Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (5): 789-92 (2009)

Short Communication

Received: May 21, 2009
Accepted: June 8, 2009

Vibrational Spectra of the Physiologically and Pharmacologically Relevant Cu(L-His)$_2$ Complex

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SUMMARY. **The infrared spectrum of the Cu(II) complex of L-histidine (L-His) of composition [Cu(L-His)2].1.5 H_2O, generated at physiological conditions, was recorded and analyzed in relation to its structural peculiarities and by comparison with the spectrum of the free amino acid. The electronic spectrum of the complex is also briefly discussed.**

INTRODUCTION

Besides iron and zinc, copper is the most ubiquitous transition metal present in living organisms, and is involved in a large number of important functions and processes. Copper metalloenzymes are utilized for electron transfer (galactose oxidase, azurins, laccases, plastocyanins), for oxygenation reactions (tyrosinase, ascorbate oxidase), for dismutations (superoxide dismutase) and even for oxygen transport in arthropods and mollusks (hemocyanin) [1-3].

On the other hand, copper compounds and, in particular, Cu(II) complexes present important pharmacological interest since several of them show an important number of effects, including anti-inflammatory, antiulcer, anticonvulsant and even anti-tumoral activity [4-8].

During the last years important efforts have been made to understand the intricate and specific mechanisms related to different aspects of copper absorption and transport, but only recently our knowledge on the molecular aspects of copper trafficking within the body began to emerge. The recent discovery of Wilson and Menkes disease genes and the identification of copper chaperones generated unprecedent advances in our understanding of these mechanisms [9-11]. L-histidine plays an essential role in copper transport before its entry into cellular transport systems and incorporation into enzymes and proteins. A small fraction of Cu(II) bound to L-histidine maintains an exchangeable pool of copper in equilibrium with albumin in human blood. The exchange of Cu(II) between L-histidine and albumin modulates the availability of this biometal to the cell [9].

Menkes disease is a genetic neurodegenerative disorder, characterized by a widespread defect in intracellular copper transport. Progressive neurodegeneration associated with psychomotor retardation and connective tissue abnormalities are the main clinical features and children usually die before the age of three [10]. It was found that the parenteral administration of copper(II)-L-histidine, when initiated very early in life, is very effective in preventing neurodegenerative problems in Menkes patients [10,12,13].

It is well known that the Cu(II)/L-histidine is a very complicated system, due to the important number of different species that are formed in solution at different pH-values, metal-to-ligand ratios and ionic strengths (cf. for example [14,15] and references therein). Due to the commented importance of this complex, in relation to copper transport and for the treatment of Menkes disease the knowledge of structural and physic-

KEY WORDS: Copper(II), Electronic spectrum, IR spectrum, L-histidine.

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ochemical properties of physiological Cu(II)/L-histidine species is of considerable interest.

Fortunately, very recently it becomes possible to isolate and perform a complete crystallographic structural analysis of the [Cu(L-His)$_2$].1.5 H_2O complex generated at physiological conditions. On the basis of this structural information we have now performed the investigation of the vibrational spectra of this complex, complemented with a brief analysis of its electronic spectrum.

MATERIALS AND METHODS

L-histidine was purchased from ICN-Biomedicals Inc., whereas all the other reagents were analytical grade products from Merck. For the preparation of the complex 0.39 g (1.5 mmol) of $CuSO_4.5H_2O$ were dissolved in 15 ml of distilled water. To this solution 0.485 g (3.0 mmol) of L-histidine were added, under continuous stirring, and the pH-value was immediately adjusted to about 7.4 with a diluted NH_4OH solution. An equal volume of dimethylformamide (DMF) was slowly added to make a 50/50 vol/vol H_2O/DMF mixture. The obtained solution was introduced in a vacuum exsiccator, containing ethanol, and air was carefully pumped off. This allows the slow diffusion of ethanol into the reaction mixture. After 8-10 days small clusters of intense blue crystals were deposited [15]. Analytical results: Found: C, 36.00; H, 4.85; N, 21.12. Calculated for $CuC_{12}H_{19}N_6O_{5.5}$ (MW: 398.87): C, 36.10; H, 4.76; N, 21.06%.

The infrared spectra in the range 4000-400 cm-1 were recorded with a FTIR-Bruker-EQUINOX-55 instrument, using the KBr pellet technique. Spectra measured with suspensions of the compounds in Nujol mulls, gave identical results. Raman spectra in the same spectral range, were measured on powdered samples using the FRA 106 Raman accessory of an IF66 Bruker spectrophotometer. Radiation from a Nd:YAG solid-state laser (1064 nm) was used for excitation.

The electronic spectra of aqueous solutions of the complex were measured with a Hewlett-Packard 8452 diode-array spectrophotometer, using 10-mm quartz cells.

RESULTS AND DISCUSSION

Structure of the complex

L-histidine (Figure 1) has four potential sites for (de)protonation and thus for metal binding, i.e. the carboxylate group, the primary amino group and the two N atoms of the imidazole ring. Notwithstanding, the deprotonation of the imidazole ring is generally not considered, regarding its extremely high pK value (pK = 14) [14]. All the three available coordination sites have been used in the investigated complex, in which the two ligand groups present a very interesting and unique coordination behavior different from all the previously postulated structures at physiological pH-values [14,15].

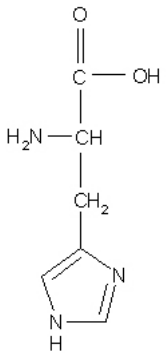

Figure 1. Schematic structure of L-histidine.

[Cu(L-His)$_2$].1.5 H_2O crystallizes in the monoclinic $P2_1$ space group with Z = 2, presenting a neutral five-coordinated distorted square pyramidal coordination [15]. As shown schematically in Figure 2 one of the ligands acts in the monoanionic bidentate form through one carboxylate O-atom and the amino N-atom. The second ligand binds as a monoanionic tridentate ligand, through the imidazole N-atom, the amino N-atom and one carboxylate O-atom, with this last atom occupying the apical position. This peculiar structural arrangement is surely the result of a combination of steric effects induced by the tridentate coordination of one of the ligands and the tendency of the Cu(II) ion toward square planar geometry. Besides, the pendant imidazole group plays an important role not only in the crystallization process but probably also in copper transport [15].

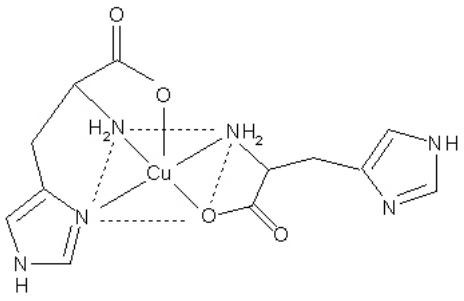

Figure 2. Schematic structure of the investigated complex, [Cu(L-His)$_2$].1.5H_2O.

Vibrational spectra

Initially, for comparative purposes and in order to facilitate the spectral assignment of the complex, we have recorded the FTIR spectrum of L-histidine. The obtained data are presented in Table 1 together with the proposed assignments which are based in some general standard references [16,17] as well as on recent studies of aqueous solutions of the amino acid [18], the known spectroscopic behavior of free imidazole [19] and other related papers [20,21].

The FTIR spectrum of $[Cu(L\text{-}His)_2].1.5H_2O$ is shown in Figure 3. Unfortunately, all the recorded Raman spectra were of relatively poor quality, presenting only a reduced number of weak and medium intensity lines. A similar behavior was also reported in the case of solution Raman spectra, which turned increasingly less informative with increasing pH values of the solution, as a result of strong darkening of the solution [14].

The proposed assignment for the complex is presented in Table 2 and was performed on the basis of the previous assignments for L-histidine.

As it can be seen from Figure 3, the spectrum of $[Cu(L\text{-}His)_2].1.5H_2O$ is relatively simple. A first overview and a comparison with the data of the free amino acid (Table 1) shows the expected disappearance of all of the NH_3^+ -modes and of some of the typical imidazole bands. Some other spectroscopic peculiarities are discussed as follows:

The typical $\nu_{as}(COO^-)$ band is better defined than in the free amino acid but is somewhat broadened, due the presence not only of the $\delta(NH_2)$ mode, but also of the deformational mode of water. The complex origin of this band is surely responsible for its appearance at somewhat higher frequency than in the amino acid. On the contrary, the corresponding symmetric carboxylate stretching shows the expected behavior after complexation, as its energy is somewhat lower in the complex.

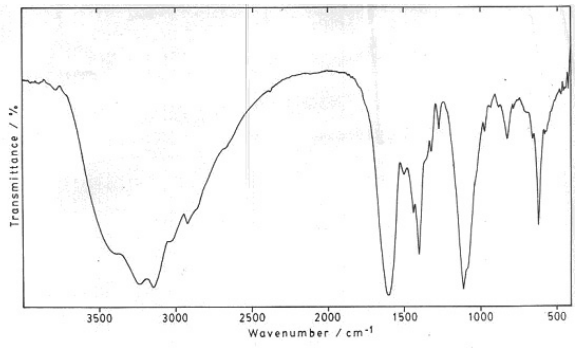

Figure 3. FTIR spectrum of $[Cu(L\text{-}His)_2].1.5H_2O$.

Infrared (cm^{-1})	Assignment
3009 vs, br	ν(NH) imidazole + $\nu_{as}(NH_3^+)$
2876 vs	ν(CH)
2711 m	$\nu_s(NH_3^+)$
2625 w	combination mode
2025 w	$\delta_{as}(NH_3^+)$
1636 vs	$\delta_{as}(NH_3^+)$
1590 s	$\nu_{as}(COO^-)$ + ν_{ring} + $\delta_s(NH_3^+)$
1500 sh, 1464 vs	$\delta(CH_2)_{sciss}$ + δ(NH) + ν_{ring}
1414 v	$\nu_s(COO^-)$
1343 vs	$\delta(CH_2)_{wagg}$ + $\delta(NH_3^+)$
1315 s	δ(CH) imidazole
1271 s	ν(CN)
1252 vs, 1220 vw	ν_{ring} + ν(CN)
1171 m	ν(CN)
1148 vs	δ(NH) imidazole + $\gamma(CH_2)$
1112 m, 1085 m, 1064 m	δ(CH) imidazole
976 s, 967 vs	ν_{ring} + $\delta(NH_3^+)$
806 m, 776 vs	δ_{ring}
731 m	$\rho(CH_2)$
685 s, 651 m	γ(CH) + γ(NH) imidazole
624 vs	$\delta(COO^-)$
538 vs	$\rho(COO^-)$

Table 1. Assignment of the FTIR spectrum of L-histidine; **vs**: very strong; **s**: strong; **m**: medium; **w**: weak; **vw**: very weak; **br**: broad; **sh**: shoulder.

Infrared (cm^{-1})	Assignments
3420 m, br	ν(OH) water
3227 s	$\nu(NH_2)$
3140 s	ν(CH) imidazole
3034 m	ν(NH) imidazole
2920 m	ν(CH)
1598 vs	$\nu_{as}(COO^-)$ + $\delta(NH_2)$ + $\delta(H_2O)$
1498 vw, 1436 vw	$\delta(CH_2)$
1401 s	$\nu_s(COO^-)$
1320 vw	δ(CH) imidazole
1271 m	ν(CN)
1112 vs, 1080 sh	δ(CH) imidazole
968 w	ν(ring) imidazole
824 m	$\delta(NH_2)$ out of plane
700 w, br	δ_{ring} + $\rho(CH_2)$
656 vw	$\delta(COO^-)$
619 s	$\rho(COO^-)$
575 vw	
466 vw, 451 vw	ν(Cu-N)

Table 2. Assignment of the FTIR spectrum of $[Cu(L\text{-}His)_2].1.5H_2O$; **vs**: very strong; **s**: strong; **m**: medium; **w**: weak; **vw**: very weak; **br**: broad; **sh**: shoulder.

Both the $\delta(COO^-)$ and the $\rho(COO^-)$ vibrations are displaced in the complex, the first one to higher frequencies and the second one to lower frequencies. Besides, the first of these vibrations also suffers an important intensity diminution.

The very well defined band triplet seen at 1112, 1085 and 1064 cm^{-1} in L-histidine is replaced by a unique and very strong feature at 1112 cm^{-1} (with a shoulder on the low-energy side) in the complex. The behavior of a ring mode located at 936 cm^{-1} in free imidazole, which is very sensitive to complexation [22], is also very interesting. Usually, after complex formation this band is displaced to higher energy and shows an important intensity lowering [23]. In the present case, it is seen as a weak feature at 968 cm^{-1}, practically at the same position as in the free amino acid, but with a marked intensity diminution.

Two very weak features at 575 and 451 cm^{-1} have been assigned to ν(Cu-N) modes, as they lie in similar positions as in other Cu(II) complexes of amino acids [24,25]. The corresponding Cu-O modes are surely located at lower frequencies [24-26].

Finally, it is interesting to mention that the FTIR spectrum recorded with the crystalline material compares reasonably well with that recorded with an aqueous solution of the complex at a pH of 8.0 [14].

Electronic spectra

The electronic spectrum of an aqueous solution of the $[Cu(L\text{-}His)_2].1.5H_2O$ complex presents a relatively broad band with a maximum at about 625 nm (ε = 60 $M^{-1}cm^{-1}$), which is characteristic for Cu(II) d-d transitions, related to square pyramidal environments [27]. We have analyzed the position of this band with the simple calculations discussed in the theoretical model of Prenesti *et al.* [28,29] and adding the suggested mean value of 36 nm for the incorporation of the axial carboxylate oxygen-atom to the square coordination sphere [29]. The obtained value of 632 nm is in excellent agreement with the experimental one.

Acknowledgements. This work has been supported by CONICET and UNLP. E.J.B. is a member of the Research Career from CONICET.

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