Vol. 58, No. 2, 1974 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

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

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Received March 3,1974

SUMMARY : Cu (II) - poly (L-arginine) (PLA) complexes have been studied using potentiometric titrations, optical absorption and circular dichroism spectra. Three different complexes have been observed. The first one (complex I) is formed up to pH 8 and results from the coordination of two guanidinium groups to the metal ion. The second and third complexes (complexes II_A and II_B) are formed between pH 8 and 11, in different proportions which are dependent on PLA : Cu molar ratio. In these two complexes two guanidinium groups and two peptide nitrogens participate as ligands around the copper ion.

The properties and mode of action of metalloproteins depend to a large extent on the amino acid residues which function as the binding ligands for the metal ions. Yet, our knowledge of the coordination sphere of the metal ion in copper proteins is still very limited. One of the simplest way of gaining some insight into the specific type of interaction between metal ions and amino acid residues is to study the formation and structure of metal-synthetic polypeptide complexes (1, 2, 3).

In this communication we report results on complex formation between Cu (II) ion and PLA in aqueous solution using potentiometric measurements, optical absorption and circular dichroism (CD) spectra. In order to eliminate any influence of conformational changes upon the metal-amino acid residue interaction PLA has been choosen, since this synthetic polypeptide does not undergo helix-coil transition in the range of pH in which the copper complexes are formed.

The formation of two successive copper complexes has been observed

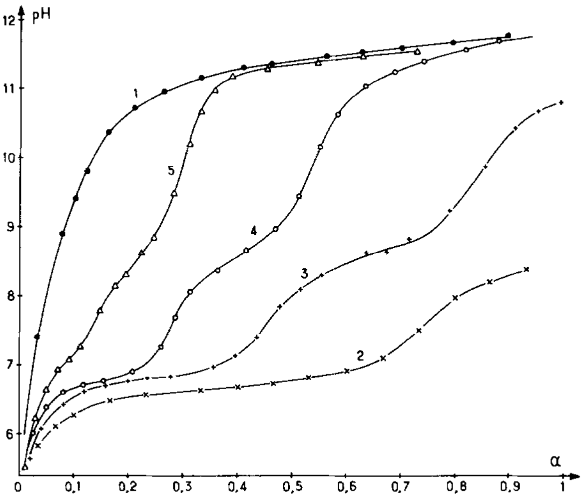

Figure 1. Titration curves of PLA - Cu (II) solutions with sodium hydroxide at different molar ratios :

Curve	1	2	3	4	5
$\|PLA\|$:	2.2×10^{-3}M	2.5×10^{-3}M	4.2×10^{-3}M	6.4×10^{-3}M	8.1×10^{-3}M
$\|Cu\|$:	0	1×10^{-3}M	1×10^{-3}M	1×10^{-3}M	5×10^{-4}M

by potentiometric titrations at different PLA : Cu molar ratios (molar residue over molar copper ion concentrations). Figure 1 shows some of the plot of pH vs α, α being the degree of neutralization as defined by the equation :

$$\alpha = \frac{|OH^-|_n}{|R|_T}$$

where $|OH^-|_n$ is the molar concentration of neutralized hydroxyl-ions and $|R|_T$ the total molar concentration of arginine residues. As can be observed, two main complexes are formed in the pH range from 5 to 11. The first - complex I - is formed up to pH 8, the second - complex II - between pH 8 and 11. Formation curves calculated by Bjerrum method as modified by Gregor (4) for polymeric acids give the following values of $\bar{n}$ - average number of arginine residues bound to a copper ion - : 2, for the first inflexion point at pH ≃ 8, and 4 for the second at pH ≃ 10,5 respectively. For-

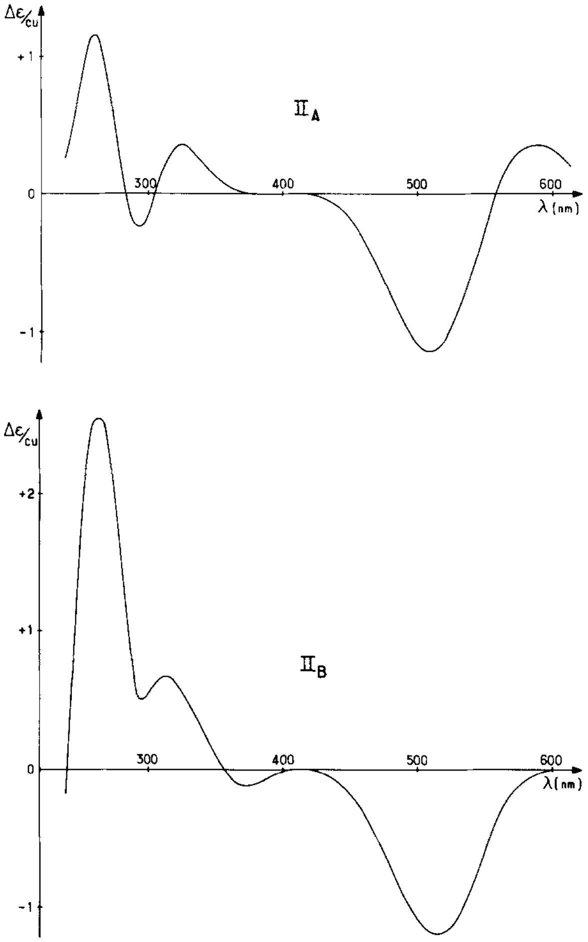

Figure 2. The CD spectra of complexes II_A and II_B.

mation curves provide, moreover, a way to calculate "complexation constants", although these constants may vary owing to the specific nature of polyions (5). The values obtained in the case of Cu (II) - PLA complexes are of the order 10^{13} $mole^{-2}$ for complex I and 10^{10} $mole^{-2}$ for complex II. These are rather high values for a formation constant; so, as it has been confirmed by experiments, all copper ions are bound to arginine residues, in such a way that there are no free copper ions in the solution when the molar ratios are greater or equal to 2, and there are no free residues when the molar ratio is smaller or equal to 2.

Visible spectra data of the two complexes are shown in table I. The

Complex I	Ab. λ_M (nm)		690						260 (sh)
	ε_M		38						2000
Complex II_A	Ab. λ_M (nm)		535				320 (sh)		260 (sh)
	ε_M		160				700		3500
	CD λ_M (nm)	590		510		325		295	260
	$\Delta\varepsilon_M$	+0.34		-1.15		+0.35		-0.24	+1.15
II_B	Ab. λ_M (nm)		525				320 (sh)		260 (sh)
	ε_M		180				1400		5000
	CD λ_M (nm)		512		375		313		264
	$\Delta\varepsilon_M$		-1.20		-0.12		+0.68		+2.55

Table I. ε the molar absorption coefficient and $\Delta\varepsilon$ the molar circular dichroism coefficient are expressed in terms of the molar concentrations of the cupric ions.
Ab. absorption, CD : circular dichroism.

values of λ_M and $\varepsilon_M/_{Cu}$ of complex I are those found in copper peptide complexes when one copper ion is bound to two nitrogens and two oxygens lying at the corners of a square (6). The values of λ_M and $\varepsilon_M/_{Cu}$ of complex II, on the other hand, correspond to copper peptide complexes containing one metal atom coordinated to four nitrogens at the corners of a square (6).

C.D. spectra indicate that the d-d copper and charge transfer transitions of complex I are optically inactive. This result strongly suggests the two guanidinium groups as being the ligands coordinated to the metal ion. Since the inductive effect is thought to be transmitted through the bonds linking the asymmetric center to the chromophore, it depends on the number of atoms between them (7). If, as we suppose, arginine residues are bound to copper ions by the guanidinium nitrogen, five atoms lie between copper and the asymmetric carbon. Accordingly, induced optical activity is not to be expected.

Complex II is optically active, indicating that the two other atoms linked to copper ion are amidic nitrogens. Analysis of C.D. spectra at several molar ratios demonstrate that two different complexes are formed between pH 8 and 10.5; they are called II_A and II_B. Their relative concentration is dependent on molar ratio : when it is smaller or equal to 2 only complex II_A is formed; as the molar ratio increases, complex II_B begins to appear and when molar ratio is greater than 10, complex II_B is the only species present. The C.D. spectra of these two complexes are shown in figure 2, the values of λ_M and $\Delta\varepsilon_M/_{Cu}$ are reported in table I.

As can be observed, the spectrum of complex II_A shows two d-d transitions at 590 and 510 nm and the spectrum of complex II_B only one at 512 nm. This difference in the number of bands can be ascribed to a lower ligand-field symmetry in complex II_A than in complex II_B . Moreover, the two complexes present the same bands at 260 and 320 nm, which we have assigned to charge transfer transitions, between copper ion and amine nitrogens for the first and between copper and amide nitrogens for the second. These two bands are present in the C.D. spectra of superoxide dismutase a metallo-enzyme that contains type (II) or "non blue" EPR detectable Cu(II) ions (8, 9). Although it has been suggested nitrogen containing ligands as binding sites for copper in these enzymes, a direct proof of this assumption is missing so far. Our spectral results on Cu-PLA complexes give a direct evidence of the presence of nitrogen atoms (amine and amidic) in the coordination sphere of "non blue" Cu(II) ions.

C.D. spectra at values of λ lower than 230 nm indicate that, from pH 5 to 11, both PLA and Cu(II) - PLA complexes have a random coil conformation.

Experimental - PLA (M.W. = 65000) has been purchased from K and K and Sigma Laboratories and used without any further purification. Water content was determined by dessication under vacuum at 130° C. All the other chemicals used were of analytical grade. Potentiometric measurements were made with a Radiometer PHM 63 apparatus provided with a Metrohm EA 147 combined Ag/AgCl - glass electrode under argon atmosphere. C.D. spectra were recorded with a Roussel-Jouan Dichrograph CD 185 and the absorption spectra with a Cary 14 spectrophotometer.

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