Tyrosinate and Lysinate as Bridging Residues in Copper(II) Dipeptide Complexes

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A pH-metric study, together with some supporting spectroscopy (u.v.–visible, c.d., and e.s.r.), was made on copper(II) complexes of L-phenylalanyl-L-tyrosine, L-tyrosyl-L-phenylalanine, L-lysyl-L-tyrosine, and L-tyrosyl-L-lysine at 25 °C and I = 0.2 mol dm^{-3} (KCl). It was established that in dilute aqueous solutions, besides metal–ligand co-ordination characteristic of simple dipeptides, there are interactions between copper(II) and the side-chain phenolate group of the tyrosine residue and/or the ε-amino group of the lysine residue. In these dimeric species, both the lysine and the tyrosine moieties can behave as bridges between monomeric complexes.

The side-chain donor groups of peptide molecules can affect their complex-forming capabilities significantly.[1] In the case of the transition-metal complexes of di- and tri-peptides the role of the aspartic acid,[2,3] cysteine,[4,5] histidine,[6] and tyrosine[7–9] residues seems to be the most important. The co-ordination properties of the side-chain groups have been found to depend considerably on their position in the peptide molecule. Cysteine or histidine in the N-terminal position hinders the co-ordination of the peptide-amide group and thus the participation of the C-terminal residue in the co-ordination is subordinate. The carboxylate group of a non-N-terminal aspartic acid in the peptide chain can also block the deprotonation and co-ordination of the remainder of the molecule. Aromatic moieties can generally interact with the empty *d* orbitals of metal ions. Strongly co-ordinating side-chain donor groups, such as phenolate, imidazolyl, or thiolate, can act as bridges between monomeric species, which leads to the formation of various oligomeric (mainly dimeric) complexes. This is more pronounced if the side-chain group is in the N-terminal position.

The role of the lysyl residue in metal-ion binding has not been widely studied. In metal complexes of lysine itself, the ε-amino group is not able to co-ordinate directly for steric reasons. Of the papers published so far[3,10–13] on the complex-forming properties of lysine-containing oligopeptides, only two have suggested the involvement of the lateral Lys-NH_2 group in metal-ion binding. In both cases the ligands were rather large biomolecules: poly(L-lysine),[12] Gly-Gly-Pro-Lys, or (Gly-Gly-Pro-Lys)$_2$.[13] Recently, *X*-ray evidence was obtained of the direct co-ordination of the ε-amino group of the lysyl residue of a small dipeptide, Lys-Tyr, to copper(II) in the solid state.[14]

Accordingly, in this work the complex-formation processes between copper(II) and various dipeptides containing Tyr and Phe or Lys, such as L-phenylalanyl-L-tyrosine (Phe-Tyr), L-tyrosyl-L-phenylalanine (Tyr-Phe), L-lysyl-L-tyrosine (Lys-Tyr), and L-tyrosyl-L-lysine (Tyr-Lys) have been studied. To determine the stoicheiometries and stabilities of the complexes formed between copper(II) and these ligands, detailed equilibrium investigations were carried out over a wide pH range, while u.v.–visible, circular dichroism (c.d.), and e.s.r. spectroscopic measurements were made in order to clarify the mode of bonding in the complexes.

Experimental

The dipeptides used were Sigma products of puriss quality. The exact concentrations of their solutions were determined by the Gran method.[15]

The stability constants of the copper(II) complexes of the ligands were determined by pH-metric titration of 5-cm^3 samples. The concentration of the ligands in the samples was 4×10^{-3} or 2×10^{-3} mol dm^{-3}, the metal ion:ligand ratio was 1:1, 1:2, or 1:4, and in each case the ionic strength was adjusted to 0.2 mol dm^{-3} with KCl. The titrations were performed over the pH range 3—11 with KOH solution of known concentration (*ca.* 0.2 mol dm^{-3}).

The pH was measured with a Radiometer pHM 84 instrument, with G202B glass and K104 calomel electrodes. The electrode system was calibrated by the method of Irvin *et al.*,[16] so that the pH-meter readings could be converted into hydrogen-ion concentrations. In all cases the temperature was 25.0 ± 0.1 °C.

To determine the dissociation microconstants[17] and to elucidate the bonding modes in the complexes formed in the metal ion–ligand systems, spectrophotometric studies were performed with a Beckman ACTA MIV double-beam recording spectrophotometer in the u.v. and visible wavelength regions. Circular dichroism spectra were measured on a JASCO-J-20 automatic recording spectropolarimeter. Spectra are expressed in terms of $\Delta\varepsilon(\varepsilon_l - \varepsilon_r)$. E.s.r. spectral measurements were made on a JEOL JES-ME-3X spectrometer at 9.12 GHz and at liquid-nitrogen temperature.

The concentration stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$ were calculated from the pH-metric titration curves by means of the PSEQUAD computer program.[18]

Results and Discussion

The dipeptides Phe-Tyr and Tyr-Phe contain three, while Lys-Tyr and Tyr-Lys contain four, dissociable protons in the measurable pH range. From a consideration of the pK values listed in Table 1, a slight overlap of the dissociations of the terminal $-NH_3^+$ and phenolic hydroxy groups (in the cases of Phe-Tyr and Tyr-Phe) and of the terminal $-NH_3^+$, phenolic hydroxy, and ε-ammonium groups (in the cases of Lys-Tyr and Tyr-Lys) cannot be excluded. Accordingly, to confirm the assignment of the pH-metrically determined macroconstants, the dissociation of the phenolic hydroxy group was measured pH-spectrophotometrically too *via* the u.v. band of phenolate. These data are also included in Table 1. It can be seen that the pH-metrically and spectrophotometrically determined pK values for the tyrosine phenolic hydroxy group agree well within

Table 1. Proton dissociation constants of the ligands at 25 °C and I = 0.2 mol dm^{-3} (KCl)

	Phe-Tyr	Tyr-Phe
pK_{CO_2H}	3.23 ± 0.02	3.18 ± 0.02
$pK_{NH_3^+}$	7.26 ± 0.02	7.34 ± 0.02
pK_{OH}	10.01 ± 0.03	10.02 ± 0.03
pK_{OH} (sp)*	10.01 ± 0.05	9.98 ± 0.05
	Lys-Tyr	**Tyr-Lys**
pK_{CO_2H}	3.02 ± 0.02	3.34 ± 0.02
$pK_{NH_3^+}$	7.47 ± 0.02	7.33 ± 0.02
pK_{OH}	9.79 ± 0.02	9.72 ± 0.02
$pK_{\varepsilon\text{-}NH_3^+}$	11.06 ± 0.04	11.01 ± 0.04
pK_{OH} (sp)*	9.82 ± 0.04	9.74 ± 0.04

* Determined spectrophotometrically (see text).

Table 2. Copper(II) complex-formation constants of Phe-Tyr and Tyr-Phe at 25 °C and I = 0.2 mol dm^{-3} (KCl)

	Phe-Tyr	Tyr-Phe
log β_{pqr} values		
$[Cu(HA)]^+$	15.23 ± 0.12	14.93 ± 0.15
$[CuA]$	11.64 ± 0.02	11.74 ± 0.02
$[CuAH_{-1}]^-$	2.49 ± 0.03	2.97 ± 0.04
$[CuAH_{-2}]^{2-}$	−7.85 ± 0.03	−7.33 ± 0.02
$[Cu(HA)(HAH_{-1})]^-$	24.24 ± 0.13	24.65 ± 0.08
$[Cu_2A_2H_{-2}]^{2-}$	6.5	8.35 ± 0.11

	Phe-Tyr	Gly-Tyr[a]	Tyr-Phe	Tyr-Gly[b]
log β'_{pqr} values[b]				
$[Cu(HA)]^+$	5.22	5.66	4.91	4.86
$[Cu(HAH_{-1})]$	1.63	1.70	1.72	1.29
$[Cu(HAH_{-1})(OH)]^-$ and $[Cu(AH_{-1})]^-$	−7.52	−7.43	−7.05	−7.56
$[Cu(HA)(HAH_{-1})]^-$	4.22	4.66	4.61	4.04
log K values				
$[Cu(HA)]^+ \rightleftharpoons [CuA] + H^+$	3.59	3.96	3.19	3.57
$[CuA] \rightleftharpoons [CuAH_{-1}]^- + H^+$	9.15	9.13	8.77	8.85
$[CuAH_{-1}]^- \rightleftharpoons [CuAH_{-2}]^{2-} + H^+$	10.34	10.42	10.30	10.29
$2[CuAH_{-1}]^- \rightleftharpoons [Cu_2A_2H_{-2}]^{2-}$	1.5	—	2.41	2.12

[a] See ref. 9. [b] Calculated from the overall stability constants (logβ_{pqr}) with the use of pK_{OH} of the ligand, since HA^- is regarded as the complex-forming species.

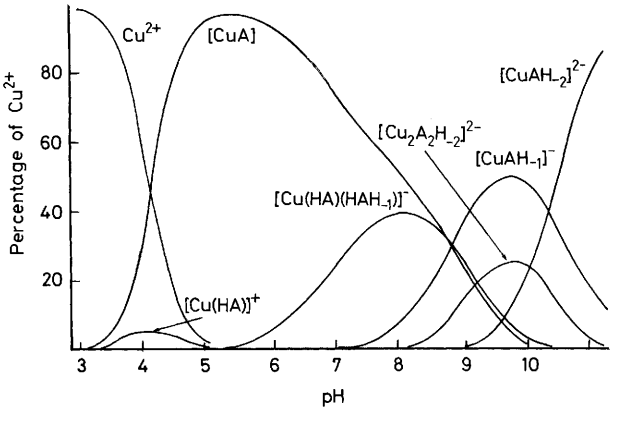

Figure 1. Concentration distribution of the complexes formed in the copper(II)–Tyr-Phe system as a function of pH. c_{Cu} = 0.004, c_{ligand} = 0.008 mol dm^{-3}

experimental error, and thus the assignment of the dissociation constants is justified. It is noteworthy that the pK of the lysyl ε-NH_3^+ group is unusually high, which can presumably be explained by electrostatic and/or hydrophobic interactions between the tyrosyl and lysyl residues.

The titration curves for the copper(II)–Phe-Tyr and –Tyr-Phe systems were evaluated by assuming the formation of the same species as were assumed earlier for the copper(II) systems with Gly-Tyr and Tyr-Gly.[9] The data, together with those obtained for Gly-Tyr and Tyr-Gly, are listed in Table 2. For the sake of easier comparison of the data, we have calculated the formation constants characteristic of the copper(II) complexes of the ligand HA^-, protonated on the least acidic phenolic hydroxy group. This group does not dissociate in the pH range of metal-complex formation, thus HA^- may be regarded as the complex-forming species. These data are also included in Table 2. The concentration distribution curves for the complexes formed in the copper(II)–Tyr-Phe system are depicted in Figure 1.

It can be seen from the data in Table 1 that the presence of an extra aromatic ring in the molecule causes practically no change in the complex-forming properties of these Tyr-containing dipeptides. Hence, similarly as for Gly-Tyr and Tyr-Gly,[7–9] the following conclusions can be drawn on the copper(II) complexes of Phe-Tyr and Tyr-Phe.

(*i*) The bonding modes in the species $[Cu(HA)]^+$, $[Cu(HAH_{-1})]$, and $[Cu(HA)(HAH_{-1})]^-$ correspond to the fundamental bonding modes in the simple dipeptides,[1] *i.e.* HA means a peptide molecule co-ordinated *via* its terminal amino and peptide-carbonyl groups, while HAH_{-1}^- means a molecule co-ordinated *via* its terminal amino and deprotonated peptide-amide groups.

(*ii*) A possible interaction between the empty *d* orbital of copper(II) and the 6π-electron system of the aromatic rings[9] favours the deprotonation and co-ordination of the peptide-NH and thus the formation of $[Cu(HAH_{-1})]$.

(*iii*) The visible and e.s.r. spectral results [the appearance of a charge-transfer band at 380 nm, characteristic of the copper(II)–phenolate interaction, and a significant decrease in intensity of the e.s.r. signal in the range pH 9—10.5] are consistent with the formation of a cyclic dimeric species of composition $[Cu_2A_2H_{-2}]^{2-}$, in which the monomeric species $[Cu(HAH_{-1})]$ are linked *via* phenolate bridges. In the case of the copper(II) complexes of Tyr-Phe, where both amino acids are aromatic, the higher log K_d value (2.41 for Tyr-Phe and 2.12 for Tyr-Gly) corresponding to the dimerization process $2[CuAH_{-1}]^- \rightleftharpoons [Cu_2A_2H_{-2}]^{2-}$ strengthens the folded structure of the dimer,[8] in which it is stabilized by stacking between the two parallel phenolate rings and by additional stacking between the closely situated aromatic rings. The formation of such a dimeric species from dipeptides containing a C-terminal tyrosyl residue is sterically hindered.

(*iv*) The stepwise deprotonation of $[Cu(HAH_{-1})]$ to $[CuAH_{-2}]^{2-}$ or more precisely to $[CuAH_{-1}(OH)]^{2-}$ takes place in parallel pathways, in which the co-ordinated water molecule and the non-co-ordinated phenolic hydroxy group dissociate in overlapping processes.

As compared with Phe-Tyr, the other two ligands studied, Lys-Tyr and Tyr-Lys, contain an extra acidic proton on the ε-ammonium group, which together with the phenolic hydroxy group, dissociates only at pH > 10. Because of the separation of the metal-ion co-ordination processes and the deprotonation processes of the non-co-ordinated phenolic hydroxy and ε-ammonium groups, the ligand H_2A may be regarded as the complex-forming species, and the formation constants of the metal complexes of H_2A were also calculated. The log β values, together with the derived equilibrium data, are listed in Table 3.

The concentration distribution curves for the complexes formed in the copper(II)–Tyr-Lys system are depicted in Figure 2.

Table 3. Copper(II) complex-formation constants of Lys-Tyr and Tyr-Lys at 25 °C and I = 0.2 mol dm^{-3} (KCl)

	Lys-Tyr	Tyr-Lys
log β_{pqr} values		
$[Cu(H_2A)]^{2+}$	26.04 ± 0.14	25.80 ± 0.15
$[Cu(HA)]^{+}$	22.30 ± 0.02	22.11 ± 0.02
$[CuA]$	13.20 ± 0.10	13.42 ± 0.07
$[CuAH_{-1}]^{-}$	3.41 ± 0.03	3.80 ± 0.05
$[CuAH_{-2}]^{2-}$	−7.88 ± 0.04	−7.15 ± 0.07
$[Cu(HA)(H_2AH_{-1})]^{+}$	45.34 ± 0.14	45.87 ± 0.11
$[Cu_2A_2]$	29.92 ± 0.11	30.11 ± 0.06
log β'_{pqr} values *		
$[Cu(H_2A)]^{2+}$	5.19	5.07
$[Cu(H_2AH_{-1})]^{+}$	1.45	1.37
$[Cu(H_2AH_{-1})(OH)]$ and $[Cu(HAH_{-1})]$	−7.65	−7.31
$[Cu(H_2A)(H_2AH_{-1})]^{+}$	3.64	4.41
log K values		
$[Cu(H_2A)]^{2+} \rightleftharpoons [Cu(HA)]^{+} + H^{+}$	3.74	3.69
$[Cu(HA)]^{+} \rightleftharpoons [CuA]^{+} + H^{+}$	9.10	8.69
$[CuA] \rightleftharpoons [CuAH_{-1}]^{-} + H^{+}$	9.69	9.62
$[CuAH_{-1}]^{-} \rightleftharpoons [CuAH_{-2}]^{2-} + H^{+}$	11.29	10.95
$2[CuA] \rightleftharpoons [Cu_2A_2]$	3.52	3.27

* Calculated from the overall stability constants (log β_{pqr}) with the use of pK_{OH} and pK_{NH_3} of the ligands, since H_2A is regarded as the complex-forming species.

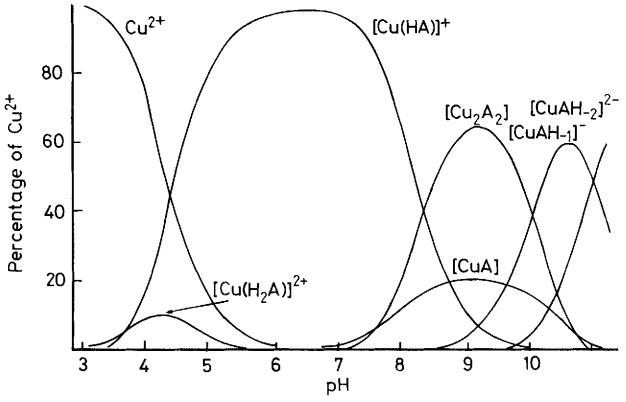

Figure 2. Concentration distribution of the complexes formed in the copper(II)–Tyr-Lys system as a function of pH. c_{Cu} = 0.004, c_{ligand} = 0.004 mol dm^{-3}

The results of the absorption, c.d., and e.s.r. spectral studies carried out to clarify the bonding modes in the copper(II) complexes of Lys-Tyr and Tyr-Lys are listed in Table 4.

The good agreement between the derived equilibrium data for the Phe- and Lys-containing dipeptides (see Tables 2 and 3) indicates the same bonding modes in the corresponding species. The stepwise loss of three protons from $[Cu(H_2AH_{-1})]^{+}$ to give $[CuAH_{-2}]^{2-}$ can be ascribed to the overlapping deprotonation of a co-ordinated water molecule and the non-co-ordinated phenolic hydroxy and ε-ammonium groups of the peptide molecule.

The dimer formation capabilities of the Phe- or Lys-containing dipeptides of tyrosine, however, are strikingly different. Both Tyr-Lys and Lys-Tyr readily form a dimeric species, $[Cu_2A_2]$, in the range pH 8—10.5. The decrease in intensity of the e.s.r. signal in the pH range of formation of the dimeric species (see Figure 2) is consistent with a strong copper(II)–copper(II) interaction. The log K_d values (see Table 3) are significantly higher than those for any other Tyr-containing dipeptides.[7–9] At the same time the intensity of the charge-transfer band at 375 nm characteristic of the copper(II)–phenolate interaction is much lower than in the case of Tyr-Phe (see Figure 3), for example, indicating a less important phenolate involvement in the metal-ion co-ordination. It is also noteworthy that, as Figure 4 shows, in the copper(II)–Lys-Tyr system in the pH range of dimer formation the *d–d* absorption band is shifted to higher energies by about 15 nm, which might suggest an increase in the number of co-ordinated N donors in the equatorial plane.[1]

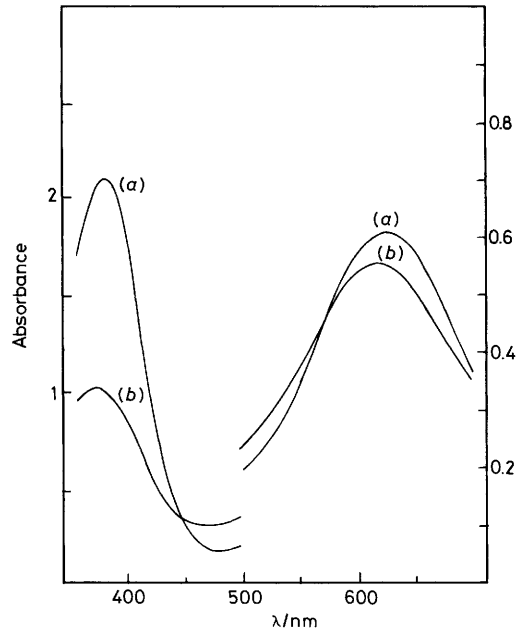

Figure 3. Visible spectra of the copper(II)–Tyr-Phe (*a*) and copper(II)–Tyr-Lys (*b*) systems at a 1:1 metal ion:ligand ratio and pH 9.2

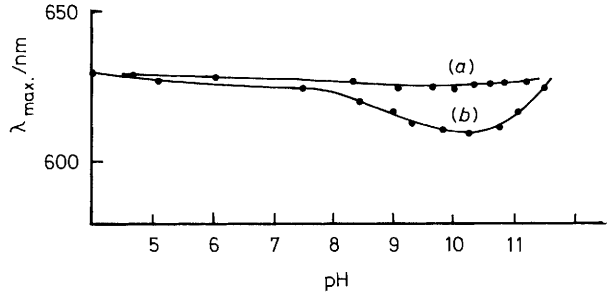

Figure 4. Changes of the *d–d* absorption in the copper(II)–Phe-Tyr (*a*) and copper(II)–Lys-Tyr (*b*) systems at 1:1 metal ion:ligand ratio as a function of pH

The c.d. data listed in Table 4 support this assumption. At pH 9.4 a broad distinct shoulder occurs at 520 nm, while some decrease in the intensity of the c.d. band at 605 nm, corresponding to the 2 N species is observed. Hence, the shoulder at 520 nm can presumably be assigned to the *d–d* transition of a new species involving 3 N in the co-ordination sphere. Furthermore, in the copper(II)–Tyr-Lys system at a 1:1 metal ion:ligand ratio, a deep violet compound precipitates from the slightly greenish solution. The visible reflectance spectrum of this solid complex shows a *d–d* absorption at 560 nm, suggesting the co-ordination of 3 N in the equatorial plane of the copper(II).[1] Precipitation does not occur in the copper(II)–Lys-Tyr system; however, the structure of a solid complex that crystallized slowly from the solution was determined by *X*-ray methods, and the co-ordination of the ε-amino group of the

Table 4. Spectral data for the copper(II)–Lys-Tyr and –Tyr-Lys systems

		U.v.–visible		C.d.			E.s.r.	
pH	Major species	$\lambda_{max.}$/nm	ε^a	$\lambda_{max.}$/nm	$\Delta\varepsilon^a$	Assignment	$g_{\|}$	$A_{\|}$/G[a]
Cu^{II}–Tyr–Lys (1:1)								
6.3	$[Cu(HA)]^+$	628	96	660	−0.53	$B + E^b$	2.251	175
				315	0.24	N^-–Cu^c		
8.8	$[Cu(HA)]^+$	618	95	660	−0.48	$B + E^b$	2.240	176
	[CuA]			520(sh)	−0.14	$B + E^b$		
	$[Cu_2A_2]$	375	114	400	−0.03	O^-–Cu^c	Very low	
				310	+0.29	N^-–Cu^c	intensity	
Cu^{II}–Lys–Tyr								
6.6	$[Cu(HA)]^+$	625	95	640	−0.99	$B + E^b$	2.241	180
9.4	[CuA]	612	90	605	−0.71	$B + E^b$	2.240	180
	$[Cu_2A_2]$			520(sh)	−0.34	$B + E^b$	Very low	
		375(sh)	84	356br	−0.40	O^-–Cu^c	intensity	
						N^-–Cu^c		
11.1	$[CuAH_{-1}]^-$	620	80	627	−0.72	$B + E^b$	2.242	158
	$[CuAH_{-2}]^{2-}$			520(sh)	−0.34	$B + E^b$		
				342	−0.46	N^-–Cu^c		

[a] The ε values are given in $dm^3\ mol^{-1}\ cm^{-1}$, G = 10^{-4} T; sh = shoulder, br = broad. [b] *d–d* transition. [c] Charge-transfer transition.

lysyl residue at the fourth equatorial site of the species $[Cu(HAH_{-1})]$ was confirmed. The monomeric units are linked *via* the lateral amino groups into a chain-polymeric structure.[14] Unfortunately, single crystals for an *X*-ray study could not be obtained from the copper(II)–Tyr-Lys system, in spite of the facile precipitation.

These results together strongly suggest that in solution, besides the side-chain phenolate, the ε-amino group of the lysyl residue in both Tyr-Lys and Lys-Tyr also plays a role in the copper(II) ion binding; in the dimeric species $[Cu_2A_2]$, both the lateral amino and the phenolate groups take part in the linking of the $[Cu(HAH_{-1})]$ monomers. It is very likely that in the case of Tyr-Lys the phenolate group is more involved in the co-ordination, while in the case of Lys-Tyr the ε-amino group is more involved.

At higher pH, however, the hydroxyl ion can displace both bridging donor groups from the equatorial plane of the copper(II), and a mixed hydroxo complex $[CuAH_{-2}]^{2-}$, or more precisely $[Cu(AH_{-1})(OH)]^{2-}$, is formed.

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