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*p*H-metric Investigation of Cu(II) Complexes with Dipeptides

M. SIVASANKARAN NAIR, M. SANTAPPA* & P. NATARAJAN
Department of Physical Chemistry, University of Madras, A. C. College Campus, Madras 600 025

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The acidity constants and stability constants of Cu(II) complexes of glycylglycine, glycyl-L-alanine, L-alanylglycine, glycyl-L-leucine, L-leucylglycine and glycinamide have been determined *p*H-metrically in aqueous perchlorate medium at $I = 0.15$ ($NaClO_4$) and 37°. The stability constant data obtained are discussed in terms of the steric factors due to side chain alkyl substituents in the dipeptide ligands. The results suggest that the amide deprotonated dipeptides coordinate in a tridentate manner with Cu(II).

CONSIDERABLE attention has been paid in recent years on the binary and mixed-ligand complexes of metal ions and in particular Cu(II) ion with amino acids and their derivatives such as peptides[1,2]. In peptides and probably also in proteins, the deprotonated amide group is one of the important binding groups for Cu(II). The labilization of peptide protons and the formation of copper-N(peptide) instead of copper-O(peptide) bonds are readily occurring in the copper complexes of dipeptides, because Cu(II) (d^9-system) has a crystal field stabilization energy which can be significantly increased by the substitution of strong-field(nitrogen) for weak-field (oxygen) donors. In a broad programme to study some mixed dipeptide-imidazole complexes of Cu(II) under physiologically important conditions with view to understanding enzyme-metal ion-substrate complexes, we report in this paper the acidity constants and stability constants of Cu(II) complexes of six dipeptides, viz. glycylglycine, glycyl-L-alanine L-alanylglycine, glycyl-L-leucine, L-leucylglycine and glycinamide at $I=0.15$ ($NaClO_4$) and 37°. Glycinamide resembles a dipeptide without the carboxyl group.

Materials and Methods

All the ligands used in this work were obtained from Fluka. $Cu(ClO_4)_2$ and other reagents were prepared and estimated as described by Ramamoorthy and Santappa[3].

The pH titrations were carried out at 37° under purified, oxygen and CO_2 free nitrogen atmosphere using a digital *p*H meter (M/s Bhagyanagar Electronics, Hyderabad) fitted with glass and calomel electrode assembly and having an accuracy of ± 0.01 *p*H unit. The *p*H standards taken were 0.05*M* potassium hydrogen phthalate (*p*H 4.02) and 0.05*M* borax (*p*H 9.08) at 37°. The electrode system was calibrated by the method of Irving *et al.*[4]. A constant ionic strength of 0.15 was maintained by the addition of sodium perchlorate. Titrations were carried out on solutions (total volume 50 ml) containing low concentrations of $Cu(ClO_4)_2$ and the ligand against standard CO_2-free sodium hydroxide.

Calculations have been restricted to systems at *p*H <8 since above this region the systems are complicated due to the hydrolysis of the complexes. All the calculations were done with the aid of the computer program[5] : MINIQUAD-75 on an IBM 37 computer. The results obtained are given in Table1.

Results and Discussion

It is generally accepted that with dipeptides Cu(II) forms complexes of the type $[CuA]^+$, $[CuAH_{-1}]$, $[CuAH_{-2}]^-$, $[CuA_2H_1]^-$ and $[Cu_2A_2H_{-3}]^-$. However, the formation of these complexes is highly *p*H sensitive and in the present study below *p*H 8, the complex species $[CuA]^+$, $[CuAH_{-1}]$ and $[CuA_2H_{-1}]^-$ in addition to [HA] and $[H_2A]^+$ were detected for all the six Cu(II)-dipeptide (A) systems in appreciable amounts. The charges of these complex species are omitted in the rest of this paper for clarity.

The solid and solution state studies on Cu(II) binary complexes of dipeptides[1,2] show that the initial complex formation between Cu(II) and a dipeptide results in a chelate involving the terminal amino moiety and the oxygen of the neighbouring amide group. Hence it may be expected that the second (bifunctional) amino acid in a dipeptide should have some effect on the stability of the complexes. The plot of log K^{Cu}_{CuA} versus $pK_{NH_3^+}$ (Fig. 1) shows that CuA complexes with glycyl (alkylglycinate) i.e. glycyglycinate, glycyl-L-alaninate, glycyl-L-leucinate and glycinamide fall in a straight line, whilethose with (alkylglycyl)glycinate, i.e. L-alanylgly cinate and L-leucylglycinate deviate from the linear plot. This indicates that while an alkyl substituent at the glycine end of the dipeptide ligand has no influence on the stability of the CuA dipeptide complex and its formation depends solely on the basicity of the terminal amino group of the dipeptide, such a substituent at its glycyl residue by steric effects decreases the stability of the complex.

At higher *p*H, the dipeptide ligand(A) in the CuA complex where it is bound in a bidentate manner

†Present address : Vice-Chancellor, S. V. Tirupati University, Tirupati.

TABLE 1 — STABILITY CONSTANTS OF Cu(II)-DIPEPTIDE SYSTEMS

[Temp. = 37°C; I = 0.15 ($NaClO_4$)]

Results	Glycyl-glycine	Glycyl-L-alanine	L-Alanyl-glycine	Glycyl-L-leucine	L-Leucyl-glycine	Glycin-amide
log β_{HA} ($pK_{NH_3}^+$)	7.99(1)	8.06(1)	8.00(1)	8.09(1)	7.94(1)	7.89(1)
log β_{H_2A}	11.26(1)	11.37(1)	11.37(10)	11.39(10)	11.26(1)	—
log β_{CuA} (log $K)^{Cu}_{CuA}$	5.70((8)	5.85(7)	5.58(7)	5.94(5)	5.34(10)	5.53(5)
log $\beta_{CuAH_{-1}}$	1.62(2)	1.76(2)	1.52(1)	1.88(1)	1.44(1)	—1.14(9)
log $\beta_{CuA_2H_{-1}}$	5.50(10)	5.50(12)	4.68(7)	5.72(7)	4.57(10)	3.18(9)
pK_{COOH}	3.27	3.31	3.37	3.30	3.32	—
pK^H_{CuA}	4.08	4.09	4.06	4.06	3.90	6.67
log $K^{CuA}_{CuA_2H_{-1}}$	—0.20	—0.35	—0.90	—0.22	—0.77	—2.35
log $K^{CuAH_{-1}}_{CuA_2H_{-1}}$	3.88	3.74	3.16	3.84	3.13	4.32
log $\beta_{CuAH_{-1}}$ minus log $K^{CuA}_{CuA_2H_{-1}}$	1.82	2.11	2.42	2.10	2.21	1.21

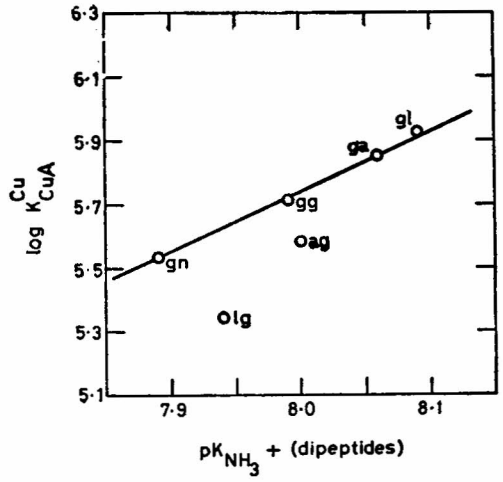


Fig. 1 — Relation between log K^{Cu}_{CuA} and $pK_{NH_3}^+$ (dipeptide) for the binary CuA complexes [gg = glycyglycine; gl = glycyl-L-leucine; ga = glycyl-L-alanine; lg = L-leucylglycine; ag = L-alanylglycine; gn = glycinamide]

undergoes deprotonation of the amide group and $CuAH_{-1}$ complex is formed. Here it may be mentioned that ionization of an amide hydrogen atom can not be distinguished unambiguously from hydrolysis of coordinated water, since both will have the same apparent stoichiometry. Such reactions may be expressed as overall formation constants (Eq. 1).

$$Cu + A \overset{\beta_{CuAH_{-1}}}{\rightleftharpoons} CuAH_{-1} + H$$
$$\text{or } Cu + A + H_2O \rightleftharpoons CuA(OH) + H \quad ...(1)$$

$$\beta_{CuAH_{-1}} = \frac{[CuAH_{-1}][H]}{[Cu][A]} \text{ or } \frac{[CuA(OH)]}{[Cu][A][H]^{-1}} \quad ...(2)$$

The overall equilibrium reaction in Eq. (1) takes place in two distinct steps(3) and (5)

$$Cu + A \overset{K^{Cu}_{CuA}}{\rightleftharpoons} CuA \quad ...(3)$$

$$K^{Cu}_{CuA} = \frac{[CuA]}{[Cu][A]} \quad ...(4)$$

$$CuA \overset{K^H_{CuA}}{\rightleftharpoons} CuAH_{-1} + H$$
$$\text{or } CuA + H_2O \rightleftharpoons CuA(OH) + H \quad ...(5)$$

$$K^H_{CuA} = \frac{[CuAH_{-1}][H]}{[CuA]} \text{ or } \frac{[CuA(OH)]}{[CuA][H]^{-1}} \quad ... (6)$$

The pK^H_{CuA} values in Table 1 for the Cu(II)-dipeptide systems in the present study are too small to be due to ionization of coordinated water molecules and hence these are attributed to the ionization of the amide hydrogen atom. The tridentate binding of the amide deprotonated dipeptides in $CuAH_{-1}$ complexes via N-amino, N-amide and O-carboxylate group may be confirmed by a comparison of the glycylglycinate and glycinamide systems with pK^H_{CuA} values of 4.08 and 6.67 respectively, i.e. the presence of the carboxylate group in glycylglycine allows the formation of a tridentate chelate after amide deprotonation and hence this ionization is favoured by a factor of 2.6 log units.

From the studies relating to Cu(II) binary complexes of amino acids[1,2,6], it is known that above pH 6 with an excess of ligand, the CuA_2 complex predominates. Accordingly in all the Cu(II) -dipeptide systems in the present study the formation of CuA_2 was assumed, but they got rejected in the final refinement of the computer based analysis using MINIQUAD-75 program. However, the CuA_2H_{-1} complex species were detected in appreciable amounts below pH 8 for all the systems. Opinions differ as to the structure of CuA_2H_{-1} complexes. In the glycinamide system log $K^{CuA}_{CuA_2H_{-1}}$ is smaller than log $\beta^{Cu}_{CuAH_{-1}}$ by about 1.2 log units, i.e. the affinity of the species CuA for AH_{-1} ion is less than that of free Cu(II) by about the amount expected on statistical and general environmental grounds assuming similar bonding in each case. In all other systems the difference is more than 2 log units, suggesting that

the deprotonated dipeptides, AH_{-1} bind more strongly to Cu(II) than to CuA. It means that in the formation of CuA_2H_{-1} species, the binding of AH_{-1} with CuA (Eq. 7) is less favoured. Hence we have to think about the other possibility, i.e. the binding of A with $CuAH_{-1}$ as given in Eq. (9).

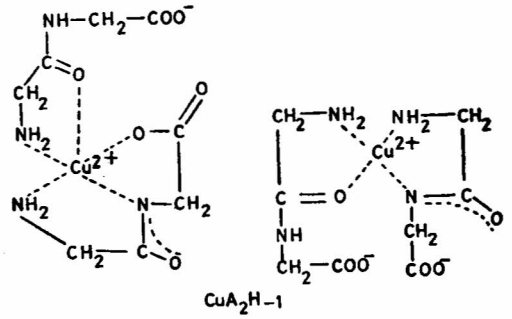
$$CuA + AH_{-1} \overset{K^{CuA}_{CuA_2H_{-1}}}{\rightleftharpoons} CuA_2H_{-1} \quad \ldots (7)$$

$$K^{CuA}_{CuA_2H_{-1}} = \frac{[CuA_2H_{-1}]}{[CuA]\,[AH_{-1}]} \quad \ldots (8)$$

$$CuAH_{-1} + A \overset{K^{CuAH_{-1}}_{CuA_2H_{-1}}}{\rightleftharpoons} CuA_2H_{-1} \quad \ldots (9)$$

$$K^{CuAH_{-1}}_{CuA_2H_{-1}} = \frac{[CuA_2H_{-1}]}{[CuAH_{-1}]\,[A]} \quad \ldots (10)$$

This is to be expected if the tridentate nature of the deprotonated dipeptide is accepted. Therefore the probable structure for CuA_2H_{-1} species seems to be I as suggested by Kaneda and Martell[7]. However, for the same species the equatorial coordination of the donor group was also considered by several workers[11,12] on the assumption that the C=O group of the second dipeptide ligand expels the CO_2^- group of the first ligand from the coordination sphere as shown in II.



I II

The concentration distribution diagrams for all the Cu(II) — dipeptide binary systems in the present study show the same qualitative features namely a progressive increase, with *p*H, in the amounts of the amide deprotonated complexes, tending to limiting values, accompanied by corresponding decrease in the concentration of free metal ion and the CuA complex. Figure 2 represents the contribution of various species in Cu(II) — glycylglycine/glycinamide systems for 1 : 2 solutions. It may be noted that the concentration distribution curve for the $CuAH_{-1}$ glycinamide species is of unusual nature, which may be due to the deprotonated (hydroxo) complex formation : the concentration of which increases with increasing *p*H, i.e. in the glycinamide system the formation of the deprotonated (hydroxo) complex is more favoured due to the fact that even after the amide deprotonation only two binding groups, viz. N-amino and N-amide are available, while in other dipeptide systems the carboxylate group is also available for coordination after amide deprotonation Unusual concentration distributions in various equili brium systems have also been reported by several workers[8-11].

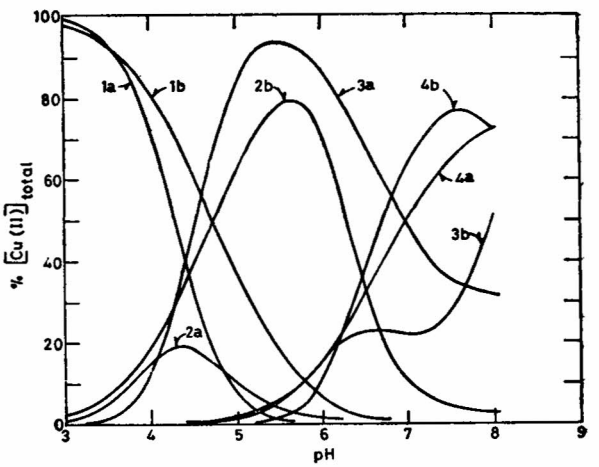


Fig. 2 — Species distribution for Cu(II) complexes of glycylglycine (a) and glycinamide (b) [$C_{Cu} = 0.003M$; $C_A = 0.006M$. (1) unbound Cu(II); (2) CuA; (3) $CuAH_{-1}$, (4) CuA_2H_{-1}]

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