Critical Chain Length for the Formation of the α-Helix in the Solid State. Synthesis and Conformation of Sequential Oligopeptides and a Polypeptide containing the Sequence L-Leucyl-L-alanyl-L-leucylglycine

By **Ryoichi Katakai**, Department of Industrial Chemistry, College of Technology, University of Gunma, Tenjin-cho, Kiryu-shi 376, Japan

A series of sequential oligopeptides and a polypeptide containing the sequence L-leucyl-L-alanyl-L-leucylglycine have been prepared by the fragment condensation method and the self-polycondensation method, respectively. The conformations of the peptides have been studied by far-i.r. spectroscopy and *X*-ray powder diffraction. The polypeptide and hexadecapeptide form an α-helix and the dodeca- and lower oligo-peptides a β-structure in the solid state. The results suggest that the critical chain length for the formation of an α-helix in the solid state is 16 residues.

THE critical chain lengths of peptides for the formation of secondary structures are of current interest.[1-10] We have demonstrated that sequential oligopeptides containing the sequences of L-alanyl-L-leucylglycine [11] and L-leucyl-L-leucylglycine [12] begin to form an α-helix at the pentadecapeptide level in the solid state when treated with solvents such as trifluoroethanol, hexafluoropropan-2-ol, dichloroacetic acid, *etc.* This critical length is greater than those of homo-oligopeptides in solution, which begin to form helices at chain lengths of from **7** to **9** residues.[13-18] The amino-acid sequence strongly influences the conformations of peptides; introduction of glycine residues sometimes destabilize the α-helical conformation of a sequence.[19,20] The greater critical chain lengths may therefore result from the presence of the glycine residues.

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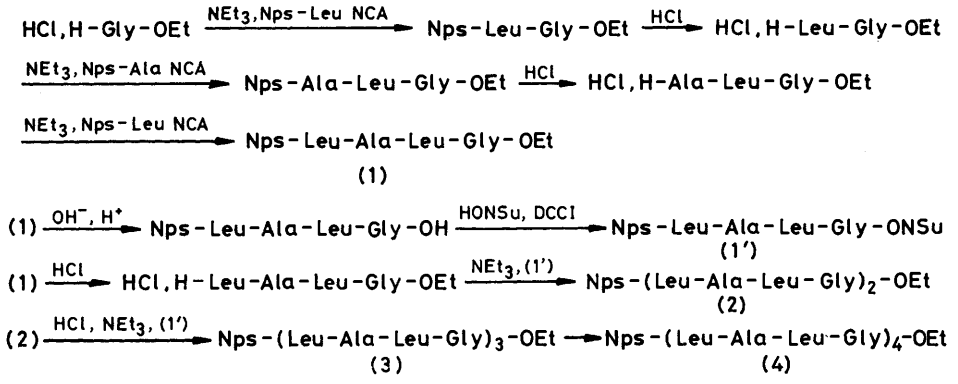
We have now extended our study to systems having a tetrapeptide sequence. A series of oligopeptides and a polypeptide containing the sequence L-leucyl-L-alanyl-L-leucylglycine was prepared, in which the proportion of glycine residues is decreased to 25%. If the glycine residues destabilize the α-helix, such peptides might be expected to begin to form the α-helix at a shorter peptide chain length than 15 residues.

The sequential oligopeptides Nps-(L-Leu-L-Ala-L-Leu-Gly)$_n$-OEt (n = 1—4) were prepared by fragment condensation of a tetrapeptide active ester. The fragment



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was prepared by stepwise elongation of the peptide chain with *o*-nitrophenylsulphenyl (Nps) *N*-carboxy-α-amino-acid anhydrides (NCAs).[21-27] The synthesis of the sequential oligopeptides is set out in the Scheme.

Glycine ethyl ester was treated with Nps-L-leucine NCA to give Nps-L-leucylglycine ethyl ester. The Nps group was removed with hydrochloric acid and the resulting hydrochloride was treated with Nps-L-alanine NCA to give Nps-L-alanyl-L-leucylglycine ethyl ester. The tripeptide was elongated by removal of the Nps group, followed by treatment with Nps-L-leucine NCA to yield Nps-L-leucyl-L-alanyl-L-leucylglycine ethyl ester. A portion of the tetrapeptide ester was used as the starting peptide for fragment condensation, and the other portion was saponified to give the free acid, which was activated by treatment with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodi-imide (DCCI). Condensation of the tetrapeptide active ester with the tetrapeptide ester produced by removal of the Nps group from the tetrapeptide derivative was carried out in *NN*-dimethylformamide for 3 days. The resulting octapeptide derivative was purified by recrystallization. Further fragment condensation was carried out by a procedure analogous to the octapeptide synthesis. All peptide derivatives obtained were demonstrated to be pure by t.l.c. The results are shown in the Table. The sequential polypeptide was prepared by self-polycondensation of the tetrapeptide active ester.[28]

The conformations of the sequential oligopeptides and the polypeptide were examined by far-i.r. spectroscopy and *X*-ray powder diffraction. Figure 1 shows the far-i.r. spectrum of the sequential polypeptide. Bands at 466, 452, and 394 cm^{-1} are characteristic of L-leucine residues associated with the α-helix and those at 522 and 370 cm^{-1} of L-alanine residues. This result demonstrates that the sequential polypeptide has the α-helical conformation. Figure 2 shows the far-i.r. spectra of the sequential oligopeptides Nps-(L-Leu-L-Ala-L-Leu-Gly)$_n$-OEt (n = 3 or 4). That of the dodecapeptide (n = 3) has bands at

Syntheses of Nps-(L-Leu-L-Ala-L-Leu-Gly)$_n$-OEt

n	Yield (%)	M.p. (°C)	$[\alpha]_D$ (°)	Found (%) C	Found (%) H	Found (%) N	Required (%) C	Required (%) H	Required (%) N
2	88	258—261 (decomp.)	−74.4 [a]	55.6	7.8	13.8	55.5	7.75	13.9
3	86	265—270 (decomp.)	−63.8 [a]	56.1	8.05	14.5	56.1	8.0	14.4
4	64	278—285 (decomp.)	−11.5 [b]	56.55	8.2	14.6	56.4	8.1	14.7

[a] *c* 0.2 in 10% hexafluoroisopropan-2-ol in dimethyl sulphoxide. [b] *c* 0.2 in 10% hexafluoroisopropan-2-ol in methanol.

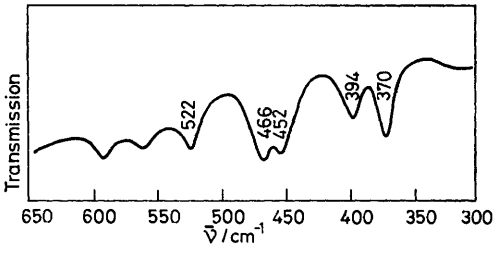

FIGURE 1 Far-i.r. spectrum of the sequential polypeptide (L-Leu-L-Ala-L-Leu-Gly)$_n$

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612, 572, 484, 449, 432, 407, and 375 cm^{-1}. The band at 449 cm^{-1} can be assigned as characteristic of L-alanine residues in the β-structure.[29] The bands observed in this spectrum also occur in that of a sequential oligopeptide with a similar sequence, Nps-(L-Leu-L-Leu-Gly)$_n$-OEt, having the β-structure, which shows bands at 612,

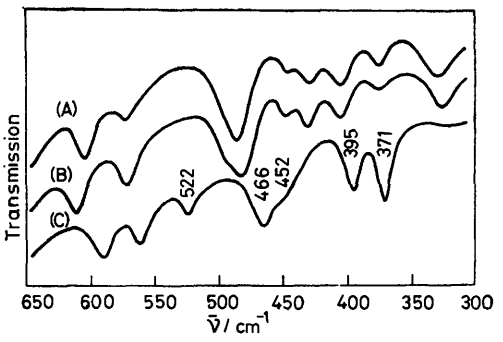

FIGURE 2 Far-i.r. spectra of the sequential oligopeptides Nps-(L-Leu-L-Ala-L-Leu-Gly)$_n$-OEt: (A) $n = 3$, (B) $n = 4$ (before treatment with hexafluoropropan-2-ol), (C) $n = 4$ (after treatment)

563, 489, 432, and 408 cm^{-1}.[12] We therefore conclude that the dodecapeptide has the β-structure. The sequential hexadecapeptide ($n = 4$) showed a spectral pattern similar to that of the dodecapeptide. This suggests that the hexadecapeptide also has the β-structure. However after treatment with hexafluoropropan-2-ol the hexadecapeptide showed a spectrum having bands at 522, 466, 452, 395, and 371 cm^{-1} characteristic of the α-helix. This conformational transformation is also accomplished by treatment with dichloroacetic acid.

Results of *X*-ray powder diffraction measurements support the above results. Figure 3 shows the *X*-ray

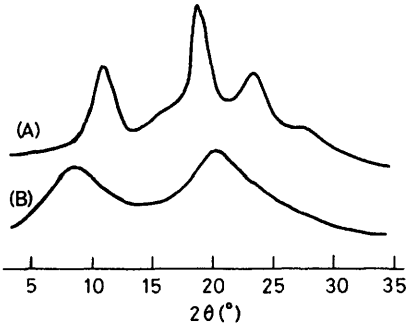

FIGURE 3 *X*-Ray powder diffraction pattern of the sequential hexadecapeptide Nps-(L-Leu-L-Ala-L-Leu-Gly)$_4$-OEt: (A) before treatment with hexafluoropropan-2-ol, (B) after treatment

diffraction pattern of the hexadecapeptide before and after treatment with the solvent. The sample before treatment showed three prominent peaks at $2\theta = 11.4$, 18.7, and 23.3°. The diffraction pattern of the dodecapeptide is similar to this pattern before and after treatment. The first and second reflections can be assigned as corresponding to the (020) and (110) planes of the orthorhombic unit cell of the peptide with the β-structure. The sample after treatment showed two prominent peaks at $2\theta = 8.4$ and 19.9°, the first of which can be assigned as corresponding to the (100) plane of the hexagonal unit cell of the peptide with the α-helix.

In general, peptides isolated from the synthetic system invariably have the β-structure, which may be transformed to the α-helix on treatment with solvents. As demonstrated in our earlier studies, this transformation is one from a non-equilibrium to an equilibrium conformation of the peptide in the solid state. Thus the hexadecapeptide assumes the α-helix as the equilibrium conformation in the solid state, but the dodeca- and lower oligo-peptides cannot do so.

Thus the critical chain length for the formation of the α-helix in the solid state is 16 residues for our sequential tetrapeptide system, not less than 15 as expected. The fact that the decreased proportion of the glycine residues does not influence the critical chain length suggests that the requirement of a greater chain length for the formation of the α-helix in the solid state than in solution may result from other factors, such as differences in phase or sequence of amino-acids.

EXPERIMENTAL

Syntheses of Sequential Oligopeptides.—*Nps-L-Leu-L-Ala-L-Leu-Gly-OEt.* Nps-L-alanyl-L-leucylglycine ethyl ester [27] (44 g) was dissolved in 2N-hydrochloric acid in dioxan (100 ml). To the solution was added diethyl ether (500 ml) and the resulting precipitate was filtered off, washed with diethyl ether until the yellow colour disappeared, and recrystallized from ethanol. The hydrochloride was dissolved in tetrahydrofuran (300 ml), and triethylamine (14 ml) and Nps-L-leucine NCA (34 g, 0.11 mol) were added. The solution was stirred for 3 h at room temperature, diluted with ethyl acetate (300 ml), then washed with 5% citric acid, 5% sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated under reduced pressure. Hexane was added to the residue to crystallize the product; this was recrystallized from tetrahydrofuran to afford pure *material* (50 g, 91%) which gave a single spot on t.l.c. (silica gel); m.p. 170—171°; $[\alpha]_D$ −64.8° (*c* 0.5 in Me_2N·CHO), R_F 0.34 [ethyl acetate–benzene (1 : 1)] and 0.75 (ethyl acetate) (Found: C, 54.3; H, 7.2; N, 12.8. $C_{25}H_{39}N_5O_7S$ requires C, 54.25; H, 7.1; N, 12.65%).

Nps-L-Leu-L-Ala-L-Leu-Gly-ONSu.—The foregoing tetrapeptide ester (27.7 g, 0.05 mol) was dissolved in acetone (200 ml) and N-sodium hydroxide (50 ml) was added. The mixture was stirred for 1 h at room temperature. The resulting solution was washed with diethyl ether and acidified with 10% citric acid to crystallize out the free acid. The product was filtered off, washed with water and diethyl ether, dried, and recrystallized from tetrahydrofuran. The free acid (26 g, 0.05 mol) was dissolved in tetrahydrofuran (500 ml) and the solution was cooled to 0 °C. To the solution were added with stirring *N*-hydroxysuccinimide (11.5 g, 0.1 mol) and dicyclohexylcarbodi-imide (15 g, 0.075 mol). The solution was stirred for 3 h at 0 °C and left overnight in a refrigerator. The resulting crystals of dicyclohexylurea were filtered off and the filtrate was diluted with ethyl acetate (300 ml). The solution was quickly washed with

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1% citric acid, 2% sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated under reduced pressure. Hexane was added to the residue to crystallize the product; this was recrystallized from tetrahydrofuran to yield pure *material* (23.6 g, 76%); m.p. 128—130°; R_F 0.14 [ethyl acetate–benzene (1 : 1)] and 0.64 (ethyl acetate) (silica gel t.l.c.); $[\alpha]_D$ −57.8° (*c* 0.5 in Me_2SO) (Found: C, 52.3; H, 6.2; N, 13.65. $C_{27}H_{38}N_6O_9S$ requires C, 52.1; H, 6.15; N, 13.5%).

Fragment Condensation.—The Nps-tetrapeptide ethyl ester (16.6 g, 0.03 mol) was dissolved in 2N-hydrochloric acid in dioxan (30 ml) and diethyl ether (300 ml) was added to precipitate the hydrochloride. The product was filtered off, washed with diethyl ether, and recrystallized from tetrahydrofuran containing a small amount of ethanol. The resulting hydrochloride of the tetrapeptide ester (13 g, 0.03 mol) was dissolved in *NN*-dimethylformamide (200 ml) and triethylamine (4.3 ml) was added. To the solution was added with stirring the Nps-tetrapeptide active ester (26 g, 0.04 mol), and the mixture was stirred for 3 days at room temperature, then was diluted with water (500 ml). The precipitate was filtered off, washed with 5% citric acid, 5% sodium hydrogen carbonate, water, methanol, ethyl acetate, tetrahydrofuran, and diethyl ether, and dried. The product was recrystallized from warm *NN*-dimethylformamide to give pure *Nps-L-leucyl-L-alanyl-L-leucylglycyl-L-leucyl-L-alanyl-L-leucylglycine ethyl ester*. Further elongation was performed by analogous treatment: removal of the Nps group and reaction with the Nps-tetrapeptide active ester. Longer chain peptide derivatives were recrystallized from warm dimethyl sulphoxide.

Synthesis of the Sequential Polypeptide.—The Nps-tetrapeptide active ester was treated with 2N-hydrochloric acid in dioxan to remove the protecting group. The resulting tetrapeptide ester hydrochloride (5.05 g, 0.01 mol) was dissolved in *NN*-dimethylformamide (20 ml). To the concentrated solution triethylamine (1.7 ml, 0.012 mol) was added with vigorous stirring, and stirring was continued for 1 day at room temperature. The mixture soon became gelatinous; it was diluted with methanol to precipitate the *polymer*, which was filtered off, washed with methanol (to remove any by-products such as cyclic peptides and the unchanged tetrapeptide) and diethyl ether, and dried (P_2O_5); yield 3.3 g (92%); η_{sp}/c = 0.21 (at 25 °C in dichloroacetic acid; 0.5 g in 100 ml) (Found: C, 57.45; H, 8.65; N, 15.85. $C_{17}H_{30}N_4O_4$ requires C, 57.6; H, 8.55; N, 15.8%).

Reprecipitation of the Sequential Oligopeptides.—The oligopeptide was dissolved in a small amount of hexafluoropropan-2-ol or dichloroacetic acid, and diethyl ether was added. The precipitate was filtered off, washed with diethyl ether, and dried (P_2O_5).

Measurements.—Far-i.r. spectra were measured with a JASCO IR-F spectrophotometer for Nujol mulls. *X*-Ray powder diffraction measurements were made with a JEOL Rotex JRX-12 *X*-ray diffractometer.

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