Potentiometric and Spectrophotometric Study of the Co-ordination Compounds formed between Copper(II) and Dipeptides containing Tyrosine

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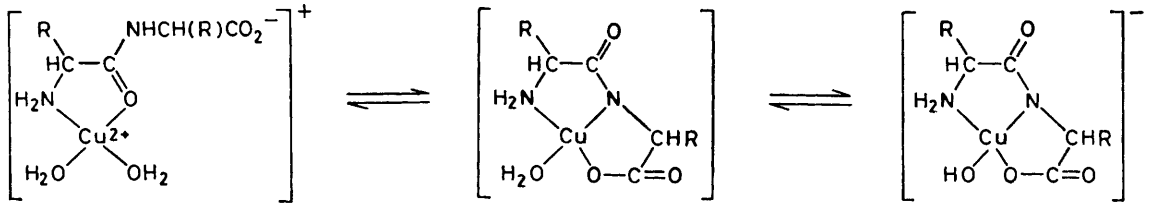
Co-ordination compounds formed between L-tyrosylglycine (H_2L), L-tyrosyl-L-leucine, L-tyrosyl-D-leucine, glycyltyrosine, L-leucyl-L-tyrosine, and L-tyrosineamide and H^+ and Cu^{2+} have been studied potentiometrically at 25 °C and I = 0.10 mol dm^{-3} ($K[NO_3]$). In addition to the expected complex compounds $[Cu(HL)]^+$, $[CuL]$, $[Cu(H_{-1}L)]^-$, $[Cu(H_{-2}L)]^{2-}$, and $[Cu(HL_2)]^-$, a binuclear complex, $[\{Cu(H_{-1}L)\}_2]^{2-}$, was found to form in the pH region 8—10.5 with tyrosylglycine and tyrosyl-D/L-leucine (giving a green solution) but was absent when tyrosine was the terminal carboxyl of the dipeptide. Spectrophotometric titrations showed this dimer to involve copper(II)–phenolate oxygen bonding. A structure for the dimer is described which explains the absence of a comparable dimer with glycyl- and leucyl-tyrosine, and the stereoselectivity found with the tyrosyl-D/L-leucine diastereoisomers.

THE principal modes of co-ordination of copper(II) ions to simple dipeptides (HA) is now well established. At low pH a $[CuA]^+$ species is formed with the dipeptide acting as a bidentate ligand (I). Around pH 5 the amide proton ionizes in the presence of Cu^{2+} allowing rearrangement of the donor centres to give structure II, the $[Cu(H_{-1}A)]$ species. At higher pH values (>9) the equatorial water molecule ionizes to form a monohydroxy-complex, III, of formula $[Cu(H_{-2}A)]^-$ {or $[Cu(H_{-1}A)(OH)]^-$}. In addition to these mono complexes, bis complexes are also well established. These include $[CuA_2]$, $[CuA(H_{-1}A)]^-$, and a polynuclear species $[Cu_2(H_{-3}A_2)]^-$. Two suggestions have been made as to the structure of the $[CuA(H_{-1}A)]^-$ complex. One is five-co-ordinate [1,2] and the other is planar.[3,4] The polynuclear complex has been detected by a number of workers as a clearly defined species although its structure is far from clear. Structures suggested include a monohydroxy-bridge [2,4-6] and a binuclear five-co-ordinate complex.[7]



I II III

When the dipeptide has side-chains containing potential donor atoms, more complicated complex species are to be expected. A number of histidine-containing dipeptides have been studied. The major species were the expected mononuclear complexes, although results did suggest the presence of binuclear complexes as minor species.[8,9]

There is little information on complexes in which the side-chain contains centres with only limited donor properties to Cu^{2+} (*e.g.* –OH) although the mode of co-ordination in complexes of the corresponding amino-acids has been a frequent subject for study, with often inconclusive results.[10]

We now report the results of a study of the complexes of tyrosylglycine (H_2L), tyrosyl-L/D-leucine, glycyltyrosine, leucyltyrosine, and tyrosineamide with H^+ and Cu^{2+}. The most interesting species to emerge from this study is a binuclear complex, $[\{Cu(H_{-1}L)\}_2]^{2-}$, causing a green colouration in solution, found with Tyr-Gly and Tyr-D/L-Leu but not with Gly-Tyr or Leu-Tyr.

EXPERIMENTAL

Dipeptides.—L-Tyrosylglycine, glycyl-L-tyrosine, and tyrosineamide were obtained from the Sigma Chemical Co. L-Tyrosyl-L-leucine and L-tyrosyl-D-leucine were synthesized from the optically pure amino-acids. The amine group of L-tyrosine was protected by forming the benzyloxycarbonyl-derivative (Z-Tyr) by the usual Schotten–Bauman procedure.[11] An active ester of Z-Tyr was synthesized using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide.[12] The resulting *N*-protected active ester was treated with L(or D)-leucine in alkaline solution. The Z-protecting group was removed by hydrogenolysis to give the dipeptide, which was recrystallized from a 1 : 1 ethanol–water mixture. L-Leucyl-L-tyrosine was made by an identical method from Z-Leu.

Analyses.—L-Tyr-L-Leu, white crystals, α (20 °C, 589 nm) = 3.0° (Found: C, 57.85; H, 7.85; N, 8.9. $C_{15}H_{22}N_2O_4 \cdot H_2O$ requires C, 57.7; H, 7.7; N, 9.0%).

L-Tyr-D-Leu, small white needles, α (20 °C, 589 nm) = 112.8° (Found: C, 57.65; H, 7.75; N, 9.0. Required values as for L-Tyr-L-Leu).

L-Leu-L-Tyr, white crystals, α(20 °C, 589 nm) = 7.3°

(Found: C, 60.15; H, 7.45; N, 9.25. $C_{15}H_{22}N_2O_4 \cdot 0.25H_2O$ requires C, 60.3; H, 7.55; N, 9.35%).

Potentiometric Studies.—Metal complex formation constants were calculated from potentiometric titration curves of the dipeptides in the absence and presence of metal ions at 25 °C and $I = 0.10$ mol dm^{-3} (K[NO_3]). Changes in pH were followed using a glass electrode and Radiometer PHM64 pH-meter calibrated in terms of hydrogen-ion concentrations. Titrations were carried out at metal : ligand ratios of 1 : 1 to 1 : 2 (although most complex species were most clearly defined in 1 : 1 mixtures) and with a range of ligand concentrations between 0.001 and 0.005 mol dm^{-3}. Formation constants were calculated from the experimental data with the help of the MINIQUAD computer program.[13] The quoted standard deviations are meaningful for the purposes of internal comparison but do not include systematic errors.

Spectrophotometric Studies.—These were performed using a Cary 14H spectrophotometer at 21 °C, over the spectral range 350—800 nm.

RESULTS AND DISCUSSION

Complex formation constants were expressed by the recognised convention as overall formation constants, β_{xyz}, where *xyz* have the values required by the formula $M_xL_yH_z$.[4] A neutral dipeptide such as tyrosylglycine (H_2L) can form the protonated species H_3L^+, H_2L, HL^-, and L^{2-}, the last proton to ionize being the phenolic proton of the tyrosine group. In the presence of Cu^{2+} the amide proton can ionize to give the species [Cu(HLH_{-1})], *i.e.* a 110 species similar to the simple [CuL]. Hence potentiometry cannot distinguish unambiguously between the various possibilities for proton ionization, including hydrolysis of co-ordinated water, since very different species may have the same apparent stoicheiometry. Since dipeptides containing tyrosine have an additional ionizable proton (the phenolic proton) compared to simple dipeptides, and since this proton will be one of the last to ionize as pH is raised, comparisons of formation constants must be made on the basis of $M_xL_yH_z$ for tyrosine-containing complexes, which is comparable to $M_xA_yH_{z-1}$ for complexes of simple dipeptides.

Proton complex formation constants are shown in Table 1, together with literature values for Gly-Tyr and Leu-Tyr, the only ligands previously studied in detail. The values for β_{011} refer predominantly to protonation of the phenolate oxygen, β_{012} also includes protonation of the amine nitrogen, and β_{013} includes carboxyl protonation as well. Hence tyrosineamide would be expected to be only dibasic. The proton complex formation constants have values close to those expected.[6,7]

TABLE 1

Proton complex formation constants at 25.0 °C and $I = 0.10$ mol dm^{-3} (K[NO_3]). Standard deviations (σ values) are given in parentheses

Dipeptide	log β_{011}	log β_{012}	log β_{013}
Gly-Tyr	10.133(3)	18.335(3)	21.390(4)
	10.31 [a]	18.68 [a]	21.69 [a]
Tyr-Gly	9.926(2)	17.613(3)	20.767(4)
L-Leu-L-Tyr	10.179(3)	18.019(3)	21.282(5)
	10.08,[b]	17.81,[b]	21.01,[b]
	10.15 [c]	17.97 [c]	21.20 [c]
L-Tyr-L-Leu	10.112(3)	17.525(3)	20.919(4)
L-Tyr-D-Leu	10.383(4)	18.243(4)	21.260(5)
Tyrosineamide	9.861(1)	17.334(1)	

log K_{031}^{011} values: L-Tyr-L-Leu = 10.81; L-Tyr-D-Leu = 10.88

$pK_{OH} = \log \beta_{011}$
$pK_{NH_2} = \log \beta_{012} - \log \beta_{011}$
$pK_{COOH} = \log \beta_{013} - \log \beta_{012}$

[a] H. Dobbie and W. O. Kermack, *Biochem. J.*, 1955, **59**, 246. [b] Ref. 7. [c] Ref. 6.

Stereoselectivity in the protonation constants of L-Tyr-D/L-Leu is close to that found generally for optically active dipeptides,[6,14] *i.e.* the *meso* form of the Zwitterion is particularly stable. Hence the carboxyl group ionizes at a lower pH in the *meso* (L-D) complex than in the optically active (L-L) complex while both the phenolic and amine protons are retained to a higher pH. The combined formation constants for the protonation of the carboxyl and amine groups are approximately the

TABLE 2

Copper complex formation constants (log β values) at 25.0 °C and $I = 0.10$ mol dm^{-3} (K[NO_3]). Standard deviations (σ values) are given in parentheses

	Gly-Tyr	Tyr-Gly	L-Leu-L-Tyr	L-Tyr-L-Leu	L-Tyr-D-Leu	Tyrosineamide [a]
β_{110}	12.096(1)	11.409(1)	12.243(1)	11.177(2)	10.854(1)	8 093(5)
	12.36 [b]		12.00,[c] 11.92 [d]			
β_{111}	16.127(8)	15.18(2)	15.51(6)	15.04(2)	15.26(1)	14.378(2)
	16.21 [b]		15.27,[c] 15.30 [d]			
β_{11-1}	3.029(3)	2.32(1)	3.088(3)	1.73(3)	1.70(1)	0.986(1)
	3.11 [b]		2.96 [c]			
	−7.70(1)	−7.890(6)	−7.388(3)	−8.506(7)	−8.722(5)	−8.990(1)
			−7.34 [c]			
β_{121}	25.53(1)	24.08(2)	25.05(1)	23.72(3)	23.91(1)	20.24(1)
β_{22-3}	−1.85(4)		−2.29(7)			−5.42(1)
			−1.9 [c]			
β_{22-2}		7.26(6)		6.88(4)	6.10(4)	
K_{111}^{011}	5.00	5.25	5.33	4.93	4.89	4.52
K_{111}^{110}	3.84	3.77	3.27	3.86	4.41	6.29
K_{110}^{11-1}	9.07	9.09	9.15	9.45	9.15	7.11
K_{11-1}^{11-2}	10.72	10.21	10.48	10.24	10.42	9.98
K_D [e]		2.62		3.42	2.70	

[a] log $\beta_{122} = 27.53(5)$. [b] H. Dobbie and W. O. Kermack, *Biochem. J.*, 1955, **59**, 246. [c] Ref. 7. [d] Ref. 6. [e] $K_D = \log \beta_{22-2} - \log \beta_{11-1}$.

same in the two diastereoisomers (log K = 10.81 and 10.87).

Formation constants for the complexes with Cu^{2+} are shown in Table 2. Since complexes of L-Leu-L-Tyr, L-Leu-D-Tyr, and D-Leu-L-Tyr had been studied previously by Nakon and Angelici [6] and by Kaneda and Martell [7] we studied L-Leu-L-Tyr only of the Leu-Tyr isomers in an attempt to detect a dimeric copper complex.

Stereoselectivity in the formation constants for L-Tyr-D/L-Leu was found to be close to that expected from a study of comparable complexes.[14] There was insignificant stereoselectivity in the 111 complexes, but it was significant in the 110 complexes (which correspond to the 11—1 complexes of simple dipeptides), the optically active species being the more stable. Comparison of the values for log K_{111}^{110} [*i.e.* ionization of the amide proton to form the major copper(II)–dipeptide species at intermediate pH] shows that the optically active complex is more acidic (3.86) than the *meso* analogue (4.41). These values may be compared with those found for comparable complexes formed from dipeptides with only one optically active centre. Results suggest that the positive stabilization found in the optically active complex may be the result of the hydrophobic interaction between the two aromatic side chains which are close together in the optically active complex, but are on opposite sides of the molecule in the *meso* complex. Such hydrophobic interaction will tend to minimise unfavourable free-energy effects at the interface between the solvent (water) and the phenyl rings. Significant stereoselectivity is also found in the $[\{Cu(H_{-1}L)\}_2]^{2-}$ complexes. This is discussed later.

The tyrosine-containing dipeptides behaved similarly to simple dipeptides below pH 9, forming $[Cu(HL)]^+$ and [CuL] species. This latter species has a formation constant comparable to that for the $[Cu(H_{-1}A)]$ species of simpler dipeptides and can be assumed to be, in reality, $[Cu(HLH_{-1})]$ (*i.e.* structure II). It is the major species in solution in the pH range 5—9 and, for most practical purposes, can be assumed to be the only species in the region of pH 6.5. Tyrosineamide also forms $[Cu(HL)]^+$ and [CuL] species. The [CuL] species $\{[Cu(HLH_{-1})]\}$ in particular is less stable than the analogous dipeptide complexes because it is unable to form a second chelate ring through the carboxyl group.

In the region of pH 8 significant differences in the behaviour of tyrosyl dipeptides compared to other dipeptides were noticed. With most dipeptides, complexes with Cu^{2+} remained royal blue as the pH was raised. With Tyr-Gly and Tyr-Leu, however, the solutions became distinctly green in the pH range 8—10.5, returning to blue above this range. Attempts to fit the experimental data with model systems based on the species normally found in copper(II)–dipeptide systems were all unsatisfactory in this pH range. However, the inclusion of a $[\{Cu(H_{-1}L)\}_2]^{2-}$ dimer immediately gave an entirely satisfactory fit, particularly in the 1 : 1, Cu^{2+} : ligand data. This dimeric species was found with Tyr-Gly and both isomers of L-Tyr-D/L-Leu. It was absent with Gly-Tyr, Leu-Tyr, and tyrosineamide. The dimeric $[Cu_2(H_{-3}L_2)]^{3-}$ species referred to earlier was generally found to be present when the $[\{Cu(H_{-1}L)\}_2]^{2-}$ dimer was absent. While the 22 — 3 dimer appears to be of general occurrence in copper(II)–dipeptide systems, the 22—2 dimer found with these tyrosyl dipeptides has not been reported previously.

When the pH was raised above 10.5 the monomeric $[Cu(H_{-2}L)]^{2-}$ became the major species in all the systems studied. It may be assumed that this species has the structure III with a co-ordinated hydroxyl group. The phenolate oxygen will be deprotonated.

The green colour found in the copper(II)–tyrosyl systems in the pH range 8—10.5 coincided exactly with the formation of the dimeric $[\{Cu(H_{-1}L)\}_2]^{2-}$ species. The colour was found to be due to an absorption band at 375 nm (ε approximately 300 $dm^3\ mol^{-1}\ cm^{-1}$) on the edge of the large charge-transfer band found in these systems. On its own this band would have given the solution a yellow colour; the green colour results from mixing with the normal royal blue colour arising from the copper *d–d* transition at 800 nm. A spectrophotometric titration showed this band at 375 nm to increase to a maximum absorbance at pH 9.5 and then to decrease coinciding exactly with formation of the $[\{Cu(H_{-1}L)\}_2]^{2-}$ dimer. An identical spectral peak was found with both isomers of Tyr-D/L-Leu but it was completely absent with Gly-Tyr, Leu-Tyr, and tyrosineamide. A species distribution graph for the copper(II)–Tyr-Gly (1 : 1) system is shown in Figure 1.

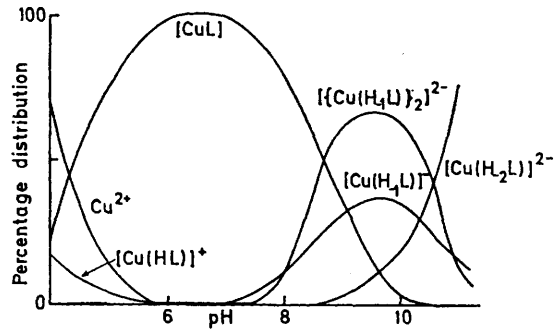


FIGURE 1 The species distribution graph for the 1 : 1 copper(II)–tyrosylglycine system (I = 0.01 mol dm^{-3})

There is good evidence that an absorption band at 375 nm indicates the presence of copper(II)–phenolate bonding. Much early work on copper(II)–tyrosine systems suggested the absence of copper(II)–phenolate interaction, and the absence of stereoselectivity in the copper(II)–D/L-tyrosine systems was interpreted as supporting this.[15] However, an absorption band at 390 nm in the copper(II)–tyrosine system above pH 10 was detected by Letter and Bauman,[16] and assigned to copper(II)–phenolate interaction. This type of interaction has also been found with catechol-type ligands * such as

* Catechol = *o*-Dihydroxybenzene; L-dopa = 3-(3,4-dihydroxyphenyl)alanine; epinephrine = 3,4-dihydroxy-α-[(methylamino)methyl]benzyl alcohol.

catechol,[17] L-dopa,[17,18] epinephrine,[17] and *NN'*-ethylenebis(2-*o*-hydroxyphenyl)glycine.[19]

Although there is dispute over the assignment of formation constants to these systems it is clear that, while L-dopa bonds glycine-like at low pH, at high pH the bonding is catechol-like through the phenolate oxygens. This bonding has been shown to give rise to absorption maxima in the visible region between 375 and 405 nm (ε *ca.* 200 dm^3 mol^{-1} cm^{-1}).[17-20] Phenolate metal interaction is important in a number of biological systems including the apoproteins ovotransferrin and transferrin. Both are colourless in the absence of metal ions but bind two copper(II) ions per molecule to give yellow complexes characterised by a strong absorption band at 440 nm (ε = 2 200 dm^3 mol^{-1} cm^{-1}).[21]

Spectrophotometric studies therefore demonstrate that the dimeric $[\{Cu(H_{-1}L)\}_2]^{2-}$ species contains copper(II)–phenolate bonding. The probable structure is shown in Figure 2. The reason for the formation of the dimer with tyrosyl dipeptides while it does not form with Gly-Tyr is not immediately obvious. However, examination of molecular models based on Figure 2 suggests that

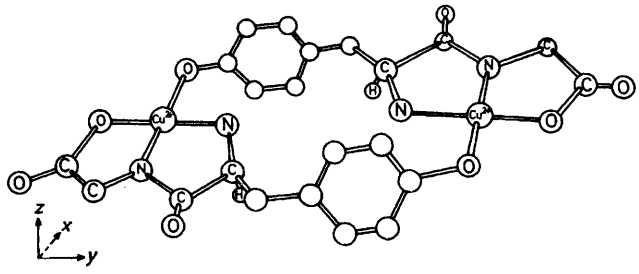


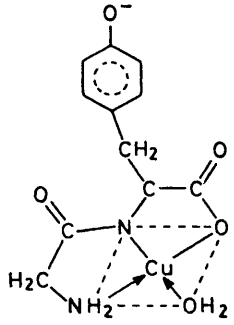
FIGURE 2 The probable structure for $[\{Cu(H_{-1}L)\}_2]^{2-}$, H_2L = tyrosylglycine

the reason for non-formation is essentially steric. Similarly steric factors can explain the stereoselectivity found in Tyr-D/L-Leu.

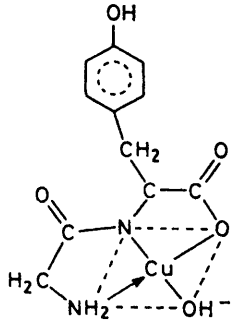
The structure shown can exist in two relatively sterically unhindered extremes. In the one shown, the two metal-ion chelate rings are approximately coplanar (*xy* plane). The two phenyl rings are then best situated sterically at 90° to this plane and parallel to each other in the *yz* plane. It is likely that this conformation is stabilized by stacking interactions between the phenyl rings. An advantage of this structure is that there are no significant destabilizing interactions, and the phenyl rings form a single compact hydrophobic region. In the other conformation extreme the structure shown in Figure 2 can be imagined as folded such that the chelate ring planes are parallel to each other in the *xz* plane. In this conformation the phenyl rings are at right angles in the *xz* and *yz* planes respectively. Hence they cannot form a compact hydrophobic region. What is more, the carboxyl groups and the metal ions are much closer together than in the conformation illustrated. As a result the structure shown is to be preferred. This structure explains why Gly-Tyr does not form a similar binuclear species. It is not possible to construct a $[\{Cu(H_{-1}L)\}_2]^{2-}$ complex with the metal ion chelate rings coplanar because the carboxyl groups are then too close from both steric and electrostatic points of view.

The $[\{Cu(H_{-1}L)\}_2]^{2-}$ dimer formed with Tyr-L-Leu was significantly more stable (0.7 log units) than with Tyr-D-Leu. Molecular models showed that the hydrophobic region can be much more compact in the optically active complex than in the *meso* analogue. Hence the origin of the steroselectivity is probably the same as that found in the $[Cu(HLH_{-1})]$ complex.

Ionization of a further proton, from a co-ordinated water molecule, normally proceeds without significant stereoselectivity. In the case of the tyrosine dipeptides ionization of the phenolate oxygen and of a co-ordinated water molecule overlap. This is clear from Figure 1 where the $[Cu(H_{-2}L)]^{2-}$ species starts to form before the $[Cu(H_{-1}L)]^-$ species has reached its maximum concentration. It is probable, therefore, that the $[Cu(H_{-1}L)]^-$ species contains contributions from both species IV and



IV



V

V. A typical value for the ionization constant of a co-ordinated water molecule in a copper(II)–dipeptide complex is pK = 9.4.[4] Values for the ionization constants of copper(II)–Tyr-Gly suggest that the $[Cu(H_{-1}L)]^-$ species is predominantly species V. However, Figure 1 demonstrates that, as the pH is raised, the sequence of complex formation is as shown below.

$$[Cu(H_{-1}L)]^- \rightleftharpoons [\{Cu(H_{-1}L)\}_2]^{2-} \rightleftharpoons [Cu(H_{-2}L)]^{2-}$$

Since it has been shown that the dimer must contain copper(II)–phenolate bonding, it is reasonable to assume that the species $[Cu(H_{-1}L)]^-$ also contains a significant, if minor, concentration of species IV. This is supported by the fact that while log K_{110}^{11-1} values are almost identical for Gly-Tyr and Tyr-Gly (9.08) the value of log $K_{11\text{-}1}^{11-2}$ for Tyr-Gly (10.2) is significantly lower than for Gly-Tyr (10.7). Table 2 shows that the ionization represented by log $K_{11\text{-}1}^{11-2}$ is stereoselective, unlike simple dipeptides. This again suggests that the macro-ionization reaction cannot be due entirely to ionization of a co-ordinated water molecule.

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