The Influence of Protecting Groups on the β-Sheet-Structure Stability of Protected Peptides[1)]

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The influence of protecting groups on the β-sheet-structure stability of protected peptides was studied in organic solvents. α-Amino groups, carboxyl groups, and side-chain functional groups of model peptides were protected by availably used protecting groups. The difference between the solubilities of model peptides was investigated by the solvent-titration method using infrared (IR) absorption spectra. The β-sheet-structure-stabilizing potentials (SPβ) of each protecting group showed similar behaviors, except for Npys, Mts, and Z_2. The result exhibits that the ⟨SPβ⟩ values of the protected peptide are almost independent of the kinds of their protecting group.

The insolubility of protected peptides in the chemical synthesis of peptides and proteins causes an intermolecular hydrogen-bonded β-sheet aggregation. The disruption of the β-sheet structure caused by sufficient solvation of a peptide chain is important in smoothly carrying out the successive reactions. Thus, an evaluation of the β-sheet-structure stability of protected peptides is essential for designing synthetic routes for peptides and proteins.

In previous paper,[2,3)] we proposed a predictive method for the solubility of protected peptides. They defined the β-sheet-structure-stabilizing potentials (SPβ) of the 20 kinds of amino acid residues from the difference between the solubilities of the protected model peptides, Boc–**X**–Val–Asp(OBzl)–Ala–Gly–OPac and Boc–**X**–Ala–Glu(OBzl)–Leu–Gly–OPac. The prediction was carried out by using the SPβ of the 20 kinds of amino acid residues in protected peptides, whose side-chain functional groups were protected by suitable groups commonly used in peptide synthesis. The β-sheet structure of protected model peptides was disrupted in DCM by increasing amounts of DMSO. The disrupted behaviors were dependent on the nature of the guest amino acid residues. According to these results, the 20 guest amino acid residues could be classified into six groups (Table 1).

The evaluation of the β-sheet-structure stability of protected peptides was performed using their ⟨SPβ⟩ values, which are defined as the arithmetic average of the SPβ of amino acid residues comprising the protected peptides. Using 77 kinds of protected tri- to heptapeptide fragments of *E. coli* ribosomal protein L7/L12, we showed that their ⟨SPβ⟩ values are useful for estimating their β-sheet-structure stability in organic solvents.[4)] On the other hand, protected peptides mentioned above were protected as follows: The α-amino group is protected by Boc, the carboxyl group is protected by Pac, and the side-chain functional groups are Bzl groups.

Table 1. The β-Sheet-Structure-Stabilizing Potential of the 20 Amino Acid Residues

SPβi	Conclusive classification
6	Arg(Mts), Asn, Val
5	Ala, Gln, Gly, His(Bom), Ile
4	Phe, Trp(CHO), Tyr(Bzl)
3	Cys(Bzl), Glu(OBzl), Leu, Lys(Z), Met(O)
2	Ser(Bzl), Thr(Bzl)
1	Asp(OBzl), Pro

In this study we investigated the influence of protecting groups on the β-sheet-structure stability of a protected peptide. The α-amino groups, carboxyl groups and side-chain functional groups of model peptides were protected by suitable groups used in peptide synthesis. The protected penta- and heptapeptides used in this study are summarized in Fig. 1.

Experimental

Materials. Boc–Ala–Glu(OBzl)–Leu–Gly–OPac was prepared in DCM by a common stepwise elongation using DCC and HOBt as coupling reagents.[5)] R^N–Leu–Ala–Glu(OBzl)–Leu–Gly–OPac (R^N = Boc, Fmoc, Npys, and Z) and Boc–**X**–Ala–Glu(OBzl)–Leu–Gly–OPac (**X** = Arg(Mts), Arg(Z_2), Asp(OBzl), Asp(OcHex), Glu(OBzl), Glu(OcHex), His(Bom), His(Tos), Lys(Z), Lys(Tos), Tyr(Bzl), and Tyr(Cl_2-Bzl)) were similarly prepared in a mixture of DCM and DMF by coupling reactions of R^N–Leu–OH and Boc–**X**–OH with HCl·H–Ala–Glu(OBzl)–Leu–Gly–OPac respectively. Boc–Thr(Bzl)–Ala–Glu(OBzl)–Leu–Gly–Ile–Ala–R^C (R^C = OBzl, OPac) were prepared in a mixture of DCM and DMF by coupling reactions of Boc–Thr(Bzl)–Ala–Glu(OBzl)–Leu–Gly–OH with HCl·H–Ile–Ala–OPac and HCl·H–Ile–Ala–OBzl. Removing the Boc group of the Boc-derivatives was treated with 3.6 M (1 M = 1 mol dm^{-3}) HCl/AcOEt. The residue, dissolved in HFIP/DCM (1 : 4), was liberalized with the addition of TEA; the free amino component of the peptide

1. Amino protecting groups

Boc - Leu-Ala-Glu(OBzl)-Leu-Gly-OPac
Z - Leu-Ala-Glu(OBzl)-Leu-Gly-OPac
Npys - Leu-Ala-Glu(OBzl)-Leu-Gly-OPac
Fmoc - Leu-Ala-Glu(OBzl)-Leu-Gly-OPac

2. Carboxyl protecting groups

Boc -Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-Ile-Ala-**OPac**
Boc -Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-Ile-Ala-**OBzl**

3. Side chain protecting groups

Boc -**X**-Ala-Glu(OBzl)-Leu-Gly-OPac

X = Arg(**Mts**), Arg($\mathbf{Z_2}$),
Asp(**OBzl**), Asp(**OcHex**),
Glu(**OBzl**), Glu(**OcHex**),
His(**Bom**), His(**Tos**),
Lys(**Z**), Lys(**Tos**),
Tyr(**Bzl**), Tyr($\mathbf{Cl_2}$-**Bzl**)

Fig. 1. The protected peptides used in this study.

(R^N = H) was obtained by precipitation with ether. Also, removing the Pac group was treated with Zn/AcOH; the free-acid component of the peptide was obtained. The synthetic route of their protected peptides is summarized in Scheme 1. All of the products gave a single peak on normal-phase HPLC, and were negative for the Kaiser test. The acid hydrolysis of the peptides was carried out with propionic acid/12 M HCl (2 : 1, v : v) at 115 °C for 5 d.[6] The amino acid ratios of the acid hydrolysates were in good agreement with the calculated values. An elemental analysis of the protected peptides is summarized in Table 2.

Infrared Absorption Spectra Measurements. The IR absorption spectra of the model peptides in solution or in the suspended state were recorded at room temperature with a JEOL Model JIR-100 FT-IR spectrometer by employing 0.5 mm-path-length cells with sodium chloride windows. The peptides, excluding Boc–**X**–Ala–Glu(OBzl)–Leu–Gly–OPac (**X** = Asp(OBzl), Asp(OcHex)), were dissolved or suspended in DCM containing a variety of concentrations of DMSO. The exclusive peptides were

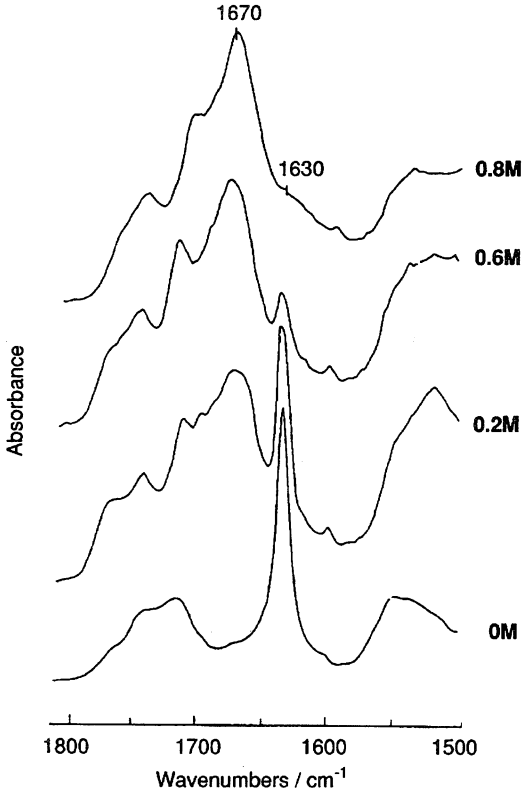
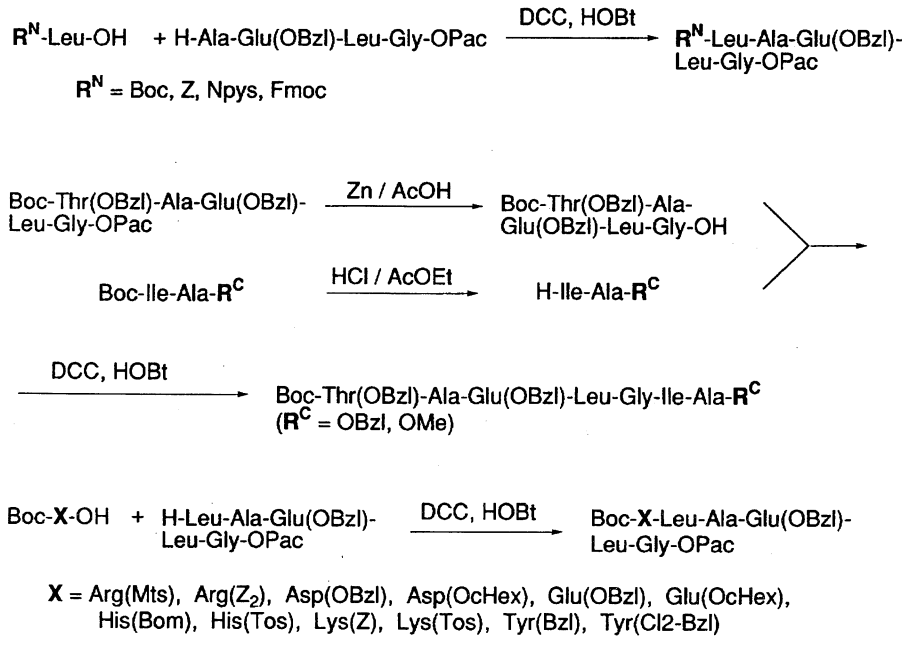


Fig. 2. IR absorption spectra in the amide I region of Boc–Thr(Bzl)–Ala–Glu(OBzl)–Leu–Gly–Ile–Ala–OPac in DCM containing various concentrations of DMSO.



Scheme 1.

Table 2. Elemental Analyses of Protected Model Peptides

Protected peptide	Formula	Found (Calcd)							
		C/%		H/%		N/%		S or Cl/%	
Boc–Leu–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{42}H_{59}N_5O_{11}$	62.12	(62.28)	7.69	(7.34)	8.73	(8.65)		
Z–Leu–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{45}H_{57}N_5O_{11}$	63.89	(64.04)	6.75	(6.81)	8.31	(8.30)		
Npys–Leu–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{43}H_{54}N_6S_1O_{13}$	57.55	(57.71)	6.01	(6.08)	8.97	(9.39)	3.82	(3.58)
Fmoc–Leu–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{52}H_{61}N_5O_{11}$	67.47	(67.01)	6.88	(6.60)	7.34	(7.51)		
Boc–Leu–Ala–Glu(OBzl)–Leu–Gly–**OBzl**	$C_{41}H_{59}N_5O_{10}$	63.19	(62.98)	7.65	(7.61)	9.29	(8.96)		
Boc–**Arg(Mts)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{51}H_{70}N_8S_1O_{13}$	59.24	(59.17)	6.63	(6.82)	11.49	(10.82)	3.49	(3.10)
Boc–**Arg(Z_2)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{58}H_{73}N_8O_{15}$	60.22	(62.56)	7.05	(6.56)	11.11	(10.00)		
Boc–**Asp(OBzl)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{47}H_{59}N_5O_{13}$	60.82	(60.84)	6.69	(6.74)	11.73	(11.35)		
Boc–**Asp(OcHex)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{46}H_{63}N_5O_{13}$	61.55	(61.80)	6.91	(7.10)	7.23	(7.83)		
Boc–**Glu(OBzl)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{48}H_{61}N_5O_{13}$	62.05	(62.94)	7.41	(6.71)	8.12	(7.65)		
Boc–**Glu(OcHex)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{47}H_{65}N_5O_{13}$	62.47	(62.17)	7.08	(7.21)	7.04	(7.71)		
Boc–**His(Bom)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{50}H_{63}N_7O_{12}$	63.73	(62.94)	7.17	(6.66)	10.06	(10.28)		
Boc–**His(Tos)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{49}H_{61}N_7S_1O_{13}$	60.39	(59.56)	6.68	(6.22)	9.79	(9.92)	3.55	(3.24)
Boc–**Lys(Z)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{50}H_{66}N_6O_{13}$	62.24	(62.62)	6.63	(6.94)	8.47	(8.76)		
Boc–**Lys(Tos)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{49}H_{66}N_6S_1O_{13}$	60.42	(60.11)	7.44	(6.79)	8.01	(8.58)	3.41	(3.27)
Boc–**Tyr(Bzl)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{52}H_{63}N_5O_{12}$	65.88	(65.74)	6.63	(6.68)	7.49	(7.37)		
Boc–**Tyr(Cl_2-Bzl)**–Ala–Glu(OBzl)–Leu–Gly–OPac	$C_{52}H_{61}N_5Cl_2O_{12}$	61.22	(61.29)	6.27	(6.03)	7.11	(6.87)	7.45	(6.96)

dissolved in CH_3CN containing various concentrations of DCM. The peptides in the suspended state were recorded by putting them between ditched sodium chloride plates. The concentration of each peptide was kept at 5.0×10^{-2} M.

Results

The β-sheet-structure stability of the protected peptides was evaluated by monitoring the β-sheet-structure-disrupted behaviors of the protected peptides in DCM or CH_3CN using a solvent-titration method.[7] DMSO or DCM was used as the titrating solvent. Figure 2 shows the IR absorption spectra of Boc–Thr(Bzl)–Ala–Glu(OBzl)–Leu–Gly–OPac in DCM containing a variety of molar concentrations of DMSO. It was evaluated by monitoring the peptide IR absorption band around 1630 cm^{-1} assigned to a β-sheet structure.[8] The behavior of the β-sheet-structure disruption was studied in CH_3CN or DCM by measuring the successive decrease in the intensity of the band around 1630 cm^{-1} concurrent with the successive addition of a titrating solvent, DCM or DMSO. The solvent-titration curves of R^N–Leu–Ala–Glu-(OBzl)–Leu–Gly–OPac (Fig. 3), Boc–Thr(Bzl)–Ala–Glu-(OBzl)–Leu–Gly–Ile–Ala–R^C (Fig. 4), and Boc–**X**–Ala–Glu-(OBzl)–Leu–Gly–OPac (Fig. 5) are depicted using the relative intensities of the bands around 1630 cm^{-1}, which were determined using the bands around 1760 and 1730 cm^{-1} as a standard and normalizing to 1.0 for each relative intensity in CH_3CN or DCM. As shown in Figs. 3, 4, and 5, the successive addition of a titrating solvent induced a dramatic

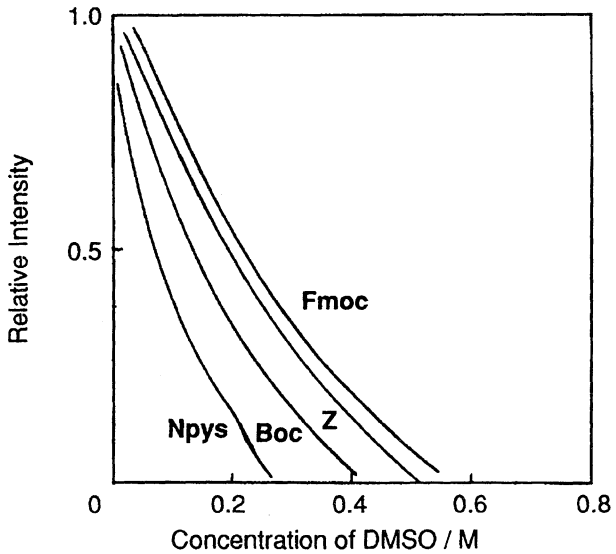


Fig. 3. The solvent titration curves of R^N–Leu–Ala–Glu-(OBzl)–Leu–Gly–OPac (R^N = Npys, Boc, Z, Fmoc) in DCM using DMSO as a titration solvent.

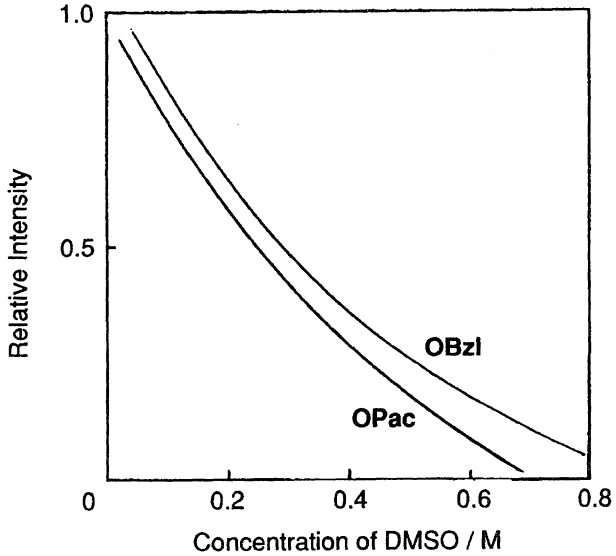


Fig. 4. The solvent titration curves of Boc–Thr-(Bzl)–Ala–Glu(OBzl)–Leu–Gly–Ile–Ala–R^C (R^C = OBzl, OPac) in DCM using DMSO as a titration solvent.

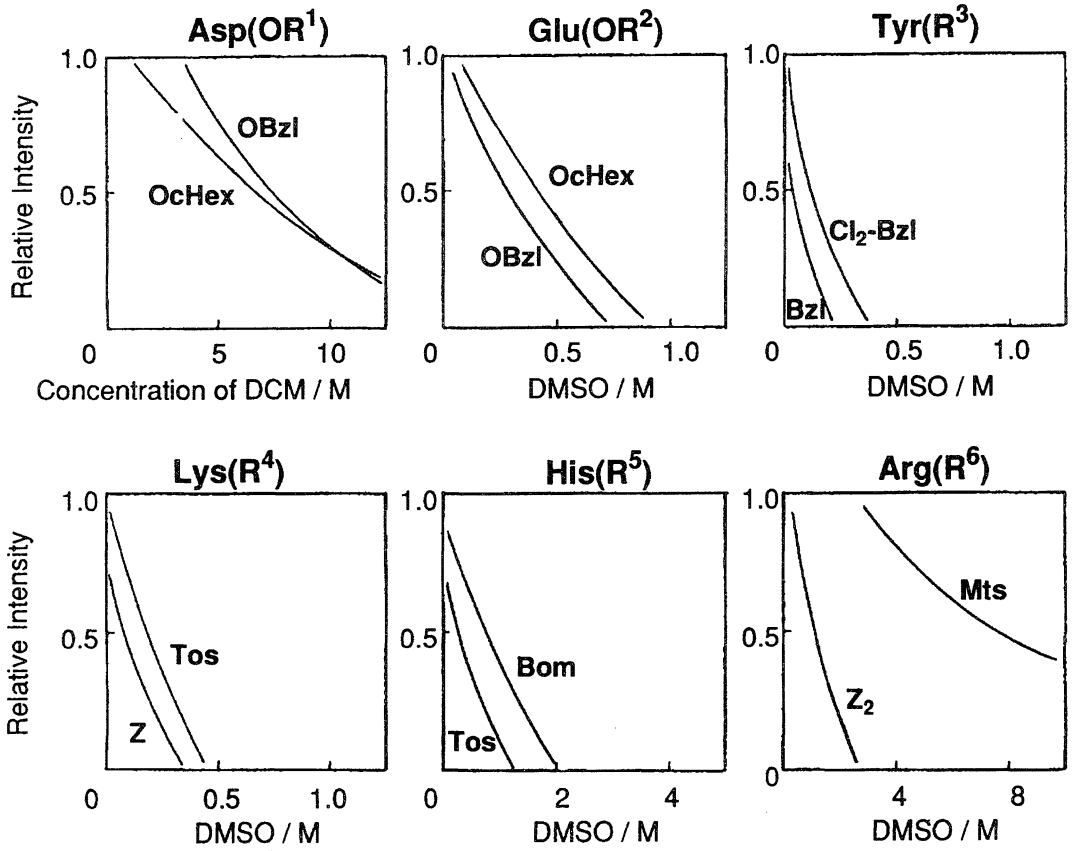


Fig. 5. The solvent titration curves of Boc–**X**–Ala–Glu(OBzl)–Leu–Gly–OPac (**X** = Asp(OBzl), Asp(OcHex)) in CH_3CN using DCM as a titration solvent and the others (**X** = Arg(Mts), Arg(Z_2),Glu(OBzl),Glu(OcHex), His(Bom), His(Tos), Lys(Z), Lys(Tos), Tyr(Bzl), Tyr(Cl_2-Bzl)) in DCM using DMSO as a titration solvent.

decrease in the band around 1630 cm^{-1}, indicating the β-sheet-structure stabilities.

Discussion

An evaluation of the β-sheet-structure stability of the protected peptides was performed by using their $\langle SP\beta \rangle$ values, which are defined as the arithmetic average of the β-sheet-structure stabilizing potentials ($SP\beta$) of the amino acid residues comprising the protected peptides. For example, the $\langle SP\beta \rangle$ values of Boc–**X**–Val–Asp(OBzl)–Ala–Gly–OPac used in this study are as follows: Arg(Mts), Asn, and Val are 4.6; Ala, Gln, Gly, His(Bom), and Ile are 4.4; Phe, Trp-(CHO), and Tyr(Bzl) are 4.2; Cys(Bzl), Glu(OBzl), Leu, Lys(Z), and Met(O) are 4.0; Ser(Bzl) and Thr(Bzl) are 3.8; Asp(OBzl) and Pro are 3.6. Because these were appreciable as relative values, we showed that their $\langle SP\beta \rangle$ values are useful for estimating their β-sheet-structure stability in organic solvents.[4] However, these protecting groups of the peptides mentioned above were limited, as follows: The α-amino group is protected by Boc, the carboxyl group is protected by Pac, and the side-chain functional groups are Bzl groups. We therefore investigated the influence of other protecting groups on the β-sheet-structure-stability of the protected peptide.

As shown in Figs. 3, 4, and 5, the solvent-titrating curves indicate that the β-sheet-structure stabilities of the protected peptides are dependent on the nature of the protecting groups. On the basis of the solvent-titration curves of R^N–Leu–Ala–Glu(OBzl)–Leu–Gly–OPac (R^N = Boc, Fmoc, Npys, and Z), as shown in Fig. 3, the β-sheet-structure-stability of the α-amino protecting group in protected pentapeptides can be derived as follows: Fmoc > Z > Boc≫Npys. Because the Npys group doesn't have a carboxyl group, the peptide protected by the Npys group is expected to show a decreased behavior of one hydrogen bond in protected peptide. Accordingly, a good agreement between this expectation and the result of the solvent-titration curve appeared. Although the peptide protected by the Boc group showed a similar behavior, the β-sheet-structure of the protected peptide containing the Fmoc and Z groups was more stable. It was also reported that the Fmoc group promoted aggregation in solid-phase peptide synthesis.[9] Therefore, in long-chain peptide synthesis, the use of the Fmoc group may cause a decrease in the yield and purity.

On the other hand, Fig. 4 shows the solvent-titration curves of Boc–Thr(Bzl)–Ala–Glu(OBzl)–Leu–Gly–Ile–Ala–R^C (R^C = OBzl and OPac). The influence of the Bzl and Pac group on the β-sheet-structure stability of the protected peptides showed a similar behavior. This result indicates that the β-sheet-structure stability of the protected peptides exists regardless of the kind of carboxyl-protecting group.

The β-sheet-structure stability of each side-chain protect-

ing groups was compared using the solvent-titrating curves of Boc–**X**–Ala–Glu(OBzl)–Leu–Gly–OPac. The influence due to a change in the side-chain protecting groups (OBzl and OcHex in Asp, OBzl, and OcHex in Glu, Bom and Tos in His, Tos and Z in Lys, and Bzl and Cl_2-Bzl in Tyr) was not nearly showed. A different conformational behavior for a peptide containing Arg was observed. Namely, a difference between Mts and Z_2 in Arg appeared. The β-sheet-structure stability of a peptide protected by Mts is rather stable. It can be presumed that the strong donor sulfonyl group of Arg(Mts) interacts with other acceptor groups of peptides by intramolecular or intermolecular hydrogen bonds, compared with the carbonyl groups of Arg(Z_2). It is therefore considered that Z_2, as a side-chain protecting group of Arg, is more useful in peptide synthesis.

The results mentioned above show that the β-sheet-structure stability of a protected peptide is almost independent of the kind of protecting group. In our previous papers, the $\langle SP\beta \rangle$ values of protected peptides were in harmony with their β-sheet-structure stability.[3,4] As the $\langle SP\beta \rangle$ value increases, the β-sheet structure of the protected peptide becomes more stable. This fact supports the idea that the β-sheet-structure stability of protected peptides is dependent on their amino acid compositions.[10]

References

1) The abbreviations for the amino acids are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). The amino acid symbols, except for Gly, denote the L-configuration. The additional abbreviations used are the following: Bzl, benzyl; Boc, *t*-butoxycarbonyl; Bom, benzyloxymethyl; Cl_2-Bzl, 2,6-dichlorobenzyl; DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; Fmoc, (9-fluorenylmethyl)oxycarbonyl; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; HOBt, 1*H*-1,2,3-benzotriazol-1-ol; Npys, 3-nitro-2-pyridinesulfenyl; OcHex, cyclohexyloxy; Pac, phenacyl; Mts, 2-mesitylenesulfonyl; Tos, *p*-toluenesulfonyl; Z, benzyloxycarbonyl.

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