Conformations and CD curves of cyclo(L- or D-Phe-L-Pro–Aca): cyclized models for specific types of β-bends

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Cyclic tripeptides cyclo(L-Phe-L-Pro-Aca) (molecule 3) (Aca, ε-aminocaproic acid) and cyclo(-D-Phe-L-Pro-Aca) (molecule 4) are designed as models of specific types of β-bend. Energy calculation and ^1H and ^{13}C NMR studies have indicated that peptides 3 and 4 form β-bend types VI and II′, respectively. Circular dichroism spectra of 4 have a double minimum negative band at the region of 200–230 nm like those of gramicidin S. The spectra of 3, forming the *cis* peptide bond just before Pro, have a negative extremum at the 210–213 nm region. The spectra are used to estimate the contribution of various bend types in peptides.

β-Bend *CD Measurement* *Conformational energy calculation* *Cyclic peptide* *Gramicidin S* *NMR measurement*

1. INTRODUCTION

In the interpretation of peptide conformation it is useful to establish characteristic CD curves corresponding to different types of β-bend. Recently, authors have reported dipeptide molecules cyclized with $-(CH_2)_5-$ as a well defined β-bend model which corresponds to the classical type I (and III) and type II bends [1]. The CD curve of cyclo(L-Ala-D-Ala-Aca) (molecule 1) has been observed with ellipticity extrema at 225–230 nm (negative) and 202–204 nm (positive). This pattern fits in with the generally accepted values for the type II bend. That of cyclo(L-Ala-L-Ala-Aca) (molecule 2) has been observed with negative ellipticity extrema at 203–208 nm and 218–222 nm. This pattern is characteristic of the type I bend. A similar α-helix-like spectrum has been observed in several cyclic hexapeptides containing the sequence of D-amino-acyl-L-Pro [2,3]. This sequence occurs in gramicidin S in which the β-bend is characterized as type II′ [4]. It is interesting to distinguish CD patterns of type I and type II′ β-bends.

We report the results of conformational analysis of cyclo(L- or D-Phe-L-Pro-Aca-) (3 or 4). Compounds 3 and 4 are designed to be models of type VI and II′ bends, respectively. It is demonstrated here that they are indeed good models of the respective bend types. The differences of the CD patterns of type I and II′ bends are discussed by referring to those of 2 and 4. The CD pattern of type VI β-bend is also discussed by referring to that of 3.

2. THEORETICAL AND EXPERIMENTAL PROCEDURES

The conformational free energy, $G = H - TS$, was evaluated by first minimizing the conformational energy, H, and then calculating the conformational entropy, S, by calculating the second derivative matrix numerically at the minimum

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Table 1

Computed low energy conformations

Conf.	ω_0	ϕ_1	ψ_1	ω_1	X_1	X_2	ψ_2	ω_2	ΔH	$-T\Delta S$	ΔG^a	$e^{-\Delta G}/kT$	Bend
c(-L-Phe-L-Pro-Aca-) (3)													
1	155	−140	141	−1	−64	101	−13	178	0.00	0.00	0.00	1.00	VI
2	160	−145	136	0	−177	91	−13	179	−0.18	0.58	0.40	0.51	VI
3	156	−145	142	−1	59	93	−11	176	0.59	0.04	0.63	0.35	VI
c(-D-Phe-L-Pro-Aca-) (4)													
1	−163	100	−132	167	60	76	−12	177	0.00	0.00	0.00	1.00	II′
2	−166	91	−127	170	176	93	−14	179	−0.67	0.94	0.27	0.64	II′
3	−178	−51	−70	174	55	75	−63	174	−1.01	1.51	0.50	0.43	III
4	−179	−51	−72	175	172	114	−62	174	−0.24	1.68	1.44	0.09	III
5	−153	131	136	164	−60	88	−15	173	1.35	1.06	2.41	0.02	II′

[a] $\Delta G = G - G_0 = (H - TS) - (H_0 - TS)_0$, where H_0, $-TS_0$ and G_0 are −12.20, 9.30 and −2.90 kcal/mol for c(-D-Phe-L-Pro-Aca-), and −10.96, 11.13 and 0.17 kcal/mol for c(-L-Phe-L-Pro-Aca-) at T = 300°K

energy conformation. Procedures of the calculation employed in the minimization are basically the same as in [5], except for the treatment of ring closure [6].

Compounds 3 and 4 were synthesized by classical solution methods, and satisfactory elemental analysis, thin layer chromatography and EI mass spectrometry data were obtained. ^1H and ^{13}C NMR spectra were recorded in dimethylsulphoxide (DMSO)-d_6 solution on a JEOL FX-200 spectrometer at 29°C. Tetramethylsilane was used as an internal reference. CD spectra were recorded in methanol on a JASCO J-40 spectropolarimeter.

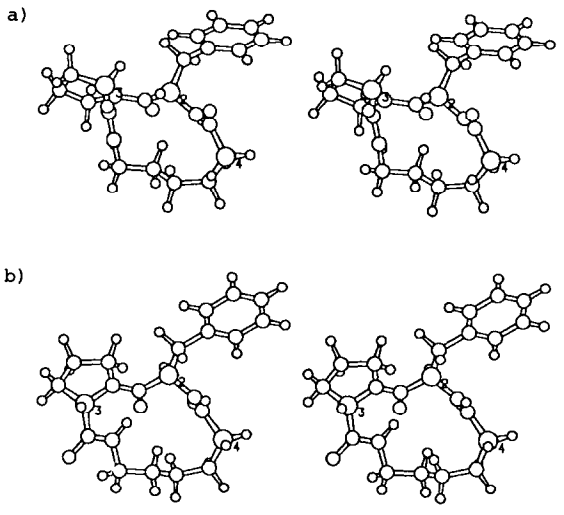

Fig. 1. Stereoscopic drawing of computed low energy structures of cyclic peptides. (a) cyclo(-L-Phe-L-Pro-Aca-) (conf. 1); (b) cyclo(-D-Phe-L-Pro-Aca-) (conf. 1)

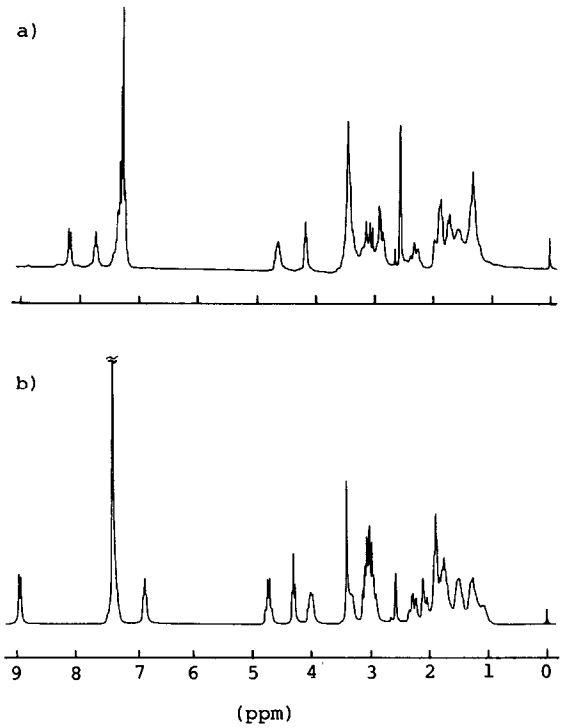

Fig. 2. ^1H-NMR Spectra of cyclic peptides. Solvent: DMSO-d_6. (a) 3; (b) 4.

Table 2

^{1}H NMR data on peptide NH-resonance in DMSO-d_6 at 29°C

Peptides	Phe			Aca	
	δ	$J_{N\alpha}$(Hz)	$\Delta\delta/\Delta T(\times 10^{-3})$	δ	$\Delta\delta/\Delta T(\times 10^{-3})$
3[a]	8.09	7.1[b]	−4.8	7.65	−3.2
4[a]	8.83	7.2[b]	−4.7	6.75	−1.4

[a] $\Delta^{\beta,\gamma}$ Values of chemical shifts of [^{13}C]proline C^β and C^γ in 3 and 4 are 9.11 and 4.23 ppm, respectively.
[b] These values of coupling constant correspond to ϕ = −36°, −84°, 83° or 157°

3.RESULTS

The free energies of the minimum conformations computed for both peptides are listed in table 1. All 5 low energy conformers of 4 have all-*trans* configuration of the peptide bonds, and correspond to bends of type II′ and III. They are populated in these two bend types in a ratio of 3:1. Three conformers of compound 3 have *cis* configuration of the peptide bond just before Pro and correspond to the type VI bend. Stereoscopic drawings of the computed low energy structures of 3 and 4 are shown in fig.1.

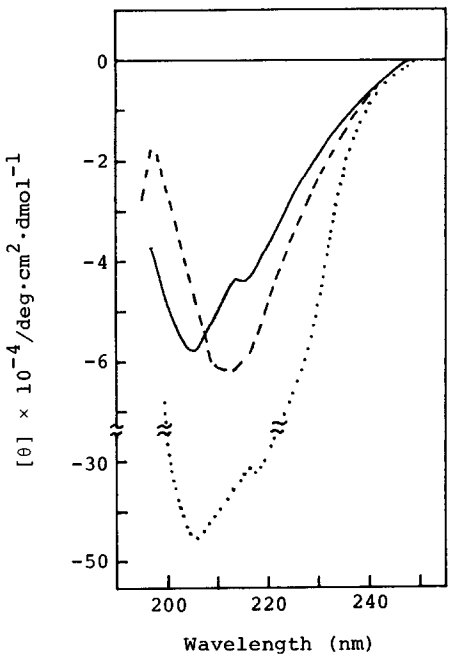

Fig. 3. CD Curves of cyclic peptides. Solvent: MeOH. Curve: (– – –) 3; (——) 4; (···) gramicidin S.

Both ^{1}H and ^{13}C NMR spectra reveal the signals of a time-averaged single conformer in both cyclic peptides (fig. 2). Chemical shifts, coupling constants and temperature coefficients ($\Delta\delta/\Delta T$) of Phe and Aca NH-groups are summarized in table 2. In compound 4, the Aca-NH occurs at higher field (6.75 ppm) than the usual peptide NH and has a low $\Delta\delta/\Delta T$ value (-1.4×10^{-3} ppm/°C) characteristic of a solvent-shielded or intramolecularly hydrogen-bonded NH group, whereas the $\Delta\delta/\Delta T$ value (-4.7×10^{-3} ppm/°C) for the Phe-NH is indicative of a solvent-exposed proton. These results are compatible with a β-bend conformation, possibly with a hydrogen-bond between the Aca-NH and the Aca-CO. In compound 3, the Aca has a somewhat low $\Delta\delta/\Delta T$ value (-3.2×10^{-3} ppm/°C) characteristic of a solvent-shielded or weaker intramolecularly hydrogen-bonded NH group.

Based on the difference ($\Delta^{\beta,\gamma}$) of chemical shifts of Pro C^β and C^γ in the ^{13}C NMR spectra, compounds 3 and 4 are found to have *cis*-L-Phe-L-Pro ($\Delta^{\beta,\gamma}$ = 9.11 ppm) and *trans* D-Phe-L-Pro ($\Delta^{\beta,\gamma}$ = 4.23 ppm) peptide bonds, respectively.

CD spectra of 3, 4 and gramicidin S in methanol are shown in fig. 3. In 3 a strong negative band ($[\theta]_M$ = 61 500) is observed at 210–213 nm.

4. DISCUSSION

Conformational energy calculation and several of the NMR parameters such as coupling constants, temperature coefficients of amide protons, and difference of Pro C^β and C^γ chemical shifts in-

Table 3

Dihedral angles and CD spectral parameters for cyclic peptides

Peptides	Dihedral angles of cyclic peptides						CD spectral parameters			
	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ω_2	λ_{min} (nm)	θ^a ($\times 10^{-3}$)	λ_{min} (nm)	θ^a ($\times 10^{-3}$)
C(-D-Phe-L-Pro-L-Val-)$_2$[b]	60	−135	−162	− 86	−15	−176	223	− 8.7	201	−16.3
C(-D-Phe-L-Pro-Aca-) (4)	100	−132	167	− 75	−12	177	215	−14.8	206	−19.0
Gramicidin S	55	−110	180	− 60	−40	180	215	−38.2	205	−44.9
C(-L-Ala-L-Ala-Aca-) (2)	− 79	− 47	176	−111	65	−179	220–2	−21	208	−20
α-Helix	− 57	− 47		− 57	−47		222		209	

[a]These values are expressed as mean residue ellipticities ($m_\theta = M_\theta/n$, where n is number of residues)
[b]Crystal structure was determined as in [7]. CD was measured in AcCN [3]

dicate that compound 4 takes the type II′ β-bend. Moreover, this CD spectrum, which has double minima in 200–230 nm, is similar in shape to that reported for gramicidin S but with much smaller ellipticity (fig. 3). These results suggest that the CD pattern of the D-aminoacyl-L-Pro sequence, which prefers type II′ β-bend, has α-helix-like double minima at 201–206 nm and 215–223 nm regions.

Compound 2 with a type I bend shows CD spectra similar to those of 4. However, there is a clear difference between the two spectra. The first trough at 201–206 nm is slightly shallower than the second at 213–225 nm region in compound 2 (type I); on the other hand, the first trough is deeper than the second in compound 4 (type II′). This means that type I and II′ β-bends, both with α-helix-like CD spectra, can be distinguished. The dihedral angles and CD parameters of several peptides showing the double minimum at 200–230 nm are listed in table 3. All of the ϕ and ψ values of cyclo(-D-Phe-L-Pro-L-Val-)$_2$, gramicidin S, and 4 are almost identical. However, the ϕ and ψ values of cyclo(-L-Ala-L-Ala-Aca-) differ from these three cyclic peptides. Comparison with the α-helical values of dihedral angles indicates that ϕ_1 and ψ_1 of 2 are very close and ϕ_2 and ψ_2 are different. Similar comparison of the values of dihedral angles in the α-helix and 4 indicates that ϕ_1 and ψ_1 are different and ϕ_2 and ψ_2 are close. These small differences may explain the fact that the CD patterns in 2 and 4 are similar to that in the α-helix but the ellipticity of the double minima in 2 and 4 has opposite relative values.

Compound 3 is characterized as a type VI bend on the basis of energy calculation and spectroscopic data. This bend structure contains a *cis* peptide bond just before Pro. This molecule takes *cis* conformation as the preferred one because of steric hindrance between the L-Phe side chain and the Pro δ-methylene group brought out by the cyclization. The CD curve of 3 shown in fig. 3 is the first one for the bend containing a *cis* peptide bond.

CD spectra of 2 and 4 are used to identify and estimate the contribution of various bend types in acyclic and cyclic peptides.

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