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SUCCESSIVE SHORT GLYCYL SEQUENCE PROHIBITING FORMATION OF ASSOCIATED β-STRUCTURE IN THE PRECIPITATION

Ryoichi KATAKAI
Department of Chemistry, College of Technology, Gunma University,
Tenjin-cho, Kiryu-shi 376

A peptide consisting of L-alanine and L-leucine, Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt takes β-structure in spite of its peptide length above the critical peptide length for the formation of α-helix in the solid state. Introduction of successive short glycyl residues to the terminal position of the peptide prevents the formation of the β-structure to develop the α-helix of the peptide.

Peptides consisting of L-alanine, L-leucine, and glycine begin forming α-helices at their critical peptide sizes for the formation of the α-helix in the solid state when they are precipitated from a solution by addition of diethyl ether.[1-3] On the contrary, peptides consisting of L-alanine and L-leucine do not form the α-helix but form the β-structure by the precipitation.[4-6] Application of shear stress to the peptides precipitated from the solution develops the α-helix if the peptides have the chain lengths longer than the critical size.[5] These facts suggest that the latter tends to associate during the rapid phase transformation from a solution to the solid states to form the β-structure. This association in the precipitation process may be deeply related with their regular arrangement of the hydrophobic side chains of L-alanine and L-leucine, because the former peptides which contain glycyl residues without the hydrophobic side chain did not form the associated β-structure in the precipitation. Destruction of the regularity of the side chains, therefore, could prevent the formation of the associated β-structure to develop the α-helix of the peptides having the chain length longer than the critical size for the formation of the α-helix.

We studied solid state conformations of Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt, Nps-(L-Leu-L-Leu-L-Ala)$_3$-Gly$_2$-OEt, and Nps-Gly$_2$-(L-Leu-L-Leu-L-Ala)$_3$-OEt. The first peptide consists of only L-alanine and L-leucine, and the second and third peptides have a diglycyl residue at the terminal position of the peptide moiety having the sequence L-Leu-L-Leu-L-Ala. The diglycyl residue was introduced to destroy the regular arrangement of the side chains of the first peptide. If our speculation is proper, the first peptide should take the β-structure and the second and third peptides should form the α-helix when they are precipitated from a solution.

Peptide syntheses were done by the fragment condensation method using dicyclohexylcarbodiimide in the presence of N-hydroxysuccinimide.[7] The tripeptide fragment Nps-L-Leu-L-Leu-L-Ala-OH was prepared by saponification of the tripeptide

ethyl ester, which had been prepared stepwise by the Nps-NCA method for peptide synthesis.[8,9] Glycyl residues were introduced stepwise by using Nps-Gly-OH and dicyclohexylcarbodiimide. The final peptides showed single spot on thin layer chromatography. Conformational characterization of these peptides was done by infrared spectra in the amide I and far-infrared regions. Since the infrared bands in these regions are known to be very sensitive to the conformational change of peptides,[10,11] we can characterize the conformations of the peptides by careful examination of the infrared bands.

Figure 1 shows infrared spectra of samples of these peptides as synthesized and reprecipitated from a solution in hexafluoropropan 2-ol (HFIP) by addition of diethyl ether. The sample as synthesized of Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt showed bands at 1698 and 1632 cm^{-1} in the amide I region, which are characteristic of the anti-parallele β-structure.[12] The spectrum of this sample in the far-infrared region has bands at 491 and 420 cm^{-1} and at 449 cm^{-1} characteristic of L-leucine and L-alanine with the β-structure, respectively.[4,13] Thus this peptide as synthesized takes the β-structure. Sample of this peptide after the reprecipitation from the HFIP solution showed similar bands to the sample as synthesized. This result demonstrates that the peptide Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt takes the β-structure after the reprecipitation. Since this peptide consists of 12 residues of L-alanine and L-leucine which should be longer than the critical peptide chain length (10 amino acid residues) for the formation of the α-helix,[4] the β-structure found in the sample precipitated from the HFIP solution suggests that this peptide formed the association of the peptide chains during the phase transformation from the solution to the solid states.

On the contrary, the peptides having the successive glycyl residues Nps-(L-Leu-L-Leu-L-Ala)$_3$-Gly$_2$-OEt and Nps-Gly$_2$-(L-Leu-L-Leu-L-Ala)$_3$-OEt as synthesized take the β-structure but change their conformation to the α-helix after the reprecipitation. The samples as synthesized of these peptides showed similar spectra to that for the sample of Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt precipitated from the HFIP solution which have the bands at 1697, 1627, 491, 449, and 420 cm^{-1} characteristic of the β-structure. After the reprecipitation from the HFIP solution, these peptides, however, showed spectra different from those of the samples as synthesized. All the bands characteristic of the β-structure disappeared and some new bands appeared at 1655 cm^{-1} in the amide I region which can be assigned to the α-helix and at 524 and 366 cm^{-1} and at 564 and 392 cm^{-1} in the far-infrared region which are characteristic of L-alanine and L-leucine with the α-helical conformation, respectively.[13] These peptides having the successive glycyl residues, therefore, form the α-helix after the reprecipitation from the HFIP solution. This α-helix formation of the peptides Nps-(L-Leu-L-Leu-L-Ala)$_3$-Gly$_2$-OEt and Nps-Gly$_2$-(L-Leu-L-Leu-L-Ala)$_3$-OEt and the failure of the α-helix formation of the peptide Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt should demonstrate the propriety of our idea that prevention of the association of the peptide chains during the phase transformation from the solution to the solid state by introduction of the successive glycyl residues to destroy the regular arrangement of the hydrophobic side chains of peptides could develop the α-helical conformation in the peptides which have the peptide chain length longer than the critical size for the formation of the α-helix.

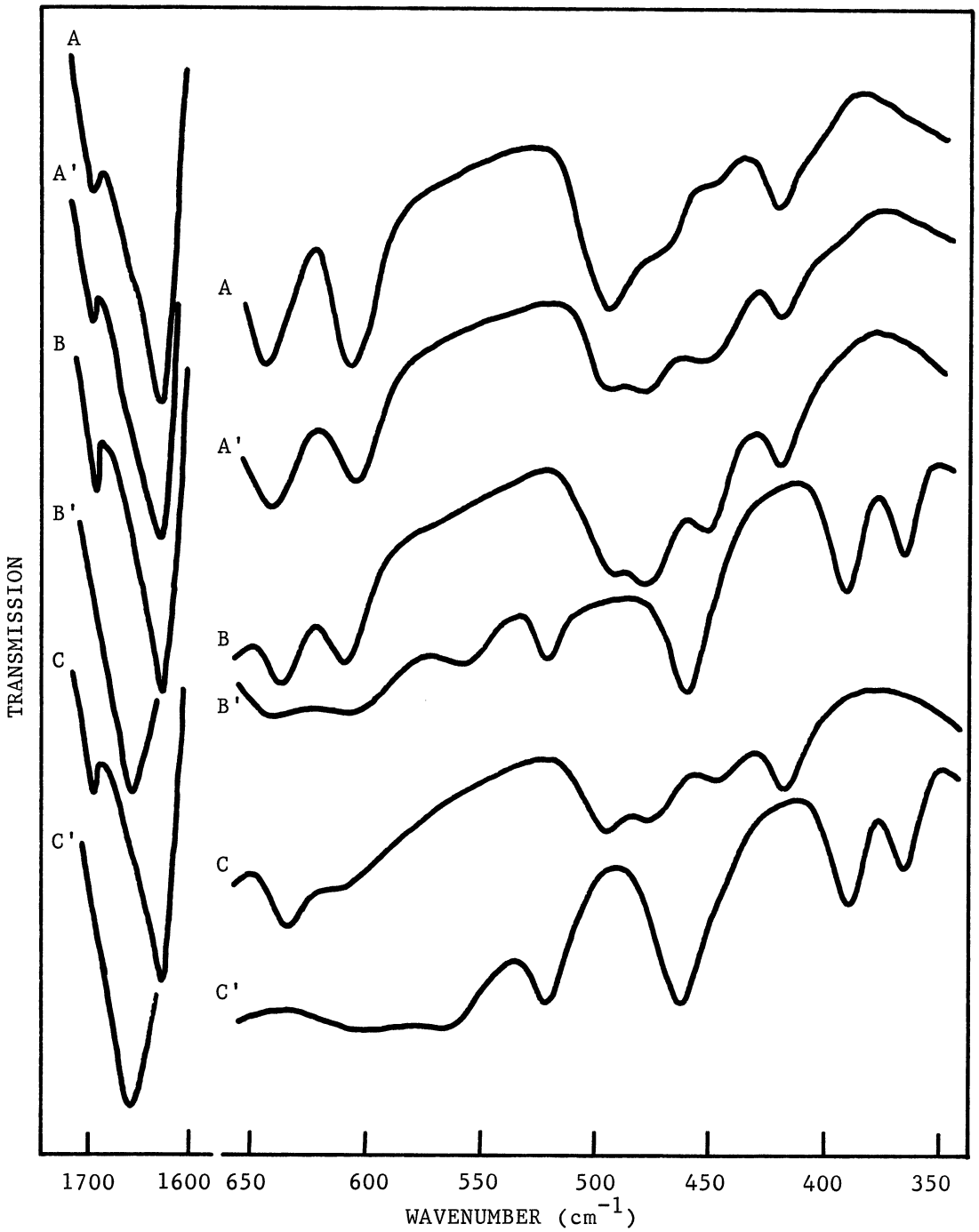

Fig.1. Infrared spectra of oligopeptides. A: Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt as synthesized, A': Nps-(L-Leu-L-Leu-L-Ala)$_4$-OEt precipitated from HFIP solution, B: Nps-(L-Leu-L-Leu-L-Ala)$_3$-Gly$_2$-OEt as synthesized, B': Nps-(L-Leu-L-Leu-L-Ala)$_3$-Gly$_2$-OEt precipitated from HFIP solution, C: Nps-Gly$_2$-(L-Leu-L-Leu-L-Ala)$_3$-OEt as synthesized, C': Nps-Gly$_2$-(L-Leu-L-Leu-L-Ala)$_3$-OEt precipitated from HFIP solution.

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