Studies on Transition-metal–Peptide Complexes. Part 6.† Influence of Side-chain Donor Group on the Equilibrium and Thermodynamics of Binary and Ternary Copper(II)–Dipeptide Complexes

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Copper(II) binary and ternary complexes of dipeptides containing amide carbonyl, alcoholic hydroxy, and carboxy groups in their side-chain have been studied. The dipeptides (A ligands) were glycyl-L-serine (GlySer), glycyl-L-asparagine (GlyAsn), glycyl-L-aspartic acid (GlyAsp), and glycyl-L-glutamic acid (GlyGlu). In the case of copper(II)–dipeptide ternary complexes, L/D-α-alanine (α-Ala), β-alanine (β-Ala), L/D-aspartic acid (Asp), L/D-ornithine (Orn), and 2,2′-bipyridyl (bipy) were used as B ligands. pH-Titrimetry and calorimetry were applied to determine the stability constants and the enthalpy and entropy changes of the species formed in the systems studied. It was assumed that the carboxylate group in the GlyAsp side-chain co-ordinates in the axial position to copper(II). Above pH 10, deprotonation of the amide carbonyl in GlyAsn takes place, accompanied by its bonding in the square plane to copper(II). A [CuAB] type complex is formed in high concentration, mainly with bipy as B ligand. However, [$CuABH_{-1}$] type deprotonated species are also formed in the case of copper(II)–dipeptide–amino-acid systems. Stereoselectivity in this complex was observed only with Asp and Orn. This effect may presumably be attributed to electrostatic interactions. Thermodynamic data support the assumption of bipy occupying two equatorial positions in [CuAB] species, and an equatorial and an axial position in [$CuABH_{-1}$].

STUDIES on binary and ternary complexes of peptides are continuing with ever greater intensity.[1,2] Attention is becoming increasingly focused on the side-chain donor groups, with regard to how they influence complex formation. Martin and co-workers [3] have established that in basic solution the side-chain amide of glycylasparagine undergoes hydrogen ionization, followed by amide nitrogen bonding to copper(II). No such effect was observed for the alcoholate group of glycylserine. However, at least in the case of L-alanyl-L-serine, Kozlowski and Siatecki [4] concluded that at high pH the carboxylate oxygen is replaced by the deprotonated alcoholate (O^-) donor atom in the bonding to copper(II). From stability data Sigel *et al.*[5] also suggested the participation of the non-deprotonated hydroxy group in the complex formation.

As regards deprotonation of the peptide NH in the copper(II)–dipeptide–2,2′-bipyridyl (bipy) ternary system, Sigel [6] found that this group varies considerably in acidity. The pK value of around 4 in the parent complexes is increased to *ca.* 8 in the ternary system. He measured the same pK values for deprotonation of the glycinamide and glycylglycine ternary complexes containing bipy. From this coincidence the conclusion was drawn that bipy prevents the co-ordination of carboxylate oxygen in the square plane to copper(II). However, Martin and co-workers [7] used X-ray diffraction to determine the structure of the glycylglycinato–1,10-phenanthroline–copper(II) complex, and reported that the glycylglycinate acts as a tridentate ligand, co-ordinating to the copper(II) in one plane *via* amino and ionized amide nitrogens and carboxylate oxygen donor atoms. Hence, the 1,10-phenanthroline occupies only one equatorial and one axial position in the co-ordination sphere of copper(II). The visible absorption spectra indicate that this structure persists in solution. The same co-ordination arrangement was suggested by Martin and co-workers [7] also for the glycylglycinato–bipy–copper(II) complex in solution.

We have already studied some binary and ternary [8] complexes of simple dipeptides containing amino-acids as B ligands, and established that complexes of type [$CuABH_{-1}$] are formed in the highest concentrations. An equatorial and an axial co-ordination were assumed for complexes containing two ligands. In mixed complexes the amino-acids were found to occupy one equatorial and one axial position. With aspartic acid, the [CuAB] type mixed complexes were formed in relatively high concentration. This phenomenon was attributed, in part, to the co-ordination of both carboxylate oxygens to copper(II), which means that one of the peptide donor atoms is displaced from the square plane of the co-ordination sphere.

To acquire more information on the co-ordination possibilities of dipeptides in their copper(II) complexes, we have dealt in this work with the following dipeptides: glycyl-L-serine (GlySer), glycyl-L-asparagine (GlyAsn), glycyl-L-aspartic acid (GlyAsp), and glycyl-L-glutamic acid (GlyGlu). In the copper(II)–dipeptide–B ligand ternary systems the B ligands were as follows: L/D-α-alanine (α-Ala), β-alanine (β-Ala), L/D-aspartic acid (Asp), L/D-ornithine (Orn), and bipy.

EXPERIMENTAL

GlySer, GlyAsn, and GlyGlu were purchased from Sigma; GlyAsp was a Fluka product. With the exception of GlyAsn, all dipeptides were used without any purification. GlyAsn was purified by recrystallization from water–ethanol and dried at 25 °C. α-Ala, β-Ala, Asp, Orn, glycinamide (Glam), and bipy were Reanal products. The amino-acids and Glam were applied without any purification. A stock solution of bipy was made after repeated recrystallization from water, followed by dissolution in two equivalents of HCl of known concentration. D-Orn was ob-

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tained from DL-Orn by resolution with the method of Alberton [9] through the enzyme, papain.

Concentrations of the dipeptide, amino-acid, and Glam stock solutions were checked by applying the Gran [10] function, as described earlier.[8] A stock solution of copper(II) was made as reported earlier.[8]

A Radiometer pHM 72 pH-meter was used to determine the stability constants. The calibration of the electrodes and the titration were performed as described previously.[8] The titrants were of known concentrations (*ca.* 0.2 mol dm^{-3} KOH and *ca.* 0.2 mol dm^{-3} HCl).

To determine the stability constants of copper(II)–dipeptide complexes, titrations were performed at five different metal : ligand ratios (1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5). Concentrations of GlySer and GlyAsn were 0.005 mol dm^{-3} and those of GlyAsp and GlyGlu, 0.006 mol dm^{-3}. The copper(II) concentrations depended on the metal : ligand ratio. Stability constants of copper(II)–amino-acid binary complexes had already been determined; [11,12] hence, during this work they were only checked, by measuring a sample series of 1 : 2 metal : ligand ratios, applying a ligand concentration of 0.006 mol dm^{-3}.

In studies on the ternary complexes, titrations were performed at metal : ligand A : ligand B ratios of 1 : 1 : 1, 1 : 1 : 2, and 1 : 2 : 1. The copper concentration was in each sample 0.006 mol dm^{-3}, while the concentrations of the ligands depended on the metal : ligand ratios.

All titrations were carried out at an ionic strength I = 0.2 mol dm^{-3} (KCl) and in the range pH *ca.* 3—11.5 at 25 °C; in each case 250—350 experimental points were utilized for the calculations.

Calorimetric measurements were performed on an LKB 8702-2 titration calorimeter, using the continuous titration method already described.[13] To obtain the formation heat for the copper(II)–bipy binary complex, the titrant was a 0.2 mol dm^{-3} bipy solution containing an equivalent amount of HCl. The titration vessel contained 0.008 mol dm^{-3} copper(II) and 0.2 mol dm^{-3} KCl. In other cases the titrant was always 0.2 mol dm^{-3} KOH solution.

The deprotonation heats of the peptide carboxy groups were found through titration after adding to them one equivalent of acid. For determination of the formation heats of parent complexes, titrations were performed at metal : ligand ratios of 1 : 1 and 1 : 2; for those of ternary complexes, metal : ligand A : ligand B ratios of 1 : 1 : 1 were used. In each case the dipeptide and Glam concentrations were 0.008 mol dm^{-3}.

In the case of copper(II) dipeptides, visible spectrophotometric examinations were carried out on a Beckman ACTA MIV double-beam recording spectrophotometer. The concentrations of the peptides were 0.005 mol dm^{-3} and the metal : ligand ratio was 1 : 2. The pH of the samples was also measured by using a flow cell.

The stability constants and the thermodynamic data were calculated as already reported.[8,13]

RESULTS AND DISCUSSION

Stability Constants and Thermodynamic Data relating to the Copper(II)–Dipeptide Systems.—The overall stability data on both the proton and copper(II) complexes of the dipeptides are listed in Table 1. For comparison, Table 1 also contains the data relating to the proton and copper(II) complexes of glycyl-DL-α-alanine (GlyAla).[8]

Table 2 presents the enthalpy and entropy changes obtained for most of the species shown in Table 1. [The values for the copper(II)–GlyAla system were also published earlier.[8]] Table 2 also includes the data for the copper(II)–bipy complexes.

It can be stated from Table 1 that the pK values of the GlySer and GlyAsn carboxy groups decrease to some extent, because of the electron-withdrawing effects of the hydroxy and amide carbonyl groups. The electron densities on the two carboxy groups of GlyAsp and GlyGlu are similar to each other. However, it can be assumed that the carboxy groups at the end of the chain are more basic than those in the chain. The values we obtained for the copper(II)–bipy system are in good agreement with those published by Anderegg.[14]

Complex formation with dipeptides begins in general with the co-ordination of the donor atoms of the terminal NH_2 group and the carbonyl oxygen of the neighbouring amide group. Nevertheless, in the cases of GlyAsp and GlyGlu, CuA complex formation and deprotonation of the carboxy group take place in the same pH range and partly overlap each other. In these cases, therefore, CuAH type complexes are also formed. The pK values for these species were evaluated as follows: 3.80 for GlyAsp (where pK_{COOH} = 4.31) and 3.64 for GlyGlu (where pK_{COOH} = 4.36). However, without a knowledge of the deprotonation micro-constants of these groups, these equilibrium data alone are not enough for con-

TABLE 1

Species and their formation constants relating to the proton and copper(II)–dipeptide [a]

	log β				
	GlySer	GlyAsn	GlyAsp	GlyGlu	GlyAla [b]
HA	8.09	8.12	8.35	8.27	8.20
H_2A	11.03	11.23	12.66	12.63	11.37
H_3A			15.45	15.49	
[CuAH]			10.41	9.95	
[CuA]	5.66	5.99	6.61	6.31	5.76
[$CuAH_{-1}$]	1.68	1.67	1.85	1.46	1.55
[$CuAH_{-2}$]	−7.67	−7.11	−7.97	−7.87	−7.94
[$CuAH_{-3}$]		−17.91			
[CuA_2H_{-1}]	4.64	4.72	4.54	4.33	4.63
[$Cu_2A_2H_{-3}$]	−3.80	−2.70	−4.20	−4.80	−4.18
[CuA_2]			*ca.*11.50		

[a] Temp. = 25 °C, I = 0.2 mol dm^{-3} (KCl); $\beta_{qpr} = [Cu_qA_pH_r]/[Cu]^q[A]^p[H]^r$. [b] See ref. 8.

clusions to be drawn on the participation of the carboxylate groups in the complex formation.

Comparison of the stability constants of the CuA type complexes, taking also into account the basicities of the terminal amino-groups, reveals that they increase in the following sequence: GlySer ~ GlyAla < GlyAsn < GlyGlu < GlyAsp (see Table 1). The charge neutralization may also play a role in this sequence (see the GlyAsp and GlyGlu ligands). However, the phenomenon is surprising for GlyAsn as neither the spectrophotometric nor the calorimetric measurements give any hint of participation of the amide carbonyl group in the complex formation. In fact, in Table 2 the ΔH values relating to Cu–GlyAsn and Cu–GlyAla are seen to be almost the same. Though the contribution of the copper(II) carboxylate bond to the ΔH value is in any case very small,[15] a weak bond may be assumed between the oxygen of the amide carbonyl or the carboxy group of the side-chain and the metal, as is the case with the copper(II)–Asp and copper(II)–Asn systems.[11,12]

On the basis of potentiometric titrations, Kittl and Rode[16] recently supposed that complexes $[CuA_2]$ ($[CuA_2H_2]$) and $[CuA_2H_{-2}]$ ($[CuA_2]^{2-}$) are also formed in the systems in the case of aliphatic dipeptides. As we have also discussed in detail,[8] $[CuA_2]$ complex formation may indeed be assumed, but its concentration is no higher than 2—5%. As for the $[CuA_2H_{-2}]$ species, calculations have shown that formation of this species to an extent of 10—15% is, in general, undetectable. These findings are supported both by data to be found in the literature[5,6,17] and by the thermodynamic data in Table 2, as well as in our earlier work.[8]

To permit more conclusions from the equilibrium data, the stepwise formation constants were derived. The values obtained are given in Table 3.

In the range pH *ca.* 4—8 the $[CuAH_{-1}]$ species exists, in general[6] in the highest concentration and is formed according to the equilibrium $[CuA] \rightleftharpoons [CuAH_{-1}] + H$. This process, as in the cases of other simple dipeptides, is well known to be accompanied by a rearrangement of the bonding: in the $[CuAH_{-1}]$ complexes the NH_2 nitrogen, the deprotonated peptide N–, and the carboxylate oxygen donor atoms are co-ordinated in the square plane to copper(II).

Concerning the deprotonation constants, there is a considerable decrease in that of the GlySer-containing CuA complex. This fact may be explained by assuming a weak equatorial co-ordination of the alcoholic group to copper(II). The increased stability makes deprotonation of the peptide NH much easier. With GlyAsn, GlyAsp, and GlyGlu, deprotonation is hindered either by side-chain donor group co-ordination in the plane, or by a higher electron density on the CuA species.

Depending on the metal : ligand ratios, complex formation takes place in different ways.

(*i*) When the metal : ligand ratio is 1 : 1, at pH *ca.* 9 a $[Cu_2A_2H_{-3}]$ type complex is formed in low concentration, presumably in the following way: when the hydroxide ion is in excess, it co-ordinates to $[CuAH_{-1}]$ in the equatorial position, and at the same time bridges two $[CuAH_{-1}]$ species to form $[Cu_2A_2H_{-3}]$. Our ^1H n.m.r. measurements show that this complex is antiferromagnetic in character. If the pH is increased to 10, *i.e.* when enough OH^- is present, the mixed hydroxo-complex $[CuAH_{-1}(OH)]$ too is formed in high concentration with these dipeptides.

TABLE 2

Thermodynamic data on the proton and copper(II)–dipeptide and –bipy systems *

	$-\Delta H$	ΔS	$-\Delta H$	ΔS	$-\Delta H$	ΔS	$-\Delta H$	ΔS	$-\Delta H$	ΔS	$-\Delta H$	ΔS
	GlySer		GlyAsn		GlyAsp		GlyGlu		bipy		GlyAla	
HA	45	4	44	8	45	9	45	7	17	26	45	6
H_2A	45	60	45	64	51	69	54	61			47	60
H_3A					56	106	64	82				
[CuAH]					85	−86						
[CuA]	29	7	28	21	22	53	17	64	47	−1	27	19
$[CuAH_{-1}]$	5	5	1	29	4	22	0	28			3	22
$[CuA_2H_{-1}]$	29	−8	29	−7	27	−4	26	−4			24	−8
$[Cu_2A_2H_{-3}]$	−50	95	−42	86	−42	60	−43	53			−37	44
$[CuAH_{-2}]$	−84	135	−64	79	−63	59	−64	64			−48	9
$[CuA_2]$									74	126		

* Temp. = 25 °C, I = 0.2 mol dm^{-3} (KCl); ΔH values are given in kJ mol^{-1} and ΔS in J K^{-1} mol^{-1}.

TABLE 3

Stepwise formation constants of the species present in the copper(II)–dipeptide systems [a]

	log K				
Equilibrium process	GlySer	GlyAsn	GlyAsp	GlyGlu	GlyAla [b]
$Cu^{2+} + HA \rightleftharpoons [CuAH]$			6.10	5.59	
$Cu^{2+} + A \rightleftharpoons [CuA]$	5.66	5.99	6.61	6.31	5.76
$[CuA] \rightleftharpoons [CuAH_{-1}] + H$	−3.78	−4.38	−4.82	−4.88	−4.22
$[CuAH_{-1}] + A \rightleftharpoons [CuA_2H_{-1}]$	2.96	3.05	2.58	2.87	3.08
$[CuAH_{-1}] + OH \rightleftharpoons [CuAH_{-1}(OH)]$	4.40	4.97	3.90	4.42	4.33
$[CuAH_{-1}(OH)] \rightleftharpoons [CuAH_{-2}(OH)] + H$		−10.80			

[a] Temp. = 25 °C, I = 0.2 mol dm^{-3} (KCl). [b] See ref. 8.

(*ii*) If the metal : ligand ratio is 1 : 2 or higher, the [CuA_2] type complex will temporarily appear at pH *ca.* 5—9, in a measurable concentration (10—15%) only in the case of the copper(II)–GlyAsp system. With all other dipeptides the [CuA_2H_{-1}] complex is the species formed in relatively high concentration (25—50%). With the exception of copper(II)–GlyAsn, the equilibrium constants for the process [$CuAH_{-1}$] + A ⇌ [CuA_2H_{-1}] are all lower than for the copper(II)–GlyAla system (see Table 3). This finding may presumably be attributed to the co-ordination of the side-chain donor group to [$CuAH_{-1}$] in its axial position. Further, [$CuAH_{-1}$] is negatively charged and hence the co-ordination of a ligand A with a charge of −2 is unfavourable.

When the pH is increased above 10, a new alkali consumption process begins in the copper(II)–GlyAsn system and at the same time the absorbance maximum is shifted considerably towards lower wavelength (from *ca.* 630 to *ca.* 570 nm). This phenomenon resembles that observed for the copper(II)–Asn system.[12] Consequently, here too the formation of a mixed hydroxo-complex is assumed, its composition being [$CuAH_{-3}$]. In this species the deprotonated amide carbonyl group displaces the carboxy group from one of the equatorial positions. It is worth mentioning that the analogous deprotonation process did not occur until pH *ca.* 11.5 in the copper(II)–GlySer system.

Ternary Copper(II)–Dipeptide Systems.—In connection with the studies of ternary complexes, in the course of this work we have determined the stability constants of binary copper(II) complexes of optically pure α-alanine, aspartic acid, and ornithine. The results obtained for both the binary and ternary complexes are given in Tables 4 and 5. The values determined in this work for the complexes of the L and D forms of amino-acids are in good agreement with both each other and those already found for the racemic ligands. The stability constants relating to the copper(II)–bipy system in Table 4 are taken from the work of Anderegg.[14]

It can be seen from Table 5 that [CuABH] mixed-ligand complexes are also formed in the case of Orn. Such ternary complexes exist at pH *ca.* 5.5—7.5. However, their concentration maxima do not reach 25% in any of the investigated systems. As regards the bonding donor atoms in the [CuABH] species, it was assumed that the amino-nitrogen and the oxygen of the amide carbonyl in the dipeptides, as well as the carboxylate oxygen and the α-amino-nitrogen, co-ordinate to copper(II) in the square plane, leaving the ω-amino-group of Orn in protonated form.

[CuAB] ternary complexes are always formed when bipy is present as the B ligand. On the other hand, with amino-acids, the [CuAB] concentration does not exceed 10—15%. Therefore, the Δ log K values defined by Sigel [18] as log K^{CuA}_{CuAB} − log K^{Cu}_{CuB} were calculated only for the system containing bipy. The Δ log K data evaluated, with the exception of GlyAsp and GlySer, vary approximately in the same way as those obtained by Sigel *et al.*[5] for the [CuAB] complexes of dipeptides with a donor group in the C-terminal side-chain. (The type of co-ordination in these complexes will be mentioned later.)

In the copper(II)–dipeptide (A)–Orn (B) systems the pK values for the equilibrium process [CuABH] ⇌ [CuAB] + H are about 7. The acidity of the NH group in other copper(II)–dipeptide mixed-ligand complexes is similarly close to 7.[5,6] From this fact, the assumption is made that the amide group deprotonates in the [CuAB] complexes containing Orn as B ligand, and the terminal amino-group is in the protonated form. Hence, the

TABLE 4

Stability constants (log β) of the proton and copper(II) complexes of amino-acids and bipy

	log β							
	HA	H_2A	H_3A	[CuAH]	[CuA]	$[CuA_2H_2]$	$[CuA_2H]$	$[CuA_2]$
L/D-α-Ala	9.71	12.04			8.06			14.74
β-Ala	10.14	13.56			6.91			12.36
L/D-Asp	9.62	13.31	15.26	12.46	8.85			15.83
L/D-Orn	10.52	19.35	21.42	17.92		34.60	25.40	15.83
bipy *	4.49				8.00			13.60

* See ref. 14.

TABLE 5

Stability constants (log β_{111}) of the mixed-ligand complexes of the copper(II)–dipeptide (A)–ligand (B) systems *

A ligand	[CuABH]	[CuAB]	[$CuABH_{-1}$]	[CuABH]	[CuAB]	[$CuABH_{-1}$]	[CuABH]	[CuAB]	[$CuABH_{-1}$]	[CuABH]	[CuAB]	[$CuABH_{-1}$]
B ligand	GlySer			GlyAsn			GlyAsp			GlyGlu		
L-α-Ala			5.24			5.38			5.07			5.04
D-α-Ala			5.16			5.32			4.96			5.13
β-Ala			5.17			5.18			4.87			4.88
L-Asp		13.52	5.53		13.55	5.81			4.85		13.48	5.10
D-Asp		13.37	5.48		13.59	5.76			4.90		13.73	5.26
L-Orn	23.19	15.78	5.65	23.09	15.77	5.76	23.67	15.53	5.07	23.42	15.73	5.59
D-Orn	23.20	15.54	5.45	23.07	15.53	5.63		15.14	4.85	23.99	15.31	4.66
bipy		13.80	5.95		13.90	6.03		14.07	5.63		14.12	5.78

* Temp. = 25 °C, I = 0.2 mol dm^{-3} (KCl); β_{111} = [CuAB]/[Cu][A][B].

[CuAB] complexes are in fact $[Cu(AH_{-1})(HB)]$ species. Otherwise, in all cases studied the $[CuABH_{-1}]$ complex is present in measurable concentration.

In contrast to all other ternary complexes studied, a noteworthy phenomenon was found when Asp and Orn were applied as B ligands. This is shown in Figures 1 and 2, where the distributions of the species formed in

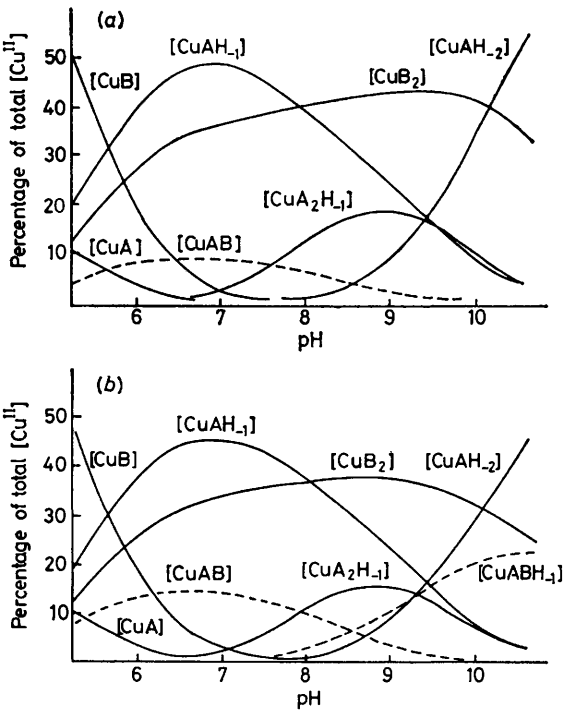

FIGURE 1 pH-dependent percentage concentration distributions of species formed in (*a*) the copper(II)–GlyGlu–L-Asp system and (*b*) the copper(II)–GlyGlu–D-Asp system in the pH range 5.5—10.5; [A ligand] = [B ligand] = [copper(II)] = 6×10^{-3} mol dm^{-3}

the copper(II)–GlyGlu–L/D-Asp and copper(II)–GlyGlu–L/D-Orn systems are represented.*

It is clear from Figure 1 that ternary complex formation in the copper(II)–GlyGlu–D-Asp system is more favoured than in the system containing L-Asp. However, the trend in ternary complex formation is the reverse with L/D-Orn as B ligands, as is evident from Figure 2. This phenomenon may presumably be explained by the different electronic interactions in the complexes, since in the case of L-Asp the less favoured ternary complex formation might arise from the repulsion between the side-chain carboxylate groups of the two ligands. On the other hand, with L-Orn, ternary complex formation is enhanced by a hydrogen bond between the side-chain carboxylate group of GlyGlu and the terminal amino-group of Orn. Naturally, a more adequate clarification of this stereoselectivity requires further investigations.

* The maximum concentration of the $[CuABH_{-1}]$ species in the copper(II)–GlyGlu–L-Asp system is less than 5%, therefore, it is not represented in Figure 1.

To characterize in more detail the equilibrium process in which the $[CuABH_{-1}]$ complex is formed, the derived constants were also evaluated; these are presented in Table 6.

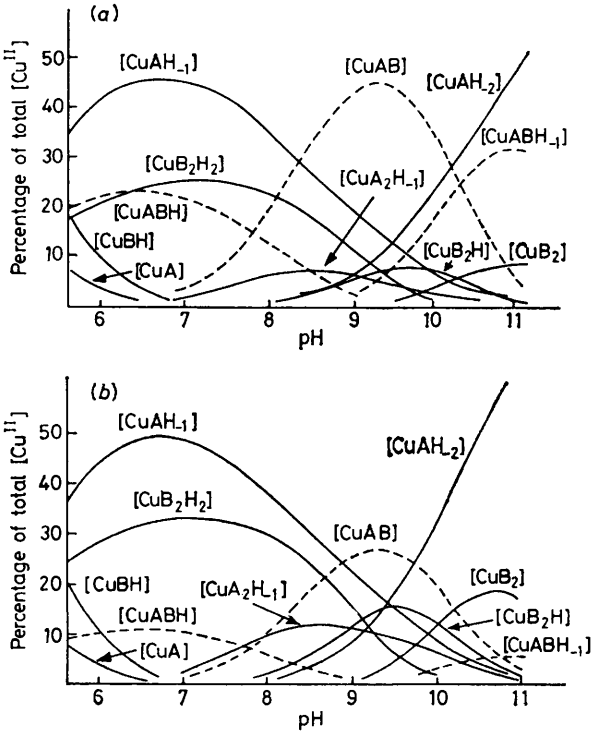

FIGURE 2 pH-dependent percentage concentration distributions of species formed in (*a*) the copper(II)–GlyGlu–L-Orn system and (*b*) the copper(II)–GlyGlu–D-Orn system in the pH range 6—11; [A ligand] = [B ligand] = [copper(II)] = 6×10^{-3} mol dm^{-3}

It is clear from Table 6 that the constants relating to the process $[CuA_2H_{-1}] + B \rightleftharpoons [CuABH_{-1}] + A$ are smaller for GlyAsp than for the other dipeptides. This observation supports the assumption that mixed-complex formation with GlyAsp is in any case less favoured. The phenomenon may be interpreted by the relatively strong co-ordination of the side-chain carboxylate group of GlyAsp in the axial position. Hence, ternary complex formation takes place only if the carboxylate is directed away from the co-ordination sphere.

Equilibrium constants for the processes $[CuAH_{-1}] + B \rightleftharpoons [CuABH_{-1}]$ are approximately 1—2 log units higher than log K_4 for copper(II)–amine systems.[2] This finding verifies both the above assumption and the conclusion that the amino-acids behave as bidentate ligands in the ternary complexes. At the same time, it is worthy of note that the values relating to the ternary complexes formed with α-Ala and β-Ala are almost the same. Consequently, from this fact it may also be concluded that the amino-acids occupy two equatorial sites in the co-ordination sphere of copper(II). If this were not the case, then the ternary complex containing α-Ala should be more stable than that of β-Ala.[2] This finding is in good agreement with our assumption [8]

TABLE 6

Derived constants for the mixed-ligand copper(II)–dipeptide (A)–ligand (B) systems

A ligand / B ligand	$[CuA_2H_{-1}] + B \rightleftharpoons [CuABH_{-1}] + A$				$[CuAH_{-1}] + B \rightleftharpoons [CuABH_{-1}]$				$[CuAB] \rightleftharpoons [CuABH_{-1}] + H$				
	GlySer	GlyAsn	GlyAsp	GlyGlu	GlySer	GlyAsn	GlyAsp	GlyGlu	GlySer	GlyAsn	GlyAsp	GlyGlu	Glam
L-α-Ala	0.60	0.66	0.53	0.71	3.56	3.64	3.22	3.58					
D-α-Ala	0.52	0.60	0.46	0.79	3.48	3.58	3.11	3.66					
β-Ala	0.53	0.46	0.33	0.55	3.49	3.44	3.02	3.42					
L-Asp	0.89	1.09	0.31		3.85	4.07	3.00		7.99	7.74			
D-Asp	0.84	1.04	0.36	0.93	3.80	4.02	3.05	3.80	7.89	7.83		8.47	
L-Orn	0.62	0.69	0.53	0.88	3.56	3.58	3.02	3.75	7.41	7.32		7.69	
D-Orn	0.31	0.22	0.31		3.27	3.27	2.70		7.66	7.54		7.68	
bipy	1.31	1.31	1.09	1.45	4.27	4.36	3.78	4.32	7.85	7.87	8.44	8.34	7.71

concerning ternary copper(II) complexes of simple dipeptides, *i.e.* co-ordination in the square plane to copper(II) by three donor atoms of the dipeptide, while donor groups of the amino-acid complete the equatorial plane and occupy one apical position.

The deprotonation constants were evaluated for all the systems studied in this work when [CuAB] and [CuABH$_{-1}$] complexes were formed in detectable concentrations. The data obtained for the equilibrium process $[CuAB] \rightleftharpoons [CuABH_{-1}] + H$ are reported in Table 6. The values relating to the copper(II)–dipeptide–bipy, copper(II)–dipeptide–amino-acid, and copper(II)–Glam–bipy systems are very similar, hence, in the first approximation, for the dipeptides, they might also suggest the same type of co-ordination in all the ternary complexes.

Calorimetric results may provide some more information on the bonding mode in the mixed species. The thermodynamic data on the copper(II)–dipeptide–bipy and copper(II)–Glam–bipy systems are given in Table 7.

TABLE 7

Enthalpy and entropy changes for the copper(II)–dipeptide–bipy mixed-ligand systems [a]

Complex	$-\Delta H$	ΔS	$-\Delta H$	ΔS
A ligand	[CuABH$_{-1}$]		[CuAB]	
GlySer	25	30	74	16
GlyAsn	21	45	71	28
GlyAsp	26	21	75	18
GlyGlu	18	50	72	29
Glam [b]	35	−6	74	11

[a] $-\Delta H$ values are given in kJ mol^{-1} and ΔS in J K^{-1} mol^{-1}. [b] $-\Delta H$ values for the proton and copper(II) complexes of Glam are −45, HA; 26, [CuA]; 45, [CuA$_2$]; −3, [CuA$_2$H$_{-1}$]; and −42 kJ mol^{-1}, [CuA$_2$H$_{-2}$].

The following conclusions may be drawn from the data of Tables 2 and 7. The values for the [CuAB] ternary complexes are approximately equal to the sum of the ΔH data relating to the [CuA] and [CuB] binary complexes. This probably means that the modes of co-ordination of both bipy and dipeptide in the [CuAB] mixed complexes are the same as those separately in the [CuA] and [CuB] binary complexes, *i.e.* both bipy and dipeptide co-ordinate as bidentate ligands in the equatorial sites. On the other hand, the ΔH values for the [CuABH$_{-1}$] complexes are about 20—25 kJ mol^{-1} less than the sum of the ΔH data corresponding to the [CuAH$_{-1}$] and [CuB] binary complexes. Since the bond between copper(II) and the carboxylate oxygen is essentially electrostatic,[15] the ΔH values reflect mainly the copper(II)–N bonds, and therefore, their decrease may be interpreted as follows: the dipeptides occupy three positions in the square plane, while one nitrogen-donor atom of the bipy creates a bond equatorially and the other co-ordinates axially to copper(II). This suggestion is strengthened by the data relating to the copper(II)–Glam–bipy system, where bipy may also create two equatorial bonds to copper(II) of the [CuABH$_{-1}$] type complex. Consequently, its ΔH value is much higher than those of the copper(II)–dipeptide–bipy complexes. These findings seem to support the assumption of Martin and co-workers [7] that in the [CuABH$_{-1}$] complex of glycylglycine and bipy the peptide co-ordinates to the metal with the three donor atoms in the square plane.

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