Chain-Length Effects on Helix-Forming Tendency in Sequential Peptides Containing Δ^2 Phe Residues

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(Received October 13, 1995)

ABSTRACT: The present paper describes chain-length effects on helix-forming tendency in sequential peptides containing Δ^2 -dehydrophenylalanine (Δ^2 Phe) residues, *i.e.*, $(X-\Delta^2\text{Phe})_n$. For sequential peptides Boc-(L-Leu- Δ^2 Phe) $_n$ -L-Leu-OMe ($n=2-6$) that take 3_{10} -helical conformation in CHCl_3 , solvent dependence on their helical structures was investigated by CD spectroscopy. CD patterns corresponding to a right-handed helix were observed for peptides $n=3-6$ in CHCl_3 , tetrahydrofuran, acetonitrile, methanol, and trimethyl phosphate. Such helical structures became less stable in dimethyl sulfoxide and trifluoroethanol. Conformational energy calculation was carried out for acetyl-(L-Ala- Δ^2 Phe) $_n$ -NHCH $_3$ ($n=1-12$). Peptides above $n=2$ were predicted to form helical structures preferentially. In addition, rigidity of the helical structure containing Δ^2 Phe residues was found to be somewhat lower than that containing α -aminoisobutyric acid residue with strong helix-forming tendency. This can be ascribed to the conformational properties inherent in Δ^2 Phe residue that induces not only helical backbones, but β -turn backbones. Thus, Δ^2 Phe residue will be useful to design functional peptides having such backbones.

KEY WORDS: Δ^2 -Dehydrophenylalanine / Sequential Peptides / Helix-Forming Tendency / Chain-Length Effect / Circular Dichroism Study / Conformational Energy Calculation / α -Aminoisobutyric Acid /

The α,β -dehydroamino acid residue has been found naturally in many peptides having biological activity and in some proteins.¹⁻⁵ This type of residue shows inherent conformational properties due to structural features, *e.g.*, planarity of $\text{C}^\alpha=\text{C}^\beta$ double bond and trigonal geometry of α -carbon atom. As for Δ^2 -dehydrophenylalanine (Δ^2 Phe), the introduction of two or three Δ^2 Phe residues into a peptide chain leads to helical structures.⁶⁻¹⁶

Thus, Δ^2 Phe residue attracts much interest from the view point of designs of helical backbones. However, fundamental properties of helical structures induced by Δ^2 Phe residues are not well known. In our previous study,¹⁷ chain-length effects on helical conformations were investigated for sequential peptides:

Boc-(L-Leu- Δ^2 Phe) $_n$ -L-Leu-OMe **I-n**, $n=2-6$
(Boc, *t*-butoxycarbonyl; OMe, methoxyl).

Peptides **I-2** to **I-6** in CDCl_3 showed a 4 $\rightarrow$ 1 intramolecular hydrogen bonding characteristic of a 3_{10} -helix.

The present paper shows the stability of helical structures, particularly how the helix-forming tendency depends on the chain length. For this purpose, CD spectroscopy and conformational energy calculations were performed for peptides **I-n**. In addition, helix-

forming tendency of the Δ^2 Phe residue was compared with Ala and other helicogenic α -aminoisobutyric acid (Aib) residues.^{18,19}

EXPERIMENTAL

Materials

Peptides **I-n** prepared in the previous study¹⁷ were used.

Spectroscopy

CD and UV spectra were recorded simultaneously, using a JASCO J-600 with Δ^2 Phe concentration of 5×10^{-5} M. Chloroform, tetrahydrofuran (THF), acetonitrile, methanol, trimethyl phosphate (TMP), dimethyl sulfoxide (DMSO), and trifluoroethanol (TFE) were used as solvents, and purified by distillation before use.

Conformational Energy Calculation

Empirical conformational energy calculations were performed using energy functions of ECEPP system.²⁰ Structural and energy parameters were determined in previous studies.^{21,22} The program PEPCON, written by Sisido²³ for conformational energy calculation²⁰ and graphics²⁴ of a given peptide, was modified to be applicable to Δ^2 Phe-containing peptides.

Conformational energy of peptides **I-n** ($n=1-6$) was calculated on Ac-(L-Ala- Δ^2 Phe) $_n$ -NMA (Ac, acetyl; NMA, *N*-methylamide), where L-Ala was used instead of L-Leu to simplify calculation and exclude the affects of undesirable orientation of Leu's side chain on main-chain conformation. This replacement should be valid, since the main-chain energy contour map of Leu res-

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idue was almost the same as that of Ala residue (data not shown). Based on crystallographic data of Δ^2 Phe-containing peptides,^{6,8,9,12} all amide groups were fixed in the *trans* conformation ($\omega=180^\circ$), and each Δ^2 Phe side-chain (χ_1) was fixed to 0° . Two methods were used to predict the conformation of peptides **I-n**.

One was to search all possible conformations by stepwise elongation of the repeating unit (Ala- Δ^2 Phe), as proposed by Oka.^{25,26} In the first step, energy minimization for dipeptide, Ac-Ala- Δ^2 Phe-NMA, was carried out. Here all combinations of energy minima of Ala and Δ^2 Phe residues (6×20) were used as starting conformations. The second step was minimization of tetrapeptide having two repeating units of Ala- Δ^2 Phe, *i.e.*, Ac-(Ala- Δ^2 Phe)₂-NMA. All minima in the first step were used as starting conformations for the second step. The third step was minimization of Ac-(Ala- Δ^2 Phe)₃-NMA using all minima in the second step as starting conformations for the third step. Likewise, Ac-(Ala- Δ^2 Phe)_{*n*}-NMA of *n*=4, 5, 6, 8, 10, and 12 were energy-minimized, using all starting conformations obtained in the minimization steps of *n*=3, 4, 5, 6, 8, and 10, respectively. Assuming helical symmetry in the sequential peptides, all minimizations were carried out with five variables, ϕ_{Ala} , ψ_{Ala} , $\phi_{\Delta^2\text{Phe}}$, $\psi_{\Delta^2\text{Phe}}$, $\chi_{2,\Delta^2\text{Phe}}$, using

the Simplex algorithm. The conformations obtained were also expressed by the conformational letter code that specifies 16 regions in conformational space.²⁷

The other method was to calculate the main-chain energy contour map of Ala (ϕ_{Ala} , ψ_{Ala}) in Ac-(Ala- Δ^2 Phe)₆-NMA of the lowest-energy right-handed helix by changing all ϕ and ψ at 5° in the region of -180° to 180° . Similarly, the energy contour map of Δ^2 Phe ($\phi_{\Delta^2\text{Phe}}$, $\psi_{\Delta^2\text{Phe}}$) was calculated. In each (ϕ , ψ), the χ_2 angle in Δ^2 Phe residue was taken as the value that gives minimal conformational energy on changing all χ_2 at 10° in 0 to 180° . For comparison, main-chain energy contour maps for Ac-(Ala-Ala)₆-NMA and Ac-(Ala-Aib)₆-NMA in a right-handed helix were calculated.

RESULTS AND DISCUSSION

Solvent Dependence on CD Spectra

Peptides **I-2** to **I-6** take a right-handed 3_{10} -type helix in CHCl_3 essentially.¹⁷ In the present study, solvent dependence of CD spectra for peptides **I-2** to **I-6** was investigated to determine how such a helical structure in CHCl_3 is affected by other solvents, and how helical stability depends on chain length.

Figures 1(a)–(e) show CD and UV spectra of peptides **I-2** to **I-6** in CHCl_3 , tetrahydrofuran (THF), acetonitrile, methanol, TMP, DMSO, and TFE. (CD spectra of peptide **I-6** in these solvents were also shown in the previous paper.¹⁷) The molar extinction coefficients (ϵ) and $\Delta\epsilon$ ($=\epsilon_L - \epsilon_R$) are expressed with respect to molar concentration of the Δ^2 Phe residue.

All UV absorption spectra showed intense maxima

Figure 1. CD (top) and UV absorption (bottom) spectra of peptides (a) **I-2**, (b) **I-3**, (c) **I-4**, (d) **I-5**, and (e) **I-6** in CHCl_3 (—), THF (---), acetonitrile (---), methanol (— · —), TMP (— · — · —), DMSO (— · — · —), and TFE (— · — · —).

around 280 nm (band I) assignable to $\Delta^2\text{Phe}$ residue. UV patterns did not change essentially in peptides **I-2** to **I-6**, and all patterns were similar to those of Boc-Leu- $\Delta^2\text{Phe}$ -Leu-OMe having a single $\Delta^2\text{Phe}$ residue (data not shown). Thus, no strong ground-state interactions between $\Delta^2\text{Phe}$ - $\Delta^2\text{Phe}$ pair(s) exist in peptides **I-2** to **I-6**. No such interaction was observed in 3_{10} -helical sequential peptides containing $\Delta^2\text{Phe}$ residues.^{17,21}

Unlike the UV spectra, the corresponding CD spectra were markedly affected by solvents and chain-length. Namely, two marked features could be seen. First, in CHCl_3 , THF, acetonitrile, methanol, and TMP, peptides **I-3** to **I-6** showed exciton couplets with negative peaks at longer wavelengths for band I, although peptide **I-2** showed different patterns in these solvents. Peptides **I-2** to **I-6** take a 3_{10} -type helix in CDCl_3 by NMR study.¹⁷ Accordingly, the CD pattern observed in CHCl_3 should be ascribed to the 3_{10} -helical structure. These splitting patterns at band I are found to be right-handed helices, by applying the exciton chirality method²⁸ to band I, whose transition moment was estimated to lie on the phenyl-carbonyl line.²¹ That peptides **I-3** to **I-6** showed similar CD patterns in CHCl_3 , THF, acetonitrile, methanol, and TMP should indicate that these helical conformations in CHCl_3 are retained in other solvents. The marked solvent dependence on CD patterns of peptide **I-2** is ascribed to conformational changes by solvents. Consequently, a right-handed helix is preferentially formed above $n=2$ in peptides **I-*n***.

Secondly, in DMSO, and TFE, CD patterns for all peptides were essentially different from those in CHCl_3 , suggesting that helical structures are broken in such solvents at least partly. The reason for this may be the stronger hydrogen-bonding nature of DMSO and TFE, which interferes with the formation of intramolecular hydrogen-bonding in helical structures. Behavior that a helical structure in CHCl_3 becomes less stable in DMSO was also observed for oligopeptides containing two $\Delta^2\text{Phe}$ residues.^{11,13} The rigidity of helical structures induced by introducing $\Delta^2\text{Phe}$ residues into a peptide backbone seems not to be extremely high. Helical structures containing other helicogenic Aib residues were found in DMSO^{18,29–31}; *e.g.*, Boc-(Aib-X)₅-OMe (X = Ala or Val) takes a 3_{10} -helical conformation in both of CDCl_3 and d_6 -DMSO.²⁹ Thus, helix-forming tendency of $\Delta^2\text{Phe}$ residue seems somewhat weaker than that of Aib residue, as suggested by Uma *et al.*¹⁸

Chain-Length Effects on Helical Stability

CD studies indicated that peptides above $n=2$ form right-handed helices preferentially. In this section, such chain-length effect on helix-forming tendency is discussed on the basis of theoretical conformational analysis. Energy-minimized conformations of Ac-(Ala- $\Delta^2\text{Phe}$)_{*n*}-NMA ($n=1$ –12) are shown in Tables I–VII, which list lowest-energy to fifth-low energy conformations. ΔE_{res} is the energy difference per residue from the lowest energy.

Peptides $n=3$ –12 showed the lowest energy conformations with the letter code AA, which corresponds to a right-handed α - or 3_{10} -helical region. These lowest-energy conformations have 5→1 hydrogen-bonding mode characteristic of a right-handed α -helix. This α -helix

is somewhat different from standard α -helix (-57° , -47°)³²; *i.e.*, average (ϕ, ψ) of $\Delta^2\text{Phe}$ residues in peptides $n=3$ –12 is (-52° , -39°), which corresponds to rather a 3_{10} -type helical one. However, the prediction that peptides $n=3$ –6 favor an α -helix essentially disagrees with the previous result: ¹H NMR and CD studies indicated that peptides **I-3** to **I-6** take a right-handed 3_{10} -type helix in CHCl_3 . α - and 3_{10} -helices take relatively similar structures: *e.g.*, (ϕ, ψ) is (-57° , -47°)³² for standard α -helix and (-60° , -30°)^{33,34} for standard 3_{10} -helix. Also, the energy difference between both helices should be small, since 3_{10} - to α -helical transitions occur with solvents and chain-lengths in several Aib-containing peptides.^{18,30,31,35–39} Thus, it seems difficult to predict both helical structures.

Here we estimated chain-length effects on helical stability theoretically rather than predict helical type. As shown in Table II, peptide $n=2$ showed AA (right-handed helical) conformation as the third low-energy conformation, and non-helical conformation (CB*) as the lowest-energy conformation. Namely, the preference for helical conformation in $n=2$ was considered not strong. In addition, five conformations were found in $\Delta E_{\text{res}} < 0.3 \text{ kcal mol}^{-1}$. The prediction that peptide $n=2$ shows such conformational versatility should agree well with CD spectral change with solvents in peptide **I-2**. Peptides $n=3$ –12 showed the right-handed helix (AA) for the lowest-energy conformation, as mentioned above. Figure 2 shows the chain-length effects on helix-forming tendency in Ac-(Ala- $\Delta^2\text{Phe}$)_{*n*}-NMA and Ac-(Ala-Ala)_{*n*}-NMA. ΔE_{AA} is defined by $(E_{\text{AA}} - E')/2n$, where E_{AA} is the lowest energy of helical conformations (AA), E' is the lowest energy of non-helical conformations, and n is the number of repeating unit (Ala- $\Delta^2\text{Phe}$) or (Ala-Ala). For (Ala- $\Delta^2\text{Phe}$)_{*n*}, ΔE_{AA} was positive in $n=1$ –2, but ΔE_{AA} was negative above $n=3$. ΔE_{AA} decreased drastically up to $n=4$, and decreased gradually above $n=4$. Consequently, peptides (X- $\Delta^2\text{Phe}$)_{*n*} have the tendency to form stable helical structures above $n=2$. This tendency is consistent with the CD results of peptides **I-*n*** as already shown.

The tendency to form a right-handed helix seems to be stronger in (Ala-Ala)_{*n*} than in (Ala- $\Delta^2\text{Phe}$)_{*n*}. This may be ascribed to the chirality of the Ala residue. However, as described in the next section, the $\Delta^2\text{Phe}$ residue decreases the tendency to form β -sheet structure inherent in Ala-type peptides.

The above conformational calculations were carried out, assuming helical symmetry in sequential peptides. To check the validity of this assumption, energy minimizations for peptides $n=1$ –12 were carried out using each variable independently: *e.g.*, peptide $n=3$ has 15 variables ($3 \times \phi_{\text{Ala}}$, $3 \times \psi_{\text{Ala}}$, $3 \times \phi_{\Delta^2\text{Phe}}$, $3 \times \psi_{\Delta^2\text{Phe}}$, $3 \times \chi_{2,\Delta^2\text{Phe}}$). The conformations in Tables I–VII were used as starting conformations. Minimized conformations retained helical symmetry in terms of conformational letter code: *e.g.*, for peptide $n=3$, the letter code of the lowest-energy conformation was AAAAAA (=AA), and that of second-low energy conformation was ABABAB (=AB). These results are also plotted on Figure 2. The tendency of chain-length effect did not change essentially, whether this assumption was used or not.

Table I. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe)-NMA

Conformational letter code	Ala		Δ^Z Phe			ΔE_{res}^a kcal mol $^{-1}$
	ϕ	ψ	ϕ	ψ	χ_2	
CB*	-69	99	126	-11	137	0.00
CB	-85	126	-126	12	45	0.63
AB	-71	-41	-125	13	43	0.74
AB*	-83	-49	125	-13	135	0.84
CB*	-154	155	126	-13	136	0.87

$$^a \Delta E_{\text{res}} = (E - E_0)/2. E_0 = 9.32 \text{ kcal mol}^{-1}.$$

Table II. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe) $_2$ -NMA

Conformational letter code	Ala		Δ^Z Phe			ΔE_{res}^a kcal mol $^{-1}$
	ϕ	ψ	ϕ	ψ	χ_2	
CB*	-70	98	125	-10	137	0.00
CB	-84	76	-126	12	44	0.13
AA	-74	-50	-54	-37	141	0.16
AB	-71	-42	-124	10	43	0.25
CB	-82	101	-125	9	48	0.28

$$^a \Delta E_{\text{res}} = (E - E_0)/4. E_0 = 24.91 \text{ kcal mol}^{-1}.$$

Table III. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe) $_3$ -NMA

Conformational letter code	Ala		Δ^Z Phe			ΔE_{res}^a kcal mol $^{-1}$
	ϕ	ψ	ϕ	ψ	χ_2	
AA	-75	-50	-53	-38	141	0.00
CB*	-71	96	125	-9	138	0.62
AB	-74	-47	-129	16	44	0.81
CB	-85	76	-127	11	44	0.83
CB	-81	99	-124	11	48	0.90

$$^a \Delta E_{\text{res}} = (E - E_0)/6. E_0 = 36.22 \text{ kcal mol}^{-1}.$$

Table IV. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe) $_4$ -NMA

Conformational letter code	Ala		Δ^Z Phe			ΔE_{res}^a kcal mol $^{-1}$
	ϕ	ψ	ϕ	ψ	χ_2	
AA	-76	-49	-53	-39	141	0.00
AB	-74	-49	-130	16	45	1.08
HB*	-71	-95	125	-9	138	1.10
A*A*	56	63	51	44	38	1.15
CB	-85	76	-127	11	44	1.25

$$^a \Delta E_{\text{res}} = (E - E_0)/8. E_0 = 46.52 \text{ kcal mol}^{-1}.$$

Table V. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe) $_5$ -NMA

Conformational letter code	Ala		Δ^Z Phe			ΔE_{res}^a kcal mol $^{-1}$
	ϕ	ψ	ϕ	ψ	χ_2	
AA	-77	-49	-52	-39	141	0.00
A*A*	56	62	51	44	38	1.18
AB	-75	-49	-130	17	45	1.23
CB	-71	95	-125	-9	137	1.30
CB	-85	76	-127	10	44	1.46

$$^a \Delta E_{\text{res}} = (E - E_0)/10. E_0 = 57.22 \text{ kcal mol}^{-1}.$$

Table VI. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe) $_6$ -NMA

Conformational letter code	Ala		Δ^Z Phe			ΔE_{res}^a kcal mol $^{-1}$
	ϕ	ψ	ϕ	ψ	χ_2	
AA	-77	-48	-52	-39	141	0.00
A*A*	56	62	51	44	38	1.23
AB	-74	-50	-130	16	46	1.35
CB*	-71	95	125	-9	138	1.51
CB	-86	75	-127	10	44	1.68

$$^a \Delta E_{\text{res}} = (E - E_0)/12. E_0 = 67.11 \text{ kcal mol}^{-1}.$$

Table VII. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe) $_{12}$ -NMA

Conformational letter code	Ala		Δ^Z Phe			ΔE_{res}^a kcal mol $^{-1}$
	ϕ	ψ	ϕ	ψ	χ_2	
AA	-78	-48	-52	-40	141	0.00
A*A*	56	61	51	45	37	1.32
A*B*	54	50	118	26	141	1.43
AB	-73	-50	-131	16	46	1.63
CE*	-72	113	131	-146	139	1.71

$$^a \Delta E_{\text{res}} = (E - E_0)/24. E_0 = 127.16 \text{ kcal mol}^{-1}.$$

Figure 2. Chain-length effects on helix-forming tendency in Ac-(Ala- Δ^Z Phe) $_n$ -NMA (○, ●) and Ac-(Ala-Ala) $_n$ -NMA (△): helical symmetry was assumed in energy minimization for plots ○ and △, and this assumption was not used for plot ●. ΔE_{AA} is defined by $(E_{\text{AA}} - E')/2n$, where E_{AA} is the lowest energy of right-handed helical conformations (AA), E' is the lowest energy of non-helical conformations, which are left-handed helix (A*A*) except ones shown in the parentheses around the corresponding plots. n is the number of repeating unit (Ala- Δ^Z Phe) or (Ala-Ala).

Preference for Helical Structure by the Δ^Z Phe Residue

Here the preference for helical structure induced by introducing Δ^Z Phe residues is discussed on the basis of a conformational energy contour map. Figures 3–5 show main-chain energy contour maps for three right-handed helices in Ac-[Ala(1)- Δ^Z Phe(2)] $_6$ -NMA, Ac-[Ala(1)-Ala(2)] $_6$ -NMA, and Ac-[Ala(1)-Aib(2)] $_6$ -NMA. The three right-handed helices were obtained by energy-minimization as described before: $\phi_{\text{Ala}(1)} = -77^\circ$, $\psi_{\text{Ala}(1)} = -48^\circ$, $\phi_{\Delta^Z\text{Phe}(2)} = -52^\circ$, and $\psi_{\Delta^Z\text{Phe}(2)} = -39^\circ$ for [Ala(1)- Δ^Z Phe(2)] $_6$; $\phi_{\text{Ala}(1)} = -68^\circ$, $\psi_{\text{Ala}(1)} = -38^\circ$, $\phi_{\text{Ala}(2)} = -68^\circ$, and $\psi_{\text{Ala}(2)} = -38^\circ$ for [Ala(1)-Ala(2)] $_6$; $\phi_{\text{Ala}(1)} = -74^\circ$, $\psi_{\text{Ala}(1)} = -43^\circ$, $\phi_{\text{Aib}(2)} = -53^\circ$, and $\psi_{\text{Aib}(2)} = -39^\circ$ for [Ala(1)-Aib(2)] $_6$. For each peptide, contour map was calculated for first ($\phi_{(1)}$, $\psi_{(1)}$) and second residues ($\phi_{(2)}$, $\psi_{(2)}$) in repeating units.

In [Ala(1)-Ala(2)] $_6$, two contour maps ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$)

Figure 3(a). Main-chain energy contour map of Ala(1) ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$) in Ac-[Ala(1)- Δ^2 Phe(2)]₆-NMA of ($\phi_{\Delta^2\text{Phe}(2)}$, $\psi_{\Delta^2\text{Phe}(2)}$) = (-52° , -39°). $\chi_{2,\Delta^2\text{Phe}(2)}$ angle was taken as the value that gives minimal conformational energy by changing all χ_2 at 10° in 0 to 180° . The contours are drawn in $0.5\text{-kcal}[\text{mol}(\text{residues})]^{-1}$ increments from the energy minimum point (-110° , -45°) to $5\text{ kcal}[\text{mol}(\text{residues})]^{-1}$.

Figure 3(b). Main-chain energy contour map of Δ^2 Phe ($\phi_{\Delta^2\text{Phe}(2)}$, $\psi_{\Delta^2\text{Phe}(2)}$) in Ac-[Ala(1)- Δ^2 Phe(2)]₆-NMA of ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$) = (-77° , -48°). $\chi_{2,\Delta^2\text{Phe}(2)}$ angle was taken as the value that gives minimal conformational energy by changing all χ_2 at 10° in 0 to 180° . The contours are drawn in $0.5\text{-kcal}[\text{mol}(\text{residues})]^{-1}$ increments from the energy minimum point (-50° , -40°) to $5\text{ kcal}[\text{mol}(\text{residues})]^{-1}$.

Figure 4(a). Main-chain energy contour map of Ala(1) ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$) in Ac-[Ala(1)-Ala(2)]₆-NMA of ($\phi_{\text{Ala}(2)}$, $\psi_{\text{Ala}(2)}$) = (-68° , -38°). The contours are drawn in $0.5\text{-kcal}[\text{mol}(\text{residues})]^{-1}$ increments from the energy minimum point (-65° , -40°) to $5\text{ kcal}[\text{mol}(\text{residues})]^{-1}$.

and ($\phi_{\text{Ala}(2)}$, $\psi_{\text{Ala}(2)}$) were almost the same as shown in Figures 4(a) and (b). Stable regions in these maps were a right-handed helical region ($\sim -65^\circ$, $\sim -40^\circ$), and a wide range region including β -sheets [parallel sheet (-119° , 113°)⁴⁰ and antiparallel sheet (-139° , 135°)⁴¹].

The contour map ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$) in [Ala(1)- Δ^2 Phe(2)]₆ diminished somewhat the β -sheet region in [Ala(1)-Ala(2)]₆. The other contour map ($\phi_{\Delta^2\text{Phe}(2)}$, $\psi_{\Delta^2\text{Phe}(2)}$) was markedly different from that ($\phi_{\text{Ala}(2)}$, $\psi_{\text{Ala}(2)}$) in [Ala(1)-Ala(2)]₆. Namely, the map ($\phi_{\Delta^2\text{Phe}(2)}$, $\psi_{\Delta^2\text{Phe}(2)}$) showed mainly three stable regions, letter code

Figure 4(b). Main-chain energy contour map of Ala(2) ($\phi_{\text{Ala}(2)}$, $\psi_{\text{Ala}(2)}$) in Ac-[Ala(1)-Ala(2)]₆-NMA of ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$) = (-68° , -38°). The contours are drawn in $0.5\text{-kcal}[\text{mol}(\text{residues})]^{-1}$ increments from the energy minimum point (-65° , -40°) to $5\text{ kcal}[\text{mol}(\text{residues})]^{-1}$.

Figure 5(a). Main-chain energy contour map of Ala(1) ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$) in Ac-[Ala(1)-Aib(2)]₆-NMA of ($\phi_{\text{Aib}(2)}$, $\psi_{\text{Aib}(2)}$) = (-53° , -39°). The contours are drawn in $0.5\text{-kcal}[\text{mol}(\text{residues})]^{-1}$ increments from the energy minimum point (-70° , -45°) to $5\text{ kcal}[\text{mol}(\text{residues})]^{-1}$.

Figure 5(b). Main-chain energy contour map of Aib(2) ($\phi_{\text{Aib}(2)}$, $\psi_{\text{Aib}(2)}$) in Ac-[Ala(1)-Aib(2)]₆-NMA of ($\phi_{\text{Ala}(1)}$, $\psi_{\text{Ala}(1)}$) = (-74° , -43°). The contours are drawn in $0.5\text{-kcal}[\text{mol}(\text{residues})]^{-1}$ increments from the energy minimum point (-55° , -35°) to $5\text{ kcal}[\text{mol}(\text{residues})]^{-1}$.

A of right-handed helix ($\sim -60^\circ$, $\sim -50^\circ$), code A* of left-handed helix ($\sim 50^\circ$, $\sim 50^\circ$), and code B of ($\sim -120^\circ$, $\sim 10^\circ$). It should be noted that conformational space in Ac-(Ala- Δ^2 Phe)₆-NMA diminished a wide region of β -sheet, a typical region in Ac-(Ala-Ala)₆-NMA. Thus, the decrease of tendency to take a β -sheet increases the helix-forming tendency of peptide.

The contour map ($\phi_{\text{Aib}(2)}$, $\psi_{\text{Aib}(2)}$) in [Ala(1)-Aib(2)]₆ (Figure 5(b)) showed two helical regions greatly restricted, *i.e.*, right- and left-handed helices (codes A and A*). The helix-forming tendency of Aib residue is stronger than that of Δ^2 Phe residue. This reflects that stability of helical structures in strong hydrogen accepting

DMSO is lower in Δ^2 Phe-containing peptides than in Aib-containing peptides, as already mentioned. Such flexibility of helical structures induced by Δ^2 Phe residues is ascribed to the presence of region B ($\sim -120^\circ$, $\sim 10^\circ$), seen as another stable region for Δ^2 Phe residue in Tables I—VII. This region for Δ^2 Phe residue has been found in several peptides containing a single Δ^2 Phe residue: *i.e.*, such peptide chains form type II β -turn,^{18,42} as indicated by the following hydrogen bond:

Here (ϕ , ψ) of the Δ^2 Phe residue takes code B. Thus, the Δ^2 Phe residue shows preference for two conformations, *i.e.*, β -turn and helical conformations.

CONCLUDING REMARKS

The present paper reveals chain-length effect on helix-forming tendency in sequential peptides $(\text{X}-\Delta^2\text{Phe})_n$. Experimentally and theoretically, helical structures were preferentially formed above $n = 2$. Such helical structures were broken partly in DMSO and TFE. The conformational rigidity induced by introducing Δ^2 Phe residues into a peptide backbone was found somewhat lower than that for Aib residues. This is ascribed to the conformational properties inherent in Δ^2 Phe residue that induces not only a helical backbone, but a β -turn backbone. Therefore, such helical and β -turn structures can be formed by introducing Δ^2 Phe residues. Here β -substituent (phenyl group) in rigid side chain of Δ^2 Phe residue can be regularly arranged along such backbone. This will be useful in the design of functional peptides using Δ^2 Phe residues.

It should be noted that the conformational properties of Δ^2 Phe residue are essentially different from those of Ala-type residues. Conformational properties inherent in Δ^2 Phe residues will attract much interest from the view point of design of functional polypeptides having new types of helical backbones. Sequential polypeptides containing non-Ala-type residues such as Pro and Gly have been theoretically predicted to form non- α -helical structures such as β - and γ -helices.^{26,43,44} Accordingly, sequential polypeptides containing Δ^2 Phe as well as other types of residues (*e.g.*, Gly, Pro, and Aib) can expand possible designs of new types of helical backbones where β -substituents are regularly arranged. To demonstrate this possibility, current efforts are being directed toward synthesis and conformational analysis of sequential polypeptides containing Δ^2 Phe residues.

Acknowledgments. We express our sincere gratitude to Professor M. Kawai in Department of Applied Chemistry, Nagoya Institute of Technology for the use of CD apparatus.

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