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The Ability of an α-Aminoisobutyric Acid Residue to Promote Helical Folding in Oligopeptides[1)]

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In order to investigate the ability of an Aib residue to promote helical folding in oligopeptides, oligo(Leu)s containing an Aib residue were prepared by stepwise elongation and fragment condensation methods. The peptides prepared were the following: Boc-Aib-Leu$_n$-OBzl (n=3—6 and 9), Boc-Leu$_n$-Aib-OBzl (n=3—6 and 9), Boc-Leu$_3$-Aib-Leu$_3$-OBzl, Boc-Leu$_4$-Aib-Leu$_4$-OBzl, Boc-Leu$_8$-Aib-Leu$_4$-OBzl, Boc-Leu$_4$-Aib-Leu$_8$-OBzl, and Boc-Leu$_8$-Aib-Leu$_8$-OBzl. The IR absorption conformational analyses of Boc-Aib-Leu$_n$-OBzl (n=3—6) in dichloromethane have shown the occurrence of incipient helical structures (α- or 3_{10}-helixes) formed by one, two, three, and so forth $i \rightarrow i$—4 or $i \rightarrow i$—3 hydrogen-bonding patterns. All the peptides except Boc-Aib-Leu$_9$-OBzl and Boc-Leu$_n$-Aib-OBzl (n=6 and 9) have also shown helical structures (α- or 3_{10}-helixes), indicating the great ability of an Aib residue to promote helical folding in peptides. This is in remarkable contrast with the fact that homologous oligo(Leu) counterparts have β-sheet structures. The solubility properties of the peptides were in good agreement with those speculated from their conformations. The initiation and stabilization mechanism of helical folding in peptides has been illustrated schematically and attributed to the restriction of the values of the backbone dihedral angles φ and ψ of an Aib residue due to steric hindrance, followed by the restriction of the values of φ and ψ of other amino acid residues due to hydrogen bonds initiated by the Aib residue. The great ability of an Aib residue to promote helical folding in peptides also suggests that the restriction of the values of the backbone dihedral angles φ and ψ (right-handed α-helix: φ=−57°, ψ=−47°) of an amino acid residue in peptide chains is one of important initiation mechanisms of α-helical folding in natural proteins. The implication of the new findings for the study of proteins containing Aib residues is presented on the basis of the stabilizing efficacy of Aib residues on helical regions of proteins.

Aib-rich peptide fragments are often found in membrane-channel-forming polypeptides like alamethicin[2-4)] and suzukacillin,[5)] and are well recognized to have 3_{10}- and α-helical structures.[6)] The wide occurrence of Aib residues in microbial, particularly in the peptaibophols, has been attributed to their role in constraining the peptide backbones. In a previous paper,[7)] we have demonstrated the usefulness of a novel strategy for solubility improvement in helical oligopeptides based on the restriction of the values of the backbone dihedral angles φ and ψ of Aib residues, and the results have been attributed to the ability of the Aib residue to promote helical folding in the peptide. We have also shown that the properties described there for an Aib residue have important implications for the study of proteins and that the introduction of a few Aib residues in place of Ala residues in helical regions of proteins offers the prospect for creating novel proteins not found in nature. In this relation, it is very interesting to investigate how many Leu residues in model oligo(Leu)s are constrained in helical folding by the introduction of an Aib residue at N- or C-terminal or at a central portion.

On the other hand, the investigation on the initiation mechanism of α-helical folding in proteins is one of the upmost interesting problems for understanding the mechanism of protein folding and the prediction of folding from amino acid sequence. Helical folding in Boc-Leu$_n$-Aib-OBzl (n=3 and 4) in the previous study[7)] apparently suggests that the restriction of the values of φ and ψ of an amino acid residue is one of the important mechanisms for the initiation of helical folding in proteins.

In this paper, we report syntheses and conformations of model oligo(Leu)s containing an Aib residue and the ability of Aib residues to promote helical folding in oligopeptides.

Experimental

General. The uncorrected capillary melting points will be reported. Analytical instruments and conditions were described in the previous paper.[7)]

General Procedure for the Stepwise Elongation Using DCC Activation. The preparation of Boc-Leu$_n$-OBzl (n=3—6) was described in a previous paper.[8)] The preparation of Boc-Aib-Leu$_n$-OBzl and Boc-Leu$_n$-Aib-OBzl (n=3—6) is essentially the same with that of Boc-Leu$_n$-OBzl. The preparation of Boc-Leu$_n$-Aib-OBzl (n=3 and 4) was actually described in the previous paper.[7)]

General Procedure for Removal of the Boc Group. A Boc-peptide Bzl ester (10 mmol) in 3 M† HCl/AcOEt (70 ml) was stirred in an ice-chilled bath for 1.5 h, and the mixture was concentrated *in vacuo*. The residue was used for a subsequent coupling reaction without further purification.

General Procedure for Removal of the Bzl Group. A Boc-peptide Bzl ester (7 mmol) was hydrogenated overnight at atmospheric pressure in a mixture of acetic acid and methanol (1/4, v/v) (100 ml) using 10% Pd/C (10 wt%) as a catalyst. After removal of Pd/C by filtration, the filtrate was concentrated *in vacuo*. The residue was triturated with water and purified by recrystallization from appropriate solvents (mainly aqueous ethanol).

General Procedure for Fragment Condensation Using DCC Activation in the Presence of HOBt. DCC (1.1 equiv) was added to an ice-chilled stirred mixture of a carboxyl component (1.1 equiv), HOBt (1.2 equiv), and an amino component (5 mmol) (obtained by treatment of the HCl salt with an equivalent NMM) in dichloromethane (100 ml). The reaction mixture was stirred in an ice-chilled bath for

† 1 M=1 mol dm^{-3}.

3 h, at room temperature overnight, and then filtered. The filtrate was washed with 10% citric acid, 5% sodium hydrogencarbonate, and water, dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by recrystallization from appropriate solvents shown in Table 1. The coupling reaction for the peptide **12** was performed in a mixture of dichloromethane and DMF (3/2, v/v) in an ice-chilled bath for 3 h and then at room temperature for 2 d, and that for the peptide **10**, in NMP on the same reaction conditions.

Results

Syntheses of Oligo(Leu)s Containing an Aib Residue. Oligo(Leu)s containing an Aib residue were prepared by stepwise elongation and fragment condensation methods. The synthetic scheme is shown below. Boc-Aib-Leu$_n$-OBzl (n=3—6: **1—4**) and Boc-Leu$_n$-Aib-OBzl (n=3—6: **6—9**) were prepared by a stepwise elongation, starting with H-Leu-OBzl and H-Aib-OBzl as amino components, respectively. The synthetic procedure was essentially the same with that described before.[7,8] Removal of the Bzl group from the peptides **6** and **7** was performed by hydrogenolyses using Pd/C as a catalyst and the products **6′** and **7′** were used as carboxyl components in subsequent fragment condensations for the preparation of Boc-Leu$_3$-Aib-Leu$_3$-OBzl (**11**) and Boc-Leu$_4$-Aib-Leu$_4$-OBzl (**12**), respectively. Boc-Aib-Leu$_9$-OBzl (**5**) and Boc-Leu$_9$-Aib-OBzl (**10**) were similarly prepared by fragment condensations of Boc-Aib-Leu$_6$-OH with H-Leu$_3$-OBzl, and of Boc-Leu$_3$-OH with H-Leu$_6$-Aib-OBzl, respectively. Boc-Leu$_8$-Aib-Leu$_4$-OBzl (**13**), Boc-Leu$_4$-Aib-Leu$_8$-OBzl (**14**), and Boc-Leu$_8$-Aib-Leu$_8$-OBzl (**15**) were also prepared by fragment condensations of Boc-Leu$_4$-OH with H-Leu$_4$-Aib-Leu$_4$-OBzl, of Boc-Leu$_4$-Aib-Leu$_4$-OH with H-Leu$_4$-OBzl, and of Boc-Leu$_4$-OH with H-Leu$_4$-Aib-Leu$_8$-OBzl, respectively. Through the preparation in the scheme, the fragment condensations were performed in high yields using DCC activation in the presence of HOBt, and were monitored by HPLC on a gel filtration column. Liberation of the amino groups from Boc-peptide Bzl esters was carried out by treatment with 3 M HCl/AcOEt followed by treatment with NMM. Removal of the Bzl groups from

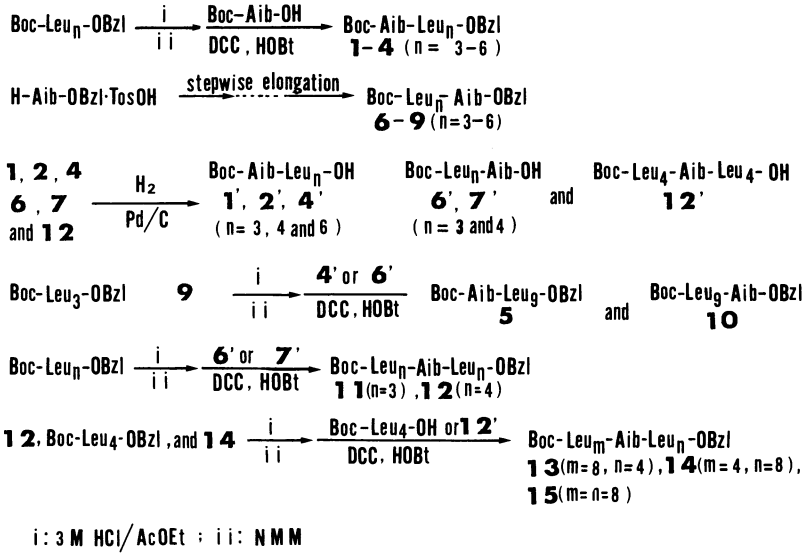

TABLE 1. SYNTHETIC RESULTS AND PHYSICAL PROPERTIES OF THE PEPTIDES **1—15**

Compound	Yield/%[a]	Recrystallization solvent	Mp(θ_m/°C)	$[\alpha]_D^{20}$ (c=1.0, MeOH)	Retention time/min
1	53	Ether/hexane (1/1)	145—147	−52.5	14.1
2	96	Ethanol/water (2/3)	188—193	−46.5	14.0
3	96	Ethanol/water (1/1)	218—222	−40.5	13.8
4	73	Ethanol/water (4/1)	over 250	−45.0	13.8
5	69	b)	over 250	—	—
6	92	Ethanol/water (6/5)	121—123	−63.0	14.0
7	94	Ethanol/water (2/1)	230—232	−58.5	13.8
8	65	Ethanol/water (1/1)	over 250	−55.5	13.7
9	70	Ethanol/water (4/1)	over 250	—	—
10	57	b)	over 250	—	—
11	84	Ethanol/water (7/3)	73—77	−49.5	13.8
12	85	Ethanol/water (7/3)	147—155	−33.0	13.8
13	83	Ethanol/water (2/1)	163—168	−47.3	13.8
14	75	Ethanol/water (2/1)	over 250	−34.5	13.8
15	67	Ethanol	over 250	—	—

a) Final coupling yield. b) Washing with hot methanol.

Table 2. Elemental and Amino Acid Analyses of the Peptides **1**—**15**

Compound	Formula	Found (Calcd)			Found (Calcd)
		C(%)	H(%)	N(%)	Leu/Aib
1	$C_{34}H_{56}N_4O_7$	64.87 (64.53)	9.29 (8.92)	8.91 (8.85)	3.20 (3.00)
2	$C_{40}H_{67}N_5O_8 \cdot 0.5H_2O$	63.60 (63.63)	9.54 (9.08)	9.32 (9.28)	4.04 (4.00)
3	$C_{46}H_{78}N_6O_9$	64.35 (64.31)	9.59 (9.15)	10.20 (9.78)	4.56 (5.00)
4	$C_{52}H_{89}N_7O_{10} \cdot 2H_2O$	62.29 (61.94)	9.21 (9.30)	9.65 (9.81)	6.09 (6.00)
5	$C_{70}H_{122}N_{10}O_{13} \cdot 2H_2O$	62.32 (62.38)	9.57 (9.42)	10.20 (10.39)	9.01 (9.00)
6	$C_{34}H_{56}N_4O_7$	64.37 (64.53)	9.28 (8.92)	8.82 (8.85)	2.98 (3.00)
7	$C_{40}H_{67}N_5O_8$	64.65 (64.40)	9.51 (9.05)	9.35 (9.39)	4.19 (4.00)
8	$C_{46}H_{78}N_6O_9$	64.26 (64.31)	9.65 (9.15)	9.98 (9.78)	4.60 (5.00)
9	$C_{52}H_{89}N_7O_{10} \cdot 0.5H_2O$	63.21 (63.65)	9.69 (9.24)	10.21 (9.99)	6.13 (6.00)
10	$C_{70}H_{122}N_{10}O_{13} \cdot H_2O$	63.03 (63.23)	9.67 (9.40)	10.66 (10.53)	8.78 (9.00)
11	$C_{52}H_{89}N_7O_{10} \cdot H_2O$	63.06 (63.07)	9.48 (9.26)	9.93 (9.90)	5.73 (6.00)
12	$C_{64}H_{111}N_9O_{12}$	63.99 (64.13)	9.69 (9.33)	10.55 (10.55)	7.70 (8.00)
13	$C_{88}H_{155}N_{13}O_{16} \cdot 1.5H_2O$	63.07 (62.97)	9.66 (9.49)	10.51 (10.85)	12.26 (12.00)
14	$C_{88}H_{155}N_{13}O_{16} \cdot H_2O$	63.18 (63.32)	9.72 (9.48)	10.86 (10.91)	11.88 (12.00)
15	$C_{112}H_{199}N_{17}O_{20} \cdot 2H_2O$	62.95 (62.86)	9.74 (9.56)	11.32 (11.03)	15.99 (16.00)

Table 3. IR Absorption Frequencies (Wave Numbers in cm^{-1}) of the Peptides **1**—**15** in Dichloromethane[a]

Compound	Amide A		Amide I		
	3600—3200		1700—1600		
1	3425 (m)	3340 (s)	1700 (w)	1672 (s)	
2	3430 (m)	3340 (s)	1700 (w)	1665 (s)	
3	3430 (w)	3330 (s)	1690 (w)	1662 (s)	
4	3430 (w)	3320 (s)	1698 (w)	1662 (s)	
5[b]		3265 (s)	1693 (m)	1660—1640 (m, sh)	1627 (s)
6	3430 (s)	3340 (s)	1700 (m, sh)	1673 (s)	
7	3430 (m)	3340 (s)	1695 (m, sh)	1665 (s)	
8	3420 (w)	3330 (s)	1690 (m, sh)	1663 (s)	
9[b]		3280 (s)	1692 (m)	1660—1640 (m, sh)	1630 (s)
10[b]		3270 (s)	1693 (m)	1660—1640 (m, sh)	1630 (s)
11	3425 (w)	3320 (s)	1690 (m, sh)	1659 (s)	
12	3425 (w)	3320 (s)	1690 (m, sh)	1659 (s)	
13	3430 (w)	3330 (s)		1659 (s)	
14	3420 (w, sh)	3330 (s)		1659 (s)	
15		3320 (s)		1659 (s)	

a) The following symbols are used: s, strong; m, medium; w, weak; sh, shoulder. b) The IR spectra were recorded in a solid state for nujor mulls.

other peptides was also performed by hydrogenolyses using Pd/C as a catalyst. Homogeneity of the peptides except the peptides **5** and **10** was easily ascertained as the peptides had a single peak on HPLC. The synthetic results and physical properties of the peptides are assembled in Table 1, and data of elemental and amino acid analyses of the peptides, in Table 2. The analytical data are in good agreement with the calculated values.

*IR Absorption Conformational Analyses of the Peptides **1**—**15** in Dichloromethane.* As a method of phase transformation of peptides from solution to a solid state greatly influences their solid-state conformational properties at critical size for development of helical structures,[9] the ability of an Aib residue to promote helical folding in oligo(Leu)s was examined in dichloromethane using IR absorption spectroscopies. Due to low solubility of the peptides **5**, **9**, and **10** in dichloromethane, these IR spectra were measured in the solid state for nujor mulls. The results in the most important spectral regions for the conformational assignments (3600—3200 cm^{-1}, amide A; 1700—1600 cm^{-1}, amide I) are listed in Table 3. As representative examples, the IR spectra of the peptides **1**—**4** are presented in Fig. 1. Those of the peptides **11**, **12**, and **15** are also shown in Fig. 2. All the peptides in solution show strong bands at 3340—3320 cm^{-1} in the amide A region and strong bands at 1673—1659 cm^{-1} in the amide I region, indicating helical structures.[10] Weak or medium-intensity bands at 3430—3420 cm^{-1} of the peptides are assigned to the free urethane and amide NH groups,[11] while the strong bands at 3340—3320 cm^{-1} to the intramolecular hydrogen-bonded NH groups (α-helix: $i \rightarrow i$—4, C_{13} or 3_{10}-helix: $i \rightarrow i$—3, C_{10}).[11] The latter absorption shifts to lower frequencies with increasing peptide chain length. In addition, the ratio of the intensity of the band of the hydrogen-bonded NH group to free NH group (A_H/A_F) increases regularly with increasing peptide chain length. The same tendency has been observed in the study of IR absorption spectra in deuteriochloroform in the 3480—3300 cm^{-1} frequency range of Z-(Aib)$_n$-O-*t*-Bu (n=3—5).[11b]

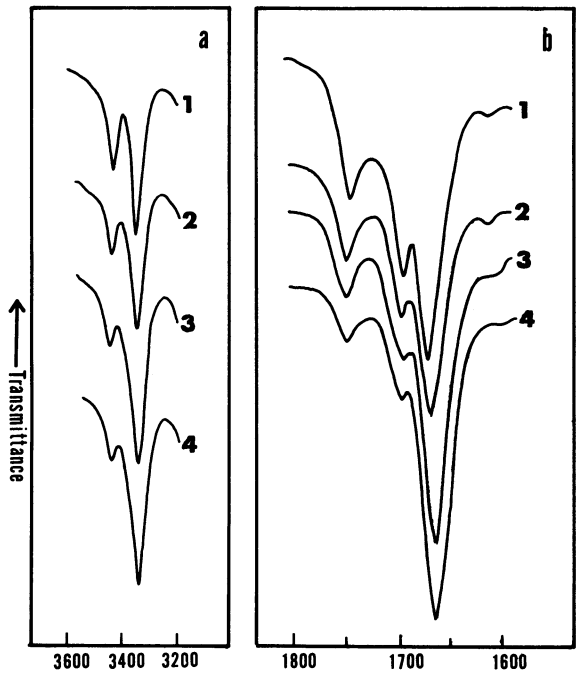

Fig. 1. IR absorption spectra of the peptides **1**—**4**. a: The amide A region; b: the amide I region.

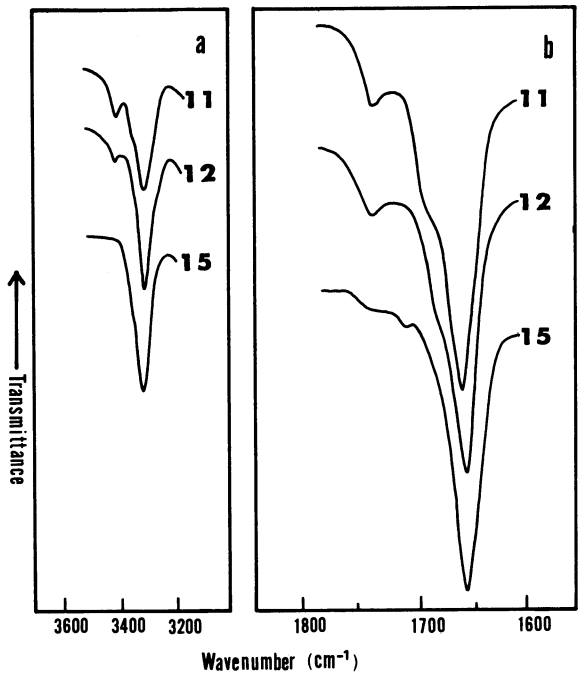

Fig. 2. IR absorption spectra of the peptides **11**, **12**, and **15**. a: The amide A region; b: the amide I region.

On the other hand, the solid-state conformations of the insoluble peptides **5**, **9**, and **10** are predominantly antiparallel β-sheet structures, judging from strong bands at 3280—3265 cm^{-1} in the amide A region and medium-intensity bands at 1692—1693 cm^{-1} and strong bands at 1630—1627 cm^{-1} in the amide I region,[10] accompanied with medium-intensity shoulders at 1660—1640 cm^{-1}, which was assigned to helical or coiled conformations.[10]

Solubility Properties. The relationship between solubilities and conformations of protected peptides has well documented and the severe insolubility of protected peptides in organic solvents results from their β-sheet aggregation.[7,8,12-14] The solubility data of the peptides **1**—**15** in various organic solvents are assembled in Table 4. The results are in agreement with those speculated from the conformations.

Discussion

The great ability of an Aib residue to promote helical folding in model oligo(Leu)s is clearly demonstrated by the IR absorption conformational analyses of the peptides **1**—**15**. The helical conformations of the peptides **1**—**4**, **6**—**8**, and **11**—**14** in dichloromethane are in remarkable contrast with the β-sheet

Table 4. Solubility properties[a] of the peptides **1**—**15** (c=1.0 g/dl)

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

a) Solubility: A, soluble at room temperature; B, soluble at 80 °C or refluxing temperature; C, partially soluble at the same temperature; D, nearly insoluble at the same temperature. b) Abbreviations: HMPA, hexamethylphosphoric triamide; DMA, *N*,*N*-dimethylacetamide; DMSO; dimethyl sulfoxide; MeOH, methanol; EtOH, ethanol; AC, acetone; CF, chloroform; DCM, dichloromethane; CT, carbon tetrachloride; BE, benzene. Others, see text.

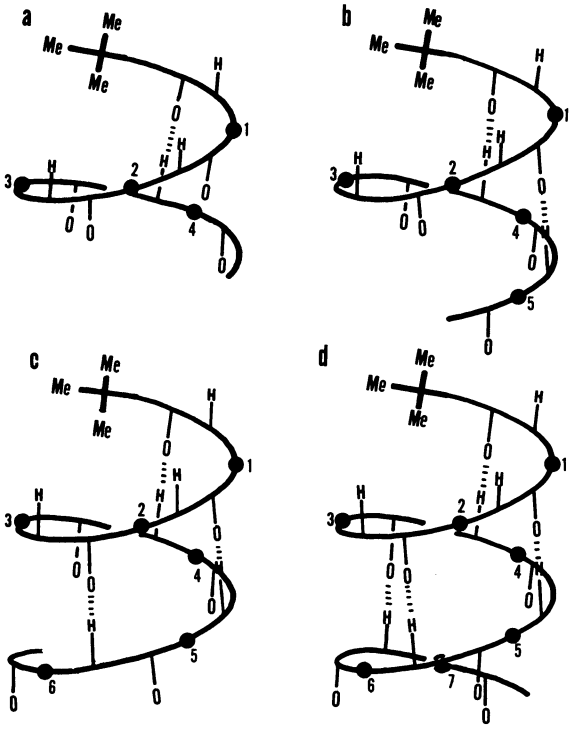

Fig. 3. Initiation and propagation of α-helical structures of the peptides **1**—**4**. a: **1**; b: **2**; c: **3**; d: **4**.

structures of the homologous oligo(Leu) counterparts.[7,15-17] Each Aib residue of these peptides apparently stabilizes helical folding in the peptides to great extent. For example, in Fig. 2, the ratio of the intensity of the amide A bands of the hydrogen-bonded NH groups to free NH groups increases regularly from the tetrapeptide **1** to the heptapeptide **4**, indicating helical propagation together with increasing peptide chain length. This is illustrated schematically in Fig. 3, where α-helical structures ($i \rightarrow i$—4 hydrogen-bonding patterns) are adopted as an example.[18] The initiation and propagation of helical structures of the peptides **1**—**4** is apparently attributed to the restriction of the Aib residue to conformations in the helical region ($\phi = \pm 60 \pm 20$, $\psi = \pm 30 \pm 20$) due to its steric hindrance,[19,20] followed by the restriction of the values of ϕ and ψ of other Leu residues due to hydrogen bonds initiated by the Aib residue. It is suggested that the restriction of the values of ϕ and ψ of an amino acid residue is one of the important mechanisms for the initiation of helical folding in proteins. Thus, we characterize this route for helical formation in protein folding as an initiation mechanism by the restriction of the values of the ϕ and ψ of an amino acid residue. The analogous idea of helix initiation was proposed by Blagdon and Goodman,[21] and characterized as terminal initiation mechanism by polar groups and by turns.

In Fig. 3, the urethane carbonyl groups of the peptides **1**—**4** participate in the 5→1 hydrogen-bonding. A shift of the frequencies of the urethane carbonyl groups to 1700—1690 cm^{-1} (Table 3) apparently supports the involvement of the carbonyl groups in the hydrogen bond.[22] In Fig. 3a, the amide carbonyl groups of the peptide **1** do not participate in a hydrogen bond, and this is supported by the observation that the peptide **1** has a strong band at 1672 cm^{-1} in the amide I region (Table 3). The α-helical structure of the peptide **1** in Fig. 3a, however, implies the restriction of the values of ϕ and ψ of the three Leu residues. The carbonyl absorption in the peptides **1**—**4** shifts to lower frequencies together with the NH absorption of the intramolecular hydrogen-bonding NH groups (Table 3), indicating propagation of helical structures with increasing peptide chain length as shown in Fig. 3. This observation is very close to the results that the X-ray diffraction and IR absorption conformational analyses of Z-$(Aib)_n$-OH (n=3—5) in the solid state have shown the occurrence of incipient 3_{10}-helixes, formed by one, two, and three type III (or type III′) β turns, respectively.[11b] In solvents of low polarity, the IR absorption data of Z-$(Aib)_n$-OH (n=3—5) were also in favor of the same intramolecular hydrogen-bonded forms as found in the solid state.[11b] The peptides **1**—**4**, however, could not be obtained as crystalline materials having helical structures, because α-helical→β conformational transformation took place during phase transformation of the peptides from solution to the solid state. Figure 4 presents the α- and 3_{10}-helical structures of the peptide **12** as an example. The α-helix in Fig. 4a appears to be more stable than the 3_{10}-helix in Fig. 4b, judging from the angles of hydrogen bonds, although it is well recognized that Aib-rich peptides favor 3_{10}-helixes.[2-6] The peptide **5** is soluble in a reaction mixture of dichloromethane during the coupling reaction, but it becomes less soluble as shown in Table 4 after obtaining as a crystalline material. The solubility data of the peptide **5** are in good agreement with the ones speculated from the β-sheet conformation.

The IR data of the peptides **6**—**8** in Table 3 also indicate that the helical structures correspond to those of the peptides **1**—**3** (Fig. 3a—c), respectively. In the peptides **6**—**10**, however, only the value of the backbone dihedral angle of ϕ of the Aib residues is restricted. Difference in helical stability between the peptides **4** and **9** appears to be illustrated by no participation of the ester group of the Aib residue of the peptide **9** in helical folding, while the Boc group bound to the Aib residue of the peptide **4** participates in helical folding. Thus, the peptides **9** and **10** are predominantly in favor of antiparallel β-sheet structures, and are less soluble in organic solvents (Table 4).

The peptides **9**—**15** are regarded to be produced by the hybridization, through an Aib residue, of the peptides having an Aib residue at C-terminal ends with those at N-terminal ends. The IR data in Table 3 clearly indicate that helical structures of the peptides **11**—**15** are efficiently stabilized by the hybridization of the

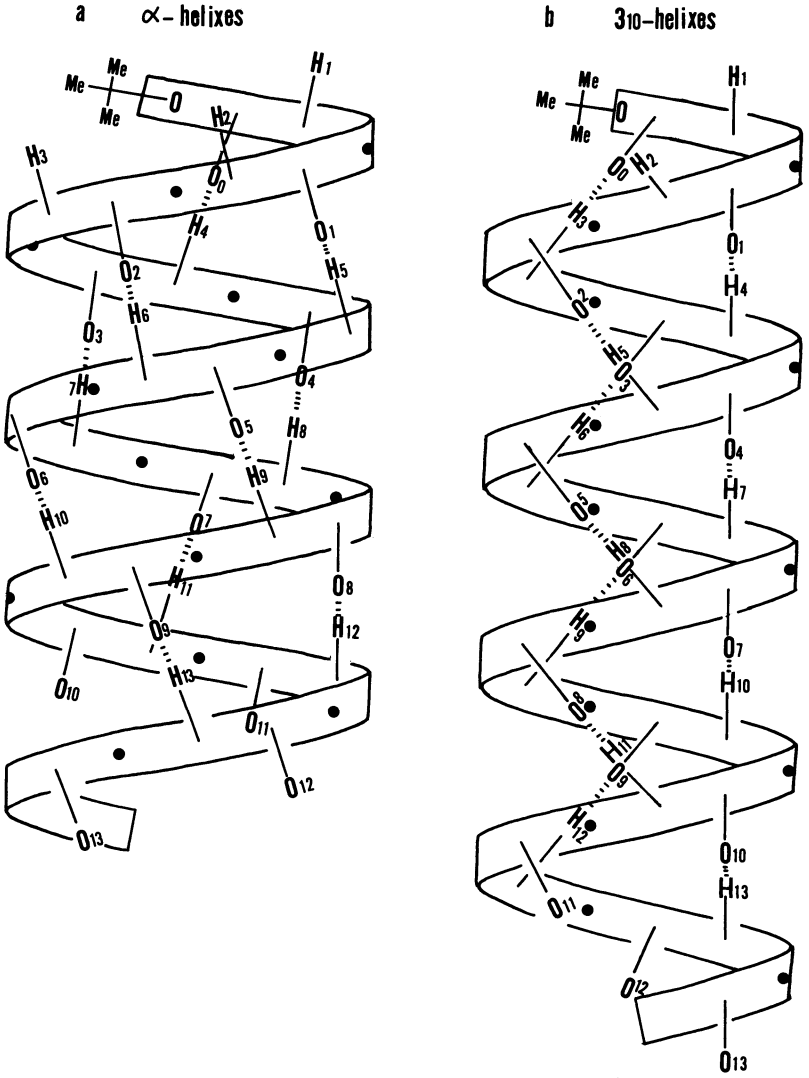

Fig. 4. α- and 3_{10}-helical structures of the peptide **12**.

corresponding two peptides. For example, the hybridization, through the Aib residue, of the peptide **1** with the peptide **6** and of the peptide **2** with the peptide **7** produces three additional hydrogen bonds in the peptides **11** and **12**, and strongly stabilizes the helical structure. This is illustrated schematically in Fig. 5, where α-helical structures are also postulated as an example. Difference in helical stability among the peptides **5**, **10**, and **13**—**15** is apparently resulted from the additional hydrogen bonds in the peptides **13**—**15**.

In this study the strategy for solubility improvement in protected peptides based on the restricted values of the backbone dihedral angles ϕ and ψ of an Aib residue[7] was also successfully applied to the model oligo(Leu)s. The solubility data of the peptides **1**—**15** directly reflect their conformations (Tables 3 and 4). The high solubility of the peptides **4** and **11**—**14** is in remarkable contrast with the observation that their homologous oligo(Leu) counterparts are barely soluble or insoluble in various organic solvents.[7,8]

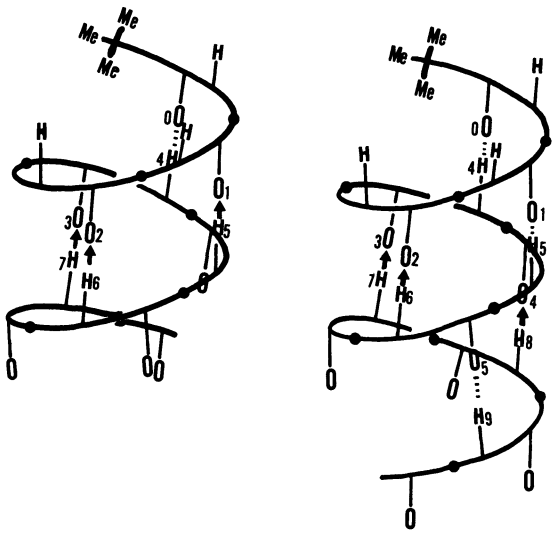

Fig. 5. Stabilization of α-helical structures of the peptides **11** and **12**. Three additional hydrogen bonds are shown by the arrow (→).

Conclusions

The great ability of an Aib residue to promote helical folding in peptides is clearly demonstrated using model oligo(Leu)s. The results make clear that the restriction of the values of the backbone dihedral angles ϕ and ψ of an amino acid residue is one of initiation mechanisms of helical folding in peptides, suggesting it to be one of important initiation mechanisms of α-helical folding in natural proteins. The stabilizing efficacy of an Aib residue on helical structures is also attributed to the restriction of the values of the backbone dihedral angles ϕ and ψ of the Aib residue, followed by the restriction of the values of those of other amino acid residues through hydrogen bonds initiated by the Aib residue. The properties of an Aib residue for stabilizing helical structures have innovative implications for the study of proteins. For example, replacement of a few Ala residue with Aib residue in helical regions of enzymes offers the prospect for creating novel enzymes, having thermostable properties and a wide range of pH optimum. The techniques offer the potential for altering protein function in ways not possible by any other method, just as mentioned in protein engineering pioneered by genetic technology.[24]

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