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Critical Peptide Size for Insolubility Caused by a β-Sheet Aggregation and Solubility Improvement in Hydrophobic Peptides by Replacement of Alanine Residues with α-Aminoisobutyric Acid Residues[1)]

Mitsuaki NARITA,* Junn-Yann CHEN, Hiroyuki SATO, and Yukio KIM
Department of Industrial Chemistry, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184
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In order to demonstrate an important role of a β-sheet aggregation on insolubility of peptides, hydrophobic peptides having partial amino acid sequences found in natural proteins have been prepared by the stepwise elongation and fragment condensation methods. In conformity with the solubility prediction for protected peptides in our previous paper, solubility data indicate that insolubility of peptides appears to begin at octa- or nonapeptide levels. In hydrophobic sequences found in proteins, the structure of peptide backbones, namely, the β-structure clearly plays an important role on insolubility of peptides, but the composition of peptides does not. Solubility improvement in hydrophobic peptides included in α-helical regions of proteins is easily achieved by replacement of Ala residues with Aib residues. High solubility of the peptides containing Aib residues is clearly explained by the observation that replacement of C^{α} hydrogen atoms with methyl groups greatly disturbs β-sheet structures, promoting helical folding in peptides. The great ability of an Aib residue to promote helical folding is distinctly revealed in the peptide fragments included in α-helical regions of proteins as demonstrated before in model oligo(Leu)s.

Peptide fragments having only hydrophobic amino acid residues are often found in signal peptides[2)] and hydrophobic regions of intrinsic membrane proteins.[3)] On the basis of our solubility prediction method in a previous paper,[4)] they are predicted to be extraordinarily less soluble in the solvents used in peptide syntheses and to be less amenable to further elongation of peptide chains. For solubility prediction in the paper,[4)] the peptides **1**—**5** in Fig. 1 were used as representative examples of hydrophobic peptide fragments contained in natural proteins and, as expected, actually found to be extraordinarily less soluble due to their β-sheet aggregations. In Fig. 1, the peptides **1**—**5** have the amino acid sequences corresponding to murine H-2K^b [287—294], murine H-2K^b [291—298], murine H-2K^b [287—298], subtilisin BPN′ [227—235], and T24 human bladder carcinoma oncogene [6—15], respectively.[5-7)] The numbers in brackets in the following trivial names represent the positions of the first and last amino acids from the N-terminals in the intact proteins.[8)]

In this paper, we describe syntheses and IR absorption conformational analyses of the peptides in Fig. 1 and investigate critical chain length for

Boc-Val Ala Val Leu Val_2 Leu Gly-OBzl
1

Boc-Val_2 Leu Gly Ala_2 Ile Val-OBzl
2

Boc-Val Ala Val Leu Val_2 Leu Gly Ala_2 Ile Val-OBzl
3

Boc-Val Ala Gly Ala_3 Leu Ile Leu-OBzl
4

Boc-Leu Val_3 Gly Ala $(Val\ Gly)_2$-OBzl
5

Boc-Val Gly Phe Gly Leu Ile Leu_2-OBzl
6

Boc-Val Ala Val Leu-OBzl
7

Boc-Leu Val_3 Gly-OBzl
8

Boc-Ala_3 Leu Ile Leu-OBzl
9

Boc-Val Leu Gly Ala_2 Ile Val-OBzl
10

Boc-Val Gly Ala $(Val\ Gly)_2$-OBzl
11

Boc-Val_2 Gly Ala $(Val\ Gly)_2$-OBzl
12

Boc-Val_3 Gly Ala $(Val\ Gly)_2$-OBzl
13

Boc-Aib Ala Ile Val-OBzl
14

Boc-Val Ala Gly Ala Aib-OBzl
15

Boc-Gly Ala Aib Ala Leu Ile Leu-OBzl
16

Boc-Val_2 Leu Gly Aib Ala Ile Val-OBzl
17

Boc-Val Ala Gly Ala Aib Ala Leu Ile Leu-OBzl
18

Fig. 1. The amino acid sequences of the peptides **1**—**18** used in this study.

insolubility of hydrophobic peptides caused by the β-sheet aggregation since we postulated that peptides smaller than heptapeptides are readily soluble in the solvents used in peptide syntheses.[4] The peptide **6** in Fig. 1 is further presented as an interesting example to have high solubility in spite of having the β-structure in solution. It has the amino acid sequence corresponding to bacteriorhodopsin [217—224].[9] The peptides **7—13** in Fig. 1 are also presented to investigate the critical chain length for insolubility. They have partial sequences of the peptides **1—5**. Recently, we also demonstrated that replacement of Ala residues with Aib residues in peptides constrained helical folding in the peptides, and that a dramatic change took place in solubility.[10,11] The implications of the strategy of solubility improvement for the chemistry of peptides and proteins were also discussed. The peptides **14—18** in Fig. 1 are presented to demonstrate the usefulness of replacement of Ala residues with Aib residues for solubility improvement in hydrophobic peptides included in α-helical regions of proteins. They have the amino acid sequences in which Ala residues in the peptides **2** and **4** and in their fragments are replaced by Aib residues.

Experimental

General. The uncorrected capillary melting points will be reported. Analytical instruments and conditions were described in the previous paper.[10] The solubility (c=1.0 g/dl) was divided into the following four classes: (A) completely soluble at room temperature, (B) completely soluble at 80 °C or refluxing temperature, (C) partially soluble at 80 °C or refluxing temperature, and (D) nearly insoluble at 80 °C or refluxing temperature.

General Procedure for the Coupling Reaction Using DCC Activation in the Presence or in the Absence of HOBt. DCC (1.1 equiv) was added to an ice-chilled, stirred mixture of a carboxyl component (1.1 equiv) and an amino component (10 mmol) in dichloromethane or DMF (100 ml). The amino components were obtained from a TosOH salt or HCl salt with an equivalent of NMM. When the carboxyl component was a peptide, HOBt (1.2 equiv) was added before the addition of DCC. Peptide chain elongation of lower peptides was performed in dichloromethane, and fragment condensation for the peptides **1—6** and **9—13** was carried out in DMF. The reaction mixture was stirred in an ice-chilled bath for 3 h, at room temperature overnight, and then filtered. The filtrate was concentrated *in vacuo*. The subsequent work-up procedure was essentially the same as those described before.[10,11]

General Procedure for Removal of the Boc Group. Boc-peptide benzyl esters (10 mmol) in 3 M† HCl/AcOEt (40 ml) were stirred in an ice-chilled bath for 1.5 h. Removal of the Boc group from the peptide **2** was carried out in TFA/dichloromethane (1/2, v/v) at room temperature for 1 h. Then, the mixture was concentrated *in vacuo*, followed by the repetition of the addition and removal *in vacuo* of dichloromethane. The residue was used for a subsequent coupling reaction without further purification.

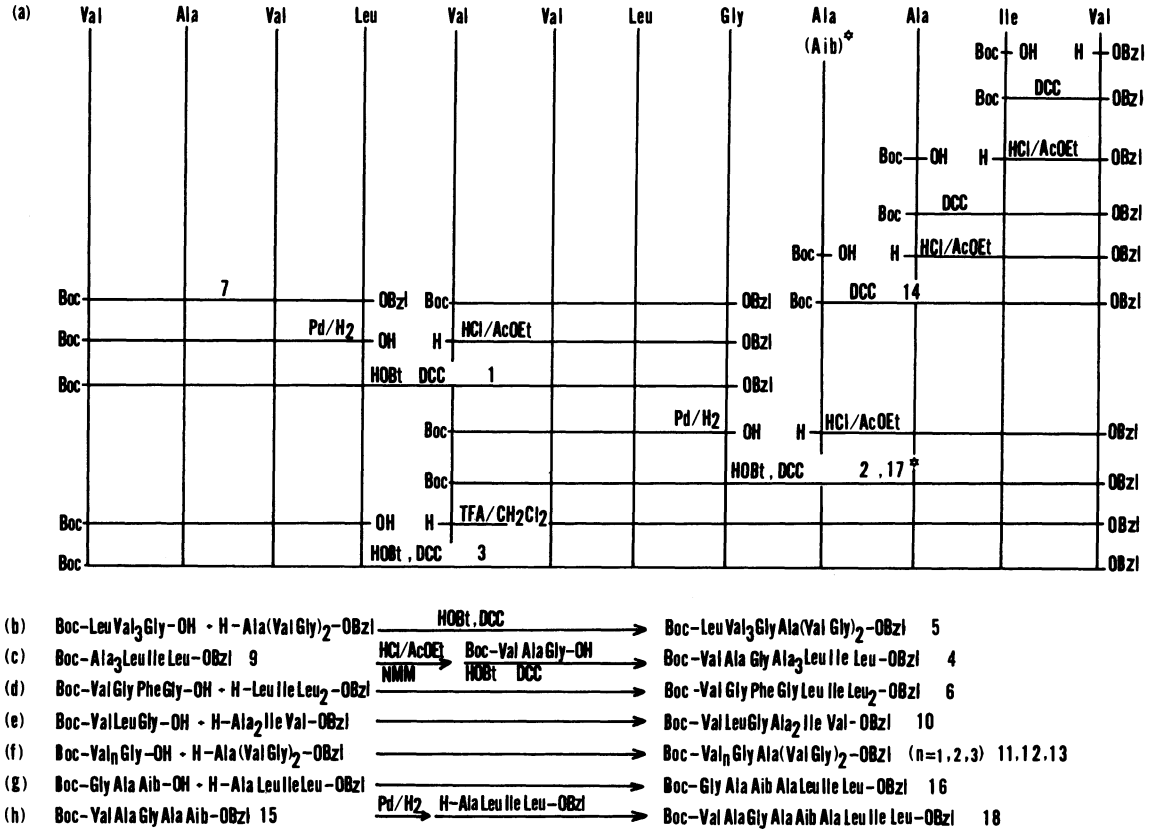
General Procedure for Removal of the Bzl Group. Boc-peptide benzyl esters (7 mmol) were hydrogenated overnight at atmospheric pressure in a mixture of AcOH/MeOH (1/4, v/v) (100 ml) using 5% Pd/C (10 wt%) as a catalyst. After the removal of Pd/C, the filtrate was concentrated *in vacuo*. The residue was washed with water and recrystallized from appropriate solvents.

Results

Syntheses and Solubilities of Hydrophobic Oligopeptides. The typical synthetic procedures are summarized in Scheme. Starting with amino acid benzyl esters as amino components, di- to pentapeptides were prepared by usual stepwise elongation of the peptide chains using DCC activation[12] in dichloromethane. Further chain elongations were performed mainly in DMF by fragment condensation with least danger of racemization using DCC activation in the presence of HOBt.[13] Liberation of the amino group from Boc-peptide benzyl esters was carried out by treatment with 3 M HCl/AcOEt followed by treatment with NMM. Removal of the benzyl group from Boc-peptide benzyl esters was performed by hydrogenolysis using Pd/C as a catalyst in a mixture of MeOH and AcOH. All the soluble peptides were purified by repeated recrystallization, and, as a result of this purification process, each peptide gave a single peak on HPLC. Insoluble peptides were purified by repeated washing with hot MeOH. Synthetic and analytical results of the peptides **1—18** are assembled in Tables 1—3. The amino acid and elemental analyses of the peptides shown in Tables 2 and 3 are in good agreement with the calculated values. Solubility of the peptides **1—18** is also summarized in Table 4. Solubility of the peptides **1—5** was previously examined and found to be extraordinarily less soluble in such solvents as DMSO, NMP, DMA, DMF, and MeOH (Table 4). For these peptides HMPA turned out to be a better solvent than the above (Table 4). On the other hand, the lower oligopeptides **7—11** are easily soluble in these solvents. The peptides **17** and **18**, which are obtained by replacement of Ala residues with Aib residues in the peptides **2** and **4**, respectively, have also high solubility in moderately and highly polar organic solvents. This is in remarkable contrast with the result that the original peptides **2** and **4** are barely soluble in these solvents.

IR Absorption Conformational Analyses of Hydrophobic Peptides in the Solid State and in Dichloromethane. Conformational analyses of hydrophobic peptides in the solid state are interesting for the purpose of elucidating the relationship between

† 1 M=1 mol dm^{-3}.



Scheme

TABLE 1. SYNTHETIC RESULTS AND PHYSICAL PROPERTIES OF THE PEPTIDES **1—18**

Compound	Yield/%[a]	Recrystallization solvent	Mp θ_w/°C	$[\alpha]_D^{20}$ ($c=1.0$, DMF)	Retention time/min
1	88	b)	over 250	c)	c)
2	76	〃	〃	〃	〃
3	81	〃	〃	〃	〃
4	71	〃	〃	〃	〃
5	87	〃	〃	〃	〃
6	74	Ethanol	〃	−33.0	13.6
7	81	AcOEt/hexane	178—180	−29.3	14.8
8	85	Methanol	over 250	−27.8	14.0
9	75	〃	〃	−44.3	14.0
10	83	〃	244—247	−28.5	13.6
11	95	b)	over 250	−19.5	13.1
12	89	〃	〃	c)	c)
13	93	〃	〃	〃	〃
14	76	d)	93—95	−14.3	14.8
15	93	AcOEt/hexane	117—120	−15.0	13.8
16	94	〃	163—165	−36.8	14.3
17	91	Acetone/water	198—201	−22.5	13.9
18	82	AcOEt/hexane	152—155	−26.3	13.7

a) Coupling yields in final steps. b) Purified by washing with hot methanol. c) Not obtained due to insolubility in DMF. d) Purified by Sephadex LH-20 column chromatography.

the conformation and solubility of hydrophobic peptides. Figure 2 shows the IR absorption spectra of the peptides **1**—**6** in the solid state in the most significant spectral regions for the conformational assignments (3600—3200 cm^{-1}, amide A; 1700—1600 cm^{-1}, amide I). As representative examples of the tetra- to hexapeptides, the IR absorption spectra of the peptides **7**—**9** in the solid state are also depicted in Fig. 3. All the peptides show strong bands at 3290—3270 cm^{-1} and 1635—1625 cm^{-1}, assigned to a typical β-sheet structure.[14-16] On the other hand, the IR absorption spectra (Fig. 4) of the peptides **14**—**18** in the solid state, which are obtained by replacement of Ala residues with Aib residues in the peptides **2** and **4** and in their fragments, have shoulder bands at about 3340 cm^{-1} and 1660—

Table 2. Elemental analyses of the peptides **1**—**18**

Compound	Formula	Found (Calcd)/%		
		C	H	N
1	$C_{49}H_{82}N_8O_{11}$	61.28 (61.36)	8.70 (8.62)	11.67 (11.68)
2	$C_{47}H_{78}N_8O_{11}$	60.59 (60.62)	8.60 (8.44)	12.03 (12.04)
3	$C_{66}H_{112}N_{12}O_{15}\cdot H_2O$	59.79 (59.53)	8.63 (8.60)	12.65 (12.62)
4	$C_{49}H_{81}N_9O_{12}$	59.51 (59.55)	8.36 (8.26)	12.72 (12.76)
5	$C_{52}H_{86}N_{10}O_{13}\cdot 1.5H_2O$	57.53 (57.49)	8.33 (8.26)	12.44 (12.89)
6	$C_{54}H_{84}N_8O_{11}\cdot 1.5H_2O$	61.86 (61.87)	8.31 (8.37)	10.86 (10.69)
7	$C_{31}H_{50}N_4O_7$	62.74 (63.03)	8.55 (8.53)	9.59 (9.48)
8	$C_{35}H_{57}N_5O_8\cdot 0.5H_2O$	61.19 (61.38)	8.33 (8.53)	10.63 (10.23)
9	$C_{39}H_{64}N_6O_9\cdot 0.5H_2O$	60.42 (60.84)	8.47 (8.51)	11.05 (10.91)
10	$C_{42}H_{69}N_7O_{10}\cdot 0.5H_2O$	60.05 (59.98)	8.35 (8.39)	11.59 (11.66)
11	$C_{36}H_{57}N_7O_{10}\cdot 0.5H_2O$	57.29 (57.19)	7.72 (7.72)	12.82 (12.95)
12	$C_{41}H_{66}N_8O_{11}\cdot 2H_2O$	55.43 (55.77)	7.53 (7.99)	12.92 (12.69)
13	$C_{46}H_{75}N_9O_{12}\cdot 2H_2O$	55.99 (56.25)	8.04 (8.11)	13.42 (12.84)
14	$C_{30}H_{48}N_4O_7$	62.31 (62.48)	8.62 (8.39)	9.82 (9.71)
15	$C_{29}H_{45}N_5O_8$	58.42 (58.87)	8.03 (7.67)	11.82 (11.84)
16	$C_{42}H_{69}N_7O_{10}$	60.30 (60.63)	8.56 (8.36)	11.53 (11.78)
17	$C_{48}H_{78}N_8O_{18}\cdot H_2O$	59.83 (59.85)	8.69 (8.58)	11.33 (11.63)
18	$C_{50}H_{83}N_9O_{12}\cdot H_2O$	58.84 (58.86)	8.38 (8.40)	12.16 (12.36)

Table 3. Amino acid analyses[a] of the peptides **1**—**18**

Compound	Found (Calcd)					
	Gly	Ala	Val	Leu	Ile	Phe
1	1.09 (1)	1.00 (1)	3.95 (4)	2.12 (2)	—	—
2	1.06 (1)	2.06 (2)	3.00 (3)	0.95 (1)	0.92 (1)	—
3	1.15 (1)	3.17 (3)	5.00 (5)	2.19 (2)	0.98 (1)	—
4	1.00 (1)	4.17 (4)	0.99 (1)	2.17 (2)	0.91 (1)	—
5	3.00 (3)	0.85 (1)	5.07 (5)	1.15 (1)	—	—
6	2.07 (2)	—	1.07 (1)	3.00 (3)	0.96 (1)	0.98 (1)
7	—	1.14 (1)	2.00 (2)	1.00 (1)	—	—
8	1.08 (1)	—	2.97 (3)	1.00 (1)	—	—
9	—	3.27 (3)	—	2.00 (2)	0.93 (1)	—
10	1.04 (1)	1.92 (2)	1.82 (2)	1.00 (1)	0.90 (1)	—
11	3.00 (3)	1.00 (1)	2.87 (3)	—	—	—
12	3.02 (3)	1.00 (1)	3.79 (4)	—	—	—
13	3.00 (3)	1.09 (1)	4.93 (5)	—	—	—
14	—	1.10 (1)	1.00 (1)	—	1.02 (1)	—
15	1.00 (1)	2.07 (2)	0.99 (1)	—	—	—
16	0.90 (1)	1.95 (2)	—	2.06 (2)	1.00 (1)	—
17	1.00 (1)	0.96 (1)	3.00 (3)	1.03 (1)	0.99 (1)	—
18	0.93 (1)	3.01 (3)	0.95 (1)	2.06 (2)	1.00 (1)	—

a) For the peptides **14**—**18**, Aib and Ala peaks often overlapped. The ratio of the peak area for Aib/Ala is 1/50.

TABLE 4. SOLUBILITY PROPERTIES[a] OF THE PEPTIDES **1**—**18** (c=1.0 g/dl)

Compound	Solvent[b]									
	HMPA	NMP	DMSO	DMA	DMF	MeOH	THF	AC	AcOEt	DCM
1	A	C	C	D	D	D	D	D	D	D
2	A	D	C	D	D	D	D	D	D	D
3	C	D	D	D	D	D	D	D	D	D
4	A	B	D	D	D	D	D	D	D	D
5	C	D	C	D	D	D	D	D	D	D
6	A	A	A	A	A	A	A	C	A	B
7	A	A	A	A	A	A	A	B	B	A
8	A	A	A	A	A	B	B	D	D	C
9	A	A	A	A	A	C	D	D	D	D
10	A	A	A	A	A	B	C	D	D	C
11	A	A	A	B	B	C	C	D	D	D
12	A	C	B	D	D	D	D	D	D	D
13	B	C	C	D	D	D	D	D	D	D
14	A	A	A	A	A	A	A	A	A	A
15	A	A	A	A	A	A	A	A	A	A
16	A	A	A	A	A	A	A	A	B	A
17	A	A	A	A	A	A	B	A	C	A
18	A	A	A	A	A	A	A	A	B	A

a) Symbols of A, B, C, and D: see experimental in text. b) Abbreviations: AC, acetone; DCM, dichloromethane. Others, see Ref. 1).

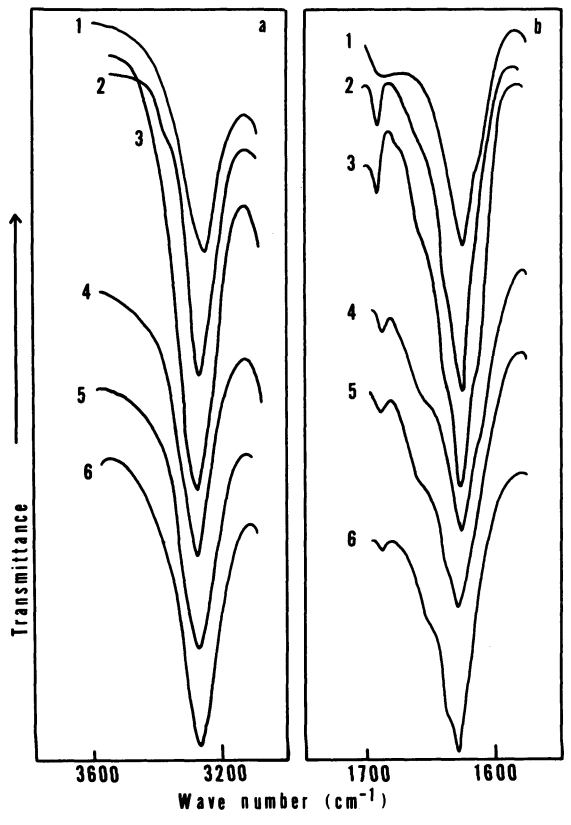


Fig. 2. IR absorption spectra of the peptides **1**—**6** in the solid state.

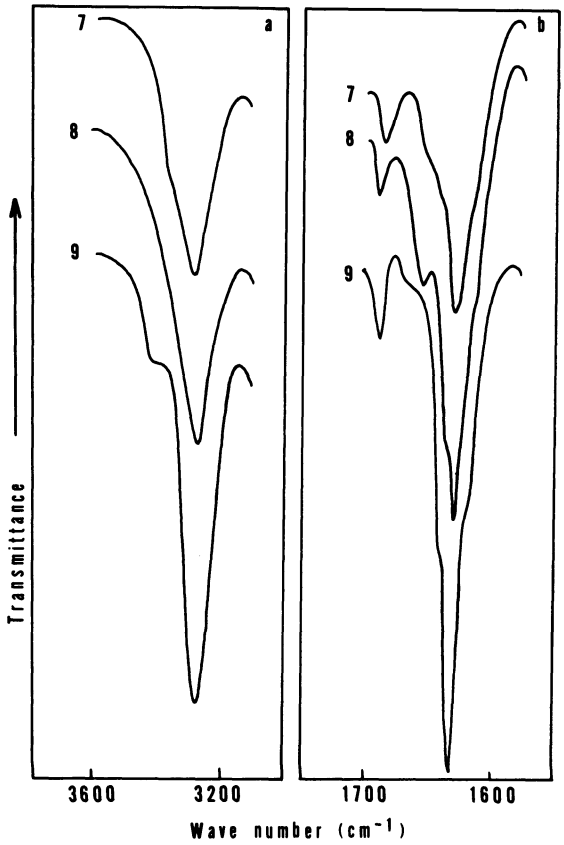


Fig. 3. IR absorption spectra of the peptides **7**—**9** in the solid state.

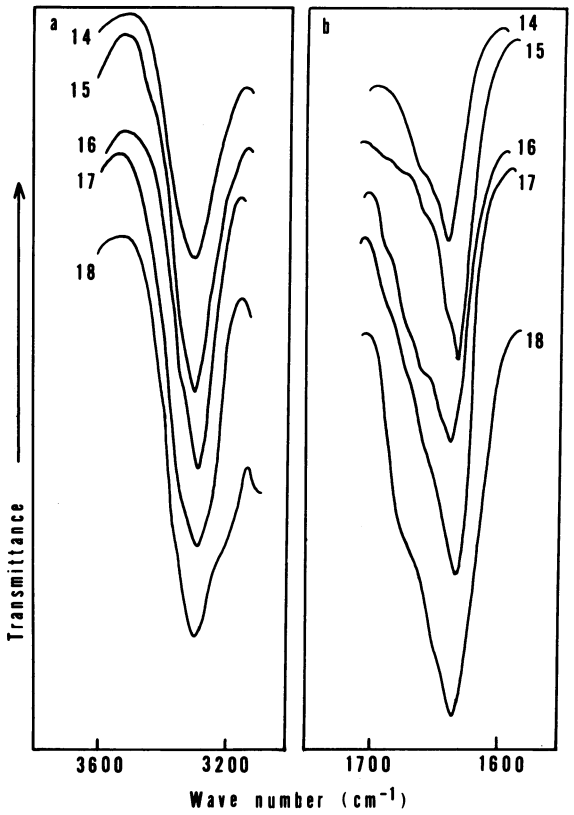


Fig. 4. IR absorption spectra of the peptides **14**—**18** in the solid state.

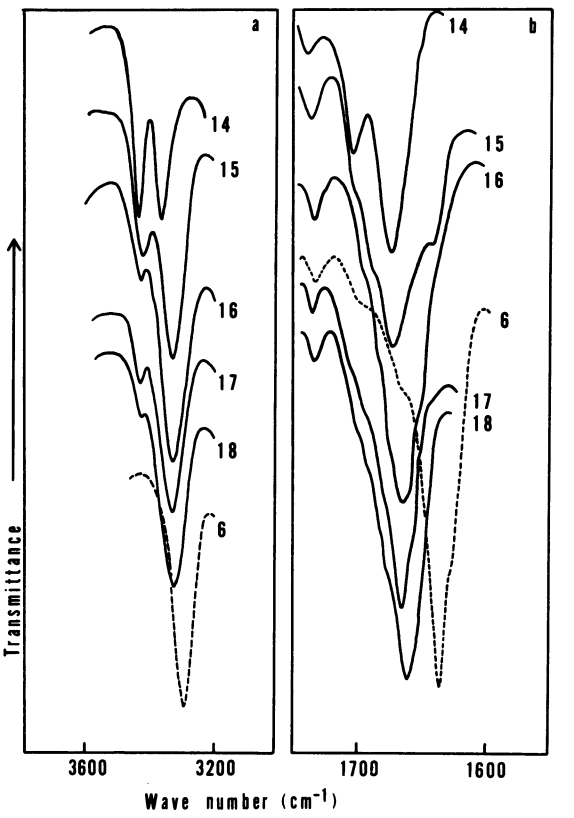


Fig. 5. IR absorption spectra of the peptides **6** and **14**—**18** in dichloromethane.

1650 cm^{-1}, indicating that β-structures are distorted on the Aib residues.

The peptides **6** and **14**—**18** are soluble in dichloromethane and their IR absorption spectra in dichloromethane are presented in Fig. 5. The spectral pattern of the peptide **6** in dichloromethane is essentially the same as that in the solid state. The peptides **14**—**18** in solution, as expected from solubility data and the nature of Aib residues, show no band at about 3280 cm^{-1} and 1630 cm^{-1}, indicating that the restriction of the values of the backbone dihedral angles ϕ and ψ of the Aib residues within the values in the helical regions ($\phi=\pm60\pm20$ and $\psi=\pm40\pm20$)[17] efficiently hinders development of the β-sheet structure. The IR absorption spectrum of the tetrapeptide **14** has two strong bands at 3430 cm^{-1} and 3350 cm^{-1} in the amide A region, indicating the existence of both the nonhydrogen bonding NH and hydrogen bonding NH bonds.[10,11,18] The IR spectrum of the peptide **14** has also a medium-intensity shoulder band at 1708 cm^{-1} and a strong band at 1672 cm^{-1} in the amide I region, assigned to the hydrogen-bonded urethane and free amide carbonyl groups, respectively.[10,11,18] Owing to a strong preference of the Aib residues to form a helical conformation (3_{10}- or α-helix), the peptides **15**—**18** show strong intensity bands at 3325—3320 cm^{-1} and 1672—1660 cm^{-1}, indicating a helical structure (3_{10}- or α-helix), although the frequencies of the amide I region are rather high.[14] Weak or medium intensity bands at 3430—3420 cm^{-1} of the peptides **15**—**18** are also assigned to the nonhydrogen bonding NH bonds, which are attributed to the NH bonds of two or three amino acid residues at the N-terminal portions.

Discussion

Preparation of the peptides **1**—**18** were performed without difficulty by the usual stepwise elongation and fragment condensation methods. The amino component peptide having the Aib residue at the N-terminal, H-Aib-Ala-Ile-Val-OBzl, reacted smoothly with the carboxyl component peptide, Boc-Val_2-Leu-Gly-OH, to produce the peptide **17** in a high yield and the carboxyl component peptide having the Aib residue at the C-terminal, Boc-Val-Ala-Gly-Ala-Aib-OH, also reacted with the amino component peptide, H-Ala-Leu-Ile-Leu-OBzl, to produce the peptide **18** in a high yield, in spite of the fact that the N- and C-terminal Aib residues were sterically hindered.[19] In conformity with the solubility prediction for protected peptides,[4] the β-sheet aggregation caused insolubility in polar solvents of hydrophobic peptides larger than octa- or nonapeptides and, in their preparation, gelation took place along with the reaction. In hydrophobic sequences found in

proteins, the structure of peptide backbones, namely, the β-structure apparently plays an important role on insolubility of peptides, but the structure of peptide side chains, namely, the composition does not.

With respect to the critical chain length for insolubility caused by the β-sheet aggregation, solubility data of the peptides **1**—**13** (Table 4) indicate that insolubility in highly polar solvents of hydrophobic peptides appears to begin at the octa- or nonapeptide levels. Solubility of the octapeptide **6** is rather high in spite of having the β-sheet structure in the solid state (Fig. 3) and 1.0 g of the peptide **6** is easily soluble at room temperature in 100 ml of such solvents as DMSO, HMPA, DMA, DMF, MeOH, and THF. The spectral pattern of the peptide **6** in dichloromethane (Fig. 5) and THF (not shown) is essentially the same as that in the solid state and indicates that the peptide **6** in both solutions exists in the β-sheet aggregation form as reported for a homohexapeptide of Glu(OBzl) in 1,2-dichloroethane.[20] Further development of interpeptide-chain interactions through hydrogen bonding will give rise to severe insolubility as pointed out for model peptides in our previous paper.[21]

Hydrophobic sequences found in natural proteins are expected to be highly organized since hydrophobic amino acid residues have high potentials for the formation of helix- and β-structures.[22] The peptides **1**—**3** are thought to be the partial sequences of a membrane-binding subregion of the murine H-2K^b molecule and the subregion is practically considered to be in the conformation of an α-helix.[23] The peptide **4** is also known to be included in an α-helical region of subtilisin BPN′.[24] On the other hand, the preferred structure, predicted by the Chou-Fasman method,[22] is the β-structure and α-helix for the peptides **1**—**3**, **5**, and **6** and for the peptide **4**, respectively (Table 5). In spite of the disparity between observed and predicted of the conformation, a conformational preference for β-structure formation in the solid state is experimentally demonstrated for the peptides **1**—**6** (Fig. 1). The result is illustrated by the observation that secondary structure formation is dependent on peptide chain length and that critical peptide size for development of the α-helix in the solid state is among decapeptide and pentadecapeptide levels.[25] Therefore, in protein syntheses by fragment condensation methods, the peptide fragments included in α-helical regions of proteins have the β-sheet structure and low solubility before they give rise to helical folding.

TABLE 5. THE PREFERRED STRUCTURE OF THE PEPTIDES **1**—**6** PREDICTED BY THE CHOU-FASMAN METHOD

Compound	P_α	P_β	Preferred structure
1	1.15	1.35	β
2	1.15	1.32	β
3	1.19	1.33	β
4	1.24	1.15	helix
5	1.01	1.29	β
6	1.04	1.23	β

Solubility improvement in hydrophobic peptides included in α-helical regions of proteins is easily achieved by the strategy of replacement of Ala residues with Aib residues.[10,11] The strategy for solubility improvement is based on the restriction of the values of the backbone dihedral angles ϕ and ψ of Aib residues within the values in the region of α- and 3_{10}-helices ($\phi=\pm60\pm20$ and $\psi=\pm40\pm20$). In fact, high solubility of the peptides containing Aib residue is clearly explained by the observation that replacement of C^α hydrogen atoms with methyl groups greatly disturbs β-sheet structures, promoting helical folding in the peptides **14**—**18** (Figs. 4 and 5). The IR spectral patterns of the peptides **14**—**18** in the solid state and in dichloromethane are nearly equivalent to those of the corresponding oligo(Leu)s containing an Aib residue described before.[11] The great ability of an Aib residue to promote helical folding is distinctly revealed in the peptide fragments included in α-helical regions of proteins as demonstrated before in the study of model oligo(Leu)s.[10,11] These results indicate that replacement of a few Ala residues with Aib residues in helical regions of proteins offers the prospect for creating novel proteins in ways not possible by any other method as mentioned previously.[10,11]

References

1) This paper forms Part III of "Peptides and Proteins Containing α,α-Disubstituted α-Amino Acid Residues" series. Part II, M. Narita, K. Ishikawa, H. Sugasawa, and M. Doi, *Bull. Chem. Soc. Jpn.*, **58**, 1731 (1985). 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: Aib, α-aminoisobutyric acid residue; Boc, *t*-butoxycarbonyl; OBzl, benzyl ester; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxy-1*H*-benzotriazole; TosOH, *p*-toluenesulfonic acid; NMM, *N*-methylmorpholine; DMF, *N*,*N*-dimethylformamide; AcOEt, ethyl acetate; TFA, trifluoroacetic acid; AcOH, acetic acid; MeOH, methanol; DMSO, dimethyl sulfoxide; NMP, *N*-methyl-2-pyrrolidone; DMA, *N*,*N*-dimethylacetamide; HMPA, hexamethylphosphoric triamide; THF, tetrahydrofuran.

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