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Infrared Absorption Study of Peptide Fragments of Human Hemoglobin α-Chain (123–136) in the Solid State[1)]

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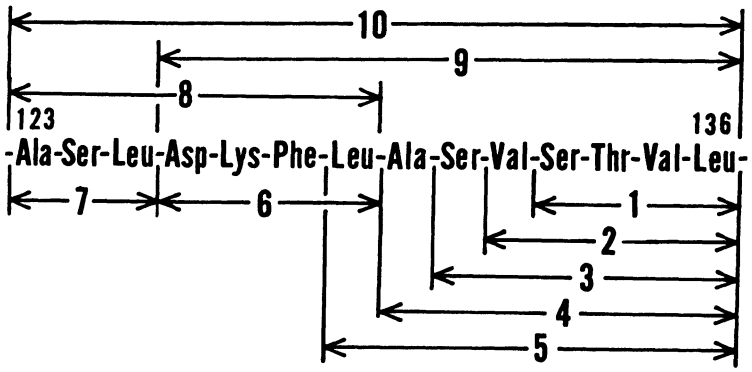
In order to evaluate the influence of polar amino acid residues on peptide conformations, IR absorption spectroscopic analysis of peptide fragments of human hemoglobin α-chain (123–136) and Boc-Leu$_n$-OBzl (n=3—6, 9, and 12) was performed in the solid state. The polar amino acid residues involved in the peptide fragments are in the following: Asp(OBzl), Lys(Z), Ser(Bzl), and Thr(Bzl). Regardless of the amino acid composition, all of the peptides equal to or larger than a pentapeptide level exhibited a high potential for the formation of a β-sheet structure in the solid state. Conformational behaviors of peptides having polar amino acid residues are similar to those of Boc-Leu$_n$-OBzl, indicating that polar side chains can not cause disturbance of the formation of β-sheet structures. The fine relationship among the conformation, solubility, and $\langle P_c \rangle$ value of the peptide fragments strongly supports the propriety of the solubility prediction method for peptide intermediates. The influence of shear stress on peptide conformations in the solid state was also investigated precisely, and it was suggested that a heptapeptide size had the potential for the development of a helical structure in the solid state.

In previous papers,[2-6)] we have demonstrated that the insolubility of peptide intermediates in high-polar solvents is essentially caused by a β-sheet aggregation formed by peptides equal to or larger than an octapeptide sequence level and that their solubility is independent of their amino acid sequences and compositions. The insolubility of peptide intermediates is a serious problem in protein synthesis. The relationship between the conformation and the solubility of peptide having polar amino acid residues has received little attention,[5,6)] since their role in the conformational behavior of the peptides is complicated.



1. Boc-Ser(Bzl)-Thr(Bzl)-Val-Leu-OBzl
2. Boc-Val-Ser(Bzl)-Thr(Bzl)-Val-Leu-OBzl
3. Boc-Ser(Bzl)-Val-Ser(Bzl)-Thr(Bzl)-Val-Leu-OBzl
4. Boc-Ala-Ser(Bzl)-Val-Ser(Bzl)-Thr(Bzl)-Val-Leu-OBzl
5. Boc-Leu-Ala-Ser(Bzl)-Val-Ser(Bzl)-Thr(Bzl)-Val-Leu-OBzl
6. Boc-Asp(OBzl)-Lys(Z)-Phe-Leu-OPac
7. Boc-Ala-Ser(Bzl)-Leu-OPac
8. Boc-Ala-Ser(Bzl)-Leu-Asp(OBzl)-Lys(Z)-Phe-Leu-OPac
9. Boc-Asp(OBzl)-Lys(Z)-Phe-Leu-Ala-Ser(Bzl)-Val-Ser(Bzl)-Thr(Bzl)-Val-Leu-OBzl
10. Boc-Ala-Ser(Bzl)-Leu-Asp(OBzl)-Lys(Z)-Phe-Leu-Ala-Ser(Bzl)-Val-Ser(Bzl)-Thr(Bzl)-Val-Leu-OBzl
11. Boc-Leu$_3$-OBzl
12. Boc-Leu$_4$-OBzl
13. Boc-Leu$_5$-OBzl
14. Boc-Leu$_6$-OBzl
15. Boc-Leu$_9$-OBzl
16. Boc-Leu$_{12}$-OBzl

Fig. 1. The amino acid sequence of human hemoglobin α-chain (123–136) and peptides **1**—**16** used in this study.

This is in contrast to the fact that conformational analyses of homo- and sequential peptides have been widely carried out.[7-9]

On the other hand, in a study of the relationship between the conformation and solubility of human proinsulin C-peptide fragments,[5] we have shown that the average coil conformation $\langle P_c \rangle$ value of each peptide fragment was effective for estimating the potential for the formation of a β-sheet structure of peptide fragments below the critical size for the development of an α-helical structure in the solid state. The result also clearly showed that a synthetic route for peptides and proteins could be designed on the basis of the solubility prediction method which we previously proposed.[3] Human proinsulin C-peptide fragments previously investigated include the polar amino acids, Asp (OBzl), Gln, Glu (OBzl), and Ser (Bzl), and their conformational analysis was performed using IR absorption spectra in the solid state and in dichlormethane solution.

In this study, we performed IR absorption spectroscopic analysis of peptide fragments of human hemoglobin α-chain (123-136) in the solid state and investigated the influence of polar amino acid residues on peptide conformdtions. For the IR analysis, we also examined the influence of shear stress on peptide conformation. Human hemoglobin α-chain (123-136) shown in Fig. 1 corresponds to the partial sequence of the helical region H of the α-chain.[10,11] Figure 1 also illustrates the protected peptide fragments **1**—**10** and Boc-Leu$_n$-OBzl ($n=3$—6, 9, and 12) **11**—**16** used in this study.

Experimental

Materials. Samples of human hemoglobin α-chain (123-136) fragments **1**—**10** and Boc-Leu$_n$-OBzl **11**—**16** are those described in previous papers.[2,6] The solid samples of the peptides **1**—**4**, **6**—**8**, and **11**—**14** were obtained by repeated recrystallization from appropriate solvents.[2,6] Those of the peptides **5**, **9**, **10**, **15**, and **16** 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 also 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. IR spectra (Figs. 2—4) of the peptides **1**—**16** under a slight shear stress were

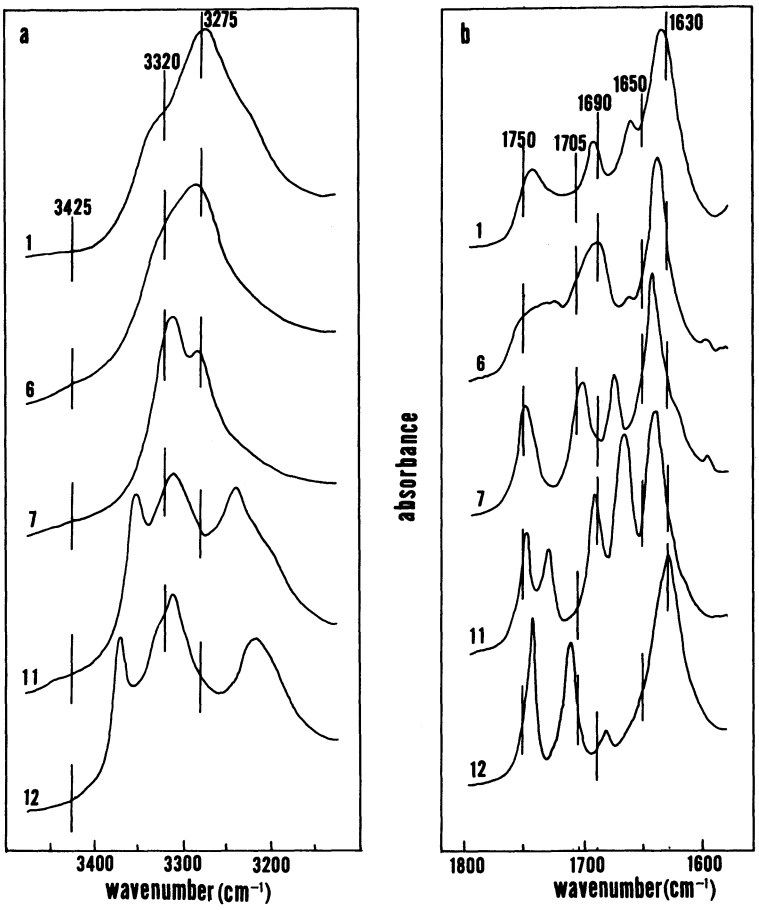


Fig. 2. IR absorption spectra of the tri- and tetrapeptides **1**, **6**, **7**, **11**, and **12** in the solid state. a: The amide A region; b: the amide I region.

obtained after the solid samples were pulverized with Nujol by weak shear stress. IR spectra (Figs. 5—7) of the peptides after application of shear stress were also obtained in a Nujol mull after the solid samples were pulverized without Nujol by strong shear stress.

Results

IR Absorption Spectra of the Tri- and Tetrapeptides 1, 6, 7, 11, and 12 in the Solid State. Figure 2 shows the IR absorption spectra of the peptides **1**, **6**, **7**, **11**, and **12** 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 few of strong bands at 3371—3215 cm^{-1} and a negligibly small band around 3425 cm^{-1} in the amide A region, suggesting that the Boc- and Z-urethane N-H bonds and peptide N-H bonds are mostly subjected to hydrogen bonding in the solid state. The spectra in the amide A region further indicate that hydrogen bonding patterns of the peptides are different from those of helical and β-sheet structures.[7-9,12,13] However, the spectra of the tetrapeptides **1** and **6** still show the strong bands at 3284—3271 and 1641—1635 cm^{-1}, indicative of a large contribution of β-sheet-like structure.[8,9,13] The assignment of each band in the amide I region is as follows: The bands around 1750 cm^{-1} are assigned to the Pac- and Bzl-ester carbonyl groups, the bands at 1736—1705 cm^{-1} to the Boc- and Z-urethane-carbonyl groups and the bands at 1695—1630 cm^{-1} mainly to hydrogen-bonded amide-carbonyl groups. The Pac ketone carbonyl group is presumed to show the band around 1685 cm^{-1}, overlapped with the amide carbonyl group.[5]

IR Absorption Spectra of the Peptides 2—5, 8—10, and 13—16 Equal to or Larger than a Pentapeptide in the Solid State. Figure 3 shows the IR absorption spectra of the pentapeptides **2** and **13** through the heptapeptides **4** and **8**. Figure 4 also presents those of the octapeptide **5** through the tetradecapeptide **10**. All of the peptides have the strong bands at 3280—3265 cm^{-1} in the amide A region, at 1635—1625 cm^{-1} in the amide I region and the weak band at 1699—1689 cm^{-1} in the amide I region, being indicative of typical antiparallel β-sheet structures, although most of them are accompanied by weak shoulder bands around 1650 cm^{-1}, assigned to other conformations. The shoulder bands around 3320

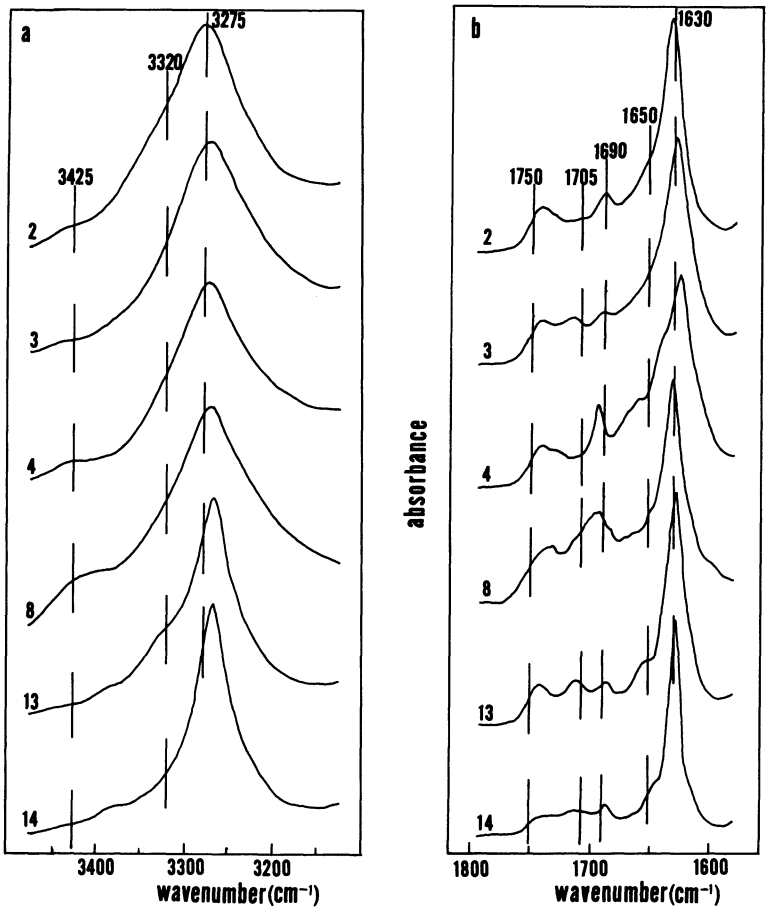


Fig. 3. IR absorption spectra of the pentapeptides **2** and **13** through the heptapeptides **4** and **8** in the solid state. a: The amide A region; b: the amide I region.

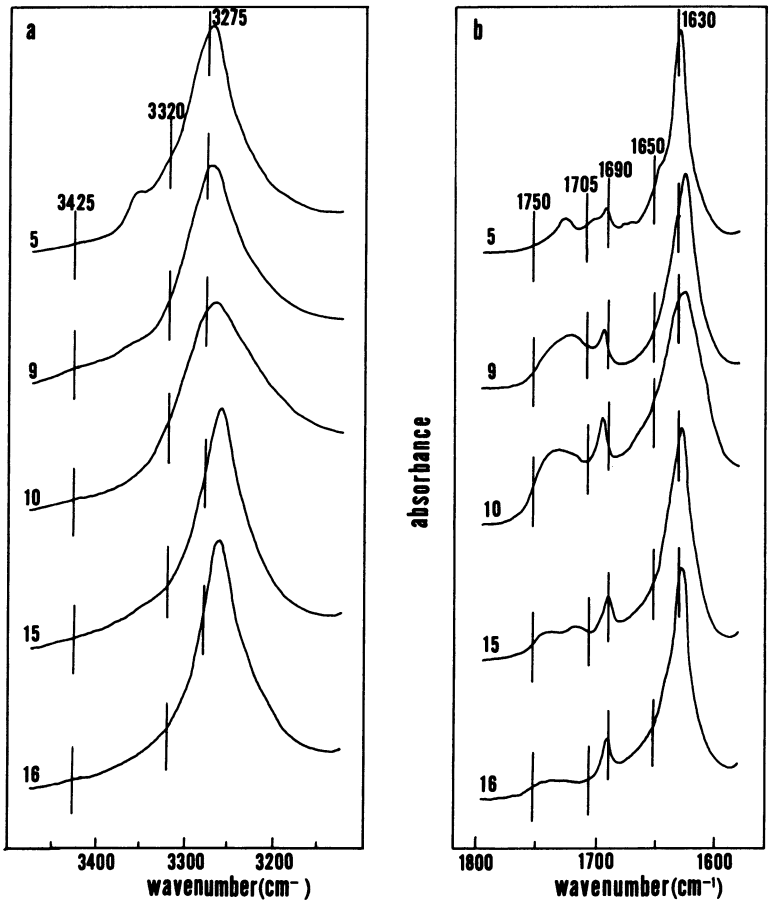


Fig. 4. IR absorption spectra of the octapeptide **5** through the tetradecapeptides **10** in the solid state. a: The amide A region; b: the amide I region.

cm^{-1} of peptides also indicate a small contribution of other conformations. The intensity of both shoulder bands increases with increasing shear stress when solid samples are pulverized with Nujol.

The Influence of Shear Stress on Peptide Conformations in the Solid State. It has been reported that β-sheet→α-helix conformational change occurs in the solid state on application of shear stress at the decapeptide level.[14] Figure 5 shows the IR absorption spectra of the tri- and tetrapeptides **1**, **6**, **7**, **11**, and **12** after application of strong shear stress and illustrates the influence of shear stress on peptide conformations in the solid state although it is difficult to estimate their specific conformational changes. For the peptides **1**—**16**, the influence of shear stress on their conformation is observed to a small or large extent. Conformational changes of the penta- and hexapeptides are relatively small although not negligible (not shown). Figures 6 and 7 exhibit the IR absorption spectra of the hepta- and octapeptides **4** and **5** and the peptides **8**—**10** after application of strong shear stress. The former spectra (Fig. 6) exhibit the influence of shear stress to a small extent, and the conformations of both peptides are essentially antiparallel β-sheet structures. On the other hand, the latter spectra (Fig. 7) are in contrast to the former. Especially, the conformational change of the heptapeptide **8** is quite drastic. The shoulder band at 3320 cm^{-1} in the amide A region and the band at 1657 cm^{-1} in the amide I region strongly suggest a β-sheet → α-helical transformation to a considerable extent. The onset of helical conformation, although to a small extent, is also evident in the undeca- and tetradecapeptides **9** and **10**.

Discussion

As pointed out, application of shear stress for solid peptides brings about a small or large extent of conformational transformation. IR spectra (Figs. 2—4) of the peptides **1**—**16** under a slight influence of shear stress are reproducibly obtained when solid samples are pulverized with Nujol by weak shear stress. Regardless of the amino acid composition, all of the peptides equal to or larger then a pentapeptide exhibit a high potential for the formation of β-sheet structures in the solid state. The conformational behavior of peptides having polar amino acid residues is essential-

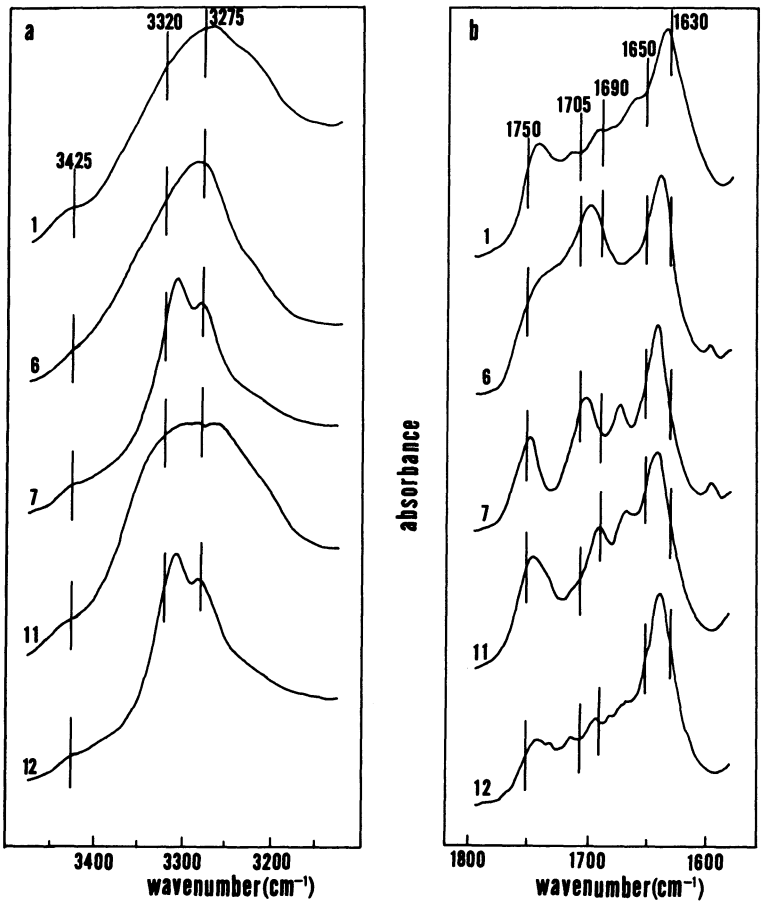


Fig. 5. IR absorption spectra of the tri- and tetrapeptides **1**, **6**, **7**, **11**, and **12** in the solid state after application of strong shear stress. a: The amide A region; b: the amide I region.

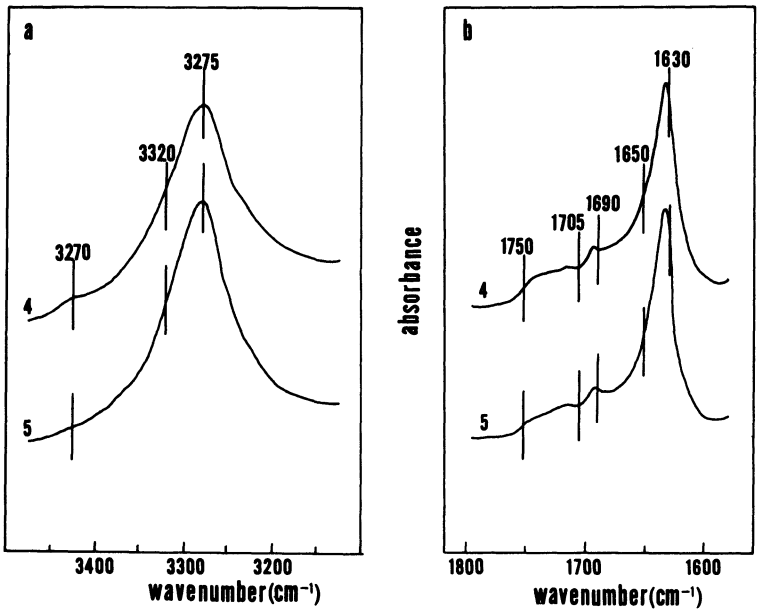


Fig. 6. IR absorption spectra of the hepta- and octapeptides **4** and **5** in the solid state after application of strong shear stress. a: The amide A region; b: the amide I region.

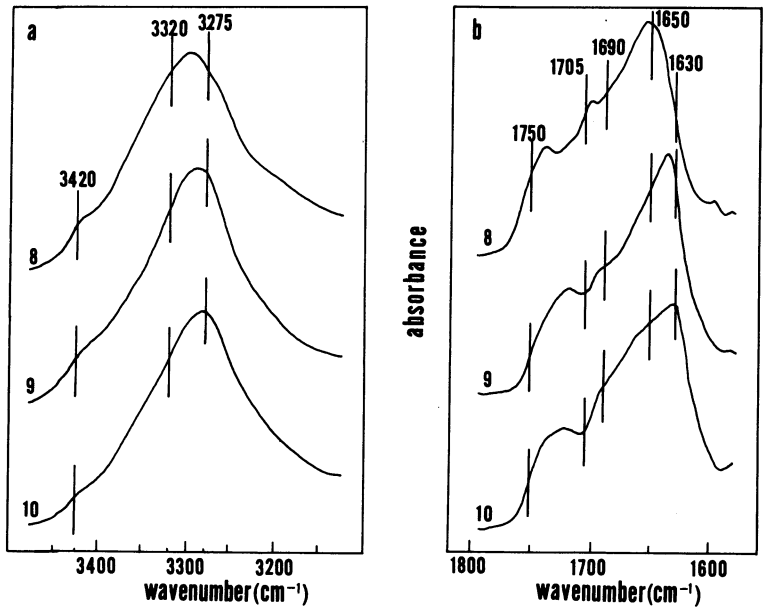


Fig. 7. IR absorption spectra of the peptides **8**—**10** in the solid state after application of strong shear stress. a: The amide A region; b: the amide I region.

ly similar to those of Boc-Leu$_n$-OBzl, indicating that polar side chains can not disturb the formation of β-sheet structures. Furthermore, as in the case of hydrophobic peptides and human proinsulin C-peptide fragments,[2-5] the peptides **5**, **9**, **10**, **15**, and **16** equal to or larger than an octapeptide are notably less soluble in high polar solvents.[2,6] These results strongly support our previous findings that the insolubility of peptide intermediates in highly polar solvents is principally caused by β-sheet aggregation formed by peptides equal to or larger than an octapeptide sequence level and that their insolubility is independent of their amino acid sequences and composition. However, apparently, this conclusion is not applicable to peptide intermediates including a Pro residue.[2,3,15]

In the Table 1, the average conformation $\langle P_c \rangle$, $\langle P_\alpha \rangle$, and $\langle P_\beta \rangle$ values of each peptide are summarized. The values of $\langle P_c \rangle$, $\langle P_\alpha \rangle$, and $\langle P_\beta \rangle$ were determined according to the literature.[3,16] The average coil conformation $\langle P_c \rangle$ values of the peptides **1**—**16** are below 0.9, suggseting that these peptides are highly structured. In fact, the peptides **2**—**5**, **8**—**10**, and **13**—**16** exhibit a high potential for the formation of β-sheet structures. This indicates that, even when the polar side chains of the Asp, Lys, Ser, and Thr residues are protected by suitable groups, the coil conformational parameters, P_c, obtained for intact amino acid residues[3] are useful for estimating the potential for the formation of a β-sheet structure of peptides smaller than critical size[14] for the development of an α-helical structure in the solid state. This result is also in good accord with our previous findings.[5] The fine relationship among the conformation, solubility,

Table 1. The Average Conformation $\langle P_c \rangle$, $\langle P_\alpha \rangle$, and $\langle P_\beta \rangle$ Values of Each Peptide

Compound	$\langle P_c \rangle$	$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$
1	0.92	1.02	1.20
2	0.86	1.05	1.29
3	0.94	1.00	1.19
4	0.90	1.07	1.16
5	0.88	1.10	1.17
6	0.89	1.13	1.01
7	0.90	1.19	0.97
8	0.90	1.16	0.99
9	0.90	1.09	1.11
10	0.90	1.11	1.08
11—16	0.68	1.34	1.22

and $\langle P_c \rangle$ value of the peptide fragments **1**—**10** of human hemoglobin α-chain (123-136) also strongly supports the correctness of our solubility prediction method for peptide intermediates.[3]

IR spectra (Figs. 5—7) of peptides after application of shear stress are also reproducibly obtained when solid samples are pulverized without Nujol by strong shear stress. The effect of shaer stress on the conformational behavior of the peptides **4**, **5**, and **8**—**10** may directly reflect their potential for the formation of an α-helical structure, and the tendency of the β-sheet $\rightarrow\alpha$-helix transformation is clearly estimated using the average helix conformation $\langle P_\alpha \rangle$ values of each peptide. In fact, the heptapeptide **8** has the high $\langle P_\alpha \rangle$ value, 1.16, and the most significant transformation is observed. The minimum size for the development of

an α-helical structure in the solid state has hitherto been recognized to be not less than the decapeptide level,[14] but this result suggests that a helical structure can be held at a heptapeptide size level in the solid state as well as in dichloromethane solution.[17] On the other hand, the average $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values of the heptapeptide **4** are 1.07 and 1.16, and those of the octapeptide **5** are 1.10 and 1.17, respectively. This suggests that the peptides **4** and **5** have a high potential for the formation of a β-sheet structure. In fact, no tendency for the β-sheet→α-helix transformation is observed in the solid state as well as in dichloromethane solution.[17] Therefore, the partial β-sheet→α-helix transformanion of the peptides **9** and **10** probably takes place at their N-terminal portions, while their C-terminal portions remain β-sheet structures.

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1) This paper forms Part VI of "Design of the Synthetic Route for Peptides and Proteins Based on the Solubility Prediction Method" series. For part V of this series, see M. Narita, M. Doi, K. Kudo, and Y. Terauchi, *Bull. Chem. Soc. Jpn.*, **59**, 3553 (1986). The abbreviations for amino acids are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). Amino acid symbols denote the L-configuration. Additional abbreviations used are the following: IR, infrared Bzl, benzyl; Z, benzyloxycarbonyl; Boc, *t*-butoxycarbonyl; Pac, phenacyl.

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