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Coordination abilities of amide oxygens in a 24-membered ring pseudopeptide toward several transition metal ions in acetonitrile

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Abstract

Stability constants of a cyclic octapseudopeptide, *cyclo*(Gly-eLL-Gly)$_2$ (eLL = *N,N'*-ethylene-bridged (*S*)-leucyl-(*S*)-leucine, Gly = glycine), for eleven kinds of transition metal ions were investigated by CD spectra in acetonitrile at 25 °C. The stability constants toward bivalent ions of first transition metal are in the order of $Mn^{2+} > Fe^{2+} > Co^{2+} > Ni^{2+} < Cu^{2+} < Zn^{2+}$ and those toward Group XII are $Zn^{2+} < Cd^{2+} > Hg^{2+}$. This trend may be explained by the cavity size of *cyclo*(Gly-eLL-Gly)$_2$, but not the hard and soft acids and bases principle. The titration curves obtained from CD data indicated the presence of only a PC (peptide:cation = 1:1) complex for Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+} and Hg^{2+}, both PC and P_2C (peptide:cation = 2:1) complexes for Fe^{3+}, and both PC and PC_2 (peptide:cation = 1:2) complexes for Fe^{2+} and Cd^{2+}. But ligand/metal ion interactions are considered to be very weak for Ag^+ and Ni^{2+} because of undetectable CD spectral changes. Moreover, ^{13}C NMR studies revealed that six amide oxygens in *cyclo*(Gly-eLL-Gly)$_2$ coordinate to Cu^{2+} and Fe^{3+} in CD_3CN.

Keywords: Transition metal ion complexes; Pseudopeptide complexes; Macrocyclic pseudopeptide; Stability constants

1. Introduction

Ionophorous macrocyclic peptides have been studied extensively by many workers [1–6]. The amide oxygens of these peptides are hard donors and they interact better with alkali or alkaline earth metal ions than with transition metal ions. Several workers [7,8] have reported that the deprotonated amide groups of peptide skeletons and functional groups such as amine nitrogens or carboxylate oxygens coordinate mainly to transition metal ions.

However, Baker [9] and Nar et al. [10] reported recently that the amide oxygen of Gly45 coordinates to copper ion weakly in a kind of metallo protein, asurin. Jackson and co-workers [11] also synthesized several pentaaminecobalt(III) complexes coordinated with the amide oxygens of formamide, *N,N*-dimethylacetamide etc., and examined their solution structures and reactivities. These facts prompted us to investigate the coordination abilities of amide oxygens in cyclic pseudopeptides toward several transition metal ions, because of the importance as a model of a metallo protein. We have synthesized and studied a series of cyclic pseudopeptides including *N,N'*-ethylene-bridged dipeptides. These cyclic pseudopeptides have no intramolecular hydrogen bond [12,13], so that their cavities are favorable for including organic substrates [14] and alkaline earth metal ions [5,6], effectively. This paper describes the apparent binding constants of a cyclic octapseudopeptide, *cyclo*(Gly-eLL-Gly)$_2$ (**L24**), (see Scheme 1), for several transition metal ions in acetonitrile. This solvent has a small Gutmann donor number [15]. Also, ^{13}C NMR measurement revealed that six amide oxygens of **L24** coordinate to metal ions.

2. Experimental

2.1. Materials

A cyclic pseudopeptide, **L24**, was prepared according to the published method [5]. Perchlorate salts and solvents used here were purchased from Nacalai Tesque Inc., Kishida Chemical Co., Wako Pure Chemical In-

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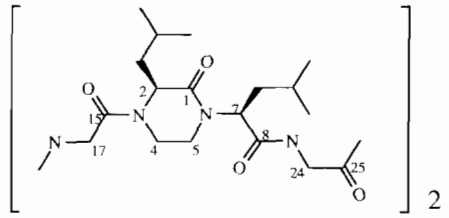
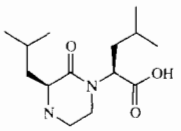


cyclo(Gly-eLL-Gly)$_2$ (**L24**)

eLL-OH;



Scheme 1.

dustries, Alfa Products, Aldrich Chemical Company, Inc., Mitsuwa Chemical Co. Ltd. and ISOTEC Inc. Metal perchlorates were used without additional purification, and their concentrations were determined by inductively coupled plasma arc emission spectrometry (ICP).

2.2. Measurements

^{13}C NMR spectra were recorded in CD_3CN at 30 °C using the nitrile carbon signal (118.2 ppm) as a reference. All signals were assigned by the two-dimensional (H–C COSY) method and the ^1H NMR data of **L24** [5]. The concentration of **L24** was 1.3×10^{-2} mol dm^{-3}. CD and UV–Vis data were obtained in CH_3CN using a quartz cell at 25 °C. The concentration of **L24** was 2.00×10^{-4} and 1.5×10^{-3} mol dm^{-3} for CD spectra in the ranges 210–250 and 260–400 nm, respectively. Job plots were carried out at 300 nm in the total concentration ([**L24**] + [Cu^{2+}]) of 2.00×10^{-3} mol dm^{-3}. A Jeol GX-400 (NMR spectra), a Jasco J-500A with a DP-500 data processor (CD spectra), a Hitachi U-3300 spectrophotometer (UV–Vis spectra) and a Shimadzu ICPS-1000III (ICP analysis) were used for the measurements.

2.3. Methods for calculating binding constants

Binding constants were calculated by methods similar to those of Bergeron et al. [16] (for K_1 only) and Baron et al. [17] (for K_1 and $K_{1/2}$ or K_1 and K_2). In the case of a 2:1 (peptide:cation) complex, the approximate procedure is as follows:

$$P + C \overset{K_1}{\rightleftharpoons} PC$$

$$PC + P \overset{K_{1/2}}{\rightleftharpoons} P_2C$$

$$K_1 = \frac{[PC]}{[P][C]}, \quad K_{1/2} = \frac{[P_2C]}{[PC][P]}$$

$$P_0 = [P] + K_1[P][C] + 2K_1K_{1/2}[P]^2[C] \quad (1)$$

$$C_0 = [C] + K_1[P][C] + K_1K_{1/2}[P]^2[C] \quad (2)$$

where P_0 and C_0 are initial concentrations of peptide and cation, respectively. Using the differences ($\Delta[\theta]_{P_2C}{}^{\lambda}$ and $\Delta[\theta]_{PC}{}^{\lambda}$) of molar ellipticities at the plateaux, the apparent differences ($\Delta[\theta]^{\lambda}$) are presented by

$$\Delta[\theta]^{\lambda} = \{2[P_2C]/P_0\}[\Delta[\theta]_{P_2C}{}^{\lambda}] + \{[PC]/P_0\}[\Delta[\theta]_{PC}{}^{\lambda}] \quad (3)$$

[P] is calculated from Eqs. (1) and (2) by the Newton–Raphson method and $\Delta[\theta]_{(calc)}{}^{\lambda}$ is derived by Eq. (3). $\Delta[\theta]_{(calc)}{}^{\lambda}$ was calculated using three different wavelengths (225, 227 and 230 nm). K_1 and $K_{1/2}$ values were determined by finding the minimum value of the total sum of square deviations:

$$S = \sum_{\lambda}\sum_{j}[\Delta[\theta]_{j(exp)}{}^{\lambda} - \Delta[\theta]_{j(calc)}{}^{\lambda}]^2$$

3. Results and discussion

The CD spectra of **L24** show the $n\rightarrow\pi^*$ negative Cotton effect of a peptide carbonyl group at 227 nm in acetonitrile (Fig. 1). When a large excess of metal perchlorate (Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}) was added to the solution of **L24** in acetonitrile, the mean residue ellipticities were drastically changed. The Cotton effect was changed in the positive direction keeping roughly the same extreme with Cu^{2+}, Fe^{2+} or Fe^{3+}. Also, the new extremes appeared at 215, 220 and 220 nm with Co^{2+}, Zn^{2+} and Mn^{2+}, respectively. These results suggest the marked conformation changes of **L24**. Stoichiometries of cation–**L24** complexes were determined by the differences between the mean residue ellipticities at one wavelength (nm) of **L24**/cation complex and that of free **L24**. The titration curves (Fig. 2) reveal the formation of a PC complex for Mn^{2+},

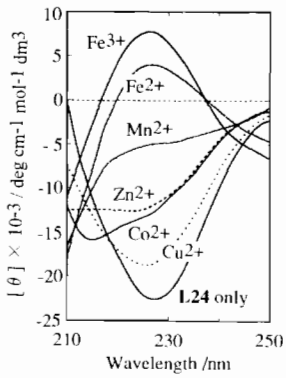


Fig. 1. CD spectra (mean residue ellipticity) of **L24** with or without metal perchlorates in acetonitrile at 25 °C. [**L24**] = 2.00×10^{-4} mol dm^{-3}; [Mn^{2+}], [Fe^{2+}], [Co^{2+}], [Cu^{2+}], [Zn^{2+}] = 1.00×10^{-2} mol dm^{-3}; [Fe^{3+}] = 1.00×10^{-3} mol dm^{-3}.

Table 1
Binding constants of **L24** with perchlorate salts in acetonitrile at 25 °C

Cation	Ionic radii (Å)	Binding constant (mol^{-1} dm^3)		
		$(P + C \overset{K_1}{\rightleftharpoons} PC)$	$(PC + P \overset{K_{1/2}}{\rightleftharpoons} P_2C)$	$(PC + C \overset{K_2}{\rightleftharpoons} PC_2)$
Mn^{2+}	(0.83)	7.4×10^3		
Fe^{2+}	(0.78)	4.2×10^3		2.3×10^2
Co^{2+}	(0.75)	1.6×10^2		
Ni^{2+}	(0.69)	very small		
Cu^{2+}	(0.73)	2.6×10^2		
Zn^{2+}	(0.74)	1.2×10^3		
Fe^{3+}	(0.65)	5.7×10^5	3.2×10^4	
Ag^{+}	(1.15)	very small		
Cd^{2+}	(0.95)	6.5×10^4		9.6×10^2
Hg^{2+}	(1.02)	5.1×10^3		
Pb^{2+}	(1.19)	7.8×10^3		
Mg^{2+},[a]	(0.72)	6.7×10^3		
Ca^{2+},[a]	(1.00)	4.2×10^6	7.6×10^4	
Ba^{2+},[a]	(1.35)	4.9×10^4		

[a]Ref. [5].

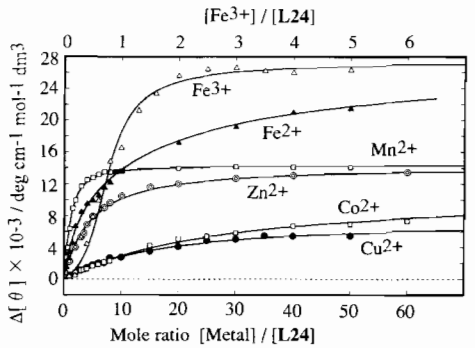


Fig. 2. Titration curves of **L24** for metal perchlorates in acetonitrile at 227 nm. $\Delta[\theta]$ indicates the difference between the mean residue ellipticity of **L24** and that of its complex ion. $[\mathbf{L24}] = 2.00\times10^{-4}$ mol dm^{-3}.

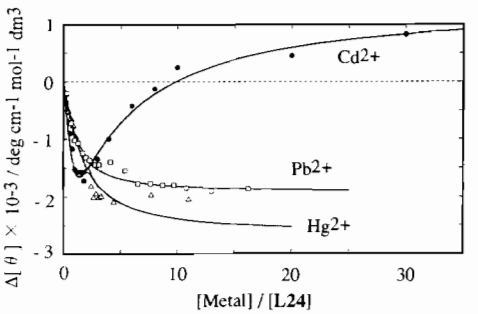


Fig. 3. Titration curves of **L24** for metal perchlorates in acetonitrile at 227 nm. $\Delta[\theta]$ indicates the difference between the mean residue ellipticity of **L24** and that of its complex ion. $[\mathbf{L24}] = 2.00\times10^{-4}$ mol dm^{-3}.

Co^{2+}, Cu^{2+} or Zn^{2+}, both PC and PC_2 complexes for Fe^{2+} or Cd^{2+}, and both PC and P_2C complexes for Fe^{3+}, where P is a cyclic peptide (**L24**) and C is a metal cation. On the other hand, the small spectral changes were observed with Cd^{2+}, Pb^{2+} or Hg^{2+}. The titration curves (Fig. 3) reveal the formation of a PC complex for Pb^{2+} or Hg^{2+}, and both PC and P_2C complexes for Cd^{2+}, indicating small conformation changes in the complex formation. The spectra of **L24** were scarcely changed with Ni^{2+} or Ag^{+}, indicating little interaction between these two ions and **L24** because of the small ion radii of Ni^{2+} and the univalent charge of Ag^{+}.

The stability constants of **L24** for transition metals were calculated using the titration data (Table 1). As shown in Figs. 2 and 3, $\Delta[\theta]_j^{\lambda}$ values (real lines) simulated using the calculated binding constants summarized in Table 1 agree well with the experimental ones (points). The K_1 values toward the bivalent ions of first transition metals are in the order of $Mn^{2+} > Fe^{2+} > Co^{2+} > Ni^{2+} < Cu^{2+} < Zn^{2+}$, and those toward Group XII are $Zn^{2+} < Cd^{2+} > Hg^{2+}$. This trend is contrary to the 'Irving–Williams series of stability' [18], and may be explained by the cavity size of **L24**. The six-coordinate effective ionic radii are Mn^{2+} (0.83) > Fe^{2+} (0.78) > Co^{2+} (0.75) > Ni^{2+} (0.69) < Cu^{2+} (0.73) < Zn^{2+} (0.74) < Cd^{2+} (0.95) < Hg^{2+} (1.02 Å) [19]. Thus, this macrocycle prefers a metal ion having ~0.95–1.00 Å radii, and this result is comparable to the results toward alkaline earth metal ions as shown in Table 1 [5].

When $Cu(ClO_4)_2$ was added to the solution of **L24** in CH_3CN, a new absorption band, which is probably due to charge transfer transition, appeared at 301 nm ($\epsilon = 131$ dm^3 mol^{-1} cm^{-1}). Job plots showed a peak at 0.5 molar ratio of $[\mathbf{L24}]/([\mathbf{L24}] + [Cu^{2+}])$, indicating the formation of a PC complex. Also, new absorption bands were observed at 366 nm ($\epsilon = 84$ dm^3 mol^{-1}

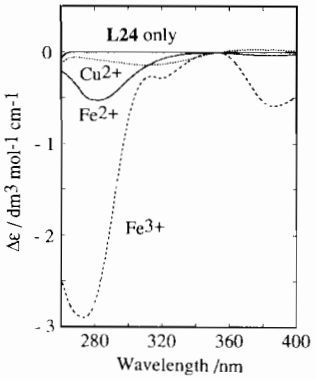


Fig. 4. CD spectra of **L24** with or without $Cu(ClO_4)_2$, $Fe(ClO_4)_2$ or $Fe(ClO_4)_3$ in acetonitrile.. **[L24]** $= 1.5 \times 10^{-3}$ mol dm^{-3}, $[MClO_4] = 1.5 \times 10^{-3}$ mol dm^{-3}.

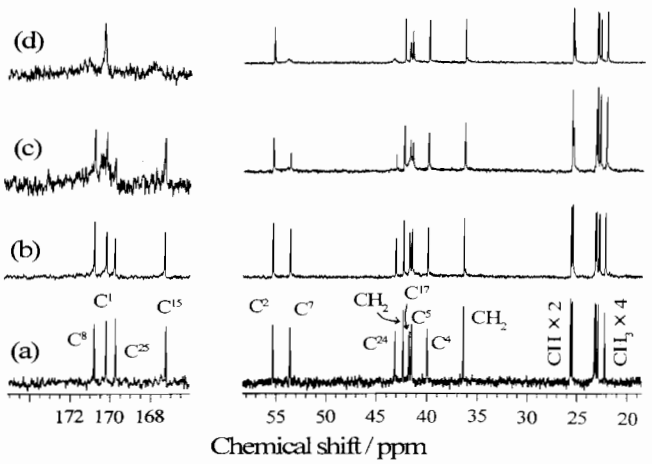


Fig. 5. ^{13}C NMR spectra of **L24** in CD_3CN with or without $Cu(ClO_4)_2$. **[L24]** $= 1.3 \times 10^{-2}$ mol dm^{-3}. The mole ratios of salt to peptide are: (a) no salt; (b) 1:20; (c) 1:16; (d) 1:15.

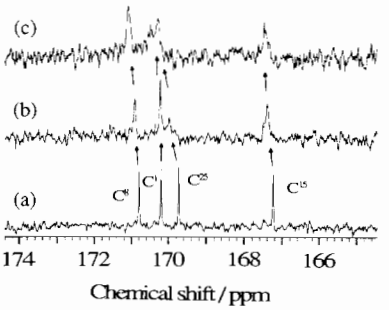


Fig. 6. ^{13}C NMR spectra of **L24** in CD_3CN with or without $Fe(ClO_4)_3$. **[L24]** $= 1.3 \times 10^{-2}$ mol dm^{-3}. The mole ratios of salt to peptide are: (a) no salt; (b) 1:8; (c) 1:4.

cm^{-1}) and 371 nm ($\epsilon = 2431$ dm^3 mol^{-1} cm^{-1}) with Fe^{2+} and Fe^{3+}, respectively. Moreover, Cotton effects were newly observed at 310 nm ($\Delta\epsilon = -0.144$ dm^3 mol^{-1} cm^{-1}) and 375 nm ($\Delta\epsilon = 0.031$ dm^3 mol^{-1} cm^{-1}) for Cu^{2+}, at 282 nm ($\Delta\epsilon = -0.532$ dm^3 mol^{-1} cm^{-1}) and 386 nm ($\Delta\epsilon = -0.034$ dm^3 mol^{-1} cm^{-1}) for Fe^{2+}, and at 273 nm ($\Delta\epsilon = -2.898$ dm^3 mol^{-1} cm^{-1}), 320 nm ($\Delta\epsilon = -0.287$ dm^3 mol^{-1} cm^{-1}) and 386 nm ($\Delta\epsilon = -0.0588$ dm^3 mol^{-1} cm^{-1}) for Fe^{3+} (Fig. 4). These results indicate the formation of chiral complexes in which chiral **L24** coordinates to Cu^{2+}, Fe^{2+} or Fe^{3+}.

Fig. 5 shows ^{13}C NMR spectra of **L24** with or without $Cu(ClO_4)_2$. Signals of C^8, C^{25}, C^{15}, C^7 and C^{24} were broadened and finally disappeared together with the increasing mole ratio of Cu^{2+} to **L24**. But those of C^1, C^2, two kinds of γ-CH, two kinds of β-CH_2 and four kinds of δ-CH_3 of leucine residues remained intact. These results indicate that the six amide oxygens (three kinds of oxygens) of **L24** coordinate to a copper ion. Similarly, the signals of C^8, C^{25} and C^{15} shifted to lower field and broadened with Fe^{3+} (Fig. 6), indicating the coordination of six amide oxygens to Fe^{3+}.

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