  Bull. Chem. Soc. Jpn., 66, 2283—2288(1993)

Evaluation of the β-Sheet-Structure-Stabilizing Potential of 20 Kinds of Amino Acid Residues in Protected Deca- and Pentadecapetides[1)]

Jin-Shik LEE, Yuka MURAKAWA, Kentarou FUJINO, and Mitsuaki NARITA*
Department of Biotechnology, Faculty of Technology, Tokyo University of Agriculture and Technology, Nakamachi, Koganei 184
(Received December 17, 1992)

The β-sheet-structure-stabilizing potential of 20 kinds of guest amino acids has been evaluated by solvent titration for the protected pentapeptide, Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac, described previously. It has been proved that the <SPβ> value was effective in evaluating the β-sheet-structure stability of protected tri- to heptapeptide in organic solvents. In order to widen the application range of the <SPβ> value, deca- and pentadecapeptides were synthesized by fragment condensation of pentapeptides. The β-sheet-structure-stabilizing potentials of individual guest amino acids determined from deca- and pentadecapeptides showed different results from the <SPβ> value. Circular dichroism showed that deca- and pentadecapeptides adopted helix and random coil structures in organic solvents. The helix structure influences the solvation mechanism of these protected peptides.

One of the great problems in the chemical synthesis of peptides and proteins is the insolubility of protected peptides in organic solvents with increasing peptide chain length. This insolubility originates in β-sheet-structure formation by intermolecular hydrogen bonding of protected peptides, and it causes difficulty in successive condensation reactions. Therefore, the estimation of the β-sheet-structure stability of protected peptides in organic solvents becomes important in the design of peptide synthetic routes to avoid the problem of insolubility.

In previous papers, we proposed an <SPβ> value to estimate the β-sheet-structure stability and solvation mechanism of protected peptides in organic solvents.[2—4)] The SPβ values are the β-sheet-structure stabilizing potentials of 20 kinds of guest amino acid residues obtained for host-guest pentapeptides whose side chain functional groups were protected by suitable groups. Here, SPβ represents single residue information; peptide segment information is represented by <SPβ>. This β-sheet-structure stability and solubility of protected peptides can be estimated by using the arithmetic average, $<SP\beta>=\Sigma SP\beta_i/\Sigma n_i$. The β-sheet-structure stability and the solubility of a protected peptide were dependent on the $SP\beta_i$ of the amino acid resudues composing the protected peptide as well as the peptide chain length. In fact, the <SPβ> value was effective for the estimation of the β-sheet-structure stability of protected tri- to heptapeptide fragments of *E. coli* ribosomal protein L7/L12.[5)]

On the other hand, it has been proposed that the secondary structure formation of protected peptides is dependent on the chain length.[6—8)] It is thought that the conformational change from β-sheet to helix proceeds when the chain length is longer than the critical chain length for the formation of a stable helix. The formation of a stable helix in the peptides which have enough peptide chain length in solution is expected to make their solubility easy in various organic solvents. Practically, we found that the decapeptide had a β-sheet structure in the solid state and the icosapeptide obtained by fragment condenstion of this decapeptide had a stable helical structure and was easily soluble in organic solvents.[9)]

In the present study, the β-sheet-structure-stabilising potential of individual amino acids was obtained for deca- and pentadecapeptides to estimate the β-sheet-structure stability of protected peptides longer than a decapeptide. The β-sheet-structure-stabilizing potential obtained here is defined as SPβ′. The difference between the SPβ value obtained previously and the SPβ′ was also investigated.

The daca- and pentadecapeptides were synthesized by fragment condensation reactions between the amino- and carboxyl components obtained from Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac (X = Ala, Arg(Mts), Asn, Asp(OBzl), Cys(Bzl), Gln, Glu(OBzl), Gly, His(Bom), Ile, Leu, Lys(Z), Met(O), Phe, Pro, Ser(Bzl), Thr(Bzl), Trp(CHO), Tyr(Bzl), or Val) which were used when the SPβ value was obtained. According to the β-sheet-structure-disrupted behaviors of deca- and pentadecapeptides which were monitored by IR absorption measurements, guest amino acids were classified into six groups. Especially, when deca- and pentadecapeptides were dissolved in organic solvents, they formed random coil structures accompanied by helix structures. The helix content of the protected peptides was obtained from circular dichloroism measurements and the helix-structure stability of guest amino acid residues was estimated from the difference in the CD spectra of the individual peptides.

Experimental

Materials. Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac was prepared by the procedure reported in our previous study.[3)] In order to prepare deca- and pentadecapeptides by fragment condensation, deprotection of the Pac group in the protected pentapeptide was performed by treatment

with Zn/AcOH to yield the carboxyl component. The removal of the Boc group was carried out by treatment with TFA/anisole (4/1, v/v) to give the amino component. The synthetic procedure for protected deca- and pentadecapeptides is shown in Scheme 1. The coupling reactions were carried out in a mixture of CH_2Cl_2 and DMF using DCC and HOBt as coupling reagents.[10,11] The reactions were monitored by the Kaiser test. After the usual work-up procedures, all the products were purified by repeated washing with hot methanol. They gave a single peak on HPLC and were negative for the Kaiser test. Acid hydrolysis of the peptides was carried out for the pentapeptide with propionic acid/12 M HCl (2/1, v/v) at 115° C for 7 d.[12] The amino acid ratios of the acid hydrolysates were in good agreement with the calculated values as reported previously for the pentapeptide.[3]

Circular Dichroism. The circular dichroism (CD) spectra were recorded using a JASCO model J-40AS spectrophotometer. The spectra were obtained using cylindrical fused quartz cells of 0.2 and 10-mm path lengths. Dry prepurified nitrogen was employed to keep the instrument oxygen-free during the experiments. The measurements were performed at a sample concentration of 7×10^{-4} M (1 M=1 mol dm^{-3}) in CH_2Cl_2/HFIP (9/1, v/v) at room temperature. The CD data represent the average values from four separate recordings. The calibration was based upon $[\theta]_{304}=1.12\times10^{-4}$ deg cm^2 $dmol^{-1}$ for a purified sample of androsterone (Tokyo Kasei) in 5×10^{-3} % dioxane solution.

IR Absorption Spectrum Measurements. The IR absorption spectra of the protected 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 or ditched sodium chloride plates. The protected peptides were dissolved or suspended in CH_2Cl_2 containing a variety of concentrations of DMSO or HFIP. The peptides in the suspended state were recorded by placing them between ditched sodium chloride plates. The concentrations of individual peptides were kept around 1.6×10^{-2} M.

Results

The IR absorption spectrum of the protected peptide showed 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.[13] The bands observed around 1670 and 3420 cm^{-1} were assigned to an unordered structure. The stability of the β-sheet structure of Boc–(X–Ala–Glu(OBzl)–Leu–Gly)$_n$–OPac (n=2,3) was evaluated by monitoring the IR absorption band around 1630 cm^{-1} in CH_2Cl_2 containing a variety of concentrations of DMSO or HFIP. The β-sheet structure of the protected peptides was disrupted in CH_2Cl_2 with increasing amounts of DMSO or HFIP, resulting in the successive increase in the intensity of the band around 1670 cm^{-1}. The behavior of the β-sheet-structure disruption of the peptides depended on the guest amino acid and the results are summarized in Tables 1 and 2. Typical IR absorption spectra in the amide I regions of the peptides are shown in Fig. 1.

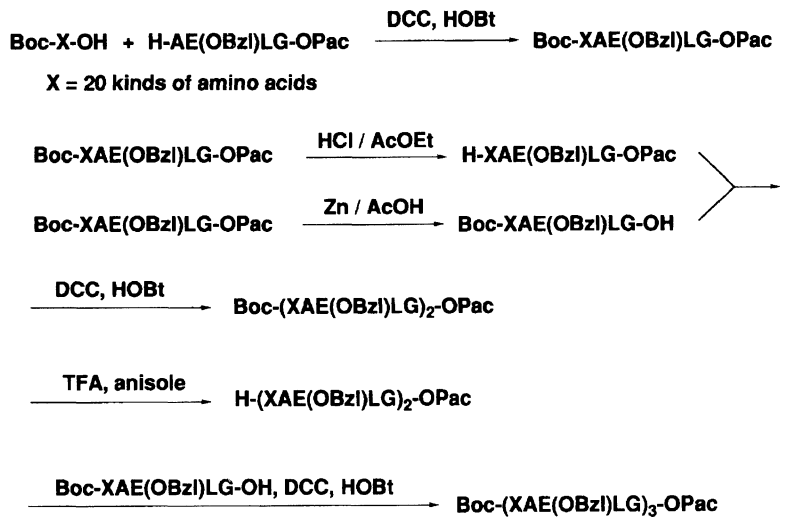
In order to summarize the results of the IR absorption spectra in Tables 1 and 2, the IR absorption spectra of the peptides were classified into five groups. The first group (Fig. 1a) showed a strong band around 1630 cm^{-1} with no or a weak shoulder band around 1670 cm^{-1}. They are assigned to β in Tables 1 and 2. The second group (Fig. 1b) exhibited a strong band around 1630 cm^{-1} and a medium shoulder band around 1670 cm^{-1} (β/r(sh) in Tables 1 and 2), the third (Fig. 1c), medium bands around both 1630 and 1670 cm^{-1} (β/r in Tables 1 and 2), and the fourth (Fig. 1d), a medium shoulder band around 1630 cm^{-1} and a strong band around 1670 cm^{-1} (β(sh)/r in Tables 1 and 2). The fifth group (Fig. 1e) showed a strong band around 1670 cm^{-1} with no or a weak shoulder band around 1630 cm^{-1} (r in Tables 1 and 2). The β-sheet-structure stability of the protected deca- and pentadecapeptides were dependent on the nature of the guest amino acid residues. On the basis of the results, the β-sheet-structure-stabilizing potentials, SPβ', of the 20 kinds of guest amino acid residues in the protected peptides were classified into six groups.

The CD spectrum of the pentadecapeptide in HFIP/CH_2Cl_2 (9/1, v/v) has two negative bands at 204 nm (the amide π–π^* transition) and 222 nm (the amide n–π^* transition). Figure 2 shows representative spectra for the Boc–(X–Ala–Glu(OBzl)–Leu–Gly)$_3$–OPac (X=Arg(Mts), Asn, Asp(OBzl), Ile, Ser(Bzl), and Thr-

Table 1. β-Sheet-Structure Dispersion[a] of Decapeptides in Mixed Solvents[b]

Protected peptide	Mixed solvents			
	1	**2**	**3**	**4**
Boc–(PAELG)$_2$–OPac	r	—	—	—
Boc–(DAELG)$_2$–OPac	—	r	—	—
Boc–(RAELG)$_2$–OPac	—	r	—	—
Boc–(KAELG)$_2$–OPac	—	r	—	—
Boc–(NAELG)$_2$–OPac	—	r	—	—
Boc–(HAELG)$_2$–OPac	—	β(sh)/r	r	—
Boc–(QAELG)$_2$–OPac	β	β(sh)/r	r	—
Boc–(CAELG)$_2$–OPac	β	β/r	r	—
Boc–(MAELG)$_2$–OPac	β	β/r	r	—
Boc–(WAELG)$_2$–OPac	β	β/r	r	—
Boc–(LAELG)$_2$–OPac	β	β/r(sh)	r	—
Boc–(YAELG)$_2$–OPac	β	β/r(sh)	r	—
Boc–(TAELG)$_2$–OPac	β	β	r	—
Boc–(EAELG)$_2$–OPac	β	β	r	—
Boc–(SAELG)$_2$–OPac	β	β	r	—
Boc–(VAELG)$_2$–OPac	β	β	β(sh)/r	—
Boc–(FAELG)$_2$–OPac	β	β	β(sh)/r	—
Boc–(IAELG)$_2$–OPac	β	β	β(sh)/r	—
Boc–(GAELG)$_2$–OPac	β	β	r	—
Boc–(AAELG)$_2$–OPac	β	β	r	—

a) Structure: β,β-sheet structure; r, random or α-helix structure; sh, shoulder. b) Mixed solvents: **1**, CH_2Cl_2 : DMSO=4 : 1 (v/v); **2**, CH_2Cl_2 : DMSO=3 : 2 (v/v); **3**, CH_2Cl_2 : DMSO=2 : 3 (v/v); **4**, CH_2Cl_2 : HFIP=95 : 5 (v/v).



Scheme 1.

Table 2. β-Sheet-Structure Dispersion[a] of Pentadecapeptides in Mixed Solvents[b]

Protected peptide	Mixed solvents 1	2	3	4
Boc–(PAELG)$_3$–OPac	—	—	r	—
Boc–(NAELG)$_3$–OPac	—	—	r	—
Boc–(RAELG)$_3$–OPac	—	—	β/r	—
Boc–(KAELG)$_3$–OPac	—	—	β/r(sh)	—
Boc–(WAELG)$_3$–OPac	—	—	β/r(sh)	—
Boc–(CAELG)$_3$–OPac	—	—	β	—
Boc–(YAELG)$_3$–OPac	—	—	β	—
Boc–(DAELG)$_3$–OPac	—	—	β	r
Boc–(QAELG)$_3$–OPac	—	—	β	r
Boc–(LAELG)$_3$–OPac	—	—	β	r
Boc–(SAELG)$_3$–OPac	—	—	β	β(sh)/r
Boc–(TAELG)$_3$–OPac	—	—	β	β(sh)/r
Boc–(MAELG)$_3$–OPac	—	—	β	β(sh)/r
Boc–(EAELG)$_3$–OPac	—	—	β	β/r
Boc–(IAELG)$_3$–OPac	—	—	β	β/r
Boc–(VAELG)$_3$–OPac	—	—	β	β/r(sh)
Boc–(HAELG)$_3$–OPac	—	—	β	β
Boc–(AAELG)$_3$–OPac	—	—	β	β
Boc–(GAELG)$_3$–OPac	—	—	β	β
Boc–(FAELG)$_3$–OPac	—	—	β	β

a) Structure: β,β-sheet structure; r, random or α-helix structure; sh, shoulder. b) Mixed solvents: **1**, CH_2Cl_2 : DMSO = 4 : 1 (v/v); **2**, CH_2Cl_2 : DMSO = 3 : 2 (v/v); **3**, CH_2Cl_2 : DMSO = 2 : 3 (v/v); **4**, CH_2Cl_2 : HFIP = 95 : 5 (v/v).

(Bzl)). Judging from the magnitude of $[\theta]$ and the position of these bands, these peptides are characteristic of α-helix and random coil conformation. The helix content was deduced by following the intensity of the minimum at 222 nm ($-[\theta]_{222}$). Figure 2 shows the helix content of each peptide. The value of $-[\theta]_{222}$ for 100% helix has been estimated at 35000.[14] By comparing the CD spectra of the protected peptides, the α-helix-structure stability of the protected pentadecapeptides was strongly dependent on the nature of the guest amino acid residues.

Discussion

The host–guest deca- and pentadecapeptides were synthesized to evaluate the β-sheet-structure stabilizing potentials of protected peptides longer than decapeptides. The SPβ′ values of 20 kinds of guest amino acid residues evaluated for Boc–(X–Ala–Glu(OBzl)–Leu–Gly)$_n$–OPac (n=2,3) was compared with the SPβ values evaluated for Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac. On the basis of the disruption of the β-sheet structure of the host–guest deca- and pentadecapeptides, the guest amino acid residues, X, were classified into six groups as shown in Table 3. When the protected peptide did not dissolve in DMSO alone, the β-sheet-structure stability was evaluated in a mixture of CH_2Cl_2/HFIP (90/10, v/v).

The amino acid residue β-sheet-structure-stabilizing potentials obtained from deca- and pentadecapeptides were compared. Ala, Arg(Mts), Asn, Cys(Bzl), Gln, Glu(OBzl), Gly, Leu, Lys(Z), Pro, Thr(Bzl), and Val are on the same rank. Asp(OBzl), Ile, Met(O), Phe, Ser(Bzl), and Tyr(Bzl) have one rank differences. The decapeptide classification is in good agreement with the pentadecapeptide classification except for His(Bom) and Trp(CHO) having two rank differences. This result shows that the β-sheet-structure-stabilizing potential of each amino acid residue is nearly unchanged even though the peptide chain length increased from a deca- to a pentadecapeptide. Thus, the β-sheet-structure-stabilizing potentials obtained from deca- and pentadecapaptides were conclusively classified as shown in Table 3.

However, when the SPβ′ values of the amino acid residues in the conclusive classification and the SPβ values obtained previously were compared, differences were observed in the classification of the amino acid residues. It is believed that the difference in the β-sheet-structure-stabilizing potentials of these amino acids between

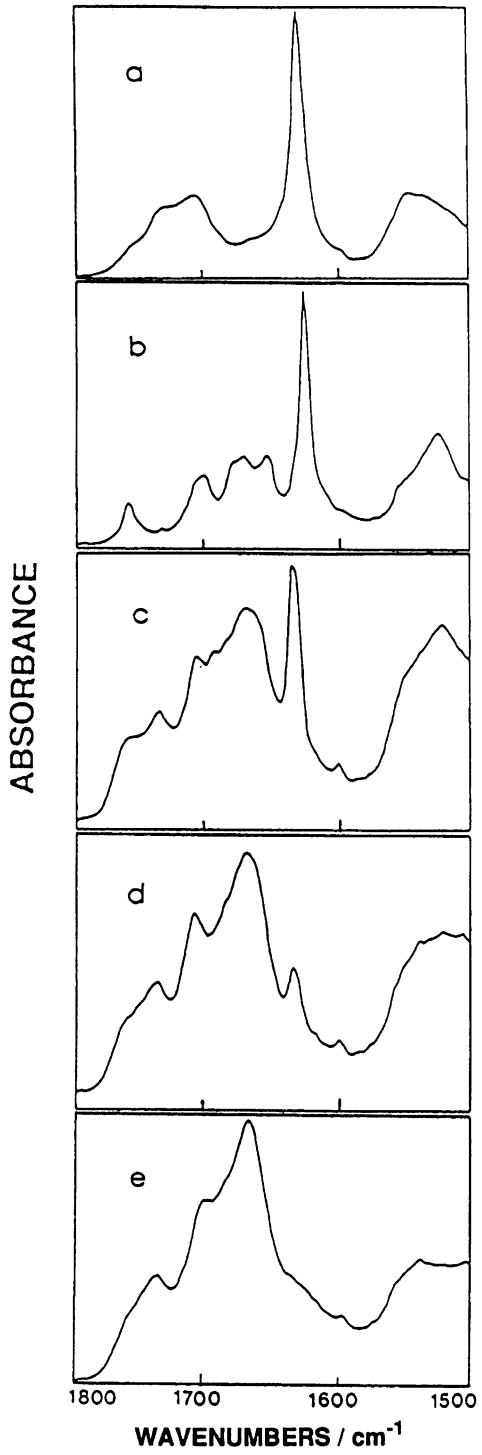


Fig. 1. The classification of typical IR absorption spectra in the amide I region of the peptides in Table 1. a) β, Boc–(QAELG)$_2$–OPac in mixed solvent **1**, b) β/r(sh), Boc–(LAELG)$_2$–OPac in mixed solvent **2**, c) β/r Boc–(MAELG)$_2$–OPac in mixed solvent **2**, d) β(sh)/r Boc–(VAELG)$_2$–OPac in mixed solvent **3**, e) r, Boc–(DAELG)$_2$–OPac in mixed solvent **2**.

SPβ and SPβ' originates from a difference in the solvation mechanism. When a peptapeptide is solvated sufficiently, it has a random coil structure. On the other

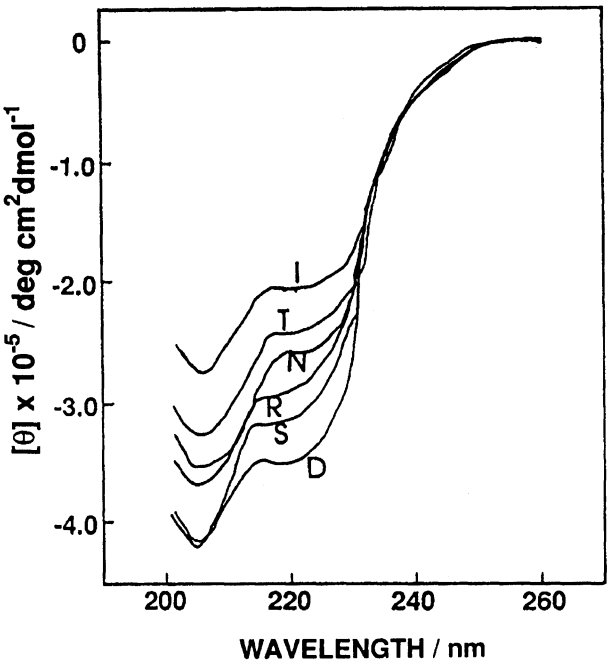


Fig. 2. CD spectra of Boc–(XAELG)$_3$–OPac (X=Arg-(Mts), Asn, Asp(OBzl), Ile, Ser(Bzl), Thr(Bzl)) in CH_2Cl_2/HFIP (90/10, v/v).

hand, when deca- and pentadecapeptides having sufficient chain lengths for helix structure formation are solvated, they have a mixture of random coil and helix structures.

In fact, the CD spectra of the deca- and pentadecapeptides measured in CH_2Cl_2/HFIP (90/10, v/v) showed the existence of helix and the results are summarized in Table 4. Also, the helix-structure-stabilizing potentials, SPα, of 20 kinds of amino acid residues were determined from the helix content of each peptide resulting from the difference in the guest amino acid residues. We classified the 20 kinds of amino acid residues into six groups based on the helix content of the peptides as determined by SPα.

As a result, Arg(Mts), Asn, and Gln have high helix-forming tendencies in spite of the fact that they have high β-sheet-structure stabilizing potentials. It is expected that the helix-forming tendency has an effect on the solvation of deca- and pentadecapeptides. On the other hand, it is confirmed that helix structure is not contained in pentapeptides. When the β-sheet structure is disrupted by solvation, it is clear that the helix-structure-stabilizing potential of each amino acid is reflected in peptide chain lengths longer than a decapeptide.

Hence, the β-sheet-structure stability of protected peptides longer than decapeptides and the solubility correlated with the β-sheet-structure stability are difficult to estimate correctly by using the <SPβ> value. A new parameter containing the influence of the helix-structure-stabilizing potentials of each amino acid residue is needed. We determined the β-sheet-struc-

Table 3. The β-Sheet-Structure-Stabilizing Potentials, SPβ and SPβ′, of the 20 Kinds of Guest Amino Acid Residues[a)]

Class	$(XAELG)_2$	$(XAELG)_3$	Conclusive classification (SPβ′)	SPβ	SPα
6	I, G, A	A, G, F	G, A, I	R, V, N	D, C, S
5	E, S, V, F	E, I, V, H	E, S, V, F	Q, G, H, A, I	E, R, Q, N
4	W, L, Y, T	L, S, T, M	L, T, Y, H	F, W, Y	A, K, T
3	H, Q, C, M	C, Y, D, Q	C, Q, W, M	C, K, L, E, M	Y, L, W, I, F
2	D, R, K, N	N, R, K, W	N, K, R, D	S, T	V, G
1	P	P	P	D, P	H, P, M

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

Table 4. The Helix Content of Protected Peptides[a)]

Model peptides	Helix content/%	
	Decapaptide (n=2)	Pentadecapeptide (n=3)
Boc-$(DAELG)_n$-OPac	33	66
Boc-$(CAELG)_n$-OPac	28	62
Boc-$(SAELG)_n$-OPac	26	61
Boc-$(EAELG)_n$-OPac	23	56
Boc-$(RAELG)_n$-OPac	24	55
Boc-$(QAELG)_n$-OPac	30	54
Boc-$(NAELG)_n$-OPac	25	51
Boc-$(KAELG)_n$-OPac	20	48
Boc-$(AAELG)_n$-OPac	21	48
Boc-$(TAELG)_n$-OPac	18	46
Boc-$(LAELG)_n$-OPac	18	44
Boc-$(YAELG)_n$-OPac	18	44
Boc-$(WAELG)_n$-OPac	17	43
Boc-$(IAELG)_n$-OPac	18	41
Boc-$(FAELG)_n$-OPac	20	40
Boc-$(VAELG)_n$-OPac	20	37
Boc-$(GAELG)_n$-OPac	10	29
Boc-$(HAELG)_n$-OPac	15	23
Boc-$(PAELG)_n$-OPac	9	18
Boc-$(MAELG)_n$-OPac	26	18

a) Helix content; $H=[\theta]_{222}/-35000$, $-[\theta]_{222}=\phi\times MW/d\times c\times 100$. c; concentration/g ml^{-1}, d; cell path length/dm, ϕ; deg, MW; molecular weight.

Table 5. The Influence of Chain Length on the β-Sheet-Structure-Stabilizing Potentials of Protected Peptides

Chain length	Solid state conformations	Comformational changes by solvation	Comformational parameters
Pentapeptides	β-Structure	β→Random	SPβ
Deca- and Pentadecapeptides	β-Structure	β→Helix+Random	SPβ′

ture-stabilizing potentials (SPβ′) of 20 kinds of amino acid residues obtained from decapeptides and pentadecapeptides. When the SPβ′ values of deca- and pentadecapeptide segments are estimated, β-sheet-structure stabilities in organic solvents and the solubilities corresponding to those shown in Table 5 can be predicted. The SPα value shows the helix-structure-stabilizing potential of each amino acid residue. We think that if the SPα value relating to the length of the peptide segment is applied, the helix-structure formation of the peptide segment can be predicted.

According to the length of the peptide segment, prediction of the β-sheet-structure stability of a peptide by using the <SPβ> and <SPβ′> values can contribute much to the design of a synthetic route. Prediction of the formation of helix structure by the <SPα> value can also contribute much to the design of the synthetic route of a peptide or protein. The prediction of the β-sheet-structure stability of protected peptides using the parameters SPβ, SPα, and SPβ′ and the relationship between them will be discussed elsewhere.

We wish to thank Professor Fukuji Higashi and Dr. Shokichi Ohuchi of our department for useful discussions.

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: DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic acid; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; Boc, *t*-butoxycarbonyl; Pac, phenacyl; Bzl, benzyl; OBzl, benzyl ester; Bom, benzyloxymethyl; CHO, formyl; Z, benzyloxycarbonyl; Mts, 2-mesitylene sulfonyl; AcOH, acetic acid; IR, infrared; DCC, dicyclohexylcarbodiimide; HOBt, 1*H*-1,2,3-benzotriazol-1-ol.

2) M. Narita and Y. Kojima, *Bull. Chem. Soc. Jpn.*, **62**, 3572 (1989).

3) M. Narita, J.-S. Lee, Y. Murakawa, and Y. Kojima, *Bull. Chem. Soc. Jpn.*, **66**, 483 (1993).

4) M. Narita, J.-S. Lee, S. Hayashi, Y. Yamazaki, and T. Sugiyama, *Bull. Chem. Soc. Jpn.*, **66**, 500 (1993).

5) M. Narita, J.-S. Lee, S. Hayashi, and M. Hitomi, *Bull. Chem. Soc. Jpn.*, **66**, 489 (1993).

6) C. Toniolo, G. M. Bonora, H. Anzinger, and M. Mutter, *Macromolecules*, **16**, 147 (1983).

7) M. Mutter, F. Maser, K.-H. Altmann, C. Toniolo, and G. M. Bonora, *Biopolymers*, **24**, 1057 (1985).

8) R. Katakai, *Biopolymers*, **25**, 1 (1986).

9) M. Narita, Y. Kojima, and S. Isokawa, *Bull. Chem. Soc. Jpn.*, **62**, 1976 (1989).

10) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

11) M. Narita, M. Doi, H. Sugasawa, and K. Ishikawa, *Bull. Chem. Soc. Jpn.*, **58**, 1473 (1985); M. Narita, K. Ishikawa, H. Sugasawa, and M. Doi, *Bull. Chem. Soc. Jpn.*, **58**, 1731 (1985).

12) F. C. Westall, J. Scotcher, and A. B. Robinson, *J. Org. Chem.*, **37**, 3363 (1972).

13) T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961); T. Miyazawa, "Poly-α-Amino Acids," ed by G. D. Fasman, Marcel Dekker, New York (1967), pp. 69—103.

14) M. Mutter, H. Mutter, and E. Bayer, "Peptides," ed by M. Goodman and J. Meienhofer, Wiley, New York (1977), p. 143.