# Copper(II) Complexes of Tyrosine-containing Tripeptides

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A pH-metric and spectroscopic (u.v.–visible and e.s.r.) study has been made of copper(II) complexes of glycyl-L-leucyl-L-tyrosine, glycyl-L-tyrosylglycine, and L-tyrosylglycylglycine at 25 °C and $I$ = 0.2 mol dm$^{-3}$ (KCl). In dilute aqueous solutions the tyrosine-containing tripeptides basically display the properties of simple tripeptides in their interactions with the copper(II) ion and, independently of the position of the tyrosine, the phenolic hydroxy group in the side-chain does not play a direct role in co-ordination to the metal ion.

Tyrosine is a constituent of many neuropeptides, and it may be assumed that it plays a fundamental role in the activity of these compounds.[1] Since the copper(II) concentration in the brain is extremely high, its interaction with the neuropeptides may be of physiological importance. For steric reasons, the phenolic group in tyrosine is not able to co-ordinate directly to copper(II), but a copper(II)–phenolate interaction has been conclusively demonstrated in various of its oligopeptides.[2–7] The nature and extent of this interaction may depend considerably on the position of the tyrosine in the peptide molecule.

Accordingly, in the present work I have studied the complex-formation processes between copper(II) and tripeptides ($H_2A$) containing tyrosine in position 1, 2, or 3: glycyl-L-leucyl-L-tyrosine (Gly-Leu-Tyr), glycyl-L-tyrosylglycine (Gly-Tyr-Gly), and L-tyrosylglycylglycine (Tyr-Gly-Gly). To determine the stoicheiometries and stabilities of complexes formed between copper(II) and these ligands, detailed equilibrium investigations have been carried out in a wide range of pH, while u.v.–visible spectrophotometric and e.s.r. spectral measurements were made in order to clarify the mode of bonding in the complexes.

## Experimental

The tripeptides used were Fluka or Sigma products of puriss. quality. The purity of the peptides and the exact concentrations of their solutions were checked and measured by the Gran method.[8]

The stability constants of the copper(II) complexes of the ligands were determined by pH-metric titration of 10-cm$^3$ samples in the pH range 3–11. The ligand concentration was $4 \times 10^{-3}$ mol dm$^{-3}$, the metal ion:ligand ratio was 1:1, 1:2, or 1:4, and the ionic strength was adjusted to 0.2 mol dm$^{-3}$ with KCl. The titrations were performed with KOH solution of known concentration (*ca.* 0.2 mol dm$^{-3}$). These measurements were made on a Radiometer pHM 64 instrument with a GK 2301 combined electrode.

To determine the proton and metal complex-formation microconstants and to elucidate the bonding mode 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.

E.s.r. spectral measurements on solutions at room temperature or frozen at liquid-nitrogen temperature were carried out on a JES-ME-3F spectrometer.

The stability constants were calculated from the pH-metric titration curves by means of the PSEQUAD computer program as reported previously.[9]

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

| | Gly-Leu-Tyr | Gly-Tyr-Gly | Tyr-Gly-Gly |
|---|---|---|---|
| $pK_{CO_2H}$ | 3.14 ± 0.02 | 3.36 ± 0.01 | 3.19 ± 0.01 |
| $pK_{NH_3^+}$ | 8.07 ± 0.01 | 7.92 ± 0.01 | 7.37 ± 0.01 |
| $pK_{OH}$ | 10.30 ± 0.03 | 9.78 ± 0.01 | 10.09 ± 0.02 |

## Results and Discussion

The acid dissociation constants of the ligands are listed in Table 1. A slight overlap of the dissociations of the terminal $^+NH_3$ and phenolic hydroxy groups of these tripeptides cannot be excluded.[7] Accordingly, to confirm the assignment of the pH-metrically determined macroconstants, the dissociation of the phenolic hydroxy group was also measured pH-spectrophotometrically at the u.v. band of phenolate. This method yielded values of 10.29 ± 0.07, 9.81 ± 0.05, and 10.03 ± 0.07 for Gly-Leu-Tyr, Gly-Tyr-Gly, and Tyr-Gly-Gly, respectively. These values agree within the limit of experimental error with the constants given in Table 1 and thus the assignment of the dissociation constants is justified.

The titration curves for the copper(II)–peptide systems were evaluated by assuming various models. The best fits between the measured and calculated titration curves were obtained by assuming the species given in Table 2. The average difference between the measured and calculated titration curves (fitting parameter) characteristic of the quality of the fit[9] was 0.0069 cm$^3$ for the copper(II)–Gly-Leu-Tyr system (calculated from 239 experimental points), 0.0065 cm$^3$ for the copper(II)–Gly-Tyr-Gly system (606 points), and 0.0041 cm$^3$ for the copper(II)–Tyr-Gly-Gly system (183 points). The tabulated data reveal that species with equivalent compositions are formed in all three copper(II)–ligand systems, and stabilities of the individual complexes are almost identical. This suggests that all the species involve the same bonding mode, independent of the ligand. This view is supported by the similar spectral (visible and e.s.r.) parameters found for the various systems (see Table 3). It is noteworthy that bis complexes are not formed in measurable concentration in any system even at high (1:4) ligand excess. The spectral characteristics of the systems are the same at any metal ion:ligand ratios above pH 7, which confirm that the bonding mode in the complexes is not affected by excess of ligand.

**Table 2.** Copper(II) complex formation constants of the ligands at 25 °C and $I$ = 0.02 mol dm$^{-3}$ (KCl)

| Species | Gly-Leu-Tyr | Gly-Tyr-Gly | Tyr-Gly-Gly | pH range (main species) |
|---|---|---|---|---|
| $[CuAH]^+$ | 14.9 ± 0.1 | 14.75 ± 0.03 | 14.8 ± 0.1 | 4.3—6.0 |
| $[CuA]$ | 9.60 ± 0.03 | 9.72 ± 0.01 | 9.77 ± 0.02 | 5.0—7.2 |
| $[CuAH_{-1}]^-$ | 3.15 ± 0.03 | 3.52 ± 0.01 | 3.57 ± 0.02 | 6.4—10.5 |
| $[CuAH_{-2}]^{2-}$ | −7.13 ± 0.04 | −6.41 ± 0.01 | −6.67 ± 0.03 | above 10.0 |
| $Cu^{2+} + HA^- \rightleftharpoons [CuHA]^+$ | 4.60 | 4.97 | 4.71 | |
| $[CuHA]^+ \rightleftharpoons [Cu(HA)H_{-1}] + H^+$ | −5.30 | −5.03 | −5.03 | |
| $[Cu(HA)H_{-1}] \rightleftharpoons [Cu(HA)H_{-2}]^- + H^+$ | −6.45 | −6.20 | −6.20 | |
| $[Cu(HA)H_{-2}]^- \rightleftharpoons [CuAH_{-2}]^{2-} + H^+$ | −10.28 | −9.93 | −10.24 | |

**Table 3.** Spectral data on the copper(II)–tripeptide complexes

| | pH | $\lambda_{max.}$/nm | ε/dm$^3$ mol$^{-1}$ cm$^{-1}$ | $g_\parallel$ | $g_\perp$ | $A_\parallel$/G* |
|---|---|---|---|---|---|---|
| Cu$^{II}$–Gly-Leu-Tyr–OH$^-$ | | | | | | |
| 1:1:2 | 5.9 | 650 | 43 | 2.259 | 2.063 | 169 |
| 1:1:3 | 8.2 | 540 | 142 | 2.201 | 2.050 | 200 |
| 1:1:4 | 10.4 | 545 | 161 | 2.201 | 2.050 | 200 |
| 1:1:6 | 11.1 | 548 | 163 | 2.203 | 2.053 | 198 |
| Cu$^{II}$–Gly-Tyr-Gly–OH$^-$ | | | | | | |
| 1:1:2 | 6.1 | 650 | 40 | 2.235 | 2.070 | 175 |
| 1:1:3 | 7.9 | 545 | 154 | 2.200 | 2.058 | 190 |
| 1:1:4 | 10.0 | 545 | 156 | 2.200 | 2.058 | 190 |
| 1:1:6 | 11.0 | 545 | 154 | 2.200 | 2.058 | 190 |
| Cu$^{II}$–Tyr-Gly-Gly–OH$^-$ | | | | | | |
| 1:1:2 | 6.0 | 635 | 48 | 2.264 | 2.065 | 167 |
| 1:1:3 | 8.3 | 550 | 143 | 2.203 | 2.048 | 193 |
| 1:1:4 | 10.3 | 547 | 146 | 2.203 | 2.048 | 193 |
| 1:1:6 | 11.1 | 548 | 143 | 2.203 | 2.048 | 193 |

* G = 10$^{-4}$ T.

The results permit the assumption that, similarly to simple di- and tri-peptides,[10] in the complex $[CuAH]^+$ the ligand is co-ordinated in a bidentate manner *via* the terminal $NH_2$ group and the peptide carbonyl oxygen, and thus the bonding mode is *N,O*.

The two overlapping deprotonation processes of the complex $[CuAH]^+$, beginning at pH ~ 4.5, can be ascribed to proton loss and co-ordination by the two peptide NH groups, accompanied by an appreciable spectral change. In the pH interval (pH 4.5—6.0) of formation of the complex [CuA] {more exactly $[Cu(HA)H_{-1}]$*} the visible and e.s.r. spectral parameters exhibit values characteristic of phenylalanine-containing dipeptides,[7] which is indicative of a tridentate bonding mode *N,N,O*, involving co-ordination of the terminal $NH_2$, the first peptide $N^-$, and the second peptide carbonyl oxygen. As the pH is raised, the second peptide NH also undergoes deprotonation and co-ordination to the metal ion,[10] this being accompanied by a considerable increase in the energy of the *d–d* transition ($\lambda_{max.}$ ~ 545 nm) and by characteristic changes in the e.s.r. parameters. In the resulting complex $[Cu(HA)H_{-2}]^-$, the bonding mode is *N,N,N,O* due to co-ordination of the terminal $NH_2$, the two peptide $N^-$ groups, and the carboxylate oxygen.

Neither the equilibrium data nor the spectral properties of the complexes indicate co-ordination of the phenolic hydroxy group or phenolate to the metal ion. In the pH interval in question, a charge-transfer band of moderate intensity, characteristic of the copper(II)–phenolate interaction, is not observed at ~400 nm with any of the ligands. In the concentration range applied (maximum 4 × 10$^{-3}$ mol dm$^{-3}$), the e.s.r. spectral data also exclude the formation of a dimeric species.[11]

A further base-consuming process is observed at pH > 9.5; however, this is not associated with a spectral change and can be assigned to the deprotonation of the tyrosine phenolic hydroxy group non-co-ordinated to the metal ion, the bonding mode thus remaining unchanged. In contrast to that observed with glycylglycylglycine,[10] replacement of the co-ordinated carboxylate group by an $OH^-$ ion in this process can practically be excluded. At a metal ion:ligand ratio of 1:1, the deprotonation constants $pK_{[Cu(HA)H_{-2}]}$, determined pH-spectrophotometrically at the u.v. band of phenolate, were 10.32 ± 0.07, 9.97 ± 0.07, and 10.16 ± 0.05 for Gly-Leu-Tyr, Gly-Tyr-Gly, and Tyr-Gly-Gly, respectively, agreeing well with the corresponding constants measured pH-metrically (see Table 2). It is noteworthy that co-ordination of the $OH^-$ ion does not even occur with the copper(II) complex of glycyl-L-phenylalanyl-L-phenylalanine. It may therefore be assumed that this is due to the presence of the aromatic hydrophobic side chains surrounding the metal ion.

The results demonstrate that tyrosine-containing tripeptides display properties typical of simple tripeptides in their interactions with the copper(II) ion. In contrast to tyrosine-containing dipeptides, where the phenolate group in the side chain can act as a bridge to link together *N,N,O*-bonded $[CuAH_{-1}]^-$ monomer units,[2,7] with tyrosine-containing tripeptides there is no possibility of this due to the development of the very stable *N,N,N,O* co-ordination. In these tripeptides, therefore, independent of the position of the tyrosine, the phenolic hydroxy group does not play a direct role in co-ordination to the metal ion.

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* In HA the H refers to the ionizable proton on the phenolic OH group.

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