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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 suitable groups commonly used in peptide synthesis. The difference of the solubilities of model peptides was investigated by the solvent-titration method by using IR absorption spectra. The β-sheet structure of model peptide in CH_2Cl_2 was easily disrupted by increasing the amounts of DMSO. The β-sheet-structure-stabilizing potentials of each protecting group showed similar behaviors except Npys, Mts and Z_2. The result exhibits that the $\langle SP_\beta \rangle$ values of protected peptides are almost independent of the kinds of their protecting groups.

Introduction

The insolubility of protected peptides in organic solvents is one of the most serious obstacles in peptide and protein syntheses. The insolubility is due to intermolecular hydrogen-bonded β-sheet aggregation. The disruption of the β-sheet structure by sufficient solvation of a peptide chain is important in carrying out the successive coupling reactions smoothly. Thus, the evaluation of the β-sheet structure stability of protected peptides is essential for the design of synthetic routes for peptides and proteins. The most important thing in selecting the appropriate solvent and deciding of synthetic route is to expect the solubility of protected peptide, and this is connected directly with the stability of β-sheet structure.

In previous paper,[2,3] we proposed a predictive method for the solubility of protected peptides. The prediction was carried out by using the β-sheet structure stabilizing potentials, SP_β, 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. Using model host-guest peptide, the β-sheet structure of protected peptides in CH_2Cl_2 was disrupted by increasing the amounts of DMSO. The disrupting 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. Arg(Mts), Val, Asn, Gln, Gly, Ala, His(Bom), Ile have high β-sheet structure formation ability, Phe, Trp(CHO), Tyr(Bzl), Cys(Bzl), Lys(Z), Glu(OBzl), Met(O) have average ability, and Ser(Bzl), Thr(Bzl), Asp(OBzl), Pro have low ability.

The evaluation of the β-sheet-structure stability of the protected peptides was performed by calculating their $\langle SP_\beta \rangle$ values, which are defined as the arithmetic average of the β-sheet-structure-stabilizing potentials, SP_β, of the amino acid residues composing the protected peptides. Using 77 kinds of protected tri- to heptapeptide fragments of *E. coli* ribosomal protein L7/L12, we showed that their $\langle SP_\beta \rangle$ values are useful for the estimation of their β-sheet-structure-stability in organic solvents.[4] The protected peptides mentioned above were protected as follows: α-Amino groups are protected by Boc, carboxyl groups are protected by Pac and side chain

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(Z)
       - His(Bom) ----- His(Tos)
       - Asp(OBzl) ----- Asp(OcHex)
       - Glu(OBzl) ----- Glu(OcHex)
       - Tyr(Bzl) ----- Tyr(Cl2-Bzl)
       - Lys(Z) ----- Lys(Tos)

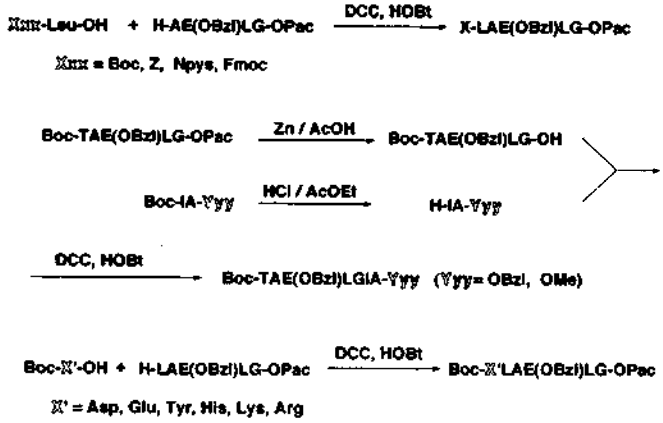
Figure 1. The protected peptides used in this study.

functional groups are protected by Bzl groups. There are many kinds of protecting groups in synthesizing protected peptides. Therefore, It is essential that we investigate the β-sheet structure formation ability of each amino acid with these kinds of protecting groups.

In this study, we investigated the influence of protecting groups on the β-sheet-structure-stability of protected peptide. 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 hepta-peptides used in this study are summarized in Figure 1.

Experimental

Materials. Boc-Ala-Glu(OBzl)-Leu-Gly-Opac was prepared in CH_2Cl_2 by common stepwise elongation using DCC and HOBt as coupling reagents.[5] Yyy-Leu-Ala-Glu(OBzl)-Leu-Gly-OPac(Yyy=Boc, Z, Npys, Fmoc) were prepared in a mixture of CH_2Cl_2 and DMF by coupling Yyy-Leu-OH with HCl. H-Ala-Glu(OBzl)-Leu-Gly-OPac. Boc-Xxx-Ala-Glu(OBzl)-Leu-Gly-OPac [Xxx=Arg(Mts), Arg(Z), His(Bom), His(Tos), Asp(OBzl), Asp(OcHex), Glu(OBzl), Glu(OcHex), Tyr(Bzl), Tyr(Cl$_2$-Bzl), Lys(Z), Lys(Tos)] were similarly prepared in a mixture of CH_2Cl_2 and DMF by coupling Boc-Xxx-OH with HCl. H-Ala-Glu(OBzl)-Leu-Gly-OPac. Boc-Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-Ile-Ala-Yyy(Yyy=OPac, OBzl) were prepared in a mixture of CH_2Cl_2 and DMF by coupling Boc-Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-OH with HCl. H-Ile-Ala-Opac and HCl. H-Ile-Ala-OBzl. The deprotection of the Pac group in the protected peptide was performed by treatment with Zn/AcOH to yield the carboxyl component. The removal of the Boc group was carried out by treatment with 3.6 M (1 M=1 moldm^{-3}) HCl/AcOEt to give amino component. The synthetic routes of protected peptides are summarized in Scheme 1. The coupling reactions were repeated until the Kaiser test became negative. After the usual work-up procedures, all the products were purified by recrystallization. They gave a single peak on HPLC and were negative for the Kaiser test. Acid hydrolysis of the peptides was carried out with propionic acid/12 M HCl (2/1, V/V) at 115 ℃ for 5 days.[6] The amino acid ratios of the acid hydrolysates were in good agreement with the calculated values as shown in Table 1.



Scheme 1. The synthetic route of protected peptides used in this study.

IR Absorption Spectra Measurements. The IR absorption spectra of the model peptides in solution or in the

Table 1. Amino acid Analyses of the Protected Peptides

Protected peptide	Found (Calcd)										
	Ala	Glu	Leu	Gly	Ile	Thr	Asp	Arg	His	Tyr	Lys
Boc-5	1.22(1)	1.00(1)	1.89(2)	0.93(1)	–	–	–	–	–	–	–
Z-5	1.11(1)	0.88(1)	2.01(2)	1.00(1)	–	–	–	–	–	–	–
Npys-5	1.20(1)	1.00(1)	2.22(2)	1.16(1)	–	–	–	–	–	–	–
Fmoc-5	1.07(1)	1.00(1)	2.16(2)	1.10(1)	–	–	–	–	–	–	–
7-OPac	2.00(2)	0.92(1)	0.88(1)	0.90(1)	0.89(1)	1.04(1)	–	–	–	–	–
7-OBzl	2.26(2)	1.22(1)	1.00(1)	1.07(1)	0.94(1)	0.90(1)	–	–	–	–	–
Glu(OBzl)-4	0.90(1)	1.85(2)	1.07(1)	1.00(1)	–	–	–	–	–	–	–
Asp(OBzl)-4	1.00(1)	1.26(1)	0.96(1)	1.22(1)	–	–	0.84(1)	–	–	–	–
Arg(Mts)-4	1.30(1)	1.23(1)	1.00(1)	1.11(1)	–	–	–	0.78(1)	–	–	–
His(Bom)-4	0.91(1)	1.00(1)	0.89(1)	0.93(1)	–	–	–	–	0.81(1)	–	–
Tyr(Bzl)-4	1.33(1)	0.86(1)	0.91(1)	0.80(1)	–	–	–	–	–	1.00(1)	–
Lys(Z)-4	0.89(1)	0.94(1)	1.00(1)	1.27(1)	–	–	–	–	–	–	1.87(2)

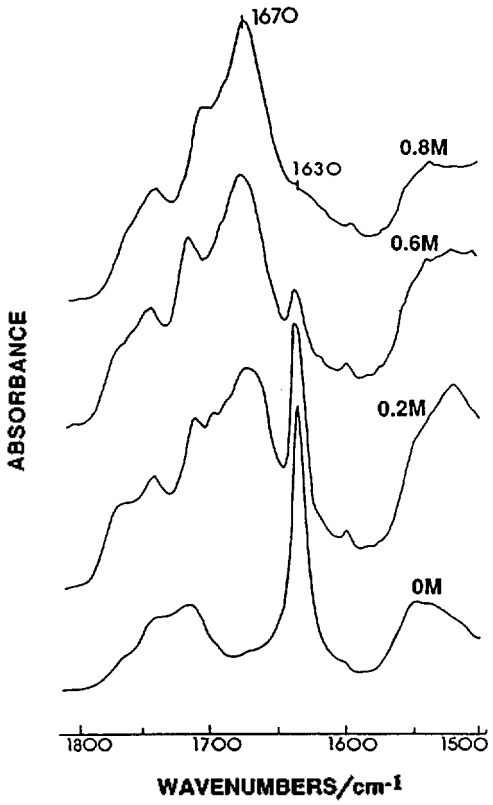


Figure 2. IR absorption spectra in the amide I region of Boc-Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-Ile-Ala-OPac in CH_2Cl_2 containing various concentrations of DMSO.

suspended state was recorded at room temperature with a JEOL Model JIR-100 FT-IR spectrometer by employing 0.5 mm-path-length cells with sodium choride windows. All the peptides except Boc-Xxx-Ala-Glu(OBzl)-Leu-Gly-OPac [Xxx =Asp(OBzl), Asp(OcHex)] were dissolved or suspended in CH_2Cl_2 containing various concentrations of DMSO. The above peptides were dissolved in CH_3CN containing various concentrations of CH_2Cl_2. The peptides in 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 and Discussion

The β-sheet-structure-stability of the protected peptides was evaluated by monitoring the β-sheet-structure-disrupting behaviors of the protected peptides in CH_2Cl_2 or CH_3CN using a solvent-titration method.[7] The β-sheet structure of the protected peptides in CH_2Cl_2 was disrupted by adding increasing amounts of DMSO. The IR absorption spectra of the protected peptides showed 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. The behavior of the β-sheet-structure disruption was monitored by a successive decrease in the intensity of the band around 1630 cm^{-1} and increase in the band around 1670 cm^{-1}, mainly assigned to an unordered structure,[8] resulting from successive addition of titrating solvent DMSO. Figure 2 shows the typical IR absorption spectra of Boc-Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-OPac in CH_2Cl_2 containing a variety of molar concentrations of DMSO. The solvent-titration curves of Yyy-Leu-Ala-Glu(OBzl)-Leu-Gly-OPac (Figure 3), Boc-Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-Ile-Ala-Yyy (Figure 4) and Boc-Xxx-Ala-Glu(OBzl)-Leu-Gly-OPac (Figure 5) are depicted using the relative intensities of the bands around 1630 cm^{-1}, which were determined by using the bands around 1760 cm^{-1} and 1730 cm^{-1} due to the ester carbonyl groups of Gly-OPac and Glu(OBzl), respectively, as a standard and normalizing to be 1.0 for each relative intensity in CH_2Cl_2 or CH_3CN alone. As shown in Figures 3, 4 and 5, the successive addition of a titrating solvent induced a dramatic decrease in the band around 1630 cm^{-1}.

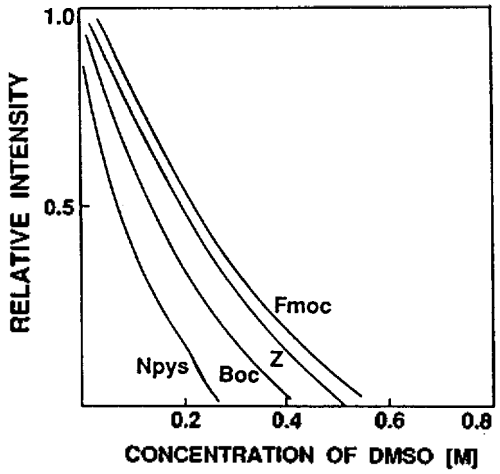


Figure 3. The solvent titration curves of Yyy-Leu-Ala-Glu(OBzl)-Leu-Gly-OPac (Yyy=Npys, Boc, Z, Fmoc) in CH_2Cl_2 using DMSO as a titration solvent.

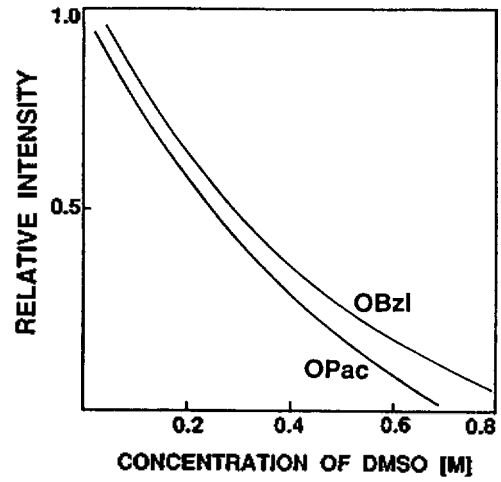


Figure 4. The solvent titration curves of Boc-Thr(Bzl)-Ala-Glu (OBzl)-Leu-Gly-Ile-Ala-Yyy(Yyy=OBzl, OPac) in CH_2Cl_2 using DMSO as a titration solvent.

The solvent-titrating curves indicate that the β-sheet-structure-stabilities of the protected peptides are related to the nature of the protecting groups. On the basis of the solvent-titration curves of Yyy-Leu-Ala-Glu(OBzl)-Leu-Gly-OPac(Yyy =Boc, Z, Npys, Fmoc) as shown in Figure 3, the β-sheet-structure-stabilizing potentials of α-amino protecting group

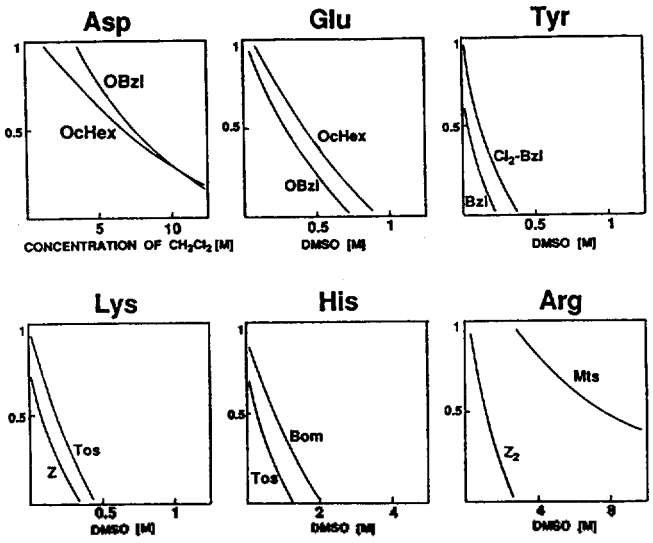


Figure 5. The solvent titration curves of Boc-Xxx(Yyy)-Ala-Glu (OBzl)-Leu-Gly-OPac [Xxx(Yyy)=Arg(Mts), Arg(Z), His(Bom), His(Tos), Asp(OBzl), Asp(OcHex), Glu(OBzl), Glu(OcHex), Tyr (Bzl), Tyr(Cl2-Bzl), Lys(Z), Lys(Tos)] Asp in CH_3CN using CH_2Cl_2 as a titration solvent and the others in CH_2Cl_2 using DMSO as a titration solvent.

in protected pentapeptides can be derived as follows: Fmoc>Z>Boc>Npys. Because Npys group don't have oxygen of carbonyl group, the peptide protected by Npys group is expected to show the decreased behaviors of one hydrogen-bond in protected peptide. Accordingly, the fine agreement between this expectation and the result of solvent-titration curve is appeared. The β-sheet structure stabilizing potentials of peptides protected by Fmoc, Z and Boc showed similar behaviors. Fmoc, Z and Boc are commonly used α-amino protecting groups in peptide synthesis and they appeared to have the same effect on β-sheet structure formation. Also, this is very useful on the design of synthetic route considered deprotect reactions.

On the other hand, Figure 4 shows the solvent-titration curves of Boc-Thr(Bzl)-Ala-Glu(OBzl)-Leu-Gly-Ile-Ala-Yyy (Yyy=Bzl, Pac). The influence of Bzl and Pac groups on the β-sheet-structure-stability of protected peptides showed similar behaviors. This result indicates that the β-sheet-structure-stability of protected peptides is independent of the kind of carboxyl protecting groups.

The β-sheet-structure-stability of each side chain protecting groups was compared by using solvent-titrating curves of Boc-Xxx-Ala-Glu(OBzl)-Leu-Gly-OPac. The side chain protecting groups are changed as follows: Bom and Tos in His, OBzl and OcHex in Asp, OBzl and OcHex in Glu, Bzl and Cl_2-Bzl in Tyr and Z and Tos in Lys. The influence by changing side chain protecting groups was not nearly showed. But the different conformational behavior for peptide containing Arg was observed. Namely, the difference between Mts and Z_2 in Arg was appeared. The β-sheet-structure-stability of the peptide protected by Mts is more prominent than by Z_2. It is considered that Z_2 as side chain protecting group of Arg is more useful on the peptide synthesis.

The β-sheet-structure-stabilizing potentials of each protecting group showed similar behaviors except Npys, Mts and Z_2. The results exhibit that the β-sheet-structure-stability of protected peptides are almost independent of the kinds of their protecting groups. In our previous paper, the $\langle SP_\beta \rangle$ values of protected peptides are in harmony with their β-sheet-structure stability. As the $\langle SP_\beta \rangle$ value increases, the β-sheet structure becomes more stable. This fact supports that the β-sheet structure stability of protected peptides is dependent on their amino acid compositions.

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1. The abbreviations for amino acids are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.* **1972**, *247*, 977. Amino acid symbols except for Gly denote the L-configration. Additional abbreviations used are the following: DMSO, dimethyl sulfoxide; DMF, N,N-dimethylformamide; IR, infrared; Boc, t-butoxycarbonyl; Pac, Phenacyl; Bzl, benzyl; OBzl, benzyl ester; DCC, dicyclohexylcarbodiimide; HOBt; 1H-1,2,3-benzotriazol-1-ol; Z, benzyloxycarbonyl; Npys, 3-Nitro-2-Pyridine sulfenyl; Fmoc, 9-fluorenyl methyloxycarbonyl; Mts, 2-mesitylenesulfonyl; Bom, benzyloxymethyl; Tos, Toluenesulfonyl; OcHex, cyclohexylester; Cl2-Bzl, 2,6-dichlorobenzyl; AcOEt, ethyl acetate;
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