Copper(II) Complexes of L-Histidylglycyl-L-histidylglycine and L-Histidyl-L-histidylglycylglycine: Coordination Mode of Histidyl Residues

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Abstract

The complex formations of L-histidylglycyl-L-histidylglycine (His-Gly-His-Gly) and L-histidyl-L-histidylglycylglycine (His-His-Gly-Gly) with copper(II) ion were studied in a slightly alkaline medium by visible absorption, circular dichroism and electron spin resonance spectroscopies. His-Gly-His-Gly coordinates to copper(II) ion via a terminal amino nitrogen, two deprotonated peptide nitrogens and an imidazole nitrogen of the histidyl residue in the third position. The copper(II) ion in the His-His-Gly-Gly complex is coordinated by three nitrogen donors; *i.e.*, a terminal amino nitrogen, an adjacent deprotonated peptide nitrogen and an imidazole nitrogen of the histidyl residue in the second position. The imidazole nitrogen at the N-terminal does not participate in the chelate formation with the copper(II) ion, but it bridges between the two monomeric complexes so that a broad ESR spectrum without the hyperfine structure is observed.

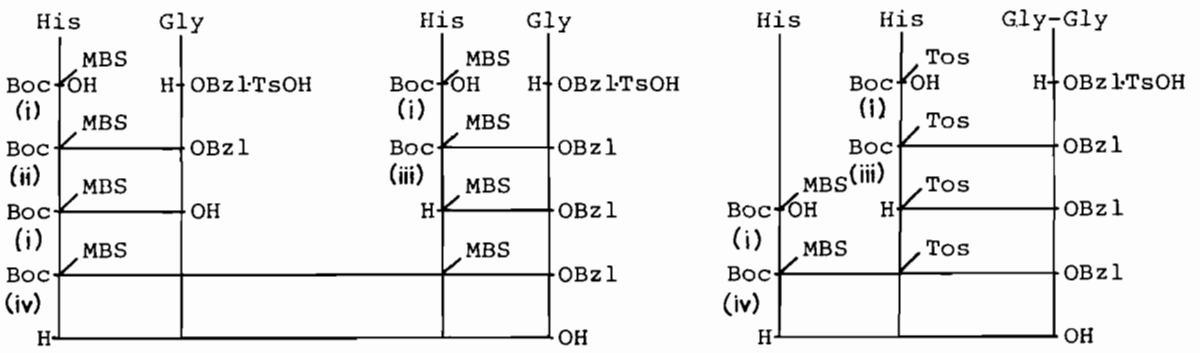
Introduction

Histidyl residues in proteins are known to participate in complexation with metal ions. The complexation of metal ions with histidine-containing peptides has been studied by various physicochemical methods [1] in an attempt to elucidate the ligation mode of the histidyl residue in metal proteins. Copper(II) complexes of oligopeptides containing one histidyl residue, e.g., L-histidylglycine (His-Gly) [2, 3, 5], glycyl-L-histidine (Gly-His) [4–6], L-histidylglycylglycine (His-Gly-Gly) [2], glycyl-L-histidylglycine (Gly-His-Gly) [4, 6, 8] and glycylglycyl-L-histidine (Gly-Gly-His) [6–9] have been extensively studied. However, few studies have been reported on the complexation of copper(II) ion with linear oligopeptides containing more than two histidyl residues [10, 11]. Histidyl side chains are expected to cooperate or compete with each other in complexing with metal ions. We consider that studies on the complexation of copper(II) ion with oligopeptides containing more than two histidyl residues should bring more information on the protein–metal interaction. This paper describes the interaction of copper(II) ion with L-histidylglycyl-L-histidylglycine (His-Gly-His-Gly) and L-histidyl-L-histidylglycylglycine (His-His-Gly-Gly) as studied by absorption, circular dichroism (CD) and electron spin resonance (ESR) spectroscopies.

Experimental

Materials

The tetrapeptides were synthesized by a liquid phase method as summarized in Scheme 1. Starting



Scheme 1. Reagents: (i) DEPC, DMF; (ii) H_2-5% Pd/C; (iii) CF_3CO_2H; (iv) HF, 0 °C.

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materials were Boc-His(MBS)-OH* [12], Boc-His-(Tos)-OH* [13], Gly-Gly-OBzl·TsOH*, and Gly-OBzl·TsOH. Diethylphosphorocyanidate (DEPC) [14] was used as a coupling reagent. The Bzl group was removed by hydrogenolysis using 5% Pd/C, and the Boc group was cleaved by trifluoroacetic acid (TFA). The fully protected tetrapeptides were treated with hydrogen fluoride (HF) in the presence of anisole [15]. The residue was treated with a column of ion-exchange resin, Dowex 1-X4 (AcO^- form), to remove the remaining HF. The peptides were lyophilized and purified by reprecipitation. *Anal.* His-Gly-His-Gly. Found: C, 45.25; H, 5.72; N, 26.00. Calc. for $C_{16}H_{22}N_8O_5 \cdot H_2O$: C, 45.27; H, 5.71; N, 26.40%. His-His-Gly-Gly. Found: C, 44.66; H, 5.65; N, 22.90. Calc. for $C_{16}H_{22}N_8O_5 \cdot AcOH \cdot H_2O$: C, 44.63; H, 5.83; N, 23.13%.

Preparation

A stock solution of copper(II) ion, which was prepared by dissolving $Cu(ClO_4)_2 \cdot 6H_2O$ (G. Frederick Smith) in doubly distilled water, was standardized by the conventional complexometric titration with EDTA [16]. An aliquot of the copper(II) solution was mixed with a 4 mol% excess peptide to ensure the complex formation. The sample solution was adjusted to the appropriate pH with dilute NaOH. Ionic strength was maintained at 0.5 M with $NaClO_4$. Boric acid and HEPES were used as buffering agents. The pH values of the solution were measured on a TOA DENPA HM-5A pH meter.

Spectral Measurements

Absorption spectra were recorded on a Union Giken SM 401 spectrometer at room temperature. Circular dichroism (CD) spectra were measured on an automatic recording spectrometer JASCO-J-20 at room temperature. ESR spectra were measured at 77 K with a JEOL PE-IX (X-band) spectrometer with 100 kHz field modulation. ESR parameters were calibrated by comparison with the standard sample of Mn^{2+} doped in MgO.

Results and Discussion

Cu(II)–(His-Gly-His-Gly) System

The fully protonized form of His-Gly-His-Gly was shown to liberate four-equivalent protons from an amino, two histidyl imidazole and a carboxylate groups by potentiometric titration with NaOH. In the presence of equimolar copper(II) to the peptide, additional two-equivalent protons were liberated which were probably derived from two peptide groups coordinated with the copper(II) ion. It is well known that the peptide group undergoes proton ionization by coordination with copper(II) or nickel(II) ions. Thus, the potentiometric study suggests that candidates for the ligand in Cu(II)–(His-Gly-His-Gly) are an amino nitrogen and two deprotonated nitrogens, and that the fourth group is either a carboxylate oxygen or an imidazole nitrogen. The former corresponds to a coordination mode for Gly-Gly-Gly and the latter for Gly-Gly-His. Spectral data might provide information about the coordination mode for the complex. The complex formed showed an absorption at 527 nm due to d–d transition as shown in Fig. 1. Empirical eqn. (1) [1] showing the relation between λ_{max} and metal-ligated atoms has been derived as follows:

$$\lambda_{max} = 10^3/[0.494n\ (\text{deprotonated peptide}) + 0.460n\ (\text{amino}) + 0.434n\ (\text{imidazole}) + 0.346n\ (\text{carboxylate}) + 0.294n\ (\text{water, carbonyl})] \quad (1)$$

where n represents the number of each type of donor atom in the complex.

The predicted λ_{max} value for the coordination mode in Cu(II)–(Gly-Gly-Gly) is 557 nm and that in Cu(II)–(Gly-Gly-His) is 531 nm. The CD spectral data of Cu(II)–(His-Gly-His-Gly) show two CD peaks as observed in Cu(II)–(Gly-Gly-His) (Fig. 1): the positive at 503 nm and the negative at 600 nm. They may be assigned to $d_{yz,xz} \rightarrow d_{x^2-y^2}$ and $d_{xy} \rightarrow d_{x^2-y^2}$ transitions, respectively [17]. The ESR spectrum of Cu(II)–(His-Gly-His-Gly) with $g_{\parallel} = 2.17$ and $A_{\parallel} = 212 \times 10^{-4}$ cm^{-1} (Fig. 2) resembles that of Cu(II)–(Gly-Gly-His) [18]. Thus, spectral data show that the His-Gly-His-Gly complex has the same coordination structure as the Cu(II)–(Gly-Gly-

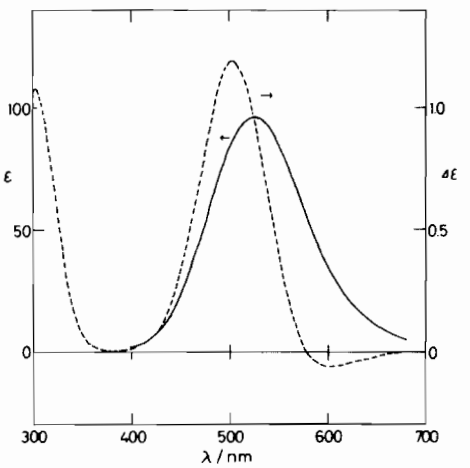

Fig. 1. Absorption (solid line) and CD spectra (broken line) of His-Gly-His-Gly in the presence of copper(II) ion at pH 8.0. [His-Gly-His-Gly] = 5.2×10^{-3} M, [Cu(II)] = 5.0×10^{-3} M.

*Abbreviations: Boc = tert-butoxycarbonyl; MBS = 4-methoxysulfonyl; Tos = *p*-toluenesulfonyl; Bzl = benzyl; TsOH = *p*-toluenesulfonic acid.

TABLE I. Visible Absorption and CD Spectral Data for Cu(II)–Peptide Complexes at pH 8.0

	Absorption λ_{max} (nm) (ϵ (cm^{-1} M^{-1}))	CD λ_{max} (nm) ($\Delta\epsilon$ (cm^{-1} M^{-1}))
Cu(II)–(His-Gly-His-Gly)	527(96)	301(1.08), 503(1.21), 600(−0.06)
Cu(II)–(His-Gly-Gly)	607(88)	582(0.25), 700(−0.03)
Cu(II)–(Gly-Gly-His)	527(97)	303(0.44), 494(0.48), 578(−0.17)

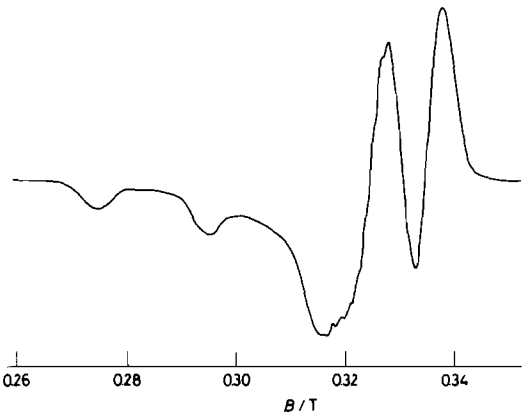

Fig. 2. ESR spectrum of copper(II)–(His-Gly-His-Gly) at pH 8.0 and 77 K.

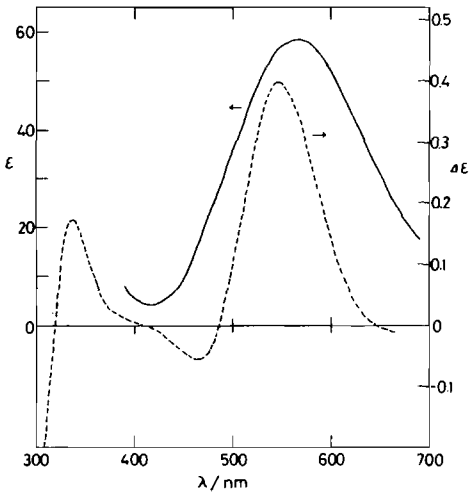

Fig. 3. Absorption (solid line) and CD spectra (broken line) of His-His-Gly-Gly in the presence of copper(II) ion at pH 8.0. [His-His-Gly-Gly] = 5.2×10^{-3} M, [Cu(II)] = 5.0×10^{-3} M.

His) complex. Spectral data of the complex are shown in Table I, along with those of the copper(II) complexes of the peptides with one histidyl residue in the first and in the third position. The structure of the Cu(II)–(Gly-Gly-His) moiety has been determined by X-ray crystal analysis [19]. His-Gly-His-Gly is shown to function as a quadridentate ligand. The copper(II) ion is chelated by four donor atoms; an amino, two deprotonated peptides, and an imidazole of the histidyl residue in the third position. The imidazole nitrogen of the histidyl residue at the amino terminal probably does not coordinate with the copper(II) ion.

Cu(II)–(His-His-Gly-Gly) System

The fully protonized His-His-Gly-Gly was shown to liberate four-equivalent protons from an amino, two histidyl imidazole, and a carboxylate groups by potentiometric titration with NaOH. In the presence of equimolar copper(II) ion to the peptide, five-equivalent protons were liberated as a whole. Liberation of one additional proton probably resulted from the coordination between the copper(II) ion and the peptide nitrogen adjacent to the amino terminal. The potentiometric study suggests that the copper(II) ion probably coordinates with the His-His-Gly moiety of the peptide via three nitrogen atoms derived from the amino, deprotonated peptide and imidazole groups as observed in Cu(II)–(Gly-His-Gly) [20, 21]. The complex formed shows an absorption band due to d–d transition with λ_{max} at 569 nm (Fig. 3). In contrast, Cu(II)–(Gly-His-Gly) has λ_{max} at 604 nm. The discrepancy between their λ_{max} values might be ascribable to the difference of the fourth ligand in the complexes. In Cu(II)–(His-His-Gly-Gly), the fourth ligand is assumed to be an imidazole nitrogen, while it is a water molecule in Cu(II)–(Gly-His-Gly). This assumption is supported by the observation that a ternary complex from Cu(II)–(Gly-His-Gly) and imidazole (abbreviated hereafter as Cu(II)–(Gly-His-Gly)–Im) displayed the λ_{max} to 576 nm. The CD spectrum of Cu(II)–(His-His-Gly-Gly) showed two peaks (Fig. 3): the positive at 547 nm and the negative at 465 nm. They may be assigned to $d_{xy} \rightarrow d_{x^2-y^2}$ and $d_{yz,xz} \rightarrow d_{x^2-y^2}$ transition. The CD spectrum of Cu(II)–(Gly-His-Gly)–Im closely resembles that of Cu(II)–(His-His-Gly-Gly) as shown in Table II.

Cu(II)–(His-His-Gly-Gly) gives a broad ESR spectrum at 77 K without a hyperfine structure due to paramagnetic copper(II) ion (Fig. 4). In contrast, the spectrum of Cu(II)–(Gly-His-Gly) shows both a hyperfine structure and a superhyperfine structure due to the Cu(II)–N interaction (Fig. 5). Those findings indicate that Cu(II)–(Gly-His-Gly) is a monomer

TABLE II. Visible Absorption, CD, and ESR Spectral Data for Cu(II)–Peptide Complexes at pH 8.0

	Absorption λ_{max} (nm)(ϵ)	CD λ_{max} (nm)($\Delta\epsilon$)	ESR $g_{\parallel}$ ($A_{\parallel}$ (10^{-4} cm^{-1}))
Cu(II)–(His-His-Gly-Gly)	569(58)	337(0.17), 465(−0.06), 547(0.41)	
Cu(II)–(His-Gly-Gly)	607(88)	582(0.25), 700(−0.03)	
Cu(II)–(Gly-His-Gly)	604(53)	335(0.21), 498(−0.05), 604(0.41)	2.21(211)
Cu(II)–Im–(Gly-His-Gly)	576(51)	327(0.24), 474(−0.04), 568(0.32)	2.20(211)

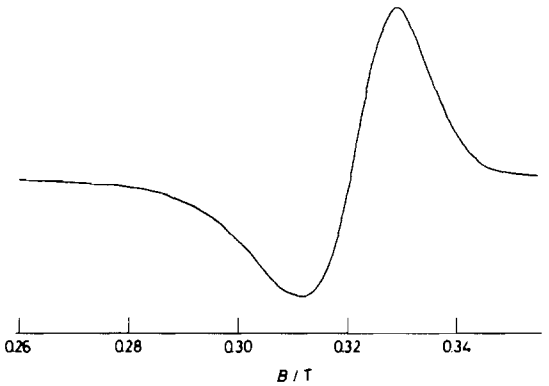

Fig. 4. ESR spectrum of copper(II)–(His-His-Gly-Gly) at pH 8.0 and 77 K.

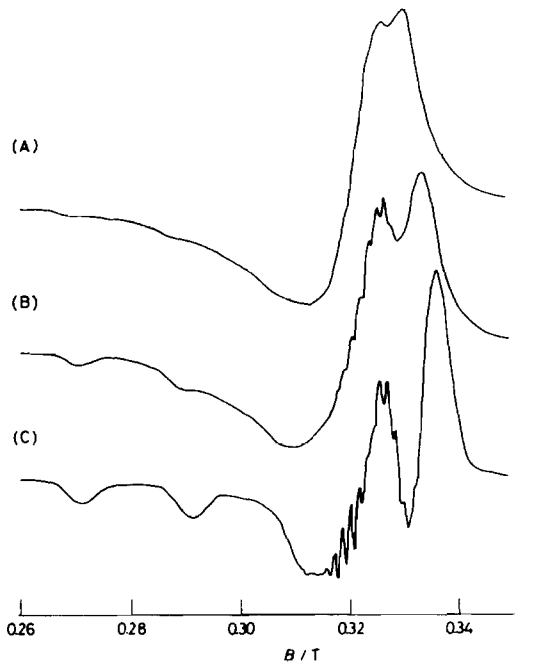

Fig. 5. ESR spectra of (a) copper(II)–(His-Gly-Gly), (b) –(Gly-His-Gly), and (c) –(Gly-His-Gly)–Im at pH 8.0 and 77 K. [Peptides] = [Imidazole] = 5.2×10^{-3} M, [Cu(II)] = 5.0×10^{-3} M.

species but that the copper(II) complex of His-His-Gly-Gly is a polynuclear species in which the imidazole group of the histidyl side chain at the amino terminal probably bridges between monomeric complexes. The copper(II) complexes of His-Gly, His-Gly-Gly, and analogous peptides also give broad ESR spectra.

Spectroscopic studies indicate that Cu(II)–(His-His-Gly-Gly) has basically the same coordination structure as Cu(II)–(Gly-His-Gly). Monomeric complexes are linked via the imidazole group of the terminal histidyl residue so that the complex formed displays a broad ESR spectrum.

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