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NOTES

Complex Formation between *cyclo*(Gly-Pro-Gly)$_2$ and Metal Ions

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Cyclic peptide–cation complexes have been widely studied to model various aspects of metal proteins, metal enzymes, or ion carriers. For example, *cyclo*(Pro-Gly)$_3$,[1-3] *cyclo*(Pro-Gly)$_4$,[4,5] *cyclo*(Gly-Pro-Gly-D-Ala-Pro),[6] *cyclo*(D-Val-Pro-Val-D-Pro)$_3$,[7] *cyclo*(Pro-Phe-Gly-Phe-Gly)$_n$ ($n=1,2$),[8] *cyclo*(Val-Gly-Gly-Pro)$_3$,[9] and *cyclo*[Glu(γ-OBzl)-Sar-Gly-(N-R)Gly]$_2$ (R = *n*-hexyl and cyclohexyl)[10] bind cations in a specific manner. The binding sites of cations in these cyclic peptides are generally carbonyl oxygens of the main-chain peptide bonds.

On the other hand, as pointed out by Blout and coworkers, binding of cations to the cyclic hexapeptides of the type *cyclo*(X-Pro-Y)$_2$ has not been studied so intensively, since the stable conformation with β-turns seems to preclude binding to cations.[11] They found, however, that *cyclo*(D-Ala-Pro-Gly)$_2$ changed its conformation upon complexation with divalent cations.[11]

Thus, we are interested in the complex formation between *cyclo*(Gly-Pro-Gly)$_2$ (**1**), which also has Type II β-turns,[12] and metal cations. In the present study, it was found by measurement of CD spectra that **1** undergoes complex formation with various metal ions, although drastic conformational changes of **1** were not observed.

EXPERIMENTAL

Cyclo(Gly-Pro-Gly)$_2$ (**1**) was synthesized in the same manner as reported.[13] $LiClO_4$ (Nakarai Chemicals), $NaClO_4$ (Kanto Chemical Co.), $Mg(ClO_4)_2$ (Nakarai Chemicals), and $Zn(ClO_4)_2$ (Soekawa Chemicals) were used as supplied.

CD spectra were taken on a JASCO J-20 spectropolarimeter at room temperature using a 1 mm cell. The concentration of **1** was 1.0×10^{-3} mol dm^{-3}. Molar ellipticity [θ] was calculated on the basis of the concentrations of amide chromophores.

RESULTS AND DISCUSSION

Figure 1 shows the changes in CD spectra of **1** in CH_3CN on adding four kinds of metal ions (Li^+, Na^+, Mg^{2+}, Zn^{2+}) in large excess. **1** has a trough in the CD spectrum at 225 nm with [θ] of *ca.* -3.4×10^3 deg cm^2 dmol^{-1}. This type of CD spectrum was assigned to type B and **1** was found to have Type II β-turns.[12] It is very interesting that the addition of Na^+, Mg^{2+} and Zn^{2+} increased the trough (Figure 1 (B)—(D)) while Li^+ decreased it (Figure 1(A)). In every case, however, the troughs of the CD spectra slightly shifted to shorter wavelengths. The binding constant between **1** and the metal ions (Li^+, Mg^{2+}, Zn^{2+}) could be estimated as follows. Assuming that **1** and the metal ion

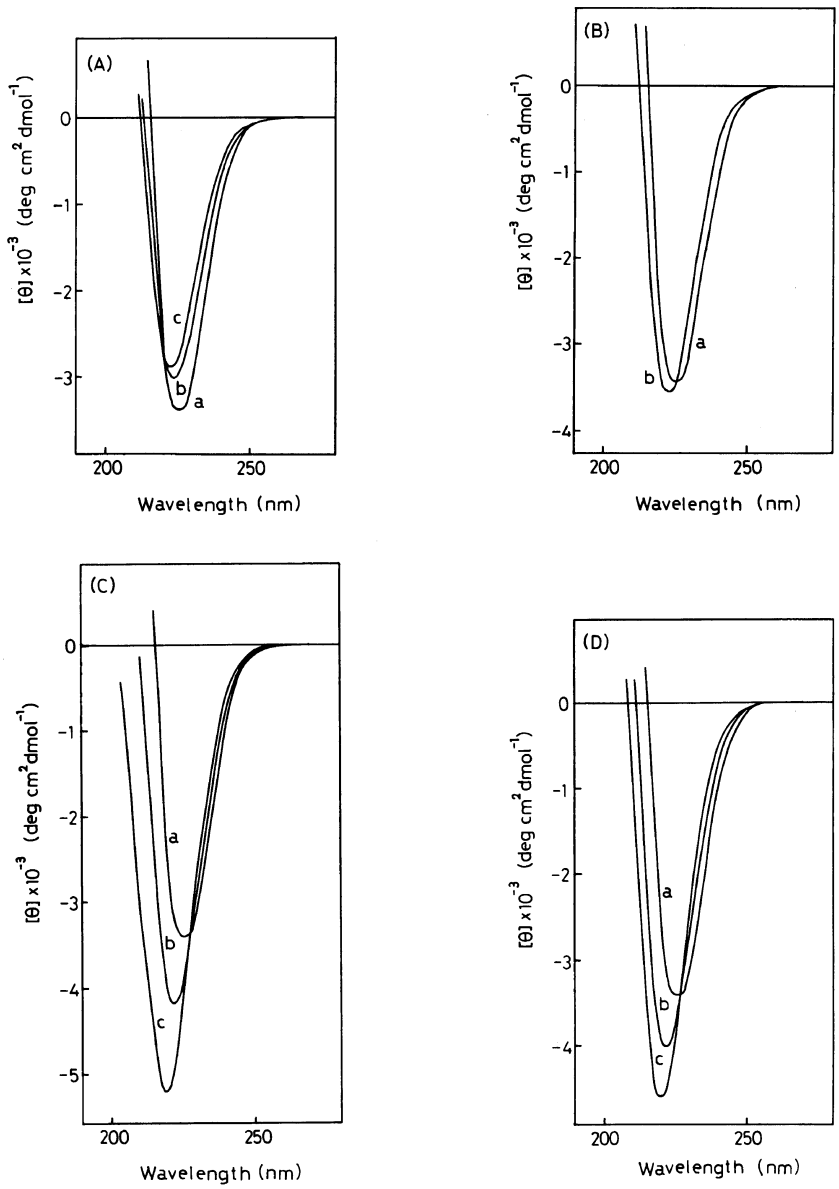

Figure 1. CD spectra of **1** (1.0×10^{-3} mol dm^{-3}) in the absence and presence of metal ions in CH_3CN. (A) $LiClO_4$: $[Li^+]/[\mathbf{1}]=0$ (a), 15 (b), 50 (c). (B) $NaClO_4$: $[Na^+]/[\mathbf{1}]=0$ (a), 100 (b). (C) $Mg(ClO_4)_2$: $[Mg^{2+}]/[\mathbf{1}]=0$ (a), 17 (b), 40 (c). (D) $Zn(ClO_4)_2$: $[Zn^{2+}]/[\mathbf{1}]=0$ (a), 40 (b), 200 (c).

form a 1 : 1 complex, eq 1 holds.

$$K = C_1/\{(C_0 - C_1)[\mathrm{M}]\} \qquad (1)$$

K, Binding constant;
C_0, Concentration of **1**;
C_1, Concentration of the complex;
[M], Concentration of the metal ions ($[\mathrm{M}] \gg C_0$).

On the other hand, the change of $[\theta]$ ($\Delta[\theta]$) upon addition of the metal ions can be represented as follows.

$$C_0\Delta[\theta]=\{C_1[\theta]_1+(C_0-C_1)[\theta]_0\}-C_0[\theta]_0 \quad (2)$$

$[\theta]_1$, Molar ellipticity of the complex;
$[\theta]_0$, Molar ellipticity of **1**.

Equations 1 and 2 give eq 3.

$$\Delta[\theta]^{-1}=\{\Delta[\theta]_0K[\mathrm{M}]\}^{-1}+\Delta[\theta]_0{}^{-1} \quad (3)$$

$$\Delta[\theta]_0\equiv[\theta]_1-[\theta]_0$$

Therefore, a linear relationship should exist between $\Delta[\theta]^{-1}$ and $[\mathrm{M}]^{-1}$. In fact, the linear relationship shown in Figure 2 could be obtained, from which K was determined as $53\,\mathrm{mol^{-1}\,dm^3}$ for Li^+, $52\,\mathrm{mol^{-1}\,dm^3}$ for Mg^{2+} and $31\,\mathrm{mol^{-1}\,dm^3}$ for Zn^{2+}. Compared with Li^+, Mg^{2+}, and Zn^{2+}, Na^+ caused much less change in the CD spectrum of **1** (Figure 1 (B)).

It may be expected that when the solvent, which can coordinate to metal ions, is used, the interaction between **1** and the metal ions will be weakened. Thus, in CH_3OH, the effects of the metal ions upon the CD spectrum of **1** were smaller than those in CH_3CN solutions (Table I). Mg^{2+} and Zn^{2+} gave larger changes in the CD spectra than Li^+.

In aqueous solutions, moreover, significantly large amounts of metal ions were necessary to bring about the changes in the CD spectrum of **1** (Table I).

As described above, **1** and the metal ions seem to form a 1:1 complex in CH_3CN solutions under the present experimental conditions. Taking into account the binding sites of more flexible cyclic peptides,[1-11] the binding sites of **1** are probably the carbonyl oxygens of the peptide bonds. Direct interactions between the carbonyl oxygens and the metal ions were substantiated by the findings that the changes in the CD spectrum of **1** were smaller in CH_3OH or aqueous

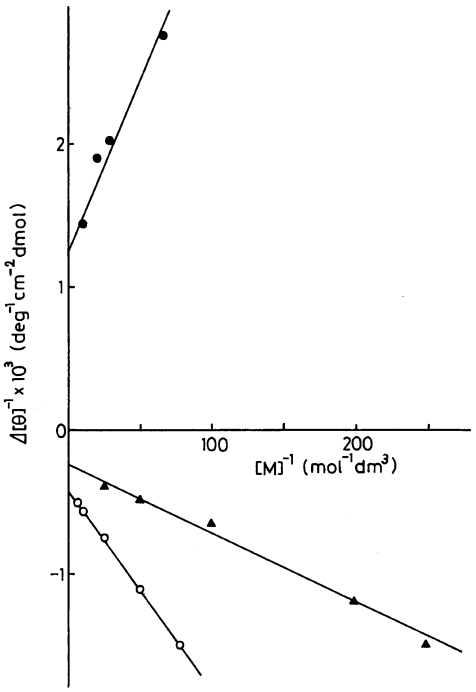

Figure 2. Relation between $\Delta[\theta]^{-1}$ and the reciprocal of the concentrations of metal ions ($[\mathrm{M}]^{-1}$) in CH_3CN solutions; $LiClO_4$ (●), $Mg(ClO_4)_2$ (▲), $Zn(ClO_4)_2$ (○). $\Delta[\theta]$ was measured at 224 nm for Li^+ and at 220 nm for Mg^{2+} and Zn^{2+}.

Table I. Characteristics of CD spectra[a]

Solvent	Metal ions	[Metal ion]/[1]	Trough	$[\theta]\times10^{-3}$
			nm	deg cm^2 d mol^{-1}
CH_3CN		0	226	−3.4
	Li^+	15	224	−3.0
	Li^+	50	222	−2.9
	Na^+	100	223	−3.6
	Mg^{2+}	17	222	−4.2
	Mg^{2+}	40	220	−5.2
	Zn^{2+}	40	222	−4.0
	Zn^{2+}	200	220	−4.6
CH_3OH		0	224	−2.5
	Li^+	200	223	−2.8
	Mg^{2+}	200	222	−3.0
	Zn^{2+}	200	222	−3.0
H_2O		0	221	−3.9
	Li^+	500	221	−4.0
	Li^+	1000	221	−4.2
	Na^+	500	221	−4.1
	Na^+	1000	221	−4.3
	Mg^{2+}	500	221	−4.3
	Mg^{2+}	1000	221	−4.6
	Zn^{2+}	420	221	−4.3
	Zn^{2+}	830	221	−4.6

[a] Concentration of **1** was $1.0\times10^{-3}\,\mathrm{mol\,dm^{-3}}$.

solutions, where the metal ions may be coordinated with the carbonyl oxygens of **1** and with the oxygens of the solvents, competitively.

In aqueous solutions, the CD minimum at *ca.* 220 nm scarcely shifted upon addition of the metal ions in spite of the increase of the trough, which is in great contrast with the CD minimum in CH_3CN solutions where it shifted to a shorter wavelength. The reason for this difference is unknown at present.

In the present report, the rigid cyclic hexapeptide, *cyclo*(Gly-Pro-Gly)$_2$, was found to undergo complex formation with the metal ions (Li^+, Na^+, Mg^{2+}, Zn^{2+}). This paper may be one of the first studies to deal with the complex formation between rigid cyclic peptides and metal ions.

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