Equilibrium and Structure of Deprotonated Bis(glycylglycinato)-copper(II) in Aqueous Solution

Noriyuki NAKASUKA,* Kenya MAKIMURA, and Hiroko KAJIURA†

Laboratory of Analytical Chemistry, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464

(Received February 27, 1981)

Potentiometric titration was carried out at 25.0±0.1 °C and at an ionic strength of 1.00 mol dm^{-3} in sodium perchlorate for a system of copper(II)–dipeptide(A)–a second ligand(B) in an aqueous solution to obtain the formation constants of mixed ligand complexes $Cu_pH_qA_rB_s$. A was glycylglycine or glycinamide, and B was ethylamine or 2-aminoethanol. The structure of $CuH_{-1}A_2$ in solution is discussed. It was found that the ligand A acts as a unidentate ligand and the other deprotonated $H_{-1}A$ as a terdentate one for the most part; only about 2% of this complex are in a bidentate-bidentate form.

Glycylglycine, one of the simplest peptides, gives rise to several metal complexes in an aqueous solution, and serves as a model compound for the complexation reactions of polypeptides and even of proteins. Thermodynamic equilibria of copper(II)–glycylglycine complexes have been studied rather extensively,[1-4] and the existence of species such as CuA, CuHA, $CuH_{-1}A$, $CuH_{-2}A$, and $CuH_{-1}A_2$ has been reported (HA represents a glycylglycine molecule). Strandberg *et al.* have elucidated by crystallographic studies the structure of the diaquaglycylglycinatocopper(II) hydrate ($CuH_{-1}A$), in which the peptide NH proton is released; they found that the ligand acts as a terdentate one.[5] For $CuH_{-1}A_2$, however, two possible structures are expected, so it is of interest to discuss the equilibrium data.

Experimental

Reagents. Glycylglycine and glycinamide hydrochloride were used as received (Tokyo Kasei Kogyo Co., G. R.).

Ethylamine hydrochloride and 2-aminoethanol were obtained from Wako Pure Chemical Ind. Ltd. The former was recrystallized from water and dried *in vacuo* at 60 °C, while the latter was vacuum distilled over sodium hydroxide.

Copper(II) perchlorate: Copper(II) carbonate was prepared by mixing copper(II) chloride (Wako Pure Chemical Ind. Ltd., G. R.) with a solution of sodium carbonate. Perchloric acid was added in small excess to the washed copper carbonate and copper perchlorate was then crystallized from the solution.

Sodium perchlorate: Commercial sodium perchlorate (Merck, *pro analysi*) was recrystallized from water. A concentrated solution was analyzed gravimetrically after heating the solution gently in a platinum crucible on a hot plate to dryness. This simple method gives very reproducible and accurate results.

Potentiometry. Measurement of potential was made with a digital pH/mV meter(Orion, model 801) using a glass electrode (Metrohm EA 109) and a reference calomel electrode (Metrohm EA 404), which contained an internal solution of sodium chloride. Each measurement was accurate to 0.2 mV. Titrations were carried out in double-walled glass cell thermostated at 25±0.1 °C under nitrogen presaturated with water vapor. A Metrohm piston burette was used for addition of 1.140 M†† sodium hydroxide solution. The ionic strength of each solution was adjusted to 1.00 M in sodium perchlorate.

† Present address: National Institute for Basic Biology, National Center for Biological Sciences, Myodaiji, Okazaki 444.

†† Throughout this paper: 1 M=1 mol dm^{-3}.

Results

Determination of Stability Constants. Complex formation of copper(II) ion (M) with a complexing agent (A) and a second ligand (B) is represented in general by the following expression:

$$pM + qH + rA + sB \rightleftharpoons M_pH_qA_rB_s, \quad (1)$$

where charges are omitted for simplicity. The formation constant β_{pqrs} is defined as follows:

$$\beta_{pqrs} = [M_pH_qA_rB_s]/m^ph^qa^rb^s, \quad (2)$$

where m, h, a, and b refer to the concentrations of the free metal ion, proton, and free A and B ligands, respectively. When B is absent from the system, only β_{par} is defined.

Table 1. Experimental conditions for the measurement of formation constants

1) Protonation constants		
No.	$10^2\ C_{ligand}$/M	Ligand
I	1.088	Glycylglycine
II	1.694	Glycinamide
III-1, III-2	1.113, 2.197	Ethylamine
IV	1.170	2-Aminoethanol

2) Glycinamide complexes		
No.	$10^2\ C_A$/M	$10\ C_M$/M
V-1	1.778	0.7054
V-2	1.449	1.033

3) Glycylglycine complexes			
No.	$10^3\ C_A$/M[a]	$10^3\ C_M$/M	$10^2\ C_B$/M
VI-1	3.727	3.299	
VI-2	8.565	4.124	
VI-3	8.698	4.124	
VI-4	12.69	4.124	
VI-5	5.159	4.124	
VI-6	8.707	8.248	
VI-7	8.704	2.062	
VII-1[b]	4.127	3.299	1.024
VII-2[b]	4.977	4.124	2.100
VII-3[b]	4.348	4.124	4.184
VIII[c]	4.284	4.124	1.247

a) A: Glycylglycine. b) B: Ethylamine. c) B: 2-Aminoethanol.

TABLE 2. FORMATION CONSTANTS β_{pqrs} ($c^{\ominus}=1$ mol dm^{-3}, 25.0±0.1 °C, $\mu=1$ ($NaClO_4$)).

1) Protonation constants

Ligand	*pqr*	log β_{pqrs}
Glycylglycine	011	8.21±0.05
	021	11.46±0.08
Glycinamide	011	8.208±0.003
Ethylamine	011	11.04±0.10
2-Aminoethanol	011	9.77±0.05

2) Glycinamide complexes

pqr	log β_{pqrs}
101	5.53±0.03
102	10.00±0.05
1$\bar{1}$2	2.72±0.05
1$\bar{2}$2	−5.75±0.08

3) Glycylglycine complexes

pqrs	log β_{pqrs}	B
101	5.54±0.05	
1$\bar{1}$1	1.31±0.05	
1$\bar{2}$1	−7.99±0.10	
1$\bar{1}$2	4.50±0.05	
1$\bar{1}$11	4.71±0.08	2-Aminoethanol
1$\bar{1}$11	4.9±0.2	Ethylamine

Lefebvre's method (Méthode de la surface potentiométrique)[6] provided compositions of species present as well as approximate values of the corresponding formation constants. These values were further refined by using a least-squares technique with the data $\bar{q}$ (average number of protons bound per mole of constituent A and B)[4] on an electronic computer FACOM 230-75 at the Computation Center of Nagoya University. A Fortran program QPOL (R.-P. Martin, Universite Claude Bernard, Lyon I) was used for this calculation after certain modifications by the present authors. Experimental conditions and the results of calculation are summarized in Tables 1 and 2.

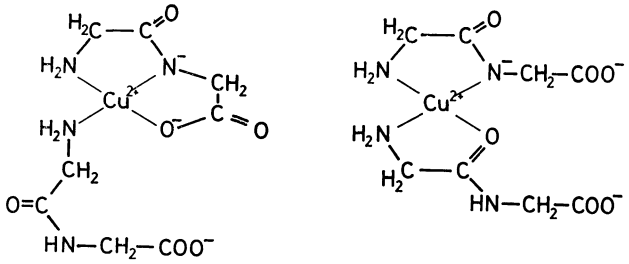
Table 2 shows a remarkable similarity between the protonation constants as well as between the complex formations of CuA for glycylglycine and glycinamide. These similarities indicate that the terminal nitrogen atoms in these ligands have the same basicity.

The values in this work for glycinamide are somewhat larger than those obtained by Dorigatti and Billo;[7] this may be accounted for in terms of different ionic media (0.10 M in $NaClO_4$). The results obtained for glycylglycine are essentially the same as those obtained by Martin *et al.*[4]

Discussion

There are two possible structures for the complex $CuH_{-1}A_2$, as shown in Fig. 1. In the first case, one peptide coordinates to the central ion as a terdentate ligand, while the other works as a unidentate: this is termed type (3,1). This type of crystal structure has already been found for a copper(II)–glycylglycine–imidazole complex.[8]

In the second complex, each ligand coordinates as a bidentate one, although the donor atoms are different.



type(3,1) type(2,2)

Fig. 1. Possible structures for bis(glycylglycinato) copper (II) $CuH_{-1}A_2$.

Therefore this is referred to as type (2,2). The principal point of interest is which predominates in an aqueous solution.

For the first type of complex, a unidentate glycylglycine A may be designated as N, because the donor atom must be the amino nitrogen. Then formation of a complex of type (3,1), $Cu_{-1}AN$, is formulated as follows:

$$CuH_{-1}A + N \overset{K^{N}_{CuH_{-1}AN}}{\rightleftharpoons} CuH_{-1}AN. \quad (3)$$

The formation constant of mixed ligand complexes has been discussed by many authors. Here the formulation of Tanaka[9] is applied, since the interactions between donor atoms are very important in this case.

$$\log K^{N}_{CuH_{-1}AN} = \log K^{N}_{CuN} + \Delta \log K_{os} + \Sigma\delta_{ij}x_i(H_{-1}A)x_j(N), \quad (4)$$

$$\Delta \log K_{os} = \log K_{os}(CuH_{-1}A, N) - \log K_{os}(Cu, N), \quad (5)$$

where K^{N}_{CuN} is the formation constant of CuN, δ_{ij} the effect of the donor atom i in the ligand $H_{-1}A$ on the donor atom j in the ligand N (mainly a repulsion term, $\delta_{NO}=-0.26$ and $\delta_{NN}=-0.35$, and x_i or x_j is the number of the donor atoms i or j in each ligand. K_{os} denotes the formation constant of an outer sphere complex, but with amino acids and their related ligands log K_{os} may be assumed to be negligible.[9]

The data for K^{N}_{CuN} is not available and therefore the formation constant of copper(II)–ammine complex is substituted for it.[10]

$$\log K^{N}_{CuH_{-1}AN} = 4.197 + 0 - 0.97 = 3.24, \quad (6)$$

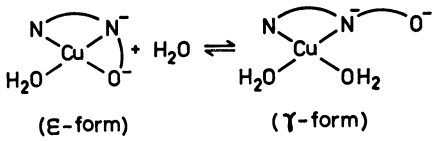
$$\beta_{1\bar{1}2}(3,1) = \frac{[CuH_{-1}A_2]}{[CuH_{-1}A][N]} \frac{[CuH_{-1}A]\,h}{m\,a} = K^{N}_{CuH_{-1}AN} \cdot \beta_{1\bar{1}1}, \quad (7)$$

$$\log \beta_{1\bar{1}2}(3,^{6}1) = 3.24 + 1.31 = 4.55. \quad (7')$$

The observed value 4.50 for log $\beta_{1\bar{1}2}$ is in good accord with the above value calculated. 2-Aminoethanol and ethylamine act only as a unidentate ligand; the formation constants of their mixed ligand complexes (log $\beta_{1\bar{1}11}$) correspond unambiguously to that of type (3,1). The experimental values of 4.71 (2-aminoethanol) and 4.9 (ethylamine) compare with the theoretical ones. Koltun *et al.* have reported the formation constant of the imidazole complex, from which log $\beta_{1\bar{1}11}$ is calculated to be 4.91 (ionic strength=0.075 M, 25 °C).[2] The terdentate nature in this complex is well established

in a solid state.[8] These findings suggest predominance of the complex of type (3,1) in solution. A similar calculation for type (2,2) is also interesting.

The structure of $CuH_{-1}A$ has been determined by the X-ray method.[5] The majority of the deprotonated ligands may play the role of terdentate ligands also in solution (ε form).



(8)

Nevertheless, there may exist a complex in which the peptide acts as bidentate ligand, though in a very small amount; this type of complex is named γ form. In fact Nakahara *et al.* have prepared dipotassium bis(glycylglycinato)cuprate(II),[11] whose structure has been determined.[12] The primary coordination around the central ion is approximately square, and two ligands bond to the copper ion through the amino and peptide nitrogen atoms alone, that is, in a γ form.

The thermodynamic constant for the equilibrium between γ and ε is not available, so we consider the complexation reactions of ethylenediamine monoacetic acid (EDMA, potentially terdentate) with copper(II).[13] The equilibrium between γ and ε forms in the EDMA complex is written as follows:

$$R = \frac{[\varepsilon]}{[\gamma]} = \frac{[\mathrm{Cu(edma)}]}{[\mathrm{Cu(edma)'}]} = \frac{[\mathrm{Cu(edma)}]}{m[\mathrm{edma}]} \frac{m[\mathrm{edma}]}{[\mathrm{Cu(edma)'}]}. \quad (9)$$

Cu(edma)′ represents a complex in which EDMA is attached to copper(II) ion as a bidentate ligand, with the ethylenediamine nitrogen atoms being donor ones. Thus the formation constant of this complex may be approximated by the Cu–ethylenediamine complex ($\beta_{\mathrm{Cu(en)}}$).[14]

$$R = \beta_{\mathrm{Cu(edma)}}/\beta_{\mathrm{Cu(en)}} = 10^{2.82} \quad (10)$$

This ratio R may be substituted for the equilibrium constant for Reaction 8 of the dipeptide complexes. The validity of this assumption is also confirmed by the following argument on the deprotonation of copper chelates:

$$\mathrm{CuA} \rightleftharpoons \mathrm{CuH_{-1}A'} + \mathrm{H}, \quad (11)$$

where $CuH_{-1}A'$ denotes the deprotonated copper complex of glycylglycine in γ form.

$$K_1 = \frac{[\mathrm{CuH_{-1}A'}]\,h}{[\mathrm{CuA}]} = \frac{[\varepsilon]\,h}{[\mathrm{CuA}]} \frac{[\gamma]}{[\varepsilon]} = \beta_{111}/(\beta_{101}R) = 10^{-7.05}. \quad (12)$$

For glycinamide, corresponding data are not available, but this equilibrium constant K_1, may be approximated by that of the deprotonation of CuA_2 giving rise to $CuH_{-1}A_2$, neglecting some effects resulting from the other ligand A.

$$K_2 = \frac{[\mathrm{CuH_{-1}A_2}]h}{[\mathrm{CuA_2}]} = \beta_{1\bar{1}2}/\beta_{102} = 10^{2.72}\,10^{-10.00} = 10^{-7.28} \fallingdotseq K_1. \quad (13)$$

The equality of K_1 to K_2 justifies experimentally the validity of the R value, because this ratio is used in calculating K_1, while K_2 is derived from the experimental data. Thus the formation constant of the glycylglycine complex of type (2,2) is rewritten as:

$$\beta_{1\bar{1}2}(2,2) = \frac{[\mathrm{CuH_{-1}A_2}]h}{m\,a} = \frac{[\mathrm{CuH_{-1}A'}]}{[\mathrm{CuH_{-1}A}]} \frac{[\mathrm{CuH_{-1}A}]h}{m\,a} \frac{[\mathrm{CuH_{-1}A_2}]}{[\mathrm{CuH_{-1}A'}]\,a} = R^{-1}\beta_{1\bar{1}1}K^{\mathrm{A}}_{\mathrm{CuH_{-1}A'A}}. \quad (14)$$

Application of the formulation from Tanaka[9] gives:

$$\log K^{\mathrm{A}}_{\mathrm{CuH_{-1}A'A}} = \log K^{\mathrm{A}}_{\mathrm{CuA}} + \log K_{\mathrm{os}} + \Sigma\delta_{ij}x_i(\mathrm{H_{-1}A})x_j(\mathrm{A}) = 5.54 + 0 - 1.22 = 4.32. \quad (15)$$

Then we have

$$\log \beta_{1\bar{1}2}(2,2) = -2.82 + 1.31 + 4.32 = 2.81. \quad (16)$$

This calculated value is about two orders of magnitude less than the observed one, while it is in good agreement with the experimental one for glycinamide ($\log \beta_{1\bar{1}2} = 2.72$). Since this amide complex can take only the geometry of type (2,2), as evident from Fig. 2, the agreement is a fair indication of the validity of such an approach.

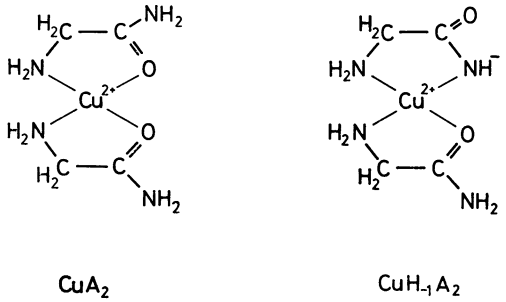


Fig. 2. Structures of bis(glycinamide) copper(II) complexes.

For the mixed ligand complex $CuH_{-1}AB$ which includes glycine as B, a similar calculation shows that $\log \beta_{1\bar{1}1}(3,1) = 4.55$ and that $\log \beta_{1\bar{1}1}(2,2) = 5.35$, while the observed value is 5.255.[4] Thus, glycine and the deprotonated dipeptide both chelate on the basal plane as bidentate ligands, in contrast to the situation in the $CuH_{-1}A_2$ complex. This may result from the much greater ability of glycine to chelate a metal ion compared with the dipeptide interacting with the non-deprotonated CONH group.

On the other hand, glycine is supposed to coordinate with an apical position in its mixed ligand copper(II) complexes with substituted iminodiacetic acids or diethylenetriamine, because its formation constants are considerably greater than those for its esters.[15,16]

For a dipeptide, however, coordination *via* carbonyl oxygen is generally very weak (type (3,1)), and its terminal carboxylate cannot interact with the apical position without considerable strain, according to the Dreiding Stereomodel (type (2,2)). Therefore the apical coordination will be unimportant in the case of

glycylglycinato copper(II) complexes, which probably account for the good agreement between the observed and calculated formation constants as shown above.

In brief, the glycylglycine complex $CuH_{-1}A_2$ of type (3,1) predominates in an aqueous solution, while about 2% of them are still present in the form of type (2,2), since $\beta(2,2)/\beta(3,1)=1.8\times10^{-2}$. This conclusion supports the proposal by Gergely and Nagypál,[17] apart from the question of the apical interaction.

The authors are greatly indebted to Prof. M. Tanaka, Nagoya University, for helpful discussions as well as for continuing encouragement in performing this investigation, and also to Mr. T. Noda, Nagoya University, for technical assistance in improvement of the glassware apparatus.

This research was financially supported in part by a Grant-in-Aid for Scientific Research No. 554184 from the Ministry of Education, Science and Culture.

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