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The Study on Peptide and Protein Syntheses. Infrared Spectroscopic Conformational Analysis of Oligo-L-leucines Containing Only One D-Amino Acid Residue[1)]

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In order to investigate the influence of a D-amino acid residue on the conformational behavior of peptides and proteins, IR spectroscopic conformational analysis of oligo-L-Leus containing only one D-Ala or D-Val residue was performed in the solid state. For the estimation of their structures, a CPK model and Ramachandran map were very helpful. The D-amino acid residue is clearly inhibited to take the values of the backbone dihedral angles ϕ and ψ for β-sheet structures formed by L-amino acid residues, while it is allowed for the D-amino acid residue to take the values ϕ and ψ for helical structures formed by L-amino acid residues. The IR absorption spectroscopic analysis of samples after application of strong shear stress was also useful for the investigation. The effect of shear stress on β-sheet-like structure→helix conformational change is larger for oligo-L-Leus containing a D-Ala residue than those containing a D-Val residue, indicating that the D-Ala residue is more favorable in a helical structure than the D-Val residue. This tendency can be also easily confirmed using CPK model. The significance of this study was discussed in relation to protein synthesis and design of three-dimensional structures of peptides using D-amino acid residues.

In our recent papers,[2-8)] we have demonstrated that a β-sheet aggregation plays an important role in the insolubility of oligopeptides larger than octa- or nonapeptide levels and that replacement of an L-amino acid residue with an Aib residue promotes helical folding in an oligopeptide to improve its solubility to a remarkable extent. The solubility improvement was clearly explained by the fact that the values of the backbone dihedral angles ϕ and ψ of the Aib residue were restricted to helical regions.[4)] The Aid residue in an oligopeptide is inhibited to take the values of ϕ and ψ corresponding to a β-sheet region, and a β-sheet structure of the oligopeptide containing the Aib residue is destroyed due to steric hindrance of the Aib residue. A D-Ala residue has the structure that the C_L^β methyl group in the Aib residue is replaced by a hydrogen atom, and the D-Ala residue in oligo-L-Leus is also inhibited to take the values of ϕ and ψ corresponding to a β-sheet structure formed by oligo-L-Leus. Therefore, it is interesting to investigate the conformational behavior of oligo-L-Leus containing only one D-Ala residue.

The concern in this paper is to investigate the influence of a D-amino acid residue on the conformational behavior of oligo-L-Leus containing only one D-Ala or D-Val residue in the solid state. The investigation is significant in the study on peptide and protein syntheses since racemization during coupling reaction is inevitable. It also gives information for the design of three-dimensional structures, especially β-turn structures, of peptides using D-amino acid residues.

Experimental

Materials. The peptides used in this study are in the following: Boc-L-Leu$_3$-D-X-L-Leu$_3$-OBzl: X=Ala **1a**, X=Val **1b**; Boc-L-Leu$_4$-D-X-L-Leu$_4$-OBzl: X=Ala **2a**, X=Val **2b**; Boc-L-Leu$_5$-D-X-L-Leu$_3$-OBzl: X=Ala **3a**, X=Val **3b**; Boc-L-Leu$_4$-L-X-L-Leu$_4$-OBzl: X=Ala **4a**, X=Val **4b**. Preparation and solubility properties of the peptides **1**—**4** will be reported elsewhere.[9)] The peptides **1a** and **3a** are soluble in dichloromethane, tetrahydrofuran, and highly polar solvents such as *N,N*-dimethylformamide and dimethyl sulfoxide. The peptides **1b** and **3b** are less soluble in dichloromethane and tetrahydrofuran and soluble in the highly polar solvents. The peptides **2** and **4** are insoluble even in the highly polar solvents. The solid samples of the peptides **1a** and **3a** were obtained by repeated recrystallization from aqueous ethanol and the solid sample of the peptide **1b**, from methanol. Those of the peptides **2**, **3b**, and **4** were obtained by repeated washing with hot methanol. The purity of the peptides was confirmed by elemental and amino acid analyses. The peptides soluble in *N,N*-dimethylformamide gave a single peak on high-performance liquid chromatography.

IR Measurements. The IR absorption spectra of the solid samples were recorded with a JEOL Model JIR-100 FT-IR spectrometer in a Nujol mull. The IR absorption spectra (Fig. 1) of the peptides **1**—**4** under slight shear stress were obtained after the solid samples were pulverized with Nujol by weak shear stress.[8,13)] The IR absorption spectra (Fig. 2) of the peptides **1**—**4** after application of shear stress were also obtained in a Nujol mull after the solid samples were pulverized without Nujol by strong shear stress.[8,13)]

Results

IR Absorption Spectra of the Peptides 1—4 in the Solid State. Figure 1 shows the IR absorption spectra of the peptides **1**—**4** in the most significant spectral regions for the conformational assignments (3500—3100 cm^{-1}, amide A; 1800—1600 cm^{-1}, amide I). All of the peptides show a strong band at 3280—3270 cm^{-1} and a negligible small band around 3425 cm^{-1} in the amide A region, suggesting that the Boc-urethane

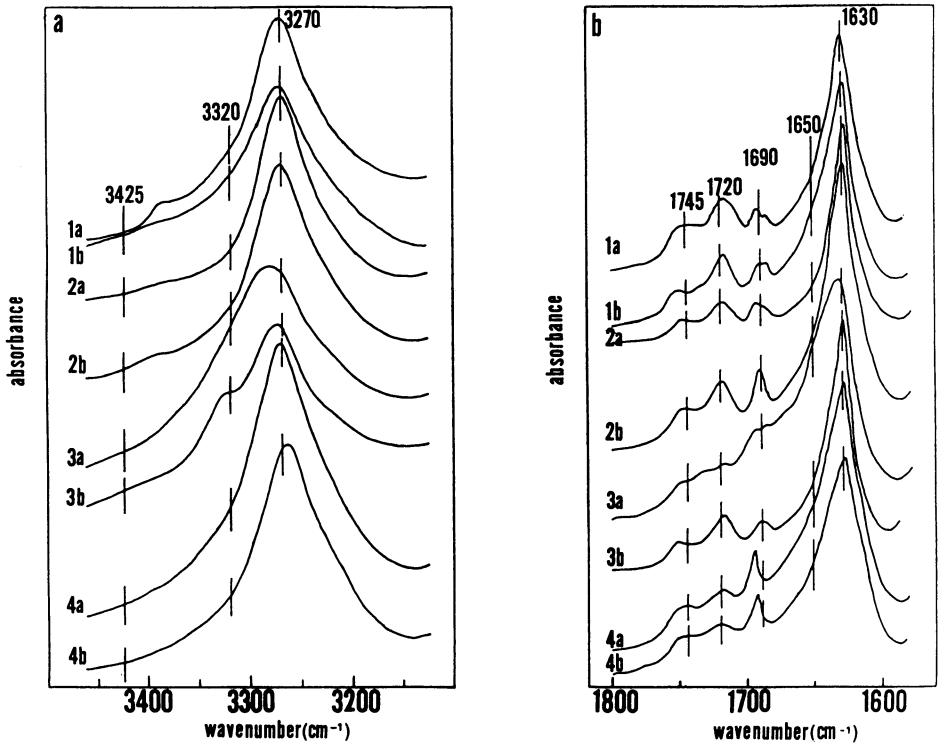

Fig. 1. IR absorption spectra of the peptides **1**—**4** in the solid state under slight shear stress. a: The amide A region; b: the amide I region.

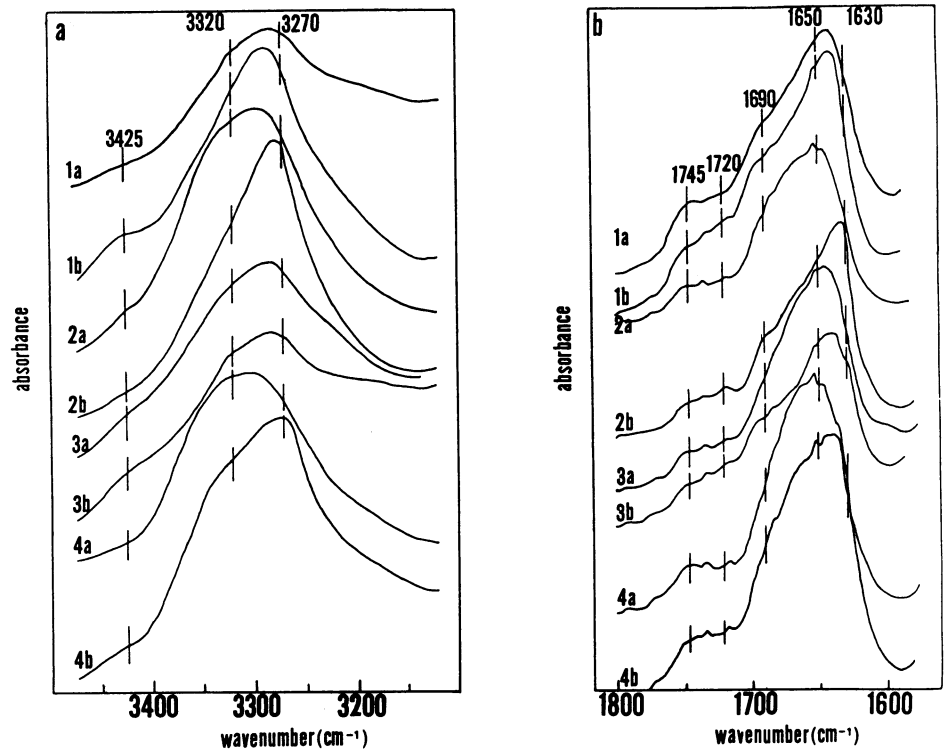

Fig. 2. IR absorption spectra of the peptides **1**—**4** in the solid state after application of strong shear stress. a: The amide A region. b: the amide I region.

N-H bond and peptide N-H bonds are mostly subjected to hydrogen bonding in the solid state. The bands around 1745 cm^{-1} and 1720 cm^{-1} are assigned to the Bzl-ester carbonyl group and the Boc-urethane carbonyl group, respectively, and the bands at 1690—1625 cm^{-1}, mainly to hydrogen-bonded amide-carbonyl groups. The strong bands at 3280—3270 cm^{-1} in the amide A region and at 1635—1625 cm^{-1} in the amide I region and the weak band at 1690 cm^{-1} in the amide I region suggest that the peptides **1—3** have an antiparallel β-sheet-like structure, while those of the peptides **4a** and **4b** containing only L-amino acid residues are indicative of an antiparallel β-sheet (β_A-sheet) structure.[10-12] The peptides **3a** and **3b** are accompanied by a shoulder band around 3320 cm^{-1}, indicating a contribution of other conformations.

The Influence of Shear Stress on Peptide Conformations in the Solid State. It has been reported that β-sheet→helix conformational change in the solid state brings about by application of shear stress.[8,13] Figure 2 shows the IR absorption spectra of the peptides **1—4** after application of strong shear stress. These spectra indicate the influence of shear stress on peptide conformations to a small or considerable extent. The increase in intensities of 3320-cm^{-1} and 1655-cm^{-1} shoulder bands of the peptides strongly suggests the onset of a helical structure although the increase in intensity of a 3425-cm^{-1} shoulder band is not negligible, which is assigned to N-H bonds free from hydrogen bonding.

Discussion

The IR absorption spectra (Fig. 1) of the peptides **1—4** under a slight influence of shear stress are reproducibly obtained when solid samples are pulverized with Nujol by weak shear stress, indicating that the peptides **1—3** have a β_A-sheet-like structure, while the peptides **4a** and **4b** have a β_A-sheet structure. The solubility of the nonapeptides **2a** and **2b** is as low as that of Boc-(L-Leu)$_9$-OBzl and the nonapeptides **4a** and **4b**, also suggesting that they have successive-strong intermolecular hydrogen bonds like β-sheet aggregation. The solubility of peptides forming successive-intermolecular hydrogen bonds is strongly dependent on peptide chain length,[2-7] and the heptapeptides **1a** and **1b** is actually more soluble than the nonapeptides **2a** and **2b**.

For the estimation of the structures of the peptides **1—3**, a CPK model and Ramachandran map[14] are quite helpful. The values of the backbone dihedral angles ϕ and ψ of an L-amino acid residue are allowed in the regions outlined by solid lines in Fig. 3a, and those of a D-amino acid residue, in the regions outlined by solid lines in Fig. 3b. The restriction of the values ϕ and ψ in Fig. 3 results entirely from the steric repulsion of $C^{\beta}_{L,i}$ and $C^{\beta}_{D,i}$ with O_{i-1}, N_{i+1}, and H_{i+1} (Fig. 4). Therefore, the D-Ala and D-Val residues in the peptides **1** and **2** clearly can not exist in β_A-sheet and parallel β-sheet (β_P-sheet) structures formed by L-amino acid residues. Furthermore, Fig. 3 suggests three possibilities of the conformation of the peptides **1** and **2**. The first one is the right handed helical (H_R) structure, where the L-Leu, D-Ala, and D-Val residues should have the values of ϕ and ψ corresponding to the H_R region. The second one is the segment-separated structure, where β-sheet structures are separated by the D-Ala or D-Val residue into two peptide segments as proposed previously for tertiary peptide bond-containing polypeptides.[2,15] The last one is the β_P- or β_A-sheet-like structure as shown in Fig. 5. In the β_P-sheet-like structure shown in Fig. 5a, the L-Leu residues have the values of ϕ and ψ corresponding to the β_P region in the L-configuration:

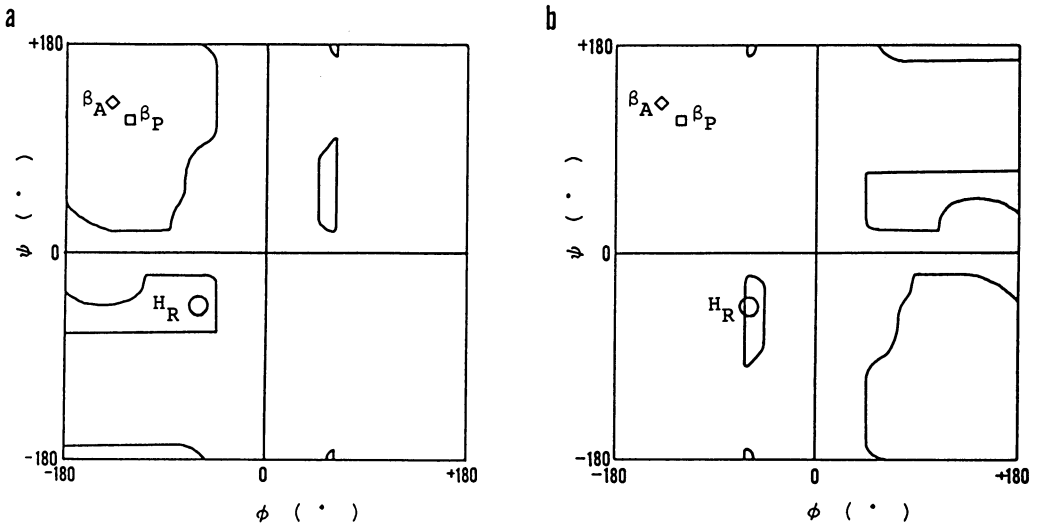

Fig. 3. Ramachandran map of amino acid residues except for glycine and proline residues. a: L-Amino acid residues; b: D-amino acid residues.

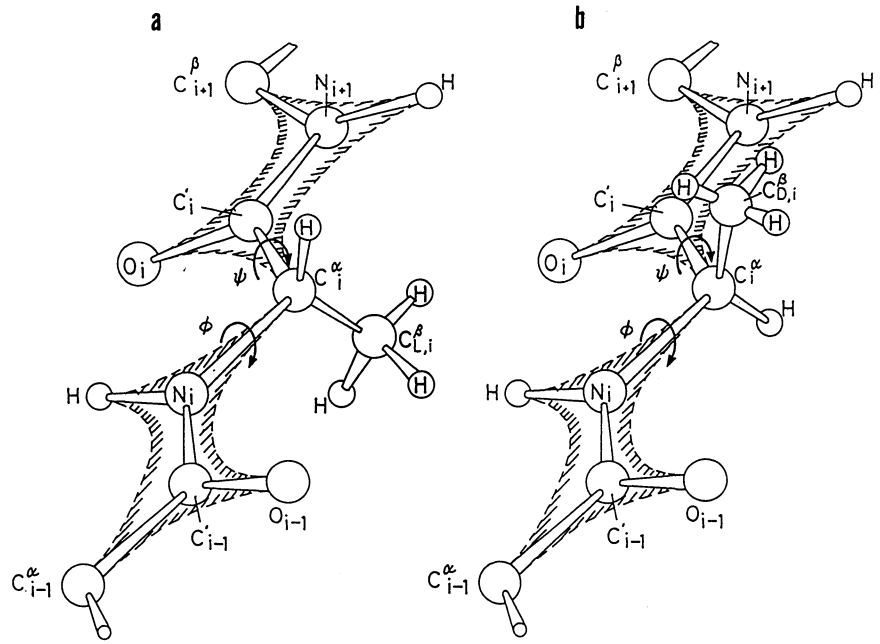

Fig. 4. The structure of peptide backbone and the backbone dihedral angles ϕ and ψ. a: L-Amino acid residues; b: D-amino acid residues.

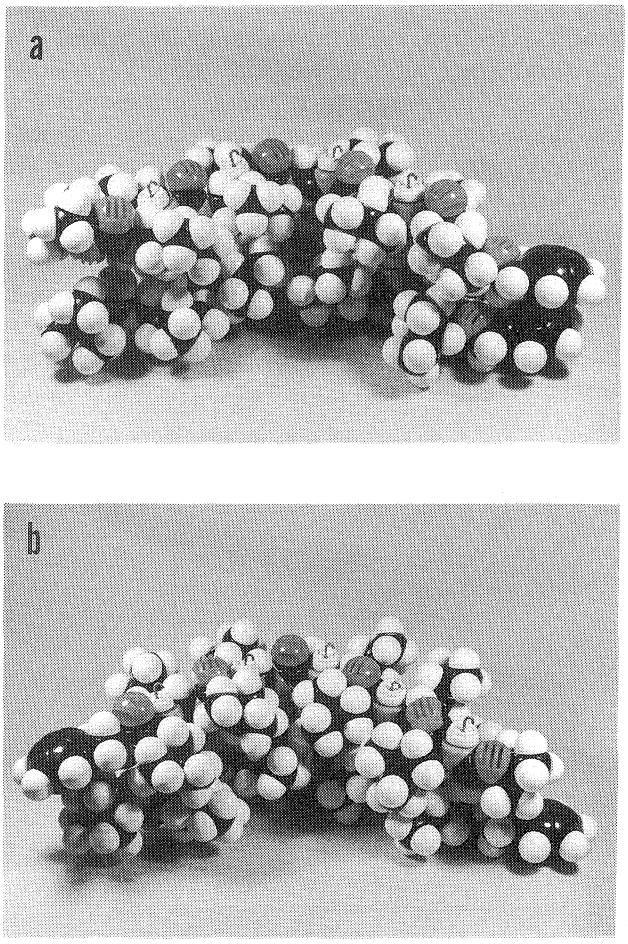

Fig. 5. The CPK model of theβ_P- and β_A-sheet-like structures of the peptide **2a**. **a**: The β_P-sheep-like structure; b: the β_A-sheet-like structure.

ϕ=−119° and ψ=+120°, while the D-Ala and D-Val residue have the other values in the regions outlined by solid lines in Fig. 3b. In the β_A-sheet-like structure shown in Fig. 5b, the L-Leu residues have the values of ϕ and ψ corresponding to the β_A region in the L-configuration: ϕ=−139° and ψ=+135°, and the D-Ala and D-Val residue have the other values in Fig. 3b. The solubility of peptides having the helical or segment-separated structure is expected to be easily soluble in organic solvents.[2,4,15] In addition to the above consideration, the IR absorption spectra (Fig. 1) and solubility properties of the peptides **1** and **2** strongly suggest that they have the β_A-sheet-like structure shown in Fig. 5b although an L-Leu residue is favorable in both β_A- and β_P-sheet structures.[16] The β_A-sheet-like structure of the peptides **1** and **2** is also supported by the solubility behavior and IR absorption spectrum (Fig. 1) of the peptide **3a**. When the peptide **3a** is favorable in a β_P-sheet-like structure having successive-intermolecular hydrogen bonds, it will be insoluble in highly polar solvents. However, the peptide **3a** is easily soluble even in medium-polar solvents such as dichloromethane and tetrahydrofuran, and its IR absorption spectrum (Fig. 1) rather suggestes the β_A-sheet-like structure containing other conformational contribution.

The IR absorption spectra (Fig. 2) of the peptides **1**—**4** after application of shear stress are also useful for investigating the influence of a D-amino acid residue on the conformational behavior of oligo-L-Leus containing only one D-amino acid residue. They are reproducibly obtained when solid samples are pulverized without Nujol by strong shear stress. The

effect of shear stress on β-sheet-like structure→helix conformational change is larger for oligo-L-Leus containing a D-Ala residue, **1a—3a**, than those containing a D-Val residue, **1b—3b**. This result indicates that the D-Ala residue is more favorable in a helical structure than the D-Val residue. In fact, this tendency can be easily confirmed using a CPK model. The difference of conformational preference in the D-Ala and D-Val residues subsequently brings about the solubility difference of the peptides **3a** and **3b**. The peptide **3a** is easily soluble in dichloromethane, while the peptide **3b** is nearly insoluble. The peptide **3a** is indeed estimated to have a helical structure in dichloromethane solution as well as Boc-(L-Leu)$_4$-Aib- (L-Leu)$_4$-OBzl and the peptide **1a**.[4,17] The same tendency of conformational transformation is observed in the peptides **4a** and **4b** having a typical β_A-sheet structure. The effect of shear stress on β-sheet→helix conformational change is also larger for the peptide **4a** having an L-Ala residue than the peptide **4b** having an L-Val residue (Fig. 2), but both peptides are insoluble even in highly polar solvents such as *N,N*-dimethylformamide and dimethyl sulfoxide. The conformational behavior and solubility properties of the peptides **3** and **4** clearly suggest that, in the case of oligopeptides containing a D-Ala residue, the inhibition of the values ϕ and ψ in the β-sheet region and the allowance of the values ϕ and ψ in the helical region promote helical folding to improve their solubility as pointed out for oligopeptides containing an Aib residue.[4]

The conformational behavior of the peptides **1—3** is significant in the study on peptide and protein syntheses. The results obtained indicate that a D-amino acid residue resulted in inevitable racemization in coupling reactions can not exist in β_A- and β_P-sheet structures of peptides and proteins and brings about remarkable conformational change shown in Fig. 5 at least in the β-sheet regions. On the other hand, Fig. 3 suggests that, due to steric hindrance, β-turn structures can be kept stable using a D-amino acid residue in the position $i+1$ or $i+2$ of β-turn structures. We are now working on the design of β-turn structures using a D-amino acid residue.

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References

1) This paper forms Part VIII of "Designs of Three-dimensional Structures of Peptides and Proteins and the Synthetic Route for Peptides and Proteins" series. This series follows "Design of the Synthetic Route for Peptides and Proteins Based on the Solubility Prediction Method Part VII," see M. Narita, M. Doi, and T. Nakai, *Bull. Chem. Soc. Jpn.*, **60**, 3255 (1987). Abbreviaions used are in the following: IR, infrared; Leu, leucine residue; Ala, alanine residue; Val, valine residue; Aib, α-aminoisobutyric acid residue; Boc, *t*-butoxycarbonyl; OBzl, benzyl ester.

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