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Macrocyclic Peptides VI. Complex Formations and Conformations of an Ionophorous Cyclic Octapeptide Containing *N,N'*-Ethylene-Bridged (*S*)-Leucyl-(*S*)-leucine and Glycine in Acetonitrile

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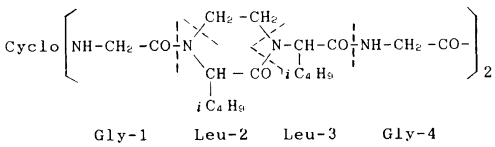
ABSTRACT: Complex formations and conformations of a synthetic cyclic octapeptide, cyclo[Gly-ELL-Gly]$_2$ (**1**), with alkali and alkaline earth metal ions were investigated in acetonitrile by CD and NMR spectroscopies. The interactions between **1** and alkali metal complex ions (Li^+, Na^+, and K^+) are considered to be very weak from small CD spectral changes. In the case of alkaline earth metal ions, titration curves obtained from CD data indicated the presence of only a PC (peptide–cation = 1 : 1) complex ion for Mg^{2+} and Ba^{2+}, and of both P_2C (peptide sandwich) and PC complex ions for Ca^{2+}. The K_1 values evaluated from the titration curves were in the order of $Ca^{2+} > Ba^{2+} > Mg^{2+}$. Furthermore, ^1H and ^{13}C NMR measurements showed that **1** and the complex ions with Ca^{2+} and Ba^{2+} have each different C_2-symmetric structure.

KEY WORDS Solution Conformation / Cation-Binding Properties / CD Spectra / Cyclic Octapeptide / Glycine / (*S*)-Leucine / ^1H and ^{13}C NMR / Piperazin-2-one /

Ionophores form stable and lipophilic complexes with alkali and alkaline earth metal ions, and are able to transport them across membranes. It has been known that various cyclic peptides[1-4] and cyclic or acyclic polyether[5] have an interesting feature to bind inorganic substrates such as alkali and alkaline earth metal ions, specifically.

The authors have studied the interactions[6] between several novel cyclic peptides including *N,N'*-ethylene-bridged dipeptides {EXX; piperazin-2-one (MKP) derivatives} and organic substrates, and also found that these cyclic peptides are useful in the investigation[7,8] of the chiral recognition of (*R*)- and (*S*)-enatiomers. However, the conformations of organic substrate-cyclic peptide complexes have not been studied in detail because of the indefinite stoichiometries and the multiple coordination modes between organic substrates and cyclic peptides in comparison with those between inorganic substrates and cyclic peptides. Therefore, the authors have attempted to study the complex formation of a cyclic octapeptide (**1**) including ELL {L = (*S*)-leucine} with inorganic substrates.



Cyclo[Gly-ELL-Gly]$_2$ = **1**; iC_4H_9 = iso-butyl

This article deals with the conformations of **1** and its Ba^{2+} complex ion, and the complex formations of **1** with alkali and alkaline earth metal ions, which were investigated in acetonitrile by CD and NMR spectroscopies, and speculated by CPK modeling.

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EXPERIMENTAL

The preparation of **1** was reported in our previous paper.[7] Reagent grade perchlorates dried *in vacuo* at 120°C were used as alkali and alkaline earth metal salts.[2] Spectrometric grade acetonitrile (DOJINDO Laboratories) was used as the solvent for CD measurement without further purification. CD spectra were recorded on a Jasco J-500A automatic recording spectropolarimeter equipped with a DP-500 data processor. The measurements were carried out in acetonitrile using a quartz cell with 0.005 dm path length over the wavelength region from 210 nm to 250 nm at 23°C. The data were represented as the mean residue ellipticities. NMR spectra were measured at 36°C in CD_3CN by a Jeol GX-400. All chemical shifts were given in ppm relative to the signals of CD_3CN (1.93 and 118.2 ppm for ^1H and ^{13}C NMR, respectively). All signals were assigned by two-dimensional (2D) and selective decoupling methods.

RESULTS AND DISCUSSION

CD Investigations

CD Spectra of Free 1 and Its Complex Ions When the large excess of alkali metal ions (Li^+, Na^+, and K^+) were added to the solution of **1**, small spectral changes were observed. The spectra of complex ions increased slightly in the intensity, and their extreme moved scarcely from 227 nm for **1**. On the other hand, the spectra changed largely in the intensity by the addition of the excess of Ca^{2+} or Ba^{2+}, as shown in Figure 1. Moreover, in the addition of the excess of Mg^{2+}, the extreme sign of the spectral bands changed from negative for free **1** to positive one. The CD variation of this complex is similar to that of a PC complex of cyclo(Pro-Gly)$_3$ with Mg^{2+} reported earlier by Blout *et al.*[1] Their extreme shifted from 227 nm for **1** to 230, 226, and 229 nm in the addition of Mg^{2+}, Ca^{2+}, and Ba^{2+}, respectively.

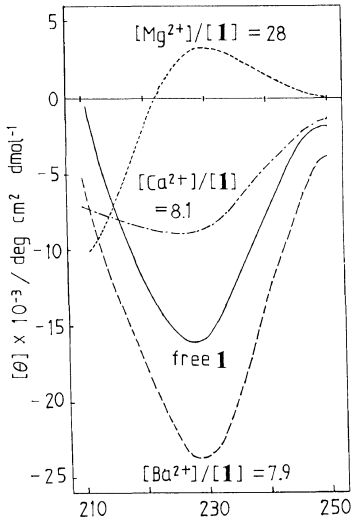


Figure 1. CD spectra of free **1** and **1** in the presence of Mg^{2+}, Ca^{2+}, and Ba^{2+} in acetonitrile. Mole ratios of salts to **1** are shown in the figure. $[\mathbf{1}] = 2.36 \times 10^{-4}$ mol dm^{-3}

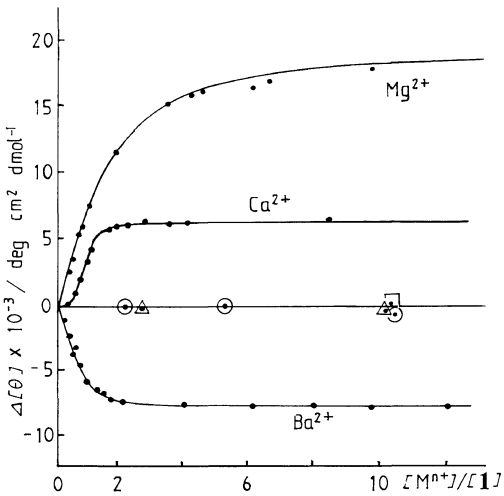


Figure 2. Titration curves of **1** for various metal perchlorates in acetonitrile at 227 nm. $\Delta[\Theta]$ indicates the difference between the mean residue ellipticity of **1** and that of its complex ion. $[\mathbf{1}] = 2.36$, 2.66 and 2.64×10^{-4} mol dm^{-3} for Mg^{2+}, Ca^{2+}, and Ba^{2+}, respectively. (□, Li^+; △, Na^+; ○, K^+.)

Titration Curves Figure 2 indicates the changes of the mean residue ellipticities at 227 nm, which were plotted against the molar ratios of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, and Ba^{2+} to **1**, because the titration curves

generated from several wavelengths (222—232 nm) showed no remarkable differences in comparison with that obtained at 227 nm. The small changes of the ellipticities with the stepwise addition of the alkali metal salts suggest their weak interactions with **1**. Both Mg^{2+} and Ba^{2+} complex ions indicate the positive and negative hyperbola type curves, respectively. Only one plateau is observed for Mg^{2+} and Ba^{2+}, suggesting the formation of a PC complex. The titration curve of Ca^{2+} shows a gradual increase of the ellipticity as the molar ratio increases, and then indicates a plateau. This observation suggests that **1** forms at least two types of complexes (PC and P_2C) with Ca^{2+}. Of course, the existence of the other species can not be excluded in the presence of larger amount of each metal salt.

Complex-Formation Constants The complex-formation constants were calculated by the methods similar to those of Bergeron *et al.*[9] (for K_1 only) and Deber *et al.*[1] (for K_1 and $K_{1/2}$). The values thus obtained are shown with $\Delta M^{\lambda}_{\theta,i}$ in Table I. In Figure 2, the values calculated using the K_1 or K_1 and $K_{1/2}$ summarized in Table I are shown as the real lines, agreeing well with the measured ones (points) for Mg^{2+}, Ca^{2+}, and Ba^{2+}. The K_1 sequences are in the order of $Ca^{2+} > Ba^{2+} > Mg^{2+}$, which may be attributed to the difference of the diameters among Ca^{2+} (1.98 Å), Ba^{2+} (2.70 Å), and Mg^{2+} (1.30 Å).

Table I. Binding constants and mean residue ellipticities of the complexes of **1** with alkaline earth metal salts in acetonitrile at 23°C

Cation	Binding constants/$mol^{-1}\,dm^3$		ΔM_θ[a] (deg cm^2 $dmol^{-1}$) $\times 10^{-3}$	
	K_1 (P+C⇌PC)	$K_{1/2}$ (PC+P⇌P_2C)	PC	P_2C
Mg^{2+}	6.7×10^3		19.8	
Ca^{2+}	4.2×10^6	7.6×10^4	6.44	0.64
Ba^{2+}	4.9×10^4		−7.60	

[a] Mean difference per residue at 227 nm.

NMR Investigations

In the preceding paper,[10] the conformational study of Boc-Gly-ELL-OH (**2**) was examined by ^1H NMR measurement in CD_3CN at 36°C in order to examine the bonding modes (*trans* or *cis*) between Gly-1 and the MKP ring. This study indicates that **2** exists as two conformational isomers (**2A**, *trans* peptide bond and **2A′**, *cis* one), and the chemical shifts values of H2 and H4e are 4.85 and 3.74 ppm for **2A** and 4.26 and 4.12 ppm for **2A′**, respectively. The difference of these chemical shifts can be explained reasonably by the magnetic anisotropy effect of Gly amide group. The above numbering of **2** follows that of **1** shown in Table II. In the present work, the data obtained for **2** are used in order to reinforce the conformational studies of **1** and its Ba^{2+} complex ion.

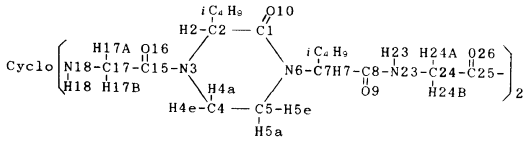
Table II shows the chemical shifts and the coupling constants of the protons of free **1** and **3** ([Ba^{2+}]/[**1**]=2). **1** and **3** show the simple spectra because of each C_2-symmetric structure. Each signal was assigned using the NOE observed between H7 and H23. Also, Table II shows that the chemical shifts of H2 of **1** and **3** are 5.15 and 5.29 ppm, and those of H4e are 3.67 and 3.90 ppm, respectively. These results suggest that the peptide bond between Gly-1 and each MKP ring for **1** and **3** is *trans*, referring the data obtained for **2A** and **2A′**. Moreover, all peptide bonds of **1** and **3** are *trans* from the above results.

A signal (3.95 ppm) of Gly-1 methylene protons (H17A and B) of **1** shows that the methylene group is mobile, while two signals (4.08 and 3.42 ppm of H24A and B) of Gly-4 ones indicate that the methylene group is rigid. One the other hand, the addition of Ba^{2+} to **1** gives relatively rigid conformation, because all signals of methylene protons of Gly-1 and -4 of **3** are splitted: the values of H17A, H17B, H24A, and H24B of **3** are 4.25, 4.01, 4.29, and 3.56 ppm, respectively. The dihedral angles between the NH(H18) and H17A-B of **3**

Table II. ^{1}H Chemical shifts and coupling constants of **1** and **3**[a] (2[Ba^{2+}]/[**1**]) in CD_3CN at 36°C

	Chemical shift, δ/ppm (Coupling constants, J/Hz)										
	H18	H17A & H17B		H2	H4e[b]	H4a[b]	H5e[b]	H5a[b]	H7	H23	H24A	H24B
1	6.71 (t, 4.3)	3.95 (d, 4.3)		5.15 (t, 7.4)	3.67 (m)		3.37 (m)		5.20 (dd, 8.6, 6.7)	7.03 (b)	4.08 (dd, 16.6, 8.1)	3.42 (dd, 16.6, 4.9)
3	7.26 (s[c])	4.25 (dd, 16.6, 4.8)	4.01 (dd, 16.1, 5.4)	5.29 (bd)	3.90 (bd, 12.5)	3.58 (td, 12.5, 3.4)	3.16 (bdd, 12.5, 2.7)	2.90 (td, 12.5, 4.9)	5.33 (dd, 8.8, 6.3)	6.54 (b)	4.29 (dd, 16.6, 8.8)	3.56 (d, 16.6)

[a] The numbering of **1** and **3**: iC_4H_9, iso-butyl.



[b] a, axial; e, equatorial.

[c] Triplet like.

estimated by the method of Bystrov *et al.*[11] are 0° and +120°, while those between H23 and H24A-B are +10° and +130° for **1** and +90° and −150° for **3**. As shown in Table II, the coupling constant (7.4 Hz) of H2 of **1** shows the existence of an equilibrium between U_I and U_{II} forms on the conformation of MKP ring, suggesting an equilibrium between a pseudo-chair form for U_I and a boat one for U_{II}, as shown in the previous study of Boc-Gly-EFF-OH(F=(*S*)-phenylalanine).[12] U_I and U_{II} corresponding to the energy minima of internal rotation around C^{α}–C^{β} bond, is available as illustrated in Figure 3. Accordingly, the signals of the MKP ring ethylenic protons of **1** appear as multiplets (see Table II). A pseudo-chair form of MKP ring of **3** was established with W-letter long range coupling between H2 and H4e in 2D COSY spectra and the coupling constants of four ethylenic proton signals observed clearly. Consequently, the conformation of the side chain on MKP ring of **3** suggests U_I form, while that outside MKP ring has U_{II} one from the coupling constants of H7 and the steric

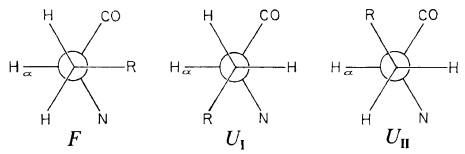


Figure 3. Conformations about the α–β bond of leucyl residue: R=$CH_2CH(CH_3)_2$.

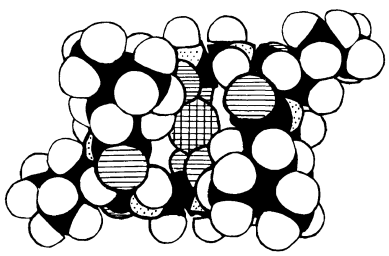


Figure 4. A proposed structure of **3** in acetonitrile ○, hydrogen atom; ◎, nitrogen atom; ⊜, oxygen atom, ●, Ba^{2+} ion; ●, carbon atom.

hindrance between the iso-butyl methylene group on and the iso-butyl methyl ones outside MKP ring. From the above results, a structure of **3** is proposed in Figure 4. Also, the down-field shifts of H18 from 6.71 ppm of **1** to 7.26 ppm of **3** and the up-field shift of

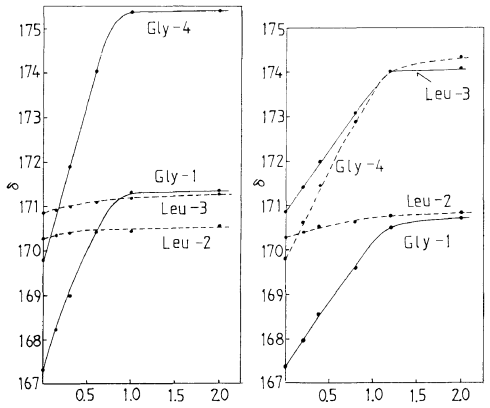


Figure 5. Shifts of amide carbonyl resonances of **1** in the addition of Ba^{2+} and Ca^{2+} in CD_3CN; [**1**]= 0.010 mol dm^{-3}.

H23 from 7.03 ppm of **1** to 6.54 ppm of **3** were observed, supporting the conformational change of **1** in the complex formation. The NMR studies of Mg^{2+} and Ca^{2+} complex ions could not be carried out in detail because of the broad signals for the former and the existence of two species for the latter.

The titrations of **1** with Ba^{2+} and Ca^{2+} were also carried out by ^{13}C NMR measurements. The amide carbons in the addition of Mg^{2+} to **1** was not observed because of the broading of the spectra. Figure 5 indicates the chemical shifts of the four amide carbons (Gly-1, Leu-2, Leu-3, and Gly-4), whose signals were assigned by a selective decoupling method. Figure 5 shows the marked downfield shifts of Gly-1 and -4 amide carbons in the addition of Ba^{2+} while Leu-2 and -3 amide ones indicate the small shifts, suggesting that Ba^{2+} is interacted with Gly-1 and -4 amide oxygens. These results also support the structure of **3** shown in Figure 4. On the other hand, the addition of Ca^{2+} to **1** shows the marked downfield shifts of Gly-1, Leu-3, and Gly-4 amide carbon signals while only Leu-2 one indicates the small shift, suggesting that Ca^{2+} is interacted with Gly-1, Leu-3, and Gly-4 amide oxygens. The difference of the coordination modes between Ba^{2+} and Ca^{2+} may be attributed to the existences of PC complex for Ba^{2+} and PC and P_2C complexes for Ca^{2+}.

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