# Copper(II) Complexes of Histidine and Its Related Compounds in Aqueous Solutions

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The copper(II) complexes of histidine and its related compounds in aqueous solution were investigated by potentiometric, spectrophotometric, and magnetic methods. The presence of a diolated binuclear copper(II) complex was indicated in the 1 : 1 histamine-copper(II) system by quantitative analysis of the titration data and the antiferromagnetic character of the complex. The titration data could not be quantitatively interpreted owing to precipitation, but the formation of a similar diolated copper(II) complex was also shown in the 1 : 1 histidine-copper(II) system by the magnetic properties of the complex. From a comparison of the spectral properties of the pertinent complexes, it was shown for the neutral 2 : 1 histidine-copper(II) complex that histidine binds to copper-(II) through the amino and imidazole groups in the chelate plane and through the carboxyl group in the apical position. In the copper(II) complexes of histidine and histamine, it was shown that ionization of the pyrrole nitrogen of the imidazole ring is promoted, causing a spectral blue shift.

Elucidation of the complex formation between copper-(II) and histidine in the aqueous solution is not easy, since histidine involves a bulkyl imidazole ring and has three or four coordination sites, and copper(II) requires a tetragonal coordination. This and the biological significance of histidine residue as a metal binding site in protein have prompted many workers to investigate copper(II)-histidine complexes. The presence of various complex species, *viz.*, protonated, normal and hydroxo complexes, has been found to be dependent on pH of medium and histidine-to-copper(II) ratio.[1-6] However, the structures of the complexes have not been completely elucidated. The coordinating groups which occupy four positions in the square coordination sp-here are not elucidated in the ligand to metal ratio 2 : 1 normal complex. In this connection, a weak coordination from the apical direction has been the subject of debate. Questions on the structure of the hydroxo complex formed in the 1 : 1 system remain undetermined and structural changes in the complexes in alkaline solution have not been fully discussed. For the purpose of obtaining further information on the structures of various complexes which may be formed in the reaction of histidine with copper(II) under various conditions, we studied the copper(II) complexes of histidine and its related compounds in aqueous solution by potentiometric, visible spectrophotometric, and magnetic methods.

## Experimental

*Materials.* L-Histidylglycine was synthesized according to the method described previously.[7] Commercial ligands and reagents were used.

*Methods.* Potentiometric titration was carried out on a solution (10 ml) containing a ligand ($10^{-2}$ M) and copper(II) ($10^{-2}$ M or $0.5\times10^{-2}$ M). Visible spectrophotometric measurements were made on solutions with copper(II) concentrations $4\times10^{-3}$ M—$1\times10^{-2}$ M. Details of experimental conditions and method have been described.[7,8] The magnetic susceptibilities of the solutions containing a ligand ($10^{-1}$ M) and copper ($10^{-1}$ M) were measured by the Gouy method at room temperature. The molar susceptibility and magnetic moment of copper(II) in each system was calculated, with the correction of the diamagnetism of water, ligand and other ions present.

## Results

*Potentiometric Titration.* Figure 1 shows the titration curves of L-histidine, histamine, and L-3-benzylhistidine in the presence of equimolar $CuCl_2$. In each 1 : 1 system a pH inflection is observed at $a=2$, followed by an additional deprotonation in neutral region, where $a$ represents moles of KOH added per diprotonated ligand.

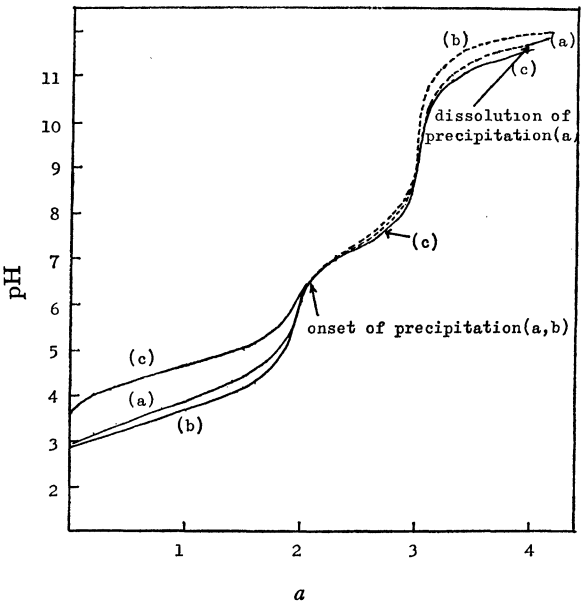



Fig. 1. Titration curves of L-histidine, L-3-benzylhistidine and histamine in the presence of equimolar $CuCl_2$:
(a) histidine, (b) 3-benzylhistidine, (c) histamine, [ligand]=[Cu(II)]=$10^{-2}$ M (at $a=0$).
The dotted line indicates precipitation.

The 1 : 1 complex ($CuL'^{2+}$) is regarded as the main species in the region, where $a$ value is about 2 in the 1 : 1 histamine-copper(II) system.[1] The following equation was assumed for the deprotonation between $a=2$ and 3 in the histamine-copper(II) system, the equilibrium constant $pK_e^1$ being calculated at each reaction point:

$$CuL'^{2+} \rightleftharpoons CuL'(OH)^{+} + H^{+}$$

$$K_c^1 = \frac{[CuL'(OH)^{+}][H^{+}]}{[CuL'^{2+}]} \quad (1)$$

where L' represents the neutral species of histamine.

The results given in Table 1 show that the equation does not hold, since the calculated values of $pK_c^1$ decrease with the increase in $a$ value. The formation of a diolated binuclear complex might be expected with the dissociation of a proton from the complex $CuL'^{2+}$.[3,9] Thus the followiug reaction was assumed and the equilibrium constant $pK_c^2$ was calculated at each point.

$$2CuL'^{2+} \rightleftharpoons Cu_2L'_2(OH)_2^{2+} + 2H^{+}$$

$$K_c^2 = \frac{[Cu_2L_2'(OH)_2^{2+}][H^{+}]^2}{[CuL'^{2+}]^2} \quad (2)$$

The values of $pK_c^2$ (Table 1) remain constant irrespective of $a$ values. In contrast, the values of $pK_c^3$, $pK_c^4$, and $pK_c^5$ which correspond to the formations of the trimer, tetramer, and pentamer respectively increase with the increase in $a$ value in the region $a$= 2.1—2.7. This suggests the formation of a dimer with the deprotonation between $a$=2 and 3.

In the histidine-copper(II) 1 : 1 system, the complex precipitates immediately when the solution of KOH was added beyond $a$=2. The amount of precipitate increased with $a$ value up to $a$=3, decreased gradually and then dissolved completely at $a$=4. Precipitation also occurred in the 3-benzylhistidine-copper(II) system, but in this case the precipitate did not dissolve at $a$=4. Due to precipitation titration data between $a$=2 and 3 in the histidine-copper(II) and 3-benzylhistidine-copper(II) systems were not interpretated quantitatively. In the 1 : 1 histidine-copper(II) and 1 : 1 histamine-copper(II) systems, pH in the region $a$=3—4 was lower than that in the systems of the ligand itself,[7] whereas in the 1 : 1 3-benzylhistidine-copper(II) system, no fall in pH was observed. The results suggest further deprotonation between $a$=3 and 4 in the former two systems. Similar falls of pH were also observed in the region $a$=2—3 in the 2 : 1 histidine-copper(II) and 2 : 1 histamine-copper(II) systems respectively.

Table 1. Quantitative analysis of the titration curve in the region $a$=2—3 in the 1:1 histamine-copper(II) system

| $a$ value | pH | $pK_c^1$ | $pK_c^2$ | $pK_c^3$ | $pK_c^4$ | $pK_c^5$ |
|---|---|---|---|---|---|---|
| 2.1 | 6.58 | 7.53 | 12.02 | 16.39 | 20.71 | 24.99 |
| 2.2 | 6.82 | 7.42 | 12.10 | 16.65 | 21.15 | 25.63 |
| 2.3 | 6.96 | 7.33 | 12.09 | 16.72 | 21.30 | 25.85 |
| 2.4 | 7.08 | 7.26 | 12.07 | 16.75 | 21.38 | 25.99 |
| 2.5 | 7.22 | 7.22 | 12.09 | 16.83 | 21.52 | 26.19 |
| 2.6 | 7.36 | 7.18 | 12.09 | 16.88 | 21.61 | 26.31 |
| 2.7 | 7.53 | 7.16 | 12.11 | 16.94 | 21.72 | 26.47 |
| 2.8 | 7.70 | 7.10 | 12.04 | 16.86 | 21.63 | 26.37 |
| 2.9 | 7.98 | 7.03 | 11.95 | 16.75 | 21.49 | 26.21 |

*Visible Absorption Spectra.* Spectral characteristics of the systems containing a ligand and copper(II) with the molar ratios of 1 : 1 and 2 : 1 are given in Table 2. Each $a$ value corresponds to that in the titration. In the 1 : 1 histamine-copper(II) system, the spectrum at $a$=2 exhibited an absorption maximum at 665 nm ($\varepsilon$=38). Addition of equimolar KOH caused a significant blue shift (665 nm→615 nm). Addition of equimolar KOH caused another blue shift to show an absorption maximum at 590 nm. In the 1 : 1 histidine-copper(II) system, similar spectral changes were observed, although the spectrum of the solution at $a$=3 could not be measured owing to the precipitation. The spectrum at $a$=4 exhibited an absorption maximum at 605 nm. The spectra of the 1 : 1 3-benzylhistidine-copper(II) system in the region $a$=3—4 could not be measured owing to the precipitation. In the 2 : 1 histamine-copper(II) and 2 : 1 histidine-copper(II) systems, blue shifts were observed in the region $a$=2—3 by the addition of KOH. No spectral change was observed by the addition of KOH in the 2 : 1 3-benzylhistidine-copper(II) system.

The spectra of the 2 : 1 normal complexes of copper(II) with some related compounds of histidine are shown in Fig. 2, together with those of some 1 : 1 : 1 mixed ligand complexes of these ligands. The charac-

Table 2. Visible absorption characteristics of 1:1 and 2:1 copper(II) complexes

| System | $a$ value | Main species | $\lambda_{max}$ (nm) | $\varepsilon_{max}$ ($cm^{-1}M^{-1}$)[b] |
|---|---|---|---|---|
| Histidine·HCl+Cu(II) | 2 | $CuL^{+}$ | 665 | 38 |
| | 3[a] | $Cu_2L_2(OH)_2$ | — | — |
| | 4 | $Cu_2X_2(OH)_2^{2-}$ | 605 | 62 |
| Histamine·2HCl+Cu(II) | 2 | $CuL'^{2+}$ | 665 | 38 |
| | 3 | $Cu_2L_2'(OH)_2^{2+}$ | 615 | 52 |
| | 4 | $Cu_2X_2'(OH)_2$ | 590 | 64 |
| 3-Benzylhistidine+HCl+Cu(II) | 2 | $CuL^{+}$ | 670 | 36 |
| | 3[a] | $Cu_2L_2(OH)_2$ | — | — |
| | 4[a] | $Cu_2L_2(OH)_2$ | — | — |
| Histidine·HCl+$^1/_2$Cu(II) | 2 | $CuL_2$ | 640 | 84 |
| | 3 | $CuX_2^{2-}$ | 625 | 96 |
| Histamine·2HCl+$^1/_2$Cu(II) | 2 | $CuL'^{2+}$ | 601 | 82 |
| | 3 | $CuX_2'$ | 580 | 91 |
| 3-Benzylhistidine+HCl+$^1/_2$Cu(II) | 2 | $CuL_2$ | 642 | 88 |
| | 3 | $CuL_2$ | 642 | 88 |

a) Complex precipitated. b) Molar absorptivity is expressed per gram atom of copper(II).

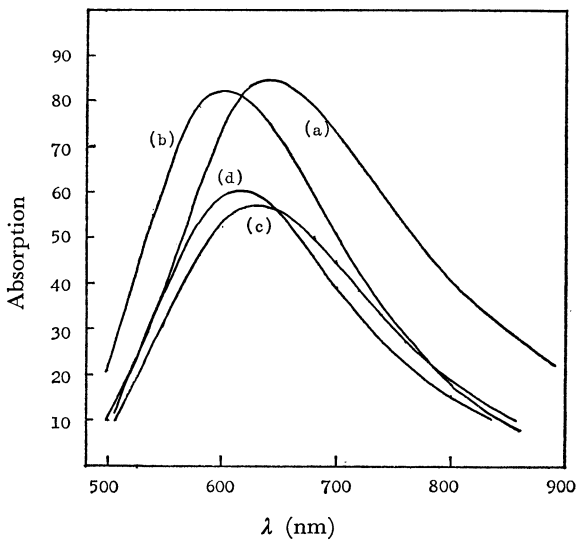



Fig. 2. Visible absorption spectra of normal 2 : 1 copper(II) complexes of histidine related compounds: (a) histidine, (b) histamine, (c) 1-methylhistidine, (d) histidine + 1-methylhistidine (1 : 1): The absorbance is expressed on a molar copper(II) basis.

Table 3. Visible absorption characteristics of 2: 1 normal copper(II) complexes

| | $\lambda_{max}$ (nm) | $\varepsilon_{max}$ ($cm^{-1}M^{-1}$) | $\Delta\lambda_{1/2}$ (nm)[a] |
|---|---|---|---|
| Histidine | 640 | 84 | 250 |
| 3-Benzylhistidine | 642 | 88 | 246 |
| Histidylglycine | 636 | 88 | 237 |
| Histidine methyl ester | 629 | 85 | 228 |
| Histidine + Histamine (1: 1) | 628 | 83 | 220 |
| Histamine | 601 | 82 | 200 |
| 1-Methylhistidine | 628 | 57 | 193 |
| Histidine + 1-Methyl-histidine(1: 1) | 615 | 60 | 194 |

a) $\Delta\lambda_{1/2}$ represents the width at the half-height of the spectra.

teristics of these spectra are given in Table 3. The molar absorptivities do not differ much from each other among the copper(II) complexes of histidine, 3-benzylhistidine, histidine methyl ester, histidylglycine and histamine. On the other hand, the molar absorptivities 2 : 1 1-methylhistidine-copper(III) and 1 : 1 : 1 1-methylhistidine-copper(II)-histidine complexes are smaller than the molar absorptivity of 2 : 1 histidine-copper (II) complex.

The order of the wavelength of the absorption maximum in the 2 : 1 complexes is : histamine histidine methyl ester, histidylglycine, histidine and 3-benzylhistidine (from shorter to longer wavelength). The width at the half-height of the spectral band increases in the same order. The broadening of the band was observed in the longer wavelength region than the absorption maximum.

*Magnetic Moment.* The molar susceptibilities and the magnetic moments of the copper(II) in the 1 : 1 ligand-copper(II) systems are shown in Table 4. The magnetic data at $a=3$ in the histidine-copper(II) and at $a=4$ in the histamine-copper(II) were not measured in aqueous solution owing to the precipitation. The magnetic moment decreases with the increase in $a$ value in each system, but the values are always normal in the glycylglycine-copper(II) system. The subnormal values were obtained in the histamine-copper(II) and in the histidine-copper(II) systems when $a$ values are 3 and 4, respectively. This suggests that antiferromagnetic interactions are present in these systems.

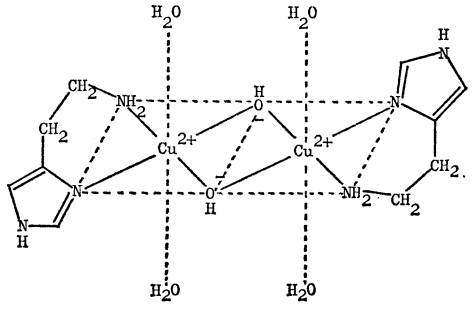
Table 4. Room temperature magnetic data on the copper(II) in the ligand-Cu(II) aqueous solutions

| System | $a$ value | $\chi_A \times 10^6$ c.g.s. units | $T$(K) | $\mu_{eff}$ (B.M.) |
|---|---|---|---|---|
| Histidine·HCl + Cu(II) | 0 | 1552 | 296 | 1.87 |
| | 1 | 1450 | 293 | 1.82 |
| | 2 | 1409 | 293 | 1.82 |
| | 3[a] | not determined | | |
| | 4 | 1001 | 296 | 1.54 |
| Histamine·2HCl + Cu(II) | 0 | 1548 | 286 | 1.88 |
| | 1 | 1474 | 286 | 1.94 |
| | 2 | 1391 | 296 | 1.81 |
| | 3 | 1104 | 288 | 1.59 |
| | 4[a] | not determined | | |
| Glycylglycine + Cu(II) | 0[b] | 1562 | 288 | 1.90 |
| | 1[b] | 1457 | 288 | 1.85 |
| | 2[b] | 1409 | 296 | 1.83 |
| | 3[b] | 1307 | 296 | 1.76 |

a) Complex precipitated. b) The values represents the number of moles of KOH added per neutral (monoprotonated) glycylglycine.

## Discussion

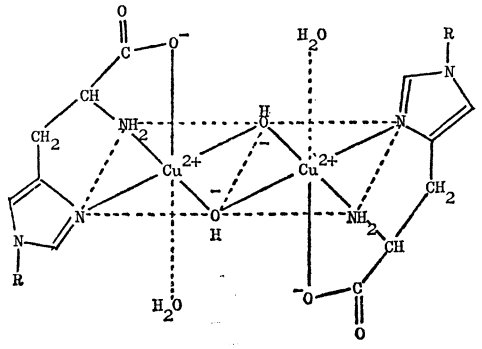
*1 : 1 Hydroxo Complexes.* Quantitative analysis of the titration data in the 1 : 1 histamine-copper(II) system suggests the formation of a dimer complex with the release of a proton in the region $a=2$—3. This conclusion is compatible with the results reported by Perrin and Sharma.[3,9] The source of the liberated proton is regarded as the coordinated water molecule and a dimer I is presumed to be formed by the olation between two molecules. The antiferromagnetic character of the 1 : 1 histamine-copper(II) system at



I, $Cu_2L_2'(OH)_2^{2+}$

$a=3$ is a strong evidence for the binuclear structure.[10] It is noteworthy that the formation of the diolated complex displaces the absorption maximum at a wavelength about 50 nm shorter than in the mother complex in spite of the lower position of $OH^-$ than $H_2O$ in the spectrochemical series.

Perrin and Sharma suggested from their quantitative analysis of the titration data that a monohydroxo complex CuL(OH), where $L^-$ represents the anionic species of histidine, is a predominant species near $a=3$ in the 1 : 1 histidine-copper(II) system.[3] However, in the 1 : 1 histidine-copper(II) system, the titration data for the region $a=2$—3 can not be analysed quantitatively owing to precipitation, and there may be some uncertainty in their conclusion. The antiferromagnetic character of the 1 : 1 histidine-copper(II) complex at $a=4$ suggests the presence of the binuclear hydroxo complex $Cu_2L_2(OH)_2$ similar to that in the case of 1 : 1 histamine-copper(II) complex. The probable structure for the 1 : 1 histidine-copper(II) complex is shown to be II. The same diolated complex will

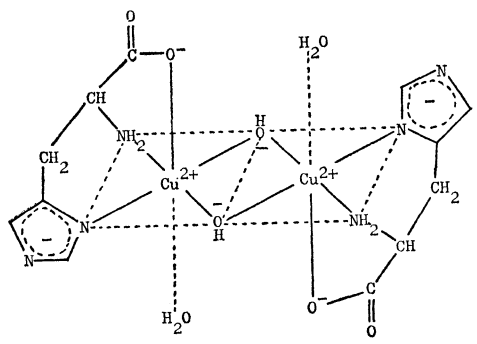


II, $Cu_2L_2(OH)_2$ R=H : histidine

R=$CH_2$-  : 3-benzylhistidine

also be formed in the 1 : 1 3-benzylhistidine-copper(II) system. The steric requirement of the bulky imidazole group is presumed to promote the formation of the binuclear complexes in these systems.[11,12]

The dissolution of the 1 : 1 histidine-copper(II) complex accompanied by deprotonation between $a=3$ and 4 suggests the formation of an anionic complex $Cu_2X_2(OH)_2^{2-}$, where $XH^-=L^-$. The evidence that the source of this proton is imino group of the imidazole ring is given by the fact that no similar behavior was observed in the 3-benzylhistidine-copper(II) system. The structure of the 1 : 1 histidine-copper(II) anionic complex is shown by III. Similarly, further deprotonation accompanied by a conspicuous blue shift in absorption spectra for the region $a=3$—4 in the 1 : 1 histamine-copper(II) system suggests the dissociation of the proton from the imino group of imidazole giving a neutral complex $Cu_2X_2'(OH)_2$, where X'H=L'.

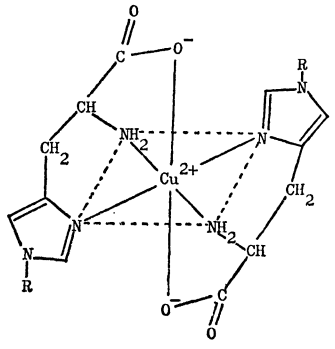


III, $Cu_2X_2(OH)_2^{2-}$

The absorption at longer wavelength at $a=4$ in the 1 : 1 histidine-copper(II) complex than in the 1 : 1 histamine-copper(II) complex can be ascribed to the relaxation of the tetragonal distortion due to the apical coordination of the carboxyl groups.[13]

2 : 1 *Complexes.* For the neutral 2 : 1 histidine-copper(II) complex, the following possibilities are taken into account for the group which occupies the four coordination positions in the copper(II) plane. (a) Two histidine molecules bind to copper(II) with the amino and imidazole groups(histamine type).[1,3,5,6,14-18] (b) Two histidine molecules bind to copper(II) with the amino and carboxyl groups (glycine type).[19-21] (c) One histidine molecule coordinates with copper(II) as in the case of histamine, and another as in the case of glycine(mixed type).[22,23]

The larger molar absorptivity of the 2 : 1 histidine-copper(II) complex than in 2 : 1 1-methylhistidine-copper(II) and 1 : 1 : 1 1-methylhistidine-copper(II)-histidine complexes suggests that the coordination is neither a glycine nor a mixed type. Possibility (a) can be accepted in view of the similarity in the molar absorptivities between the 2 : 1 complexes of histidine, 3-benzylhistidine, histidylglycine, histidine methyl ester, and histamine. The presence of the two six-membered chelate ring composed of the amino and imidazole groups in the copper(II) plane seems to give a similar absorptivity. The order of the wavelength of the absorption maximum and that of the increase of the width of the spectral band in these 2 : 1 copper(II) complexes seem to reflect the extent of tetragonal distortion of the copper(II) complexes,[13] since these orders are in agreement with the order of strength of apical coordination among the coordination groups, *viz.*, $-COO^- > \rangle C{=}O > H_2O$. This provides an evidence for apical coordination of the carboxyl group in the histidine-copper(II) and 3-benzylhistidine-copper(II) complexes. A smaller tetragonal distortion of the 2 : 1 histidine-copper(II) complex than in the 1 : 1 : 1 histamine-copper(II)-histidine complex sug-



IV, $CuL_2$

R=H : histidine

R=$CH_2$-  : 3-benzylhistidine

gests that both carboxyl groups coordinate to copper(II) at apical position in the former, as shown by IV.

Further deprotonation accompanied by the blue shift in the absorption spectra in the range $a=2$—3 in the 2 : 1 histamine-copper(II) and histidine-copper(II) systems, also indicates the ionization of the imino group. No deprotonation and the spectral shift in the 2 : 1 3-benzylhistidine-copper(II) system in the same condition support the conclusion. The relatively easier ionization of the imino group in the 1 : 1 and 2 : 1 copper(II) complexes of histidine and histamine would be due to the electron-withdrawing effect of copper(II) which binds to the pyridine type nitrogen.

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