained by the self-condensation reaction of the peptide *N*-succinimide ester containing the required sequence, following the procedure reported by Lontan *et al.*⁴ The synthetic procedure is summarized as follows.

Benzyloxycarbonyl-L-alanine *N*-hydroxysuccinimide ester (I) was coupled with D-alanine to form benzyloxycarbonyl-L-alanyl-D-alanine (II), which was converted to L-alanyl-D-alanine hydrogen bromide salt (III) on treatment hydrogen bromide in acetic acid (25%). *t*-Butoxycarbonyl-L-alanine *N*-hydroxysuccinimide ester was coupled with the dipeptide (III) to form *t*-butoxycarbonyl-L-alanyl-L-alanyl-D-alanine (IV). The tripeptide (IV) was converted to the *N*-hydroxysuccinimide ester (V) by the dicyclohexylcarbodiimide method in tetrahydrofuran. The *t*-butoxycarbonyl group

Table I.^a Summary of synthetic procedure of (Ala-Ala-D-Ala)_n

^a The following abbreviation are used: Z, benzyloxycarbonyl group; HOSu, *N*-hydroxysuccinimide; DCC, dicyclohexylcarbodiimide; Boc, *t*-butoxycarbonyl group; TEA, triethylamine.

of (V) was cleaved by the treatment with 2 *N* HCl in dioxane to form L-alanyl-L-alanyl-D-alanine *N*-hydroxysuccinimide ester hydrogen chloride salt (VI). The active ester (VI) was dissolved in absolute dimethylformamide (13%), and triethylamine was added (1.2 equiv). The solution, which became very turbid immediately after the addition of triethylamine, was shaken mechanically. After at intervals of 24 hours, dimethylformamide was added to dilute the reaction mixture to 1%. After 6 days, the mixture was poured into ether. The white precipitate was collected by centrifugation, washed with methanol, and dried *in vacuo* at room temperature. The methanol-soluble portion was collected by evaporating the solvent. The methanol-insoluble part of

(Ala-Ala-D-Ala)_n was suspended in distilled water and left at room temperature for two days with stirring. By centrifugation the water-insoluble portion was collected and dried *in vacuo* at room temperature. The water-soluble portion of (Ala-Ala-D-Ala)_n was obtained by lyophilization and then fractioned into oligopeptides by gel filtration on a Sephadex G-25. The crude products of (Ala-Ala-D-Ala-D-Ala)_n, (Ala-Ala-Ala-D-Ala)_n and (Ala-D-Ala-Ala-D-Ala)_n were synthesized, using the procedure similar to that described above. The methanol-soluble, water-soluble, and water-insoluble fractions of each polypeptide were obtained by the same method as that used for (Ala-Ala-D-Ala)_n.

* An Ala residue except D-Ala is of the L-conformation. The abbreviations used in this paper are those recommended by the IUPAC-IUB commission on Biochemical Nomenclature, published in *J. Biol. Chem.*, **247**, 977 (1972).

Fractionation of the Crude Products

The methanol-soluble parts of (Ala-Ala-D-Ala)_n, (Ala-Ala-D-Ala-D-Ala)_n, (Ala-Ala-Ala-D-Ala)_n, and (Ala-D-Ala-Ala-D-Ala)_n were fractioned into the

composite materials by gel filtration on a column (3.1 cm in diameter and 186 cm in length) of the Sephadex G-15 (particle size 40–120 μ m) using 0.01 *N* HCl as eluent. The results showed that the samples contained not only the polymer but also appreciable amounts of the monomer, *i.e.*, the tri or tetrapeptide *N*-hydroxysuccinimide ester corresponding to each sequential polypeptide, and small amounts of the dimers, (Ala-Ala-D-Ala)₂, (Ala-Ala-D-Ala-D-Ala)₂, (Ala-Ala-Ala-D-Ala)₂ or (Ala-D-Ala-Ala-D-Ala)₂.

The water-soluble portions of (Ala-Ala-D-Ala)_{*n*}, (Ala-Ala-D-Ala-D-Ala)_{*n*}, (Ala-Ala-Ala-D-Ala)_{*n*}, and (Ala-D-Ala-Ala-D-Ala)_{*n*} were fractionated into oligopeptides by gel filtration on a column (3.1 cm diameter and 184 cm in length) of a Sephadex G-25 (particle size 50–150 μ m) using distilled water as eluent. Figures 1 and 2 show the elution curves for the water-soluble portions of (Ala-Ala-D-Ala)_{*n*} and (Ala-Ala-D-Ala-D-Ala)_{*n*}. The ordinate of each figure indicates the absorbance at 220 nm (cell-length, 1 cm) for each fraction (fraction volume, 10 ml). The relation between the elution volume and molecular weight was obtained from the elution volume measured for samples of (Ala-Ala-D-Ala)₁, (Ala-D-Ala-Ala-D-Ala)₁, (Ala-Ala-D-Ala-D-Ala)₂, (Ala-Ala-D-Ala)₃, and (Ala-Ala-D-Ala-D-Ala)₃, synthesized by a step-wise procedure. From the results we obtain,

$$\log_{10} M_r = 4.53 - 1.24 (V_e/V_0)$$

where V_0 and V_e are the retention volume of the column (592 ml) and the elution volume, respectively, and M_r the molecular weight of the sample. This equation is applicable to oligopeptides with molecular weights larger than that of (Ala-Ala-D-Ala-D-Ala)₃ and can be used for estimating the degree of polymerization of the samples obtained from fractions I–IV in Figures 1 and 2. The estimated degree of polymerization is given in the legends of Figures 1 and 2. Each of the cut fractions were lyophilized, dissolved into 0.1 *N* HCl, and lyophilized again. The solid samples thus obtained were chromatographed on a column (3.1 cm, 186 cm) of a Sephadex G-15, using 0.1 *N* HCl as eluent. The elution peak corresponding to the sample and a small peak assignable to *N*-hydroxysuccinimide were observed. The sample fraction was collected and lyophilized, and each oligomer was obtained as a white powder. The *N*-

Figure 1. Chromatography of (Ala-Ala-D-Ala)_{*n*} on a column (3.1 cm $\times$ 184 cm) of Sephadex G-25. Eluent, water at *ca.* 50 ml hr⁻¹. The ordinate denotes the value of absorbance at 220 nm (cell length, 1 cm) of each fraction (volume, 10 ml). The approximate values of the degree of polymerization (*n*) of the polypeptides contained by the cut fractions I–IV are as follows: fraction I, *n* = 8–5; fraction II, *n* = 4, 3; fraction III, *n* = 2; fraction IV, *n* = 1. The fraction V contains small peptides which can not be identified. This may be a by-product in the polymerization process.

Figure 2. Chromatography of (Ala-Ala-D-Ala-D-Ala)_{*n*}. The column eluent and the value denoted by the ordinate are the same as those in Figure 1. The approximate *n* values of the polypeptides contained in each cut fraction are as follows: fraction I, *n* = 6 and 5; fraction II, *n* = 4 and 3; fraction III, *n* = 2; fraction IV, *n* = 1. The fraction V contains small peptide which can not be identified.

and C-terminal groups of the oligomer so obtained should have the NH_3^+ and COOH forms, respectively.

The oligomers, each with a well-defined degree of polymerization, were also obtained from the water-soluble portion of $(\text{Ala-Ala-Ala-D-Ala})_n$ and $(\text{Ala-D-Ala-Ala-D-Ala})_n$, using a procedure similar to that for $(\text{Ala-Ala-D-Ala})_n$ and $(\text{Ala-Ala-D-Ala-D-Ala})_n$.

Further purification of the water-insoluble fractions of $(\text{Ala-Ala-D-Ala})_n$, $(\text{Ala-Ala-D-Ala-D-Ala})_n$, $(\text{Ala-Ala-Ala-D-Ala})_n$, and $(\text{Ala-D-Ala-Ala-D-Ala})_n$ was not carried out. It is assumed that the degree of polymerization of the water-insoluble portion of each copolymer is larger than the maximum degree of polymerization of the water-soluble fraction of the sample.

Measurement of Infrared Spectra

Infrared spectra in the $1800\text{--}1400\text{ cm}^{-1}$ region were measured by using a Hitachi G-3 type infrared spectrometer and those in the region from 700 to 200 cm^{-1} by a JASCO IR-F far-infrared spectrometer. The spectra of the water-soluble fractions (lyophilized samples) were measured in the nujol mull state or the film state, the latter was obtained by casting an aqueous solution of the sample on calcium fluoride plates (the $1800\text{--}1400\text{ cm}^{-1}$ region) or polyethylene plates (the $700\text{--}200\text{ cm}^{-1}$ region). The measurement was also made on the films treated with ethanol. This treatment was consisting in soaking the films in ethanol for about 30 minutes and drying them *in vacuo* at room temperature. The infrared spectra of the water-insoluble portions were also measured in the nujol mull state. The spectra were also measured for the water-insoluble portion of $(\text{Ala-Ala-Ala-D-Ala})_n$ treated with dichloroacetic acid by the following procedure. The sample was dissolved in a minimum amount of dichloroacetic acid. Absolute ether was then added to the solution and the white precipitate was collected by centrifugation. The solvent was extracted from the sample with ether, using a Soxhlet extractor for three days.

Measurement of Raman Spectra

Raman spectra were obtained with a JASCO R-800 type laser Raman spectrometer. The 5145 Å excitation line was generated by a Spectra-Physics model 164 argon-ion laser. The powder samples were pressed into disks and placed at angles of 60°

with respect to the excitation line. Before each measurement, the sample was illuminated by the excitation line for at least two hours in order to reduce any background influence the luminescence of impurities.

RESULTS

Infrared Spectra

Figures 3—6 show the infrared spectra in the $700\text{--}200\text{ cm}^{-1}$ region of the sequential polypeptides, $(\text{Ala-Ala-D-Ala})_n$, $(\text{Ala-Ala-D-Ala-D-Ala})_n$, $(\text{Ala-Ala-Ala-D-Ala})_n$, and $(\text{Ala-D-Ala-Ala-D-Ala})_n$. Figures 7—10 give the spectra in the $1800\text{--}1400\text{ cm}^{-1}$ region for the same series.

The spectra in the $700\text{--}200\text{ cm}^{-1}$ region of $(\text{Ala-Ala-D-Ala})_1$ (Figure 3(A)) show only a doublet at 474 and 466 cm^{-1} , which could not be observed in the spectra of $(\text{Ala-Ala-D-Ala})_n$ ($n \geq 2$) (Fig. 3(B)—(E)). $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$) in the film state gives rise to two bands at about 520 and 370 cm^{-1} (Figures 3(B), (C) and (D)). These bands can not be observed in the spectrum of $(\text{Ala-Ala-D-Ala})_1$ (Fig. 3(A)), indicating that they can be assigned to the deformation vibration of polypeptide backbones. The α -helical poly(L-alanine) shows well-defined bands at 526 and 371 cm^{-1} .¹² From the analysis of the infrared spectra of various sequential polypeptides containing L-alanine residues, we^{9,10} concluded that these two bands are ascribed to the local α -helix-like conformations of the L-alanine residues. The bands at about 520 and 370 cm^{-1} in Figures 3(B), (C), and (D) correspond to these two bands. Therefore, it can be concluded that appreciable amounts of the L- and D-alanine residues of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$) in the film state have (ϕ, ψ) angles near $(-57^\circ, -47^\circ)$ (the L-residues) and $(+57^\circ, +47^\circ)$ (the D-residues).⁸ Figures 7(B)' and (C) show that the films of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$) give rise to the amide I and II bands at 1653 and about 1545 cm^{-1} , respectively. The frequency values of these bands correspond to those observed for the α -helical or random-coil polypeptides.

The films of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$) treated with ethanol show the spectra given in Figures 3(C)', and (D)', and 7(B)'', and 7(C)', which are quite different from those of the samples before the treatment. Figures 3(C)' and (D)' do not show any bands near 520 and 370 cm^{-1} but new bands at 625 ,

611 (a doublet), 469, and 417 cm^{-1} , indicating that the backbone conformations of the samples change by treatment with ethanol. As shown in Figures

7(B)'' and (C)', the amide I bands are observed at 1697 and about 1627 cm^{-1} and the amide II at about 1530 cm^{-1} . These spectra are virtually

Figure 3. Infrared spectra in the $700\text{--}200\text{ cm}^{-1}$ region of $(\text{Ala-Ala-D-Ala})_n$: (A), $n=1$ (film cast from the aqueous solution); (B), $n=2$ (film cast from the aqueous solution); (C), $n=3,4$ (film cast from aqueous solution); (C)', $n=3$ and 4 (film treated with ethanol); (D), $n=5\text{--}8$ (film cast from aqueous solution); (D)', $n=5$ and 8 (film treated with ethanol); (E), $n\geq 9$ (powder, Nujol mull).

identical with the spectrum of the polypeptide backbone having an antiparallel pleated sheet structure. The 1697 cm^{-1} band can be assigned to

the $\nu(o, \pi)$ mode of the amide I and the 1627 cm^{-1} to the $\nu(\pi, o)$ mode.^{13,14} Therefore, by the treatment with ethanol, the backbone conformation of (Ala-

Figure 4. Infrared spectra in the $700\text{--}200\text{ cm}^{-1}$ region of $(\text{Ala-Ala-D-Ala-D-Ala})_n$: (A), $n=1$ (film cast from the aqueous solution); (B), $n=2$ (film cast from the aqueous solution); (C), $n=3, 4$ (film cast from the aqueous solution); (D), $n=5, 6$ (powder, Nujol mull); (D'), $n=5, 6$ (film cast from the aqueous solution); (D''), $n=5, 6$ (film treated with ethanol); (E), $n \geq 7$ (powder, Nujol mull).

Ala-D-Ala_n ($2 \leq n \leq 8$) changes from that containing the local α -helix-like structure to the extended β -form conformation in which the polypeptide chains

are in the anti-parallel by interchain hydrogen-bonds. The two bands near 618 cm^{-1} in Figure 3(C)' and (D)' are observed also in the spectra of the

Figure 5. Infrared spectra in the $700\text{--}200 \text{ cm}^{-1}$ region of $(\text{Ala-Ala-Ala-D-Ala})_n$: (A), $n=1$ (film cast from aqueous solution); (B), $n=2$ (film cast from the aqueous solution); (C), $n=3, 4$ (film cast from aqueous solution); (C)', $n=3, 4$ (film treated with ethanol); (D), $n=5\text{--}7$ (film cast from aqueous solution); (E), $n \geq 8$ (powder, Nujol mull); (E)', $n \geq 8$ (powder treated with dichloroacetic acid).

anti-parallel β -form poly(L-alanine).¹⁵ The 469 and 417 cm^{-1} bands in Figures 3(C)' and (D)' can be assigned to the deformation vibrations of the L- and D-alanine residues incorporated into the extended

conformation. As shown in Figure 7(B), (Ala-Ala-D-Ala)₃ and 4 (the lyophilized samples) in the Nujol mull state give a strong amide I band at 1627 cm^{-1} and II at 1530 cm^{-1} with shoulder bands at 1656

Figure 6. Infrared spectra in the 700–200 region of $(\text{Ala-D-Ala-Ala-D-Ala})_n$: (A), $n=1$ (film cast from the aqueous solution); (B), $n=2$ (film cast from the aqueous solution); (C), $n=3, 4$ (film cast from the aqueous solution); (C)', $n=3, 4$ (film treated with ethanol); (D), $n \geq 6$ (powder, Nujol mull).

Figure 7. Infrared spectra in the 1800–1400 cm^{-1} region of $(\text{Ala-Ala-D-Ala})_n$: (A), $n=1$ (film cast from the aqueous solution); (B), $n=3, 4$ (powder, Nujol mull); (B)', $n=3, 4$ (film cast from the aqueous solution); (B)'', $n=3, 4$ (film treated with ethanol); (C), $n=5-8$ (film cast from the aqueous solution); (C)', $n=5-8$ (film treated with ethanol); (D), $n \geq 9$ (powder, Nujol mull). The dashed line shows the absorption due to Nujol.

(amide I) and 1546 (amide II) cm^{-1} . This indicates that there exist both the extended β -form and the local α -helix-like structure in the lyophilized samples of $(\text{Ala-Ala-D-Ala})_3$ and 4. The low-frequency spectrum of each sample is composed of bands resulting from the extended β -form (a doublet centered at 620 cm^{-1} and the bands at 469 and 417 cm^{-1}) and bands ascribable to the local α -helix-like conformations, (bands at 522 and 374 cm^{-1}).

The water-insoluble fraction of $(\text{Ala-Ala-D-Ala})_n$ ($n \geq 9$) gives the spectra shown in Figures 3(E) and 7(D). These spectra are more complex than those of the water-soluble samples. The bands at 517 and 370 cm^{-1} in Figure 3(E) can be considered characteristic of the local α -helix-like conformation. The 614, 449, and 255 cm^{-1} bands were observed also for the β -form poly(L-alanine).¹⁵ From the analysis of the infrared spectra of the sequential polypeptides containing the L-alanine residues,^{9,10} it was noted that bands near 440 cm^{-1} are characteristic of the local β -form structure of the L-alanine residues (the conformation with the (ϕ, ψ) values near $(-139^\circ, +135^\circ)$ for the L-alanine residue and the conformation with the values near $(+139^\circ, -135^\circ)$ for the D-residue).⁸ Therefore, the spectrum shown in Figure 3(E) indicates that $(\text{Ala-Ala-D-Ala})_n$ ($n \geq 9$) has no ordered structure but is composed partly of α -helix-like structures and partly of β -form-like structures. This is confirmed by the fact that the amide I of the sample (Figure 7(D)) is a superposition of the 1655 and 1629 cm^{-1} bands. The spectra of the films cast from aqueous solutions of $(\text{Ala-Ala-D-Ala-D-Ala})_n$ ($2 \leq n \leq 6$) (Figures 4(B), (C), and (D)') show two bands at about 520 and 380 cm^{-1} , indicating that the samples contain certain amounts of the L- and D-alanine residues whose local conformations do not deviated much from those with the (ϕ, ψ) values of $(-57^\circ, -47^\circ)$ and $(+57^\circ, +47^\circ)$, respectively. These values are similar to those of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$). As shown in Figures 8(B) and (C)', the spectra of the films of $(\text{Ala-Ala-D-Ala-D-Ala})_n$ ($2 \leq n \leq 6$) have the amide I band centered at 1652 and II at about 1540 cm^{-1} .

The films of $(\text{Ala-Ala-D-Ala-D-Ala})_5$ and 6 treated with ethanol give the spectrum in the 700–200 cm^{-1} region as shown in Figure 4(D)''. In this spectrum, the bands near 520 and 330 cm^{-1} are very weak, and those at 612, 460, and 410 cm^{-1} are observed. The latter three bands can be observed

Figure 8. Infrared spectra in the 1800–1400 cm^{-1} region of $(\text{Ala-Ala-D-Ala-D-Ala})_n$: (A), $n=1$ (film cast from the aqueous solution); (B), $n=3, 4$ (film cast from the aqueous solution); (B'), $n=3, 4$ (film treated with ethanol); (C), $n=5, 6$ (powder, Nujol mull); (C'), $n=5, 6$ (film cast from the aqueous solution); (C''), $n=5, 6$ (film treated with ethanol); (D), $n \geq 7$ (powder, Nujol mull). The dashed line shows the absorption due to Nujol.

also for the films of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 6$) treated with ethanol, indicating that the ethanol treatment converts the backbone conformation of $(\text{Ala-Ala-D-Ala-D-Ala})_5$ and 6 from the structure containing the local α -helix-like conformation to the extended β -form. The spectrum in the 1800–1400 cm^{-1} region of $(\text{Ala-Ala-D-Ala-D-Ala})_5$ and 6 treated with ethanol gives the amide I and II bands at 1697 and 1625, and 1526 cm^{-1} , respectively, which are characteristic of the anti-parallel β -form structure. (Figure 8(C)'). As can be seen in Figure 8(B)', $(\text{Ala-Ala-D-Ala-D-Ala})_3$ and 4 treated with ethanol still show the amide I and II bands at 1650 and 1545 cm^{-1} , respectively, in addition to the β -form bands. Therefore, the conformational change induced by the ethanol treatment occur only partially in the case of $(\text{Ala-Ala-D-Ala-D-Ala})_3$ and 4 . On the other hand, the water-insoluble fraction of $(\text{Ala-Ala-D-Ala-D-Ala})_n$ ($n \geq 7$) gives a typical β -form spectrum in the amide I and II region (Figure 8(D)). The spectrum in the 700–200 cm^{-1} region of the sample (Figure 4(E)) shows well-defined peaks at 614, 465, and 413 cm^{-1} , all of which are observed in the spectra of the extended β -form of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$) and $(\text{Ala-Ala-D-Ala-D-Ala})_5$ and 6 . This indicates that the formation of the extended β -form of $(\text{Ala-Ala-D-Ala-D-Ala})_n$ ($n \geq 2$) depends not only on the preparation conditions but also on the degree of polymerization. The larger the n values, the more this form is stabilized. Figure 4(D) shows that the spectrum of the nujol mull of $(\text{Ala-Ala-D-Ala-D-Ala})_5$ and 6 (the lyophilized sample) is composed of 523 and 380 cm^{-1} bands (due to the α -helix-like conformation) and the 615, 462, and 412 cm^{-1} bands (due to the extended β -form).

The spectrum of $(\text{Ala-Ala-Ala-D-Ala})_1$ observed in the 700–200 cm^{-1} region (Figure 5(A)) is almost identical with those of $(\text{Ala-Ala-D-Ala})_1$ and $(\text{Ala-Ala-D-Ala-D-Ala})_1$ (Figures 3(A) and 4(A)), suggesting that the backbone conformation of $(\text{Ala-Ala-Ala-D-Ala})_1$ is similar to those of the latter two compounds. The films cast from aqueous solutions of $(\text{Ala-Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 7$) show the spectra (Figures 5(B), (C), and (D)) exhibiting broad bands at about 520 and 380 cm^{-1} . Therefore, as in the case of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$) and $(\text{Ala-Ala-D-Ala-D-Ala})_n$ ($2 \leq n \leq 6$) in the film state, appreciable amounts of the L- and D-alanine residues of $(\text{Ala-Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 7$) take the local conformations with (ϕ, ψ) values near $(-57^\circ, -47^\circ)$ and

Figure 9. Infrared spectra in the $1800\text{--}1400\text{ cm}^{-1}$ region of $(\text{Ala-Ala-Ala-D-Ala})_n$: (A), $n=1$ (film cast from the aqueous solution); (B), $n=3, 4$ (film cast from the aqueous solution); (B)', $n=3, 4$ (film treated with ethanol); (C), $n \geq 8$ (powder, Nujol mull); (C)', $n \geq 8$ (powder treated with dichloroacetic acid, Nujol mull). The dashed line shows the absorption due to Nujol.

Figure 10. Infrared spectra in the $1800\text{--}1400\text{ cm}^{-1}$ region of $(\text{Ala-D-Ala-Ala-D-Ala})_n$: (A), $n=1$ (film casted from the aqueous solution); (B), $n=3, 4$ (film cast from the aqueous solution); (B)', $n=3, 4$ (film treated with ethanol); (C), $N \geq 6$ (powder, Nujol mull); (C)', $n \geq 6$ (powder treated with dichloroacetic acid, Nujol mull). The dashed line shows the absorption due to Nujol.

($+57^\circ$, $+47^\circ$), respectively. (Ala-Ala-Ala-D-Ala)₃ and ₄ give the amide I and II bands at 1655 cm^{-1} and 1542 cm^{-1} , respectively (Figure 9(B)). By the treatment with ethanol, the backbone conformations of (Ala-Ala-Ala-D-Ala)₃ and ₄ changes from that mentioned above to the extended β -form. This is evident from Figure 5(C)', which shows bands at 628 and 610 cm^{-1} (doublet), 454 and 410 cm^{-1} . The spectrum in the amide I and II region also confirms this (Figure 9(B)').

Figures 5(E) and (E)', and 9(C) and (C)' are the spectra of the water-insoluble fraction of (Ala-Ala-Ala-D-Ala)_n ($n \geq 8$) before and after treatment with dichloroacetic acid. The sample before treatment gives a spectrum in the 1800 – 1400 cm^{-1} region which is characteristic of the anti-parallel pleated sheet structure (Figure 9(C)). In the low-frequency spectrum (Figure 5(E)) the sample shows well-defined bands at 615 , 448 , and 253 cm^{-1} , which can all be observed in the spectrum of the β -form poly(L-alanine).¹⁵ If the D-residues are incorporated into this structure, they must assume the local conformation with (ϕ , ψ) values around (-139° , $+135^\circ$).⁸ This structure, however, is almost prohibited because of a large steric repulsion between the methyl groups of the side chains and the backbone.¹⁶ Therefore, the (ϕ , ψ) values of the D-alanine residues must differ from these values cited above. The weak bands at 530 , 490 , and 386 cm^{-1} in Figure 5(E) may be assigned to the unstable local structure of D-alanine residues. By treatment with dichloroacetic acid, these bands disappear and bands at 525 and 375 cm^{-1} appear. This is accompanied also by the appearance of shoulder bands at 1655 cm^{-1} (amide I) and 1543 cm^{-1} (amide II) (Figure 9(C)). The implication is that treatment with dichloroacetic acid converts the unstable local structure of the D-alanine residues, to which the 530 , 490 , and 386 cm^{-1} bands in Figure 5(E) can be assigned, to the local α -helix-like structure, to which the 525 and 375 cm^{-1} bands in Figure 5(E)' can be assigned.

The spectrum in the 700 – 200 cm^{-1} region of (Ala-D-Ala-Ala-D-Ala)₁ is quite different from those of (Ala-Ala-D-Ala)₁, (Ala-Ala-D-Ala-D-Ala)₁ and (Ala-Ala-Ala-D-Ala)₁. Thus the backbone of (Ala-D-Ala-Ala-D-Ala)₁ takes on a conformation different from those of other monomers. Figures 6(B), (C), and (D), (Ala-D-Ala-Ala-D-Ala)_n ($n \geq 2$) show nearly identical spectra. Treatment with ethanol does not cause any change in the infrared spectra

(Figure 6(C)' and 10(B)'). The bands observed at about 510 and 370 cm^{-1} in Figures 6(B), (C), and (D) may correspond to the 520 and 370 cm^{-1} bands characteristic of the L-alanine residues having right-handed α -helix-like conformations and the bands at 455 and 407 cm^{-1} correspond to those observed for (Ala-Ala-D-Ala)_n, (Ala-Ala-D-Ala-D-Ala)_n and (Ala-Ala-Ala-D-Ala)_n with extended β -form structures. The bands at about 598 , 480 , and 424 cm^{-1} in Figures 6(B), (C), and (D) correspond to the 580 , 478 , and 420 cm^{-1} bands observed in the spectra of copoly(D,L-alanine) with various D-residue contents.¹² The latter three bands have been assigned to the D-alanine residues incorporated into the right-handed α -helix and the L-residues contained in the left handed α -helix.¹² The constituent amino-acid residues of the samples of (Ala-D-Ala-Ala-D-Ala)_n ($n \geq 2$), therefore, have various local geometries as listed above, and it is not plausible that the backbones of the samples have any kind of ordered structure. The amide I and II bands of the samples are composed of two components at about 1659 and 1630 cm^{-1} , and about 1550 and 1530 cm^{-1} bands, respectively. Although dichloroacetic-acid treatment seems to increase the intensities of the 1630 and 1530 cm^{-1} bands (Figure 10(C)'), any apparent change in the low-frequency spectra could not be detected.

Raman Spectra

Figures 11 and 12 show the Raman spectra of (Ala-Ala-D-Ala)₃ and ₄ (the lyophilized sample) and (Ala-D-Ala-Ala-D-Ala)_n ($n \geq 6$) in the solid state. The other sequential polypeptides discussed in the preceding sections do not give spectra of high quality because of high background interference resulting from the luminescence of impurities.

The Raman spectrum of (Ala-Ala-D-Ala)₃ and ₄ (Figure 11), which is very similar to that observed by Frushour and Koenig¹⁷ for copoly(D,L-alanine) (freeze-dried sample), shows peaks at 1235 , 1075 , and 968 cm^{-1} . These peaks can be assigned to the β -form,^{17–19} so that there exists an appreciable amount of the β -form in the sample. This result is consistent with that obtained from the infrared study (Figure 7(B)). Oligo(L-alanine)s and poly(L-alanine) with the anti-parallel pleated sheet structure show the Raman scattering at about 440 cm^{-1} .^{18–20} The peak observed at 450 cm^{-1} in

Figure 11. Raman spectrum of $(\text{Ala-Ala-D-Ala})_3$ and 4 (lyophilized sample) in the solid state.

Figure 12. Raman spectra of $(\text{Ala-D-Ala-Ala-D-Ala})_n$ ($n \geq 6$) in the solid state.

Figure 11 corresponds to that mentioned above. The amide I peak at 1672 cm^{-1} in Figure 11 is also indicative of the β -form conformation,²¹ although, according to Frushour and Koenig,¹⁷ the frequency value does not necessarily suggest the existence of that conformation in copoly(D,L-alanine). The 1243

cm^{-1} peak in Figure 11 indicate that an appreciable amount of random-coil structure also exists in the lyophilized sample of $(\text{Ala-Ala-D-Ala})_3$ and 4. The peaks with weak intensities at 522 and 375 cm^{-1} can be assigned to the local α -helix-like structure of L- and D-alanine residues¹¹ indicating that some of the

Figure 13. The model of the sequential polypeptides constructed by assuming the (ϕ, ψ) angle of $(-57^\circ, -47^\circ)$ for the L-alanine residues and that of $(+57^\circ, +47^\circ)$ for the D-residues. The C=O and N-H groups are almost perpendicular to the plane of the paper. The hydrogen atoms of the methyl groups are omitted. L and D in the figure denote the L- and D-alanine residues respectively.
(A), $(\text{Ala-Ala-D-Ala})_n$; (B), $(\text{Ala-Ala-D-Ala-D-Ala})_n$.

constituent residue of $(\text{Ala-Ala-D-Ala})_3$ and 4 take on local conformations. The Raman spectrum of the water-insoluble fraction of $(\text{Ala-D-Ala-Ala-D-Ala})_n$ ($n \geq 6$) shows the amide I peak at 1662 cm^{-1} with a shoulder at about 1670 cm^{-1} (Figure 12). In the amide III region of the spectrum, there are two peaks at 1278 and 1247 cm^{-1} . The former is ascribable to the α -helical structure and the latter to the random-coil.²¹ As can be seen from Figure 12, the spectrum below 1000 cm^{-1} shows many well-defined peaks. Although these peak can not be assigned definitely at the present time, there are indication that, as already concluded from the infrared results (Figures 6(B)–(D)), the constituent amino-acid residues of these samples take on many kinds of local conformations.

DISCUSSION

Figure 13 shows the models for the backbones of $(\text{Ala-Ala-Ala-D-Ala})_n$ and $(\text{Ala-Ala-D-Ala-D-Ala})_n$, which have been constructed assuming that the L- and D-residues take on the local geometries with (ϕ, ψ) angles of $(-57^\circ, -47^\circ)$ and $(+57^\circ, +47^\circ)$, respectively. The models have structures too loose to accomodate intramolecular hydrogen-bonds, and

it is impossible for the polypeptide backbones to have the structures depicted in Figure 13. The backbone of $(\text{Ala-Ala-D-Ala})_3$ and 4 and $(\text{Ala-D-Ala-Ala-D-Ala})_n$ ($n \geq 6$) have a considerable amount of random-coil structure as shown by a strong Raman scattering peak at about 1245 cm^{-1} ²¹ (Figures 11 and 12); the other series of water-soluble sequential polypeptides are expected to assume the random-coil structure. (The 1245 cm^{-1} peak was also detected in the Raman spectrum obtained initially for $(\text{Ala-Ala-D-Ala-D-Ala})_5$ and 6 (lyophilized sample).) In addition, all the films cast from aqueous solutions of $(\text{Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 8$), $(\text{Ala-Ala-D-Ala-D-Ala})_n$ ($2 \leq n \leq 6$) and $(\text{Ala-Ala-Ala-D-Ala})_n$ ($2 \leq n \leq 7$) give broad infrared bands at about 650 cm^{-1} (Figures 3(B), (C) and (D), 4(B), (C), and (D)', and 5(B), (C) and (D')), which can be assigned to the amide V mode of the random-coil structure.²² Therefore, it is concluded that the water-soluble fractions of polypeptides take on disordered conformations, but it contains the amount of the residue with α -helix-like local the conformations in which is enough to make the 520 and 370 cm^{-1} bands observable in the low-frequency infrared spectra. This conclusion is consistent with the conformational-energy calculation for the L-alanyl

residues in the random-coil structure,²³ showing that there is a deep energy minimum at the point corresponding to the right-handed α -helix.

As described in the previous section, the ethanol treatment of the films of (Ala-Ala-D-Ala)_n ($2 \leq n \leq 8$), (Ala-Ala-D-Ala-D-Ala)_n ($2 \leq n \leq 6$), and (Ala-Ala-Ala-D-Ala)_n ($2 \leq n \leq 7$) induces a conformational change from the disordered structure to the extended β -form. The infrared spectra of the latter form shown in Figures 7(B)'' and (C)', 8(C)'' and (D), and 9(B)' suggest that the samples have highly ordered structures. The fiber repeat distances in the case of the extended β -form of (Ala-Ala-D-Ala)_n ($2 \leq n \leq 8$) and (Ala-Ala-D-Ala-D-Ala)_n ($n \geq 5$) are expected to be larger than those of the ordinary β -form, because otherwise the steric replution between the polypeptide backbone and the side chains is not observed. The deviation of the extended β -form of (Ala-Ala-D-Ala)_n ($2 \leq n \leq 8$) and (Ala-Ala-D-Ala-D-Ala)_n ($n \geq 5$) from the ordinary β -form can be deduced from the spectra of these forms, since they show no band near 440 cm^{-1} (Figures 3(C)', (D)'', 4(D)', and (E)) which can be observed in the spectra of the β -forms of poly(L-alanine) and the sequential polypeptides containing L-alanine residue.¹⁵

Lontan *et al.*,⁴ who studied the conformation of (Ala-D-Ala-Ala-Ala)_n ($n \geq 4$), reported that appreciable helix content was observed in both water and in 90% aqueous trifluoroethanol.

Dellacherie *et al.*⁷ reported that the conformation of poly(D-methylonyl-L-methyonine) was in a β -anti-parallel folded structure in which the linear polypeptide segment were near a planar extension. However, the samples of (Ala-Ala-Ala-D-Ala)_n used in this study did not take on the α -helical conformation in the solid state.

Acknowledgement. The authors should like to express their acknowledgement to JASCO Co., Ltd. for allowing the use of the laser Raman spectrometer. Thanks are also due to Dr. Akira Shōji of Gunma University for his useful comments.

REFERENCES

1. H. Heitz and G. Spach, *Macromolecules*, **4**, 429—432 (1971).
2. F. A. Bovey, J. J. Ryan, G. Spach, and F. Heitz, *Macromolecules*, **4**, 433—434 (1971).
3. F. Th. Hesselink and H. A. Sheraga, *Macromolecules*, **5**, 455—463 (1972).
4. N. Lotan, F. Th. Hesselink, H. Benderly, J. F. Yan, I. Schechter, A. Berger, and H. A. Sheraga, *Macromolecules*, **6**, 447—453 (1973).
5. K. Itoh, T. Ozaki, K. Nagayama, A. Wada, and M. Tsuboi, *Macromolecules*, **6**, 654—656 (1973).
6. F. Ascoli, G. De Angelis, F. Del Bianco, and P. De Santis, *Biopolymers*, **14**, 1109—1114 (1975).
7. E. Dellacherie, J. Neel, and F. Colonna-Cesari, *Biopolymers*, **14**, 1447—1463 (1975).
8. IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471—3479 (1970).
9. K. Itoh, H. Katabuchi, and T. Shimanouchi, *Nature, New Biology*, **239**, 42—43 (1972).
10. K. Itoh and H. Katabuchi, *Biopolymers*, **12**, 921—929 (1973).
11. K. Itoh, T. Hinomoto, and T. Shimanouchi, *Biopolymers*, **13**, 307—312 (1974).
12. K. Itoh, T. Nakahara, T. Shimanouchi, M. Oya, K. Uno, and Y. Iwakura, *Biopolymers*, **6**, 1759—1766 (1968).
13. T. Miyazawa, *J. Chem. Phys.*, **32**, 1647—1652 (1960).
14. T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712—719 (1961).
15. K. Itoh and H. Katabuchi, *Biopolymers*, **11**, 1595—1605 (1972).
16. G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, *J. Mol. Biol.*, **7**, 96—99 (1963).
17. B. G. Frushour and J. L. Koenig, *Biopolymers*, **14**, 363—377 (1975).
18. B. Fanconi, *Biopolymers*, **12**, 2759—2776 (1973).
19. B. G. Frushour and J. L. Koenig, *Biopolymers*, **13**, 455—474 (1974).
20. P. Sutton and J. L. Koenig, *Biopolymers*, **9**, 615—634 (1970).
21. B. G. Frushour and J. L. Koenig, in "Advances in Infrared and Raman Spectroscopy," Vol. 1, R. J. H. Clark and R. E. Hester, Ed., Heyden, 1975, p 35.
22. T. Miyazawa, K. Fukushima, S. Sugano, and Y. Masuda, in "Conformations of Biopolymers," Vol. II, Ramachandran, G. N., Ed., Academic Press., New York, N.Y., 1967, p 557.
23. P. J. Flory, in "Statistical Mechanics of Chain Molecules," Interscience Publishers, New York, N.Y., p 261.