Critical chain length for the formation of the α-helix in the solid state: synthesis and conformation of sequential oligopeptides and polypeptide having L-leucyl-L-leucylglycine sequence

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Synthesis and conformation in the solid state of a novel series of sequential oligopeptides and polypeptides having the sequence of L-leucyl-L-leucylglycine have been studied to demonstrate critical chain length for the formation of the α-helix of the peptide. The far infra-red spectra and X-ray powder diffraction patterns suggest that the dodeca and lower peptides take the β-structure and the pentadeca and higher peptides form the α-helix in the solid state. This result leads to the conclusion that the formation of the α-helix of this peptide system begins at the pentadecapeptide. This is consistent with the result of our earlier study on the sequential oligopeptide Nps-(L-Ala-L-Leu-Gly)$_n$-OEt.

INTRODUCTION

We have reported in a previous paper[1] that the formation of the α-helix of peptides having the sequence of L-alanyl-L-leucylglycine in the solid state begins from the pentadecapeptide and the lower oligopeptides take the β-structure. This finding, which is very interesting as the first example of a critical chain length for the formation of the secondary structure in the solid state, prompts us to develop the study for finding the critical length of the formation of the α-helix of other peptide systems. The critical length may vary with factors such as the nature of the amino-acids comprising the sequence, and the length of the sequence. The first aim of the development is to clarify that the critical length, pentadecapeptide, for the formation of the α-helix found on the peptide system Nps-(L-Ala-L-Leu-Gly)$_n$-OEt may be relevant for other peptide systems having the sequence *X*-*Y*-Gly, where *X* and *Y* are the amino-acid residues forming the α-helix in the homopolypeptides[2].

In this study we prepared a novel series of sequential oligopeptides and polypeptides having the sequence L-leucyl-L-leucylglycine. A conformational study of these oligopeptides revealed that the formation of the α-helix of the peptides in the solid state begins at the same chain length, pentadecapeptide, as the oligopeptide, Nps-(L-Ala-L-Leu-Gly)$_n$-OEt.

EXPERIMENTAL

Synthesis of the sequential oligopeptides

The sequential oligopeptides, Nps-(L-Leu-L-Leu-Gly)$_n$-OEt (n = 1–6), were prepared by a stepwise fragment-condensation method. The sequential tripeptide unit, L-leucyl-L-leucylglycine, was prepared by a method[3] using *o*-nitrophenylsulphenyl (Nps) *N*-carboxy α-amino-acid anhydrides (NCAs).

Nps-L-Leu-L-Leu-Gly-OEt

Nps-L-leucylglycine ethyl ester prepared by the Nps-NCA method[4], 37 g (0.1 mol), was dissolved in 50 ml of 4 N hydrochloric acid in dioxane, and 500 ml of diethyl ether was added to give an oil. The yellow oil was extracted with diethyl ether until the yellow colour disappeared, and redissolved in 300 ml of tetrahydrofuran. To the solution was added 15 ml of triethylamine and 34 g (0.11 mol) of Nps-L-leucine NCA. The solution was stirred for 2 h at room temperature. After the reaction, the solution was concentrated under reduced pressure to give an oily residue. The oil was dissolved in 400 ml of ethyl acetate. The solution was washed with 5% citric acid, 5% sodium bicarbonate, and water, and dried over sodium sulphate. The solution was concentrated under reduced pressure. To the residue was added n-hexane to give a crystalline product. The product was recrystallized from ethyl acetate to give a pure Nps-L-leucyl-L-leucylglycine ethyl ester. The yield was 41 g (86%) $[\alpha]_D$ – 59.8 (c 1.0, tetrahydrofuran), R_f 0.63 (silica-gel thin layer, ethyl acetate:benzene = 1:1). The calculated analysis for $C_{22}H_{34}N_4O_6S$ was: C, 54.76; H, 7.10; N, 11.61. The analysis found was: C, 54.68; H, 7.18; N, 11.65.

Nps-L-Leu-L-Leu-Gly-OH

The tripeptide ester, 24 g (0.05 mol), was dissolved in a mixture of 50 ml of acetone and 50 ml of methanol, and 50 ml of 1 N sodium hydroxide was added. The solution was stirred for 1 h at room temperature. The solution was concentrated under reduced pressure, diluted with 100 ml of water, and extracted with 50 ml of diethyl ether. The aqueous solution was acidified to pH 2 with 10% citric acid

and extracted three times with 200 ml of ethyl acetate. The combined extract was washed with water and dried over sodium sulphate. The solution was concentrated to give an oil. Addition of n-hexane to the oil give a crystalline free acid. The crystals were recrystallized from ethyl acetate. The yield was 18 g (79%). $[\alpha]_D$ − 60.2 (*c* 1.0, tetrahydrofuran), R_f 0.61 (tetrahydrofuran:benzene = 10:1).

Nps-L-Leu-L-Leu-Gly-ONSu

The tripeptide free acid, 23 g (0.05 mol), was dissolved in 250 ml of tetrahydrofuran. To the solution was added 8.6 g (0.075 mol) of *N*-hydroxysuccinimide and 13.2 g (0.065 mol) of dicyclohexylcarbodiimide. The solution was stirred for 3 h at −5°C and an additional 20 h at 0°C. The crystals of urea were removed by filtration. The solution was diluted with 250 ml of ethyl acetate. The solution was washed with 1% citric acid, 2% sodium bicarbonate, and water, and dried over sodium sulphate. The solution was concentrated under reduced pressure at 30°C to give an oil. The oil was crystallized by addition of n-hexane. The product was purified by recrystallization from tetrahydrofuran. The yield was 25 g (91%). $[\alpha]_D$ − 60.9 (*c* 1.0, tetradydrofuran), R_f 0.60 (ethyl acetate:benzene = 2:1). The calculated analysis for $C_{24}H_{33}N_5O_8S$ was: C, 52.26; H, 6.03; N, 12.70. The analysis found was: C, 52.18; H, 6.14; N, 12.77.

Stepwise fragment elongation of the tripeptide unit

The peptide chain was elongated stepwise by sequential treatments of HCl·H-L-Leu-L-Leu-Gly-OEt as a starting tripeptide ester with Nps-L-Leu-L-Leu-Gly-ONSu.

The Nps protecting group of the tripeptide ester was removed by treating it with hydrochloric acid in dioxane. The resulting hydrochloride was allowed to react with Nps-L-Leu-L-Leu-Gly-ONSu in the presence of triethylamine in dimethylformamide for a day at room temperature. The reaction system was diluted with a large amount of water to precipitate the product. The precipitate was collected by filtration, washed with 5% citric acid, 5% sodium bicarbonate, and water, dried, and recrystallized from warm dimethylformamide to give a pure Nps-L-Leu-L-Leu-Gly-L-Leu-L-Leu-Gly-OEt. The protecting group of the hexapeptide ester was removed by the action of hydrochloric acid. The resulting hydrochloride was treated with the tripeptide active ester for 2 days to give the nonapeptide derivative. Further elongation of the tripeptide unit was carried out analogously. Every peptide derivative was purified by recrystallization from dimethylformamide or dimethyl sulphoxide, and/or washing with methanol, ethyl acetate and tetrahydrofuran. The purified product gave a single spot on silica gel thin layer chromatography. Results of the syntheses of all peptides are shown in *Table 1*.

Synthesis of the sequential polypeptides.

The protecting group of the tripeptide active ester was removed by dissolving it in 1 N hydrochloric acid in dioxane. To the solution was added diethyl ether to precipitate the active ester hydrochloride. The product was isolated by filtration, washed with diethyl ether, and recrystallized from methanol. The hydrochloride was dissolved in dimethylformamide at a concentration of 0.2 mol/l. To the concentrated solution was added 1.2 equivalent of triethylamine. The system was stirred for 2 days at room temperature. The system was diluted with diethyl ether to precipitate the product. The precipitate was collected by filtration, washed adequately with methanol and diethyl ether, and dried over P_2O_5 to give the sequential polypeptide $(L\text{-Leu-L-Leu-Gly})_n$ in 72% yield.

Treatment of the peptides with solvents

The sequential oligopeptides were dissolved in hexafluoroisopropanol, dichloroacetic acid, and trifluoroacetic acid at a concentration of 100 mg/ml, and reprecipitated by addition of methanol or diethyl ether. The precipitate was isolated by filtration, washed with diethyl ether, and dried over P_2O_5. The sequential polypeptide was analogously reprecipitated from dichloroacetic acid.

Measurements

Infra-red spectra in the far-infra-red regions were measured with a JASCO IR-F spectrophotometer. Nujol mulls were used. X-ray powder diffractions were measured with a JEOL Rotex JRX-12 X-ray diffractometer.

RESULTS AND DISCUSSION

The conformations of the sequential polypeptide and oligopeptides having the L-leucyl-L-leucylglycine sequence were studied with far-infra-red spectroscopy and X-ray diffraction measurement. Far-infra-red spectra are especially useful for the study of the conformations of polypeptides in the solid state[5,6] because the bands in the far-infra-red regions are very sensitive to the conformational changes[7]. It has been found[8] that *L*-leucine residues associated with the α-helix show bands near 465 and 395 cm^{-1}. We have successfully used these bands for elucidating the conformation of the sequential oligopeptides, $Nps\text{-}(L\text{-Ala-L-Leu-Gly})_n\text{-OEt}$, in the previous study[1]. These bands may also be used for characterizing the conformation of the oligopeptides,

Table 1 Results of syntheses of $Nps\text{-}(L\text{-Leu-L-Leu-Gly})_n\text{-OEt}$

					Elemental analysis					
					Calc. (%)			Found (%)		
n	Yield (%)	M_p (°C)	$[\alpha]_D$ (*c* 1.0)	R_f	C	H	N	C	H	N
2	62	215–217	−64.5[a]	0.80[e]	56.45	7.78	12.80	56.54	7.83	12.77
3	65	251–252	−23.8[b]	0.78[f]	57.23	8.07	13.35	57.16	8.15	13.28
4	84	279–282 (dec)	−16.8[c]	0.54[g]	57.68	8.24	13.66	57.59	8.30	13.68
5	41	294–295 (dec)	−6.6[c]	0.52[h]	57.97	8.37	13.87	58.01	8.43	13.80
6	55	296–300 (dec)	−0.5[d]	0.51[h]	58.17	8.44	14.01	58.08	8.48	14.12

Solvent systems for the measurements of optical rotation and thin layer chromatography are as follows: a, tetrahydrofuran; b, *N,N*-dimethylformamide; c, hexafluoroisopropanol/methanol (1:9); d, hexafluoroisopropanol/methanol (2:8); e, tetrahydrofuran/benzene (2:1); f, tetrahydrofuran; g, trifluoroethanol/benzene (1:1); h, trifluoroethanol/benzene (10:1)

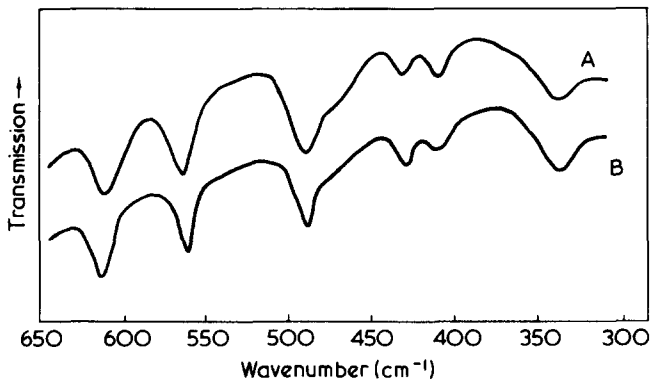

Figure 1 Far-infra-red spectrum of Nps-(L-Leu-L-Leu-Gly)$_3$—OEt. A, sample as synthesized; B, sample after treatment with hexafluoroisopropanol

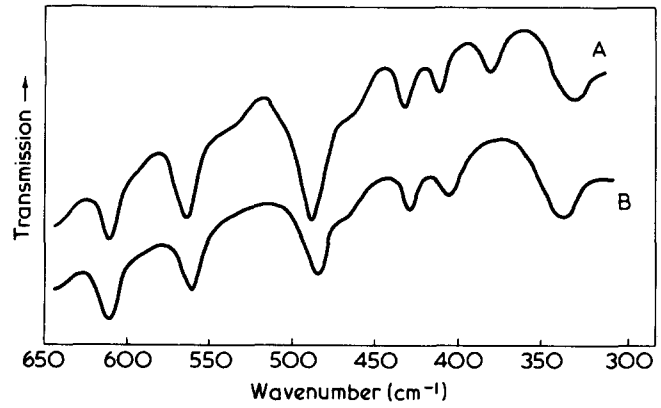

Figure 2 Far-infra-red spectrum of Nps-(L-Leu-L-Leu-Gly)$_4$—OEt. A, sample as synthesized; B, sample after treatment with hexafluoroisopropanol

Nps-(L-Leu-L-Leu-Gly)$_n$—OEt, in this study. In the previous study, the β-structure of the sequential oligopeptides, Nps-(L-Ala-L-Leu-Gly)$_n$—OEt, could be identified by the far-infra-red band at 441 cm^{-1}, which has been assigned as that of L-alanine residues with the structure. Unfortunately, the characteristic bands of L-leucine residues have not been found. The oligopeptides, Nps-(L-Leu-L-Leu-Gly)$_n$—OEt, taking the β-structure cannot therefore be characterized by the far-infra-red bands. The far-infra-red spectra, however, differ from the conformations of peptides. We identified the β-structure of the peptides, Nps-(L-Leu-L-Leu-Gly)$_n$—OEt, by a characteristic whole spectral pattern together with the result of analysis of X-ray powder diffractions.

Figure 1 shows the far-infra-red spectrum of the sequential oligopeptide, Nps-(L-Leu-L-Leu-Gly)$_3$—OEt, and *Figure 2* shows that of the sequential dodecapeptide, Nps-(L-Leu-L-Leu-Gly)$_4$—OEt. In these Figures, the upper lines are the spectra of the samples as synthesized and untreated with solvents and the lower lines are those of the samples after treatment with hexafluoroisopropanol. The spectra of these octa- and dodecapeptides showed the same pattern, which has bands at 612, 560, 486, 429, 410 and 336 cm^{-1} before and after the treatment. This result suggests that the treatment does not change the conformation of these oligopeptides. We regard the spectral pattern as that of the β-structure of the peptides, though characteristic bands cannot be assigned. This is demonstrated to be reasonable by measurements of the X-ray powder diffractions of these oligopeptides. *Figures 3* and *4* show the X-ray diffraction patterns of the sequential nona- and dodecapeptides, respectively. The nonapeptide as synthesized showed three prominent peaks at 2θ = 11.0°, 18.5°, and 22.2°. The diffraction pattern of the sample after reprecipitation from hexafluoroisopropanol has the prominent peaks at the same positions as that of the sample as synthesized. The first and second reflections can be assigned as the (020) and (110) planes of the orthorhombic unit cell of the peptide taking the β-structure. The unit cell with the dimensions a = 4.98, b = 17.68, and c = 6.89 Å are slightly larger than that of the oligopeptide Nps-(L-Ala-L-Leu-Gly)$_n$—OEt with a = 4.86, b = 15.65, and c = 6.89 Å, perhaps because of the bulkiness of the side chain of the N-terminal L-leucine residue. The sequential dodecapeptide after reprecipitation showed a similar diffraction pattern to that of the nonapeptide with three prominent peaks at 2θ = 11.0°, 18.5°, and 22.2°. These results demonstrate that the sequential nonapeptide and dodecapeptide take the β-structure before and after the treatment with the solvent.

The conformations of higher oligopeptides, Nps-(L-Leu-L-Leu-Gly)$_n$—OEt (n = 5 and 6) could be identified by the far-infra-red spectra. *Figures 5* and *6* show the far-infra-red

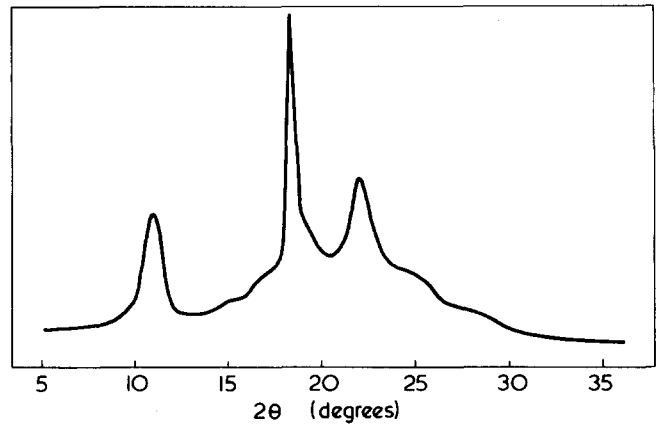

Figure 3 X-ray powder diffraction pattern of Nps-(L-Leu-L-Leu-Gly)$_3$—OEt as synthesized

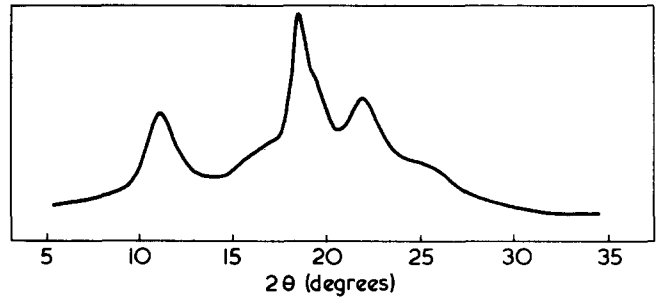

Figure 4 X-ray powder diffraction pattern of Nps-(L-Leu-L-Leu-Gly)$_4$—OEt after treatment with hexafluoroisopropanol

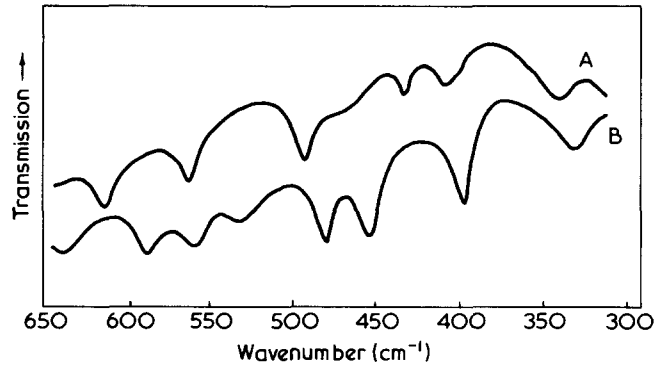

Figure 5 Far-infra-red spectrum of Nps-(L-Leu-L-Leu-Gly)$_5$—OEt. A, sample as synthesized; B, sample after treatment with hexafluoroisopropanol

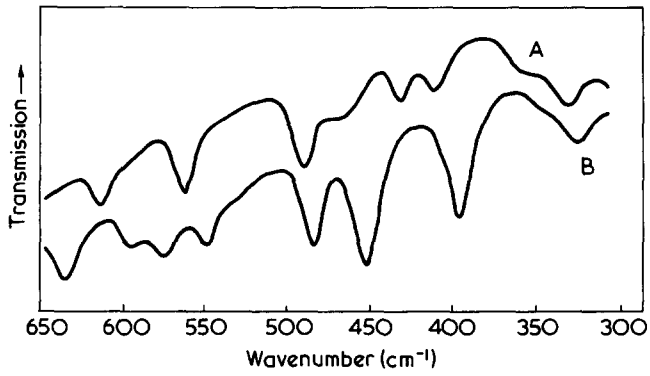

Figure 6 Far-infra-red spectrum of Nps-(L-Leu-L-Leu-Gly)$_6$—OEt. A, sample as synthesized; B, sample after treatment with hexafluoroisopropanol

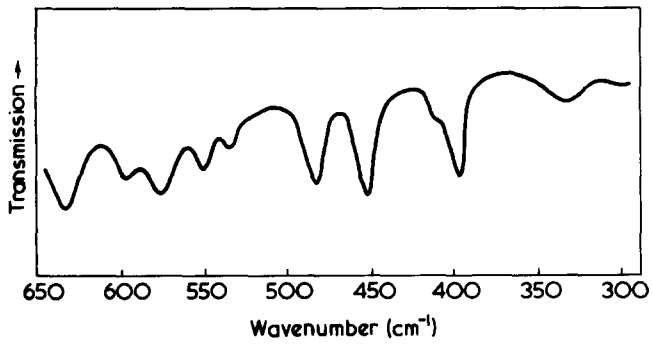

Figure 7 Far-infra-red spectrum of the sequential polypeptide (L-Leu-L-Leu-Gly)$_n$ as synthesized

spectra of the sequential pentadeca- and octadecapeptides, respectively. These peptides as synthesized showed bands at 612, 560, 486, 429, 410 and 336 cm^{-1}, which are characteristic of the β-structure. In contrast, the samples after reprecipitation from hexafluoroisopropanol showed a completely different spectral pattern from those of the β-structure. The strong bands which appeared at 455 and 395 cm^{-1} are the characteristic key bands of the α-helix of L-leucine residues.

These results suggest that the higher oligopeptides transform the conformation from the β-structure to the α-helix by precipitation from hexafluoroisopropanol. As the transformation of the conformation of the higher peptides was found with precipitation from dichloroacetic and trifluoroacetic acids as well as hexafluoroisopropanol, we concluded that the transformation occurs generally and that the α-helical conformation is the most stable one for the higher oligopeptides in the solid state. The lower dodeca- and nonapeptides did not change conformation with the treatment. Therefore the β-structure is the equilibrium conformation for the lower peptides.

The conformational study mentioned above draws the conclusion that the formation of the α-helix of the peptides with the sequence L-leucyl-L-leucylglycine begins at the pentadecapeptide in the solid state. The onset of the secondary structure of this series of peptides is consistent with that of the peptides having the sequence L-alanyl-L-leucylglycine. We therefore suppose that pentadecapeptide as a critical chain length for the formation of the α-helix in the solid state may be generally applicable for other peptide systems having the sequence of amino-acids *X*–*Y*–Gly, where *X* and *Y* are the amino-acid residues taking the α-helical conformation in the homopolypeptides.

Finally, we show the far-infra-red spectrum of the sequential polypeptide (L-Leu-L-Leu-Gly)$_n$ in *Figure 7*. The spectrum has bands at 452 and 395 cm^{-1}. These bands are the key bands of L-leucine residues characteristic of the α-helix. Thus the sequential polypeptide takes the α-helical conformation when it is isolated from the polycondensation system. This is interesting, because the α-helical conformation is only found in the sample of sequential polypeptides isolated from the polycondensation system. The sequential polypeptides take the β-structure in the isolated sample and transform the conformation to the α-helix by reprecipitation from solvents such as dichloroacetic acid. We may explain this by supposing that the polycondensation of this polymer proceeds as a solution state. The polycondensation of sequential polypeptides, in general, proceeds as a gelatinous state and the polymer precipitates out as the associated β-structure from the polycondensation system. Thus the conformation of the polymer does not change on addition of non-solvents to isolate it from the polycondensation system. In the case of the sequential polypeptide (L-Leu-L-Leu-Gly)$_n$, it is reasonable to consider that the conformation which may generate in isolation of it by addition of the non-solvent is the α-helix, as the most stable one in the solid state.

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