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Structure of the Glycyl-L-histidyl-L-lysine–Copper(II) Complex in Solution†

Jonathan H. Freedman,*,‡ Loren Pickart, Boris Weinstein, W. B. Mims, and J. Peisach

ABSTRACT: Optical, electron paramagnetic resonance, and electron spin-echo envelope spectroscopies were used to examine the structure of the Cu(II) complex of glycyl-L-histidyl-L-lysine (GHL) in solution. At neutral pH, GHL forms a mononuclear 1:1 Cu(II) compound having an EPR spectrum resembling that of Cu(II) equatorially coordinated by two or three nitrogen atoms. Electron spin-echo studies demonstrate that one of these is located in the histidyl imidazole ring. A pH titration of Cu(II)–GHL shows three optical transitions with apparent p*K*s of 3.6, 9.2, and 11.4 and molecularities, with respect to protons, of 2, 2, and 1, respectively. At the lowest p*K*, GHL binds Cu(II), forming the species present at physiological pH. At elevated pH, spectroscopic experiments suggest that an alteration of the Cu(II) structure occurs, yet the bound imidazole is retained. These solution studies are consistent with nitrogen coordination of Cu(II) in Cu(II)–GHL, but the solid-state polymeric structure, with oxygen-bridged Cu(II) pairs as previously determined by X-ray crystallographic analysis [Pickart, L., Freedman, J. H., Loker, W. J., Peisach, J., Perkins, C. M., Steinkamp, R. E., & Weinstein, B. (1980) *Nature (London) 288*, 715–717; C. M. Perkins, N. J. Rose, R. E. Steinkamp, L. H. Jensen, B. Weinstein, and L. Pickart, unpublished results], does not exist in solution.

We have previously reported the isolation and characterization of the human serum tripeptide glycyl-L-histidyl-L-lysine, which, in nanomolar concentrations, stimulates the growth or enhances the viability of a variety of cultured cells and organisms (e.g., fungi, hepatocytes, lymphocytes, fibroblasts, T-strain mycoplasma, and *Ascaris* larvae) (Pickart, 1981; Fouad et al., 1981; Castillo & Roberts, 1980; Schlesinger et al., 1977). GHL[1] coisolates from serum with copper in near equimolar quantities, and it is the Cu(II)–GHL complex that is believed to exert the observed bioactivity of the molecule (Pickart & Thaler, 1979, 1980).

X-ray diffraction analysis of crystalline Cu(II)–GHL prepared at near neutral pH (Pickart et al., 1980; C. M. Perkins, N. J. Rose, R. E. Steinkamp, L. H. Jensen, B. Weinstein, and L. Pickart, unpublished results) shows a polymeric structure, where GHL forms a planar, tridentate complex involving (1) the N-terminal amino group of glycine, (2) the nitrogen atom of the glycylhistidyl amide bond, and (3) the imino nitrogen of the histidyl imidazole ring. The fourth and fifth ligands are oxygenous and are bound to Cu(II) in an adjoining molecule forming a binuclear metal complex, reminiscent of crystalline copper(II) acetate (Bleaney & Bowers, 1952a,b; van Niekerk & Schoening, 1953). This structural assignment is in disagreement with that obtained from a potentiometric titration and a comparison of the relative binding constants of Cu(II) with glycyl-L-histidine, glycyl-L-histidylglycine, and GHL, where the metal ion coordination is believed to involve the ϵ-amino of lysine in addition to the histidyl imidazole nitrogen and the amide nitrogen (Lau & Sarkar, 1981).

In view of the broad physiological activity of this peptide and the ambiguous description of the complex as it exists in solution, we have determined the structure of Cu(II)–GHL by employing optical, electron paramagnetic resonance, and electron spin-echo envelope spectroscopies. Using these probes, we have shown that at neutral pH, the tripeptide forms a monomeric complex with a single bound Cu(II) atom. An analysis of the EPR spectrum (Peisach & Blumberg, 1974) suggests that three nitrogen atoms are coordinated to the copper as equatorial ligands. Electron spin-echo spectroscopy demonstrates that one of these is derived from a histidine imidazole. These experiments indicate that the structural assignment derived from spectroscopic measurements of the complex in solution is consistent with the three nitrogen

† From the Department of Molecular Pharmacology and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461 (J.H.F. and J.P.), the Virginia Mason Research Center, Seattle, Washington 98101 (L.P.), the Department of Chemistry, University of Washington, Seattle, Washington 98195 (B.W.), and Bell Laboratories, Murray Hill, New Jersey 07974 (W.B.M. and J.P.). *Received March 8, 1982.* Communication No. 453 from the Joan and Lester Avnet Institute of Molecular Biology. This investigation was supported in part by U.S. Public Health Service Grants HL-13399 (J.P.), NIAMDD-AM-17702 (J.H.F.), and CA-28858 (L.P.).

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[1] Abbreviations: GHL, glycyl-L-histidyl-L-lysine; GH, glycyl-L-histidine; EPR, electron paramagnetic resonance; DET, diethylenetriamine; shf, superhyperfine lines; NMR, nuclear magnetic resonance.

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structure based on the earlier X-ray diffraction analysis of the crystalline complex, but is inconsistent with a binuclear Cu(II) structure.

Experimental Procedures

Materials. Previous preparations of GHL (Pickart et al., 1973) were carried out by solid-phase synthesis according to the method of Stewart & Young (1969) as modified by Gutte & Merrifield (1971). In this, the first synthesis reported in solution, the following method was employed: N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-(benzyloxycarbonyl)-L-histidine was coupled to benzyl-N^{ϵ}-(benzyloxycarbonyl)-L-lysine by a mixed-anhydride procedure using isobutyl chloroformate and *N*-methylmorpholine to give benzyl[N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-(benzyloxycarbonyl)-L-histidyl]-N^{ϵ}-(benzyloxycarbonyl)-L-lysine (mp 97–98 °C). Cleavage of the N^{α} protecting group with hydrogen chloride in methanol produced benzyl[N^{im}-(benzyloxycarbonyl)-L-histidyl]-N^{ϵ}-(benzyloxycarbonyl)-L-lysinate hydrochloride (hydroscopic solid). This salt was neutralized with triethylamine, and the resulting dipeptide amine was joined to N^{α}-(benzyloxycarbonyl)glycine by a mixed-anhydride method to form benzyl[N^{α}-(benzyloxycarbonyl)glycyl][N^{im}-(benzyloxycarbonyl)-L-histidyl]-N^{ϵ}-(benzyloxycarbonyl)-L-lysinate (mp 151–152 °C). Microanalysis of the protected material was performed. Anal. Calcd for $C_{45}H_{48}N_6O_{10}$ (M_r 832.87): C, 64.89; H, 5.81; N, 10.07. Found: C, 64.62; H, 5.74; N, 10.07. Hydrogenolysis of the tripeptide in methanol–acetic acid produced the deblocked compound, which was chromatographed over silica gel to yield crystalline glycyl-L-histidyl-L-lysine acetate. The final product was characterized by infrared and ^{1}H NMR (60 MHz in D_2O) spectroscopies, thin-layer chromatography on silica gel (1-butanol–pyridine–NH_4OH–H_2O, 20:12:3:15; R_f 0.66), and melting point (146–148 °C) and compared well with commercial material purchased from Vega Biochemical and Calbiochem.

Glycyl-L-histidine (free base) was purchased from Sigma Chemical Co. The Cu(II)–GHL and Cu(II)–GH complexes were prepared by combining aqueous solutions of ligand and cupric acetate. For some EPR studies, Cu(II)–GHL and Cu(II)–GH were prepared with hydrated $^{65}CuCl_2$, which was the generous gift of Dr. P. Aisen. Single-imidazole, double-imidazole, and tetraimidazole Cu(II) models used in the spin-echo study were prepared in ethylene glycol–water (1:1 v/v), pH 7.5, and contained 10.0 mM Cu(II). The single-imidazole model consisted of 10.0 mM Cu(II)–diethylenetriamine to which 16.0 mM imidazole was titrated (Mondovì et al., 1977); the double-imidazole model was prepared by dissolving washed freshly precipitated Cu(II)–oxalate with 20.0 mM imidazole; the tetraimidazole model contained 0.4 M imidazole (Mims & Peisach, 1976).

Optical pH Titration. Optical spectra were recorded at room temperature (~25 °C) with a Cary 14R spectrophotometer with 1-cm path-length cuvettes. A 3.0 mM Cu(II)–GHL solution was prepared, and the pH, measured with a Radiometer PHM-52 meter equipped with an Ingold microelectrode, was adjusted with either HCl or NaOH. The absorbance at 600 nm vs. pH was fit by iterated least-squares analysis to the equation

$$Z = A + \frac{B}{1 + 10^{n_1(X-pK_1)}} + \frac{C}{1 + 10^{n_2(X-pK_2)}} + \frac{D}{1 + 10^{n_3(X-pK_3)}}$$

where Z is the absorbance at pH X, A is the maximal absorbance in the titration, and B, C, and D are the absorbance differences due to three chromophoric transitions that occur at pK_1, pK_2, and pK_3, respectively. The molecularities of each transition, with respect to protons, are n_1, n_2, and n_3.

EPR Spectra. EPR spectra were obtained at X band (~9 GHz) with a Varian E-12 spectrometer equipped with a Varian E-231 multipurpose cavity. A Systron–Donner frequency counter and a Varian NMR gaussmeter were used to measure microwave frequency and magnetic field strength, respectively. Spectra of 1.0 mM Cu(II)–GHL in ethylene glycol–water (1:1 v/v), prepared at different values of pH, were obtained at 77 K. These samples, unless otherwise noted, contained ~10% excess GHL.

EPR spectroscopy was used in a pH titration of Cu(II)–GHL. The low-field hyperfine line of Cu(II)–aquo at $g = 2.82$ is distinct from the hyperfine lines of Cu(II)–GHL and was used to quantitate the amount of Cu(II) bound by the tripeptide. The relative intensity of the $g = 2.82$ feature was applied to the equation

$$Z = A + \frac{B}{1 + 10^{n_1(X-pK_1)}}$$

where Z is the intensity of the $g = 2.82$ feature at pH X, while A, B, and n_1 are the same parameters as defined above.

EPR was also used to examine the stoichiometry of Cu(II) binding to GHL. A 1.0 mM GHL solution in ethylene glycol–water (1:1 v/v), prepared at pH 7.5, was titrated with increasing amounts of Cu(II). After each Cu(II) addition the pH was readjusted to 7.5, an aliquot was removed, and the EPR spectrum was determined. The amplitude of the $g_{\perp}$ feature at 2.06 was used to quantitate the amount of Cu(II) bound to the tripeptide.

Electron Spin-Echo Spectroscopy. Samples were prepared containing 5.0 mM GHL and 4.5 mM Cu(II) in ethylene glycol–water (1:1 v/v) at different values of pH. Electron spin-echo decay envelopes were obtained at 4.2 K with an instrument previously described (Peisach et al., 1979; Mims & Peisach, 1976, 1979a). To identify the ligands bound to the Cu(II), we employed both two-pulse and three-pulse methods. Discussions of the theory and systems design for electron spin-echo spectroscopy can be found in the review by Mims & Peisach (1981) and in Fee et al. (1981). A Fourier cosine transform of the data permitted the conversion of the echo envelope from the time domain to the frequency domain, as previously described (Shimizu et al., 1979; Lerch et al., 1981).

Results and Discussion

Stoichiometry of Cu(II) Binding to GHL. The demonstration of the stoichiometry of Cu(II) binding to GHL at physiological pH is shown in Figure 1. With increasing Cu(II) concentration, there is a concomitant increase, with a slope near unity, in the intensity of Cu(II)–GHL EPR signal. The maximum intensity is observed when 1 equiv of Cu(II) is added/equiv of GHL. Excess Cu(II) at pH 7.5 has no effect on the EPR spectrum because it forms an aggregated, magnetically concentrated, insoluble complex and is thus EPR silent.

These results suggest that a single equivalent of Cu(II) is bound to GHL forming a 1:1 complex. Were a binuclear Cu(II) complex formed, new resonances, especially near $g = 4$, or a decrease in signal intensity, due to spin–spin coupling (Abragam & Bleaney, 1970), would have been observed. We find no evidence of binuclear Cu(II) coordination in the Cu(II)–GHL complex.

EPR Study of Cu(II)–GHL Complex. The EPR spectrum of ^{65}Cu(II)–GHL prepared at pH 7.0 is presented in Figure 2. It is that of a single species with $g_{\parallel} = 2.24$, $g_{\perp} = 2.04$,

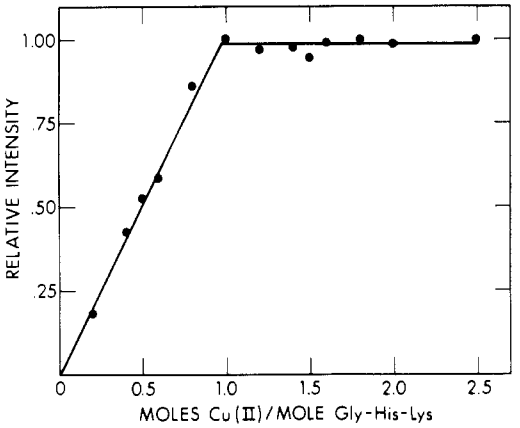

FIGURE 1: Stoichiometry of Cu(II) binding to GHL. Stoichiometry of binding was determined by titrating a 1.0 mM GHL–ethylene glycol–H_2O (1:1 v/v) solution with Cu(II) at pH 7.5. The relative intensities of the signal were normalized by comparing the magnitudes of the EPR feature at $g = 2.06$ to that seen for 1.0 mM Cu(II)–GHL. The lines represent individual least-squares fits to straight line functions for the first five and for the last eight data points. At the intersection the value of the abscissa is 0.98, indicating Cu(II)–GHL forms a 1:1 complex. Excess Cu(II) has no effect on the EPR spectrum after a single equivalent of metal ion is added.

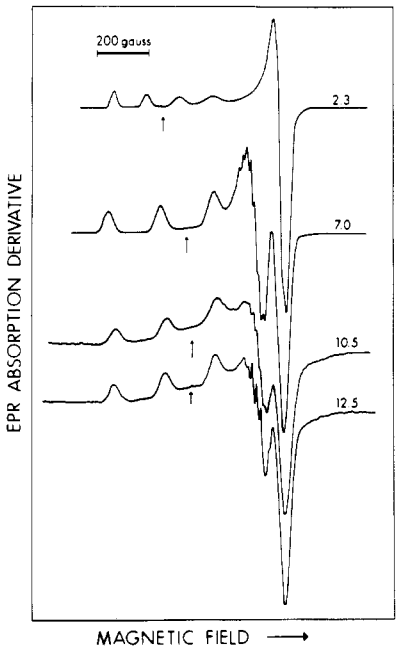

FIGURE 2: Effect of pH on the EPR spectrum of Cu(II)–GHL. Samples of 1.0 mM Cu(II)–GHL were prepared in ethylene glycol–water (1:1 v/v), to ensure good glass formation, prior to pH adjustment. The spectrum of Cu(II)–GHL at pH 7.0 was obtained by using a sample prepared with $^{63}CuCl_2$. The other samples did not contain isotopically pure Cu(II). The arrows indicate the positions of $g_\parallel$ for each spectrum.

and $A_\parallel = 20.7$ mK. In addition, one observes seven superhyperfine lines with a 12.7-G splitting at $g_\perp$. No shf structure can be detected at $g_\parallel$. From a comparison of the $g_\parallel$ and $A_\parallel$ values with those of model compounds (Peisach & Blumberg, 1974) we suggest that the equatorial ligands consist of either two or three nitrogen atoms, with oxygen occupying the remaining site(s). Although not conclusive, the presence of seven shf lines at $g_\perp$ is consistent with N_3O equatorial ligation (Maki & McGarvey, 1958a,b), although coordination by fewer nitrogen atoms cannot be ruled out.

Electron Spin-Echo Study of Cu(II)–GHL. The electron spin-echo decay envelope contains periodicities that arise from

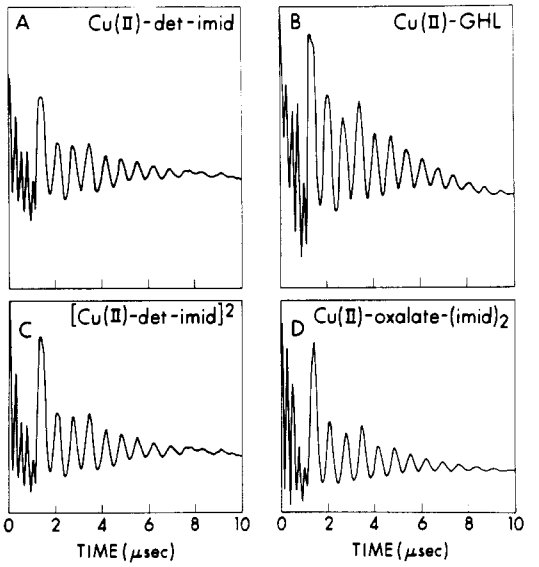

FIGURE 3: Three-pulse spin-echo envelopes of Cu(II) complexes with imidazole ligands. (A) is the echo envelope for Cu(II)–diethylenetriamine–imidazole while (C) is the square of the function shown in (A). Note that the depth of modulation in (C) is comparable to that for Cu(II)–oxalate–imidazole$_2$ shown in (D). (B) is the envelope for Cu(II)–GHL. The intensity of the modulation pattern in (B) is similar to that in (A), suggesting that a single imidazole is coordinated to Cu(II) in the tripeptide complex. For these studies, the spectrometer frequency was 9.221 GHz, the magnetic field setting was 3220 G, and the value of τ was 364 ns.

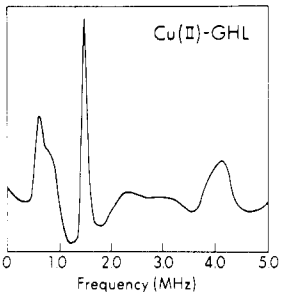

FIGURE 4: Fourier cosine transformation of the three-pulse spin-echo envelope of Cu(II)–GHL at pH 8.3. The peaks near 0.7, 1.5, and 4.0 MHz arise from the interaction between Cu(II) and the remote nitrogen atom of bound imidazole (Mims & Peisach, 1979b).

interactions between the paramagnetic center and close lying nuclei. However, ^{14}N nuclei that are directly coordinated with Cu(II), such as those from ligands containing amino or amide groups, do not in general contribute to the echo envelope (Mims & Peisach, 1978). The observed periodicities (Figure 3) are attributable to the remote protonated ^{14}N of ligated imidazole; the frequencies, which are obtained by Fourier transformation (Figure 4), are principally determined by the Zeeman field, by a contract interaction term $\boldsymbol{AI}\cdot\boldsymbol{S}$, and by the nuclear quadrupolar interactions, as explained elsewhere (Mims & Peisach, 1978). Thus, the appearance of frequencies near 0.7, 1.5, and 4.0 MHz confirms the presence of at least a single imidazole as a ligand to Cu(II) in the GHL complex.

In principle it should be possible to obtain some additional information regarding the number of imidazoles bound to Cu(II) from the depth of the modulation pattern. It has been shown theoretically (Rowan et al., 1965; Mims, 1972) that, for a single crystal sample in which there is only one kind of center and only one orientation of the center with respect to the magnetic field, the modulation pattern due to several nuclei coupled to the same electron is simply the product of the modulation patterns calculated for each nucleus considered

separately. Thus, in a sample of this kind, if there were to be a center with two chemically identical ligands, coupled in the same way with the electron spin and similarly oriented with respect to the magnetic field, the resulting modulation pattern would be given by the square of the modulation due to just one of the ligands. The modulation pattern would be deeper for two ligands than for one.

For samples prepared in frozen solution, the depth of modulation has been shown to be related to the number of interacting nuclei and their distance from the paramagnetic center by assuming a spherical average for all orientations (Dikanov et al., 1977; Mims et al., 1977; Mims & Peisach, 1979a,b). Most of the work in this area has involved hydrogen and deuterium modulation in hydrated complexes, i.e., a case in which there are many equivalent nuclei situated at the same distance from the paramagnetic ion (Kevan et al., 1975; Mims & Davis, 1976).

The case that concerns us in the present work—that of ^{14}N nuclei in imidazole ligands—is more involved, since the modulation pattern and its depth depend on the orientation of the quadrupolar axes, as well as on the orientation of the electron–nuclear anisotropy tensor. The statistical weights associated with these orientations would be strongly conditioned by steric factors and would also tend to restrict the relative orientations of two or more imidazole ligands, thus making it considerably harder here, than in the case of hydrated ions, to justify the adoption of spherical averages or products of spherical averages. In view of the difficulty of presenting an adequate theoretical treatment of this case, we have relied on an experimental demonstration.

Figure 3A shows a three-pulse echo envelope for the one-imidazole copper complex Cu(II)–DET–imidazole. The computed square of the data is seen in Figure 3C, and it may be compared with the three-pulse echo envelope, obtained under similar conditions, for the two-imidazole copper complex Cu(II)–oxalate–imidazole$_2$ shown in Figure 3D. As can be seen, the depth of the modulation, as visualized from the ratios of peak height to base line as compared to trough depth to base line for all the features of the echo envelope, is roughly related to the number of imidazole nitrogens ligated to Cu(II); the more imidazoles bound, the greater the modulation depth. It will be noted that there is a close if not exact agreement between the curves in panels C and D of Figure 3, indicating that for these complexes, and for any which are closely related to them, the product theorem provides an adequate approximation. Similar comparisons have been made between the square of the function in Figure 3D and the three-pulse echo envelope for Cu(II)–tetraimidazole. Agreement is less good here, possibly because the modulation is relatively deep for Cu(II)–tetraimidazole and the accuracy of the base-line determination becomes a critical factor [see, e.g., Figure 8 in Mims & Peisach (1978)]. However it appears to be relatively easy to distinguish between the one-imidazole and two-imidazole cases, and we are able, by comparing panel B with panel A of Figure 3, to confirm that only one imidazole coordinates to Cu(II) in GHL.

pH Titration of Cu(II)–GHL. Optical and EPR spectroscopies were used to examine the effect of pH on the structure of Cu(II)–GHL. A least-squares analysis of the optical absorption as a function of pH (Figure 5) shows three transitions with apparent pKs of 3.6, 9.2, and 11.4 and molecularities of 2, 2, and 1, respectively. In addition, as the pH increases there is a shift in the absorption maximum. At pH 5–9, Cu(II)–GHL absorbs maximally at 595 nm. However, above pH ~9, λ_{max} shifts to 565 nm. The formation of the 565-nm absorbing species is accompanied by a proportional decrease in the intensity of the 595-nm chromophore.

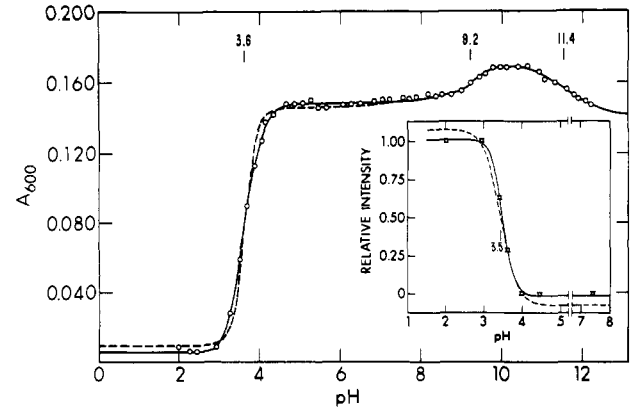

FIGURE 5: Optical and EPR pH titration of Cu(II)–GHL. Reaction mixtures for the optical titration contained 3.3 mM GHL and 3.0 mM Cu(II). The optical absorbance at 600 nm, A_{600}, was fitted as a function of pH to an equation describing a titration with three transitions as described under Experimental Procedures. The calculated pKs, 3.6, 9.2, and 11.4, have molecularities of 2, 2, and 1, respectively. The dashed line is a theoretical fit to the data where the molecularity of the pK_1 = 3.6 titration is 3. The mean-weight error is double for the n = 3 fit to the data as compared to an n = 2 fit and is therefore not as good. The inset contains an analysis of an EPR pH titration near the lowest pK of 1.0 mM Cu(II)–GHL prepared in ethylene glycol–water (1:1 v/v). The intensity of the g = 2.82 feature was normalized relative to the value obtained from the EPR spectrum of a 1.0 mM Cu(II)–aquo solution. The data were fit to the function described under Experimental Procedures and show a pK of 3.5 with n = 3 for the solid line and n = 2 for the dashed line. The standard error bars indicate the deviation of the data from the computer-generated function (n = 3) and are twice as large for the n = 2 fit.

An EPR study shows that at pH 3.5, Cu(II) is present as Cu(II)–aquo and as the Cu(II)–GHL complex seen at neutral pH. When the pH is lowered to 2.1, Cu(II)–aquo ($g_\parallel$ = 2.42, $g_\perp$ = 2.08, $A_\parallel$ = 13.89 mK) predominates in the sample (Figure 2), while at pH 5 only the resonance attributable to Cu(II)–GHL is observed. From a titration of the EPR data (Figure 5 inset) we find the conversion of Cu(II)–aquo to Cu(II)–GHL occurs with an apparent pK of 3.5 and with n = 3. This pK is in excellent agreement with that obtained from the optical titration, although the difference in the n value with that obtained optically is not simply explained from this data. However, Lau & Sarkar (1981) have reported the displacement of three protons from the peptide when Cu(II) is chelated.

Electron spin-echo spectroscopy was used to study the effect of pH on imidazole coordination in Cu(II)–GHL. In a three-pulse study at pH 2.3 no ^{14}N modulation is seen, although one could still observe in a two-pulse experiment a ^1H modulation pattern due to the interaction of the unpaired spins of Cu(II) with ligated and bulk water (Mims & Peisach, 1976). When the pH is raised to 3.5, the apparent pK of Cu(II) ligation by the peptide, a ^{14}N modulation pattern arising from coordinated imidazole is seen, but the depth of the pattern is far less than can be observed at neutral pH. The shallowness of the modulation at pH 3.5 implies structural heterogeneity, as was demonstrated optically and by EPR. At pH 6–8 the ^{14}N modulation becomes maximal (Figure 3B) and is attributed to single-imidazole coordination of Cu(II).

At high pH, pH 10–12.5, there are no significant changes in $g_\parallel$ and $A_\parallel$ in the EPR spectrum although changes are seen in the EPR line shape (Figure 2). In a complementary electron spin-echo experiment the periodicities associated with imidazole coordination do not change in this same pH range.

However, the modulation becomes shallower. These experiments suggest the formation of the 565-nm chromophore may be accompanied by an alteration of the coupling to the ^{14}N of ligated imidazole. Also, changes in axial ligation may be taking place that affect the optical chromophore but do not affect the EPR, which to first order is sensitive only to equatorial ligation (Getz & Silver, 1974).

Cu(II)–GHL vs. Cu(II)–GH. The results presented here concerning the solution structure of Cu(II)–GHL are consistent with a monomeric, tridentate Cu(II) complex involving (1) the imino nitrogen of the histidine imidazole, (2) the N-terminal group of glycine, and (3) either the amide nitrogen of the glycyl–histidyl peptide bond (Pickart et al., 1980; C. M. Perkins, N. J. Rose, R. E. Steinkamp, L. H. Jensen, B. Weinstein, and L. Pickart, unpublished results) or the ϵ-amino group of lysine (Lau & Sarkar, 1981). To help determine which of these structures is present in solution, we have examined the Cu(II) complex of glycyl-L-histidine. A study of the stoichiometry at pH 7 suggests that 1 equiv of Cu(II) is bound/mol of GH, forming a 1:1 complex. The EPR spectrum of $^{65}Cu(II)$–GH, at pH 7.4, is that of a single species with $g_{\parallel} = 2.23$, $g_{\perp} = 2.06$, $A_{\parallel} = 19.7$ mK, and seven shf lines at $g_{\perp}$ and is nearly the same as that for Cu(II)–GHL. There is no indication in the spectrum of weakly coupled binuclear Cu(II), as might be expected in a 2:2 complex, while the EPR signal intensity is that of a mononuclear $s = {}^1/_2$ species.

Cu(II)–GH was also studied by electron spin-echo spectroscopy. The three-pulse spin-echo envelope contains the periodicities due to interactions with the remote ^{14}N of bound imidazole, thus confirming the presence of this ligand. The depth of the modulation pattern is that associated with the coordination by a single imidazole.

These results are essentially the same as those observed for Cu(II)–GHL, suggesting that in the latter case the lysyl residue may not be equatorially coordinated. Furthermore, Dreiding models of Cu(II)–GHL, made with plastic hemispheres for the various hydrogen atoms, indicate that van der Waals interactions will inhibit the binding of the lysyl side chain amino nitrogen to the Cu(II), suggesting again that it is not bound to the metal ion. The lysyl residue, however, has been shown to be essential for the biological activity of Cu(II)–GHL (Pickart et al., 1979; Pickart & Thaler, 1979). For that reason, it has been suggested (Pickart, 1981) that the glycyl and histidyl residues function as Cu(II) chelators, whereas lysine may be involved in the recognition of a cell surface receptor.

Acknowledgments

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