Studies on Transition-metal–Peptide Complexes. Part 12.† Copper(II) Complexes of Dipeptides containing Phenylalanine and Tyrosine

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The thermodynamic quantities relating to the copper(II) complexes of glycyl-L-phenylalanine, glycyl-L-tyrosine, L-phenylalanylglycine, and L-tyrosylglycine have been determined pH-metrically and calorimetrically at 25 °C and at an ionic strength of 0.2 mol dm^{-3} (KCl). From these data and the u.v., visible, and e.s.r. spectra of the complexes, it has been established that, besides metal–ligand co-ordination characteristic of simple aliphatic dipeptides, there are interactions between the *d*-electron orbitals of the copper(II) and the 6π-electron system of the aromatic amino acid, between the hydrophobic parts of the molecule, and between the copper(II) and the phenolate group of the ligand.

Besides the fundamental bonding modes of the simple dipeptides, the possibility of further, weaker interactions must be taken into consideration in metal complexes of dipeptides of aromatic amino acids: interactions between the aromatic ring of the side-chain and the metal ion, between the hydrophobic parts of the peptide, and between the donor group of the aromatic side-chain and the metal ion. The potential metal ion-binding sites on this side-chain may play a particularly important rôle in the development of the active centres of certain metalloenzymes in biological systems.

The e.s.r. spectral studies by Kozlowski and co-workers[1,2] on the comparatively widely investigated tyrosine-containing dipeptides (H_2A) suggested that in the complex $[Cu(HA)H_{-1}(OH)]^-$, where the ligand can co-ordinate *via* three donor atoms, there is an interaction between the aromatic ring and the axial *d* orbital of the copper(II) ion. For the L,L and D,L diastereomers of certain phenylalanine-and tyrosine-containing dipeptides, Nakon and Angelici[3] explained the different extents of formation of copper(II) complexes in terms of hydrophobic interactions between the side-chain and the peptide amide group or between the side-chains.

With certain copper(II)–tyrosyl–dipeptide systems, Hefford and Pettit[4] and Kozlowski and co-workers[5] concluded that dimeric species exist, through the formation of copper(II)–phenolate bonds. It was noteworthy that, when tyrosine was the C-terminal amino acid in the dipeptide, such species were not formed. This result was explained by steric effects. These findings have been confirmed by the detailed equilibrium and spectral studies of Yamauchi *et al.*[6]

For tyrosine-containing peptides, as with the amino acid itself,[7,8] the dissociations of the terminal amino group and of the phenolic hydroxy group of the side-chain slightly overlap. Similar micro-processes may be assumed for the complex $[Cu(HA)H_{-1}]$, through the overlapping deprotonation of the phenolic hydroxy group and a co-ordinated water molecule.[4]

Accordingly, we set out to determine the stoicheiometries and thermodynamic data for the complexes formed in the equilibrium systems containing copper(II), and the following dipeptides of glycine with phenylalanine or tyrosine: glycyl-L-phenylalanine (Gly-Phe), L-phenylalanylglycine (Phe-Gly), glycyl-L-tyrosine (Gly-Tyr), and L-tyrosylglycine (Tyr-Gly). U.v., visible, and e.s.r. spectroscopic studies were carried out to examine the bonding mode of the ligands and the possibility of the above-mentioned micro-processes.

† Part 11 is E. Farkas, J. Tözser, and A. Gergely, *Magy. Kem. Foly.*, 1986, **92**, 49.

Experimental

Chemicals and Experimental Conditions.—The dipeptides used were Fluka or Sigma products of puriss. quality. The exact concentrations of their solutions were determined by the method of Gran.[9]

The stability constants of the copper(II) complexes of the ligands were determined by pH-metric titration of 25-cm^3 samples. The concentration of the ligands in the samples was 6×10^{-3} or 4×10^{-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 range pH 3—11 with KOH solution of known concentration (*ca.* 0.2 mol dm^{-3}).

The enthalpy changes accompanying the complex-formation processes were determined calorimetrically (LKB 8700-1 reaction and solution calorimeter), under similar conditions, with a continuous titration technique.[10]

The pH was measured with a Radiometer pHM 64 instrument, with G202B glass and K104 calomel electrodes. The electrode system was calibrated by the method of Irving *et al.*,[11] so that the pH-meter readings could be converted into hydrogen-ion concentrations. In all cases the temperature was 25.0 ± 0.1 °C.

For determination of the proton and metal complex-formation micro-constants, pH–spectrophotometric titrations were performed as described previously.[8,12] A Beckman ACTA MIV double-beam recording spectrophotometer was used for measurements in the visible and u.v. range.

E.s.r. spectral measurements at room temperature or for solutions frozen at liquid-nitrogen temperature were carried out as described earlier,[13] using a JES-ME-3F spectrometer.

Calculations.—The complexes formed in these systems can be characterized by the general equilibrium process (1), with

$$pM + qA + rH \rightleftharpoons M_pA_qH_r \qquad (1)$$

the stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$. The stability constants were calculated from the titration curves with the PSEQUAD computer program, as described previously.[14]

Results and Discussion

The pH-metrically and calorimetrically determined thermodynamic data for the ligands are given in Table 1. Comparison of the thermodynamic quantities relating to the dipeptides

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

Ligand		pK	ΔG	ΔH	ΔS
Gly-Phe	CO_2H	2.99 ± 0.02	17.1	1.7	−51.7
	$^+NH_3$	8.08 ± 0.02	46.1	43.9	−7.4
Phe-Gly	CO_2H	3.12 ± 0.02	17.8	1.6	−65.3
	$^+NH_3$	7.46 ± 0.02	42.6	43.2	+2.0
Gly-Tyr	CO_2H	3.03 ± 0.01	17.3	1.2	−54.0
	pK_1	8.10 ± 0.02	46.2	43.2	−10.1
	pK_2	9.96 ± 0.02	56.3	25.2	−104.3
Tyr-Gly	CO_2H	3.13 ± 0.02	17.9	1.8	−54.0
	pK_1	7.54 ± 0.02	43.0	41.7	−4.4
	pK_2	9.86 ± 0.02	56.3	22.7	−112.7

* Uncertainty in ΔH ± 0.3 kJ mol^{-1} and in ΔS ± 0.5 J K^{-1} mol^{-1}.

containing C- or N-terminal aromatic amino acids led to the following conclusions.

The pK_{CO_2H} and pK_1 values for Gly-Phe and Gly-Tyr, and also the corresponding enthalpy changes, are the same, within the limits of error. This is in accordance with expectations if one bears in mind the considerable distance of the phenolic hydroxy group in Gly-Tyr from the carboxyl and terminal $^+NH_3$ groups. Thus, the third dissociation process of Gly-Tyr (pK_2) can presumably be ascribed exclusively to the phenolic hydroxy group.

In the comparison of Phe-Gly and Tyr-Gly, the shorter distance between the donor groups means that the electron-releasing effect of the phenolic hydroxy group is manifested in the value of pK_1, and causes a similar difference in the pK_1 values to that observed when comparing phenylalanine and tyrosine.[8] Hence also in the case of Tyr-Gly it is probable that the third proton is lost from the phenolic hydroxy group.

There appears to be insignificant overlap of the dissociations of the $^+NH_3$ and phenolic hydroxy groups. The clear-cut assignment of the pK_1 and pK_2 values therefore appears justified. For a definite confirmation of this, we also monitored the dissociation of the phenolic hydroxy group by observing its u.v. band. The pK_2 values of 9.89 ± 0.07 and 9.85 ± 0.05 obtained in this way for Gly-Tyr and Tyr-Gly, respectively, agree well with the pH-metric data. It may therefore be stated that the pK_1 values given for Gly-Tyr and Tyr-Gly in Table 1 are characteristic of the terminal $^+NH_3$ group, while the pK_2 values pertain to the phenolic hydroxy group.

The pH-metrically and calorimetrically determined thermodynamic data for the copper(II) complexes of the four ligands are given in Tables 2 and 3. When allowance is made for differences in ionic strength, the tabulated stability constants are in satisfactory agreement with literature results.[4,6] Compared with the Phe-containing dipeptides (HA), those containing Tyr (H_2A) have an additional dissociable proton on the phenolic hydroxy group; however, this begins to dissociate only at high pH. For ease of comparison of Gly-Tyr and Tyr-Gly, we have used the overall thermodynamic quantities and data on the dissociation of the phenolic hydroxy group (Table 1) to calculate quantities characteristic of the formation of the metal complexes of the ligand HA^-, protonated on the phenolic hydroxy group. These data are also given in Tables 2 and 3.

Visible and e.s.r. spectral studies were carried out to clarify the bonding mode in the complexes. Table 4 presents the spectral parameters for the different systems at various pH values, at a metal ion:ligand ratio of 1:1.

The thermodynamic and spectral data for the C-terminal (Gly-Phe and Gly-Tyr) and the N-terminal (Phe-Gly and Tyr-Gly) aromatic amino acid-containing dipeptides were

Table 2. Thermodynamic data for copper(II) complexes of Gly-Phe and Gly-Tyr at 25 °C and I = 0.2 mol dm^{-3} (KCl)*

Species	log β	$-\Delta G$	ΔH	ΔS
Glycyl-L-phenylalanine (A^-)				
$[CuA]^+$	5.59 ± 0.02	31.9	−27.2	16
$[CuAH_{-1}]$	1.73 ± 0.01	9.9	0.4	36
$[CuAH_{-2}]^-$	−7.63 ± 0.01	−43.6	46.9	−11
$[CuA_2H_{-1}]^-$	4.87 ± 0.02	27.8	−16.3	39
$[Cu_2A_2H_{-3}]^-$	−3.60 ± 0.02	−20.6	36.8	−54
Glycyl-L-tyrosine (A^{2-})				
$[CuAH]^+$	15.62 ± 0.04	89.2	−52.3	124
$[CuA]$	11.66 ± 0.01	66.6	−25.9	137
$[CuAH_{-1}]^-$	2.53 ± 0.01	14.4	7.0	72
$[CuAH_{-2}]^{2-}$	−7.89 ± 0.01	−45.0	50.2	−17
$[CuA_2H]^-$	24.58 ± 0.03	140.3	−65.7	250
$[Cu_2A_2H_{-1}]^-$	15.4 ± 0.1	88.1	—	—
Glycyl-L-tyrosine (HA^-)				
$[Cu(HA)]^+$	5.66	32.3	−27.1	17
$[Cu(HA)H_{-1}]$	1.70	9.7	−0.7	30
$[Cu(HA)H_{-2}]^-$	−7.43	−42.4	32.2	34
$[Cu(HA)_2H_{-1}]^-$	4.66	26.6	−15.3	38
$[Cu_2(HA)_2H_{-3}]^-$	−4.5	−25.6	—	—

* Uncertainty in ΔH ± 0.5 kJ mol^{-1} and in ΔS ± 1 J K^{-1} mol^{-1}.

Table 3. Thermodynamic data on copper(II) complexes of Phe-Gly and Tyr-Gly at 25 °C and at I = 0.2 mol dm^{-3} (KCl)*

Species	log β	$-\Delta G$	ΔH	ΔS
L-Phenylalanylglycine (A^-)				
$[CuA]^+$	4.93 ± 0.04	28.1	−26.8	4
$[CuAH_{-1}]$	1.26 ± 0.01	7.2	6.4	46
$[CuAH_{-2}]^-$	−8.00 ± 0.01	−45.7	54.2	−29
$[CuA_2H_{-1}]^-$	4.04 ± 0.04	23.1	−14.4	29
$[Cu_2A_2H_{-3}]^-$	−4.26 ± 0.04	−24.3	37.0	−43
L-Tyrosylglycine (A^{2-})				
$[CuAH]^+$	14.72 ± 0.06	84.0	−51.8	108
$[CuA]$	11.15 ± 0.01	63.7	−16.9	157
$[CuAH_{-1}]^-$	2.30 ± 0.02	13.1	22.2	118
$[CuAH_{-2}]^{2-}$	−7.99 ± 0.01	−45.6	48.5	−10
$[CuA_2H]^-$	23.76 ± 0.04	135.6	−55.2	270
$[Cu_2A_2H_{-2}]^{2-}$	6.93 ± 0.03	39.6	−17.2	75
L-Tyrosylglycine (HA^-)				
$[Cu(HA)]^+$	4.86	27.7	−29.1	5
$[Cu(HA)H_{-1}]$	1.29	7.4	5.8	44
$[Cu(HA)H_{-2}]^-$	−7.56	−43.2	44.9	−6
$[Cu(HA)_2H_{-1}]^-$	4.04	23.1	−9.8	45

* Uncertainty in ΔH ± 0.5 kJ mol^{-1} and in ΔS ± 1 J K^{-1} mol^{-1}.

compared in pairs on the basis of Tables 2—4. The very good agreement of the data reveals that the bonding modes of the species $[CuA]^+$ and $[Cu(HA)]^+$, $[CuAH_{-1}]$ and $[Cu(HA)H_{-1}]$, and $[CuA_2H_{-1}]^-$ and $[Cu(HA)_2H_{-1}]^-$ formed at pH <8 are similar, and in accord with what was observed for the simple aliphatic dipeptides.[15] Thus, the spectral parameters obtained at a copper(II):ligand:OH^- ratio of 1:1:2 are practically identical (see Table 4). At pH >8, however, the deprotonation of the phenolic hydroxy group causes differences in the complex-forming properties of the ligands, as manifested in the stability constants and the spectral properties.

For a more exact understanding of the rôle of the aromatic side-chain, the equilibrium data for the individual complex-formation steps were obtained from the overall thermodynamic

Table 4. Spectral data for the copper(II)–dipeptide complexes*

System		pH	g_0	$g_\parallel$	$g_\perp$	A_0	$A_\parallel$	$A_\perp$	A_N	$\lambda_{max.}$	ε
Cu^{II}–Gly-Phe–OH	1:1:2	5.9	2.123	2.245	2.057	77	177	33	13	622	84
	1:1:4	11.5	2.123	2.243	2.055	66	156	31	13	625	82
Cu^{II}–Phe-Gly–OH	1:1:2	6.0	2.125	2.254	2.059	75	175	29	14	630	85
	1:1:4	11.5	2.127	2.250	2.068	65	153	33	13	630	83
Cu^{II}–Gly-Tyr–OH	1:1:2	6.3	2.124	2.249	2.058	78	182	34	14	623	84
	1:1:3	10.0	significantly decreased intensity							625	81
										385 (sh)	35
	1:1:5	11.7	2.128	2.250	2.061	66	158	31	—	625	82
Cu^{II}–Tyr-Gly–OH	1:1:2	6.7	2.124	2.255	2.060	72	178	31	—	628	89
	1:1:3	10.1	significantly decreased intensity							628	110
										380	270
	1:1:5	11.7	2.129	2.252	2.068	66	153	34	—	628	95
										378	157
	1:1:10	12.3	2.129	2.253	2.068	66	153	34	—	630	94

* The A values are given in G (10^{-4} T), $\lambda_{max.}$ in nm, and ε in $dm^3\ mol^{-1}\ cm^{-1}$; sh = shoulder.

Table 5. Thermodynamic data for the process $[CuA]^+ \rightleftharpoons [CuAH_{-1}] + H^+$ in the copper(II)–dipeptide systems*

Peptide	log K	ΔG/kJ mol^{-1}	ΔH/kJ mol^{-1}	ΔS_1/J K^{-1} mol^{-1}
Gly-Ala	−4.22	24.1	30.3	21
Gly-Phe	−3.86	22.0	27.6	19
Gly-Tyr	−3.96	22.6	26.4	13
Ala-Gly	−3.91	22.3	28.2	20
Phe-Gly	−3.67	21.0	33.2	41
Tyr-Gly	−3.57	20.4	34.9	49

* In the case of Gly–Tyr and Tyr–Gly, due to the presence of the phenolic hydroxy group, the thermodynamic data refer to the process $[Cu(HA)]^+ \rightleftharpoons [Cu(HA)H_{-1}] + H^+$.

Table 6. Thermodynamic data for the process $[CuAH_{-1}] + A^- \rightleftharpoons [CuA_2H_{-1}]^-$ in the copper(II)–dipeptide systems*

Dipeptide	log K	$-\Delta G$/kJ mol^{-1}	$-\Delta H$/kJ mol^{-1}	$-\Delta S$/J K^{-1} mol^{-1}
Gly-Ala	3.08	17.6	27.3	−32
Gly-Phe	3.14	17.9	16.7	4
Gly-Tyr	2.96	16.9	14.6	7
Ala-Gly	2.60	14.8	24.1	−31
Phe-Gly	2.78	15.9	20.8	−16
Tyr-Gly	2.75	15.7	15.6	0

* In the case of Gly–Tyr and Tyr–Gly, due to the presence of the phenolic hydroxy group, the thermodynamic data refer to the process $[Cu(HA)H_{-1}] + HA^- \rightleftharpoons [Cu(HA)_2H_{-1}]^-$.

Table 7. Thermodynamic data for the process $[CuAH_{-1}] + OH^- \rightleftharpoons [CuAH_{-1}(OH)]^-$ in the copper(II)–dipeptide systems*

Dipeptide	log K	$-\Delta G$/kJ mol^{-1}	$-\Delta H$/kJ mol^{-1}	$-\Delta S$/J K^{-1} mol^{-1}
Gly-Ala	4.33	24.7	12.3	42
Gly-Phe	4.40	25.1	10.2	50
Gly-Tyr	4.63	26.4	23.8	9
Ala-Gly	4.31	24.6	12.9	39
Phe-Gly	4.50	25.7	8.9	56
Tyr-Gly	4.91	28.0	17.6	35

* In the case of Gly–Tyr and Tyr–Gly, due to the presence of the phenolic hydroxy group, the thermodynamic data refer to the process $[Cu(HA)H_{-1}] + OH^- \rightleftharpoons [Cu(HA)H_{-1}(OH)]^-$.

quantities given in Tables 2 and 3. These are presented in Tables 5—7. In the interest of comparability, the Tables also contain earlier data relating to the corresponding simple aliphatic dipeptides, glycyl-DL-alanine and DL-alanylglycine.[15]

Table 5 lists thermodynamic data on the deprotonation and co-ordination of the peptide NH group. It may be seen that the log K values found for the dipeptides of the aromatic amino acids are generally a few tenths of a log unit larger than those for the corresponding aliphatic dipeptides. Hence, deprotonation and the accompanying structural rearrangement are favoured. A part may be played in this by the interaction between the empty d orbital of the copper(II) and the 6π-electron system of the aromatic ring, but an interaction is also likely between the hydrophobic parts of the co-ordinated peptide.[16] It is noteworthy that their X-ray diffraction studies led Helm and Franks[17] to suggest an interaction between copper(II) and the aromatic ring in a complex of Gly-Leu-Tyr in the solid phase.

The above assumptions are supported by the thermodynamic data on the co-ordination of the second ligand (Table 6). If the difference in pK values of the terminal $^+NH_3$ groups of the ligands are taken into consideration (Gly-Ala, pK = 8.20; Ala-Gly, pK = 8.19[15]), the derived log K − pK_{NH_3} values, characteristic of the relative stability of $[CuA_2H_{-1}]^-$, are generally smaller for the aromatic dipeptides than for Gly-Ala and Ala-Gly. For steric reasons, the secondary interactions in the complex $[CuAH_{-1}]$ may inhibit the presumably equatorial–axial co-ordination of the second ligand.[15] This is also supported by the fact that the anthalpy changes accompanying the process are less exothermic in these cases.

The data in Table 7 were calculated by ascribing the deprotonation of $[CuAH_{-1}]$ or $[Cu(HA)H_{-1}]$ at pH >9 to a co-ordinated water molecule. The outstanding thermodynamic quantities obtained for Gly-Tyr and Tyr-Gly indicate that this assumption does not hold for these ligands, *i.e.* the process also involves deprotonation of the phenolic hydroxy group.

As revealed by the concentration distribution curves for the copper(II)–Gly-Tyr and –Tyr-Gly systems in Figures 1 and 2, there is a substantial difference in the complex-forming properties of these two ligands in the interval pH 8—10.5.

In contrast with the other dipeptides, Tyr-Gly forms a dimeric species of composition $[Cu_2A_2H_{-2}]^{2-}$, which is accompanied by a change in the colour of the solution from blue to green. For this complex, the charge-transfer band of medium intensity at around 380 nm suggests the formation of a direct copper(II)–phenolate interaction. Figure 3 depicts the visible spectra of the copper(II)–Tyr-Gly and –Gly-Tyr systems at a metal ion:ligand ratio of 1:1, after the addition of 3 equivalents of base. On the basis of the shoulder at around 380 nm, a similar interaction cannot be excluded in the copper(II)–Gly-Tyr

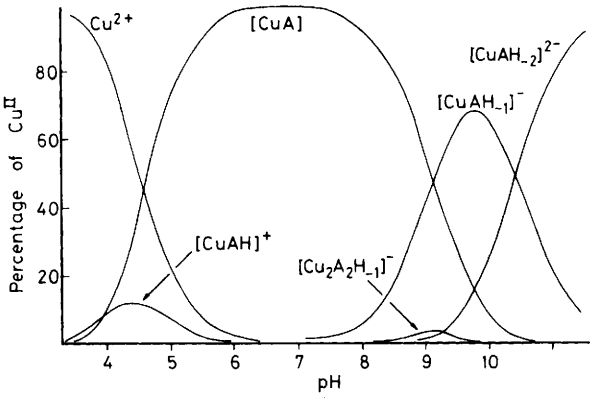

Figure 1. Concentration distribution of the complexes formed in the copper(II)–Gly-Tyr system as a function of pH. c_{Cu} = 0.004, c_{ligand} = 0.004 mol dm^{-3}

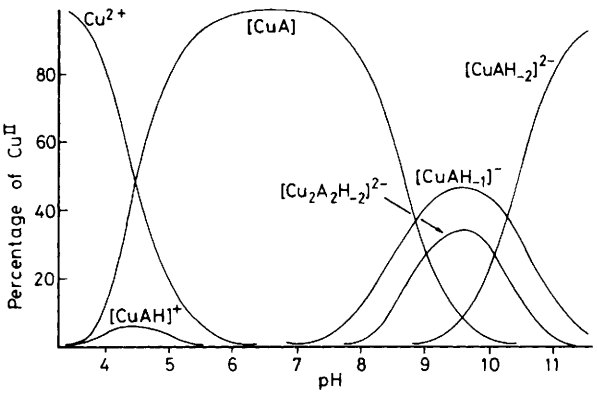

Figure 2. Concentration distribution of the complexes formed in the copper(II)–Tyr-Gly system as a function of pH. Details as in Figure 1

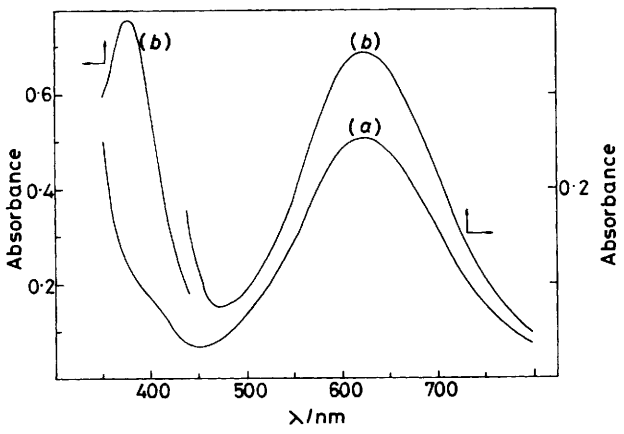

Figure 3. Visible spectra of the (*a*) copper(II)–Gly-Tyr and (*b*) copper(II)–Tyr-Gly systems at a 1:1 metal ion:ligand ratio and pH 10.0

system but this species is formed in such low concentration that it cannot be detected *via* the pH-metric titration data. The ratio of the absorbances measured at 380 nm indicates that the concentration of the dimer is <8%. The different tendencies of these two ligands to form the dimeric complex $[Cu_2A_2H_{-2}]^{2-}$

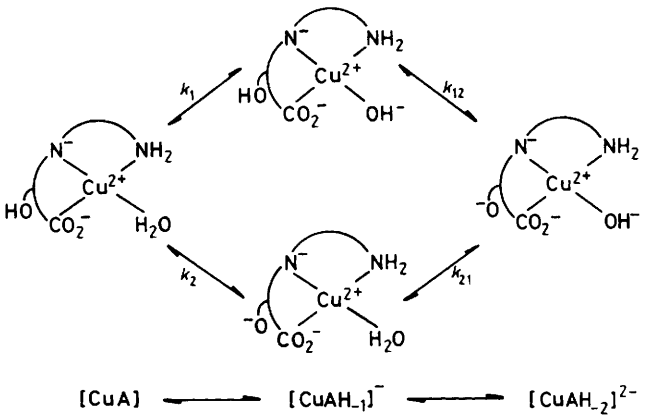

Figure 4. Micro-processes in the deprotonation of $[Cu(HA)H_{-1}]$ formed in the copper(II)–Gly-Tyr and –Tyr-Gly systems

Table 8. Macro- and micro-constants for the deprotonation of the complex $[Cu(HA)H_{-1}]$ of Gly-Tyr at 25 °C and I = 0.2 mol dm^{-3} KCl

$pK_{[Cu(HA)H_{-1}]}$	9.13 ± 0.01	$pK_{[CuAH_{-1}]^-}$	10.42 ± 0.01
pk_1	9.4 ± 0.1	pk_{12}	10.1 ± 0.1
pk_2	9.5 ± 0.1	pk_{21}	10.0 ± 0.1

appear to be explained most readily in terms of steric effects as suggested by Hefford and Pettit.[4] At the same time, it is noteworthy that the two metal ion–ligand systems exhibit similar e.s.r. spectral behaviour (see Table 4). Following the addition of 3 equivalents of base, the signal recorded at room temperature at pH *ca.* 10 is broadened, and its intensity is slightly decreased. In frozen solution, at 77 K, the very poorly resolved signal assigned to the transition $\Delta M = 1$ displays a larger intensity decrease, and at g *ca.* 4 another, low-intensity signal without hyperfine structure appears, which is not observed at lower pH. In both systems, therefore, the e.s.r. spectral properties point to a copper(II)–copper(II) interaction. That there is a significant amount of dimeric complex present also in the copper(II)–Gly-Tyr system in frozen solution can presumably be explained by the fact that formation of $[Cu_2A_2H_{-2}]^{2-}$ is an exothermic process (see the data relating to Tyr-Gly in Table 3), and thus a decrease in temperature will shift the monomer–dimer equilibrium in the direction of dimer formation.

With further elevation of the pH, a monomeric species, $[CuAH_{-2}]^{2-}$, again becomes predominant (Figures 1 and 2). As mentioned above, its formation involves ionization of a co-ordinated water molecule and deprotonation of the phenolic hydroxy group, and is therefore described by the micro-processes outlined in Figure 4. A possibility arises for the determination of the microconstants for these processes through independent spectral study of the deprotonation of the phenolic hydroxy group.

Figure 1 demonstrates that there are no other spectral changes to be considered in the copper(II)–Gly-Tyr system at a metal ion:ligand ratio of 1:1 in the interval pH 8—10. The species $[Cu_2(HA)_2H_{-3}]^-$ is formed in very low concentration and, as a consequence of its hydroxo-bridged structure,[15] it contains a protonated phenolic hydroxy group, so that its absorbance is not appreciable in the wavelength interval where phenolate absorbs. The complex $[Cu_2A_2H_{-2}]^{2-}$, detected by means of visible spectral studies (see Figure 3), does contain a deprotonated phenolate group, but its low concentration

permits its contribution to be neglected. In the copper(II)–Tyr-Gly system, however, this latter species is formed in significant amounts (see Figure 2). Since its spectral properties in the region of phenolate absorbance are not known, we have no means of correcting the measured u.v. absorbances for it. Hence, the equilibrium constants for the micro-processes outlined in Figure 4 could be estimated only for the copper(II)–Gly-Tyr system. Similarly, as for the determination of the dissociation microconstants of the ligands, the individual microconstants were calculated by evaluation of the mole fraction–pH curves determined pH–spectrophotometrically for the phenolate group.[8] The resulting values are given in Table 8. The larger errors in the constants can be attributed to the assumptions made above.

The data in Table 8 reveal that the acidity of the phenolic hydroxy group of the Gly-Tyr bound in the complex (pk_2 = 9.5) is considerably higher than that of the free ligand (pK_{OH} = 9.96). This can be explained by the assumed interaction between the copper(II) and the aromatic ring of the ligand, as a consequence of which the electron density on the ring decreases, leading to an electron shift extending to the phenolic hydroxy group. At the same time, the interaction between the d_{z^2} orbital of the copper(II) and the 6π-electron system of the ring, and through this the electron-releasing effect of the phenolic hydroxy group, are not manifested in the equatorial plane, and thus have practically no effect on the ionization of the co-ordinated water molecule (pk_1 = 9.4) (see Table 2). It appears, however, that the electron-releasing effect of the phenolate group is so large that the electron shift extends to the Cu^{II}–OH_2 bond in the equatorial plane, and thus the pk_{21} value is about 0.6 log unit higher than pk_1.

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