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Infrared Spectroscopic Conformational Analysis of Polystyrene Resin-Bound Human Proinsulin C-Peptide Fragments.[1] β-Sheet Aggregation of Peptide Chains during Solid-Phase Peptide Synthesis

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Conformational analysis of human proinsulin C-peptide fragments bound to cross-linked and soluble polystyrene resin matrices was performed using IR absorption spectroscopy. The conformational analysis of octa- and tridecapeptides in the swollen and solid states revealed that both peptides easily interacted with each other through intermolecular hydrogen bonding to form a β-sheet aggregation even at a low loading of peptide chains on the matrices. The high potential of the resin-bound peptides for the β-sheet formation was compatible with that of the corresponding peptides free from macromolecular protecting groups and was predicted using the average coil conformational value, $\langle P_c \rangle$, of each peptide. The significance of the results is discussed in connection with solid-phase peptide synthesis. Effects of shear stress on conformational transformations of resin-bound peptides was also examined in the solid state.

Peptide synthesis by fragment condensation on cross-linked and soluble polymer supports has been expected to be one of the most promising methods for syntheses of large peptides and proteins.[2,3] However, a serious problem in the method is the low yields in some fragment condensation reactions on resin supports.[4] In previous papers,[5-7] we demonstrated that decrease in coupling yields occurred on a copoly(styrene-2% divinylbenzene) support due to the restricted permeability of carboxyl-component peptides into resin matrices. We also verified that the permeability was further restricted by additional cross-linking caused by a β-sheet formation of amino-component peptides on resin matrices. Thus, the conformational analysis of cross-linked polystyrene resin-bound oligopeptides is of utmost importance in connection with solid-phase peptide synthesis. However, very few investigations have been reported of the conformational analysis of cross-linked polystyrene resin-bound oligopeptides.[8-10] In order to elucidate the hydrogen-bonding behavior of resin-bound oligopeptides, we studied the conformational properties of cross-linked polystyrene resin-bound oligo(Leu)s and showed that the peptide chains had various conformations depending on the peptide chain length.[8,9]

In this paper, we report IR absorption spectroscopic analysis of cross-linked and soluble polystyrene resin-bound human proinsulin C-peptide fragments and demonstrate that a β-sheet aggregation through intermolecular hydrogen bonds is commonly achieved in solid-phase peptide synthesis. The resin-bound oligopeptides used in this study are illustrated in Fig. 1. Cross-linked and soluble resin-bound peptides **1a** and **1b**, **2a** and **2b**, and **3a** and **3b** correspond to human proinsulin C-peptide (9-13), (6-13), and (1-13), respectively. The numbers in parentheses in the following C-peptide represent the positions of the first and last amino acids from the N-terminal in human proinsulin C-peptide.[11]

Experimental

Materials. Copoly(styrene-1% divinylbenzene) beads of 200—400 mesh, Bio-Beads S-X1, were purchased from Bio-

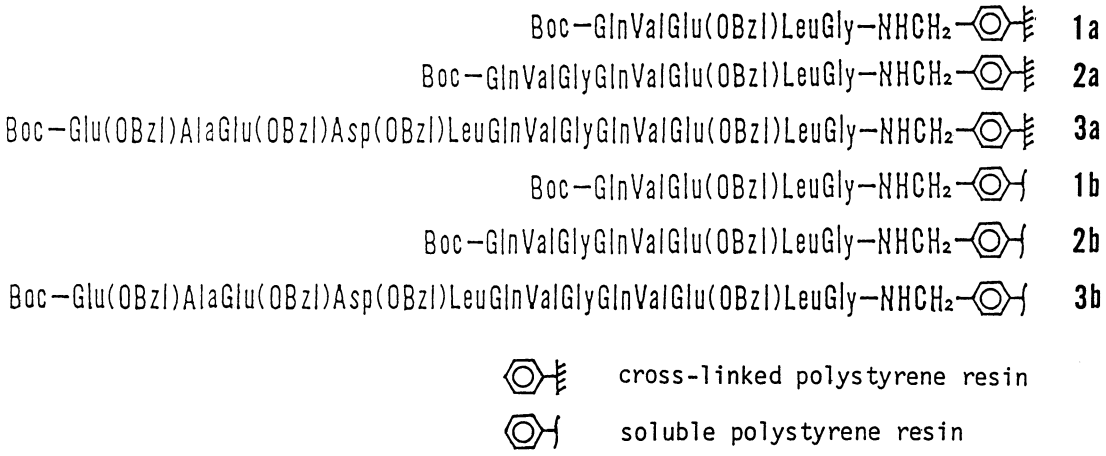

Fig. 1. The cross-linked and soluble polystyrene resin-bound peptides **1a—3a** and **1b—3b**.

Rad Laboratories. The aminomethylation was performed according to the method described previously.[12] Soluble aminomethylated polystyrene was prepared by the copolymerization of styrene and a mixture of *m*- and *p*-(phthalimidomethyl)styrene, followed by hydrazinolysis.[13] Human proinsulin C-peptide fragments, Boc-Gln-Val-Glu(OBzl)-Leu-Gly-OH, Boc-Gln-Val-Gly-OH, and Boc-Glu(OBzl)-Ala-Glu(OBzl)-Asp(OBzl)-Leu-OH, were those prepared before.[14] These peptide fragments were successively coupled with aminomethylated polystyrene resins in a mixture of dichloromethane and *N*-methylpyrrolidone (1/1, v/v) using DCC as a coupling reagent in the presence of HOBt.[2,8] The reaction using DCC and HOBt as coupling reagents is well-known to be nearly free from racemization.[15] Amino acid analyses of acid hydrolysates of the resin-bound peptides showed that the mole ratios of the component amino acid are close to the values calculated (Table 1).

IR Measurements. The IR absorption spectra of resin-bound peptides were recorded at room temperature with a JEOL Model JIR-100 FT-IR spectrometer. IR measurements in the swollen state were performed by holding the samples between potassium bromide windows after resin-bound peptides were swollen in each solvent overnight. IR measurements in solution were recorded by employing 0.1 mm path length cells with potassium bromide windows. The concentration of the solution was kept near 50 mg ml^{-1}. Only the soluble resin-bound peptide **1b** was soluble in dichloromethane and tetrahydrofuran, and the soluble resin-bound peptides **2b** and **3b** were insoluble in both solvents. The peptides **1b—3b** were insoluble in carbon tetrachloride, and the IR absorption spectra of these insoluble peptides were obtained in the swollen state. IR measurements in the solid state for the soluble resin-bound peptides **1b—3b** were carried out on films cast from their dichloromethane solution or gel in dichloromethane. IR absorption spectra without influence of shear stress (Fig. 6) were obtained using these samples. IR absorption spectra of the cross-linked resin-bound peptides **1a—3a** in the solid state under slight shear stress (Fig. 6) were obtained in a Nujol mull after the solid samples were weakly pulverized with Nujol by hand using an agate mortar.[8,16] The IR absorption spectra of the peptides **1a—3a** and **1b—3b** after application of shear stress (Fig. 7) were also obtained in a Nujol mull after the solid samples were strongly pulverized without Nujol by hand using an agate mortar.[8,16]

Amino Acid Analyses. The amino acid composition of acid hydrolysate was determined using Shimadzu HPLC LC-3A all amino acid analysis system. The conditions of acid hydrolyses of resin-bound peptides were the same to those described before.[14]

Results and Discussion

Oligopeptides Bound to Cross-Linked and Soluble Polystyrene-Resin Matrices. The copoly(styrene-1% divinylbenzene) resin support (Bio-beads S-X1), used as a starting resin bead, has commonly been used for solid-phase peptide synthesis, and the aminomethylated form is expected to have a uniform distribution of aminomethyl groups, based on the reaction mechanism of aminomethylation.[12] Soluble aminomethylated polystyrene was prepared by the copolymerization of styrene and a mixture of *m*- and *p*-(phthalimidomethyl)styrene, followed by hydrazinolysis.[13] The aminomethyl groups are also thought to be randomly distributed on the basis of the copolymerization curve. The first peptide, Boc-Gln-Val-Glu(OBzl)-Leu-Gly-OH, was directly coupled with aminomethyl groups using DCC and HOBt as coupling reagents. The peptide contents were 106 μmol g^{-1} of resin (ca. 1.1 mol-% per styrene unit) for the cross-linked resin-bound peptide **1a** and 104 μmol g^{-1} of resin for the soluble resin-bound peptide **1b**, respectively. These initial peptide contents were selected in order to get rational information for peptide chain interactions in the conventional solid-phase method.

Conformational Analysis of the Cross-Linked and Soluble Resin-Bound Peptides Swollen or Soluble in Dichloromethane. The IR absorption spectra of the cross-linked resin-bound peptides **1a—3a** swollen in dichloromethane are presented in Fig. 2 in the most significant spectral regions for conformational assignments (3500—3100 cm^{-1}, amide A; 1800—1600 cm^{-1}, amide I). Figure 3 illustrates those of the soluble resin-bound peptides **1b—3b** soluble or swollen in dichloromethane. The peptide **1b** dissolved in dichloromethane, while the peptides **2b** and **3b** were in a gel. Except for the soluble resin-bound peptide **1b**, the resin-bound peptides **1a—3a**, **2b**, and **3b** show strong bands around 3280 cm^{-1} in the amide A region and 1630 cm^{-1} in the amide I region, assigned to a β-sheet structure.[8,9,17-19] The results of the cross-linked resin-bound peptides **1a—3a** indicate that cross-linked resin-bound peptides swollen in dichloromethane, a most common solvent in conventional solid-phase peptide synthesis, can easily interact with each other through

Table 1. Amino Acid Analyses of the Resin-Bound Peptides **1a—3a** and **1b—3b**[a]

Resin-bound peptide	Asp	Glu(Gln)[b]	Gly	Ala	Val	Leu
1a	—	1.79 (2)	1.08 (1)	—	0.98 (1)	1.00 (1)
2a	—	2.96 (3)	1.90 (2)	—	2.03 (2)	1.00 (1)
3a	0.97 (1)	4.84 (5)	2.12 (2)	0.92 (1)	2.28 (2)	2.00 (2)
1b	—	1.88 (2)	1.08 (1)	—	1.00 (1)	1.06 (1)
2b	—	2.75 (3)	1.86 (2)	—	2.00 (2)	1.15 (1)
3b	0.93 (1)	4.74 (5)	2.06 (2)	0.90 (1)	2.17 (2)	2.00 (2)

a) The values in parentheses are the calculated ones. b) The values are the total ones of Glu and Gln residues.

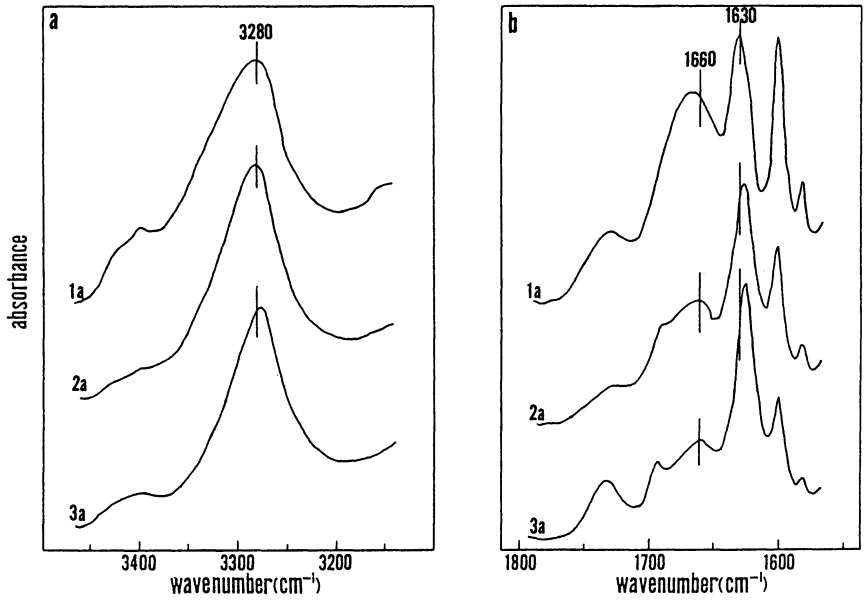

Fig. 2. IR absorption spectra in the amide A (a) and amide I (b) regions of the cross-linked resin-bound peptides **1a—3a** swollen in dichloromethane. The absorption band at 1603 cm^{-1} is due to aromatic rings of the polystyrene supports.

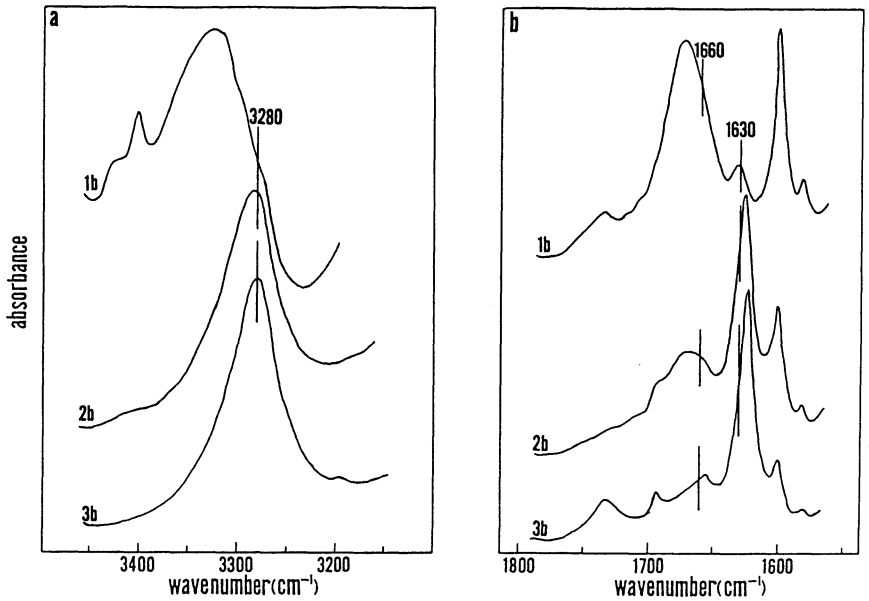

Fig. 3. IR absorption spectra in the amide A (a) and amide I (b) regions of the soluble resin-bound peptides **1b—3b** soluble or swollen in dichloromethane. For assignment of the absorption band at 1603 cm^{-1}, see Fig. 2.

peptide main chains to form intermolecular hydrogen bonds even at a low loading of peptide chains on the matrices. Especially, the result of the peptide **1a** clearly shows that the intraresin site separation of the peptide chains is not achieved even at a pentapeptide level. The conformational behavior of the soluble resin-bound peptides **2b** and **3b** is essentially the same as that of the cross-linked resin-bound peptides **2a** and **3a**. On the other hand, the soluble resin-bound peptide **1b** shows a weak band at 1630 cm^{-1} and a strong band at 1675 cm^{-1}in the amide I region, indicating that the resin-bound peptide **1b** is nearly free from intermolecular hydrogen bonds. As pointed out before,[8,9] the high potential for the β-sheet formation of the cross-linked resin-bound peptide **1a** swollen in dichloromethane is attributed to cross-linking by divinylbenzene, that is, the cross-linking has a concentrating effect. The conformational behavior of the cross-

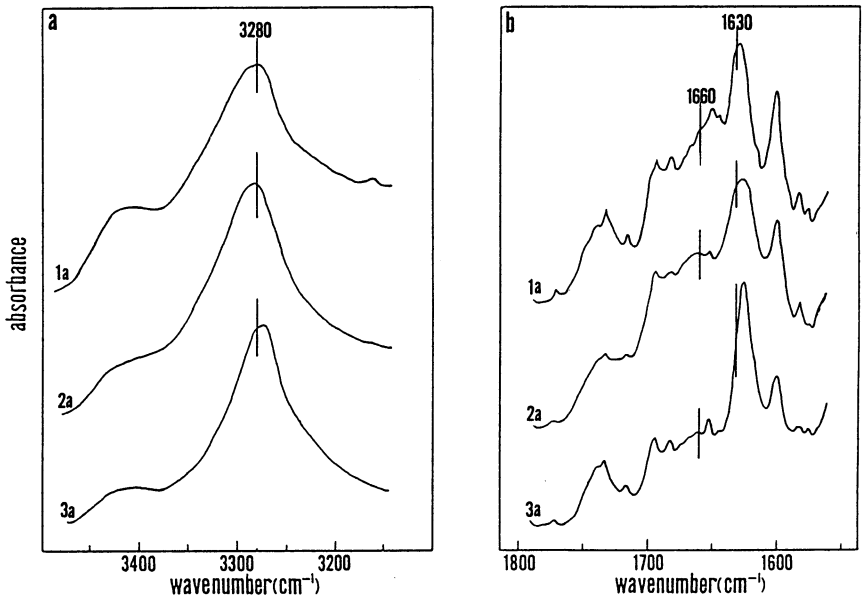

Fig. 4. IR absorption spectra in the amide A (a) and amide I (b) regions of the cross-linked resin-bound peptides **1a—3a** swollen in carbon tetrachloride. For assignment of the absorption band at 1603 cm^{-1}, see Fig. 2.

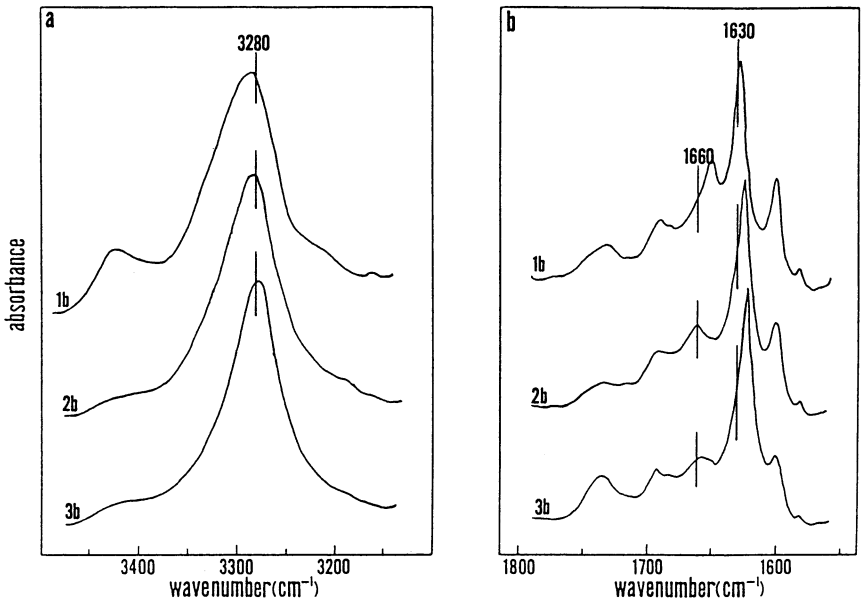

Fig. 5. IR absorption spectra in the amide A (a) and amide I (b) regions of the soluble resin-bound peptides **1b—3b** swollen in carbon tetrachloride. For assignment of the absorption band at 1603 cm^{-1}, see Fig. 2.

linked and soluble resin-bound peptides **1a—3a** and **1b—3b** in tetrahydrofuran (not shown) is essentially the same as that in dichloromethane, and the β-sheet aggregation in tetrahydrofuran is also easily achieved at a pentapeptide level in a cross-linked resin-bound peptide. Although it had been assumed that the cross-linked resin matrix isolated the growing peptide chains at a low loading, these results show that site-site reactions between pendant peptide chains do occur even at a pentapeptide level in solid-phase peptide synthesis.

The results obtained for the human proinsulin C-peptide fragments bound to resin matrices are essentially the same as those obtained for resin-bound oligo (Leu)s[9] and the corresponding human proinsulin C-peptide fragments free from macromolecular protecting groups,[18] indicating that polar side chains can not disturb the formation of the β-sheet structure in solid-

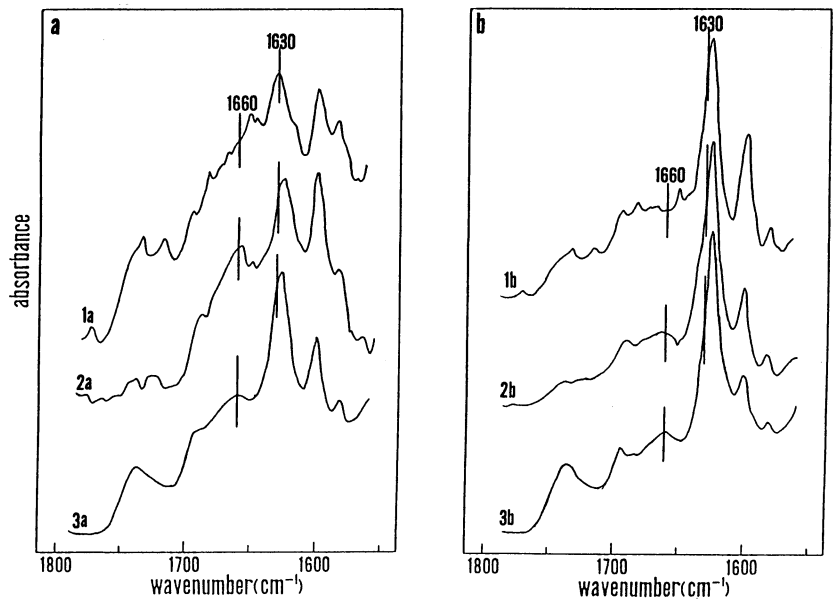

Fig. 6. IR absorption spectra in the amide I regions of the cross-linked resin-bound peptides **1a—3a** (a) and the soluble resin-bound peptides **1b—3b** (b) in the solid state. For assignment of the absorption band at 1603 cm^{-1}, see Fig. 2.

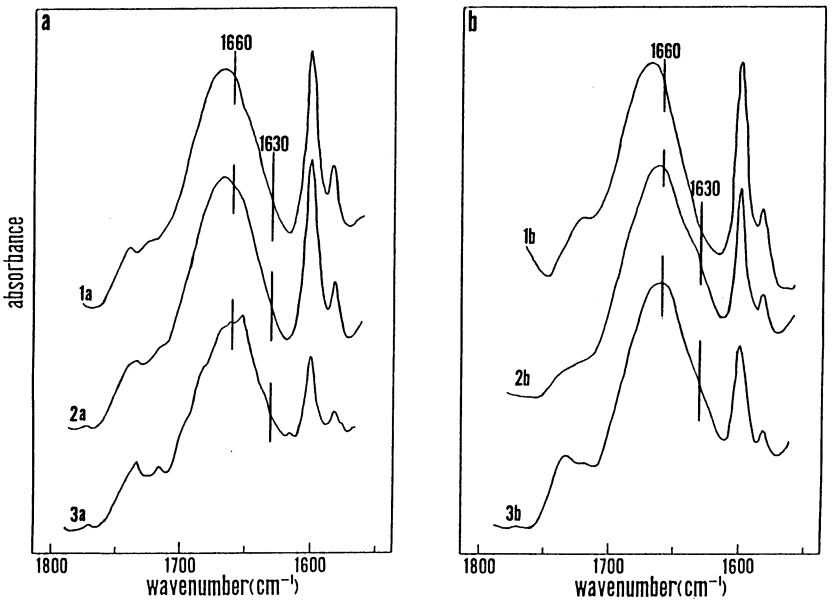

Fig. 7. IR absorption spectra in the amide I regions of the cross-linked resin-bound peptides **1a—3a** (a) and the soluble resin-bound peptides **1b—3b** (b) in the solid state after application of strong shear stress. For assignment of the absorption band at 1603 cm^{-1}, see Fig. 2.

phase peptide synthesis. Since the potential for a β-sheet formation of peptide fragments has successfully been predicted using an average coil conformational value, $\langle P_c \rangle$, calculated from the coil conformation parameter, P_c, for each amino acid,[18,20,21] the potential for a β-sheet formation of resin-bound peptides can be predicted by this method.

Conformational Analysis of the Cross-Linked and Soluble Resin-Bound Peptides Swollen or Soluble in Carbon Tetrachloride. Figures 4 and 5 illustrate the IR absorption spectra in the amide A and amide I regions of the peptide resins **1a—3a** and **1b—3b**, respectively. All of the peptides show strong bands around 3280 and 1630 cm^{-1}, assigned to a β-sheet structure.[8,9,16-18] The conformational behavior of the soluble resin-bound pentapeptide **1b** swollen in carbon tetrachloride is different from that in dichloromethane and tetrahydrofuran. It is attributed to the high poten-

tial of carbon tetrachloride for promoting a β-sheet formation of peptides. The β-sheet formation of the resin-bound peptides **1a—3a** and **1b—3b** in carbon tetrachloride indicates that the peptide phase aggregated through intermolecular hydrogen bonding behaves like a suspended crystalline, while the polystyrene part swells well in carbon tetrachloride. The conformational behavior of the resin-bound peptides **1a—3a**, **2b**, and **3b** in dichloromethane and tetrahydrofuran also indicates that a microphase separation occurs in the solvents. These results indicate that the insolubility of the peptide part in these solvents, causing the restricted permeability of carboxyl components and incomplete coupling reactions, becomes a serious problem in solid-phase peptide syntheses as well as in the classical solution method, although the insolubility of the peptide phase has been out of consideration in solid-phase peptide synthesis.

Conformational Analysis of Cross-Linked and Soluble Resin-Bound Oligopeptides in the Solid State. Figure 6 shows the IR absorption spectra of the peptide resins **1a—3a** and **1b—3b** in the amide I region. The spectra of the soluble resin-bound peptides **1b—3b** show a strong band at 1630 cm^{-1} characteristic of a β-sheet structure.[8,9,17–19] They are accompanied by weak shoulder bands around 1660 cm^{-1}, assigned to other conformations or amide carbonyl groups of Gln residues: those of the cross-linked resin-bound peptides **1a—3a** are accompanied by stronger shoulder bands around 1660 cm^{-1}. The latter spectra are resemble those of the corresponding oligopeptides free from macromolecular protecting groups.[17] The difference in the conformational behavior between the peptides **1a—3a** and **1b—3b** may be caused by added shear-stress in preparing IR samples. In fact, the β-sheet structures of the cross-linked and soluble resin-bound peptides **1a—3a** and **1b—3b** are easily disrupted by application of strong shear stress as shown in Fig. 7. The easy disruption of the β-sheet structure for the resin-bound peptides **1a—3a** and **1b—3b** reflects the potential of the peptide parts for the formation of an α-helical structure.[16]

The conformational behavior of the peptide resins **1a—3a** and **1b—3b** in the solid state resembles that of resin-bound oligo (Leu)s.[8] The easy formation of the β-sheet structure for the resin-bound peptides **1a—3a** and **1b—3b** in the solid state also indicates that resin-bound peptide chains having polar side chains can easily interact with each other through peptide main chains to form intermolecular hydrogen bonds even in polymer networks as well as in resin-bound hydrophobic peptide chains.

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1) This paper forms Part III of "Syntheses and Properties of Resin-Bound Oligopeptides" series. For part II of this series, see M. Narita, Y. Tomotake, S. Isokawa, T. Matsuzawa, and T. Miyauchi, *Macromolecules*, **17**, 1903 (1984). 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: OBzl, benzyl ester; Boc, *t*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxy-1*H*-benzotriazole.

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