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The Easy Disruption of the β-Sheet Structure of Resin-Bound Human Proinsulin C-Peptide Fragments by Strong Electron-Donor Solvents[1)]

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The β-sheet structure disruption of cross-linked polystyrene resin-bound human proinsulin C-peptide fragments containing Pro residues at suitable intervals was investigated by a solvent titration method in CH_2Cl_2, mainly using TFE and HMPA as titrating solvents. The easy disruption of the β-sheet structure of resin-bound peptides in CH_2Cl_2 by TFE and HMPA strongly suggested that the concept of "the peptide segment separation by tertiary peptide bonds" was useful for estimating the β-sheet-structure-disrupted behavior and that the β-sheet structure of the protected peptides having high $<P_c>$ values could easily be disrupted in a medium electron-acceptor solvent of CH_2Cl_2 by the addition of a strong electron-donor solvent of HMPA in spite of the electron donor-acceptor interaction between solvents. The present results indicate that effective solvents for solid-phase peptide synthesis by a fragment condensation procedure should be searched by taking the average helix, β-sheet, and coil conformation values, $<P_\alpha>$, $<P_\beta>$, and $<P_c>$, of peptide segments into consideration.

The β-sheet aggregation of protected peptides bound to a cross-linked polystyrene resin in the swollen state is one of the serious obstacles in solid-phase peptide synthesis because it reduces the efficient coupling reactions of the carboxyl component peptides with the pendant amino component peptides due to the restriction of the permeability of carboxyl components into pendant peptides.[2,3)] Thus, an investigation into the β-sheet structure disruption of resin-bound peptides is indispensable to achieve efficient coupling reactions. In previous studies[4,5)] we demonstrated that the β-sheet structure of resin-bound peptides as large as or larger than an octapeptide was disrupted in CH_2Cl_2 only by the addition of a strong electron-acceptor solvent of HFIP and that the disruption was strongly dependent on the electron-donating and -accepting properties[6,7)] of added solvents, peptide chain length, and average helix, β-sheet, and coil conformation values, $<P_\alpha>$, $<P_\beta>$, and $<P_c>$, of peptides. These values are determined on the basis of the three-dimensional structures of native proteins which were resolved by X-ray crystallography;[8,9)] they should be corrected for an estimation of the conformational behavior of protected peptides in organic solvents. Nevertheless, they were useful for the purpose of the estimation of the conformation.[4)] Practically, the β-sheet structure disruption of peptides having a $<P_c>$ value larger than 0.85 and/or a high potential for the β-sheet → helix transformation proceeded smoothly upon adding increasing amounts of HFIP; that of peptides having a low potential for the randomness as well as for the β-sheet → helix transformation did not.

Moreover, we found that the β-sheet structure disruption of cross-linked polystyrene resin-bound peptides was more difficult than that of peptides to be free from a macromolecular protecting group due to the concentrating efficacy of a cross-linked resin.[5)] As a result, the β-sheet structure of resin-bound peptides, which were as large as or larger than an octapeptide and had a $<P_c>$ value lower than 0.90, could not be disrupted by strong electron-acceptor solvents except for HFIP and by strong electron-donor solvents. This result was quite different from that of the corresponding peptides, which were free from a macromolecular protecting group.[7)]

Recently, we also found that the β-sheet structure of resin-bound human proinsulin C-peptide fragments which contained Pro residues at suitable intervals was easily disrupted by the addition of only a slight amount of HFIP, resulting in efficient coupling reactions in solid-phase peptide synthesis by a fragment condensation procedure.[10)] Thus, the investigation into solvents having a high potential for the β-sheet structure disruption of resin-bound peptides is of practical significance for solid-phase peptide synthe-

Boc-S(Bzl)LQPLALE(OBzl)GS(Bzl)LQ-OCH$_2$-Pam-resin 1

Boc-GGPGAGS(Bzl)LQPLALE(OBzl)GS(Bzl)LQ-OCH$_2$-Pam-resin 2

Boc-QVE(OBzl)LGGGPGAGS(Bzl)LQPLALE(OBzl)GS(Bzl)LQ-OCH$_2$-Pam-resin 3

Boc-QVGQVE(OBzl)LGGGPGAGS(Bzl)LQPLALE(OBzl)GS(Bzl)LQ-OCH$_2$-Pam-resin 4

Boc-E(OBzl)AE(OBzl)D(OBzl)LQVGQVE(OBzl)LGGGPGAGS(Bzl)LQPLALE(OBzl)GS(Bzl)LQ-OCH$_2$-Pam-resin 5

Fig. 1. Resin-bound peptides **1**—**5** used in this study.

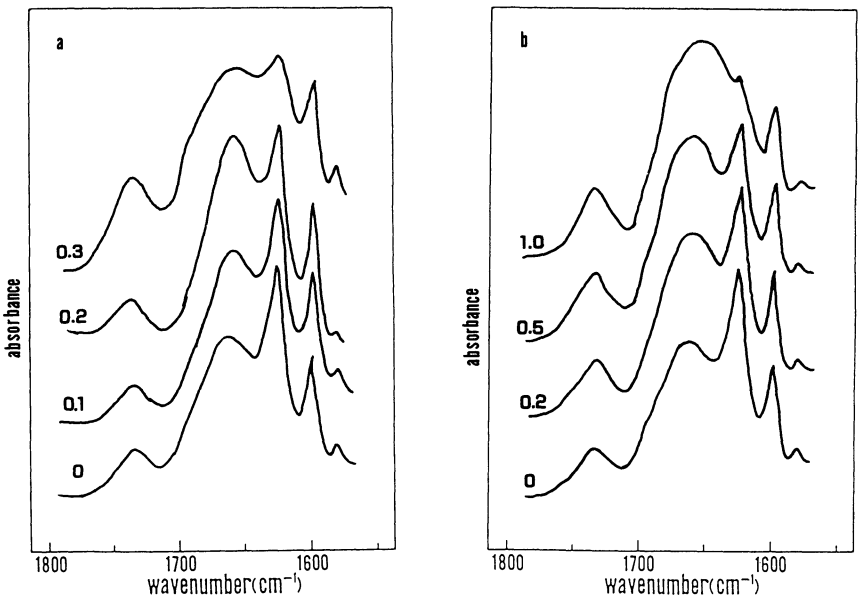

Fig. 2. Tpical IR absorption spectra in the amide I region of resin-bound peptide **4** swollen (a) in a mixture of TFE and CH_2Cl_2 and (b) in a mixture of HMPA and CH_2Cl_2. The numerals in Fig.2 indicate molar concentrations of TFE and HMPA (M).

sis. In the present paper, we report that the β-sheet structure of resin-bound human proinsulin C-peptide segments having $<P_c>$ values larger than 0.98 is marvelously disrupted in CH_2Cl_2, even by the addition of strong electron-donor solvents such as HMPA and DMSO. We also show that the concept of "the peptide segment separation by tertiary peptide bonds"[11] is useful for estimating the β-sheet-structure-disrupted behavior. Resin-bound peptides **1**—**5** used in this study are shown in Fig. 1, where amino acid residues are represented by one-letter symbols.[12]

Experimental

Resin-bound peptides **1**—**5** were prepared in a previous study.[10] The IR absorption spectra of resin-bound peptides **1**—**5** 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 CH_2Cl_2 containing TFE, HMPA, DMSO, or MeOH at various concentrations.

Results

The β-sheet structure disruption of cross-linked polystyrene resin-bound peptides **1**—**5** in CH_2Cl_2 was investigated by a solvent-titration method.[13] It was monitored by following a successive decrease in the intensity of the amide I band around 1630 cm^{-1} due to the β-sheet structure together with the successive addition of a titrating solvent. As a representative example, the IR absorption spectra of the resin-bound peptide **4** in CH_2Cl_2 containing TFE or HMPA over a wide range of concentration are presented in Fig. 2. Figures 3 and 4 illustrate the solvent-titration curves of resin-bound peptides **1**—**5** using TFE and HMPA as titrating solvents, respectively. The relative intensities in Figs. 3 and 4 as well as Fig. 5 were obtained using the band at 1603 cm^{-1} due to the aromatic ring of the resin support as a standard and were normalized to 1.0 for each relative intensity in the absence of a titrating solvent. The results indicate that the β-sheet structure of resin-bound peptides **1**—**5** swollen in CH_2Cl_2 is easily disrupted by adding increasing amounts of TFE and HMPA and that the β-sheet structure disruption

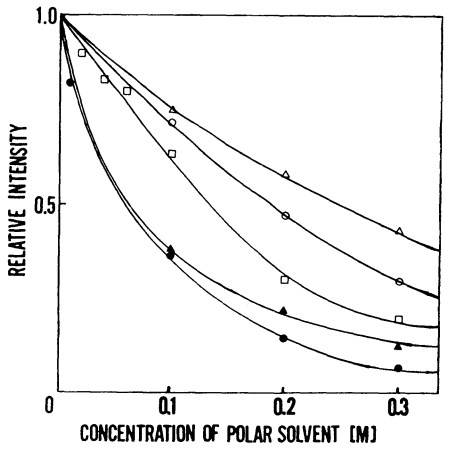

Fig. 3. The solvent-titration curves of resin-bound peptides **1**—**5** in CH_2Cl_2 using TFE as a titrating solvent. Peptide **1**, —▲—; peptide **2**, —●—; peptide **3**, —□—; peptide **4**, —△—; and peptide **5**, —○—.

of peptide **4** is the most difficult in peptides **1**—**5**. In order to elucidate the relationship between the β-sheet structure disruption of peptide **4** and electron-donating and -accepting properties of titrating solvents, the solvent-titration curves of peptide **4** in a medium electron-acceptor solvent of CH_2Cl_2 using strong electron-acceptor solvents of HFIP, TFE, and MeOH and strong electron-donor solvents of HMPA and DMSO as titrating solvents (Fig. 5) were obtained. The solvent-titration curve of peptide **4** in a medium electron-donor solvent of THF using HMPA as a titrating solvent is also shown in Fig. 5.

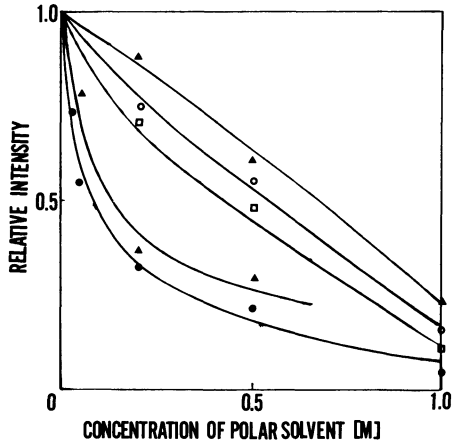

Fig. 4. The solvent-titration curves of resin-bound peptides **1**—**5** in CH_2Cl_2 using HMPA as a titrating solvent. Peptide **1**, —▲—; peptide **2**, —●—; peptide **3**, —□—; peptide **4**, —△—; and peptide **5**, —○—.

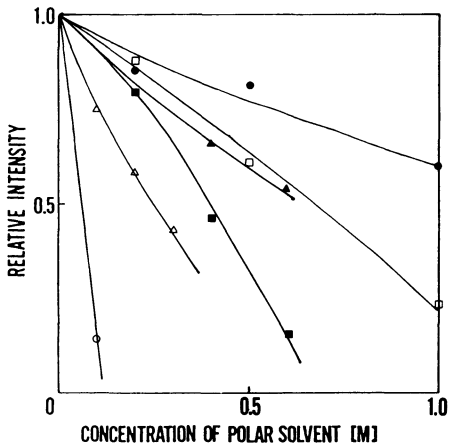

Fig. 5. The solvent-titration curves of resin-bound peptides **4** in CH_2Cl_2 using HFIP, TFE, MeOH, HMPA, and DMSO as titrating solvent. HFIP, —○—; TFE, —△—; MeOH, —▲—; HMPA, —□—; and DMSO, —●—. The solvent-titration curve of peptide **4** in THF using HMPA as a titrating solvent, —■—.

Discussion

Resin-bound peptides **1**—**5** contain Pro residues at suitable intervals, and Pro residues in peptides generally play a role in stopping the development of helix and β-sheet structures. Thus, the structures of resin-bound peptides **1**—**5** could be regarded as being assembled with peptide segment structures separated by Pro residues;[10] we called the role "peptide segment separation by tertiary peptide bonds."[11] In the swollen state in CH_2Cl_2, in fact, most parts of segment 1 (PLALEGSLQ) and a part of segment 2 (PGAGSLQ), namely, Ser(Bzl)-Leu-Gln, were estimated to form a relatively stable helix and N-terminal segment 3 appeared to form a relatively stable β-sheet structure, although the conformation of segment 2 was not clear.[10] Namely, the β-sheet structure of resin-bound peptides **1**—**5** can be regarded as being constructed by peptide segments having short peptide chain lengths. Thus, the β-sheet structure of resin-bound peptides **1**—**5** swollen in CH_2Cl_2 was expected to be disrupted by the addition of a variety of titrating solvents. Practically, it was easily disrupted by a strong electron-acceptor solvent of TFE and a strong electron-donor solvent of HMPA (Figs. 3 and 4), being in marked contrast with that of resin-bound peptides reported previously.[4,5] Especially, the disruption is clearly independent of the chain length of whole peptides, indicating that, for the evaluation of the β-sheet structure disruption of resin-bound peptides containing Pro residues, we should take account of the chain length of peptide segments instead of whole peptides. This is in harmony with the concept that "peptide segment separation by tertiary peptide bonds" is effective for estimating the conformational development of protected peptides in organic solvents.[10]

Resin-bound dodecapeptide **1** has a relatively stable helix in CH_2Cl_2 and is accompanied by a small amount of a β-sheet structure,[10] which is easily disrupted by TFE and HMPA. The disrupted behavior can be regarded as corresponding with that of segment 1. Resin-bound octadecapeptide **2** is assem-

Table 1. The Average Conformational Values, $<P_\alpha>$, $<P_\beta>$, and $<P_c>$ of Peptide Segments

Peptide segment[a]	Number of segment	$<P_\alpha>$	$<P_\beta>$	$<P_c>$
PLALEGSLQ	1	1.06	0.92	0.82
PGAGSLQ	2	0.89	0.86	1.16
QVELGGG	3	0.94	0.96	1.07
QVGQVELGGG	3	0.93	1.03	1.05
EAEDLQVGQVELGGG	3	1.07	0.91	0.98

a) Amino acid residues are represented by one-letter symbols.[12]

bled with segments 1 and 2 and its β-sheet structure is also easily disrupted by TFE and HMPA, since segment 2 has a high $<P_c>$ value (1.16). Especially, the easy disruption of resin-bound peptides **3—5** in CH_2Cl_2 by TFE and HMPA is marvelous and appears to be attributed to the high $<P_c>$ values of segments 3, suggesting that, for the evaluation of the β-sheet structure disruption of resin-bound peptides containing Pro residues, it is pertinent to use the $<P_\alpha>$, $<P_\beta>$, and $<P_c>$ values of peptide segments instead of whole peptides. The $<P_\alpha>$, $<P_\beta>$, and $<P_c>$ values of peptide segments are determined by their amino acid compositions,[8,9] and are summarized in Table 1. The easiness of the β-sheet structure disruption of peptides **3—5** by TFE and HMPA is in the following order: **3**>**5**>**4**. The most easy disruption of the β-sheet sturcture of peptide **3** is explained by both the short chain length and high $<P_c>$ value (1.07) of its segment 3. Segment 3 of peptide **5** also has a high $<P_c>$ value (0.98), and its chain length is close to the critical size for a stable helix formation of cross-linked polystyrene resin-bound peptides in CH_2Cl_2.[14] Furthermore, its $<P_\alpha>$ value (1.07) is larger than its $<P_\beta>$ value (0.91). The easy disruption of the β-sheet structure of segment 3 of peptide **5** is in good agreement with the observation that the β-sheet structure disruption of peptides having a $<P_c>$ value larger than 0.85 and having a high potential for the β-sheet → helix transformation proceeded smoothly upon adding increasing amounts of HFIP.[4] In addition, the easy disruption of the β-sheet structure of peptide **4** in CH_2Cl_2 by TFE, MeOH, HMPA, and DMSO (Fig. 5) clearly indicates that the consideration of $<P_c>$ values of peptide segments is important for a search for effective solvents to be used in solid-phase peptide synthesis.

In a study of the β-sheet structure-disruption of Boc-Val-Gly-Phe-Gly-Leu-Ile-Leu-Leu-Ala-NH-resin in a mixture of a strong electron-donor solvent of HMPA and a medium electron-acceptor solvent of CH_2Cl_2,[5] the electron donor-acceptor interaction between solvents occurred predominantly and the β-sheet structure disruption did not proceed. Thus, the result that the β-sheet structure of resin-bound peptide **4** in CH_2Cl_2 is disrupted by the addition of HMPA and DMSO strongly suggests that the β-sheet structure of resin-bound peptides having high $<P_c>$ values could be disrupted in CH_2Cl_2 even by a strong electron-donor solvent in spite of the electron donor-acceptor interaction between solvents. Contrary to CH_2Cl_2, THF is a medium electron-donor solvent and a weak electron-acceptor solvent.[6] Thus, the electron donor-acceptor interaction between HMPA and THF becomes negligible, and HMPA in THF shows a high potential for the β-sheet structure disruption of resin-bound peptide **4** (Fig. 5). As reported previously,[7] the potential of hydrogen-donor and -acceptor solvents for the β-sheet structure disruption of resin-bound peptide **4** was in the good relationship with their electron-acceptor and -donor numbers (AN and DN), respectively (Fig. 5). The AN values of HFIP, TFE, and MeOH are 88, 59, and 41.3, and the DN values of HMPA and DMSO, 38.8 and 29.8, respectively.[6,7] As AN and DN of titrating solvents are larger, their β-sheet-structure-disrupting potential becomes higher.

In conclusion, the β-sheet-structure-disrupted behavior of resin-bound peptides **1—5** suggests that concept of "peptide segment separation by tertiary peptide bonds" is useful for estimating their β-sheet-structure-disrupted behavior. In solid-phase peptide synthesis by a fragment condensation procedure, the β-sheet structure disruption in CH_2Cl_2 by the addition of HMPA and DMSO is more favorable than that of HFIP and TFE because the latter is nucleophilic and consumes a carboxyl component peptide in a coupling reaction.[15] Thus, the easy disruption of resin-bound peptides **1—5** in CH_2Cl_2 by HMPA is of practical significance for solid-phase peptide synthesis, indicating that effective solvents for solid-phase peptide synthesis should be searched by taking the $<P_\alpha>$, $<P_\beta>$, and $<P_c>$ values of the peptide segments into consideration.

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1) 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: TFE, 2,2,2-trifluoroethanol; HMPA, hexamethylphosphoric triamide; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; Boc, *t*-butoxycarbonyl; Bzl, benzyl; Pam, phenylacetamidomethyl; IR, infrared; DMSO, dimethyl sulfoxide; MeOH, methanol; THF, tetrahydrofuran.

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