Mixed-ligand Complex Formation by Copper(II) with Imidazole Derivatives and Dipeptides in Aqueous Solution †

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The equilibria involved in 12 new mixed-ligand systems, Cu^{II}–A–B (A = histamine or L-histidine; B = glycylglycine, glycyl-L-alanine, L-alanylglycine, glycyl-L-leucine, L-leucylglycine, or glycinamide), have been investigated in an aqueous perchlorate medium by pH titrimetry at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$). In addition to the species HA, H_2A, CuAH, CuA, CuA_2H, CuA_2, HB, H_2B, CuB, $CuBH_{-1}$, and CuB_2H_{-1} (also H_3A and CuA_2H_2 for A = L-histidine), three complexes of stoicheiometry CuABH, CuAB, and $CuABH_{-1}$ have been detected in these systems. The site of protonation in the CuABH species is the primary amino-group of the histamine or L-histidine (A) ligand. The stability-constant data obtained indicate the stability-enhancing effect of the ligands (A) on copper(II) mixed-ligand complex formation with dipeptides (B). The influence of the alkyl substituents in the ligands (B) on the stabilities of CuAB species follows the trends observed previously for CuB dipeptide binary complexes. In the $CuABH_{-1}$ species, the amide-deprotonated dipeptides are bidentate.

INVESTIGATIONS on mixed-ligand complexes of Cu^{II} containing peptide as a ligand are of considerable interest because, in peptides and probably also in proteins, the deprotonated amide group is one of the important binding groups for Cu^{II}. Sarkar and co-workers [1-4] reported detailed studies involving binary and mixed-ligand complexes of peptides and amino-acids with the aim of furthering the understanding of the chemistry of mixed co-ordination by proteins and related substances. As a model of the mixed-ligand complex L-histidine–copper(II)–albumin, which acts as an intermediate in the exchange of Cu^{II} in blood between a macromolecule such as albumin and a low-molecular-weight substance such as an amino-acid, Kruck and Sarkar [1] studied the system L-histidine–copper(II)–diglycyl-L-histidine. The co-ordination behaviour of dipeptides was demonstrated by Sigel and co-workers [4-6] for 2,2′-bipyridyl–copper(II)–dipeptide systems, and the effects of the structures of amino-acids on the formation equilibria of mixed-ligand complexes of dipeptides have also been reported.[4,7,8]

However, few studies have been made on mixed-ligand complexes of Cu^{II} containing imidazole and peptide, although such complexes are ' of biological interest.' [9,10] We have previously described [11] the co-ordination behaviour of dipeptides in copper(II) mixed complexes in the presence of a unidentate ligand such as unsubstituted imidazole. In the present paper, results obtained by pH titrimetry are reported for some copper(II)–histamine or L-histidine (A)–dipeptide (B) mixed-ligand systems in an aqueous perchlorate medium at I = 0.15 mol dm^{-3} ($Na[ClO_4]$) and 37 °C. The dipeptides (B) used are glycylglycine, glycyl-L-alanine, L-alanylglycine, glycyl-L-leucine, L-leucylglycine, and glycinamide (2-aminoacetamide). Glycinamide resembles a dipeptide without a carboxyl group.

EXPERIMENTAL

All the ligands used in this work were obtained from Fluka. The salt $Cu[ClO_4]_2$ and other reagents were prepared and estimated as described earlier.[12]

The pH titrations were carried out at 37 °C under a nitrogen atmosphere (freed from oxygen and CO_2) with the apparatus and procedure described previously.[12] A constant ionic strength of 0.15 mol dm^{-3} was maintained by the addition of sodium perchlorate. Titrations were done on aliquots (50 cm^3) of solutions containing low concentrations of $Cu[ClO_4]_2$, histamine [4-(2-aminoethyl)-imidazole] or L-histidine (A), and dipeptide (B) in 1 : 1 : 1 and 1 : 2 : 2 ratios with known volumes of standard CO_2-free Na[OH].

Calculations have been restricted to systems below pH 8 since the region above this pH is complicated due to hydrolysis. All the calculations were done with the aid of the computer program [13] MINIQUAD-75 on an IBM 370 computer, the stability constants for the complex species HA, H_2A, CuAH, CuA, CuA_2H, CuA_2, HB, H_2B, CuB, $CuBH_{-1}$, and CuB_2H_{-1} (also H_3A and CuA_2H_2 for the systems with A = L-histidine) estimated under identical conditions [12,14] being treated as non-refinable parameters. The results obtained are recorded in Tables 1 and 2.

RESULTS AND DISCUSSION

In the copper(II)–histamine or L-histidine (A)–dipeptide (B) mixed-ligand systems three mixed species of stoicheiometry CuABH, CuAB, and $CuABH_{-1}$ were evident in addition to the binary species mentioned above. The protonated CuABH species did not appear to be present in all cases and it was always of minor importance. It was present in all the six mixed systems with A = histamine, except the one with B = glycinamide, and in the copper(II)–L-histidine (A)–dipeptide (B) systems with B = glycyl-L-alanine, L-leucylglycine, and glycinamide. In all these systems, less than 10% of the total Cu^{II} was present in this form and hence the precision of the corresponding experimentally obtained formation constants in Tables 1 and 2 is low. However, the pK values (Tables 1 and 2) for monoprotonation of the CuAB species are nearly identical to the corresponding values of 4.22 or 4.11 respectively for the CuA histamine or L-histidine complexes, where the proton in the binary complexes is attached to the

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primary amino-group of the histamine or L-histidine ligands.[12] Hence it may be concluded that in the CuABH mixed species also the proton is attached to the primary amino-group of the histamine or L-histidine (A) primary ligands. The possibility for the attachment of the proton in the CuABH species to the dipeptide (B) secondary ligand is ruled out because no protonated species were detected [14] for the copper(II)–dipeptide (B) binary systems.

TABLE 1

Stability constants for the mixed copper(II)–histamine (A)–dipeptide (B) systems at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$). The figures in parentheses are standard deviations in the first decimal figure

		Dipeptide, B					
Parameter		Glycylglycine	Glycyl-L-alanine	L-Alanylglycine	Glycyl-L-leucine	L-Leucylglycine	Glycinamide
log β_{CuABH}		19.11(26)	19.77(8)	19.29(12)	19.83(11)	19.43(8)	
log β_{CuAB}		15.04(5)	15.35(3)	14.91(4)	15.52(4)	14.66(5)	14.47(3)
log $\beta_{CuABH_{-1}}$		7.49(9)	7.64(7)	7.34(6)	8.06(8)	7.84(8)	7.08(4)
pK_{CuABH}		4.07	4.42	4.38	4.31	4.77	
log K_{CuAB}	*a*	5.80	6.11	5.67	6.28	5.42	5.23
	b	9.34	9.50	9.33	9.58	9.33	8.96
Δ log K		0.10	0.26	0.09	0.34	−0.08	−0.30
log β_{CuAB} (calc.)		14.09	14.23	13.96	14.32	13.72	13.91
Δ log β		0.95	1.12	0.95	1.20	0.94	0.56
pK_{CuAB}′ [c]		7.55	7.71	7.57	7.46	7.82	7.39

[a] For CuA + B ⇌ CuAB. [b] For CuB + A ⇌ CuAB. [c] For CuAB ⇌ $CuABH_{-1}$ + H.

$$Cu + A \rightleftharpoons CuA;\ K_{CuA} \quad (1)$$

$$Cu + B \rightleftharpoons CuB;\ K_{CuB} \quad (2)$$

$$Cu + A + B \rightleftharpoons CuAB;\ \beta_{CuAB} \quad (3)$$

$$CuA + CuB \rightleftharpoons CuAB + Cu \quad (4)$$

$$\Delta\log K = \log \beta_{CuAB} - (\log K_{CuA} + \log K_{CuB}) \quad (5)$$

$$\Delta\log \beta = \log \beta_{CuAB}\ (expt.) - \log \beta_{CuAB}\ (calc.) \quad (6)$$

The parameters [15] Δlog K and Δlog β [equations (1)—(6)] have been calculated for all the mixed-ligand systems in the present study. For Cu^{II}, which usually has a co-ordination number of four, the expected value of Δlog K on statistical considerations [15] is −0.6 log units. Values greater than this indicate that mixed-ligand complex formation is more favourable. All the mixed-ligand systems in Tables 1 and 2 have Δlog K values much greater than −0.6, demonstrating their high stability of the CuAB mixed species. The plot in Figure 1 of log K_{CuAB} (for CuA + B ⇌ CuAB) *vs.* p$K(NH_3^+)$ of the dipeptides shows that the CuAB complexes with glycyl(alkylglycines), *i.e.* glycylglycine, glycyl-L-alanine, and glycyl-L-leucine, and glycinamide dipeptide ligands (B) fall on a straight line, while those

stabilities. The positive Δlog K values obtained for some of the copper(II)–histamine (A)–dipeptide (B) systems (Table 1) show that the dipeptide secondary ligands prefer to add to the histamine binary complex CuA rather than to aquated Cu^{II}. The positive Δlog β values (Tables 1 and 2) which range from 0.5 to 1.2 also support the view that mixed-ligand complex formation is more favourable. The π-acceptor property of the imidazole ring in the histamine or L-histidine (A) primary ligands may be the vital factor for this stability enhancement. The higher log β_{CuAB} values for the mixed systems with A = L-histidine (Table 2) compared to those for the histamine systems (Table 1) suggest that histidine functions in a tridentate manner.

Regarding the binding of the dipeptide secondary ligands, it is well established [9,10] that the initial complex formation between Cu^{II} and a dipeptide is at the amino-moiety. Hence it may be expected that the nature of the second (bifunctional) amino-acid in the dipeptide secondary ligands should have some effect on the

TABLE 2

Stability constants for the mixed copper(II)–L-histidine (A)–dipeptide (B) systems at 37 °C and I = 0.15 mol dm^{-3} ($Na[ClO_4]$). The figures in parentheses are standard deviations in the first decimal figure

		Dipeptide, B					
Parameter		Glycylglycine	Glycyl-L-alanine	L-Alanylglycine	Glycyl-L-leucine	L-Leucylglycine	Glycinamide
log β_{CuABH}			20.20(15)			19.54(45)	20.13(21)
log β_{CuAB}		15.77(5)	15.85(5)	15.66(6)	16.00(6)	15.39(5)	15.46(6)
log $\beta_{CuABH_{-1}}$		8.71(6)	8.70(7)	8.22(6)	8.74(8)	8.07(7)	8.17(8)
pK_{CuABH}			4.35			3.95	4.67
log K_{CuAB}	*a*	5.50	5.58	5.39	5.73	5.12	5.19
	b	10.07	10.00	10.08	10.06	10.04	9.93
Δ log K		−0.21	−0.27	−0.19	−0.21	−0.22	−0.34
log β_{CuAB} (calc.)		15.25	15.39	15.12	15.48	14.88	15.07
Δ log β		0.52	0.46	0.54	0.52	0.51	0.39
pK_{CuAB} [c]		7.06	7.15	7.44	7.26	7.32	7.29

[a,b,c] See Table 1.

with (alkylglycyl)glycines, *i.e.* L-alanylglycine and L-leucylglycine, ligands (B) deviate greatly from this line. This demonstrates that an alkyl substituent at the glycine end of the dipeptide (B) ligand has no influence on the stability of the CuAB mixed species and their formation depends solely on the basicity of the terminal amino-group of the dipeptide, but that such a substituent in its glycyl residue decreases their stability because of steric effects. The same trends have also

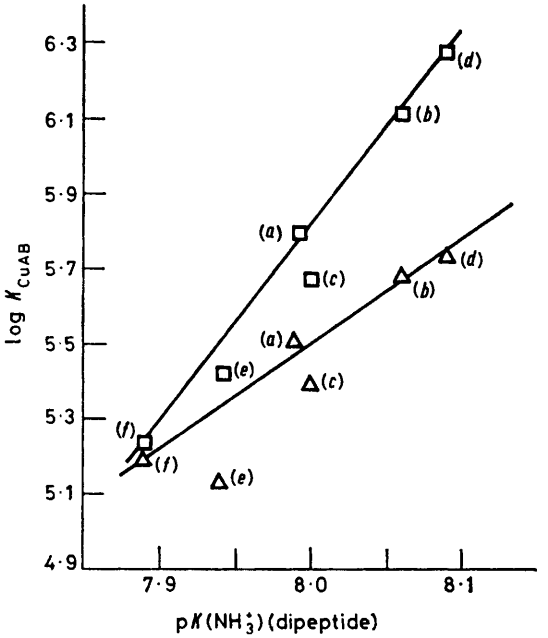

FIGURE 1 Relation between log K_{CuAB} (for CuA + B $\rightleftharpoons$ CuAB) and pK(NH$_3^+$) of the dipeptides for the CuAB complexes in the systems (△) copper(II)–L-histidine (A)–dipeptide (B) and (□) copper(II)–histamine (A)-dipeptide (B) mixed-ligand systems. Peptides: (*a*), glycylglycine; (*b*), glycyl-L-alanine; (*c*), L-alanylglycine; (*d*), glycyl-L-leucine; (*e*), L-leucylglycine; and (*f*), glycinamide

been noticed in binary and ternary complexes of Cu^{II} containing dipeptides.[5,6,11,14]

All the copper(II)–histamine or L-histidine (A)–dipeptide (B) systems formed appreciable amounts of the deprotonated mixed species CuABH$_{-1}$ below pH 8. It is noteworthy that Brookes and Pettit [7] could not detect such a species in copper(II)–D- or L-histidine (A)–dipeptide (B) systems with B = glycyl-L-valine and L-valyl-L-valine even at pH 9. It was suggested that the driving force for the ionization of the amide proton in the dipeptide secondary ligands in these mixed systems is drastically reduced due to the tridentate character of the histidine (A) primary ligand. However, our results indicate that, in the presence of a bidentate ligand such as histamine or a tridentate ligand such as L-histidine, the amide-deprotonated dipeptides (BH$_{-1}$) in the CuABH$_{-1}$ species bind in a bidentate mode. This conclusion was drawn by a comparison of the pK_{CuAB}' values of (*i*) 7.55 and 7.39 for the copper(II)–histamine (A)–glycylglycinate and –glycinamide (B) and (*ii*) 7.06 and 7.29 in the copper(II)–L-histidine (A)–glycylglycine and –glycinamide (B) systems (Tables 1 and 2). Thus the nearly identical values of pK_{CuAB}', within the limits of experimental error, for both the glycylglycine and glycinamide (B) secondary ligand systems clearly indicate that the carboxylate group of the amide-deprotonated glycylglycine (BH$_{-1}$) secondary ligand does not bind with Cu^{II} in the CuABH$_{-1}$ species. This behaviour may easily be explained when account is taken of the fact that, out of the four positions of the square plane around Cu^{II}, two are already occupied by the primary amino- and imidazole nitrogens of the histamine or L-histidine (A) ligand and the two remaining positions may be occupied by the *N*-amino- and *N*-peptide groups of the deprotonated dipeptide (BH$_{-1}$). Similar conclusions have been drawn by Sigel and co-workers [5,6] in their study of copper(II)–2,2'-bipyridyl (A)–dipeptide (B) mixed-ligand systems in aqueous solution.

However, a recent crystal-structure analysis of the CuABH$_{-1}$ (A = 1,10-phenanthroline, B = glycylglycine) mixed species by Lim *et al.*[16] contradicts these observations. Thus, in the solid state, it was shown that the amide-deprotonated glycylglycine (BH$_{-1}$) co-ordinates with all three possible binding groups, *i.e.* including the carboxylate group in the square plane around Cu^{II}. One of the phenanthroline nitrogens completes the square, while the other occupies a tilted apical position giving a distorted square-pyrimidal

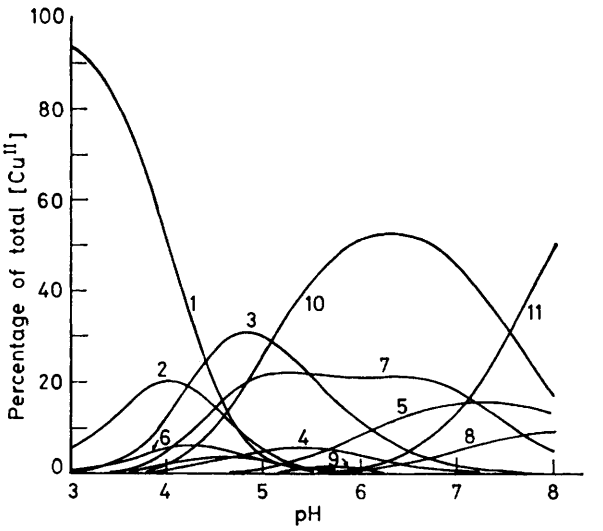

FIGURE 2 Species distribution for the copper(II)–histamine (A)–glycylglycine (B) system with $c_{Cu} = c_A = c_B = 0.003$ mol dm^{-3}. Species: (1) free Cu^{II}; (2), CuAH; (3), CuA; (4), CuA$_2$H; (5), CuA$_2$; (6), CuB; (7), CuBH$_{-1}$; (8), CuB$_2$H$_{-1}$; (9), CuABH; (10), CuAB; and (11), CuABH$_{-1}$

geometry about Cu^{II}. On the basis of the visible spectra, these workers suggested that an appreciable, perhaps predominant, fraction of the complexes in aqueous solution has the structure found in the crystal. However, the interpretation of the absorption spectra of such systems at higher pH is difficult [6] due to the presence of many hydrolysed species such as Cu(BH$_{-1}$)-

(OH), $Cu_2(BH_{-1})_2(OH)$, *etc.* Hence, without detailed knowledge of the concentrations of these complex species from their absorption maxima and absorption coefficients one cannot easily draw any conclusions

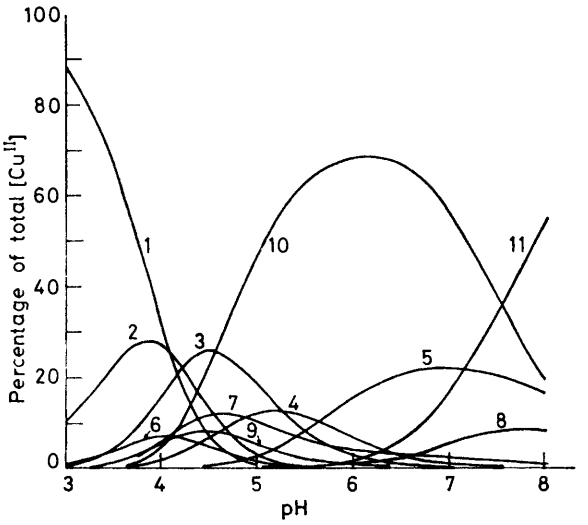

FIGURE 3 Species distribution for the copper(II)–histamine (A)–glycylglycine (B) system with $c_{Cu} = 0.003$ and $c_A = c_B = 0.006$ mol dm^{-3}. Species as in Figure 2

regarding their structures. Again, if tridentate co-ordination of the amide-deprotonated dipeptides (BH_{-1}) in the $CuABH_{-1}$ (A = histamine or L-histidine) species is expected, the nature of the third possible binding group, *viz.* carboxylate, should also be reflected in the pK_{CuAB}' values as is the case for the $CuBH_{-1}$ species in Cu^{II}–dipeptide (B) binary systems [14] or the $CuABH_{-1}$ and CuA_2BH_{-1} mixed species in the copper(II)–imidazole

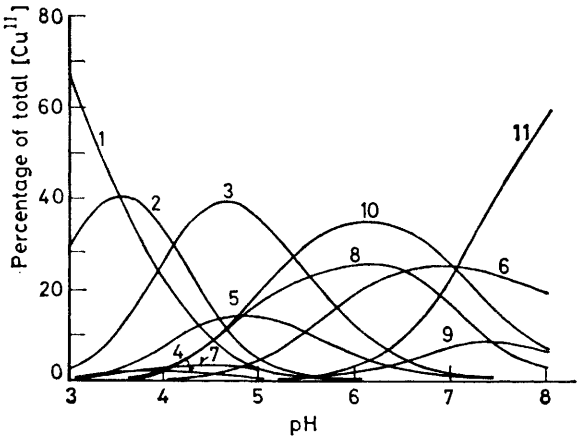

FIGURE 4 Species distribution for the copper(II)–L-histidine (A)–glycylglycine (B) system with $c_{Cu} = c_A = c_B = 0.003$ mol dm^{-3}. Species: (1) free Cu^{II}; (2), CuAH; (3), CuA; (4), CuA_2H_2; (5), CuA_2H; (6), CuA_2; (7), CuB; (8) $CuBH_{-1}$; (9), CuB_2H_{-1}; (10), CuAB; and (11), $CuABH_{-1}$

(A)–dipeptide (B) systems.[11] So we still favour bidentate co-ordination of the amide-deprotonated dipeptides (BH_{-1}) in the $CuABH_{-1}$ species with A = histamine or L-histidine. It has been suggested [11] that the presence of tridentate binding of the amide-deprotonated dipeptides in copper(II)–imidazole (A)–dipeptide (B) systems is due to the involvement of the unidentate imidazole (A) primary ligand.

The distribution of the various binary and mixed-ligand complex species as a function of the total copper(II) present and of pH was obtained for solutions of all the copper(II)–histamine or L-histidine (A)–dipeptide (B) systems in both 1 : 1 : 1 and 1 : 2 : 2 ratios. In all our mixed-ligand systems the CuAB species occur in greater concentrations than the corresponding CuB species in copper(II)–dipeptide (B) binary systems.[14] This demonstrates the stability-enhancing effect of histamine or L-histidine (A) ligands in the formation of the mixed-ligand complexes of Cu^{II} with dipeptides (B). The concentration distributions for the glycylglycine (B) mixed-ligand systems are given in Figures 2—5. It

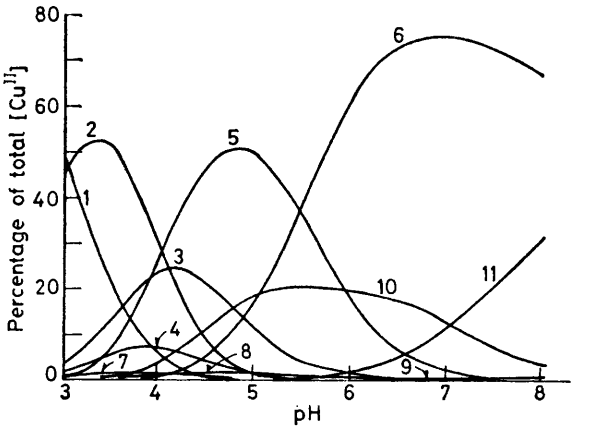

FIGURE 5 Species distribution for the copper(II)–L-histidine (A)–glycylglycine (B) system with $c_{Cu} = 0.003$ and $c_A = c_B = 0.006$ mol dm^{-3}. Species as in Figure 4

may be noted that the CuAB species in the copper(II)–histamine (A)–glycylglycine (B) system is more favoured in the 1 : 2 : 2 than in the 1 : 1 : 1 solution (Figures 2 and 3). However, in the corresponding L-histidine (A) system, the opposite effect is observed, the histidine binary complex CuA_2 predominating in the 1 : 2 : 2 solution (Figures 4 and 5). This may be explained by the fact that, depending on the conditions, the histidines in the CuA_2 complex can co-ordinate histamine-like as well as glycine-like *via* six- and five-membered chelate rings, a bonding mode which is especially favoured for Cu^{II}.[12] This type of binding is possible because in the 1 : 2 : 2 solutions an excess of histidine is available and hence the concentration of the CuA_2 (A = histidine) complex is enhanced.

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