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The Formation and Structure of a Cu(II)–Poly(L-Lysine, L-Tyrosine) Complex. Absorption and Resonance Raman Spectral Evidence of Phenolate Coordination

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The involvement of the phenolate oxygen of tyrosine residues at the metal binding sites of Fe(III), Cu(II) and several trivalent metal transferrins has been inferred using spectrometric techniques like ultraviolet difference spectra [1], nuclear magnetic resonance [2] and fluorescence [3]. The comparison of the resonance Raman (RR) spectra of metal transferrins with that of model compounds has corroborated these results and enabled the assignement of the enhanced RR bands to phenolate vibrations [4, 5]. However, none of those model compounds contained the tyrosine moiety but were either *ortho* or *para* phenolic derivatives. Boggess and Martin, on the other hand, have been able to obtain a Cu(II)–(DL-*o*-tyrosine)$_2$ complex with an *ortho*-phenolate oxygen coordinated to the metal [6]. Yet, as these authors pointed out, in the Cu(II)–(L-*p*-tyrosine)$_2$ complex, tue *para*-phenolate oxygen of tyrosine does not participate in binding to Cu(II). So far, to our knowledge, it appears that the *para*-phenolate oxygen of tyrosine acts as a ligand only in metal transferrins [4]. In this communication we report the results of a study on a Cu(II)–poly(L-Lysine, L-Tyrosine) complex using potentiometric, absorption, circular dichroism (CD) and RR data which provide positive evidence of phenolate participation in metal binding.

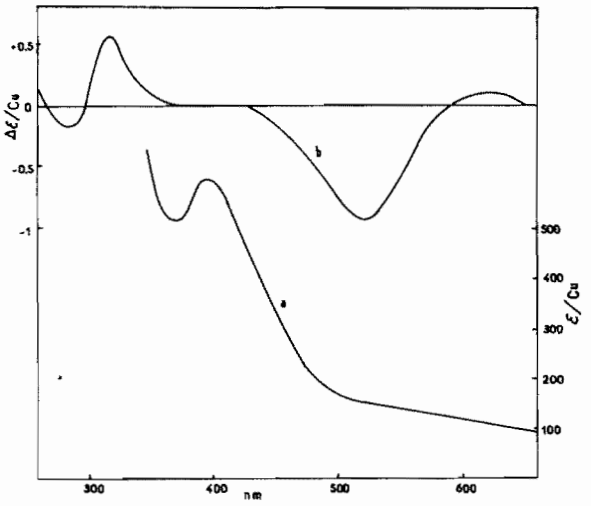

Figure 1. Absorption (curve a) and CD (curve b) spectra of Cu(II)–(L-Lys, L-Tyr)$_n$ complex in aqueous solution. [LyTy]/[Cu] = 4, [Cu] = 10^{-3} *M*, pH = 7.8.

In a previous study we have shown that the copolymer (L-Lysine, L-Tyrosine)$_n$·HBr 1:1 (Miles and Co., Lot LyTy 4), molecular weight 25,000, is soluble in water and precipitates at pH 9.2 at concentrations of the order of 10^{-3} *M* [7]. In the presence of Cu(II) ions and at molar ratios ([LyTy]/[Cu]) varying from 4 to 6 a complex is formed fron pH 6 on, as is made evident by changes in absorption and CD spectra. This complex (labelled I) is fully defined at pH 7.8, before precipitation occurs at pH 8.5 In Figure 1

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TABLE I. Absorption and CD Spectra of Cu(II) Complex of (L-Lys, L-Tyr)$_n$ at pH 8.0 and [LyTy]/[Cu] = 4 and of Cu(II) Complex of (L-Lys)$_n$ at pH 10.5 and [Lys]/[Cu] = 4.

Cu(II)–(L-Lys, L-Tyr)$_n$ Complex								
Ab	λ (nm)		550		390		L_b	transition
	ε		140		600	(±100)		
CD	λ (nm)	600		520		320	290	255
	Δε	+0.15		−0.90		+0.55	–	+
Cu(II)–(L-Lys)$_n$ Complex								
Ab	λ (nm)		530			320		260
	ε		120			1200		3600
CD	λ (nm)	590		510		320	290	255
	Δε	+0.10		−0.95		+0.55	−0.45	+1.7

the absorption and CD spectra of complex I are presented in curves a and b respectively. Spectral data are reported in Table I.

Potentiometric titrations of the (L-Lys, L-Tyr)$_n$–Cu(II) system at molar ratios ([LyTy]/[Cu]) equal or higher than 4 show that up to pH 7.8 five protons per cupric ion are released indicating the involvement of five coordination sites in complexation. A good amount of information on the nature of the ligands concerned and the symmetry of complex I is provided by comparing the CD spectrum of Figure 1 with that of the complex obtained in the system (L-Lys)$_n$–Cu(II) at pH 10.5, at molar ratios equal or higher than 4 [8]. In Table I are reported the spectral data of this complex. As can be noticed the CD spectral patterns of both complexes are virtually identical. The differences in the absorption spectra, instead, are quite significant. The strong absorption at 390 nm superimposes partially on the d–d transition at 550 nm (see Fig. 1). Moreover the L_b transition [9] of the tyrosyl residue masks the bands at 320, 290 and 255 nm. On the basis of spectrometric findings and stereochemical considerations we have shown that the cupric ion is bound to four nitrogens in the Cu(II)–(L-Lys)$_n$ complex. The nitrogens, lying at the corners of the coordination square, originate from two amino groups of lateral chains and two peptide groups [8]. In addition, we have assigned the bands at 320 and 255 nm to the two ligand–metal charge transfer (c.t.) transitions, the first from an amido-nitrogen and the second from an amino-nitrogen [8, 10, 11].

From the strong resemblance shown by the CD spectra of both complexes of Table I in the d–d transition region, we can infer a similar arrangement of nitrogen ligands around the metal. As is known, in copper complexes the ligand field is determined primarily by the four ligands lying at the corners of the coordination square, *i.e.* those closer to the metal. The ligands in apical position are practically without influence [12]. Furthermore, the presence of the band at 390 nm strongly suggests the involvement of, at least, a phenolate oxygen in complexation [4, 6, 13]. Both the absorption spectra of Cu(II)–transferrins [4, 5] and that of a model compound containing a phenolate bound to the copper in a planar position [5] present an absorption at 440 nm. Boggess and Martin, on the other hand, found a band at 393 nm in the absorption spectrum of Cu(II)–(DL-*o*-tyrosine)$_2$ [6]. These bands have been assigned to a phenolate to metal c.t. transition [4–6, 13]. Since the four positions in the coordination plane are already filled by nitrogen atoms in complex I, there are good grounds for phenolate apical coordination. As Barnes and Day have pointed out [14], a greater internuclear distance increases the energy of the charge transfer transition with respect to that of the same donor with shorter bond length. The much higher energy of the band at 390 nm in comparison with that of the model compound mentioned earlier [5] can be ascribed to a longer bond length compatible with apical coordination. A longer bond length does not allow for extensive overlap between donor and acceptor orbitals and this is in agreement with the rather low intensity of the 390 nm transition as compared to that of metal transferrins and the model compound of reference 5. Note that the transition at 390 nm in the spectra of complex I does not display optical activity (see Fig. 1) in contrast with that of copper transferrins [13, 15]. A longer bond length presumably accounts for this behaviour as well.

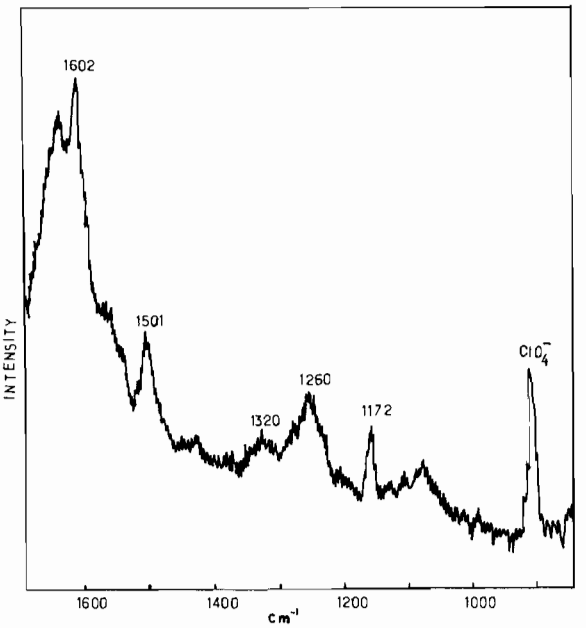

Figure 2. Raman spectrum of the Cu(II)–(L-Lys, L-Tyr)$_n$ complex in aqueous solution. [LyTy]/[Cu] = 4, [Cu] = 0.83 × 10^{-3} *M*, pH = 7.9 using 457.9 nm excitation (110 mW). Instrument conditions: spectral slit width, 4 cm^{-1}; time constant, 2.5 sec; scanning rate, 50 cm^{-1}/min.

Resonance enhanced Raman spectra of complex I can be obtained upon excitation with laser radiations the wavelengths of which fall within the contour of the absorption band at 390 nm. Figure 2 shows a typical RR spectrum of complex I. The spectral pattern is characteristic of metal transferrins [4, 5], except for an additional band at 1320 cm^{-1}, confirming phenolate coordination to copper. The frequencies of the other resonance enhanced bands are practically those of Cu(II)–ovotransferrin [5], namely: 1172, 1260, 1501 and 1602 cm^{-1}. These bands have been assigned mostly to a ring C–H bending, C–O phenolate stretch, ring "quadrant" stretch and symmetric ring stretch, respectively [4, 5]. The frequencies of the two ring stretches are

those characteristic of phenolate in the infrared spectra of the fully deprotonated form of the polymer [7]. There are some minor differences between the RR spectra of complex I and that of metal transferrins. The C–O stretching vibration is always the strongest in the latter and a broad shoulder, ranging from 1320–1350 cm^{-1} approximately, is always present. Two well defined bands of medium intensity at 1260 and 1320 cm^{-1} are observed in the RR spectrum of complex I instead, both being enhanced together.

As the band at 1260 cm^{-1}, the one at 1320 cm^{-1} might contain some contributions of C–O stretch, C–C ring stretch and C–H ring binding [5] in different proportions. Some alterations in the intensity pattern of Raman lines are by no means unexpected since they reflect a change in the environmental symmetry of the chromophore [16].

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