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Individuality of Amino Acid Residues in Protected Peptides.[1] Conformational and β-Sheet Structure-Disrupted Behaviors of Resin-Bound Peptides

Mitsuaki NARITA,* Shizuko ISOKAWA, Shinya HONDA, Hiroshi UMEYAMA, Hideaki KAKEI, and Satoshi OBANA
Department of Industrial Chemistry, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei 184
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The conformational behavior of cross-linked polystyrene resin-bound peptides swollen in CH_2Cl_2 was analyzed using IR absorption spectroscopy. All of the resin-bound peptides easily aggregated with each other through intermolecular hydrogen bonding to form a β-sheet structure. The disruption of the β-sheet structure was further investigated by a solvent titration method in order to clarify the individuality of amino acid residues. At a pentapeptide level, the β-sheet structure was easily disrupted by the addition of a small amount of HFIP. On the other hand, at a larger peptide level, the disrupted behavior of the β-sheet structure was different from each other according to the amino acid composition. Based on the view that the secondary structure of globular proteins reflects the individuality of the 20 kinds of amino acid residues in proteins, we attempted to evaluate how easily the β-sheet structure of peptides is disrupted by HFIP using the average conformation values: $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$. As a result, the β-sheet structure-disruption of peptides having a low potential for a β-sheet→helix transformation as well as for the randomness was difficult by adding increasing amounts of HFIP. On the other hand, that of the peptides having a $\langle P_c \rangle$ value larger than 0.85 and/or a high potential for the β-sheet→helix transformation proceeded smoothly upon adding increasing amounts of HFIP.

The 20 kinds of amino acid residues in proteins must reflect their individuality to form native three-dimensional structures of proteins. In order to predict the secondary structure of native globular proteins, Chou and Fasman determined the conformational parameters (P_α, P_β, and P_c) for the 20 kinds of amino acid residues on the basis of the three-dimensional structures of 15 kinds of proteins which were resolved by X-ray crystallography.[2] Thus, we have the view that parameters P_α, P_β, and P_c reflect the individuality of the 20 kinds of amino acid residues in proteins. In fact, in previous studies[3-6] we successfully applied the coil conformational parameters P_c to solubility predictions for protected peptides. The solubility-prediction method is based on the assumption that the larger P_c values of hydrophilic amino acid residues than those of hydrophobic ones reflect the tendency that the polar side-chain groups interact with their neighboring peptide bonds through hydrogen bonds, resulting in a disruption of helix and β-sheet structures.

In the solubility prediction, we also showed the usefulness of the average coil conformation value, $\langle P_c \rangle$, for estimating the potential of protected peptides for a β-sheet formation.[4,7,8] Furthermore, in a conformational transformation of protected peptides with an application of strong shear stress or with an increasing peptide chain length, we showed the usefulness of their average helix and β-sheet conformation values, $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$, for estimating their potential for the β-sheet→helix transformation.[8-10]

On the other hand, we found that the β-sheet structure of Boc–Val–Gly–Phe–Gly–Leu–Ile–Leu_2–OBzl in CH_2Cl_2 solution is easily disrupted by the addition of HFIP, a strong electron-acceptor solvent.[11]

In this paper, we demonstrate the usefulness of the $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values for estimating the conformation of resin-bound peptides and the stability of their β-sheet structure, and discuss the individuality of amino acid residues included in protected peptides. The resin-bound peptides used in this study are shown in Fig. 1.

Boc-ValAlaValAla-Gly-OCH_2-Pam-resin A_1
Boc-(ValAlaValAla)$_2$-Ile-OCH_2-Pam-resin A_2
Boc-(ValAlaValAla)$_3$-Ile-OCH_2-Pam-resin A_3
Boc-ValIleValIle-Gly-OCH_2-Pam-resin B_1
Boc-(ValIleValIle)$_2$-Gly-OCH_2-Pam-resin B_2
Boc-(ValIleValIle)$_3$-Gly-OCH_2-Pam-resin B_3
Boc-ValGlyPheGlyLeuIleLeuLeu-Ala-OCH_2-Pam-resin C_1
Boc-(ValGlyPheGlyLeuIleLeuLeu)$_2$-Ala-OCH_2-Pam-resin C_2
Boc-(ValGlyPheGlyLeuIleLeuLeu)$_3$-Ala-OCH_2-Pam-resin C_3
Boc-GlnValGlu(OBzl)LeuGly-$NHCH_2$-resin D_1
Boc-GlnValGlyGlnValGlu(OBzl)LeuGly-$NHCH_2$-resin D_2
Boc-Glu(OBzl)AlaGlu(OBzl)Asp(OBzl)LeuGlnValGlyGlnValGlu(OBzl)LeuGly-$NHCH_2$-resin D_3

Fig. 1. The resin-bound peptides used in this study.

Table 1. Solid-Phase Peptide Synthesis Cycle for the Addition of One Oligopeptide[a)]

Function	Solvent and/or reagent (amount)	Time/min	Number of application
1. Swelling	NMP[b)] (30 ml)	10	1
2. Peptide	Tetra- or octapeptide (3.0 or 2.0 equiv) HOBt (3.0 or 2.0 equiv)	30	1
3. Coupling	DCC (3.0 or 2.0 equiv) in NMP[b)] (5 ml)	3 days	1
4. Washing[c)]	NMP, EtOH and CH_2Cl_2	5	3
5. Termination	Pyridine–acetic anhydride (Vol. ratio 1:1, 30 ml)	2 h	1
6. Washing[c)]	CH_2Cl_2, EtOH, and CH_2Cl_2	5	3
7. Deprotection	TFA/CH_2Cl_2 (Vol. ratio 2:3, 30 ml)	2 h	1
8. Washing[c)]	CH_2Cl_2, EtOH, and NMP	5	3
9. Neutralization	TEA (20 equiv) in NMP (20 ml)	10	2
10. Washing[c)]	NMP, EtOH, CH_2Cl_2	5	3
11. Drying			

a) The resins used were 2.0 g, respectively. b) For assembly of the octapeptide (resin-bound peptides C_1—C_3), a mixture of NMP and CH_2Cl_2 (1:1, v/v) was used. c) Washing was carried out using each solvent.

Table 2. The Peptide Contents and Graft Ratios of the Resin-Bound Peptides

Resin-bound peptide	Peptide content[a)]	Graft ratio[b)]
A_1	133	1.4
A_2	89	1.0
A_3	66	0.77
B_1	116	1.3
B_2	50	0.58
B_3	34	0.40
C_1	83	0.95
C_2	56	0.68
C_3	48	0.61
D_1	106	1.2
D_2	97	1.1
D_3	79	1.0

a) $\mu mol\ g^{-1}$ of resin. The content does not include the terminated peptides. b) The ratio of grafted peptide to 100 styrene unit.

Experimental

Materials. Copoly(styrene/1% divinylbenzene) beads of 200—400 mesh, Bio-beads S-X1, were purchased from Bio-Rad Laboratories and used as polymer supports. Resin-bound peptides D_1, D_2, and D_3 were prepared before.[9)] Other resin-bound peptides were prepared virtually using the cycle summarized in Table 1. The acid hydrolyses of resin-bound peptides A_1, A_2, A_3, B_1, B_2, and B_3 were carried out with propionic acid/12 M HCl (2:1, v/v; 1 M=1 mol dm^{-3})[12)] for 10 days at 115 °C in evacuated and sealed tubes; those of resin-bound peptides C_1, C_2, and C_3, were carried out for 2 days at 115 °C. The amino acid composition of acid hydrolysates was determined using a Shimadzu HPLC LC-3A all amino acid analysis system. The peptide contents and graft ratios of the resin-bound peptides are shown in Table 2.

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 overnight in CH_2Cl_2 or a mixture of HFIP and CH_2Cl_2.

Results

The Conformation of the Resin-Bound Peptides Swollen in CH_2Cl_2. The IR absorption spectra of the resin-bound peptides, except for D_1, D_2, and D_3, swollen in CH_2Cl_2 are presented in Figs. 2 and 3. They show significant spectral regions for conformational assignments (3500—3200 cm^{-1}, amide A; 1750—1600 cm^{-1}, amide I). All of the resin-bound peptides show strong bands around 3280 cm^{-1} in the amide A region and around 1630 cm^{-1} in the amide I region, assigned to a β-sheet structure.[10,13)] This result is essentially the same as that of resin-bound peptides D_1, D_2, and D_3. Since the initial peptide contents of the resin-bound peptides were selected in order to obtain rational information for peptide chain interactions in solid-phase peptide synthesis, these results indicate that hydrogen bonding between pendant peptide chains do commonly occur in solid-phase peptide synthesis, as reported before.[9,10)]

The Disruption of the β-Sheet Structure of the Resin-Bound Peptides by HFIP. The disruption of the β-sheet structure by HFIP was investigated using a solvent titration method.[14)] It was monitored by a successive decrease in the intensity of the band around 1630 cm^{-1}, together with a successive addition of HFIP. Figure 4 shows the typical IR absorption spectra of resin-bound peptides A_1, A_3, B_1, and B_3 in a mixture of HFIP and CH_2Cl_2. Figures 5 and 6 illustrate the solvent-titration curves of resin-bound peptides A_1—A_3 and B_1—B_3, respectively, in CH_2Cl_2 using HFIP as a titrating solvent. The relative intensities shown in Figs. 5 and 6 as well as Figs. 7 and 8 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 be 1.0 for each relative intensity in the absence of HFIP. In the case of resin-bound peptides A_1—A_3 and B_1, a successive addition of HFIP to CH_2Cl_2 induces a dramatic decrease in the strong band around 1630 cm^{-1} and an increase in the broad

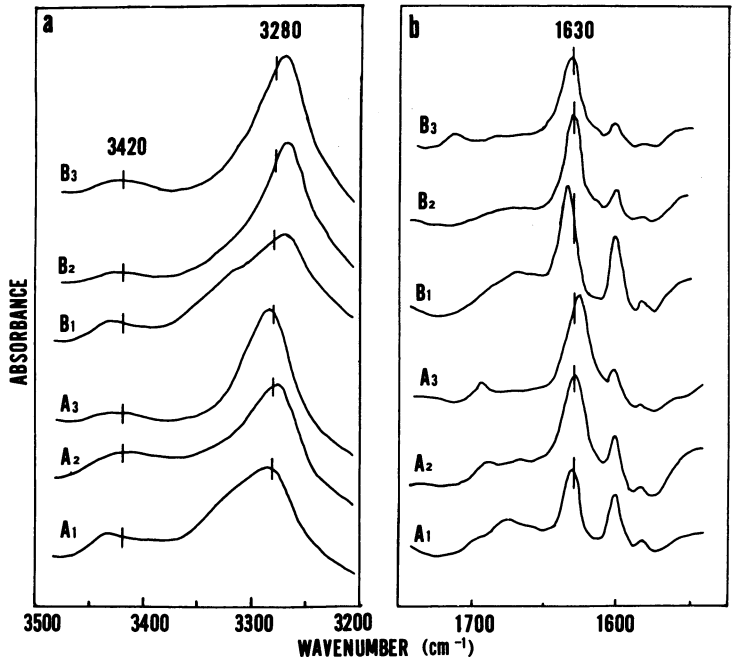

Fig. 2. IR absorption spectra (a) in the amide A and (b) in the amide I regions of the resin-bound peptides A_1—A_3 and B_1—B_3 swollen in CH_2Cl_2. The absorption band at 1603 cm^{-1} is due to aromatic rings of the polystyrene supports.

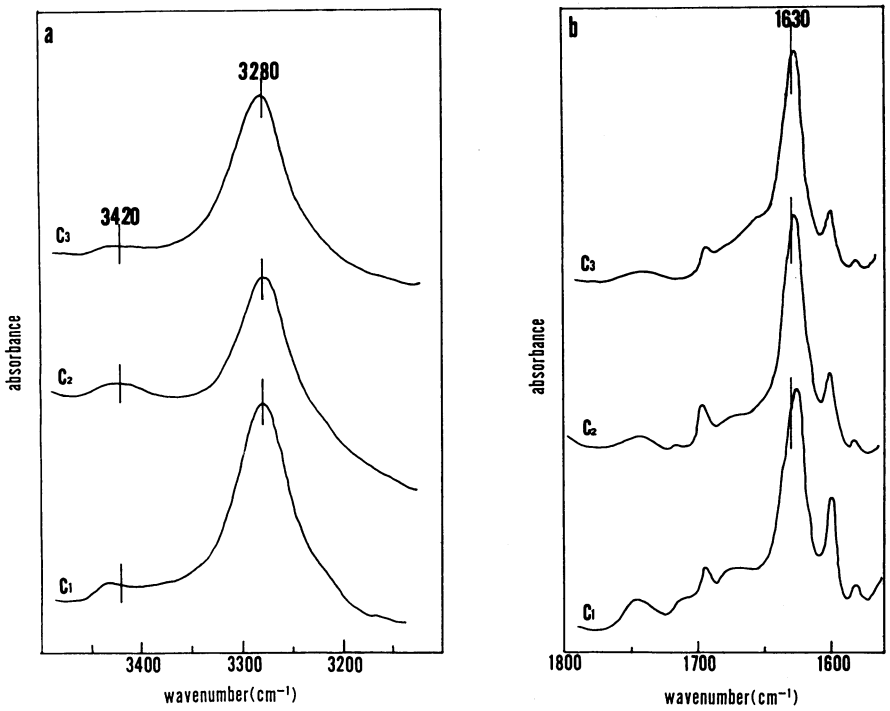

Fig. 3. IR absorption spectra (a) in the amide A and (b) in the amide I regions of the resin-bound peptides C_1—C_3 swollen in CH_2Cl_2.

band around 1655 cm^{-1}, indicating that the β-sheet aggregation of resin-bound peptides A_1—A_3 and B_1 is easily disrupted by increasing the amounts of HFIP. On the other hand, in the case of resin-bound peptides B_2 and B_3, the amide I carbonyl stretching band at 1632 cm^{-1} only shifts at 1637 cm^{-1} with broadening

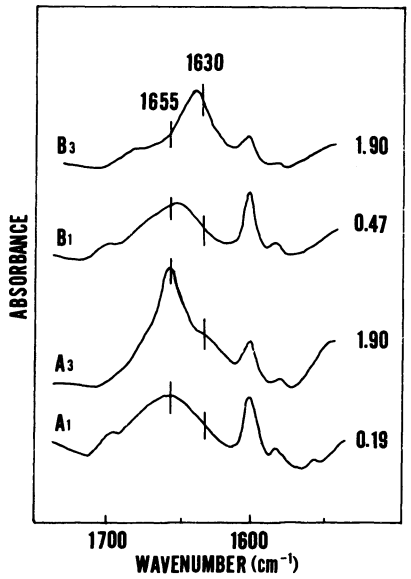

Fig. 4. Typical IR absorption spectra in the amide I region of the resin-bound peptides A_1, A_3, B_1, and B_3 swollen in HFIP-CH_2Cl_2. The numerals in Fig. 4 indicate concentrations of HFIP (M).

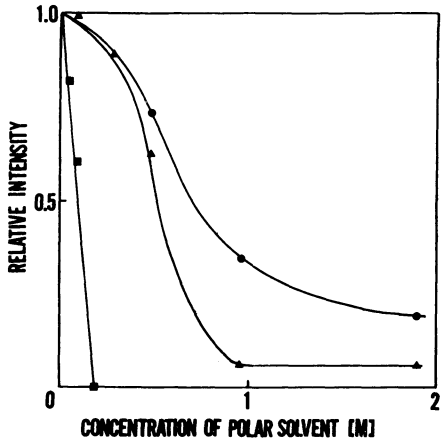

Fig. 5. The solvent-titration curves of the peptides A_1—A_3 in CH_2Cl_2 using HFIP as a titrating solvent. The peptide A_1, —■—; the peptide A_2, —▲—; the peptide A_3, —●—.

and a slight decrease in the intensity. This result strongly suggests that the β-sheet structure of the sequence of $(Val\text{-}Ile\text{-}Val\text{-}Ile)_n$ (n=2 and 3) is stable even in the presence of HFIP, although it is not clear why the band at 1632 cm^{-1} shifts at 1637 cm^{-1}. Figures 7 and 8 also present the solvent-titration curves of resin-bound peptides C_1—C_3 and D_1—D_3, respectively, in CH_2Cl_2 using HFIP as a titrating solvent. The result indicates that, regardless of an increasing peptide chain length, the β-sheet aggregation of resin-bound peptides C_1—C_3 and D_1—D_3 is easily disrupted by increasing the amounts of HFIP.

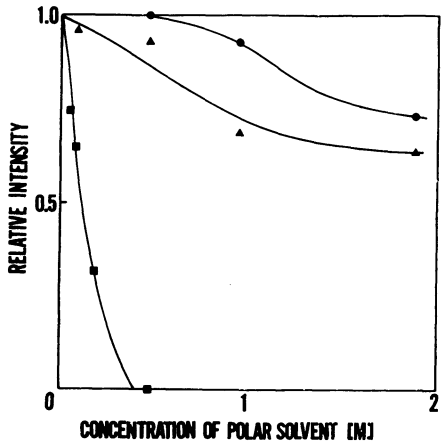

Fig. 6. The solvent-titration curves of the peptides B_1—B_3 in CH_2Cl_2 using HFIP as a titrating solvent. The peptide B_1, —■—; the peptide B_2, —▲—; the peptide B_3, —●—.

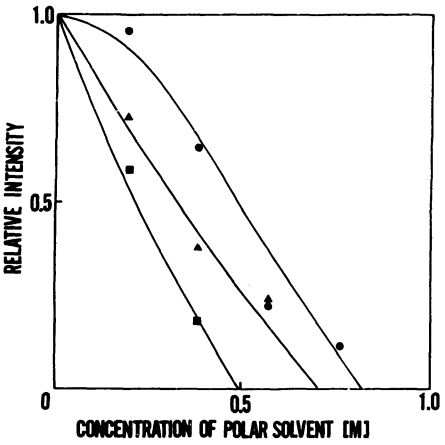

Fig. 7. The solvent-titration curves of the peptides C_1—C_3 in CH_2Cl_2 using HFIP as a titrating solvent. The peptide C_1, —■—; the peptide C_2, —▲—; the peptide C_3, —●—.

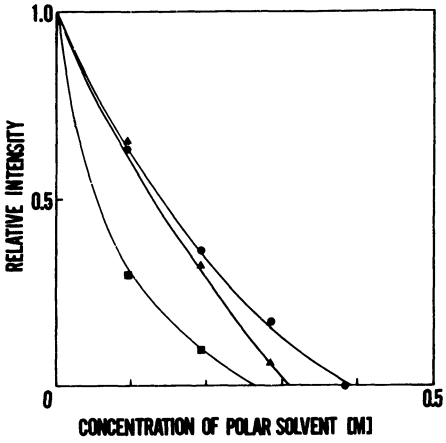

Fig. 8. The solvent-titration curves of the peptides D_1—D_3 in CH_2Cl_2 using HFIP as a titrating solvent. The peptide D_1, —■—; the peptide D_2, —▲—; the peptide D_3, —●—.

Discussion

The conformational behaviors of protected peptides must reflect the individuality of amino acid residues included in the peptides. We have the view that the conformational parameters, P_α, P_β, and P_c, for amino acid residues determined by Chou and Fasman[2] reflect the individuality of the 20 kinds of amino acid residues in proteins because the prediction method for the secondary structure of globular proteins using their $<P_\alpha>$ and $<P_\beta>$ values, proposed by Chou and Fasman,[15] is based on the three-dimensional structures of 15 kinds of proteins which are determined by X-ray crystallography.

In fact, the individuality of each amino acid residue reflected in the P_α, P_β, and P_c values is as follows. (1) The P_β values of the C^β-branched amino acid residues, that is, 1.60 of Ile, 1.20 of Thr, and 1.65 of Val are significantly larger than their P_α values, 1.00 of Ile, 0.82 of Thr, and 1.14 of Val, respectively. These values reflect the fact that the C^β-branched amino acid residues resist being included in a helical structure. (2) The values of P_α (0.53) and P_β (0.81) of the Gly residue are low and the P_c (1.50) value is extremely high. These values have been explained by the great freedom of the backbone dihedral angles, ϕ and ψ, of the Gly residue.[16] (3) The values of P_α (0.59) and P_β (0.62) of the Pro residue are low and P_c (1.59) is extremely high. These values clearly reflect the absence of an N–H bond of the Pro residue in the peptide bond.[17] (4) The P_c values of the hydrophilic amino acid residues (Arg, 1.04; Asn, 1.35; Asp, 1.20; Cys, 1.18; Gln, 0.86; Glu, 0.83; His, 1.06; Lys, 0.98; Ser, 1.32; Thr, 1.07; Tyr, 1.06) are larger than those of hydrophobic amino acid residues, except for the Pro residue, (Ala, 0.70; Ile, 0.66; Leu, 0.68; Met, 0.58; Phe, 0.71; Trp, 0.75; Val, 0.62) since the polar side-chain groups have a tendency to form hydrogen bonds with their neighboring peptide bonds, resulting in a disruption of the helical and β-sheet structures. (5) The P_c values of the hydrophilic amino acid residues having polar groups at their C^β atoms (Asn, 1.35; Asp, 1.20; Cys, 1.18; Ser, 1.32; Thr, 1.07) are larger than those of the other hydrophilic amino acid residues (Arg, 1.04; Gln, 0.86; Glu, 0.83; His, 1.06; Lys, 0.98; Tyr, 1.06). These values probably reflect the tendency that the former interacts more easily with the neighboring peptide bonds than the latter. (6) In the P_c values of the hydrophilic amino acid residues having polar groups at their C^β atoms, the P_c value of the Thr residue is the lowest due to the low freedom of the hydroxyl group. With respect to the P_c parameters for the 20 kinds of amino acid residues, our P_c parameters[3] used in this study are different from those obtained by Chou and Fasman.[2] Chou and Fasman determined them on the basis of data concerning 15 kinds of globular proteins, while we determined them on the basis of data concerning 29 kinds of globular proteins.

Table 3. The Average Conformation Values, $<P_\alpha>$, $<P_\beta>$, and $<P_c>$, of the Resin-Bound Peptides

Resin-bound peptide	$<P_\alpha>$	$<P_\beta>$	$<P_c>$
A_1—A_3	1.30	1.31	0.66
B_1—B_3	1.07	1.63	0.64
C_1—C_3	1.04	1.23	0.88
D_1	1.14	1.03	0.90
D_2	1.07	1.11	0.93
D_3	1.18	0.95	0.90

The $<P_\alpha>$, $<P_\beta>$, and $<P_c>$ values of the peptides A_1—A_3, B_1—B_3, and C_1—C_3 do not include their C-terminal amino acid residues.

Thus, the $<P_\alpha>$ and $<P_\beta>$ values of protected peptides must reflect their potential for helix and β-sheet formations, respectively, as much as their $<P_c>$ values reflect their potential for randomness. In Table 3, the average conformation $<P_\alpha>$, $<P_\beta>$, and $<P_c>$ values of the resin-bound peptides are summarized. The values of $<P_\alpha>$, $<P_\beta>$, and $<P_c>$ are necessarily determined, regardless of the amino acid sequence of the peptides.[15] They are only dependent on their amino acid composition.

With respect to the conformational behavior of resin-bound peptides, the fact that the resin-bound heptadecapeptide C_2 and pentacosapeptide C_3 swollen in CH_2Cl_2 have a β-sheet structure is quite different from that of H-Leu_n-Phe-resins (n=15 and 20), showing a helical structure in the swollen state in CH_2Cl_2.[10] Regardless of the increase in the peptide chain length, the amino acid sequence of peptides C_2 and C_3 has a high potential for a β-sheet formation, though a low potential for a helix formation, corresponding to the fact that their $<P_\alpha>$ and $<P_\beta>$ values are 1.04 and 1.23, respectively. In a conformational study of human hemoglobin α-chain fragments in the solid state,[8] we have shown that the potential of protected peptides for the β-sheet→helix transformation is clearly estimated using their $<P_\alpha>$ and $<P_\beta>$ values when the β-sheet→helix transformation occurs in the solid state under the application of shear stress. The conformational behavior of the resin-bound peptides C_1—C_3 indicates that the retention of the high potential for a β-sheet formation, regardless of an increase in the peptide chain length, can also be estimated using their $<P_\alpha>$ and $<P_\beta>$ values. Nevertheless, we should continuously make efforts to determine key sequences which dominate the peptide conformations, since the conformation of peptides is estimated to be essentially dependent on the key sequence. Concerning the sequence of peptides A_2 and A_3, its helical structure may be unstable due to repulsion between C^β-branched Ile and Val residues in the sequence of -Ile-Leu-Leu-Val-, as previously discussed in the sequence of

-Val-Ser-Thr-Val-,[8] as well as the great freedom of the backbone dihedral angles ϕ and ψ of two Gly residues.[15] Although $\langle R_\alpha \rangle$ and $\langle P_\beta \rangle$ values of protected peptides are necessarily determined regardless of the amino acid sequences of peptides and are only dependent on their amino acid compositions, they are still effective for estimating the potential for the β-sheet→helix transformation of peptides with increasing peptide chain length as well as with the application of shear stress.[8]

With regard to the β-sheet structure-disruption, Figs. 5—8 clearly show that, at a pentapeptide level, the β-sheet structure of resin-bound peptides is easily disrupted, regardless of their $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values. This corresponds to the fact that protected peptides of a pentapeptide level have high solubility in highly polar solvents, regardless of their $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values. In the case of peptides as long as or larger than an octapeptide, the β-sheet structure-disruption by HFIP is dependent on their $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values. The $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values of peptides A_2 and A_3 containing the Val and Ala residues are 1.30, 1.31, and 0.66, respectively, and their β-sheet structure is disrupted together with increasing amounts of HFIP (Fig. 5). On the other hand, the values of $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ of peptides B_2 and B_3 containing the Val and Ile residues are 1.07, 1.63, and 0.64, respectively, and their β-sheet structure-disruption is difficult, even with increasing amounts of HFIP (Fig. 6). As reflected in the $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values, peptides B_2 and B_3 have the sequence -(Val-Ile-Val-Ile)$_n$-, which can not form a helical structure due to the steric hindrance of side chains; however, peptides A_2 and A_3 have the sequence of -(Val-Ala-Val-Ala)$_n$- which can form a helical structure. Furthermore, the $\langle P_c \rangle$ values of both series of peptides are low, indicating that these peptides have a low potential for randomness. The result of the conformational behaviors of peptides A_2, A_3, B_2, and B_3 indicates that the β-sheet structure of a peptide having a high potential for a β-sheet→helix transformation, which is estimated from the $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values, can be disrupted by adding increasing amounts of HFIP. On the other hand, the β-sheet structure disruption of peptides is difficult when they have a low potential for a β-sheet→helix transformation, as well as for randomness. The $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values of peptides C_1—C_3 are 1.04, 1.23, and 0.88, indicating a low potential for the β-sheet→helix transformation and a high potential for randomness; their β-sheet structure is disrupted regardless of an increase in peptide chain length (Fig. 7). The $\langle P_c \rangle$ values of peptides D_2 and D_3 are 0.93 and 0.90, respectively, and their β-sheet structure is also disrupted, regardless of any increase in the peptide chain length (Fig. 8). Peptide D_3 has been estimated to have a high potential for the β-sheet→helix transformation, while peptide D_2 has not been. Peptides D_2 and D_3 contain protected Asp and Glu residues and the use of the P_α, P_β, and P_c parameters of unprotected Asp and Glu residues for an evaluation of the $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values of peptides D_2 and D_3 causes trouble. This is because side-chain protection clearly reduces the interaction of side chains with neighboring peptide bonds. Nevertheless, the $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_c \rangle$ values of resin-bound peptides D_2 and D_3 are also useful for estimating the potential for their β-sheet structure-disruption.

The results in this paper strongly suggest the adequacy of the solubility prediction method for protected peptides using their $\langle P_c \rangle$ values, previously proposed by us.[3]

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References

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 except for Gly denote the L-configuration. Additional abbreviations used are the following: HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; Boc, *t*-butoxycarbonyl; OBzl, benzyl ester; Pam, phenylacetamidomethyl; NMP, *N*-methylpyrrolidone; HOBt, 1-hydroxy-1*H*-benzotriazole; DCC, dicyclohexylcarbodiimide; EtOH, ethanol; TFA, trifluoroacetic acid; TEA, triethylamine; IR, infrared.

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