Critical Chain Length for Development of the α Helix of the Peptide Having the Sequence of L-Alanyl-L-leucylglycine in the Solid State

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Abstract: Conformation of a series of oligopeptides and polypeptides having the sequence of L-alanyl-L-leucylglycine in the solid state has been studied with far-infrared and infrared spectroscopies and x-ray powder diffraction measurement. It was found that the sequential pentadeca and higher oligopeptides form the α helix and the dodeca and lower oligopeptides take the β structure when they are precipitated from various solvent systems. These results suggest that the formation of the α helix of the peptide in the solid state begins at the pentadecapeptide.

Examination of the critical size of peptide derivatives required for the development of secondary structure is very important with relation to the stability of the secondary structure and the biological activity of polypeptides and proteins.[1] Though there have been published many studies on these interests using polyamino acids as model compounds,[2] the results obtained by these studies, sometimes, may not be exactly applied to explain the role of a helical and other structural parts in proteins, because the polydispersity of the synthetic polyamino acids makes the results less strict. The study of well-defined oligopeptides, therefore, is very important to obtain informations which are applicable with higher precision to the explanation of the role of the structural parts in proteins. Goodman and his co-workers[3] have extensively studied the conformations of oligopeptides in solution and found that the formation of helix[4] and the β structure in solution begins at heptapeptide and penta- or heptapeptides, respectively. The onset of the formation of the secondary structures in solution, however, varies from the nature of solvents in which the oligopeptides are dissolved, because of the possible interactions of the peptides and the solvents. Though a critical chain length of peptides may be found in the solid state as well as in solution, no direct evidence on the onset of the formation of the α helix has been found in the solid state.

We wish to report the first finding of the critical peptide size of the development of the α helix in the solid state. We have studied the conformations of a series of oligopeptides and polypeptide with the sequence of L-alanyl-L-leucylglycine[5] and found that the development of the α helix in the solid state begins from the pentadecapeptide.

Experimental Section

Materials. A series of sequential oligopeptides, Nps-(L-Ala-L-Leu-Gly)$_n$-OEt[6] (n = 1–6), was prepared by stepwise elongation of the tripeptide sequence from HCl·H-L-Ala-L-Leu-Gly-OEt by the sequential treatments with the tripeptide active esters, Nps-L-Ala-L-Leu-Gly-ONSu. Every product was obtained in highly optical and chemical pure state. The sequential polypeptide, (L-Ala-L-Leu-Gly)$_n$, was prepared by selfpolycondensation of the tripeptide active ester. Detailed accounts of the syntheses of the oligopeptides and polypeptide used in this study will be published elsewhere.[7] A part of the sample of the oligopeptide was dissolved in trifluoroethanol and other solvent systems at a concentration of 50 mg/ml and reprecipitated by addition of 200 ml of diethyl ether. The precipitate was isolated by filtration and extracted adequately with diethyl ether. A part of the sample of the sequential polypeptide was dissolved in dichloroacetic acid and reprecipitated by addition of diethyl ether. The precipitate was isolated by filtration and extracted with diethyl ether in a Soxhlet's extractor for a day.

Measurements. Infrared spectra in the far-infrared regions were measured by using a Japan Spectroscopic Co. IR-F spectrophotometer. Nujor mulls were used. Infrared spectra in the amide I and II regions were measured by using a Japan Spectroscopic Co. IR-A spectrophotometer. KBr disks were used. X-ray powder diffraction patterns were measured with a JEOL Rotex JRX-12 x-ray diffractometer.

Results and Discussion

The conformational study of the peptides in the solid state was mainly done by analysis of far-infrared spectra, which are very sensitive to the conformational changes of polypeptide backbones.[8] X-ray powder diffraction measurement and infrared spectroscopy were also used. The usefulness of the far-infrared spectroscopy to the conformational study in the solid state has been demonstrated by Itoh et al.[9] They found characteristic key bands of individual secondary structures of polypeptides in the far-infrared regions and established the far-infrared analysis for the conformations of polypeptides in the solid state. We successfully applied the far-infrared analysis to study the conformations of the sequential oligopeptides. Figure 1 shows the far-infrared spectra of the sequential polypeptide (L-Ala-L-Leu-Gly)$_n$ and the sequential oligopeptides Nps-(L-Ala-L-Leu-Gly)$_n$-OEt (n = 3–6). The far-infrared study of the sequential polypeptide was done to get the characteristic spectrum of the peptide having the sequence of L-alanyl-L-leucylglycine associated with the α helix and the β structure. The sequential polypeptide as synthesized showed bands at 617, 562, 442, 405, 328, and 300 cm^{-1}. The band at 442 cm^{-1} is assigned as the characteristic key band of the L-alanine residue associated with the β structure.[9] In the spectrum of the polypeptide treated with dichloroacetic acid,[10] the bands appeared at 665, 643, 545, 526, 465, 395, 371, and 332 cm^{-1}, and the band at 442 cm^{-1} disappeared. The bands at 526 and 371 cm^{-1} are the key bands of the L-alanine residue with the α helix and the bands at 465 and 395 cm^{-1} are those of L-leucine. These results suggest that the sequential polypeptide takes the β structure when it is isolated from the polymerization system and transforms the conformation to the α helix by treatment with dichloroacetic acid, and the key bands of the peptides having the L-alanyl-L-leucylglycine sequence are 442 cm^{-1} with the β structure and 526, 465, 395, and 371 cm^{-1} with the α helix, which are in accordance with the assignment on the sequential polypeptides containing L-alanine and L-leucine by Itoh et al.[9] The far-infrared spectral patterns of the sequential nonapeptide and dodecapeptide Nps-(L-Ala-L-Leu-Gly)$_n$-OEt (n = 3 and 4) resemble that of the sequential polypeptide as synthesized and have the band at 441 cm^{-1} characteristic of the β structure. They do not change by treatment with trifluoroethanol. These results suggest that the sequential oligopeptides (n = 3 and 4) take the β structure before and after treatment with trifluoroethanol. On the

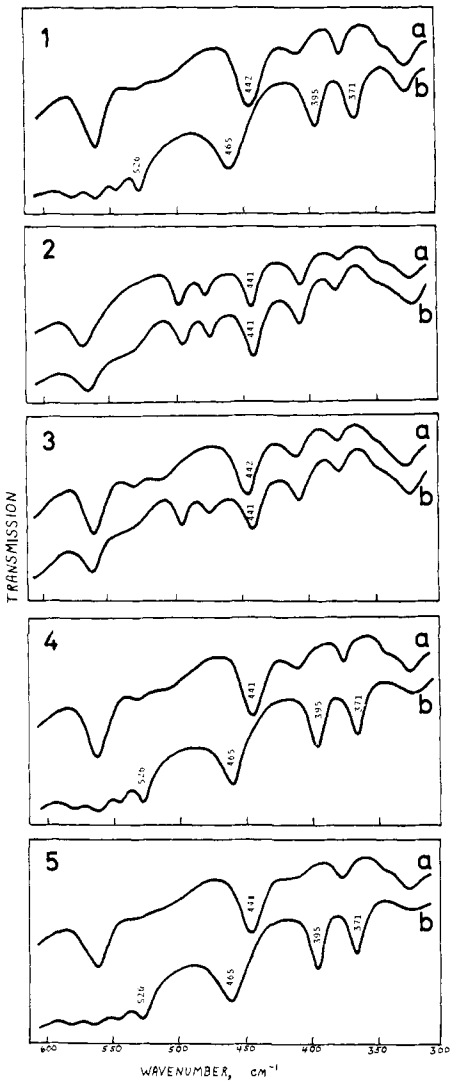

Figure 1. Far-infrared spectra of the sequential polypeptide and oligopeptides: 1, the sequential polypeptide (L-Ala-L-Leu-Gly)$_n$; 2, the sequential nonapeptide Nps-(L-Ala-L-Leu-Gly)$_3$-OEt; 3, the sequential dodecapeptide Nps-(L-Ala-L-Leu-Gly)$_4$-OEt; 4, the sequential pentadecapeptide Nps-(L-Ala-L-Leu-Gly)$_5$-OEt; 5, the sequential octadecapeptide Nps-(L-Ala-L-Leu-Gly)$_6$-OEt; a, the sample as synthesized; b, the sample precipitated from a solvent.

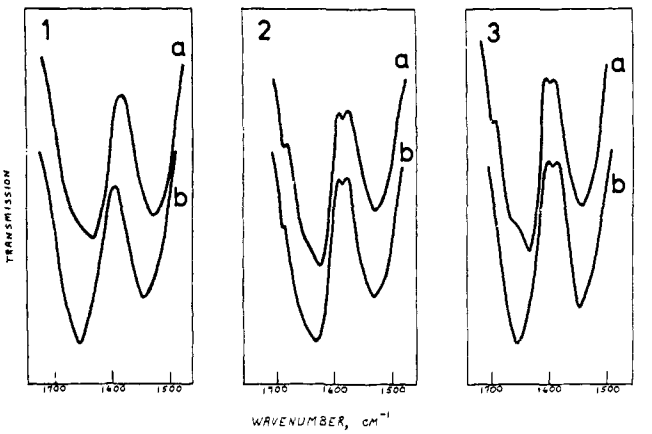

Figure 2. Infrared spectra in the amide I and II regions of the sequential polypeptide and oligopeptides: 1, the sequential polypeptide; 2, the sequential dodecapeptide; 3, the sequential pentadecapeptide; a, the sample as synthesized; b, the sample precipitated from a solvent.

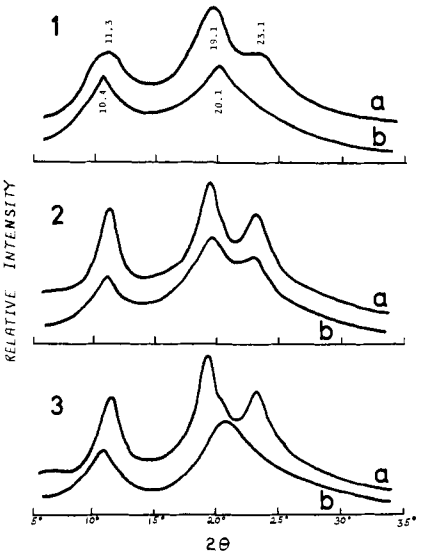

Figure 3. X-ray powder diffraction patterns of the sequential polypeptide and oligopeptides: 1, the sequential polypeptide; 2, the sequential dodecapeptide; 3, the sequential pentadecapeptide; a, the sample as synthesized; b, the sample precipitated from a solvent.

contrary to the lower oligopeptides, the sequential pentadeca- and octadecapeptides (n = 5 and 6) showed a dramatic change of the far-infrared spectral pattern before and after treatment with trifluoroethanol. The samples as synthesized showed the strong band at 441 cm^{-1} characteristic of the β structure. After treatment with trifluoroethanol, the band at 441 cm^{-1} disappeared completely and some new bands appeared at 526, 465, 395, and 371 cm^{-1}, all of which are characteristic of the α helix, and moreover the whole spectral patterns were identical with that of the sequential polypeptide with the α-helical conformation.

The facts on the sequential oligopeptides obtained by the far-infrared spectroscopy are summarized as follows. The sequential nona- and dodecapeptides are in the β structure before and after treatment with trifluoroethanol and the sequential pentadeca- and octadecapeptides are in the β structure when they are synthesized but transform the conformation to the α helix by the treatment.

These results are further demonstrated by infrared spectra in the amide I and II regions and x-ray powder diffraction patterns. Figure 2 shows the infrared spectra of the sequential polypeptide and dodeca- and pentadecapeptides. The polypeptide as synthesized showed bands at 1630 and 1530 cm^{-1} characteristic of the β structure,[10] and the same sample after treatment with dichloroacetic acid showed bands at 1655 and 1545 cm^{-1} characteristic of the α helix.[11] The sequential dodecapeptide did not change the spectral pattern which has bands at 1695, 1630, and 1530 cm^{-1} characteristic of the β structure by treatment with trifluoroethanol. On the contrary, the sequential pentadecapeptide showed bands at 1695, 1630, and 1530 cm^{-1} of the β structure before the treatment but at 1655 and 1545 cm^{-1} of the α helix after the treatment.

Figure 3 shows the x-ray diffraction patterns. The sequential polypeptide as synthesized showed three prominent peaks at 2θ = 11.3, 19.1, and 23.1°. The first and second reflections can be assigned as the (020) and (110) planes of the orthorhombic unit cell of the polypeptide taking the β structure.[12] The polypeptide after treatment with dichloroacetic acid showed two prominent peaks at 2θ = 10.4 and 20.1°. The peak at 10.4° can be assigned as the (100) plane of the hexagonal unit cell of the polypeptide with the α helix.[13] The sequential dodecapeptide showed a similar diffraction pattern having the three prominent peaks at 2θ = 11.3, 19.1, and 23.1° to that of the sequential polypeptide with the β structure. The pattern did

Table I. Conformation in the Solid State of Nps-(L-Ala-L-Leu-Gly)$_n$-OEt Precipitated with Various Pairs of Solvent-Nonsolvent[a]

n	TFA-ether	DCA-ether	HFIP-ether	TFE-ether	TFE-ethanol	TFE-methanol-ether
3	β	β	β	β	β	β
4	β	β	β	β	β	β
5	α	α	α	α	α	α
6	α	α	α	α	α	α

[a] Abbreviations: TFA, trifluoroacetic acid; DCA, dichloroacetic acid; HFIP, hexafluoro-2-propanol; TFE, trifluoroethanol; ether, diethyl ether.

not change by treatment with trifluoroethanol. This shows that the sequential dodecapeptide takes the β structure before and after the treatment. The sequential pentadecapeptide showed the prominent peaks at 2θ = 11.3, 19.1, and 23.1° before the treatment and at 2θ = 10.4 and 20.1° after the treatment. This shows that the sequential pentadecapeptide transforms the conformation from the β structure to the α helix by treatment with trifluoroethanol.

All these conformational studies draw an interesting conclusion that the formation of the α helix of the peptide having the sequence of L-alanyl-L-leucylglycine in the solid state begins at the pentadecapeptide, under a condition where the peptide is precipitated from trifluoroethanol by addition of diethyl ether. It is, however, known that the solid-state conformation frequently depends on the method of casting from various solvents. This problem is concerned with a nonequilibrium conformation of the sample. If the α-helical conformation found in the present pentadeca and higher oligopeptides by precipitation from trifluoroethanol is the nonequilibrium one, the conformation may vary with other solvents from which the peptides are precipitated. We have studied the precipitation of the peptides from various solvent systems to clarify this problem. We carefully chose trifluoroacetic acid, dichloroacetic acid, hexafluoro-2-propanol, trifluoroethanol, and a mixture of trifluoroethanol and methanol as solvent systems in which the peptides will take various possible conformations. Trifluoroacetic acid was chosen as a solvent in which the peptides have no secondary structures. Trifluoroethanol and a mixture of trifluoroethanol and methanol were chosen as solvent systems in which the peptides can form their secondary structures.[3] Results of the precipitation experiments are shown in Table I. The pentadeca and higher peptides precipitated in the α helix and the dodeca and lower peptides in the β structure. The fact that the secondary structures established by the precipitation do not depend on the nature of the solvents and therefore on the conformations in solution demonstrates that the secondary structures are the equilibrium conformations. Then the conclusion that the formation of the α helix of the peptide with L-alanyl-L-leucylglycine sequence begins at the pentadecapeptide is not one for a special case but to be generally applicable for the peptides with the sequence.

We last refer to the β structure of the pentadeca and higher peptides which is found in the samples as synthesized. The conformation first established in these samples differs in nature from that of the peptides precipitated from solvents. The former is concerned with the preparation step of the samples. The large peptides used in this study are prepared by the condensation of short peptide fragments, which take the β structure in a reaction system. As the condensation reaction proceeds, the system soon became gelatinous. This suggests that the large peptide molecules have already precipitated out from the reaction system. It is, therefore, reasonable to consider that the conformation first established in the large peptides is strongly affected by the associated β structure of the reactant short peptide fragments and is not an equilibrium one.

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(4) The word "helix" does not necessarily refer to the α helix.
(5) In such conformational studies of oligopeptides as this one, homooligopeptides are usually used as a peptide system, because there are no complicated effects resulting from the various side chains of amino acids. These homooligopeptides having longer peptide units, however, have not been synthesized. The low solubility of these peptides prevents the successful synthesis. We chose the sequential oligopeptide having a very high solubility in the reaction solvent and succeeded to prepare a series of the sequential oligopeptides having high molecular weights.
(6) Abbreviations are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **11**, 1726 (1972).
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