Design of Novel Helical Backbone. Conformational Energy Calculation and Synthesis of Sequential Polypeptide Containing Z-Dehydrophenylalanine and Proline Residues

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ABSTRACT: Theoretical conformational analysis was carried out on periodic polypeptides containing Z-dehydrophenylalanine (Δ^Z Phe) and Pro residues, poly(X- Δ^Z Phe-Pro) (X = Ala, Leu, Gly, D-Ala, and aminoisobutyric acid (Aib)), using the procedure of conformational energy minimization. The lowest-energy conformations were found to be a right-handed $\beta^{11,9}$ -helix for X = Ala, and a right-handed $\beta^{5,1}$ -helix for X = Gly. For X = Leu, D-Ala, and Aib, the lowest-energy conformation was found to be a left-handed $\beta^{6,1-6,2}$ -helix: the nearest phenyl-phenyl pairs took a center-to-center distance of 6.6–6.8 Å and an edge-to-edge orientation. On the whole, phenyl groups were arranged in C_2 -type symmetry on the helical wheel. Poly(Leu- Δ^Z Phe-Pro) was synthesized by polymerizing the corresponding tripeptide with diphenylphosphoryl azide. The polypeptide in chloroform and in tetrahydrofuran showed a positive CD signal around 280 nm, which is ascribed to the absorption band of Δ^Z Phe residue. This CD pattern means that the screw angle between neighboring Δ^Z Phe transition moments is around 0° or 180°, suggesting the β^6 -helical conformation predicted above.

KEY WORDS Sequential Polypeptide / Z-Dehydrophenylalanine / Proline / Conformational Energy Calculation / Synthesis / CD Study / β -Helix /

A synthetic helical polypeptide provides a rigid and regular backbone for arranging functional groups, which keep a specific distance and orientation between the neighboring ones. Thus the polypeptide has been widely applied to the construction of a model system¹ for investigating a biological phenomena, and to the development of a new polymeric material.² In most cases, such helical structure means α -helix. Accordingly, molecular design in an α -helical polypeptide has to be limited to its structural features (e.g., 3.6 residues per one turn). Helical backbones other than α -helix are very important to design a wide variety of functional polypeptides.

Such other types of helices have been found experimentally and theoretically in periodic polypeptides: β^6 -helix^{3,4} for poly(Ala-D-Ala); γ -helix,⁵ β -helix,⁶ and β -spiral⁷ for elastin model polypeptides such as poly(Val-Pro-Gly-Gly) and poly(Val-Pro-Gly-Val-Gly); $\beta^{6,8}$ -helix for poly(Gly-Pro)⁸; γ -helix for poly(Ala-Pro).⁹ Here β^X -helix is defined as a helix having spiral structure with X residues per turn, and γ -helix is defined as a helix that cannot be categorized to the α - and β -helices.^{4,5,10} In the above polypeptides, it should be noted that the repeating units contain non-L-Ala type of residues such as D-Ala, Gly, and Pro, which essentially differ from L-Ala-type of residues in conformational preference.

Recently we have focused on the conformational preference in Z-dehydrophenylalanine (Δ^Z Phe) from the view point of peptide design.^{11–15}

This residue shows inherent conformational properties due to structural features, e.g., planarity of $C^\alpha=C^\beta$ double bond and trigonal geometry of C^α . Thus, periodic polypeptides containing dehydroresidues as non-L-Ala type of residues will be able to create new types of helical backbones. Also the Δ^Z Phe residue has a specific conformational space for its side chain.^{13–15} The freedom of side chain would be smaller than those of naturally-occurring amino acids, such as Phe, Asp, Glu, and Lys, because the variable side-chain dihedral angle is regarded as single due to prohibited rotation about the $C^\alpha=C^\beta$ double bond. Accordingly, β -phenyl groups are tightly bound to a peptide backbone. Here β -phenyl group of Δ^Z Phe residue can be replaced by another functional group using DL- β -substituted Ser for a starting material in peptide synthesis.¹³ Therefore, β -substituted α,β -dehydroalanines in periodic polypeptides are expected to be an unique residue for designing novel helical backbones to carry β -substituents in a spatially specific manner.

We already designed a right-handed 3_{10} -helix using periodic (poly)peptides, (Ala- Δ^Z Phe-Aib)_n^{14,15} (Aib, aminoisobutyric acid) and (Leu- Δ^Z Phe)_n.^{11,12} In the present paper, periodic polypeptides containing Δ^Z Phe and Pro residues, poly(X- Δ^Z Phe-Pro), were adopted to demonstrate the possibility to form novel helical backbones. Theoretical conformational analysis was carried out on poly(X- Δ^Z Phe-Pro) (X = Ala, Leu, Gly, D-Ala, and Aib), using the procedure of conformational energy minimization. Also, poly(Leu- Δ^Z Phe-Pro) **I** was synthesized and its CD spectra were measured:

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EXPERIMENTAL

Conformational Energy Calculation

Empirical conformational energy calculations were performed using energy functions of ECEPP system.¹⁶ Structural and energy parameters of Δ^2 Phe were determined in previous studies.^{14,15} The program PEPCON, written by Sisido¹⁷ for conformational energy calculation¹⁶ and graphics¹⁸ of a given peptide, was modified to be applicable to Δ^2 Phe-containing peptides.

Conformational energy of poly(X- Δ^2 Phe-Pro) (X = Ala, Leu, Gly, D-Ala, and Aib) was calculated on Ac-(X- Δ^2 Phe-Pro)₈-NMA (Ac, acetyl; NMA, *N*-methylamide). Energy minimizations were carried out using the Simplex algorithm¹⁹ with the following variables: for the main chain, (ϕ , ψ) of X, (ϕ , ψ) of Δ^2 Phe, ψ of Pro, and for the side chain, χ^2 of Δ^2 Phe and χ 's of X residue except for Gly (χ^1 for Ala and D-Ala, χ^1 , χ^2 , $\chi^{3,1}$ and $\chi^{3,2}$ for Leu, and $\chi^{1,1}$ and $\chi^{1,2}$ for Aib). ψ of Pro was fixed to -75° .²⁰ Peptide bond of Δ^2 Phe-Pro linkage was taken to *cis* ($\omega=0^\circ$) or *trans* conformation ($\omega=180^\circ$). Based on crystallographic data of Δ^2 Phe-containing peptides,^{21–24} the other peptide bonds were fixed to the *trans* conformation ($\omega=180^\circ$), and each Δ^2 Phe side-chain (χ^1) was fixed to 0° .

The detailed procedure is shown on poly(Leu- Δ^2 Phe-Pro) as follows. All possible conformations were searched by stepwise elongation of the repeating unit (Leu- Δ^2 Phe-Pro), as proposed by Oka.^{4,5,8,9} In the first step, energy minimization for tripeptide, Ac-Leu- Δ^2 Phe-Pro-NMA, was carried out. Here all combinations of energy minima of Leu, Δ^2 Phe, and Pro residues (81x20x6) were used as starting conformations. The second step was minimization of hexapeptide having two repeating units of Leu- Δ^2 Phe-Pro, *i.e.*, Ac-(Leu- Δ^2 Phe-Pro)₂-NMA. All minima in the first step were used as starting conformations for the second step. Also, the third step was minimization of Ac-(Leu- Δ^2 Phe-Pro)₄-NMA using all minima in the second step as starting conformations for the third step. Finally, the minimization of Ac-(Leu- Δ^2 Phe-Pro)₈-NMA was carried out using all minima in the third step. The conformations obtained were also expressed by the conformational letter code that specifies 16 regions in conformational space.²⁵

Measurement

¹H NMR spectra were recorded using a Varian UNITY plus 400 (400 MHz) and a Hitachi R-90 spectrometer (90 MHz). The measurements were carried out at peptide concentration of 20–40 mg ml⁻¹ in CDCl₃ at 298 K. All chemical shifts in parts per million (ppm) were determined using tetramethylsilane as an internal standard. The pulse sequence for one-dimensional (1-D) 400 MHz ¹H NMR is as follows: 11.0- μ s pulse width, 5.5-s acquisition time, and 7.5-s interval. Correlation spectroscopy (COSY) was performed using the standard

Varian software library, using 16 scans per t_1 , 2 K data points in the t_2 domain, and 512 points in the t_1 domain. IR spectra were recorded for samples cast on an NaCl plate using a Jasco IR Report 100 and a Nicolet Fourier transform infrared (FT-IR) spectrometer. CD and UV spectra were simultaneously recorded by using a JASCO J-600 in chloroform and in tetrahydrofuran (THF). These solvents were purified by distillation before use.

Materials

Polypeptide I was synthesized by polymerizing the corresponding tripeptide, H-Leu- Δ^2 Phe-Pro-OH, with diphenylphosphoryl azide (DPPA).²⁶ The *N*-protected peptide, Boc-Leu- Δ^2 Phe-Pro-OH (Boc, *t*-butoxycarbonyl), was prepared by ring-opening Boc- Δ^2 Phe azlactone with amino group of Pro. The tripeptide was checked for purity by ¹H NMR and IR spectroscopy, thin-layer chromatography (TLC), and gel permeation chromatography (GPC). Solvent systems for TLC were (A) methanol, (B) chloroform–methanol (9:1), (C) *n*-butanol–acetic acid–water (7:2:1), and (D) ethyl acetate. The GPC was recorded on a Tosoh HLC-803-D equipped with G1000-, G2500-, and G3000-HXL columns in series, by using THF eluent. Single spot in the TLC and single peak in the GPC were obtained.

Boc-Leu- Δ^2 Phe Azlactone

The azlactone was prepared according to ref 22. mp 122–125°C (123–125°C).²³

Boc-Leu- Δ^2 Phe-Pro-OH

A precooled solution of Pro (0.21 g, 1.8 mmol) in an aqueous solution of NaOH (1.0 mol ml⁻¹, 1.8 ml) was added to a solution of Boc-Leu- Δ^2 Phe azlactone (0.50 g, 1.4 mmol) in THF (4 ml) at 0°C. The mixture was stirred for 24 h at room temperature, and then neutralized with 5% KHSO₄ solution. The solvent was concentrated *in vacuo*, and the residue was acidified with 5% KHSO₄ solution and extracted with ethyl acetate. The extract was washed with saturated NaCl solution, dried over MgSO₄, and evaporated *in vacuo* to give a white solid. Yield 0.65 g (98%). mp 110–115°C; $R_f^A=0.90$; $R_f^B=0.28$; $R_f^C=0.91$; $R_f^D=0–0.16$. 90 MHz ¹H NMR (δ in CDCl₃): 8.38 (1H, s, NH Δ^2 Phe), 7.36 (5H, s, phenyl H Δ^2 Phe), 6.24 (1H, s, C ^{β} H Δ^2 Phe), 5.12 (1H, brs, NH Leu), 4.60 (1H, t, C ^{α} H Pro), 4.24 (1H, m, C ^{α} H Leu), 3.72 (2H, m, C ^{δ} H Pro), 2.2 (2H, m, C ^{γ} H₂ Pro), 2.0 (2H, m, C ^{β} H₂ Pro), 1.6 (3H, m, C ^{β} H₂ + C ^{γ} H Leu), 1.42 (9H, s, 3 $\times$ CH₃ Boc), and 0.92 (6H, d, 2 $\times$ CH₃ Leu). IR (cm⁻¹, on NaCl): 3300 (NH), 1680/1640/1610 (C=O), and 1500 (NH).

Polymerization

Boc-Leu- Δ^2 Phe-Pro-OH (0.97 g, 2.0 mmol) was dissolved in HCOOH (12 ml) at 0°C and allowed to stand for 22 h at room temperature. The mixture was concentrated *in vacuo* to give HCOOH·H-Leu- Δ^2 Phe-Pro-OH. The HCOOH salt was dissolved in a dioxane solution of HCl (0.20 mol ml⁻¹, 25 ml), and the mixture was evaporated *in vacuo*. This procedure was carried out again, and the resulting HCl salt was dried *in vacuo*. (Formyl proton at *ca.* 8.0 ppm in ¹H NMR spectrum of the residue was completely disappeared.)

To a mixture of the salt and *N,N*-dimethylformamide (DMF) (1.5 ml) were added DPPA (0.57 ml, 2.7 mmol) and triethylamine (0.65 ml, 4.7 mmol) at 5–10°C. The mixture was stirred vigorously at 5–10°C for 2 h, and stirring was continued for 140 h. To the mixture was added a large volume of water, and the precipitate was collected by centrifugation, and dried *in vacuo*. Yield 0.70 g (93%). The obtained polypeptide was fractionated into species of MW = 0.9×10^4 – 1.8×10^4 (degree of polymerization = 25–50) by column of TOYOPEARL HW-55/DMF. The COSY spectrum is shown in Figure 1. 400 MHz ^1H NMR (δ in CDCl_3): 9.36 (1H, s, NH Δ^Z Phe), 8.43 (1H, s, NH Leu), 7.23 (2H, s, *o*-H of phenyl), 6.83 (2H, s, *m*-H of phenyl), 6.43 (1H, s, *p*-H of phenyl), 6.11 (1H, s, C^β H Δ^Z Phe), 4.64 (1H, s, C^α H Leu), 4.40 (1H, s, C^α H Pro), 3.70 (2H, d, C^δ H₂ Pro), 2.50 (2H, s, C^β H₂ Pro), 2.2–1.8 (3H, m, C^β H₂– C^γ H Leu), 1.81

(2H, s, C^γ H₂ Pro), and 1.12 (6H, d, $\text{CH}_3 \times 2$ Leu). FT-IR (cm^{-1} , on KBr): 3300 (NH), 1652/1614 (C=O), and 1530/1501/1483 (NH).

RESULTS AND DISCUSSION

Stable Conformations of poly(*X*- Δ^Z Phe-Pro)

Tables I–V show energy-minimized conformations for poly(*X*- Δ^Z Phe-Pro) of *X* = Ala, Leu, Gly, D-Ala, and Aib, respectively. Here the lowest- to 6th low-energy conformations are shown with the conformational letter code, helix-type, translation along helical axis per residue (*d*), and energy difference per residue from the lowest energy (ΔE_{res}). All the conformations in Tables I–V corresponded to β - or γ -helix, indicating poly(*X*- Δ^Z Phe-Pro) will be able to form a novel helical structure. The lowest-energy conformation for *X* = Ala was predicted

Figure 1. 400 MHz COSY spectrum of polypeptide I in CDCl_3 .

Table I. Energy-minimized conformations for Ac-(Ala- Δ^Z Phe-Pro)₈-NMA

Conformational letter code	Helix type ^a	d^b	R^c	Ala		Δ^Z Phe			Pro ^d	ΔE_{res}^e
		Å	Å	ϕ	ψ	ϕ	ψ	χ^2	ψ	kcal mol ⁻¹
DHC	$\beta^{11.9}$ (R)	0.43	5.2	-157	82	-40	-85	143	95	0.00
A*H*F	$\beta^{6.2}$ (L)	1.10	6.7	58	35	122	105	142	150	0.24
FD*A	$\beta^{4.7}$ (R)	1.56	9.8	-90	141	126	-89	136	-50	0.76
FD*F ^f	$\beta^{11.4}$ (R)	1.15	9.0	-72	132	131	-77	137	139	0.78
ED*A	$\beta^{4.7}$ (R)	1.36	10.5	-132	121	127	-81	137	-23	0.83
CH*A ^f	γ	1.44	9.1	-64	126	129	109	135	-41	0.85

^aL and R are left- and right-handed helix sense, respectively. ^bTranslation along helical axis per residue. ^cCenter-to-center distance between the nearest β -phenyl pairs. ^d $\phi = -75^\circ$. ^e $\Delta E_{\text{res}} = [E - E_0(\text{the lowest-energy})]/24$. ^f*cis*-Peptide bond of Δ^Z Phe-Pro linkage.

Table II. Energy-minimized conformations for Ac-(Leu- Δ^2 Phe-Pro)₈-NMA

Conformational letter code	Helix type ^a	d^b	R^c	Leu ^d		Δ^2 Phe			Pro ^e	ΔE_{res}^f
		Å	Å	ϕ	ψ	ϕ	ψ	χ^2	ψ	kcal mol ⁻¹
A*H*F	$\beta^{6.1}$ (L)	1.11	6.7	59	34	123	105	142	148	0.00
FD*A	$\beta^{4.7}$ (R)	1.48	10.2	-106	133	122	-91	139	-29	0.42
ED*A	$\beta^{4.7}$ (R)	1.37	10.5	-124	122	126	-83	137	-25	0.51
DHC	$\beta^{9.7}$ (L)	0.99	9.6	-156	98	-38	-68	140	86	0.52
ADA	γ	1.36	9.6	-77	-56	-131	99	48	-46	0.52
DH*A	γ	1.22	7.8	-145	65	127	120	137	-18	0.77

^a L and R are left- and right-handed helix sense, respectively. ^b Translation along helical axis per residue. ^c Center-to-center distance between the nearest β -phenyl pairs. ^d For each letter code, the most stable orientation in Leu's side chain (χ^1 , χ^2 , $\chi^{3.1}$, and $\chi^{3.2}$) is shown here. ^e $\phi = -75^\circ$. ^f $\Delta E_{\text{res}} = [E - E_0(\text{the lowest-energy})]/24$.

Table III. Energy-minimized conformations for Ac-(Gly- Δ^2 Phe-Pro)₈-NMA

Conformational letter code	Helix type ^a	d^b	R^c	Gly		Δ^2 Phe			Pro ^d	ΔE_{res}^e
		Å	Å	ϕ	ψ	ϕ	ψ	χ^2	ψ	kcal mol ⁻¹
A*EC	$\beta^{5.1}$ (R)	1.07	7.8	80	23	-130	115	48	77	0.00
A*H*F	$\beta^{6.1}$ (L)	1.13	6.8	70	29	129	109	137	137	0.01
AAA	γ	1.46	7.2	-65	-43	-42	-72	134	-17	0.16
A*HC	γ	1.50	8.2	87	40	-125	-97	40	106	0.38
G*D*C	$\beta^{5.3}$ (R)	1.05	8.3	140	58	125	-105	138	82	0.59
A*H*C	$\beta^{11.7}$ (L)	1.12	5.7	88	13	31	78	46	100	0.59

^a L and R are left- and right-handed helix sense, respectively. ^b Translation along helical axis per residue. ^c Center-to-center distance between the nearest β -phenyl pairs. ^d $\phi = -75^\circ$. ^e $\Delta E_{\text{res}} = [E - E_0(\text{the lowest-energy})]/24$.

Table IV. Energy-minimized conformations for Ac-(D-Ala- Δ^2 Phe-Pro)₈-NMA

Conformational letter code	Helix type ^a	d^b	R^c	D-Ala		Δ^2 Phe			Pro ^d	ΔE_{res}^e
		Å	Å	ϕ	ψ	ϕ	ψ	χ^2	ψ	kcal mol ⁻¹
A*H*F	$\beta^{6.2}$ (L)	1.10	6.7	74	28	127	104	138	138	0.00
D*DF ^f	γ	1.36	8.9	158	-67	-132	101	46	168	0.48
G*D*C	$\beta^{5.4}$ (R)	1.02	7.7	138	55	122	-109	137	90	0.61
C*DC	$\beta^{6.1}$ (L)	1.41	8.5	90	-70	-127	108	43	118	0.87
E*AC	$\beta^{6.6}$ (R)	1.06	7.6	163	-165	-40	-66	140	81	1.01
C*D*F	$\beta^{8.4}$ (L)	0.78	7.6	103	-67	128	-109	131	161	1.13

^a L and R are left- and right-handed helix sense, respectively. ^b Translation along helical axis per residue. ^c Center-to-center distance between the nearest β -phenyl pairs. ^d $\phi = -75^\circ$. ^e $\Delta E_{\text{res}} = [E - E_0(\text{the lowest-energy})]/24$. ^f *cis*-Peptide bond of Δ^2 Phe-Pro linkage.

Table V. Energy-minimized conformations for Ac-(Aib- Δ^2 Phe-Pro)₈-NMA

Conformational letter code	Helix type ^a	d^b	R^c	Aib		Δ^2 Phe			Pro ^d	ΔE_{res}^e
		Å	Å	ϕ	ψ	ϕ	ψ	χ^2	ψ	kcal mol ⁻¹
A*H*F	$\beta^{6.2}$ (L)	1.08	6.6	55	37	122	108	142	153	0.00
ADA	$\beta^{9.6}$ (L)	0.97	9.0	-54	-41	-127	92	41	-53	0.46
A*DC	$\beta^{8.1}$ (L)	1.08	8.1	61	40	-128	108	68	88	1.27
AH*C	$\beta^{16.0}$ (R)	0.48	8.2	-50	-46	37	-111	127	85	1.92
AHA	$\beta^{9.2}$ (R)	1.53	6.3	-55	-36	-35	113	140	-20	1.93
ADF	γ	1.63	12.2	-51	-44	-128	96	45	174	2.07

^a L and R are left- and right-handed helix sense, respectively. ^b Translation along helical axis per residue. ^c Center-to-center distance between the nearest β -phenyl pairs. ^d $\phi = -75^\circ$. ^e $\Delta E_{\text{res}} = [E - E_0(\text{the lowest-energy})]/24$.

to be a letter code of DHC, which is a right-handed $\beta^{11.9}$ -helix, as shown in Figure 2. The nearest phenyl-phenyl pairs took a center-to-center distance of 5.2 Å and a face-to-face orientation. On the whole, phenyl groups were arranged in C₄-type symmetry on the helical wheel. For X = Gly, the lowest-energy conformation was a letter code of A*EC, which is a right-handed $\beta^{5.1}$ -helix,

as shown in Figure 3. A left-handed $\beta^{6.1-6.2}$ -helix appeared as the lowest-energy conformation for X = Leu, D-Ala, and Aib, as shown in Figure 4 (X = Leu). Similar β^6 -helix was also seen as 2nd low-energy conformation for X = Ala and Gly. The nearest phenyl-phenyl pairs took a center-to-center distance of 6.6–6.8 Å and an edge-to-edge orientation. On the whole, phenyl groups

Figure 2. The lowest-energy conformation (a right-handed $\beta^{11.9}$ -helix) of poly(Ala- Δ^2 Phe-Pro): (a) side-view; (b) top-view.

Figure 3. The lowest-energy conformation (a right-handed $\beta^{5.1}$ -helix) of poly(Gly- Δ^2 Phe-Pro): (a) side-view; (b) top-view.

Figure 4. The lowest-energy conformation (a left-handed $\beta^{6.1}$ -helix) of poly(Leu- Δ^2 Phe-Pro): (a) side-view; (b) top-view.

were arranged in C_2 -type symmetry on the helical wheel. For X=Leu, D-Ala, and Aib, marked energy difference between the lowest- and 2nd low-energy helical conformations ($>0.4 \text{ kcal mol}^{-1}$) was seen, suggesting that the lowest-energy conformation is relatively stable. Thus, polypeptide I for X=Leu was synthesized here.

Novel helical conformations found in poly(X- Δ^2 Phe-Pro) should be mainly ascribed to the presence of Pro residue in the repeating units, since the lowest-energy conformation of Ac-(Ala- Δ^2 Phe-Aib)₈-NMA¹⁵ and Ac-(Ala- Δ^2 Phe)₁₂-NMA¹² showed an α -helical conformation. Table VI shows energy-minimized conformations of Ac-(Phe-Pro-Ala)₈-NMA,²⁷ in which Δ^2 Phe residue in Ac-(Ala- Δ^2 Phe-Pro)₈-NMA is replaced by the cor-

responding saturated residue. The lowest-energy conformation was a left-handed $\beta^{10.5}$ -helix (DAA), being different from $\beta^{11.9}$ -helix of the lowest-energy for Ac-(Ala- Δ^2 Phe-Pro)₈-NMA. On the other hand, the $\beta^{11.9}$ -helix resembles a left-handed $\beta^{12.0}$ -helix of the lowest-energy for poly(Ala-Pro-Phe).²⁸ From comparison of Tables I and VI, energy-minimized conformations of Ac-(Ala- Δ^2 Phe-Pro)₈-NMA differs from Ac-(Phe-Pro-Ala)₈-NMA essentially. Therefore, the introduction of Δ^2 Phe residue to Pro-containing polypeptides should vary their conformational space to create a novel helical backbone.

Table VI. Energy-minimized conformations for Ac-(Phe-Pro-Ala)₈-NMA²⁷

Conformational letter code	Helix type ^a	d^b Å	Phe		Pro ^c ψ	Ala		ΔE_{res}^f kcal mol ⁻¹
			ϕ	ψ		ϕ	ψ	
DAA	$\beta^{10.5}$ (L)	1.02	-140	79	-16	-71	-37	0.00
DCA	$\beta^{13.8}$ (R)	0.48	-146	82	86	-71	-35	0.63
DCG	$\beta^{8.7}$ (L)	1.54	-154	79	82	-159	-57	0.64
DCG	$\beta^{8.1}$ (L)	1.43	-146	79	68	-169	-53	0.84
DFA*	$\beta^{11.6}$ (R)	0.72	-158	93	178	57	53	0.98
DCA*	$\beta^{8.2}$ (L)	1.22	-152	84	96	62	50	1.02
DBA	$\beta^{10.1}$ (R)	1.24	-156	82	-9	-60	-45	1.04
EAG	γ	1.74	-152	158	-27	-143	-57	1.34

^a L and R are left- and right-handed helix sense, respectively. ^b Translation along helical axis per residue. ^c $\phi = -75^\circ$. ^d $\Delta E_{\text{res}} = [E - E_0(\text{the lowest-energy})]/24$.

CD Spectra of I

Figure 5 shows CD and UV spectra of polypeptide **I** in chloroform and in THF. Polypeptide **I** was partly or not soluble in polar solvents such as methanol and acetonitrile. 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. UV absorption spectra showed intense maxima around 280 nm assignable to Δ^2 Phe residue. The UV pattern was essentially similar to that of Boc-Leu- Δ^2 Phe-Pro-OH **II**, indicating that no strong ground-state interaction between Δ^2 Phe- Δ^2 Phe pairs exists in polypeptide **I**.

The corresponding CD spectra of polypeptide **I** in the two solvents showed a positive band around 280 nm. Compared with polypeptide **I**, peptide **II** showed somewhat smaller CD intensity and more marked solvent dependence on its CD pattern, suggesting that polypeptide **I** takes a more defined conformation. Poly-[Lys(Z)- Δ^2 Phe-Aib] [Lys(Z), N^ϵ -(benzyloxy)carbonyl]-L-lysine] that takes a right-handed 3_{10} - or α -helix in chloroform showed an exciton splitting in correspondence of the 280-nm transition, with a negative peak at longer wavelengths.¹⁵ Thus, polypeptide **I** should take a conformation other than 3_{10} - or α -helix essentially. Non-split CD pattern indicates that dipole-dipole interactions between Δ^2 Phe residues are weak in polypeptide **I**. The direction of the 280-nm transition moment is assigned to the intramolecular charge-transfer between the styryl and carbonyl groups in Δ^2 Phe residue.^{14,29} The transition moment was estimated from molecular orbital calculation to lie on the phenyl-carbonyl line, as shown in Figure 6(a).¹⁴ By applying the exciton chirality method³⁰ to the present system, non-split CD pattern should mean that the skew angle between neighboring transition moments (τ) is $\sim 0^\circ$ or $\sim 180^\circ$, i.e., a parallel arrangement of the moments along peptide backbone as shown in Figure 6(b). This arrangement might correspond to the spatial arrangement of Δ^2 Phe side chains on the β^6 -helical, as shown in Figure 4(b).

The parallel arrangement estimated from non-split

Figure 5. CD (top) and UV absorption (bottom) spectra of polypeptide **I** and peptide **II**: **I** (—) and **II** (····) in chloroform; **I** (---) and **II** (- - -) in THF.

Figure 6. (a) The location of the 280 nm-transition moment of Δ^2 Phe residue. (b) The relative orientation between neighboring moments on a helical wheel ($\tau = \sim 0^\circ$ and $\sim 180^\circ$).

CD pattern was supported by CD spectrum of Boc-Val- Δ^2 Phe-Phe-Ala-Leu-Ala- Δ^2 Phe-Leu-OMe that take an α -helical structure in chloroform.³¹ Namely, only an intense positive band was observed for the above peptide in chloroform.³² Here the two Δ^2 Phe side chains are located nearly in the opposite site of α -helical wheel, i.e., $\tau \sim 0^\circ$.

In the above discussion, peptide bond of Δ^2 Phe-Pro linkage has been fixed at 180° (*trans*). The NMR spectrum of polypeptide **I** (Figure 1) showed a single peak for Pro H^α at 4.40 ppm, indicating that Δ^2 Phe-Pro bond was taken to be either *cis*- or *trans*-form.³³ The position was close to 4.43 ppm of Pro H^α for 3_{10} -helical Boc-

(Val)₂-Aib-*trans*-Pro-(Val)₃-OMe in CDCl₃.³⁴ The *trans*-form of Δ²Phe-Pro linkage was also supported by the conformational energies between Ac-(Leu-Δ²Phe-*cis*-Pro)₈-NMA and Ac-(Leu-Δ²Phe-*trans*-Pro)₈-NMA: the lowest-energy *cis*-form was 0.96 kcal mol (residues)⁻¹ higher than the lowest-energy *trans*-form, being markedly unstable.

Further evidence is necessary to confirm the presence of β-helix in polypeptide I. We will perform NMR and X-ray analysis to determine more precise conformation. However, the present study demonstrated the possibility to design and synthesize a novel helical backbone using sequential polypeptides containing Δ²Phe and Pro residues.

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