Copper(II) Complexes of L-Histidylglycine and L-Histidylglycylglycine in Aqueous Solution

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Equilibrium constants were determined in the complex formations of L-histidylgylcine (HG) and L-histidylglycylglycine (HGG) with copper(II) ion in aqueous solution by potentiometric titration, and the structures of the complexes were discussed based on the observations in the titration and infrared and electronic absorption spectra. In the acid region, a cationic chelate, in which copper(II) binds to nitrogen atoms of amino and imidazole groups, is formed. In the neutral region, deprotonation of the peptide amide group occurs to form a dimer, in which four coordination positions of copper(II) are occupied by nitrogen atoms of deprotonated amide and amino groups and an oxygen atom of carboxyl or carbonyl group in one molecule and a nitrogen atom of imidazole group of another molecule. In HGG–Cu(II) system, the dimer is decomposed in the alkaline region with further deprotonation of the second peptide amide group to form an anionic chelate as in the case of the glycylglycylglycine-Cu(II) complex.

The complex formation of some histidine-containing peptides has been studied by various physico-chemical methods,[1–12] based on the importance of the histidyl residue as a binding site with metal ions in protein. However, the experimental results, such as the data on the potentiometric titration, the spectral measurement and X-ray analysis, are not always sufficiently detailed to discuss the behavior of these peptides in the interaction with the metal ions in aqueous solution. The copper(II) complex of L-histidylglycine has been studied by the potentiometric titration[12] and the visible and CD spectra,[5] but the behavior and the structure of the complex in aqueous solution were not interpreted in detail with the experimental results. The complex formation of L-histidylglycylglycine has not been investigated so far.

In the preceding paper,[13] we reported the syntheses and the acid dissociation constants of some histidine-containing peptides, and also the formation constants of some 2: 1 (ligand: metal) metal complexes of L-histidylglycine and L-histidylglycylglycine (abbreviated as HG and HGG hereafter). Further, the dimer formation in the reaction of HG and HGG with copper(II) ion was briefly reported.[14] This paper deals with the detailed description of the study and the discussions on the complex formation of these peptides with copper(II) ion in aqueous solution.

Experimental

Materials. The preparations of HG and HGG were described in the preceding paper.[13] All other reagents were commercially obtained.

Potentiometric Titrations. The details of the method and the experimental conditions have been described previously.[13] Titrations were carried out in the solutions (10 ml) containing a ligand (10^{-3}M), HCl (2×10^{-3}M) and $CuCl_2$ (10^{-3}M) with 0.1 M KOH.

Infrared Absorption Spectra. The infrared absorption spectra were recorded on a Koken DS-301 infrared spectrophotometer equipped with sodium chloride prisms. Measurements were made on the D_2O solutions of a ligand with or without copper(II) ion, in a KRS-5 cell. The concentration of the ligand was approximately 0.3 M.

Visible Absorption Spectra. The visible absorption spectra were recorded on a Shimadzu Double 40-R spectrophotometer. Spectral measurements were made on the solutions of a ligand containing copper(II) ion with the concentration ranging from 4×10^{-3}M to 1×10^{-2}M.

Results and Discussion

Potentiometric Titrations. Titration curves for the solutions containing 1: 1 molar ratio of copper(II) ion to ligand in the presence of two equivalents of HCl are given in Figs. 1 and 2. Both curves show sharp pH inflections at $a=3$ and $a=4$, where a represents moles of KOH added per mole of the ligand. Another pH inflection is observed at $a=5$ in the HGG–Cu(II) system. The pH inflection at $a=3$ suggests the formation of the 1: 1 ligand-copper(II) complex in both systems. Therefore, the following reactions were assumed in the region where a value is between 1 and 3.

$$LH_3^{2+} \rightleftharpoons LH_2^{+} + H^{+}$$

$$K_{COOH} = [LH_2^{+}][H^{+}]/[LH_3^{2+}] \quad (1)$$

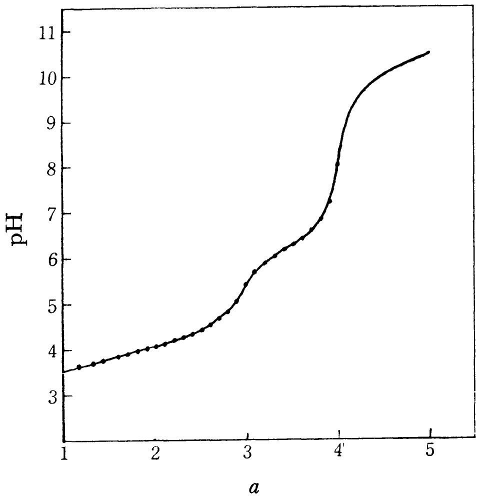


Fig. 1. Titration curve of HG in the presence of equimolar $CuCl_2$ and two equivalents of HCl.
At $a=0$, $[HG]_T=[Cu]_T=10^{-3}$M. The line is experimental curve. The points are the values calculated from equilibrium constants.

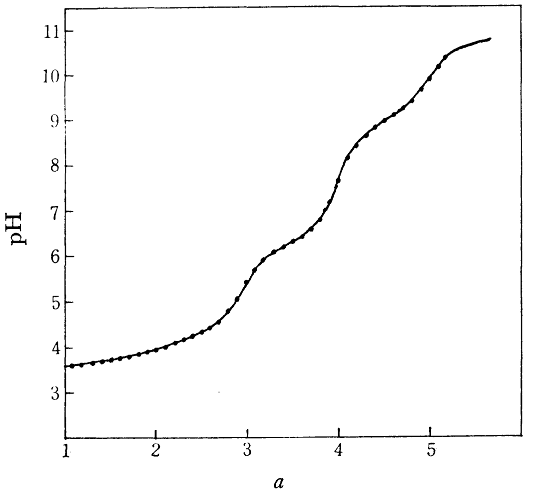


Fig. 2. Titration curve of HGG in the presence of equimolar $CuCl_2$ and two equivalents of HCl.
At $a=0$, $[HGG]_T=[Cu]_T=10^{-3}M$. The line is experimental curve. The points are the values calculated from equlibrium constants.

$$LH_2^+ \rightleftharpoons LH + H^+$$

$$K_{Im} = [LH][H^+]/[LH_2^+] \qquad (2)$$

$$LH \rightleftharpoons L^- + H^+$$

$$K_{NH_3} = [L^-][H^+]/[LH] \qquad (3)$$

$$Cu^{2+} + L^- \rightleftharpoons CuL^+$$

$$K_1 = [CuL^+]/[Cu^{2+}][L^-] \qquad (4)$$

Electroneutrality requires Eq. (5).

$$2[Cu^{2+}] + [CuL^+] + 2[LH_3^{2+}] + [LH_2^+] + [K^+] + [H^+] = [L^-] + [Cl^-] + [OH^-] \qquad (5)$$

The total concentrations of copper(II) and the ligand are expressed as Eqs. (6) and (7).

$$[Cu]_T = [Cu^{2+}] + [CuL^+] \qquad (6)$$

$$[L]_T = [CuL^+] + [LH_3^{2+}] + [LH_2^+] + [LH] + [L^-] \qquad (7)$$

Since copper(II) ion was added as chloride and two equivalents of HCl per ligand were added, the concentration of Cl^- is expressed as Eq. (8).

$$[Cl^-] = 2[Cu]_T + 2[L]_T \qquad (8)$$

Equation (9) is derived from these equations.

$$K_1 = \beta(\beta[Cu]_T-\alpha\gamma)/\alpha\gamma^2, \qquad (9)$$

where

$$\alpha = 1+[H^+]/K_{NH_3}+[H^+]^2/K_{Im}K_{NH_3}+[H^+]^3/K_{COOH}K_{Im}K_{NH_3},$$

$$\beta = [H^+]/K_{NH_3}+2[H^+]^2/K_{Im}K_{NH_3}+3[H^+]^3/K_{COOH}K_{Im}K_{NH_3},$$

$$\gamma = [Cu]_T+2[L]_T+[OH^-]-[H^+]-[K^+].$$

The formation constant, K_1, was calculated at each point of the experiments from Eq. (6), based on the data of the titration in the region where a value is between 1 and 3. The results are given in Table 1. Fairly good constant values of log K_1 were obtained with varying a values in each system. These values are in good agreement with those we obtained in the 2: 1 systems.[13] In CuL^+, the nitrogen atoms of α-amino and imidazole groups may be bound to the copper atom, as we mentioned previously.[13]

The deprotonation in the region where the a value is between 3 and 4 can not be interpreted by a simple ionization (Table 2). On the other hand, the spectral features of the complexes in the visible region suggested the formation of a polymer (Cu_nX_n) accompanying the

TABLE 1. log K_1 CALCULATED ACCORDING TO Eq. (9)

a value	His–Gly–Cu(II) system		His–Gly–Gly–Cu(II) system	
	pH	logK_1	pH	logK_1
1.2	3.67	9.06	3.65	8.64
1.3	3.72	9.04	3.70	8.59
1.4	3.76	9.05	3.74	8.58
1.5	3.81	9.03	3.77	8.60
1.6	3.86	9.01	3.81	8.60
1.7	3.90	9.03	3.85	8.60
1.8	3.95	9.02	3.90	8.58
1.9	4.01	9.00	3.94	8.59
2.0	4.06	9.01	3.98	8.62
2.1	4.11	9.02	4.04	8.59
2.2	4.18	9.00	4.10	8.59
2.3	4.25	9.00	4.16	8.59
2.4	4.32	9.01	4.22	8.62
2.5	4.41	9.01	4.40	8.61
2.6	4.50	9.04	4.52	8.63
2.7	4.63	9.05	4.70	8.65
2.8	4.81	9.06	5.02	8.65
Average	9.03±0.03		8.61±0.04	

TABLE 2. pK_c^n CALCULATED ACCORDING TO Eq. (10)

a	His–Gly–Cu(II) system						a	His–Gly–Gly–Cu(II) system					
	pH	pK_c^1	pK_c^2	pK_c^3	pK_c^4	pK_c^5		pH	pK_c^1	pK_c^2	pK_c^3	pK_c^4	pK_c^5
3.1	5.67	6.61	9.53	12.32	15.06	17.77	3.1	5.77	6.72	9.73	12.12	15.46	18.27
3.2	5.83	6.43	9.45	12.35	15.20	18.02	3.2	5.95	6.55	9.69	12.71	15.68	18.62
3.3	5.98	6.35	9.46	12.45	15.39	18.30	3.3	6.09	6.46	9.68	12.78	15.83	18.85
3.4	6.12	6.29	9.48	12.55	15.56	18.54	3.4	6.22	6.40	9.68	12.85	15.96	19.04
3.5	6.23	6.23	9.45	12.54	15.58	18.60	3.5	6.36	6.36	9.71	12.93	16.10	19.25
3.6	6.37	6.19	9.45	12.59	15.68	18.73	3.6	6.47	6.29	9.65	12.89	16.08	19.23
3.7	6.53	6.16	9.46	12.63	15.75	18.84	3.7	6.62	6.25	9.64	12.90	16.11	19.29
3.8	6.73	6.13	9.45	12.64	15.79	18.90	3.8	6.82	6.22	9.63	12.91	16.15	19.35
3.9	7.03	6.08	9.39	12.59	15.73	18.85	3.9	7.10	6.15	9.53	12.80	16.01	19.20

Average value of pK_c^2 between a=3.2 and a=3.8; His–Gly–Cu(II): 9.46±0.02, His–Gly–Gly–Cu(II): 9.67±0.04.

deprotonation, as shown later. Therefore, the equilibrium constants for the polymer formations were calculated based on Eq. (10), where $\mathrm{L}^- = \mathrm{XH}^-$.

$$n\mathrm{CuL}^+ \rightleftharpoons \mathrm{Cu}_n\mathrm{X}_n + n\mathrm{H}^+$$

$$K_c{}^n = [\mathrm{Cu}_n\mathrm{X}_n][\mathrm{H}^+]^n/[\mathrm{CuL}^+]^n \qquad (10)$$

Table 2 represents the results of the calculations for $\mathrm{p}K_c{}^n$, where n is from 1 to 5. The values of $\mathrm{p}K_c{}^1$ which corresponds to the simple ionization decrease with the increase of the a value, while the values of $\mathrm{p}K_c{}^n$ for the trimer, tetramer, and pentamer increase with the increase of the a value. On the other hand, values of $\mathrm{p}K_c{}^2$ which are applied to the formation of a dimer remain constant over the wide range. These facts support the presence of a dimer produced by the release of a proton from each CuL^+ complex.

As shown later by visible absorption spectra, further deprotonation in the region where the a value is between 4 and 5 in the HGG–Cu(II) system seems to be accompanied by the formation of an anionic chelate, CuY^-, as well as in the case of glycylglycylglycine.[16] For this deprotonation, the following reaction is assumed, where $\mathrm{YH}_2{}^- = \mathrm{XH}^- = \mathrm{L}^-$.

$$\mathrm{Cu}_2\mathrm{X}_2 \rightleftharpoons 2\mathrm{CuY}^- + 2\mathrm{H}^+$$

$$K_{c'}{}^2 = [\mathrm{CuY}^-]^2[\mathrm{H}^+]^2/[\mathrm{Cu}_2\mathrm{X}_2] \qquad (11)$$

The values of $\mathrm{p}K_{c'}{}^2$ calculated according to Eq. (11) are roughly constant as seen in Table 3. Slight scatter among the values in Table 3 suggests that the deprotonation overlaps to some extent with the preceding deprotonation.

TABLE 3. $\mathrm{p}K_{c'}{}^2$ CALCULATED ACCORDING TO Eq. (11)

a	pH	$\mathrm{p}K_{c'}{}^2$
4.1	7.85	20.37
4.2	8.29	20.60
4.3	8.55	20.72
4.4	8.75	20.80
4.5	8.01	20.86
4.6	9.05	20.89
4.7	9.18	20.90
4.8	9.31	20.90
4.9	9.47	20.89

Average value of $\mathrm{p}K_{c'}{}^2$ between $a=4.4$ and $a=4.9$: 20.87 ± 0.07.

In the reactions mentioned above, the proton is presumed to be liberated from the amide group of the peptide. However, the pH values of the region where the deprotonations occur are rather higher than those in the glycylglycine–Cu(II)[15] and glycylglycylglycine–Cu(II)[16] systems. The difficulty of the deprotonation in HG–Cu(II) and HGG–Cu(II) complexes may be due to the higher stability of the complexes, CuL^+ and $\mathrm{Cu}_2\mathrm{X}_2$, because the cleavage and recombination of the coordination bonds are necessary to cause these deprotonations. This structural conversion will be described later. The second deprotonation in the region where the a value is between 4 and 5 is also observed in the HG–Cu(II) system. Since this deprotonation occurs in the high pH region, equilibrium constants could not be determined accurately. The apparent ionization constant, which is the pH value at the half-neutralization point, is 10.00. The proton may be liberated from the imino group of the imidazole ring to give an anionic chelate, $\mathrm{Cu}_2\mathrm{Y}_2{}^{2-}$. It has been reported that the deprotonation of imino group occurs at the extremely high pH region in free imidazole and histidine[17] ($\mathrm{p}K_a \approx 14$). In the HG–Cu(II) complex, it is presumed that the electron-withdrawing effect of Cu(II) which binds to pyridine-type nitrogen of imidazole ring weakens the basicity of imino group.

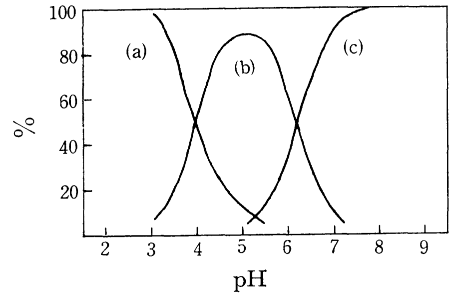


Fig. 3. Distribution of complex species for the 1 : 1 system of HG and copper(II):
(a) Cu^{2+}, (b) CuL^+, (c) $\mathrm{Cu}_2\mathrm{X}_2$.

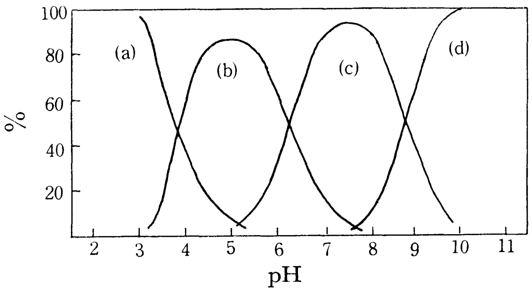


Fig. 4. Distribution of complex species for the 1 : 1 system of HGG and copper(II):
(a) Cu^{2+}, (b) CuL^+, (c) $\mathrm{Cu}_2\mathrm{X}_2$, (d) CuY^-.

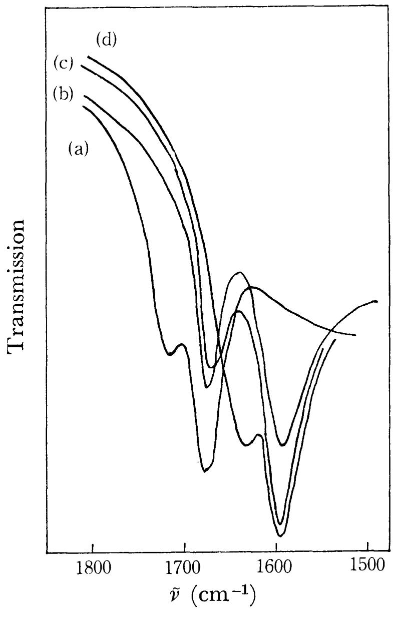


Fig. 5. Infrared spectra of HG in $\mathrm{D_2O}$ solutions. $[\mathrm{HG}]_\mathrm{T} = \sim 0.3$ M, (a) HG+2DCl, (b) HG+DCl, (c) HG, (d) HG+NaOD.

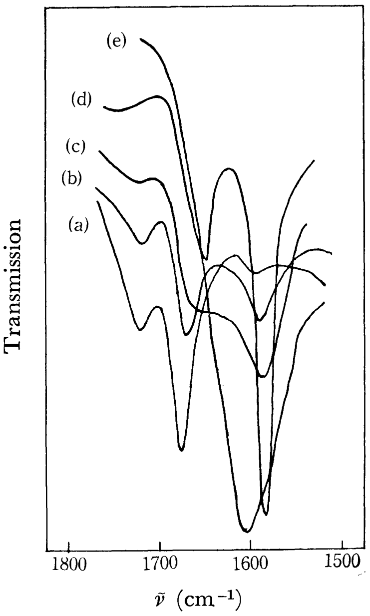


Fig. 6. Infrared spectra of HG–Cu(II) complexes in D_2O solution (1: 1): $[HG]_T = [Cu]_T = \sim 0.3$ M, (a) HG+Cu(II)+2DCl, (b) HG+Cu(II)+DCl, (c) HG+Cu(II), (d) HG+Cu(II)+NaOD, (e) HG+Cu(II)+2NaOD.

From the equilibrium constants obtained, the titration curves were re-calculated and they were compared with the experimental curves. Coincidence of both titration curves as seen in Figs. 1 and 2, supports the validity of these constants. Based on the constants, the distribution ratios of various complexes were calculated at various pH. The results shown in Figs. 3 and 4, indicate that main species in the region of pH 5 is a cationic chelate, CuL^+. The distribution ratio for the dimer, Cu_2X_2, increases with the increase of pH and reaches its maximum at the neutral region. In the alkaline region, anionic chelates, $Cu_2Y_2^{2-}$ or CuY^-, are predominant species.

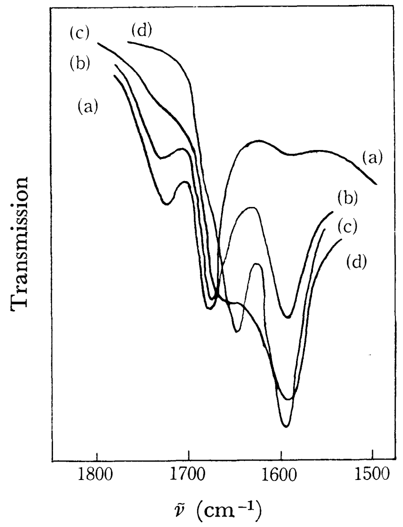


Fig. 7. Infrared spectra of HG–Cu(II) complexes in D_2O solution (2: 1): $[HG]_T = \sim 0.3$ M, $[Cu]_T = \sim 0.15$ M, (a) HG+1/2Cu(II)+2DCl, (b) HG+1/2Cu(II)++DCl, (c) HG+1/2Cu(II), (d) HG+1/2Cu(II)+NaOD.

Infrared Absorption Spectra. Infrared absorption spectra of D_2O solutions of HG in the absence and the presence of copper(II) ion were measured in the carbonyl absorption region to provide the information for the copper(II) binding sites. Figure 5 shows the infrared absorption spectra of HG. Figures 6 and 7 show the spectra of the 1: 1 and 2: 1 HG–Cu(II) complexes. The carbonyl and carboxylate absorption bands are observed in both spectra. The assignments of these bands were made by reference to those in the infrared absorption spectra of glycine peptides[18)] and their Cu(II) complexes.[15,16)] The results are given in

TABLE 4. CARBONYL AND CARBOXYLATE BANDS IN INFRARED ABSORPTION SPECTRA OF HG AND ITS Cu(II) COMPLEXES IN D_2O SOLUTION

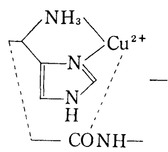
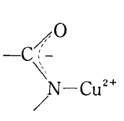
System	*a* value	Absorption band cm⁻¹ -COOH	-CONH-			-COO⁻
HG	0	1720	1680			
	1		1680			1593
	2		1675			1595
	3		1632			1595
HG+Cu(II)	0	1722	1677			1595 (w)
	1	1722	1677	1647 (w)		1595
	2		1670	1647 (sh)		1595
	3			1647		1595
	4				1608	1590 (sh)
HG+½Cu(II)	0	1725	1680			1595 (w)
	1	1725	1680	1647 (w)		1595
	2		1675	1645 (sh)		1595
	3			1645		1595

Table 4. For the convenience of the interpretation of the spectra, a values which are equivalent to those in the titrations are used.

In Fig. 5 two bands assigned to be due to undissociated carboxyl and peptide carbonyl groups respectively are observed at 1720 and 1680 cm^{-1}, in the spectrum before the addition of alkali. In the region where the a is larger than 1, the band of carboxylate appears at 1595 cm^{-1}. The bands at 1675 and 1632 cm^{-1} observed at $a=2$ and $a=3$ respectively, are assigned to the peptide carbonyl group. These shifts of carbonyl band to the lower frequency will be attributed to the deprotonation of imidazole and α-amino groups. The inductive effect of the α-amino group is much stronger than that of imidazole group. The spectral features of HG described above are similar to those of glycylglycine.[18)]

In the 1 : 1 HG–Cu(II) system, a new band appears at 1647 cm^{-1} in the region where CuL$^+$ is predominantly present. This band will be assigned to the peptide carbonyl group in CuL$^+$. The appearance of the band of carbonyl group at the higher frequency in the complex CuL$^+$ than in the ligand is worth noticing. In the glycylglycine–Cu(II) system,[15)] the band of carbonyl group moves to the lower frequency (1632 cm^{-1}→ 1625 cm^{-1}). The shift of the carbonyl band may be controlled by two contrastive effects, namely the inductive effect of Cu(II) and the reduction of the bond order of the carbonyl group based on bonding between Cu(II) and carbonyl oxygen. The shift to the higher frequency observed in the HG–Cu(II) system suggests that the latter effect is very weak. This finding is in accord with the conclusion that the peptide carbonyl group does not bind to Cu(II) in the plane. In the 2 : 1 HG–Cu(II) system, the same band appears at 1645 cm^{-1} in the region where the a value is between 1 and 3 and this band may be attributed to the complexes CuL$^+$ and CuL$_2$.

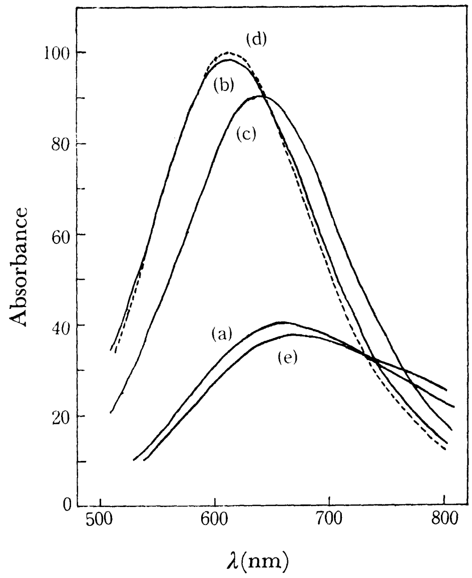


Fig. 8. Electronic spectra of HG–Cu(II) complexes in aqueous solution (1 : 1):
(a) HG+Cu(II)+KOH, (b) HG+Cu(II)+2KOH, (c) GG+Cu(II)+2KOH, (d) GG+Cu(II)+Im+2KOH, (e) His+Cu(II)+KOH.
Absorbance is expressed on a molar copper basis.
GG=glycylglycine, His=L-histidine, Im=imidazole.

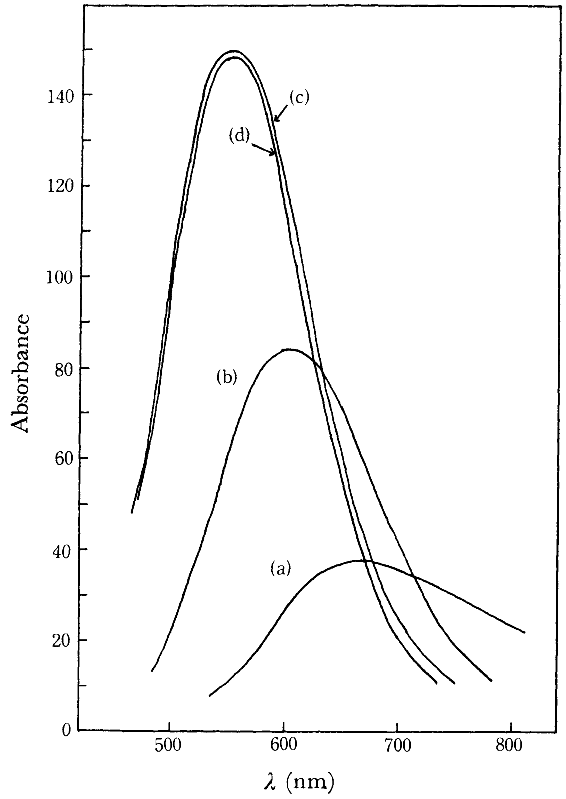
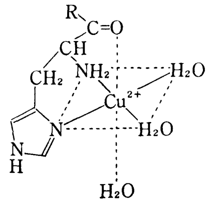


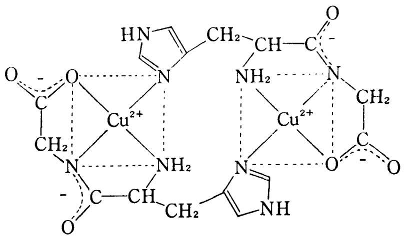
Fig. 9. Electronic spectra of HGG–Cu(II) complexes in aqueous solution (1 : 1):
(a) HGG+Cu(II)+KOH, (b) HGG+Cu(II)+2KOH, (c) HGG+Cu(II)+3KOH, (d) GGG+Cu(II)+3KOH. GGG=glycylglycylglycine.

At $a=4$ in the 1 : 1 system, where the dimer, Cu$_2$X$_2$, is the predominant species, a new band appears at 1608 cm^{-1} with a shoulder at ~1570 cm^{-1}. The band at 1608 cm^{-1} is assigned to the carbonyl group of the peptide which involves deprotonated peptide nitrogen bound to Cu(II).[15)] Accordingly, it is proved that the neutral dimer Cu$_2$X$_2$ has metal-peptide nitrogen bonds.

Visible Absorption Spectra. The visible absorption spectra of Cu(II) complexes in solution were studied to provide further evidence for the structures of the species formed in solution. Figures 8 and 9 show the spectra obtained on the solutions of 1 : 1 ligand–Cu(II) systems. For the convenience of the interpretation of the spectra a values which are equivalent to those in the titrations are used. The absorption spectra of the HG–Cu(II) and HGG–Cu(II) systems at $a=3$ are similar to the absorption spectrum of histidine–Cu(II) system. This observation suggests that the coordination structures of these complexes are similar each other. Consequently in this region, HG and HGG seem to bind to copper(II) through the nitrogen atoms of amino and imidazole group, as also presumed from the results of the titrations and infrared spectra. The structure of CuL$^+$ is given by **I**.

In the HG–Cu(II) system a shift of the absorption maximum to the shorter wavelength with a remarkable

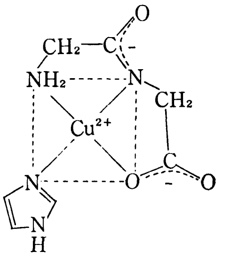




(I) (II)

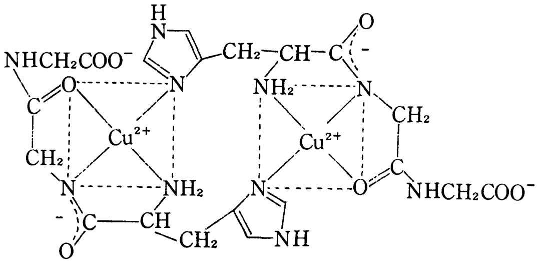
(I), CuL^+, $R=NHCH_2COO^-$: HG-Cu(II)
$R=NHCH_2CONHCH_2COO^-$: HGG-Cu(II)
(II), Cu_2X_2, HG-Cu(II)

increase of the absorbance was observed when the *a* value increases from 3 to 4. An absorption maximum appears at 613 nm (ε=99). This spectral change suggests the coordination of the copper(II) to the peptide nitrogen with a displacement of the proton and the formation of a neutral chelate, similar to glycylglycine–Cu(II) complex. However, it is noteworthy that the absorption maximum is observed at the shorter wavelength and the absorbance is larger than that in the case of glycylglycine–Cu(II) system (Fig. 8). These spectral features of HG–Cu(II) complex can be explained in terms of the following assumption. One imidazole group dissociates from the copper(II) with the deprotonation of the peptide amide group and then occupies the fourth coordination position of another copper atom to form a polymer. This structural change is reasonable from the molecular model, because the simultaneous coordination of one copper(II) ion to the nitrogen atoms of amino, imidazole, and deprotonated amide groups in one molecule of peptide are sterically unfavorable. The coordination of the imidazole group of the other molecule to the fourth position will form a somewhat stronger ligand field around copper(II) ion than in the case of the glycylglycine–Cu(II) complex. Consequently, the absorption maximum appears at the shorter wavelength than in glycylglycine–Cu(II) complex. The larger absorbance may be attributed to the steric strain caused by the coordination of the imidazole group. The structural change mentioned above leads to the formation of a polymer through imidazole group. The formation of a dimer is already indicated by the quantitative analysis on the results of the potentiometric titrations. The absorption spectrum of the 1 : 1 : 1 solution of glycylglycine, copper(II) ion and imidazole agrees with that of the HG–Cu(II) system. This agreement suggests the similarity of the coordination structures of both systems and supports the assumption mentioned above strongly. The structures for the HG–Cu(II) and glycylglycine–Cu(II)–imidazole complexes are given by **II** and **III**. It is interesting that the dimer structure is also proposed in β-alanyl-L-histidine–Cu(II) complex.[1]



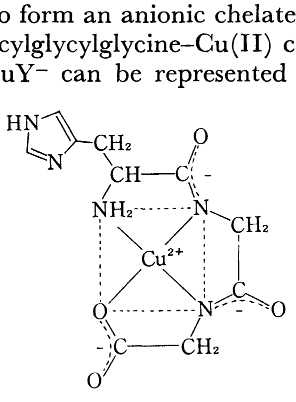
(III), GG-Cu(II)-Im

In the HGG–Cu(II) system, similar spectral features are observed at *a*=4 (Fig. 9). Therefore, similar dimer complex seems to form in this region, as shown by **IV**.



(IV), Cu_2X_2, HGG–Cu(II)

In this system, further shift of the absorption maximum to the shorter wavelength with a remarkable increase of the absorbance is observed where the *a* value is larger than 4. The spectrum at *a*=5 shows the absorption maximum at 555 nm (ε=150) and is similar to that of the 1 : 1 glycylglycylglycine–Cu(II) system under the same condition. This fact suggests that in this region the dimer is decomposed with the dissociation of the hydrogen of the second peptide amide group to form an anionic chelate, CuY^-, similar to the 1 : 1 glycylglycylglycine–Cu(II) complex.[16] The structure of CuY^- can be represented by **V**.



(V), CuY^-, HGG–Cu(II)

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