Complexation between Copper(II) Ion and Large Excess Histidine in Aqueous Solution

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The complexation between copper(II) ion and large excess histidine has been studied by visible absorption, proton and C-13 NMR spectral methods, in comparison with histamine–copper(II) system. The predominant species were considered to be ML_2H^+ and $ML_2H_2^{2+}$ at $a=0$ and ML_2 at $a=1$ and 2, respectively from the spectral data in visible region, where a represents moles of base added per mole of diprotonated ligand. In ML_2H^+, one histidine is bound histamine-like with a carboxylate coordinated in apical position and the other glycine-like with a protonated imidazolium group, and in $ML_2H_2^{2+}$, both histidine molecules are bound glycine-like. Two histidine molecules are both tridentate with apical carboxylates in ML_2. In histamine–copper(II) system, the coordination of the third histamine molecule in the apical position was suggested at $a=1$ and 2. The proton and the C-13 NMR spectra were reasonably interpreted in view of the above situation. A difference in the line-broadening of PMR signals was observed between histidine–copper(II) and histamine–copper(II) systems. This is mainly due to the difference in the exchange rates of the coordinated ligands of two systems.

On account of the importance of interaction between imidazole group and copper(II) ion in biological systems, copper(II)–histidine complexes in aqueous solution have been studied by many workers. Most of the experiments were performed at stoichiometric conditions such as ligand to copper(II) ion ratio of 2: 1 or 1: 1. Sigel and McCormick investigated the interaction at ligand to metal ion ratio of 2×10^3 by means of proton NMR spectroscopy.[1] By comparing the line-broadening of imidazole proton signals of neutral histidine with that of its derivatives, they concluded that the predominant species is 2: 1 complex where one histidine is bound histamine-like and the other glycine-like. On the other hand, based on a kinetic consideration Sundberg and Martin have suggested that histidine may interact with copper(II) ion in only a monodentate mode at extraordinarily large ratios of ligand to metal ion.[2] The purpose of the present study is to obtain detailed information about the mode of complexation in the presence of large excess histidine by means of NMR and visible spectral methods.

Experimental

Materials. L-Histidine monohydrochloride monohydrate, histamine dihydrochloride, and $CuCl_2\cdot2H_2O$ were obtained from Nakarai Chemicals, Ltd., Kyoto. The deuterated compounds, D_2O, NaOD, and TSP (sodium 3-trimethylsilyltetradeuteriopropionate) were obtained from E. Merck AG, Darmstadt.

Visible Absorption Spectra. A Shimadzu Double 40-R spectrophotometer was used to obtain the visible absorption spectra. The measurements were carried out on the solutions containing a ligand (4×10^{-1} M) and copper(II) ion (2×10^{-3} M) or a ligand (2×10^{-2} M) and copper(II) ion (10^{-2} M) at room temperature. The spectra were obtained at various pH values corresponding to each a value, in which a represents moles of base added per mole of diprotonated ligand.

NMR Spectra. The proton NMR spectra were measured on a Varian A-60 NMR spectrometer. The spectra were obtained on the D_2O solutions of a ligand (0.25 M) in the absence and the presence of 1/500- or 1/2000-fold copper(II) ion at each a value. TSP was used as an internal reference The temperature variation was made with a Varian temperature variable accessory unit (V-6040) calibrated by ethylene glycol. The C-13 NMR spectra were measured on a Nippon Electric Varian NV-21 NMR spectrometer. The spectra were obtained on D_2O solutions of histidine (0.4 M) in the absence and the presence of 1/4000-fold copper(II) ion at each a value. Dioxane was used as an internal reference.

Results and Discussion

Visible Absorption Spectra. The spectral characteristics at each a value of the 200:1 ligand–copper(II) system are shown in Table 1 together with those of 2: 1 complexes at $a=2$.

TABLE 1. VISIBLE ABSORPTION CHARACTERISTICS OF 200:1 AND 2:1 LIGAND–COPPER(II) SYSTEMS

	a value[a]	λ_{max}(nm)	ε_{max}(cm^{-1} M^{-1})
Histidine–Cu(II) 200: 1	0 (3.66)	623	60
	1 (7.81)	640	86
	2 (11.70)	635	94
Histamine–Cu(II) 200: 1	0 (3.03)	800	20
	1 (8.09)	610	96
	2 (11.68)	610	105
Histidine–Cu(II) 2: 1	2 (7.60)	640	86
Histidine–Cu(II)–1-methylhistidine 1: 1: 1	2 (7.52)	615	60
1-Methylhistidine–Cu(II) 2: 1	2 (7.50)	628	57
Histamine–Cu(II) 2: 1	2 (7.85)	600	85

a) The value of a represents moles of KOH added per diprotonated ligand. The values in parentheses are pH values.

In the 200: 1 histidine–copper(II) system at $a=0$, an absorption maximum was observed at 623 nm, which is an intermediate between that in 1: 1: 1 histidinate–Cu(II)–1-methylhistidinate complex and that in 2: 1 1-methylhistidinate–Cu(II) complex. This finding sug-

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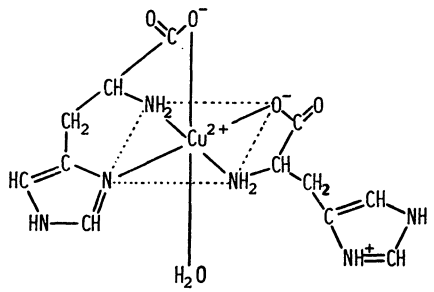
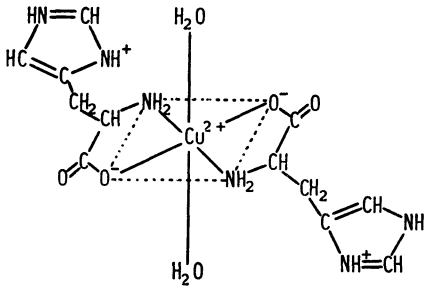
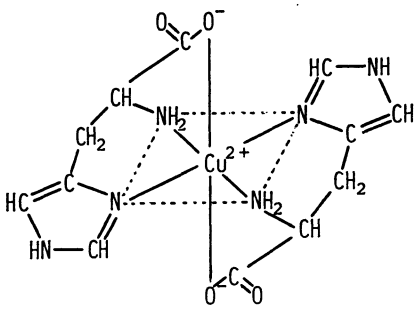


I, ML_2H^+

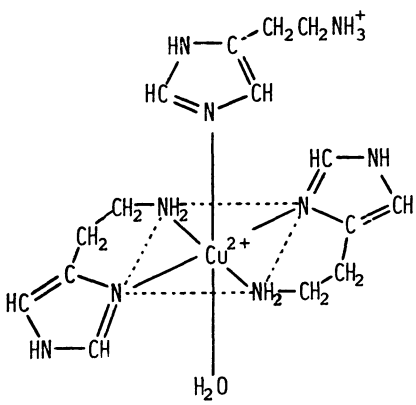


II, $ML_2H_2^{2+}$

gests the presence of the complex ML_2H^+(I), where one histidine is bound histamine-like with a coordinated apical carboxylate and the other glycine-like with a protonated imidazolium group and the complex $ML_2H_2^{2+}$(II), where both histidine molecules are bound glycine-like. Such complexes are known to be present in a weak acid pH region in the stoichiometric histidine–copper(II) systems, too.[2-4] On the other hand, the very weak ligand field (λ_{max}=800 nm) observed at a=0 in the 200: 1 histamine–copper(II) system means little complex formation between histamine and copper-(II) ion. This remarkable difference in the degree of the complexation at a=0 between two systems suggests that the carboxylate group plays an important role in the complexation in the histidine–copper(II) system.



III, ML_2



IV, ML_2LH^{3+}

The spectral characteristic, λ_{max}=640 nm (ε=86), at a=1 in the 200: 1 histidine–copper(II) system agrees completely with that of the 2: 1 histidinate–copper(II) complex ML_2. This means the formation of ML_2 as an exclusively predominant species in the 200: 1 histidine–copper(II) system. The structure of the 2: 1 complex ML_2, which is shown by III, has been discussed in detail.[5] The spectrum at a=1 in the 200: 1 histamine–copper(II) system exhibits an absorption maximum at 610 nm (ε=96), which is longer than that of 2: 1 normal histamine–copper(II) complex. This result suggests the coordination of the third histamine molecule in the apical position as shown by IV (ML_2LH^{3+}).

A little blue shift of the absorption maximum at a=2 in 200: 1 histidine–copper(II) system shows the partial deprotonation from the pyrrole nitrogen of the coordinated histidine molecules.[5] In comparison of the absorption spectrum of the 200: 1 histamine–copper(II) system at a=2 with that at a=1 the λ_{max} was the same whereas the absorptivity was higher. This observation may be interpreted by the strengthening of the apical coordination due to the deprotonation of the ammonium group and the partial deprotonation from the pyrrole nitrogen.

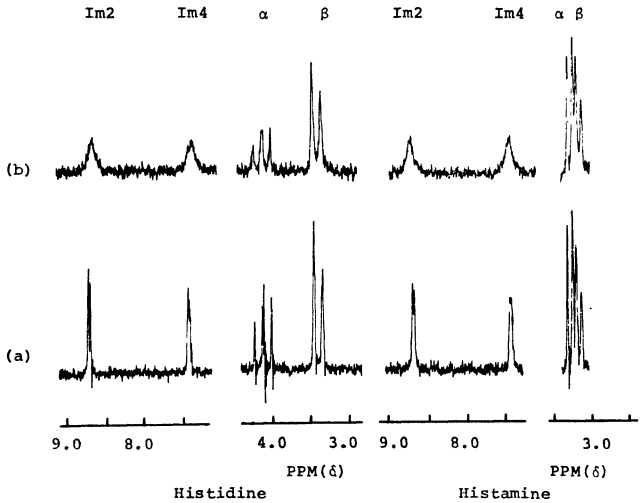


Fig. 1. PMR spectra of histidine and histamine at a=0 in the absence and the presence of 1/500-fold copper-(II) ion: concentration of ligand=0.25 M;
(a) ligand alone, (b) ligand+1/500 $CuCl_2$.

Proton Magnetic Resonance Spectra. The proton magnetic resonance spectra of histidine and histamine at a=0 in the absence and the presence of 1/500-fold copper(II) ion are shown in Fig. 1. In the 2000: 1 ligand–copper(II) systems, no evident broadening of the signals is observed. The signal of α-methine proton is broadened together with the signals of imidazole-2 and -4 protons in the 500: 1 histidine–copper(II) system, suggesting the involvement of the amino and the imidazole groups in the complex formation. This observation corresponds fully to the mode of complexation estimated by the visible spectral properties. The signals of the imidazole protons are broadened in the 500: 1 histamine–copper(II) system in the same manner

as in the 500: 1 histidine–copper(II) system. It is noteworthy that the broadening of imidazole signals is observed in the same degree in both systems in spite of the remarkable difference in the degrees of complex formation. This observation may be based on the different exchange rates in both systems.

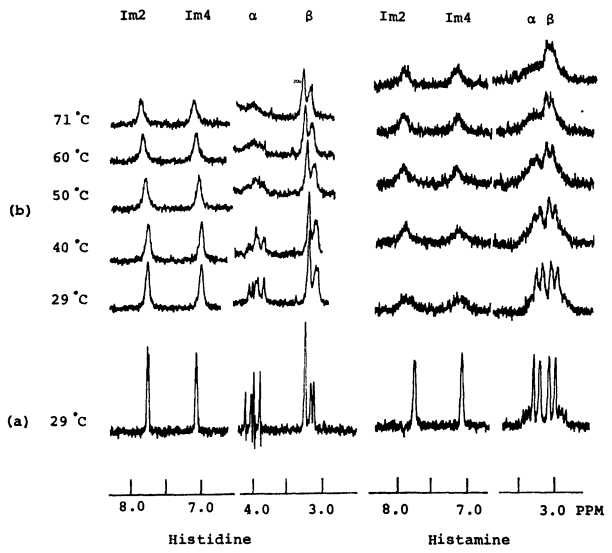


Fig. 2. PMR spectra of histidine and histamine at $a=1$ in the absence and the presence of 1/2000-fold copper (II) ion: concentration of ligand=0.25 M;
(a) ligand alone, (b) ligand+1/2000 $CuCl_2$.

Figure 2 shows the PMR spectra with temperature variation at $a=1$ in the histidine–copper(II) and the histamine–copper(II) systems. The broadening of the PMR signals is evident in both systems, giving remarkably different degrees of the broadening on the imidazole proton signals. This difference was observed on the solutions of histidine and histamine at pD 8.1 by Sigel and McCormick.[1] We measured the PMR spectra at constant a value, where the dissociation steps of both ligands are equal. In our experiment, a more remarkable difference in the broadening was observed. The previous workers concluded that such a difference is based on the different degrees of the participation of the imidazole group in complex formation, assuming that the exchange rates of the coordinated ligand are the same in both systems.[1] However, the validity of such assumption is difficult to justify. In the histidine–copper(II) system the line width of all the signals increases with increasing temperature. On the other hand, in the histamine–copper(II) system the line width of the imidazole proton signals decreases slightly with increasing temperature. This result suggests that the exchange rates of the coordinated ligands are different between two systems. In the presence of copper(II) ion, the line width at half height of signal $1/T_2$ is given by the following equation, if only one paramagnetic environment is present in solution.[6,7]

$$1/T_2 = 1/T_{2L} + P_M/P_L(1/(T_{2M}+\tau_M)) \quad (1)$$

Here, T_{2L} is the transverse relaxation time for the uncoordinated ligand, T_{2M} is that for the coordinated ligand, P_M/P_L is the ratio of the concentration of the coordinated ligand to that of the uncoordinated ligand, and τ_M is the life-time for chemical exchange of the coordinated ligand. Equation 1 is applicable to the histidine–copper(II) and the histamine–copper(II) systems to calculate the values of $1/(T_{2M}+\tau_M)$, because the presence of the main complex species, ML_2 for the histidine–copper(II) and ML_2LH^{3+} for the histamine–copper(II) systems are shown by the visible absorption spectra. We calculated the values of $1/(T_{2M}+\tau_M)$ using the line-broadening data on the imidazole-2 and -4 protons. In the histidine–copper(II) system, $1/(T_{2M}+\tau_M)$ is nearly equal to $1/\tau_M$, since the line width increases with increasing temperature and in this case τ_M is much larger than T_{2M}. The exchange rate $1/\tau_M$ is calculated to be about $3—5\times10^3\ s^{-1}$. On the other hand in the histamine–copper(II) system, $1/(T_{2M}+\tau_M)$ is calculated to be about $2—3\times10^4\ s^{-1}$. Since the line width of the imidazole proton signals in the histamine–copper(II) system decreases with increasing temperature, T_{2M} should be larger than τ_M.[6] Therefore, the exchange rate $1/\tau_M$ is much larger than $2—3\times10^4\ s^{-1}$. These results suggest that the difference in the line broadening in both systems is mainly due to different exchange rates. The relatively slower exchange rate histidine–copper(II) complex can be accounted for its coordination structure, that is, the copper(II) complex, where two histidine molecules are tridentate with apical carboxylates, is not easily accessible for an incoming ligand molecule.

In the PMR spectra at $a=2$, the signals are more broadened than those at $a=1$, especially in the histidine–copper(II) system, and the line width decreases slightly with increasing temperature. This may be explained on the basis of faster exchange rate caused by the deprotonation of the ammonium group.

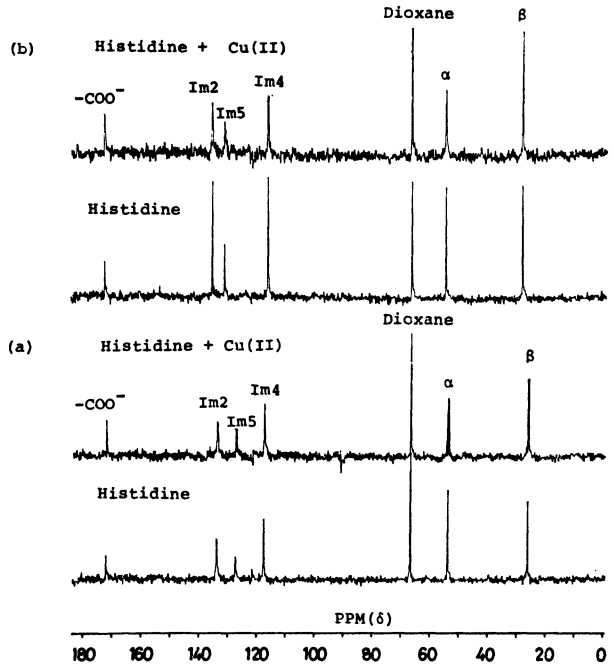


Fig. 3. C-13 NMR spectra of histidine in the absence and the presence of 1/4000-fold copper (II) ion: concentration of histidine=0.4 M;
(a) spectra at $a=0$, (b) spectra at $a=1$.

TABLE 2. THE RELATIVE SIGNAL HEIGHTS OF C-13 NMR SPECTRA OF HISTIDINE IN THE PRESENCE AND IN THE ABSENCE OF COPPER(II) ION

	$a=0$		$a=1$	
	Chemical shift (ppm)	Ratio	Chemical shift (ppm)	Ratio
Dioxane	67.40	1.00	67.40	1.00
β	26.69	1.00	28.90	1.00
α	54.44	0.66	55.54	0.53
Im4	118.51	0.89	117.62	0.44
Im5	128.24	1.16	132.67	0.56
Im2	134.75	0.85	136.87	0.40
$-COO^-$	173.02	1.53	174.35	0.95

C-13 Magnetic Resonance Spectra. Figure 3 shows C-13 NMR spectra of histidine in the absence and the presence of 1/4000-fold copper(II) ion at $a=0$ and 1 together with the assignments by Freedman *et al.*[8] The effect of copper(II) ion on the C-13 NMR signals is observed at each a value. To estimate the effect quantitatively, the relative signal heights in the presence and in the absence of copper(II) ion were compared. Table 2 shows the ratios of these heights, in which the smaller value means the larger effect of copper(II) ion, except for the carboxyl and the imidazole-5 carbons. The values for these signals are larger than 1.00 at $a=0$. This is probably due to their relatively long spin lattice relaxation times resulting from the lack of bound hydrogen atoms which can act as dipolar relaxers. By the addition of paramagnetic substance such as copper(II) ion, spin lattice relaxation time decreases and unsaturated signals may be recorded. At $a=0$ the value with the signal of α-methine carbon is smaller than those of the imidazole-2 and -4 carbons. On the other hand, the values with these three signals are roughly equal each other at $a=1$. These results give another support for the presence of ML_2H^+ and $ML_2H_2^{2+}$ at $a=0$ and ML_2 at $a=1$.

It the NMR line broadening experiments, histidine was suggested to interact with copper(II) ion in only a monodentate mode.[2] However, the present data show that histidine molecules bind to copper(II) ion in the tridentate or bidentate modes at extraordinarily large ratios of ligand to metal ion in a similar manner as at the stoichiometric conditions. The difference in the complexation between such two conditions has been found in the histamine–copper(II) system. The more remarkable difference has been observed in glycine-like ligands, which possess one amino and one carboxyl groups.[9]

NMR spectroscopic technique has been utilized to obtain structural information about copper(II) complexes. However, in NMR experiments attention should be called to the presence of large excess ligand. The mode of complexation at extraordinarily large ratios of ligand to metal ion may not be the same as those at stoichiometric ratios.[2] In addition, recently, Martin *et al.* have pointed out some criteria or disadvantage in the NMR line broadening method.[2,10] The use of visible spectral method together with NMR method will compensate such disadvantage and will give further detailed information about the mode of complexation of copper(II) ion in the presence of large excess ligand.

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