FORMATION AND STRUCTURE OF Cu(II)-POLY(L-LYSINE) COMPLEXES IN AQUEOUS SOLUTION

Arlette GARNIER[+,++] and Lucia TOSI[+]

[+] *DEPARTEMENT DE RECHERCHES PHYSIQUES, LABORATOIRE ASSOCIE AU CNRS N° 71*
UNIVERSITE PIERRE ET MARIE CURIE, 4 Place Jussieu, 75230 PARIS Cedex 05
[++] *U.E.R. DE MEDECINE ET BIOLOGIE HUMAINE, UNIVERSITE PARIS-NORD BOBIGNY*

Received December 6,1976

SUMMARY : Cu(II)-poly(L-lysine) complexes have been studied using potentiometric titrations, optical absorption and circular dichroïsm spectra. As in the Cu(II)-poly(L-arginine) system studied previously potentiometric and spectral data consistently show that two types of complexes are formed. The first formed below pH 7.6 contains two amine nitrogens and two oxygen from water molecules at the corners of a square in which the metal occupies the center. The second is obtained at pH above 7.6 when the oxygen atoms are replaced by two adjacent peptide nitrogens.

The catalytic properties of metalloproteins depend to a large extent on the nature of the amino acid residues functioning as binding sites for metal ions and on the availability of one or more coordination sites for the substrate molecule. The study of the formation and structure of metal homopolypeptide complexes has been undertaken by many authors in order to gain some understanding of the specific interaction between metal ions and amino acid residues and of the catalytic efficiency of the complexes formed as well (1-8). Sigel and Blauer, in particular, have shown that catalytic activity in the decomposition of H_2O_2 by the Cu(II)-$(Lys)_n$ system is comparable or higher than that of Cu(II)ethylendiamine(1:1) strongly suggesting the presence of two available coordination positions in the complex which enable the formation of peroxo complexes (8).

In recent reports we have demonstrated that depending on pH, $(Arg)_n$ gives two different types of complexes with cupric ions (6,7). The first of them obtained at pH<8 contains two guanidinium nitrogens and two oxygens of water molecules at the corners of the coordination square around the copper. At pH>8 the second complex is formed by replacement of the two oxygens by amide nitrogens.

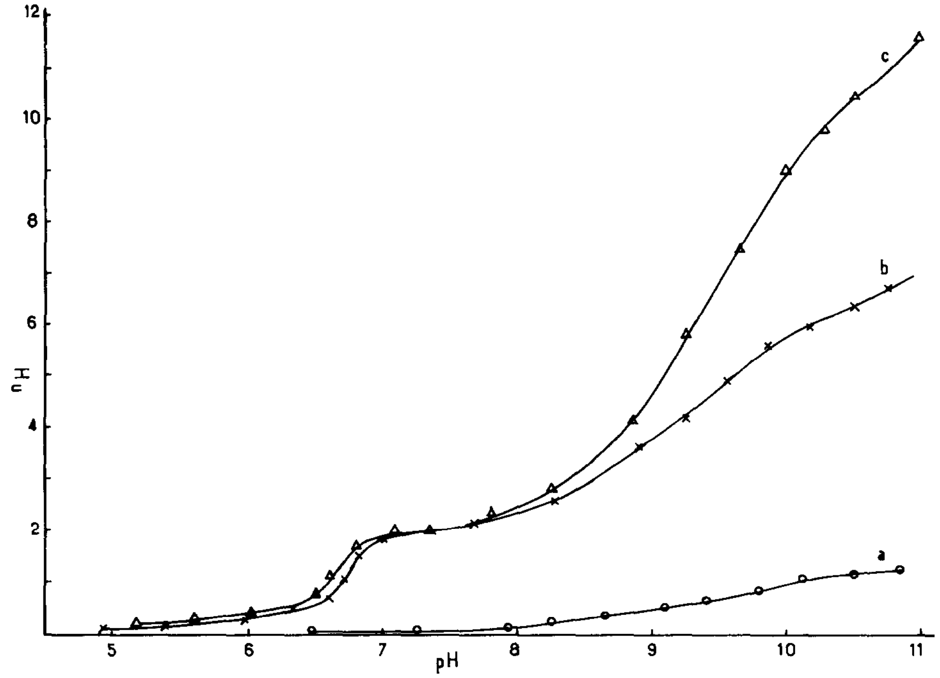

Figure 1. Potentiometric titrations curves of $(Lys)_n$ in the absence (curve a) and in presence (curves b and c) of cupric ions.
xxxx : |Lys|/|Cu| = 4 ; ΔΔΔΔ : |Lys|/|Cu| = 8 ; |Lys| = 2.10^{-4}M

In this communication we present the results of a similar investigation on the formation and properties on Cu(II)-$(Lys)_n$ complexes. This system has been already studied by Hatano et al. (2). Their spectroscopic results, however, were not consistent with their potentiometric data and with the structure assigned to the species obtained.

The results of potentiometric titrations of the Cu(II)-$(Lys)_n$ system at two values of the residue to copper molar ratios (|Lys|/|Cu|) are shown in Figure 1. Curve a gives the number of protons neutralized per Lys residue as a function of pH, curves b and c the number of protons neutralized per copper at molar ratios equalling 4 and 8 respectively. As can be noticed, when $(Lys)_n$ is titrated in the absence of cupric ions the releasing of protons starts above pH 8, and at pH ∿ 5.5 in the presence of copper. Up to pH 7.6 and independently of molar ratio

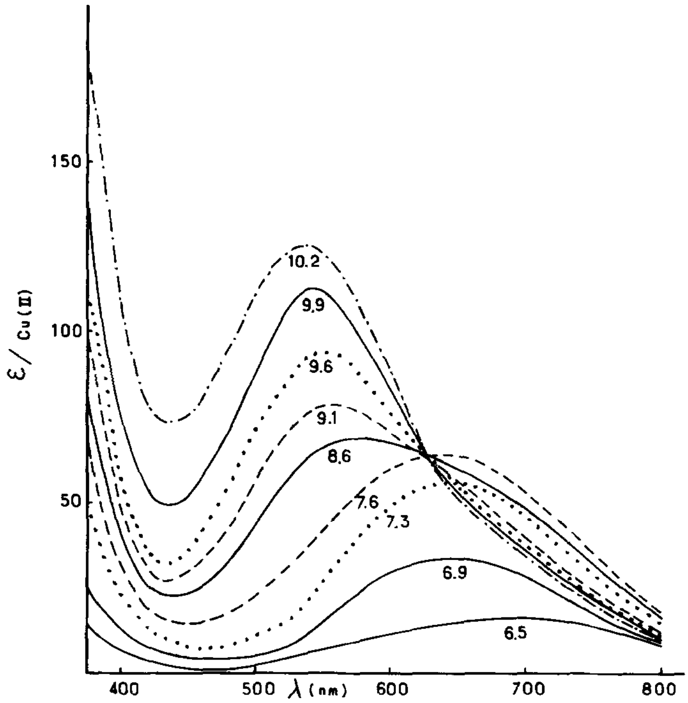

Figure 2. Optical spectra of the Cu(II)-$(Lys)_n$ system at |Lys|/|Cu| = 4 |Cu| = 0.5×10^{-4}M ; |Lys| = 2.0×10^{-4}M. Figures above curves indicate pH values.

(from |Lys|/|Cu| = 2 to 20) two protons per copper are removed yielding a first complex that contains two Lys residues coordinated to the metal. From pH 7.6 to 10.25 a second complex is formed and the number of protons released per cupric ion is always x + 2, x being the molar ratio. Since at this pH all the ammonium groups are deprotonated (one per residue as indicated by curve a) the two additional protons removed originate from the peptide group. These results do not agree with those of Hatano and coworkers which obtained a first complex below pH 8 corresponding to the loosing of four protons per copper and a second complex above pH 9 after releasing of two additional protons. They assigned to the first complex a tetragonal symmetry with the cupric ion coordinated to four amino-nitrogens from lateral chains, and to the second an octahedral symmetry with one peptide nitrogen and one hydroxyl group in apical positions (3-5).

Absorption and circular dichroïsm (CD) spectra confirm our findings and give additional information on the nature of the ligands involved in complexation. Figure 2 gives the plots of molar extinction coefficients per copper (ε/Cu) at different pH for the Cu(II)-$(Lys)_n$ system at |Lys|/|Cu| = 4. As the spectral patterns clearly show, a first complex is formed below pH 7.6 whose absorption spectrum presents a maximum at 640 nm (ε_M = 65). The second complex which is formed above pH 7.6 and until pH 10.25 exhibits another absorption whose λ_{max} lies at 530 nm (ε_M = 120). From pH 7.6 on an isosbestic point at 620 nm indicates that above this pH only the two species, complex I and complex II, are present in solution. The values of λ_{max} for the two complexes compare with those obtained by us in the case of the Cu(II)-$(Arg)_n$ system (6,7) and agree roughly with those found by Hatano et al. (3,5). Furthermore, they are consistent with copper coordination to two nitrogen and two oxygen atoms of water molecules lying at the corners of the coordination square in complex I (9) and with copper binding to four nitrogens in approximately square planar symmetry in complex II (10) as potentiometric data indicated. An absorption maximum at 645 nm, as obtained by Hatano et al. for complex I (3,5) is not consistent with copper coordination to four nitrogens in nearly square planar symmetry as assumed by these authors. Besides, the spectral shift to shorter wavelengths in going from complex I to complex II corresponds to an increase in the ligand field and this is primarily determined by donors in the coordination square (10).

The d-d transitions of complex I do not display optical activity which implies that the two nitrogen atoms bound to copper are those of the amino groups of lateral chains. In that case five atoms should lie between the assymetric carbon and the chromophore so, optical activity is not to be expected (11). Yet, the copper chromophore is rendered optically active as soon as complex II forms, showing that the two nitrogen atoms that replace the water oxygens are supplied by peptide groups. This type of binding is more plausible than the apical coordination of one peptide nitrogen suggested by Nozawa and Hatano (4,5) which is rather unusual even in the solid state (10). It has been pointed out that che-

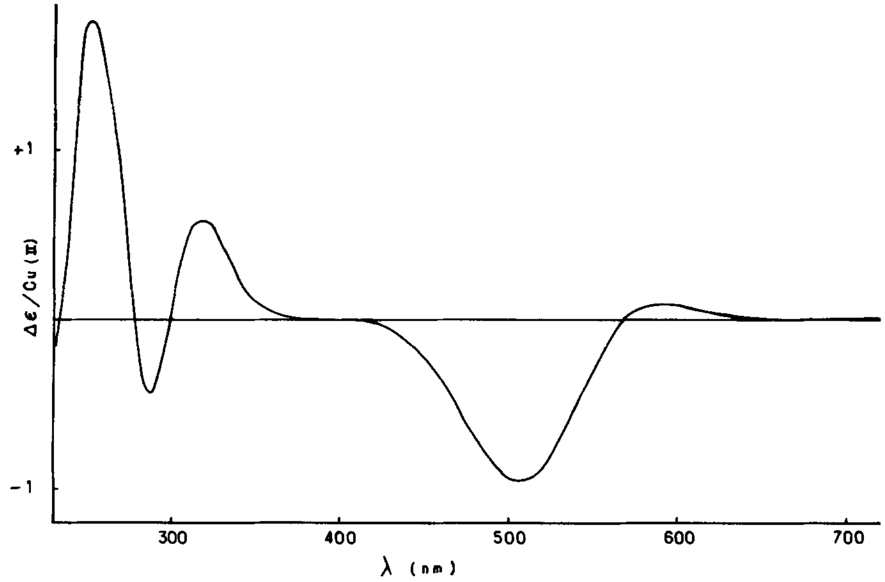

Figure 3. CD spectra of complex II.

---

lation of the optical active ligand rather than simple coordination is required for the effect on d-d transitions to be found (12,13). It can therefore be assumed that two adjacent peptide nitrogens must bind to the metal ion to form a five membered chelate ring in complex II. The CD spectrum of complex II is shown in Figure 3. It presents strong similarities with those obtained for complexes II_A and II_B in the Cu(II)-$(Arg)_n$ system and is characterized by a negative trough at 510 nm (Δε/Cu = 0.95), a positive peak at 590 nm, (+ 0.10), and three other bands at 255 (+ 1.7), 290 (-0.45) and 320 (+0.55). These three latter have been assigned by us to ligand to metal charge transfer transitions from a σ-amino nitrogen orbital for the first and from a Π-peptide orbital for the second and third,on grounds of spectroscopical data and stereochemical considerations (7). The spectrum of Figure 3 reminds that obtained by Hatano and coworkers (3) although there are minor differences. In addition, the frequency of the negative trough at 510 nm is independent of pH and molar ratio. This result is in striking contrast with Hatano et al. observations. These authors reported a shift of the 510 nm trough to lower frequencies as pH increases, which obviously indicates that other species are being formed, presumably complexes containing terminal amino nitrogens bound to copper (14). Moreover, at molar ratios equal or higher than 4 the circular dichroism at 510 nm is independent of molar ratio. At molar ratios lower than 4 the circular dichroism at 510 decreases proportionally to molar ratio. This behaviour which is characteristic of the Cu(II)-$(Orn)_n$ (14) system has not been observed in the case of Cu(II)-$(Arg)_n$ complexes (6,7).

In the absence of copper $(Lys)_n$ is fully ordered at pH 11. CD data at wavelengths lower than 250 nm show that copper binding partially inhibits conformational transition and the amount of ordered structure formed is proportional to molar ratio. At $|Lys|/|Cu| = 2$ the polymer remains in the non periodic form even at high pH. As molar ratio increases some ordered structure appears and, at molar ratios higher than 20, the Cu(II)-$(Lys)_n$ system contains practically the same percentage of ordered form as $(Lys)_n$ alone. At this molar ratio the proportion of amide nitrogen bound to the metal is negligible and this finding strongly suggests that copper coordination to the polypeptide hinders ordered structure locally.

From the foregoing results it can be concluded that the formation of a Cu(II)-$(Lys)_n$ complex at pH 7.6 containing one cupric ion coordinated to two oxygens of water molecules and two amino nitrogens is in perfect agreement with Sigel conclusions on the catalytic properties of Cu(II)-$(Lys)_n$ complexes, since water molecules may be easily replaced by the $O_2^=$ ion. In fact, the accessibility of two coordination sites to form peroxo complexes implies that the structure of complex I is cis.

EXPERIMENTAL : $(Lys)_n$.HBr (M.W. = 140.000) was purchased from Miles-Yeda Ltd and used without purification. All other chemical used were of analytical grade. All solutions were prepared with bidistilled water. Concentrations of $(Lys)_n$ solutions were determined by a micro-Kjeldahl method; copper content by EDTA. $(Lys)_n$ concentrations were equal or lower than 2×10^{-4}M in order to avoid precipitation at pH above 10.5 Potentiometric titrations were performed on a Tacussel Isis 2000 pHmeter provided with a Metrohm EA 147 combined Ag/AgCl glass electrode under Ar atmosphere. CD spectra were recorded with a Jobin-Yvon Dichrograph Model Mark II and the absorption spectra with a Cary 14 spectrometer. All measurements were made at 25°C.

Acknowledgements

We are very much indebted to Dr. Spassky of Laboratoire de Chimie Macromoléculaire, Université Pierre et Marie Curie, who put at our disposal the Jobin-Yvon model Mark III Dichrograph.

References

1. PECHT, I., LEVITZKI, A., and ANBAR, M. (1967) J. Am. Chem. Soc. 89, 1587-1591.
2. LEVITZKI, A., PECHT, I., and BERGER, A. (1972) J. Am. Chem. Soc. 94, 6844-6849.
3. HATANO, M., NOZAWA, T., IKEDA, S., and YAMAMOTO, T. (1971) Makromol. Chem. 141, 1-9, 11-19.
4. NOZAWA, T., and HATANO, M., (1971) Makromol. Chem., 141, 21-29.
5. HATANO, M., and NOZAWA, T., (1972) Progress in Polymer Sci. 4, 223-278.
6. TOSI, L., and GARNIER, A., (1974) Biochem. Biophys. Res. Comm. 58, 427-432.
7. GARNIER, A., and TOSI, L., (1975) Biopolymers, 14, 2247-2262.
8. SIGEL, H., and BLAUER, G., (1968) Helv. Chim. Acta, 51, 1246-1256.
9. GURD, F.R., and BRYCE, G.F., (1966) in The Biochemistry of Copper, PEISACH, J., AISEN, P., and BLUMBERG, W.E., Eds., Academic Press, New-York, p. 115-129.
10. FREEMAN, H.C., (1966) in The Biochemistry of Copper, PEISACH, J., AISEN, P., and BLUMBERG, W.E., Eds., Academic Press, New-York, p. 77-113.
11. HAWKINS, C.J. (1971) Absolute Configuration of Metal Complexes, p. 177-180, Wiley Interscience, New-York.
12. BHATNAGAR, D.C., and KIRSCHNER, S. (1964) Inorg. Chem. 3, 1256-1263.
13. LARSEN, E., and OLSEN, I. (1964) Acta Chem. Scand. 18, 1025-1031.
14. To be published.