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FORMATION OF A DIMER IN THE REACTION OF L-HISTIDYLGLYCINE OR L-HISTIDYLGLYCYLGLYCINE WITH COPPER(II) IN AQUEOUS SOLUTION

Akira YOKOYAMA, Hiroji AIBA, and Hisashi TANAKA

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto

In complex formation of L-histidylglycine or L-histidylglycylglycine with copper(II), formation of dimer through deprotonation of peptide amide group was indicated in neutral region, by electronic spectra and pH titration. Possible structure is proposed, in which imidazole-nitrogen coordinates to one copper(II) and other coordination sites bind to another copper(II).

The complex formations of some peptides such as those containing glycine and alanine with copper(II) ion have been actively studied, whereas those on the peptides containing histidine have not been studied extensively, despite of their biological significance. This communication deals with the characteristic reactions of L-histidylglycine (HG) and L-histidylglycylglycine (HGG) with copper(II) ion in aqueous solution.

Experimental : HG and HGG were prepared by azide method.[1] Other reagents were obtained commercially. The electronic spectra were recorded on a Shimadzu Spectrophotometer Double 40-R. Spectral measurements were made on solutions ranging in copper(II) ion concentration from 4×10^{-3} M to 1×10^{-2} M at room temperature. Potentiometric titrations were carried out with a Radiometer Titrator TTT 1 and Titrigraph at 25° C. The concentrations of ligands were 10^{-3} M.

Results and Discussion

As seen in Fig. 1, the electronic spectrum at "a" = 1 is similar to that of the solution of histidine and copper(II) ion and this fact suggests that HG coordinates to copper through the amino-nitrogen and the imidazole-nitrogen in this region. The addition of one equivalent base displayed some shift of the absorption maximum to shorter wavelength with a remarkable increase of the absorbance. The formation of a neutral chelate which is formed by the release of a proton from amide group as in the case of glycylglycine[2] is suggested, but it may be noteworthy that the absorption maximum at shorter wavelength and a little larger absorbance was observed than in the case of glycylglycine-copper(II) complex. These spectral features of HG complex may be explained by the following assumptions. Imidazole-nitrogen of histidyl group dissociates from copper with the deprotonation of the amide group of the peptide bond and then coordinates with the fourth coordination position of another copper to form a polymer. This structural change is considered to be reasonable with the release of a proton from the amide group, because the simultaneous coordination of one copper atom with amino-nitrogen, imidazole-nitrogen of histi-

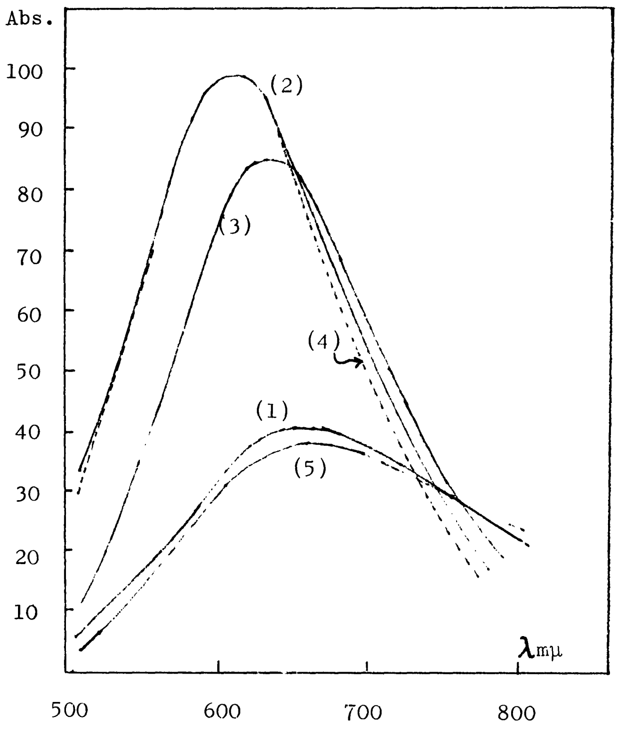

Fig. 1 Absorption Spectra

	λmax
(1) HG:Cu(II):KOH=1:1:1	660
(2) HG:Cu(II):KOH=1:1:2	614
(3) GG:Cu(II):KOH=1:1:2	638
(4) GG:Cu(II):Im:KOH=1:1:1:2	613
(5) His:Cu(II);KOH=1:1:1	665

Absorbance is expressed on a molar copper basis.
GG=glycylglycine, Im=imidazole, His=L-histidine

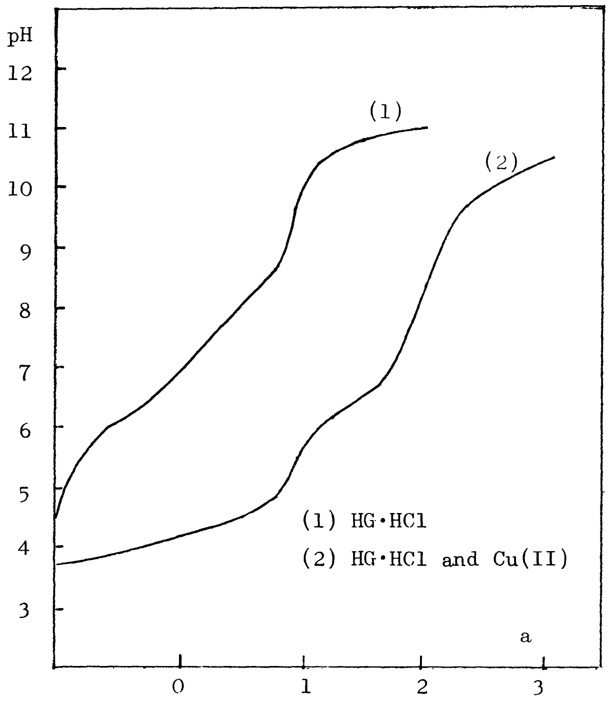

Fig. 2 Titration Curve of the Solution Containing HG and Copper(II) with Molar Ratio of 1 : 1

dyl group and deprotonated amide-nitrogen becomes sterically unfavorable. The ligand field of copper may be strengthened by this change in coordination of copper. The larger absorbance may be explained as a reflection of the steric strain caused by the coordination of imidazole group. Based on the spectral properties mentioned above the formation of a polymer may be reasonably assumed. The absorption spectrum of the 1 : 1 : 1 solution of glycylglycine, copper(II) ion and imidazole agreed with that of the 1 : 1 solution of HG and copper(II) ion. This spectral similarity supports the assumption mentioned above strongly. As shown in Fig. 2, a deprotonation in the region where "a" value lies between 1 and 2 is proved from the potentiometric titration. The equilibrium constants were calculated according to the equation 1, the formation of a polymer being assumed.

$$nCuL^+ \rightleftharpoons Cu_nL_n + nH^+ \qquad K^n = \frac{[Cu_nL_n]\,[H^+]^n}{[CuL^+]^n} \quad ----- (1)$$

The results presented in Table I indicate the formation of a dimer, because the values of pK^2 which are calculated on the basis of the formation of a dimer are closest each other among the values based on the formations of monomer and various polymers. The observations mentioned above support that the formation of a dimer produced by the release of a proton from amide group. Fig.3 shows a probable structure of the dimer, in which four coordination positions of copper(II) are occupied with amino-nitrogen, deprotonated amide-nitrogen and carboxyl-oxygen of one ligand and with imidazole-nitrogen of another ligand. In the case of the complex formation of HGG with copper with the molar ratio of 1 to 1, the formation of

Table I Equilibrium Constants in HG-Copper(II) System

a value	pK^1	pK^2	pK^3	pK^4	pK^5
1.1	6.61	9.53	12.32	15.06	17.77
1.2	6.43	9.45	12.35	15.20	18.02
1.3	6.35	9.46	12.45	15.39	18.30
1.4	6.29	9.48	12.55	15.56	18.54
1.5	6.23	9.45	12.54	15.58	18.60
1.6	6.19	9.45	12.59	15.68	18.73
1.7	6.16	9.46	12.63	15.75	18.84
1.8	6.13	9.45	12.64	15.79	18.90
1.9	6.08	9.39	12.59	15.73	18.85

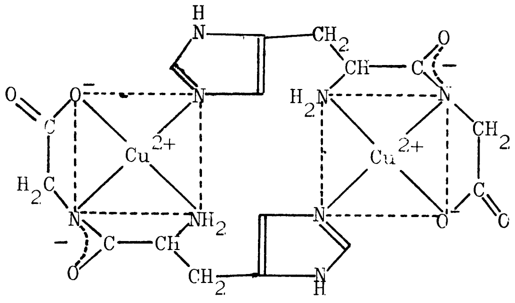

Fig. 3 Probable Dimer Structure of HG-Copper(II) Complex

a dimer is also revealed from the same discussions as in the case of HG. However, in this case, the dimer is decomposed with the dissociation of the hydrogen of another peptide group to form an anionic chelate as in glycylglycylglycine, in the region where "a" value is larger than 2. The similar dimer structure was proposed for copper(II) complex of L-carnosine (β-alanyl-L-histidine) by the x-ray diffraction study.3) We have also studied on the complex formation of glycyl-L-histidine with copper(II) ion and observed the formation of a polymer. Recently, Morris et al. proposed the formation of a tetramer in glycyl-L-histidine-copper(II) system based on the result of the titration.4) The formation of the polymer in the complex formation with copper(II) ion may be regarded as a significant feature of the histidine-containing peptides. The full details of the present study will be published later along with the results of more extensive studies on the behaviors of the imidazole group in the complex formation.

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