The Classification of Twenty Common Amino Acid Residues in Protected Peptides by Their β-Sheet-Structure-Stabilizing Potentials, SP_β, and Its Application to Peptide Synthesis[1)]

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A host-guest approach was devised to evaluate the β-sheet-structure-stabilizing potentials, SP_β, of the 20 common 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 host-guest pentapeptides, namely, Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac and Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac in which X stands for guest amino acid residues, was easily disrupted in CH_2Cl_2 by increasing amounts of DMSO. The disrupted behaviors were strongly dependent on the nature of the guest amino acid residues and the 20 guest amino acid residues could be classified into six groups. Therefore, we arbitrarily defined SP_β as values of one to six. A method for estimating the β-sheet-structure stability of protected peptides by their arithmetic average $<SP_\beta>$ values was proposed, and it was applied to peptide synthesis. The significance of the estimation method in the design of synthetic routes for peptides and proteins is briefly discussed.

One of the most serious obstacles in peptide and protein syntheses is the insolubility of protected peptides in organic solvents which causes difficulty in successive reactions. As the insolubility may be due to intermolecular hydrogen-bonded β-sheet aggregation, the disruption of the β-sheet structure caused by sufficient solvation of a peptide chain is significantly important to carry out the successive reactions smoothly. Thus, the estimation of the β-sheet-structure disruption of protected peptides is essential for the design of synthetic routes for peptides and proteins. In previous papers,[2,3)] we proposed a predictive method for the solubility of protected peptides equal to or larger than an octapeptide in highly polar solvents commonly used in peptide synthesis. The prediction was carried out using average coil conformation $<P_c>$ values of protected peptides and successfully employed in the design of synthetic routes for peptides and proteins.[3)] Recently, the predictive method was applied to the identification of difficult sequences in solid-phase peptide synthesis.[2—9)] Here, the $<P_c>$ value of a protected peptide is defined as the arithmetic average of the coil conformational parameters, P_c, of the amino acid residues composing the protected peptide. It should be emphasized that the P_c parameters were originally determined for the 20 intact amino acid residues on the basis of the three-dimensional structures of 29 kinds of native globular proteins.[2)] But, they were not necessarily suitable for amino acid residues with protected side chains.

In recent studies,[10—13)] we found that the β-sheet-structure disruption of protected peptides by organic solvents was strongly dependent on their electron-acceptor numbers (AN) and electron-donor numbers (DN), and that a solvent having a large AN or DN value had a high potential for the β-sheet-structure disruption. The β-sheet-structure-disrupted behaviors of resin-bound host-guest peptides were also examined, and from the results it became possible to evaluate the β-sheet-structure-stabilizing potentials of guest amino acid residues with protected side chains.[14)]

In the present study, we have attempted to evaluate the β-sheet-structure-stabilizing potentials, SP_β, of the 20 kinds of amino acid residues in protected peptides, and classify them into six groups having SP_β values of one to six. The utilization of SP_β for the estimation of the β-sheet-structure stability of protected peptides is finally proposed. The host-guest pentapeptides used in this study were Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac and Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac, in which X stands for guest amino acid residues as follows: 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), and Val.

Experimental

Materials. Boc–Val–Asp(OBzl)–Ala–Gly–OPac and Boc–Ala–Glu(OBzl)–Leu–Gly–OPac were prepared in CH_2Cl_2 by common stepwise elongation using DCC and HOBt as coupling reagents.[15)] Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac and Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac were similarly prepared in a mixture of CH_2Cl_2 and DMF by coupling reactions of Boc–X–OH with HCl·H–Val–Asp(OBzl)–Ala–Gly–OPac and with HCl·H–Ala–Glu(OBzl)–Leu–Gly–OPac, respectively, which were obtained from the corresponding Boc-derivatives by treatment with 3.6 M (1M=1 mol dm^{-3}) HCl/AcOEt. The coupling reactions were repeated until the Kaiser test became negative, using excess amounts of Boc–X–OH and DCC. 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 hydrolyses of the host-guest peptides were carried out with propionic acid/12 M HCl (volume ratio, 2/1) at 115°C for 5 d.[16)] Except for Ser, Thr, and Trp residues, the amino acid ratios of the acid hydrolysates were in good agreement with the calculated values.

Elemental analyses were performed for Boc–(X–Val–Asp(OBzl)–Ala–Gly)$_3$–OPac and Boc–(X–Ala–Glu(OBzl)–Leu–Gly)$_3$–OPac (X=Ser(Bzl), Thr(Bzl), and Trp(CHO)) and they were in good agreement with the calculated values. The pentadecapeptides will be used for the evaluation of the helical structure-stabilizing potentials, SP_α, of the 20 common amino acid residues in a following paper.

IR Absorption Spectra Measurements. The IR absorption spectra of the host-guest pentapeptides 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–Val–Asp(OBzl)–Ala–Gly–OPac (X=Asp(OBzl), Glu(OBzl), His(Bom), Leu, Pro, Ser(Bzl), and Thr(Bzl)) and Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac (X=Asp(OBzl) and Pro) were dissolved or suspended in CH_2Cl_2 containing a variety of concentrations of DMSO. The exclusive peptides were dissolved in CH_3CN containing various concentrations of CH_2Cl_2. The concentration of each peptide was kept at 5.0×10^{-2} M.

Results

The β-sheet-structure-stabilizing potentials of the 20 common amino acid residues were evaluated by monitoring the β-sheet-structure-disrupted behaviors of the host-guest pentapeptides in CH_2Cl_2 or CH_3CN using a solvent-titration method introduced by Toniolo et al.[17] DMSO or CH_2Cl_2 was used as a titrating solvent. Side chain functional groups of guest amino acid residues were protected by suitable groups commonly used in peptide synthesis. Figure 1 shows the IR absorption spectra of Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac (X=Val) in CH_2Cl_2 alone at 1.0×10^{-2}, 3.0×10^{-2}, and 5.0×10^{-2} M. The behavior of the β-sheet-structure disruption of the peptide in CH_2Cl_2 alone depended on its concentration, and in this study, IR absorption measurements were carried out at 5.0×10^{-2} M. The IR absorption in CH_2Cl_2 alone of the host-guest pentapeptides other than Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac where X is Asp(OBzl), Glu(OBzl), His(Bom), Leu, Pro, Ser(Bzl), and Thr(Bzl) and Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac where X is Asp(OBzl) and Pro 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.[18] In some cases, they were accompanied by a band around 1670 cm^{-1}, mainly assigned to an unordered structure.[18] The peptides whose β-sheet structure was disrupted in CH_2Cl_2 alone were kept in CH_3CN. Thus, the behavior of the β-sheet-structure disruption was studied in CH_2Cl_2 or CH_3CN by measuring the successive decrease in the intensity of the band around 1630 cm^{-1} concurrent with the successive addition of a titrating solvent, DMSO or CH_2Cl_2. Figure 2 shows a typical IR absorption spectra of Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac (X=Ala) in CH_2Cl_2 containing a variety of molar concentrations of DMSO. The solvent-titration curves of Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac (Fig. 3) and Boc–X–Ala–Glu(OBzl)–

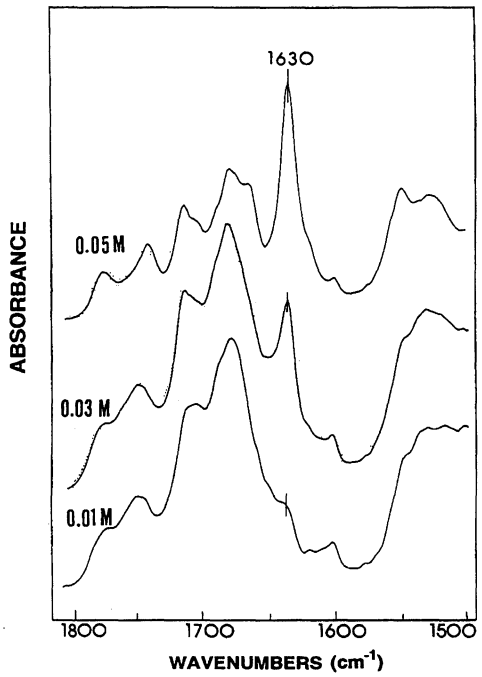

Fig. 1. IR absorption spectra of a host-guest pentapeptide (X=Val) in CH_2Cl_2 alone. The numbers in the Figure indicate the molar concentration of peptide.

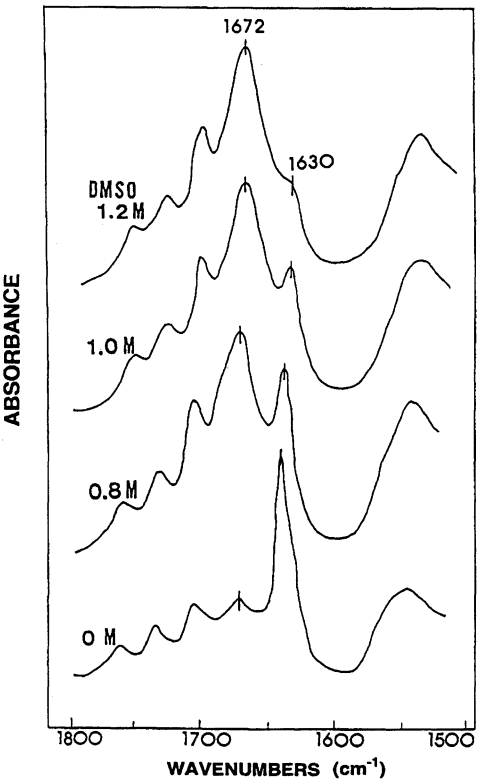

Fig. 2. Typical IR absorption spectra in the amide I region of a host-guest pentapeptide (X=Ala) in CH_2Cl_2 containing various molar concentration of DMSO.

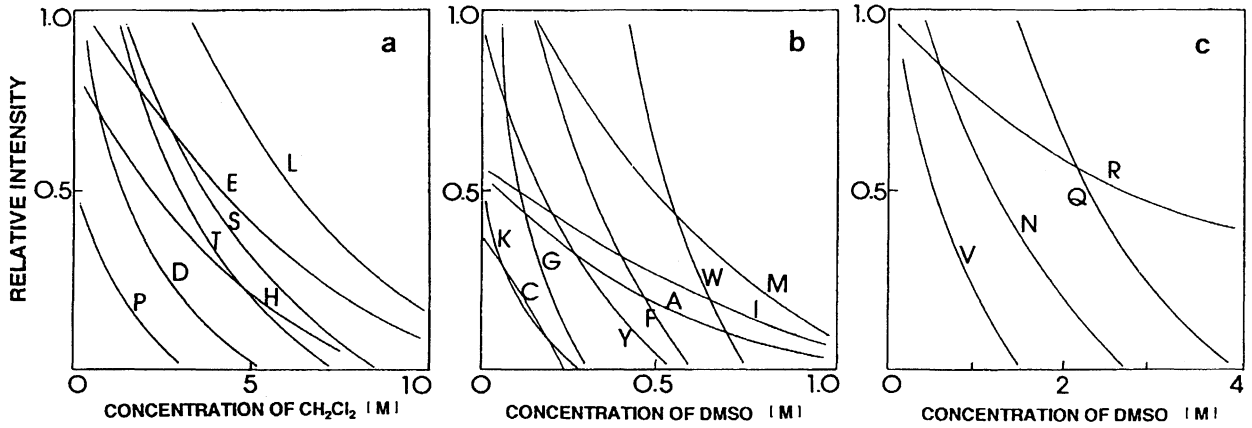

Fig. 3. The solvent titration curves of Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac (a) in CH_3CN using CH_2Cl_2 as a titration solvent and (b), (c) in CH_2Cl_2 using DMSO as a titration solvent. A guest amino acid residue for each titration curve is shown in the Figure using one-letter symbol.[22] R=Arg(Mts), Q=Gln, N=Asn, V=Val, M=Met(O), I=Ile, W=Trp(CHO), A=Ala, F=Phe, Y=Tyr(Bzl), G=Gly, K=Lys(Z), C=Cys(Bzl), L=Leu, E=Glu(OBzl), S=Ser(Bzl), T=Thr(Bzl), H=His(Bom), D=Asp(OBzl), P=Pro.

Leu–Gly–OPac (Fig. 4) 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} due to the ester carbonyl groups of Gly–OPac and Asp(OBzl) or Glu(OBzl) as a standard and normalizing to 1.0 for each relative intensity in CH_2Cl_2 or CH_3CN. As shown in Figs. 3 and 4, the successive addition of a titrating solvent induced a dramatic decrease in the band around 1630 cm^{-1} and an increase in the broad band around 1670 cm^{-1}, indicating that the β-sheet-structure stabilities of the host-guest pentapeptides are strongly dependent on the nature of the guest amino acid residues in both series of protected peptides.

Discussion

On the basis of the solvent-titration curves of Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac, the scale for the β-sheet-structure-stabilizing potentials of the 20 kinds of guest amino acid residues in the peptides can be derived as follows: Arg(Mts) > Gln > Asn > Val > Met(O) > Ile > Trp(CHO) > Ala > Phe > Tyr(Bzl) > Gly > Lys(Z) > Cys(Bzl) > Leu > Glu(OBzl) > Ser(Bzl) > Thr(Bzl) > His(Bom) > Asp(OBzl) > Pro. Except for Arg(Mts), Met(O), Trp(CHO), His(Bom) and Cys(Bzl), this is also the case for resin-bound host-guest hexapeptides, Boc–Val–Ile–X_2–Val–Ile–resin.[14] We selected DMSO, CH_2Cl_2 and CH_3CN as the titrating solvents because DMSO and CH_2Cl_2 are used in coupling reactions and DMSO, CH_2Cl_2 and CH_3CN have no IR absorption band around 1630 cm^{-1}. The host-guest pentapeptides were randomly chosen without any consideration of amino acid composition and sequence in this study, while the resin-bound peptides have been designed to be suitable for the evaluation of the β-sheet-structure-stabilizing potential of guest amino acid residues. Namely, C^{β}-branched Val and Ile residues are expected to stabilize the β-sheet structure of peptides,[10,19] and the sequence of Ile–X_2–Val resists helix formation due to the repulsion between Ile and Val residues in a helix.[11,20] Accordingly, the fine agreement between the scales of amino acids derived from two series of host-guest peptides supports the propriety of the evaluation. On the other hand, the scale derived from the solvent-titration curves of Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac is as follows: Arg(Mts) > Ala > Gly > Trp(CHO) > Asn > Gln > His(Bom) > Phe > Ser(Bzl) > Ile > Val > Cys(Bzl) > Glu(OBzl) > Met(O) > Leu > Lys(Z) > Thr(Bzl) > Tyr(Bzl) > Asp(OBzl) > Pro. Using the above three scales, we attempted to evaluate the β-sheet-structure-stabilizing potentials of the guest amino acid residues and classify them into six groups as shown in Table 1.

The conclusive classification in Table 1 was carried out as follows. When an amino acid residue in a certain group was in at least two series of host-guest peptides, it was assigned to that group. Thus, Arg(Mts), Val, and Asn are in the sixth group, Gln and His(Bom) in the fifth, Phe in the fourth, Leu, Cys(Bzl), Glu(OBzl), and Met(O) in the third, Ser(Bzl) and Thr(Bzl) in the

Table 1. The β-Sheet-Structure-Stabilizing Potential of the 20 Kinds of Guest Amino Acid Residues[a]

$SP_{\beta i}$	Host-Guest peptides			Conclusive classification
	XVDAG	XAELG	VIX_2VI-resin	
6	R, Q, N, V	R, A, G	I, V, N	R, V, N
5	M, I	W, N, Q, H	H, Q, C, Y	Q, G, A, H, I
4	W, A, F, Y	F, S, I, V	F, G, K, L, R	F, W, Y
3	G, K, C, L	C, E, M, L	T, A, M, E	C, K, L, E, M
2	E, S, T, H	K, T, Y	W, S	S, T
1	D, P	D, P	D, P	D, P

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

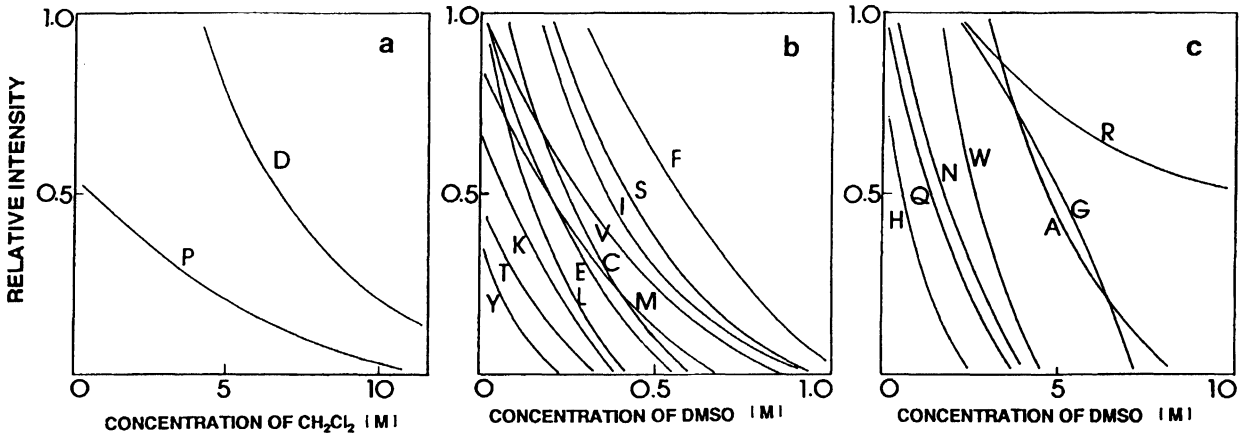

Fig. 4. The solvent titration curves of Boc–X–Ala–Glu(OBzl)–Leu–Gly–Opac (a) in CH_3CN using CH_2Cl_2 as a titration solvent and (b), (c) in CH_2Cl_2 using DMSO as a titration solvent. A guest amino acid residue for each titration curve is shown in the Figure using a one-letter symbol.

second, and Asp(OBzl) and Pro in the first. Lys(Z) belongs to the second, third, and fourth groups in the corresponding host peptieds and was finally assigned to the third group of the average. The groups of Ala, Gly, Tyr(Bzl), and Trp(CHO) were determined by their group average numbers.

The β-sheet-structure-stabilizing potentials of these four amino acid residues appeared to be strongly influenced by the amino acid composition of the peptide. The classification in the case of Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac is in harmony with the conclusive classification in regard to the fourteen amino acid residues and the deviation of one group is for Gln, Ala, and Glu(OBzl) and that of two groups is for Met(O) and Gly. The classification in the case of Boc–Val–Ile–X_2–Val–Ile–resin is compatible with the conclusive classification in regard to the ten amino acid residues and the deviation of one group is for Tyr(Bzl), Gly, Ile, Lys(Z), Leu, and Thr(Bzl) and that of two groups is for Cys(Bzl), Arg(Mts), Ala, and Trp(CHO). In fact, the labile Ala, Cys(Bzl), Gly, Trp(CHO), and Tyr(Bzl) have a deviating tendency. On the other hand, the result of Boc–X–Ala–Glu(OBzl)–Leu–Gly–OPac appears to reflect more or less the nature of the host peptide, namely, its helix-forming tendency. The deviation over two groups is for Ser(Bzl), Val, and Tyr(Bzl). The helix-structure-stabilizing potentials, SP_α, of the 20 common amino acid residues in protected peptides will be reported elsewhere. As discussed in a previous paper[10,20] for the P_α, P_β, and P_c parameters of the 20 kinds of intact amino acid residues, the β-sheet-structure-stabilizing potentials of the 20 kinds of amino acid residues in protected peptides are assumed to reflect the nature of each amino acid residue whose side chain functional group is protected. The relationship among chemical structure, SP_α, and SP_β of each amino acid residue will be discussed elsewhere.

To confirm the propriety of the conclusive classification, it was compared with the classification in the case of Boc–X_2–Val–Ile–X_2–Val–Ile–resin (Table 2). When guest amino acid residues in resin-bound octapeptides were Arg(NO_2), Glu(OBzl), Cys(Bzl), Lys(Z), Tyr(Bzl), Trp(CHO), Leu, and Phe, the behaviors of the β-sheet-structure disruption of the octapeptides were so similar that their group was treated as one group in Table 2. The conclusive classification is also in fine agreement with the classification in the case of the host-guest octapeptides except for Thr(Bzl) Arg(NO_2) and Met(O). The results obtained above suggest that the conclusive classification can be used for the estimation of the β-sheet-structure stability of protected penta- to octapeptides. Table 2 also gives the classification of intact amino acid residues into six groups based on the coil conformational parameters, P_c, and the β-sheet-structure conformational parameters, P_β. With regard to the P_c parameters, the deviation over two groups is for Met, Leu, Arg, His, Asn, and Gly, indicating that the use of the conclusive classification for the estimation of the β-sheet-structure stability is more suitable than that of the average coil conformation $<P_c>$ values. Furthermore, with regard to the P_β parameters, the deviation over two groups extends to Met, Cys, Thr, Ala, Arg, Gly, Asp, His, Asn, and Glu.

For the design of synthetic routes for peptides and proteins, the estimation of the β-sheet-structure stability of protected peptides in organic solvents is essential because the insolubility of protected peptides may be due to β-sheet aggregation and causes difficulty in successive reactions. Accordingly, it is significant that an estimation method for the β-sheet-structure stability of protected peptieds is devised based on the conclusive classification. Hence, we attempted to assign the numerical value, SP_β, of the group number to the β-sheet-structure-stabilizing potential of each amino acid

Table 2. The β-Sheet-Structure-Stabilizing Potential of the 20 Kinds of Guest Amino Acid Residues and P_c, P_β[a)]

$SP_{\beta i}$	XVDAG XAELG VIX_2VI-resin	X_2VIX_2VI-resin	P_c	P_β
6	R, V, N	V, I, N	M, V, I	M, V, I
5	Q, G, A, H, I	Q, G, A, H, T	L, F, A	C, Y, F
4	F, W, Y	[R, E, C, K]	W, E, Q	Q, L, T, W
3	C, K, L, E, M	[Y, W, L, F]	K, R, H, Y, T	A, R, G, D, K
2	S, T	M, S	C, D, S, N	S, H, N
1	D, P	D, P	G, P	P, E

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

residue. In previous papers,[9—12] we demonstrated that the β-sheet-structure stability was strongly dependent on both the amino acid compositions and peptide chain lengths of protected peptides and weakly dependent on their amino acid sequences. Thus, using $<SP_\beta>$ values of protected peptides defined by Eq. 1, their β-sheet-structure stability is expected to be estimated for each peptide chain length up to an octapeptide,

$$< SP_\beta >= \Sigma n_i SP_{\beta i} / \Sigma n_i \quad (1)$$

where n_i stands for the number of the ith amino acid residue in the peptide and $SP_{\beta i}$, the numerical value of the β-sheet-structure-stabilizing potential of the ith amino acid residue, namely, the group number for the ith amino acid residue in the conclusive classification. For example, the $<SP_\beta>$ values of Boc–X–Val–Asp(OBzl)–Ala–Gly–OPac for X=Val, Ala, Phe, and Asp(OBzl) are 4.6, 4.4, 4.2, and 3.6, respectively. Equation 1 is based on the assumption that the β-sheet-structure stability of protected peptides is determined only by the potential SP_β of the amino acid residues composing protected peptides and that it is not influenced by their amino acid sequences. Equation 1 also does not contain any information about the peptide chain length. Thus, the $<SP_\beta>$ values obtained from Eq. 1 can be used for the comparison of the β-sheet-structure stability among protected peptides having the same peptide chain length. Practically, the application of $<SP_\beta>$ values of protected tri- to heptapeptides to the estimation of the β-sheet-structure stability in organic solvents were examined using 75 kinds of protected peptide fragments of *E. coli* ribosomal protein L7/L12. The results clearly show that their $<SP_\beta>$ values are useful for the estimation of their β-sheet-structure stability in organic solvents.[21] They also indicate that solvents used for coupling reactions between protected peptides having peptide chain lengths below a heptapeptide can be properly determined using the $<SP_\beta>$ values of the peptides. This will be reported in a following paper.

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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 except for Gly denote the L-configuration. Additional abbreviations used are the following: DMSO, dimethyl sulfoxide; DMF, *N,N*-dimethylformamide; Boc, *t*-butoxycarbonyl; Pac, Phenacyl; Bzl, benzyl; OBzl, benzyl ester; Bom, benzyloxymethyl; CHO, formyl; Z, benzyloxycarbonyl; Mts, 2-mesitylenesulfonyl; IR, infrared; DCC, dicyclohexylcarbodiimide; HOBt, 1*H*-1,2,3-benzotriazol-1-ol.

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