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Absorption, Circular Dichroism and Resonance Raman Spectra of Cu(II)–Poly(L-Glutamic, L-Tyrosine) Complexes. Evidence of Phenolate Coordination

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The phenolate oxygen of tyrosine does not participate in bonding to Cu(II) even at high pH [1]. However, metal–phenolate oxygen coordination has been well established in Fe(III), Cu(II) and several trivalent metal transferrins [2, 3]. Recently, we have shown that although poly (L-Tyr) does coordinate to Cu(II) at pH higher than 9, coordination takes place through peptide nitrogen, whereas phenolate oxygen binding is not detected [4]. However, when the random copolymer poly(L-Lys, L-Tyr) 1:1 interacts with Cu(II), phenolate oxygen is able to participate in binding once amino and peptide nitrogens have been already coordinated to the metal [4, 5].

In this report we present spectral results (absorption, circular dichroism and resonance Raman) on the interaction of Cu(II) with a random copolymer containing acid (glutamic) and tyrosine residues which give evidence of phenolate binding to the metal.

The copolymer poly(L-Gly, L-Tyr) 1:1, Miles and Co., lots 8 and 10, mW 38700 and 58300 respectively, have been used. At pH higher than 6.5 the copolymer is soluble in water. In the presence of Cu(II) at residue-to-copper molar ratios equal or higher than 8, a complex is formed (complex I) which remains in solution down to pH 4.8–5. Evidence of complex formation is provided by absorption data as is shown in Fig. 1. A band at 700 nm is observed from pH 4.8 on, which reaches maximum intensity at pH 5.2, the intensity being a function of molar ratio and becoming independent of it at molar ratios equal or higher than 8. The transition at 700 nm does not display any detectable optical activity.

These results are strictly analogous to those obtained by Takesada *et al.* [6] in the case of the Cu(II)–poly(L-Glu) system. As was further demonstrated a complex is formed in this system containing four carboxylate oxygens at the corners of the coordination square [7, 8]. As pH raising proceeds, the intensity of the 700 nm band decreases reaching its minimum at *ca.* pH 7.4 (see Fig. 1). A similar behavior was observed in the Cu(II) poly(L-Glu) system [6]. As was later proved the complex does not dissociate on increasing pH since copper is still bound to the polymer up to pH 7. In fact, the decrease in intensity at 700 nm accompanies the conformational transition from α helix to non periodic which is shifted to higher pH upon complexation [6]. Presumably, the ordered form imposes a rather distorted symmetry on the metal site making the d–d transitions more al-

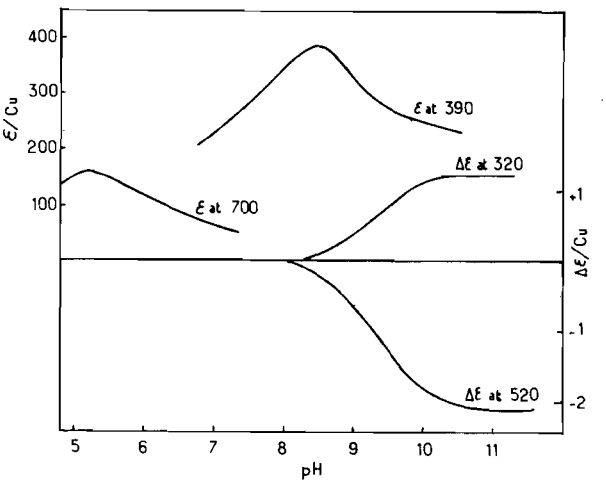

Fig. 1. Variation of molar absorption and molar circular dichroism coefficients as a function of pH at [Glu Tyr]/[Cu] = 10, [Cu] = 6.6×10^{-4} *M*. Figures upon curves indicate wavelengths of the different bands.

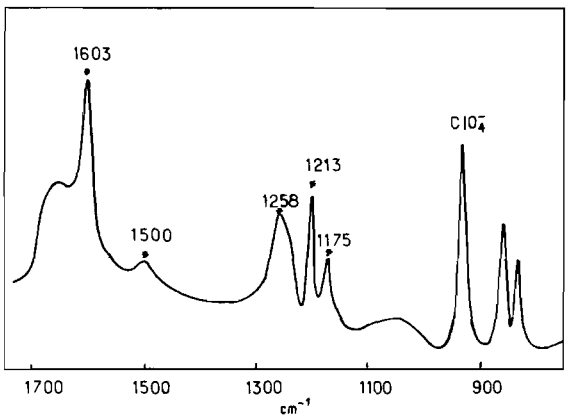

Fig. 2. Raman spectrum of the Cu(II)–poly(L-Glu, L-Tyr) system. [Ly Ty]/[Cu] = 8, [Cu] = 3×10^{-3} *M*, [ClO_4] = 0.01 *N*, pH 8.2. Excitation line 454.5 nm, 70 mW. The asterisk indicates the enhanced Raman peaks. Instrumental conditions: spectral slit width 5 cm^{-1}; time constant 2.5 sec; scanning rate 25 cm^{-1}/min.

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lowed. A similar behavior seems to take place in the Cu(II)–poly(L-Glu, L-Tyr) system. The copolymer which is ordered below pH 7 takes a non periodic conformation above this pH. In the presence of Cu(II) the periodic-to-non periodic transition is shifted to higher pH [9].

At pH 7 a new band (in fact a shoulder at the low frequency side of the L_b tyrosine transition) is apparent at 390 nm which reaches its maximum intensity at pH 8.4 (see Fig. 1). Again, the intensity at 390 nm is a function of molar ratio but is independent of it at molar ratios equal to or higher than 8. This transition does not show any discernable optical activity. An analogous absorption was noticeable in the Cu(II)–poly(L-Lys, L-Tyr) system and was assigned by us to a phenolate oxygen to copper charge transfer transition, an assignment confirmed by resonance Raman data [4, 5]. This finding clearly indicates the appearance of a new complex (complex II). Since the band at 700 cm^{-1} is still present, one must postulate phenolate apical coordination as in the Cu(II)–poly(L-Lys, L-Tyr) complex [4, 5]. Thus, in complex II, five oxygens at least are coordinated to the metal: four from carboxylates at the corners of the coordination square and one (or two) phenolate oxygens in apical position.

Note that in contrast with the Cu(II)–poly(L-Lys, L-Tyr) complex, complex II is formed in two steps. Moreover, as Fig. 1 indicates, it does not appear to be fully defined at pH 8.4 since its molar absorption coefficient is lower (380 l mol^{-1} cm^{-1}) than that of the former (600 l mol^{-1} cm^{-1}) [4, 5]. Despite this fact, resonance enhanced Raman spectra using the exciting lines of an Ar^+ laser that fall within the contour of the shoulder at 390 nm can be obtained. One typical example is illustrated in Fig. 2. The spectral pattern is very similar to that of the Cu(II)–poly(L-Lys, L-Tyr) complex [4, 5] and is characteristic of metal transferrins and phenolate coordination [2, 3]. The bands preferentially enhanced lie practically at the same frequencies: 1603, 1500, 1258 and 1175 cm^{-1}. In addition, the tyrosine ring fundamental at 1213 cm^{-1} is also observed and shows a similar pattern of enhancement as the band at 1175 cm^{-1}. Owing to the high polymer concentration (2.4×10^{-2} in Glu Tyr residues) two strong tyrosine fundamentals at 858 and 835 cm^{-1} are still present although they do not show resonance enhancement.

From pH 8.6 on the intensity at 390 nm decreases whereas two new bands become noticeable at 320 and 520 nm which display optical activity (Fig. 1). A new complex is thus formed at this stage (complex III) which reaches completion at pH 10.2. The two bands at 320 and 520 nm are characteristic of peptide nitrogen binding to the metal [10]. A similar behavior was observed in the Cu(II)–poly(L-Lys, L-Tyr) system; once peptide nitrogen coordination starts, Cu(II)–O(phenolate) bonds are broken and the absorption at 390 nm disappears [4, 5]. In the present system, however, peptide nitrogen binding starts before phenolate binding to metal ions has been completed.

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