# Spectroscopic and Potentiometric Study of Protonation and Copper(II) Complex Formation of 3-Amino-L-tyrosine

**Pál Sipos**
*Department of Inorganic and Analytical Chemistry, Attila József University, H-6720 Szeged, Hungary*
**Tamás Kiss** *
*Department of Inorganic and Analytical Chemistry, Kossuth University, H-4010 Debrecen, Hungary*

A pH-metric and spectroscopic (u.v.–visible and e.s.r.) study has been made of the proton and copper(II) complexes of 3-amino-L-tyrosine at 25 °C and $I$ = 0.2 mol dm$^{-3}$ (KCl). The acid–base chemistry of the ligand has been characterised at both macroscopic and molecular levels by determining the microscopic constants of the overlapping protonation processes. It has been established that the ligand displays a marked ambidentate character in the copper(II) complexes. Accordingly, various monomeric complexes involving aminocarboxylate- and aminophenolate-type co-ordination, and dimeric complexes involving simultaneous metal-ion co-ordination at both binding sites, are formed.

A number of studies have focused on the co-ordination behaviour of tyrosine and tyrosine analogues.[1–4] Through a combination of the usual potentiometric, calorimetric, and group-specific methods such as u.v.–visible spectrophotometry,[1–3] optical rotatory dispersion (o.r.d.),[4] and n.m.r.[5] techniques it was possible to reveal the protonation and some metal-ion co-ordination processes of various tyrosine derivatives at both macroscopic and molecular levels.

One of the newest tyrosine derivatives, 3-amino-L-tyrosine (atyr), as a biomolecule, is formed in the degradation of pheomelanin in living organisms.[6–9] It is known to exert antibacterial[10] and antifungal[11] activity.

Its proton and metal-ion binding behaviour is rather complicated since, similarly to a hydroxy derivative of tyrosine, L-3-(3,4-dihydroxyphenyl)alanine (L-dopa), atyr contains two chelate-forming donor-group pairs separated within the molecule. Further, some of the potential donor groups have similar acidities, and thus their deprotonation takes place in overlapping processes. The deprotonation constants of the aromatic acidic groups of atyr determined by u.v. ($pK_{OH}$ = 10.0)[12] and fluorometric ($pK_{NH_3^+}$ = 4.4, $pK_{OH}$ = 10.0)[13] methods are partly erroneous, as the overlap between the deprotonation of the phenolic hydroxy group and that of the side-chain ammonium group was neglected.

The aim of our study was to obtain exact data on the deprotonation of atyr at macroscopic and molecular levels, and to acquire information on the stabilities and bonding modes of the complexes formed with copper(II) ion.

## Experimental

3-Aminotyrosine was an Aldrich product of puriss. quality. Its purity and the exact concentration of its solution were checked and measured by the Gran method.[14]

The proton dissociation and copper(II) complex formation constants were determined by pH-metric titration of samples (5 cm$^3$) in the range pH 3—11, or until precipitation. Precipitation occurred at pH ≈ 7—7.5 at metal ion:ligand ratios equal to or higher than 1:2, and pH ≈ 10 at a ratio of 1:4. The ligand concentration was $4 \times 10^{-3}$ mol dm$^{-3}$, the metal ion:ligand ratio was 0:1, 2:1, 1:1, 1:2, or 1:4, and the ionic strength was adjusted to 0.2 mol dm$^{-3}$ with KCl. These measurements were made on a Radiometer pHM64 instrument with G2040B glass and K4040 calomel electrodes. Since the ligand tends to undergo oxidation, all measurements were performed in a TTA 80 titration unit in an argon atmosphere. The electrode system was calibrated by the method of Irving *et al.*,[15] so the pH-meter readings could be converted into hydrogen-ion concentrations. In all cases the temperature was 25.0 ± 0.1 °C.

Absorption spectra in the u.v.–visible region were recorded on a Beckman ACTA MIV spectrophotometer, e.s.r. spectra on a JES-ME-3F spectrometer ($X$-band) at 77 K. The measurements were performed under an argon atmosphere, with sample concentrations similar to those used in the potentiometric studies.

The concentration stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$ were calculated with the aid of the PSEQUAD computer program.[16]

## Results and Discussion

*Protonation Processes.*—The pH-metric titrations of atyr led to the macroscopic deprotonation constants $pK_1$ = 1.95 ± 0.05, $pK_2$ = 4.48 ± 0.01, $pK_3$ = 9.09 ± 0.01, and $pK_4$ = 10.19 ± 0.03. To elucidate the protonation–deprotonation microprocesses, pH-spectrophotometric titrations were also carried out. The u.v. absorption of atyr exhibits a well defined bathochromic shift from 276 to 289 nm in the range pH 3—6, due to dissociation of the aromatic ammonium group. The deprotonation constant obtained from the spectral data was $pK$ = 4.46 ± 0.04, in good agreement with $pK_2$ obtained pH-metrically. Accordingly, it can be stated that the side-chain carboxylic group and the anilinic ammonium group deprotonate in fully separated stepwise processes and $pK_1$ can be ascribed to the former process and $pK_2$ to the latter. Additional spectral changes (a shift of the absorption band from 289 to 302 nm and an increase of the molar absorptivity by ≈40%) were found in the range pH 7.5—11.5, due to deprotonation of the phenolic hydroxy group. Because of the overlap between the deprotonation of the phenolic hydroxy group and that of the side-chain ammonium group, the macroscopic constant $pK_3$ and $pK_4$ are composites of the individual acidity constants of the two groups.[3] The group-specific spectral changes characteristic of the protonation state of the phenolic hydroxy group were used to determine the deprotonation microconstants as described in ref. 3. The final results of these calculations are presented in Figure 1.

Although the four chemically different protonating groups of atyr allow 16 different protonation isomers,[17] only six are

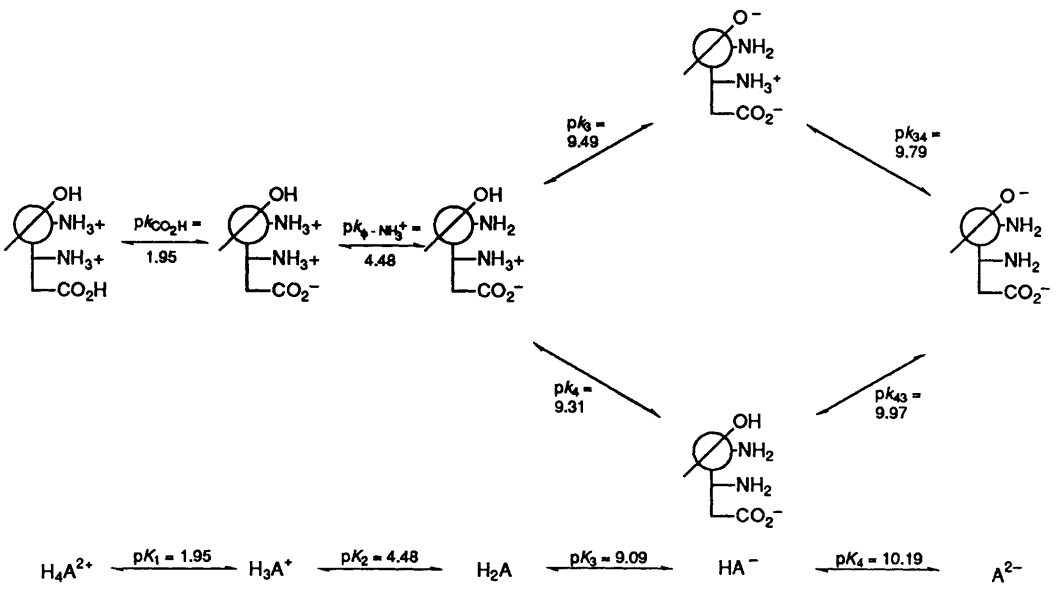



**Figure 1.** Deprotonation scheme and macroscopic and microscopic protonation constants of 3-amino-L-tyrosine

**Table.** Stability constants of the copper(II) complexes of 3-amino-L-tyrosine at 25 °C and $I$ = 0.2 mol dm$^{-3}$ (KCl)

| Species | log $\beta_{pqr}$ |
|---|---|
| $[Cu(H_2A)]^{2+}$ | 22.34 ± 0.05 |
| $[Cu(HA)]^{+}$ | 18.29 ± 0.09 |
| $[CuA_2]^{2-}$ | 16.62 ± 0.09 |
| $[Cu_2A_2H]^{+}$ | 35.58 ± 0.08 |
| $[Cu_2A_2]$ | 31.32 ± 0.03 |

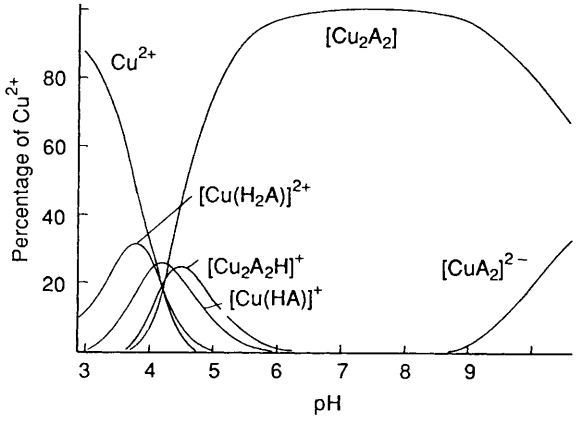


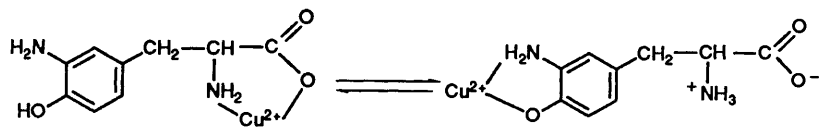

**Figure 2.** Concentration distribution of the complexes formed in the copper(II)–3-amino-L-tyrosine system as a function of pH. $c_{Cu}$ = 0.001, $c_{ligand}$ = 0.004 mol dm$^{-3}$

present in measurable concentration, and only six microconstants are obtained experimentally. No overlap was observed between the first two steps, and thus p$K_1$ and p$K_2$ could be regarded as microconstants characteristic of the true acidity of the $CO_2H$ group and the aromatic $NH_3^+$ group. The microconstants p$k_3$ and p$k_4$ indicate that the side-chain ammonium group is more acidic than the phenolic hydroxy group, as reported for tyrosine and its derivatives.[1,3,4] The interactivity parameter, which is characteristic of the change in electron-withdrawing effect of a given group due to the change in its protonation state, is p$k_3$ − p$k_{43}$ = p$k_4$ − p$k_{34}$ = 0.48. This value for tyrosine and other derivatives has been found to be ≈0.4.

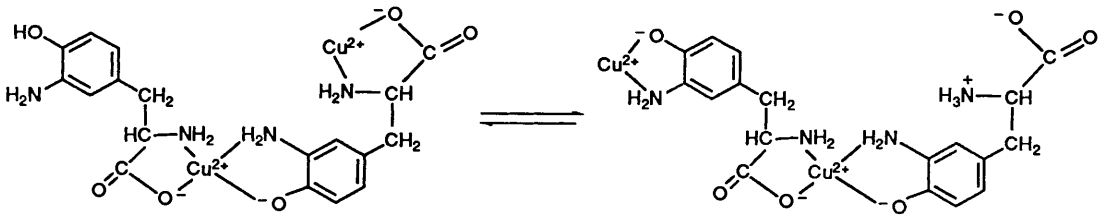
*Copper(II) Complex Formation.*—3-Aminotyrosine contains two separated chelate-forming donor-group pairs. Accordingly, copper(II) ion can co-ordinate *via* the side-chain donor groups to form ($CO_2^-$,$NH_2$)-co-ordinated complexes, or it can bind *via* the aromatic donor groups to form ($NH_2$,$O^-$)-co-ordinated species. Besides these monomeric species, there is a possibility for simultaneous metal-ion co-ordination at both metal binding sites, with the formation of various dimeric species too. The pH-metric titration curves for the copper(II)–atyr system were evaluated by assuming various speciation models on the basis of the results obtained for the copper(II)–L-dopa system.[2] The best fit between the measured and calculated titration curves was obtained by assuming the species given in the Table. The average difference between the measured and calculated titration curves (fitting parameter), characteristic of the quality of the fit,[16] was 0.0025 cm$^3$ (calculated from 268 experimental points). Other species such as $[CuA_2H_4]^{2+}$, $[CuA_2H_3]^{+}$, $[CuA_2H_2]$, $[CuA_2H]^{-}$, $[Cu_2A]^{2-}$, and $[Cu_2A_2H_2]^{2+}$ were also assumed, but they were all rejected by the computer program.

It can be seen from the species distribution curves (see Figure 2) that complex formation starts with the species $[Cu(H_2A)]^{2+}$; in this complex, copper(II) ion is co-ordinated to the amino acid side-chain donor groups. The e.s.r. parameters $g_{\|}$ = 2.315 and $A_{\|}$ = 158 cm$^{-1}$ agree very well with those for $[CuA]^{+}$ species of α-alanine (α-Ala), $g_{\|}$ = 2.31 and $A_{\|}$ = 160 cm$^{-1}$,[2] and seem to indicate that one nitrogen is bound to the copper(II) ion. The participation of the aromatic donor groups in the co-ordination occurs at pH ≈ 3.5 [at much lower pH than in the case of the copper(II)–L-dopa system, due to the higher overall acidity of the aminophenol moiety as compared to that of the catechol moiety], which is indicated unambiguously by the appearance of charge-transfer bands at ≈420 (sh) and 345 nm characteristic of the copper(II)–phenolate and –aromatic $NH_2$ interactions.[2] As the aminophenolate-type co-ordination and the deprotonation of the aromatic $NH_3^+$ group in the ($CO_2^-$, $NH_2$)-co-ordinated $[Cu(H_2A)]^{2+}$ species take place in the same pH range, equilibrium of the ($CO_2^-$,$NH_2$)- and ($NH_2$,$O^-$)-co-ordinated complexes (see Scheme 1) is highly probable for the complex $[Cu(HA)]^{+}$.

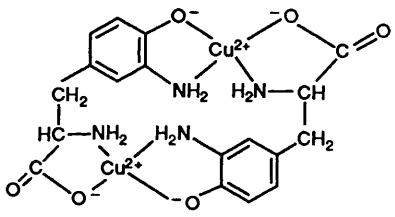
At high ligand excess and at pH > 9.5 the complex $[CuA_2]^{2-}$ is formed in relatively low concentration, this complex presumably involves purely aminophenolate-type co-ordination.



Scheme 1. $[Cu(HA)]^+$



$[Cu_2A_2H]^+$



$[Cu_2A_2]$

Scheme 2.

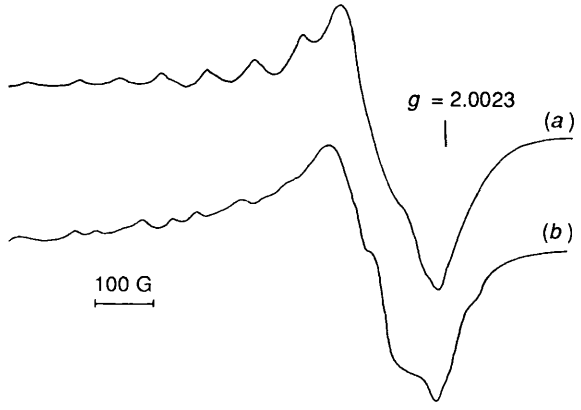



Figure 3. E.s.r. spectra (G = $10^{-4}$ T) of the copper(II)–L-dopa (*a*) and copper(II)–atyr (*b*) systems at 1:1 metal ion to ligand ratio, pH ≈ 7, and 77 K

Aminophenolate-type co-ordination is less favoured in atyr than in L-dopa, because of the much stronger metal-ion binding ability of the catecholate moiety in the latter ligand.

The overlapping of the pH ranges of aminocarboxylate and aminophenolate co-ordination makes the formation of dimeric species (with simultaneous metal-ion co-ordination at both binding sites) very favourable. Hence, as in the copper(II)–L-dopa system,[2] an open-chain dimer $[Cu_2A_2H]^+$ and a cyclic dimer $[Cu_2A_2]$ are formed; the latter is the dominant species in a wide pH range, even in the case of excess of ligand (see Figure 2). In the open-chain dimer $[Cu_2A_2H]^+$ a second copper(II) ion is co-ordinated to a $(CO_2^-,NH_2)(NH_2,O^-)$ 1:2 complex of mixed binding type at the free aminocarboxylate or aminophenolate moiety, and thus an equilibrium mixture of two species can be ascribed to the composition $[Cu_2A_2H]^+$ (see Scheme 2).

The cyclic dimer $[Cu_2A_2]$ is formed *via* ring closure of the open-chain dimer. The exclusive formation of this complex in a fairly wide range of pH or metal ion:ligand ratio is indicated by the unchanged u.v.–visible and e.s.r. spectral behaviour of solutions containing copper(II) ion and atyr in 1:1, 1:2, or 1:4 ratio at $6 < pH < 10$. The magnetic coupling between the copper(II) centres is strong enough to give rise to a rather complicated spectrum, with splitting of the e.s.r. signal, similar to that observed in the copper(II)–L-dopa system,[2] which confirms the suggested bonding mode (see Scheme 2). However, the differences in the e.s.r. behaviour of the two $[Cu_2A_2]$ complexes (see Figure 3), namely a well resolved equidistant seven-line signal with an intensity relation of approximately 1:2:3:4:3:2:1 for the complex formed in the copper(II)–L-dopa system, and a more complex spectra with more than seven non-equidistant lines in the $g_{||}$ region for the dimer formed in the copper(II)–atyr system, make it probable that in the latter complex the best planes of the donor atoms around the two copper(II) centres are not coplanar.

## Acknowledgements

The authors thank Dr. A. Rockenbauer (Central Chemical Research Institute, Budapest) for help in the explanation of the spectral results and Mrs. Á. Gönczy for assistance in the experimental work. This work was financially supported by the Hungarian Ministry of Education (Project 46/86).

## References

1 R. B. Martin, J. T. Edsall, D. B. Wetlaufer, and B. R. Hollingworth, *J. Biol. Chem.*, 1958, **233**, 1429.
2 A. Gergely and T. Kiss, *Inorg. Chim. Acta*, 1976, **16**, 51; 1983, **78**, 247.
3 T. Kiss and B. Tóth, *Talanta*, 1982, **29**, 539.
4 R. Benhallam, E. Collange, and M. R. Paris, *Bull. Chim. Soc. Fr.*, 1985, **6**, 1159.
5 R. F. Jameson, G. Hunter, and T. Kiss, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1105.

6 D. G. Patil and M. R. Chedekel, *J. Org. Chem.*, 1984, **49**, 997.
7 P. J. Angiolillo, L. A. Donoso, R. Folberg, J. J. Augsburger, and M. Wax, *Cancer Biochem. Biophys.*, 1985, **8**, 61.
8 J. Soshuke and K. Fujita, *Biochemistry*, 1985, **14**, 527.
9 S. Chaskes and R. L. J. Tyndall, *J. Clin. Microbiol.*, 1978, **7**, 146.
10 Y-K. Lin and J-J. Hwa, *Proc. Natl. Sci. Counc. Rep. China*, 1981, **5**, 75.
11 M. A. de Waard and J. G. H. van Nistelrooy, *Pestic. Biochem. Phys.*, 1919, **10**, 219.
12 M. Sokolowsky, J. F. Riordan, and B. L. Vallee, *Biochem. Biophys. Res. Commun.*, 1967, **27**, 20.
13 R. W. Cowgill, *Photochem. Photobiol.*, 1971, **13**, 183.
14 G. Gran, *Acta Chem. Scand.*, 1950, **29**, 599.
15 H. Irving, M. G. Miles, and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475.
16 L. Zékány and I. Nagypál, in 'Computational Methods for the Determination of Stability Constants,' ed. D. Leggett, Plenum, New York, 1985.
17 B. Noszál, *J. Phys. Chem.*, 1986, **90**, 4104.

*Received* 8*th February* 1990; *Paper* 0/00579G