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Mixed-Ligand Complex Formation by Cu(II) with Imidazole and Dipeptides

M. SIVASANKARAN NAIR, M. SANTAPPA and P. NATARAJAN

Department of Physical Chemistry, University of Madras, A. C. College Campus, Madras-600 025, India

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The species distribution and stability constants were evaluated for six mixed imidazole (A)–dipeptide (B) complexes of Cu(II) in aqueous perchlorate medium at I = *0.15* M *($NaClO_4$) and 37 °C from pH titration data. The dipeptides used are glycylglycine, glycyl-L-alanine, L-alanylglycine, glycyl-L-leucine, L-leucylglycine and glycinamide. In addition to the binary complex species CuA, CuA_2, CuA_3, CuA_4, CuB, $CuBH_{-1}$, CuB_2H_{-1}, three mixed complexes CuA_2B, CuA_2BH_{-1} and $CuABH_{-1}$ were detected in each system. However in the glycinamide secondary ligand (B) system, the mixed species CuAB formed instead of $CuABH_{-1}$. The stability order found for these complexes is discussed in terms of the steric effects due to side chain substituents in the dipeptide (B) ligands. The results suggest that mixed-coordination enhances the stability of the complexes. The amide deprotonated dipeptides (B) appear to be tridentate in the CuA_2BH_{-1} and $CuABH_{-1}$ complexes.*

Introduction

Studies on mixed-ligand complexes of Cu(II) containing imidazole and dipeptides are of considerable interest because such complexes may be considered as models for enzyme–metal ion–substrate complexes [1–3]. Several workers [4–6] carried out investigations on Cu(II)–imidazole (A)–glycylglycine (B) mixed-ligand system. Koltun *et al.* [4] reported the formation of $CuABH_{-1}$ mixed species in this system. Driver and Walker [5] studied the structures of the complex species CuA_2B, $CuABH_{-1}$ and Cu_2AB_2 by physicochemical methods. From solid state studies, Bell *et al.* [6] concluded that glycylglycine (B) coordinates in bidentate manner through N-amino and O-amide groups in CuA_2B and tridentate manner through N-amino, N-amide and O-carboxylate groups in $CuABH_{-1}$. However, no reports have been made by these workers regarding the distribution of various binary and mixed-ligand complex species as a function of pH. So a detailed study on the stability and species distribution in Cu(II)–imidazole (A)–glycylglycine (B) mixed-ligand system is reported in this paper for the first time and the same work has been extended to the other Cu(II)–imidazole (A)–dipeptide (B) systems.

Experimental

All the ligands used in this work were obtained from Fluka. $Cu(ClO_4)_2$ and other reagents were prepared and estimated as described earlier [7, 10].

The pH titrations were carried out at 37 °C under nitrogen (freed from oxygen and CO_2), with a digital pH meter (M/s Bhagyanagar Electronics, Hyderabad, India) with glass and calomel electrode assembly with an accuracy of ±0.01 of a pH unit. The pH standards taken were 4.02 for 0.05 *M* potassium hydrogen phthalate and 9.08 for 0.05 *M* borax at 37 °C. The electrode system was calibrated by the method of Irving *et al.* [12]. A constant ionic strength of 0.15 *M* was maintained by the addition of sodium perchlorate. Titrations were done on 50 ml portions of solutions containing low concentrations of $Cu(ClO_4)_2$, imidazole (A) and dipeptides (B) in 1:1:1 and 1:2:2 ratios with known volumes of standard CO_2-free NaOH.

Calculations have been restricted to systems at pH < 8, since above this region is complicated due to the hydrolysis of the complex. The range of pH chosen for the calculation is 3.00 to 8.00 for all the mixed systems in our study except for the 1:1:1 solution in the Cu(II)–imidazole–glycinamide system where the calculation was done for the pH region 3.00 to 7.00 due to the precipitation problem at pH 7.10. All the calculations were done with the aid of the computer program [8]: MINIQUAD-75 on an IBM 370 computer, fixing the acid dissociation constants of the ligands and the parent binary stability constants estimated under identical conditions [7, 9] as non-refinable parameters. The results are recorded in Table I.

Results and Discussion

It was shown both by crystallographic and solution studies [1–3] that the initial complex formation between Cu(II) and a peptide results in a chelate involving the terminal amino moiety and the oxygen of the neighbouring amide group. Hence, the kind of the second (bifunctional) amino acid in a peptide should have little influence on the stability of the

TABLE I. Stability Constants for the Mixed Imidazole (A)–Dipeptide (B) Systems of Cu(II) [37 °C, $I = 0.15\ M\ (NaClO_4)$]. The Figures in Parentheses are the Standard Deviations in the Last Decimal Figure.

Results	Dipeptides, B					
	Glycyl-glycine	Glycyl-L-alanine	L-Alanyl-glycine	Glycyl-L-leucine	L-leucyl-glycine	Glycin-amide
$\log \beta^{Cu}_{CuAB}$	–	–	–	–	–	9.49(4)
$\log \beta^{Cu}_{CuA_2B}$	14.29(9)	14.72(13)	14.23(11)	14.90(26)	14.31(6)	12.83(5)
$\log \beta^{Cu}_{CuABH_{-1}}$	5.65(8)	5.70(9)	5.46(13)	5.86(9)	5.43(8)	–
$\log \beta^{Cu}_{CuA_2BH_{-1}}$	8.77(16)	9.03(12)	8.86(14)	9.17(13)	9.02(9)	5.72(6)
$\log K^{CuBH_{-1}}_{CuABH_{-1}}$	4.03	3.94	3.94	3.98	3.99	–
$\Delta \log K$	−0.18	−0.27	−0.27	−0.23	−0.22	–
$pK^{H}_{CuA_2B}$	5.52	5.69	5.37	5.73	5.29	7.11

complexes. This effect on the formation of mixed-ligand complexes may be illustrated from the results obtained for the Cu(II)–imidazole (A)–dipeptide (B) systems in the present investigation. The dipeptide (B) ligands used can be grouped into two *viz.*, (i) glycyl(alkylglycinates) *i.e.* glycylglycinate, glycyl-L-alaninate and glycyl-L-leucinate ligands and (ii) (alkylglycyl)glycinates *i.e.* L-alanylglycinate and L-leucylglycinate ligands. The log $\beta^{Cu}_{CuA_2B}$ values in Table I show that the CuA_2B mixed complexes in the L-alanylglycinate and L-leucylglycinate secondary ligand (B) systems are respectively less stable than those in the glycyl-L-alaninate and glycyl-L-leucinate secondary ligand (B) systems. This suggests that an alkyl substituent at the glycyl residue of the dipeptide (*i.e.* $-CH_3$ and $-CH_2CH(CH_3)_2$ alkyl substituents at the glycyl residues respectively in the L-alanylglycinate and L-leucylglycinate dipeptides) secondary ligands (B) in CuA_2B complexes decrease their stability, while such substituents in the glycine end of the dipeptide (*i.e.* glycyl-L-alaninate, glycyl-L-leucinate) secondary ligands (B) have no influence on the stability of the mixed complexes CuA_2B. The same trends have also been noticed in the Cu(II)–dipeptide (B) binary [9, 13] and Cu(II)–2,2′-bipyridyl [13]/histamine or L-histidine [10] (A)–dipeptide (B) mixed-ligand systems. The detection of CuA_2B mixed complexes in all the systems in the present study indicates clearly that Cu(II) prefers to have tetra-coordination, the four sites being occupied by two imidazole nitrogens in the two imidazole ligands (A), and N-amino and O-amide groups in the dipeptide ligand (B).

At higher pH, the dipeptide ligand (B) in the CuA_2B complex, where it is bound in a bidentate manner undergoes deprotonation of the amide group and forms CuA_2BH_{-1} mixed complex. The tridentate mode of binding of this amide deprotonated dipeptide (BH_{-1}) may be observed if a comparison is made between the $pK^{H}_{CuA_2B}$ values of 5.52 and 7.11 respectively for the glycylglycinate and glycinamide secondary ligand (B) systems for the reaction (1).

$$CuA_2B \rightleftharpoons CuA_2BH_{-1} + H^+ \qquad (1)$$

But this observation is contradictory to the conclusions arrived for the mixed-ligand systems of Cu(II) involving a bidentate primary ligand (A) like 2,2′-bipyridyl [13, 14]/histamine [10] or a tridentate primary ligand (A) like L-histidine [10] where the amide deprotonated dipeptide secondary ligand (BH_{-1}) in the $CuABH_{-1}$ mixed species bind in a bidentate mode in aqueous solution. So the tridentate mode of binding of the amide deprotonated dipeptides (BH_{-1}) in the CuA_2BH_{-1} species in Cu(II)–imidazole (A)–dipeptide mixed systems may be attributed to the involvement of the monodentate imidazole primary ligand (A). However, the small difference* of 1.6 log units between the $pK^{H}_{CuA_2B}$ values in the glycylglycinate and glycinamide secondary ligand systems (Table I) suggests that the tridentate binding is present only to an appreciable extent *i.e.* after the deprotonation of the amide group, the dipeptide secondary ligands in CuA_2BH_{-1} may equilibrate between two structures where in one structure, it binds in a tridentate manner *via* N-amino, N-amide and O-carboxylate groups as in the $CuBH_{-1}$ dipeptide binary complexes [9] and in the other structure, it binds in a bidentate manner *via.*, N-amino and N-amide groups as in the $CuABH_{-1}$ (A = hista-

*A comparison of the $CuBH_{-1}$ species with pK^{H}_{CuB} values of 4.08 and 6.67 respectively in the Cu(II)–glycylglycine and Cu(II)–glycinamide systems [9] shows that the presence of carboxylate group in glycylglycine allows the formation of a tridentate chelate after the ionization of amide proton and favours therefore this ionization by a factor of $10^{2.6}$. But, in the CuA_2BH_{-1} complex (A = imidazole, B = glycylglycine), this type of ionization is favoured only by a factor of $10^{1.6}$.

mine/L-histidine, B = dipeptide) mixed complexes [10]. The concentration of the CuA_2BH_{-1} complex with the dipeptide binding in a tridentate manner would be more because this structure involves two five membered chelate rings, whereas in the other structure only one chelate ring is involved.

In the case of the species $CuABH_{-1}$ we may consider the equilibria (2) and the corresponding expression for $\Delta\log K$ as in equation (3) [11]. Since more coordination site are available for bonding

$$CuA + CuBH_{-1} \rightleftharpoons CuABH_{-1} + Cu \quad (2)$$

$$\Delta\log K = \log \beta^{Cu}_{CuABH_{-1}} - (\log \beta^{Cu}_{CuA} + \log \beta^{Cu}_{CuBH_{-1}}) \quad (3)$$

the first ligand to a metal ion than for the second ligand, $\Delta\log K$ should in general be negative. With Cu(II) usually having a coordination number of four, the expected value for $\Delta\log K$ would be –0.6, values greater than this demonstrating the stabilization of the mixed-ligand complex. The $\Delta\log K$ values for the $CuABH_{-1}$ complexes in Table I are considerably less negative indicating their high stabilities. The same results become more obvious if it is noted that the $\log K^{CuBH_{-1}}_{CuABH_{-1}}$ values which lie between 3.94 and 4.03 (Table I) are much higher than the $\log K^{CuA_3}_{CuA_4}$ value of 2.19 [7]. In the Cu(II)–imidazole (A)–glycylglycine (B) system, it may be mentioned that $\log K^{CuBH_{-1}}_{CuABH_{-1}}$ value of 3.85 reported by Koltun *et al.* [4] is in good agreement with our value of 4.03. The absence of the $CuABH_{-1}$ species with B = glycinamide may be due to the precipitation problem in a 1:1:1 solution containing Cu(II), imidazole (A) and glycinamide (B) at pH 7.10, this type of species being predominated in other systems only above pH 7. Due to the absence of this $CuABH_{-1}$ species (hence its pK^{H}_{CuAB} value is not available to compare with that for the $CuABH_{-1}$ complex with B = glycylglycine), it is not possible to suggest anything about the mode of binding of the amide deprotonated dipeptide (B) ligands in the $CuABH_{-1}$ complexes. However, we favour their tridentate binding in the solution state also as shown in the solid state studies [5, 6]. This is possible because out of the four positions in the square plane of Cu(II) in $CuABH_{-1}$ complex, only one position is being occupied by the imidazole ligand (A) and the remaining three positions may be completed by the N-amino, N-amide and O-carboxylate groups of the amide deprotonated dipeptide ligand (B).

The concentration distribution diagrams for all the Cu(II)–imidazole (A)–dipeptide (B) mixed-ligand systems show the same qualitative features namely a progressive increase, with pH, in the amounts of the deprotonated-, mixed- and bis complexes, tending to limiting values, accompanied by corresponding decreases in the concentrations of free metal ion and the 1:1 complexes. The $CuABH_{-1}$ species accounted for more than 50% of the total Cu(II) in a 1:1:1 solution of Cu(II), imidazole and dipeptide. The formation of CuA_2B and CuA_2BH_{-1} complexes was found to be more favoured in 1:2:2 solutions, where more than 20 and 60 percentage of the total metal were respectively present in their form. The distribution diagrams for the Cu(II)–imidazole–glycylglycine system in 1:1:1 and 1:2:2 solutions are given in Figs. 1 and 2.

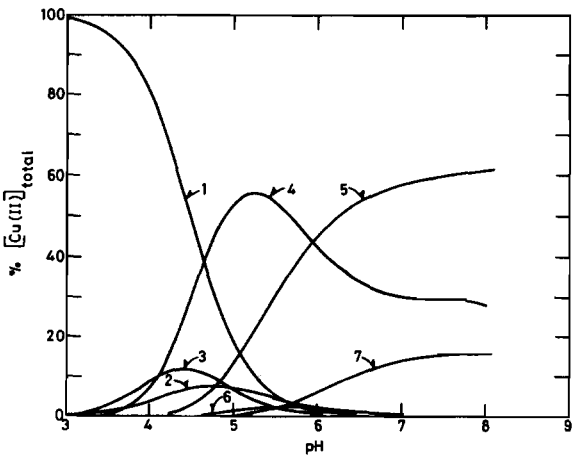

Fig. 1. Species distribution for Cu(II)–imidazole (A)–glycylglycine (B) system; $C_{Cu} = C_A = C_B = 0.003$ *M*. 1, unbound Cu(II); 2, CuA; 3, CuB; 4, $CuBH_{-1}$; 5, $CuABH_{-1}$; 6, CuA_2B; 7, CuA_2BH_{-1}. The CuA_2, CuA_3, CuA_4 and CuB_2H_{-1} are not represented in the Figure because of their very low concentration.

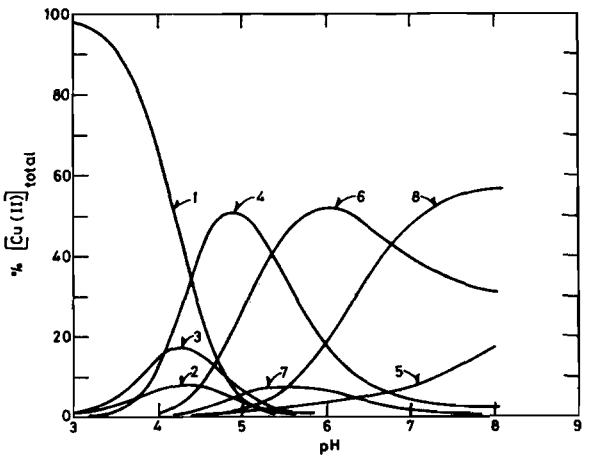

Fig. 2. Species distribution for Cu(II)–imidazole (A)–glycylglycine (B) system; $C_{Cu} = 0.003$ *M*, $C_A = C_B = 0.006$ *M*. 1, unbound Cu(II); 2, CuA; 3, CuB; 4, $CuBH_{-1}$; 5, CuB_2H_{-1}; 6, $CuABH_{-1}$; 7, CuA_2B; 8, CuA_2BH_{-1}. The CuA_2, CuA_3 and CuA_4 species are not represented in the Figure because of their very low concentration.

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