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AN ANALOGOUS LIGAND OF BLUE COPPER ACTIVE SITES : SYNTHESIS, ELECTRON SPIN RESONANCE CHARACTERISTICS OF ITS COPPER(II) COMPLEX, AND ROLE OF PROLINE RESIDUE

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Summary : In order to clarify a role of the proline residue at near cysteine and histidine positions of plastocyanin and azurin, N-mercaptoacetylglycyl-L-prolyl-L-histidine has been synthesized as an analogous ligand of blue copper sites and the spectroscopic properties of its Cu(II) complex compared with those of the N-mercaptoacetylglycylglycyl-L-histidine-Cu(II) complex. In the present tetrapeptide-Cu(II) complexes, the exchange of the glycine of the third position by the proline residue effects a red shift(80 nm) of the visible absorption and a decrease($192 \rightarrow 75 \times 10^{-4}$ cm^{-1}) of the copper hyperfine splitting. The introduction of proline residue induces a change of the complex geometry from D_{4h} to T_d symmetries.

The spectroscopic and model studies have indicated a tetrahedral copper site which involves Cu-S(cysteine) and Cu-N(histidine imidazole) coordinations for blue copper active centers.[1] In fact, very recent X-ray crystallographic analysis of poplar plastocyanin at 2.7 Å resolution confirmed that the blue copper atom has a highly distorted tetrahedral coordination geometry and is coordinated by a cysteine thiol group, a methionine thioether group, and two histidine imidazole groups.[2] Similar crystallographi model has been proposed for azurin from Pseudomonas aeruginosa on the basis of 3 Å resolution electron density map.[3] The amino acid sequences of plastocyanin and azurin from several sources, contain commonly Cys^{84}-X-Pro-His^{87}(X=Ser, Ala, Gln; plastocyanin) and Cys^{112}-Ser-Phe-Pro-Gly-His^{117}(azurin).[4] In addition, stellacyanin from lacquer tree also shows the sequence of Cys^{59}---------Pro^{90}-Lys-His^{92}.[5]

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We synthesized an analogous ligand of blue copper chromophore, N-mercaptoacetyl-L-prolyl-L-histidine(MAGPH), in order to clarify the importance of the above-mentioned molecular sequences for blue copper coordinations. In particular, a role of the proline residue at near cysteine and histidine positions of plastocyanin and azurin was estimated by comparison of the Cu(II) complexes between the model ligands, MAGPH and N-mercapto-acetylglycylglycyl-L-histidine(MAGGH).

Experimental Section

New tetrapeptides, MAGPH and MAGGH, were synthesized by making use of the Schotten-Bauman reaction between chloroacetyl chloride and glycyl-L-prolyl-L-histidine(or glycyl-glycyl-L-histidine) followed by a condensation with thiobenzoic acid and then hydrolysis in an ammonia solution.[6] These new peptides were checked by elemental analysis, infrared, and proton magnetic resonance spectra. Glycyl-L-prolyl-L-histidine was prepared according to Chart 1, and glycylglycyl-L-histidine supplied from Protein Research Foundation.

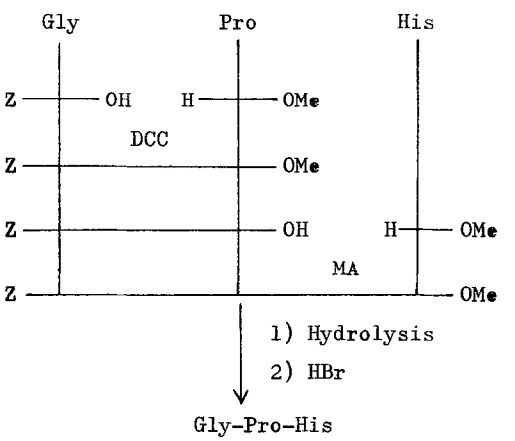


Chart 1 Synthetic Route of Glycyl-L-prolyl-L-histidine

Z=-CO-O-CH_2- 

The standard Cu(II) solution was prepared by dissolving Cu(II) nitrate in water and standardized with EDTA solution. All other reagents used were of commercial reagent grade.

Visible absorption and circular dichroism(CD) spectra were measured in an aqueous solution(pH 9.5) at 20°C with a Shimadzu recording spectrophotometer(Double 40R) and a

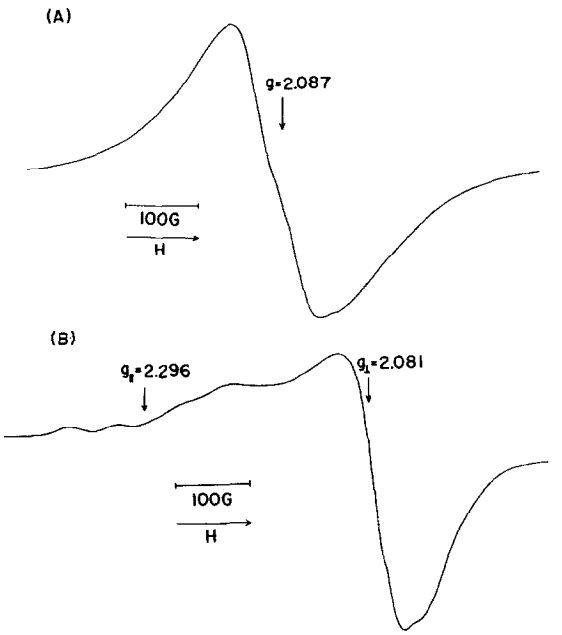


Figure 1 ESR spectra of MAGPH-Cu(II) complex at 293 K(A) and 77 K(B)

Jasco J-20 spectropolarimeter, respectively. X-Band electron spin resonance(ESR) spectra were obtained at 77 and 293 K with a JES-FE-3X spectrometer. The evaluation of ESR parameters for these peptide-Cu(II) complexes was achieved by a previously reported method.[7]

Results and Discussion

The 1:1 MAGPH-Cu(II) complex shows an absorption maximum at 590 nm(ε 190) and CD extrema at 706 nm($\Delta\varepsilon$ -0.10), 590 nm(-0.04), 517 nm(-0.12), and 408 nm(+0.08), while the 1:1 MAGGH-Cu(II) complex at 510 nm(310) and 590 nm(-0.58), 496 nm(+0.79), and 390 nm (-0.02). The λ_{max} value of the latter complex clearly shifts to a shorter wavelength than that of the former complex, suggesting the order of MAGGH > MAGPH in the magnitude of the ligand field around the central Cu(II). The visible absorption maximum of the 1:1 MAGGH-Cu(II) complex is similar to that(513 nm) of the 1:1 N-mercaptoacetyl-DL-histidyl-DL-histidine(MAHH)-Cu(II) complex which consists of 6-5-5-6 chelate ring members with donor set of $\left[S(N_p)_2N_{Im}\right]N_{Im}$. It is known that MAHH has Cu(II) binding site of a square-pyramidal configuration with axial histidine imidazole coordination.[7] On the other hand, the λ_{max} value of the 1:1 MAGPH-Cu(II) complex is close to that(598 nm) of the 1:1

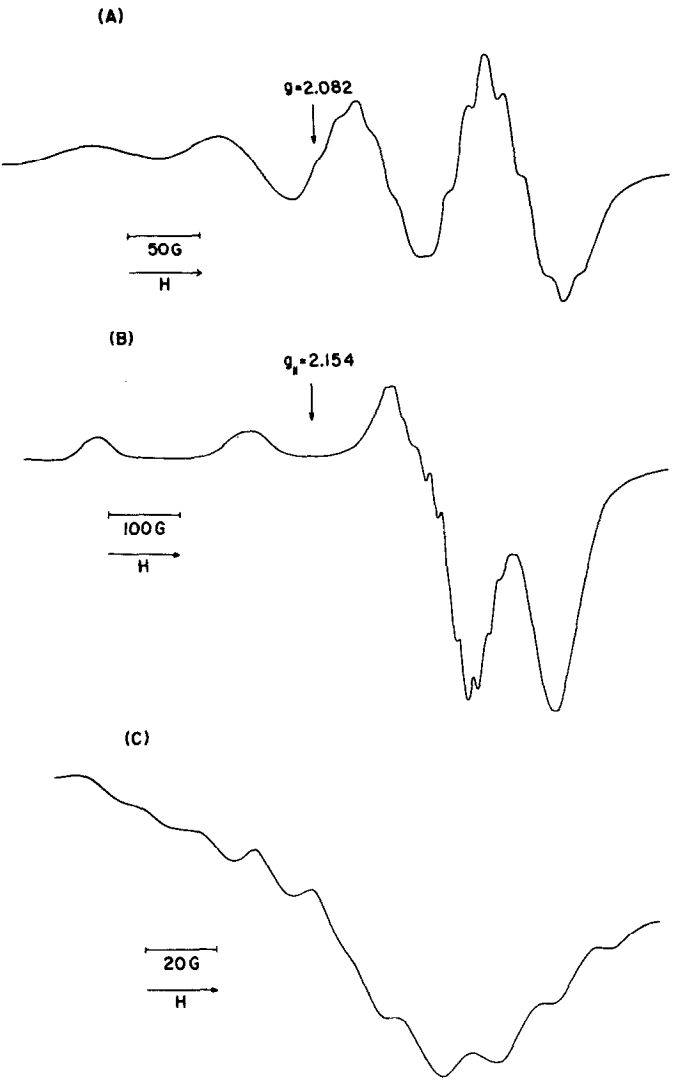
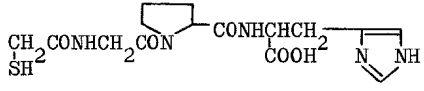
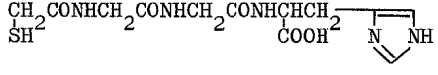
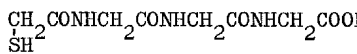
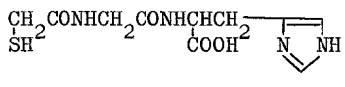
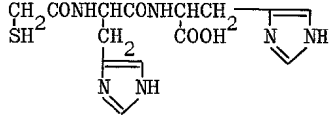
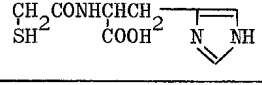


Figure 2 ESR spectra of MAGGH-Cu(II) complex at 293 K(A) and 77 K(B,C) Spectrum C shows a nitrogen nuclear superhyperfine structure.

N-mercaptoacetyl-L-histidine(MAH)-Cu(II) complex which consists of two chelate rings with $[S(N_p)N_{Im}]$ donor set.[6] In the present tetrapeptide-Cu(II) complexes, the exchange of the proline residue of the third position by the glycine residue gives a blue shift of 80 nm.

The ESR spectra for the 1:1 Cu(II) complexes of MAGPH and MAGGH observed at 293 and 77 K are shown in Figures 1 and 2. The sample concentration was 1.0-10 mM in an aqueous solution. No ESR signals were detected at half field, g=4, resulting from the spin

Table I ESR Parameters of Cu(II) Complexes of Sulfhydryl- and Imidazole-Containing Tetrapeptides

Ligand	Donor Set	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}(cm^{-1})$	$A_N(cm^{-1})$
	SN_PN_{Im}	2.296	2.081	$75x10^{-4}$	———
	$S(N_P)_3N_{Im}$	2.154	2.041	$192x10^{-4}$	$12.57x10^{-4}$
	$S(N_P)_3$	2.155	2.046	$199x10^{-4}$	$12.04x10^{-4}$
	$S(N_P)_2N_{Im}$	2.206	2.079	$195x10^{-4}$	$12.61x10^{-4}$
	$S(N_P)_2(N_{Im})_2$	2.183	2.060	$180x10^{-4}$	$12.70x10^{-4}$
	SN_PN_{Im}	2.301	2.069	$100x10^{-4}$	———

forbidden Δ_m=2-transition in a spin-coupled Cu(II) dimer. The MAGGH-Cu(II) complex clearly shows the superhyperfine interaction with neighboring nuclei, namely nitrogen (^{14}N, I=1) atoms, in contrast with the MAGPH-Cu(II) complex. The well-defined nine lines of nitrogen nuclear superhyperfine splitting strongly indicate the coordination of four nitrogen atoms from the MAGGH ligand. The relative amplitude ratios of the nitrogen splittings in the MAGGH-Cu(II) complex are appreciably different from the expected value (1:4:10:16:19:16:10:4:1) for four magnetically equivalent nuclei, and reveal the presence of non-equivalent(equatorial and axial) nitrogen atoms. Similar result has been observed in the 1:1 MAHH-Cu(II) complex.[7] In addition, the space filling molecular model also supports a square-pyramidal configuration with axial imidazole coordination from the terminal histidine residue for the MAGGH-Cu(II) complex.

On the other hand, the 1:1 MAGPH-Cu(II) complex has the ESR parameters which are different from those of the 1:1 MAGGH-Cu(II) complex(see Table I). In particular, the remarkably small copper hyperfine splitting($A_{||}$) of the MAGPH-Cu(II) complex is comparable to those of blue copper proteins. In the case of the 1:1 MAGPH-Cu(II) complex, the absence of nitrogen superhyperfine splittings is likely to reflect a distortion from the square-planar configuration, since a nitrogen-coordinated Cu(II) site with high square-planar geometry shows well-defined nitrogen splittings. In addition, a lower field shift of the $g_{||}$ value noted in the MAGPH-Cu(II) complex may be interpreted in terms of a distortion from square-planar to tetrahedral geometries.

The bonding parameters of these tetrapeptide-Cu(II) complexes were estimated by the method of Kiverson and Neiman,[8] who wrote the molecular orbital of Cu(II) complexes under D_{4h} symmetry. The results are compared with those of other sulfhydryl- and imidazole-containing peptide-Cu(II) complexes in Table II. The obtained unusual bonding parameters of the MAGPH-Cu(II) complex as well as the MAH-Cu(II) complex, strongly suggest an apparent deviation from D_{4h} to T_d symmetries. The ESR and bonding parameters of the MAGGH-Cu(II) complex are intermediate between those of MAHH- and MAGGG-Cu(II) complexes. It is known that σ-covalency of the Cu(II) site is effectively enhanced by axial imidazole coordination in a square-pyramidal configuration.[7]

Table II Bonding Parameters of Cu(II) Complexes of Sulfhydryl- and Imidazole-Containing Tetrapeptides

Ligand	According to eq(1) from $A_{\parallel}$† α^2	$(\alpha')^2$	According to eq(6) from A_N† α^2	$(\alpha')^2$	β_1^2	β^2	$\tilde{\nu}$, cm^{-1} $B_{1g} \leftarrow E_g(E_{xz})$	$B_{1g} \leftarrow B_{2g}(E_{xy})$	Ref.
MAGPH	0.58	0.52	—	—	1.23	1.52	16950	14200	This work
MAGGH	0.70	0.39	0.70	0.39	0.60	0.70	19610	16950	This work
MAGGG	0.77	0.32	0.75	0.34	0.54	0.76	20410	16700	7
MAGH	0.82	0.27	0.81	0.28	0.67	1.19	19920	16950	7
MAHH	0.75	0.34	0.80	0.29	0.65	0.96	19490	16700	7
MAH	0.65	0.45	—	—	1.19	1.12	16720	15870	6

† The detailed calculations are described in reference 7.

In conclusion, the spectroscopic properties(λ_{max}=590 nm and $A_{\parallel}=75\times10^{-4}cm^{-1}$) of the 1:1 MAGPH-Cu(II) complex are close to those of blue copper sites, though its extinction coefficient near 600 nm is small. In the Cu(II) complexes of the present tetrapeptides, MAGPH and MAGGH, the exchange of the glycine residue of the third position by the proline residue effects a red shift(80 nm) of the visible absorption and a decrease($192\rightarrow75\times10^{-4}cm^{-1}$) of the ESR $A_{\parallel}$ value. Presumably, the introduction of the proline residue induces a change of the complex geometry from D_{4h} to T_d symmetries. The present model results give an useful information for a role of the proline residue at near cysteine and histidine positions of blue copper proteins such as plastocyanin and azurin.

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