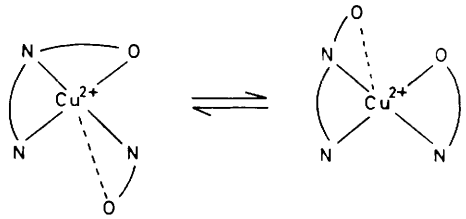
Effects of Mixed-ligand Complex Formation on Deprotonation of Amide Groups in Acid Amides and Peptides

Imre Sovago, Bela Harman, and Arthur Gergely
Department of Inorganic and Analytical Chemistry, Lajos Kossuth University, H-4010 Debrecen, Hungary
Barbara Radomska
Institute of Chemistry, University of Wroclaw, 50-383 Wroclaw, Poland

The stability constants of the parent complexes of the A ligands glycinamide, glycylglycinamide, and *N*-acetylhistidine with copper(II), and of the mixed complexes formed with the B ligands glycine, 2,3-diaminopropionic acid, tiron, histamine, L-histidine, and 2,2'-bipyridyl, have been determined from pH-metric measurements. It has been found that glycinamide forms mixed-ligand complexes of composition [CuAB] and [$CuABH_{-1}$] (charges omitted), and most favourably with histamine, L-histidine, or 2,2'-bipyridyl. In the glycylglycinamide complexes of type [$CuABH_{-1}$] the B ligand is co-ordinated to a considerable extent *via* two equatorial sites, while the bonding in the complex [$CuABH_{-2}$], similarly to the other dipeptides, is mainly axial–equatorial. In the parent complexes of copper(II) with *N*-acetylhistidine and with *N*-acetylhistamine, deprotonation and co-ordination of the amide group could not be detected. It has been found, however, that the presence of B ligands containing an aromatic N donor permits deprotonation of the amide groups in both *N*-acetylhistidine and *N*-acetylhistamine.

In earlier publications[1-3] we dealt with the complex-forming properties of histidine-containing di- and tri-peptides. In the case of the copper(II) ion, the co-ordination conditions of simple di- and tri-peptides are influenced significantly by the side-chain imidazole-N donor atom. With glycyl-L-histidine, at all metal ion:ligand ratios, formation of the complex [$CuAH_{-1}$] is favoured and one of the co-ordination sites is occupied by the deprotonated peptide-N atom. Although co-ordination of the peptide-N similarly occurs in equimolar solutions of copper(II) and L-histidylglycine, a ligand excess suppresses deprotonation, and complexes of type [CuA_2] are formed, involving histamine-like co-ordination.[1] In accordance with earlier findings,[4] therefore, the deprotonation of the amide group and its participation in co-ordination depend to a considerable extent on the other donor groups present. The data recently surveyed by Sigel and Martin[5] indicate that the determining factors are the roles of the imidazole and the thiol side-chain. However, the presence of B ligands forming stable bis complexes may be similarly important. The mixed-ligand complexes formed at the same time provide a better approach for the modelling of multicomponent biological systems.

Mixed complexes of the peptides and their derivatives have been studied by a number of authors.[5] Most data are available on the copper(II)–dipeptide–2,2'-bipyridyl and copper(II)–dipeptide–amino acid systems.[1,2,6-10] These studies revealed that the presence of the B ligand leads to a significant increase in the value of p*K* for the process [CuAB] $\rightleftharpoons$ $[CuABH_{-1}]^- + H^+$ relative to that for the peptide parent complex, *i.e.* it inhibits deprotonation of the peptide-NH group. The total concentration of the species [$CuAH_{-1}$] and $[CuABH_{-1}]^-$ is considerable in the whole pH range, *i.e.* peptide-N co-ordination is present in the mixed-ligand systems containing 2,2'-bipyridyl or amino acids. From multidirectional studies relating to the structure of $[CuABH_{-1}]^-$,[6,10,11] it is clear that the chelate-forming B ligand may bind in two different ways to the complex [$CuAH_{-1}$] as illustrated below by (**I**). Data relating to the solid state indicate that axial–equatorial co-ordination of the B ligand prevails.[11] In solution an equilibrium probably exists between the two forms,[5] and for the mixed-ligand complexes of the dipeptides this equilibrium is shifted appreciably in the direction of (**Ia**).[10]



(*Ia*) (*Ib*)

In the present paper, the effect of mixed-ligand complex formation is studied from the aspect of how the presence of various B ligands influences the deprotonation of amide groups in different environments in peptides and acid amides, and also their participation in co-ordination. Accordingly, studies have been made on the parent and mixed-ligand complexes of copper(II) with glycinamide. In these systems the acid amide is co-ordinated only through two functional groups, and thus there is a possibility for the exclusive equatorial binding of the B ligand. Similarly to glycinamide, *N*-acetylhistidine has only two functional groups. In the absence of the terminal amino-group, the NHCO group of *N*-acetylamino acids does not in general take part in co-ordination.[5] However it has also been assumed that the co-ordination of the imidazole-N atom in *N*-acetylhistidine at pH ~ 10 induces deprotonation of the amide group.[12] Although this finding is not corroborated by earlier results,[13] a similar process is assumed in a bis(*N*-acetylhistidinato)cobalt(II) complex.[14]

By means of the present investigations, therefore, we hoped to obtain new data on the deprotonation processes for the parent and mixed-ligand systems of copper(II) with *N*-acetylhistidine and *N*-acetylhistamine. A study was also made on the mixed-ligand complexes of glycylglycinamide with copper(II). This compound binds in the equatorial plane through N,N,O or N,N,N co-ordination. For purposes of comparison, therefore, studies were carried out on the corresponding mixed-ligand complexes of glycylglycine.

Experimental

Glycinamide hydrochloride, glycylglycinamide hydrochloride, and DL-2,3-diaminopropionic acid hydrochloride were products of Fluka, while *N*-acetylhistidine and *N*-acetylhistamine were products of Sigma. Glycine, glycylglycine, L-histidine hydrochloride, histamine dihydrochloride, 2,2′-bipyridyl, and tiron (disodium 4,5-dihydroxybenzene-1,3-disulphonate) were products of Reanal.

In the studies on the parent complexes, the ligand concentration, c_A, was (1—8) × 10^{-3} mol dm^{-3}, while the metal ion:ligand ratio ranged from 1:1 to 1:4. For *N*-acetylhistidine, measurements were also made at copper(II)–ligand ratios of 1:6 and 1:8. pH-Metric titrations on the mixed-ligand systems were made at Cu^{2+}:A:B ratios of 1:1:1, 1:1:2, and 1:2:1. All measurements were carried out at 25 °C, at an ionic strength of 0.2 mol dm^{-3} (KCl). Details on the pH-metric measuring procedure used, and on the method of evaluating the data, were reported previously.[15] The error analysis suggests that the standard deviations are ±0.02 in p*K* and log *K* values and ±0.05 in the stability constants of mixed-ligand complexes. Measurements were made with a Radiometer pHM 64 pH-meter, with G202B glass and K401 calomel electrodes, and an ABU13 automatic burette.

The spectra of the copper(II)–*N*-acetylhistidine complexes were recorded in the visible spectral range on a Beckman Acta MIV spectrophotometer, under the conditions given in Table 5. E.s.r. spectra were recorded on a JEOL JES-ME-3x spectrometer at 120 K (9.12 GHz). Circular dichroism (c.d.) spectra were measured on a JASCO-J-2D automatic spectropolarimeter.

Results and Discussion

The stability constants of the copper(II) complexes of glycinamide (ga), glycylglycinamide (gga), *N*-acetylhistidine (nahd), and *N*-acetylhistamine (nahm) are listed in Table 1 where A denotes the fully deprotonated forms of the ligands (monoanion for gg and nahd; neutral for ga, gga, and nahm).

Allowing for the different experimental conditions, the values found earlier for ga,[16–18] gga,[6,17] and nahd [13] agree well with the present experimental results. The data show that ga first forms complexes $[CuA]^{2+}$ and $[CuA_2]^{2+}$ by co-ordination through the amine and carbonyl groups. On increasing the pH, the acid amide groups undergo deprotonation and the complexes $[CuA_2H_{-1}]^+$ and $[CuA_2H_{-2}]$ are formed (pK_1 = 7.07, pK_2 = 8.33). The binding in these complexes involves the amino-group and the deprotonated amide-N atom.

In the complex $[CuA]^{2+}$, the co-ordination of gga is similar to that of glycylglycine.[5] Subsequently, through deprotonation of the peptide-NH group, the species $[CuAH_{-1}]^+$ is formed. The p*K* value of 5.27 for this process is higher than the corresponding values for glycylglycine (4.23) and glycylhistidine (4.33).[15,1] This can be ascribed to the fact that beside the amino and the peptide-N, the carbonyl in the equatorial plane forms a weaker bond than does the carboxyl. With increasing the pH, the acid amide group is also deprotonated at p*K* = 8.08, and thus gga becomes co-ordinated *via* three N donor atoms in the complex $[CuAH_{-2}]$.[17] At pH > 10 a further base-consuming process appears; similarly as for glycylglycine, this can be ascribed to deprotonation of the co-ordinated water molecules. It should be noted that, as with copper(II) complexes of other dipeptides,[1,15] in a ligand excess the bis complexes $[CuA_2H_{-1}]^+$ and $[CuA_2H_{-2}]$ are also formed with gga.

In the copper(II)–nahd and copper(II)–nahm systems, a base consumption in excess of that required for the formation of complexes of the type $[CuA_n]$ was not observed at any metal ion:ligand ratio. At pH > 8, a precipitate was formed in all cases; this did not dissolve in excess base. With increasing the pH, a shift in colour towards violet was not observed. Thus, the precipitate formation can probably be attributed to hydrolysis of free metal ions. Accordingly, our observations do not support the earlier conception that complexes of the type $[Cu(AH_{-1})_n]^{(2n-2)-}$ are formed in appreciable concentration in the Cu^{II}–nahd system.[12] However, the stability constant data determined for the species of type $[CuA_n]^{(n-2)-}$ agree well with the results of Martin and Edsall,[15] and strongly suggest monofunctional co-ordination of nahd *via* the imidazole-N atom.

The stability constants of the mixed-ligand complexes of glycinamide are given in Table 2, where B denotes the fully

Table 1. Stability constants of the copper(II) complexes * of glycinamide (ga), glycylglycinamide (gga), *N*-acetylhistidine (nahd), and *N*-acetylhistamine (nahm); I = 0.2 mol dm^{-3} (KCl); T = 298 K; $pM + qA + rH \rightleftharpoons M_pA_qH_r$, $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$

	log β_{pqr}			
	ga	gga	nahd	nahm
HA	8.01	7.84	7.01	7.06
H_2A	—	—	9.82	—
[CuA]	5.30	5.09	4.23	3.97
$[CuA_2]$	9.56	—	7.76	7.05
$[CuA_3]$	—	—	10.42	10.12
$[CuA_4]$	—	—	12.12	12.14
$[CuAH_{-1}]$	—	−0.18	—	—
$[CuAH_{-2}]$	—	−8.26	—	—
$[CuAH_{-3}]$	—	−18.13	—	—
$[CuA_2H_{-1}]$	2.49	3.40	—	—
$[CuA_2H_{-2}]$	−5.84	−5.36	—	—
pK_1	7.07	5.27	—	—
pK_2	8.33	8.08	—	—

* Charges are omitted.

Table 2. Stability constants of mixed-ligand complexes of glycinamide; I = 0.2 mol dm^{-3} (KCl); T = 298 K; $pM + qA + rB + sH \rightleftharpoons M_pA_qB_rH_s$, $\beta_{pqrs} = [M_pA_qB_rH_s]/[M]^p[A]^q[B]^r[H]^s$

Ligand B[a]	log β_{1110}	log β_{111-1}	Δ log β_{1110}[b]	Δ log β_{111-1}[c]	pK[d]
Glycine	12.45	4.79	−0.05	−0.01	7.66
2,3-Diamino-propionic acid	14.97	7.25	−0.02	−0.04	7.72
Tiron	17.88	9.52	0.26	−0.40	8.36
Histamine	14.46	5.97	1.35	0.56	8.49
L-Histidine	14.98	6.75	0.99	0.46	8.23
2,2′-Bipyridyl	12.91	4.99	1.03	0.81	7.92

[a] Stability constants of parent complexes are given in ref. 20 for glycine, tiron, histamine, and L-histidine; in A. Gergely, E. Farkas, I. Nagypál, and E. Kas, *J. Inorg. Nucl. Chem.*, 1978, **40**, 1709, for 2,3-diaminopropionic acid; and in G. Anderegg, *Helv. Chim. Acta*, 1963, **46**, 2397, for 2,2′-bipyridyl. [b] Δ log β_{1110} = log β_{1110} − ½(log β_{1200} + log β_{1020}) + 0.3. [c] Δ log β_{111-1} = log β_{111-1} − ½(log β_{120-2} + log β_{1020}) + 0.3. [d] pK = log β_{1110} − log β_{111-1}.

Table 3. Stability constants of mixed-ligand complexes of glycylglycinamide (gga) and glycylglycine (gg); I = 0.2 mol dm^{-3} (KCl); T = 298 K

	gga			gg		
	Glycine	Histamine	Histidine	Glycine[a]	Histamine	Histidine
$\log \beta_{1110}$	—	14.26	14.65	—	15.00	15.31
$\log \beta_{111-1}$	5.37	5.77	6.02	5.29	6.12	6.66
$\log \beta_{111\ 2}$	—	−4.60	−4.44	—	—	—
pK_1[b]	—	8.49	8.63	—	8.88	8.65
pK_2[c]	—	10.37	10.46	—	—	—
$\log K_1'$[d]	5.55	5.95	6.20	3.96	4.79	5.33
$\log K_2'$[e]	—	3.66	3.82	—	—	—

[a] See ref. 15. [b] $pK_1 = \log \beta_{1110} - \log \beta_{111-1}$. [c] $pK_2 = \log \beta_{111-1} - \log \beta_{111-2}$. [d] $K_1' = [MABH_{-1}]/([MAH_{-1}][B])$. [e] $K_2' = [MABH_{-2}]/([MAH_{-2}][B])$.

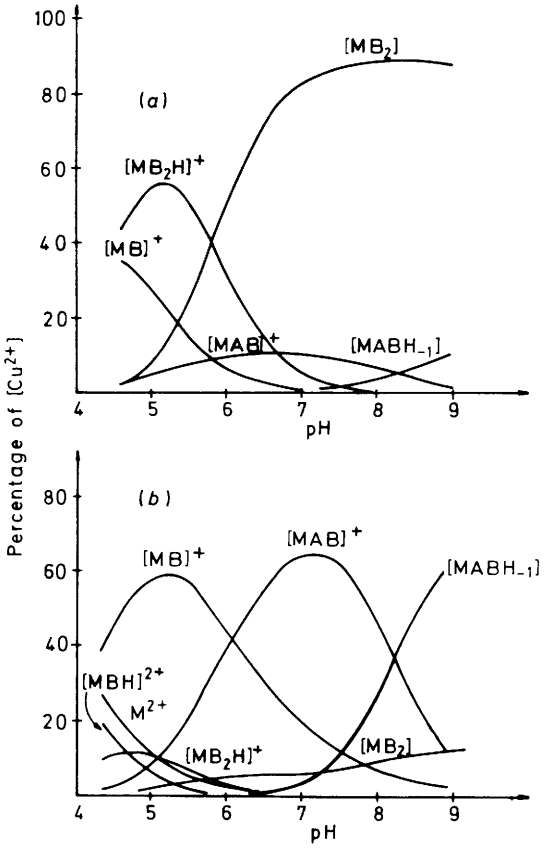


Figure 1. Concentration distribution of complexes formed in the copper(II)–glycinamide L-histidine system as a function of pH: (*a*) $c_{Cu^{2+}} = c_A = 2 \times 10^{-3}$ $c_B = 4 \times 10^{-3}$ mol dm^{-3}; (*b*) $c_{Cu^{2+}} = c_A = c_B = 2 \times 10^{-3}$ mol dm^{-3} (A = ga, B$^-$ = L-histidinate monoanion)

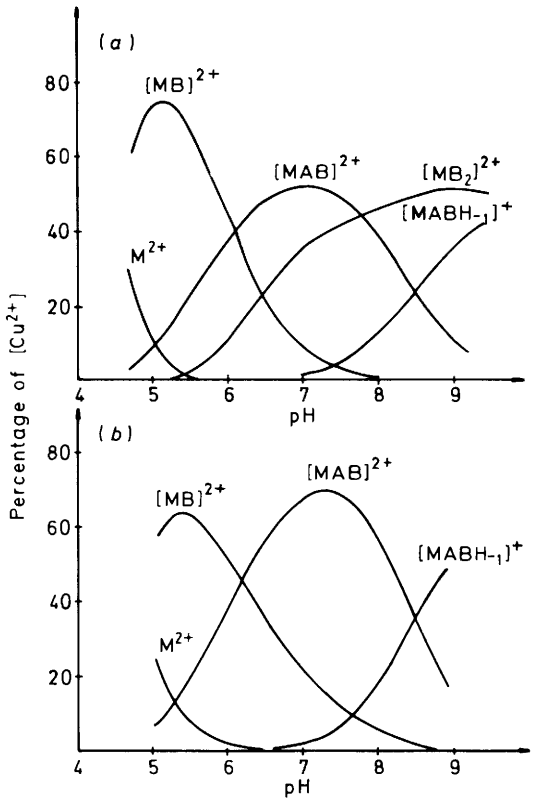


Figure 2. Concentration distribution of complexes formed in the copper(II)–glycinamide–histamine system as a function of pH: (*a*) $c_{Cu^{2+}} = c_A = 2 \times 10^{-3}$, $c_B = 4 \times 10^{-3}$ mol dm^{-3}; (*b*) $c_{Cu^{2+}} = c_A = c_B = 2 \times 10^{-3}$ mol dm^{-3} (A = ga, B = histamine)

deprotonated forms of the ligands (dianion for tiron; monoanion for glycine, L-histidine, and 2,3-diaminopropionic acid; neutral for histamine and 2,2′-bipyridyl). It follows from these data that there is considerable formation of mixed-ligand complexes for each of the B ligands. At lower pH values, complexes of the type [CuAB] are formed. Then, as the pH is increased, the species containing a deprotonated amide group, [CuABH$_{-1}$], are produced. The $\Delta \log \beta_{1110}$ values for [CuAB] are largest in the cases of histamine, L-histidine, and 2,2′-bipyridyl. This is in accordance with the findings[19,20] that coordination of ligands containing an aromatic N atom primarily promotes the binding of ligands containing O donor atoms. The extent of the stability increase is much smaller in the complexes of the type [CuABH$_{-1}$], which may be explained by the coordination of the glycinamide *via* two N atoms.[21] The formation of [CuABH$_{-1}$] becomes particularly unfavourable when tiron is the B ligand. Since the reaction between the copper(II) ion and the phenolic OH group is accompanied by the appearance of an intense charge-transfer band ($O^- \longrightarrow Cu^{2+}$), the result can only be interpreted by assuming a mutually weakening charge shift in the same direction between the deprotonated amide-N atom and the copper(II) ion.[22]

In the copper(II)–glycinamide–L-histidine and the copper(II)–glycinamide–histamine systems (Figures 1 and 2 respectively), there are striking differences between the concentration distributions of the individual systems at the different metal ion : ligand ratios. In the range pH > 6, at a Cu^{2+} : A : B ratio of 1 : 1 : 1, the presence of mixed-ligand complexes prevails for both B ligands,

Table 4. Stability constants of mixed-ligand complexes of *N*-acetylhistidine and *N*-acetylhistamine; I = 0.2 mol dm^{-3} (KCl); T = 298 K

	$\log \beta_{1110}$		$\Delta \log \beta_{1110}$		$\log \beta_{111-1}$		pK	
Ligand B	nahd	nahm	nahd	nahm	nahd	nahm	nahd	nahm
Glycine	11.96	11.78	0.36	0.53	—	—	—	—
2,3-Diaminopropionic acid	13.89	—	−0.20	—	—	—	—	—
Tiron	16.95	17.63	0.23	1.26	—	—	—	—
Histamine	13.14	—	0.93	—	5.28	—	7.86	—
Histidine	13.67	13.35	0.58	0.61	5.68	5.74	7.99	7.61
2,2′-Bipyridyl	12.13	11.71	1.15	1.07	4.25	4.47	7.88	7.24

and accordingly there is appreciable deprotonation and co-ordination of the amide. However, as Figure 1(*a*) shows, a histidine excess almost completely suppresses the co-ordination of glycinamide, and the copper(II)–histidine complex [CuB_2] is mainly formed. A similar phenomenon was observed earlier for the mixed complexes of glycyl-L-histidine with histidine.[2] With tiron and 2,3-diaminopropionic acid as the B ligands, which, similarly to histidine, form stable bis complexes, suppression of the deprotonation and co-ordination of glycinamide is likewise observed. At the same time, as Figure 2 shows, an excess of histamine does not exclude the formation of the species [CuAB]$^{2+}$ and [$CuABH_{-1}$]$^{+}$ in considerable concentration. However, the large log K_1/K_2 value of the copper(II)–histamine parent complex indicates that the co-ordination of the second histamine molecule in the bis complex is only slightly favoured.[2,3] The significant degree of mixed-ligand complex formation is therefore caused by the lower stability of the bis complex and by the considerable difference between the log K_1/K_2 values.[2,1]

The stability constants of the mixed-ligand complexes of glycylglycinamide and glycylglycine (gg) are given in Table 3. These data reveal that deprotonation of the peptide amide group occurs in both cases and that complexes of composition [$CuABH_{-1}$]$^{+}$ are formed. However, whereas the acidity of the peptide-NH group falls by about three orders of magnitude in the case of gga, with gg there is a decrease of more than four orders of magnitude. In both cases this is appreciably larger than the value of about one order of magnitude for ga, which explains the different co-ordination. For glycylglycine, structure (**I**) indicates that there is only one free equatorial site for binding of the B ligand. The situation is the same for gga, where the third co-ordination site in [$CuAH_{-1}$]$^{+}$ is occupied by a carbonyl group. However, the smaller increase of the pK_1 value compared to that for gg suggests that the equilibrium as in structure (**I**) is more shifted in the direction of (**Ib**). This assumption is supported by the log K_1' values for the process [$CuAH_{-1}$]$^{+}$ + B, this constant being substantially larger for gga than for gg. Glycylglycinamide, therefore, co-ordinating *via* the carbonyl group, permits the equatorial co-ordination of the B ligands to a greater extent than in the case of the simple dipeptides.

For gga at pH > 10, there is also a possibility for the formation of complexes [$CuABH_{-2}$]. Since this process is accompanied by a considerable blue shift of the spectrum, it is obvious that not hydrolysis, but co-ordination of gga through three N atoms occurs. Because of the relevant high pK value (pK_2), however, since the process coincides with the range of formation of [$CuAH_{-3}$]$^{-}$, the stability constants can be determined only with a relatively large error. In accordance with this, in the case of the B ligand glycine, which gives the complexes of lowest stability, the formation of [$CuABH_{-2}$]$^{-}$ is negligible and at pH > 10 the appearance of [$CuAH_{-3}$]$^{-}$ predominates. In the cases of histamine and histidine as the B ligands, the log K_2' values for the binding of B are substantially smaller than the log K_1' values. On this basis it is probable that in [$CuABH_{-2}$] the B ligands are predominantly in an axial–equatorial co-ordination, in accordance with the higher bond strength of the amide group compared to the carbonyl.

Table 5. Results of a spectrophotometric study of the copper(II)–*N*-acetylhistidine–histidine system; I = 0.2 mol dm^{-3} (KCl); T = 298 K

$10^3 c_{Cu^{2+}}$/ mol dm^{-3}	$10^3 c_{nahd}$/ mol dm^{-3}	$10^3 c_{histidine}$/ mol dm^{-3}	$c_{KOH}/c_{Cu^{2+}}$	$\lambda_{max.}$/nm
2	4	0	2	704
2	0	4	2	637
2	2	2	2	647
			3	617
2	2	4	3	637
			4	635

It must also be noted that the conditions outlined above hold only at a Cu^{2+}:A:B ratio of 1:1:1. Similarly to that observed for ga, an excess of the B ligand histidine almost completely displaces the peptide molecules (gga and gg) from the co-ordination sphere of the copper(II) ion.

As already mentioned in connection with the parent complexes, we could not demonstrate deprotonation and co-ordination of the amide groups in the copper(II)–nahd and copper(II)–nahm systems. However, a study of the mixed-ligand complexes led to very interesting results. The relevant equilibrium data are given in Table 4. The data show that the B ligands can be classified in two groups. In accordance with expectations, for glycine, 2,3-diaminopropionic acid, and tiron (which do not contain an aromatic N donor) only the mixed-ligand complexes of type [CuAB] are formed in which the amide groups are not co-ordinated. At the same time, in the cases of histamine, histidine, and 2,2′-bipyridyl a new base-consuming process appears; this can be ascribed to formation of the species of the type [$CuABH_{-1}$] (charges omitted).

The spectrophotometric data in Table 5 confirm that the base consumption is not the result of hydrolysis; in the presence of these B ligands, deprotonation and co-ordination of the amide groups occur. The absorption maxima of the [CuA_2] complexes of nahd and of histidine were found at 704 and 637 nm, respectively. In agreement with this, after total titration of the ligands in the 1:1:1 copper(II)–nahd–histidine system, the absorption maximum appears at 647 nm; this can be ascribed to the complex [CuAB]. The subsequent base-consuming process is accompanied by a considerable blue shift ($\lambda_{max.}$ = 617 nm), which therefore excludes the possibility of hydrolysis.

The c.d. and e.s.r. measurements likewise point to the co-ordination of four N atoms in the range 8 ⩽ pH ⩽ 10 in the 1:1:1 copper(II)–nahd–histidine system. The characteristic parameters in the e.s.r. spectrum attributable to the species [$CuABH_{-1}$]$^{-}$ are $A_{\parallel}$ = 186 G, $g_{\parallel}$ = 2.242, and $g_{\perp}$ = 2.09. The spectrum clearly reveals the nine-line superhyperfine splitting, originating from the four N atoms, with A_N = 15 G.

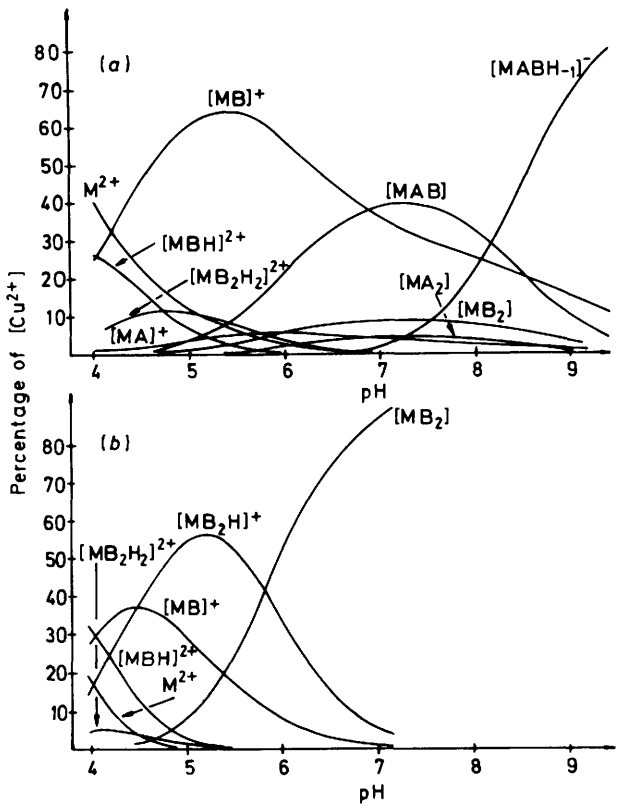


Figure 3. Concentration distribution of complexes formed in the copper(II)–nahd–L-histidine system as a function of pH: (*a*) $c_{Cu^{2+}} = c_A = c_B = 2 \times 10^{-3}$ mol dm^{-3}; (*b*) $c_{Cu^{2+}} = c_A = 2 \times 10^{-3}$, $c_B = 4 \times 10^{-3}$ mol dm^{-3} (A^- = nahd, B^- = L-histidine)

Three characteristic transitions can be identified in the corresponding c.d. spectrum. The broad band at 300 nm ($\Delta\varepsilon = -0.1$ dm^3 mol^{-1} cm^{-1}) includes the charge-transfer transition $N^- \longrightarrow Cu^{II}$. At the same time, the transitions at 705 nm ($\Delta\varepsilon = +0.24$ dm^3 mol^{-1} cm^{-1}) and 520 nm ($\Delta\varepsilon = -0.015$ dm^3 mol^{-1} cm^{-1}), together with the visible spectrum at relatively low energy (617 nm), suggest a distortion from the planarity exhibited by the other Cu^{II}–4N complexes.[24]

Our results indicate that the presence of certain B ligands in the co-ordination sphere of the copper(II) ion allows the deprotonation and co-ordination of the acid amide groups in nahd and nahm. A comparison of the data in Table 4 with those of the mixed-ligand complexes of glycinamide (Table 2) shows that in both cases the co-ordination of the acid amide is favoured over that of ligands containing an aromatic N atom. Since a charge transfer in the direction metal ion ⟶ ligand (back co-ordination) may be assumed in the copper(II) complexes of histamine, histidine, and 2,2′-bipyridyl,[20] the charge transfer amide-N ⟶ copper(II) is also probable. The high stability of the copper(II)–nahd–2,2′-bipyridyl (or histidine) complexes can therefore be interpreted in a similar way as for the copper(II)–2,2′-bipyridyl(or histidine)–catechol complexes.[16]

Figure 3 depicts the concentration distribution at various metal ion:ligand ratios in the copper(II)–nahd–histidine system. It demonstrates that the complex $[CuABH_{-1}]^-$ is formed only at a ratio of 1:1:1, and a histidine excess completely suppresses the deprotonation and co-ordination of the amide group. In accordance with this, excess base consumption cannot be observed in solutions with the composition 1:1:2, and Table 5 indicates that the spectrum of the solution is identical with that of the $[CuA_2]$ complex of copper(II)–histidine.

The results obtained previously[2] and in the present work, show that the metal ion-induced deprotonation of the acid amides is facilitated by the presence of an equivalent amount of B ligands which form stable bis complexes (*e.g.* histidine, histamine, or 2,2′-bipyridyl), but inhibited by an excess of them. As the biological systems may contain stable chelate-formers in comparatively high concentration, besides the peptide-bonded molecules, this observation may be one of the possible explanations of why the metal ion–peptide–N bond arises only rarely under biological conditions.

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