

Ternary Complexes from Cu(II)–Oligopeptide and *N*-Acetyl-L-histidine, as Studied by Circular Dichroism Spectroscopy

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The Cu(II) complexes of oligopeptides, $Cu(H_{-i}L)$; $i = 1$, 2, or 3, reacted with *N*-acetyl-L-histidine (AHis) to form ternary complexes, $Cu(H_{-j}L)(AHis)$; $j = 1$ or 2. The $Cu(H_{-1}L)$ species formed $Cu(H_{-1}L)(AHis)$, which was either dimerized to $[Cu(H_{-1}L)]_2(AHis^-)$ or converted stepwise to $Cu(AHis)_n$; $n = 3$, 4. The reaction of the $Cu(H_{-2}L)$ or $Cu(H_{-3}L)$ species with AHis led to the formation of $Cu(H_{-2}L)(AHis)$, and the formation constants of the ternary complexes were small, on the magnitude of 10^{-1}–10^{1} M^{-1}.

The importance of histidyl residues as metal-binding sites in biological systems is well recognized. Many of those systems are enzymes and proteins that contain copper or iron as prosthetic groups, and facilitate oxygen transport, the transport and storage of metal ions, electron transfer, and the dioxygen activation toward the oxidation and oxygenation of drugs and physiologically active substances.[1–4] The imidazole group of the histidyl residue has the ability not only to coordinate to the metal ion, but also to bridge between the metal ions of the two complexes.[4] The binuclear complexes bridged by the imidazole molecule have been studied by using X-ray crystallographic and ESR spectroscopic methods,[5,6] but those bridged by the imidazole group of histidyl residue are yet unknown.

We attempted to inspect the formation of ternary complexes from Cu(II)–oligopeptide and the imidazole group of the histidyl side chain. In order to selectively detect and identify the ternary complexes, the use of the chiral ligand is convenient, because, upon forming the ternary complexes, chirality is induced into the ternary complex. As a chiral and imidazole-containing ligand, *N*-acetyl-L-histidine (AHis) was used, and the circular dichroism (CD) spectrum was used as a probe for pursuing the Cu(II) and the AHis interaction. The formation of the imidazole-bridged dimeric complex was examined by ESR spectroscopy.

Experimental

The oligopeptides, purchased from BACHEM Feinkemikalien AG (Switzerland), were pure as checked, by liquid-chromatography. Copper(II) perchlorate hexahydrate, $Cu(ClO_4)_2 \cdot 6H_2O$, obtained from G. Frederick Smith Chem. Co. (Columbus, Oh), was used after recrystallization. AHis was a product of Sigma Chemical Co. (St. Louis, Mo). All other chemicals were of reagent grade and used without further purification.

Solutions of the peptide complexes, which were freshly prepared prior to use, were mixed with various concentrations of AHis solutions. The concentration of Cu(II) was fixed in 5.00×10^{-3} M ($1\ M = 1\ mol\ dm^{-3}$). The ionic strength (I) was maintained at 0.5 M and 1 M $NaClO_4$ for the absorption spectrum and ESR measurements, respectively.

The CD spectrum was recorded at room temperature (23–26 °C) on a JASCO J-20B automatic spectropolarimeter, which was normalized against a Ni–tartarate solution before the measurement.[7] The ESR spectrum was recorded at 77 K on a JEOL JES-RE-1X spectrometer with 100 kHz field modulation. The magnetic field was calibrated by a comparison with 1,1-diphenyl-2-picrylhydrazyl (DPPH) at 77 K.

The formation constant K of $Cu(H_{-2}L)(AHis)$ was calculated by the following equation:

$$K = \frac{[Cu(H_{-2}L)(AHis)]}{[Cu(H_{-2}L][AHis]} = \frac{\alpha/(1-\alpha)}{(n-\alpha)[Cu(H_{-i}L)]_o}, \quad (1)$$

where the term α denotes the fraction of $Cu(H_{-2}L)(AHis)$, i.e., $[Cu(H_{-2}L)(AHis)]/[Cu(H_{-2}L)(AHis)]_{max}$, which is proportional to θ/θ_{max} (θ; ellipticity), and n is the molars AHis to $Cu(H_{-2}L)$. The equilibrium constant K can be determined from the slope of a straight line upon plotting $(n-\alpha)$ against $\alpha/(1-\alpha)$. The K values for $Cu(H_{-1}L)(AHis)$ could be determined in the same manner.

Results and Discussion

Coordination of AHis to Cu(II) induced chirality in the complex and the CD spectrum with λ_{max} at 550–600 nm appeared. The CD spectra of the $Cu(H_{-i}$penta-Gly)/AHis system at pH 7.4 and pH 10.5 are shown in Fig. 1, where $i = 1$, 2, or 3. At pH 7.4, where $Cu(H_{-2}$penta-Gly) was predominant, the λ_{max} was at 590 nm in the range of $[AHis]/[Cu(H_{-i}\text{penta-Gly})] \leqq 3$. As the $[AHis]/[Cu(H_{-i}\text{penta-Gly})]$ ratio increased, λ_{max} shifted to longer-wavelengths and a negative CD extremum was newly appeared. The CD spectrum at $[AHis]/[Cu(H_{-i}\text{penta-Gly})] = 15$, which exhibited a negative extremum at around 540 nm and a positive extremum at around 650 nm, could be identified as $Cu(AHis)_n$, where n was estimated to be less than 4 by a molar-ratio plot. On the contrary, at pH 10.5, where $Cu(H_{-3}$penta-Gly) was predominant, λ_{max} was observed at 545 nm irrespective of the variation in [AHis]. Thus, the line-shape and λ_{max} were likely to vary relative to the coordination mode of the penta-Gly in the ternary complex and the ratio of the concentration, $[AHis]/[Cu(H_{-i}\text{penta-Gly})]$.

The imidazole of AHis replaces the donor atom in the carboxylate end, namely in the fourth binding site of $Cu(H_{-2}L)$,

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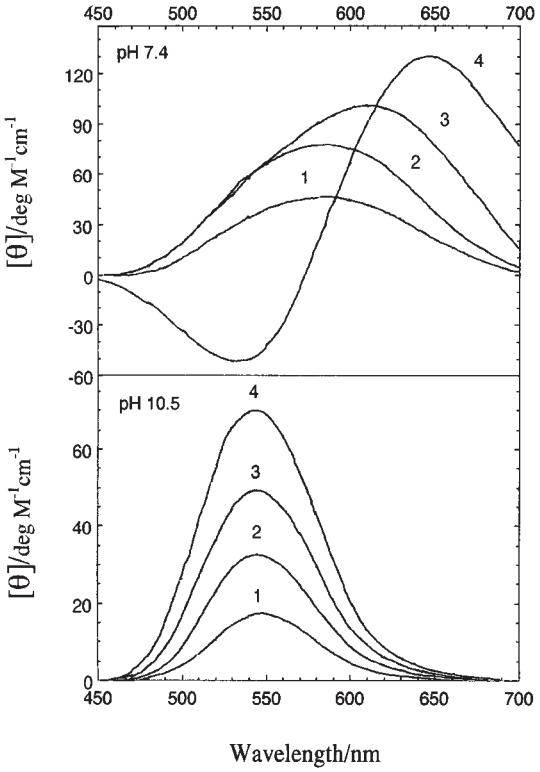


Fig. 1. CD spectra of the Cu(H$_{-i}$penta-Gly)/AHis system at pH 7.4 and pH 10.5. [AHis]/[Cu(H$_{-i}$penta-Gly)], (1) 0.5, (2) 1.0, (3) 4, and (4) 15 at pH 7.4, [AHis]/[Cu-(H$_{-i}$penta-Gly], (1) 4, (2) 8, (3) 16, and (4) 30 at pH 10.5.

forming a ternary complex, Cu(H$_{-2}$L)(AHis). The coordination modes of Cu(H$_{-i}$L), $i = 1$, 2, or 3, were found to be closely related to the λ_{max} value, which could be estimated by the "rule of the average environment".[8–10] The GlyGly-δ-Ava complex (pK_{c2} 8.75) at pH 10, composing the 5–5 membered fused-chelate from the two amino acid residues at the amino terminus (Scheme 1), can be abbreviated as Cu(H$_{-1}$GlyGly-δ-Ava)-(OH$^-$).[11] Since the fourth-site in Cu(H$_{-1}$GlyGly-δ-Ava)-(OH$^-$), as well as in Cu(H$_{-1}$GlyGly)(OH$^-$), was occupied by a freely exchangeable OH$^-$ ion, the Cu(H$_{-1}$L)(AHis) species was easily formed and the formation constants (K) had a magnitude of 10^3, or bigger:

$$\mathrm{Cu(H_{-1}L)(OH^-) + AHis + H^+} \overset{K}{\rightleftarrows} \mathrm{Cu(H_{-1}L)(AHis) + H_2O}, \quad (2)$$

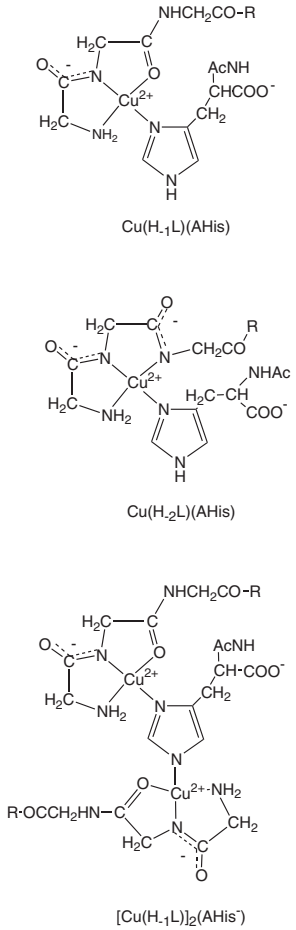
where the electric charge in AHis is omitted.

Those Cu(H$_{-1}$L)(AHis) would be labile, and either dimerize each other or undergo further ligand-exchange with AHis to yield Cu(His)$_n$; $n = 3$, 4. A significant amount of dimeric species, [Cu(H$_{-1}$L)]$_2$(AHis$^-$), was formed under the conditions at around [AHis]/[Cu(H$_{-1}$L)(OH$^-$)] $\fallingdotseq$ 0.5:

$$\mathrm{Cu(H_{-1}L)(AHis) + Cu(H_{-1}L)(OH^-)} \rightleftarrows \mathrm{[Cu(H_{-1}L)]_2(AHis^-) + H_2O}. \quad (3)$$

The dimeric species was identified by the $\Delta M = 2$ term in the ESR spectrum, as shown in Fig. 2.

As the concentration of AHis increased over 2–3 moles to



Scheme 1.

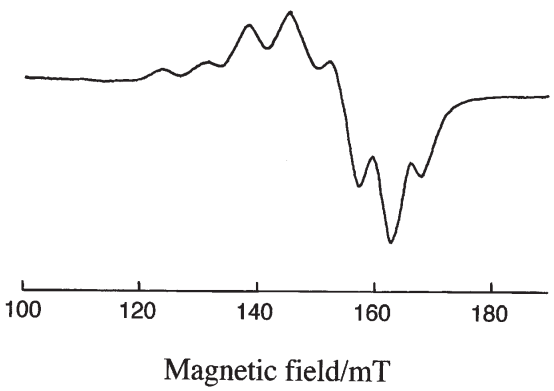


Fig. 2. ESR spectrum at 77 K of [Cu(H$_{-1}$GlyGly)]$_2$(AHis) at [AHis]/[Cu(H$_{-1}$GlyGly)] = 0.5 and pH 10.5.

Cu(H$_{-1}$L), Cu(H$_{-1}$L)(AHis), [Cu(H$_{-1}$L)]$_2$(AHis$^-$), or both were apt to stepwise change to Cu(His)$_n$,

$$\mathrm{Cu(H_{-1}L)(AHis) + (}n-1\mathrm{)AHis + H^+} \rightleftarrows \mathrm{Cu(AHis)}_n + \mathrm{L}. \quad (4)$$

The Cu(H$_{-2}$L) species, which had the fused-chelate structure from three amino-acid residues at the amino terminus, resisted being replaced by the imidazole. Then, the K values for the Cu(H$_{-2}$L)(AHis) formation were smaller than those of

Table 1. Formation Constant K of the Ternary Complexes, Cu(H_{-1}L)(AHis) and Cu(H_{-2}L)(AHis)

Cu(II)–peptide complex	$\log K/M^{-1}$	pH
Cu(H_{-1}GlyGly)(OH^-)	3.50	7.4
Cu(H_{-1}GlyGly)(OH^-)	3.31	10.0
Cu($H_{-1,-2}$GlyGlyGly)[a)]	2.97	7.4
Cu(H_{-2}GlyGlyGly)	1.77	10.0
Cu(H_{-2}GlyGly-β-Ala)	0.55	10.0
Cu(H_{-2}GlyGly-γ-Aba)	2.21	10.0
Cu(H_{-1}GlyGly-δ-Ava)(OH^-)	3.24	10.0
Cu(H_{-2}Gly-β-AlaGly)	−0.16	10.0
Cu($H_{-2}\beta$-Ala-GlyGly)	−0.16	10.0
Cu($H_{-2,-3}$penta-Gly)[b)]	3.14	7.4
Cu(H_{-3}penta-Gly)	0.99	10.5

a) A 1:4 mixture from Cu(H_{-1}GlyGlyGly) and Cu(H_{-2}GlyGlyGly), Ref. 11. b) A 4:1 mixture from Cu(H_{-2}penta-Gly) and Cu(H_{-3}penta-Gly), Ref. 13.

Cu(H_{-1}L)(AHis); $\log K < 2.2$, and arranged as follows: Cu(H_{-2}GlyGly-γ-Aba)(AHis); (5–5–7, 7.80) > Cu(H_{-2}GlyGlyGly)(AHis); (5–5–5, 6.73) > Cu(H_{-2}GlyGly-β-Ala)(AHis); (5–5–6, 5.93), where within the parentheses are given the size of the fused-chelate rings and pK_{c2} of the parent complexes,[11]

$$\text{Cu}(\text{H}_{-2}\text{L}) + \text{AHis} \overset{K}{\rightleftarrows} \text{Cu}(\text{H}_{-2}\text{L})(\text{AHis}). \quad (5)$$

This series of K values was closely related to the instability of the fused-chelate structure of Cu(H_{-2}L).[11] The Cu(H_{-2}L) species involving the 5–6–5- and 6–5–5-membered fused-rings, such as the Gly-β-AlaGly and β-AlaGlyGly complexes, hardly formed the ternary complexes; $\log K < 0$. The formation constants of the ternary complexes are summarized in Table 1.

As the pH decreased, the Cu(H_{-2}L)(AHis) species was protonated to form Cu(H_{-1}L)(AHis). In addition, pK_{c2} of Cu(H_{-1}L) is likely to remarkably increase, more than 1.0, upon forming a ternary complex.[10,12] The pK_{c2} of Cu(H_{-1}penta-Gly) is 7.00.[13] Upon forming the ternary complex, the pK_{c2} would shift to 8 or greater. Accordingly, the main species at a neutral pH region may be Cu(H_{-1}penta-Gly)(AHis), which is easily converted to Cu(AHis)$_n$ as Cu(H_{-1}GlyGly)(AHis). This is a reason why Cu(AHis)$_n$ can be formed in the Cu(H_{-i}penta-Gly)/AHis system at pH 7.4, as shown in Fig. 1.

Abbreviations

γ-Aba, γ-aminobutyric acid; δ-Ava, δ-aminovaleric acid; L, neutral form of the peptide (^+H_3N-$(CH_2CO)_i$-COO^-), AHis, *N*-acetyl-L-histidine.

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