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Nature of Metal Binding Sites in Cu(II) Complexes with Histidine and Related N-Coordinating Ligands, As Studied by EXAFS

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Knowledge of the complexes formed by N-coordinating ligands and Cu(II) ions is of relevance in understanding the interactions of this ion with biomolecules. Within this framework, we investigated Cu(II) complexation with mono- and polydentate ligands, such as ammonia, ethylenediamine (en), and phthalocyanine (Pc). The obtained Cu–N coordination distances were 2.02 Å for [Cu(NH₃)₄]²⁺, 2.01 Å for [Cu(en)₂]²⁺, and 1.95 Å for CuPc. The shorter bond distance found for CuPc is attributed to the macrocyclic effect. In addition to the structure of the first shell, information on higher coordination shells of the chelate ligands could be extracted by EXAFS, thus allowing discrimination among the different coordination modes. This was possible due to the geometry of the complexes, where the absorbing Cu atoms are coplanar with the four N atoms forming the first coordination shell of the complex. For this reason multiple scattering contributions become relevant, thus allowing determination of higher shells. This knowledge has been used to gain information about the structure of the 1:2 complexes formed by Cu(II) ions with the amino acids histidine and glycine, both showing a high affinity for Cu(II) ions. The in-solution structure of these complexes, particularly that with histidine, is not clear yet, probably due to the various possible coordination modes. In this case the square-planar arrangements *glycine-histamine* and *histamine-histamine* as well as tetrahedral coordination modes have been considered. The obtained first-shell Cu–N coordination distance for this complex is 1.99 Å. The results of the higher shells EXAFS analysis point to the fact that the predominant coordination mode is the so-called *histamine-histamine* one in which both histidine molecules coordinate Cu(II) cations through N atoms from the amino group and from the imidazole ring.

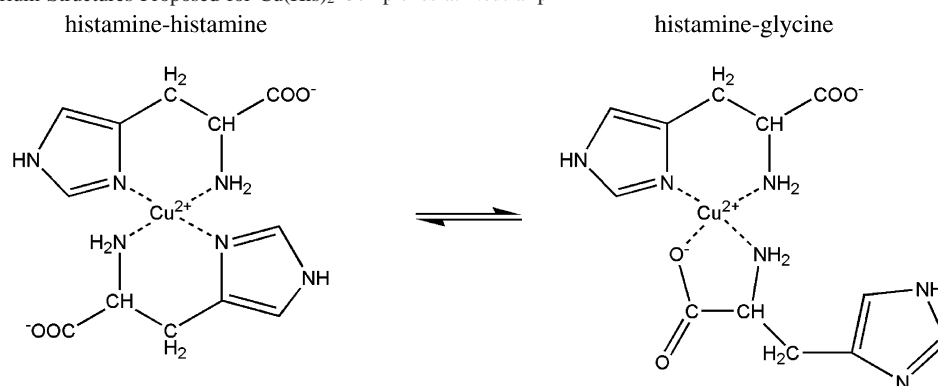
Introduction

Metal ions play a vital role in a vast number of widely differing biological processes.¹ The biochemical function of the metal ion in all processes is a matter of fundamental importance. Most metal cations in living organisms interact with proteins; thus, knowledge of metal–protein binding sites has attracted the attention of many research groups.² Each

of the naturally occurring amino acids forming the proteins may form stable five-membered chelate rings with metal ions, where the donor groups are amino N_{am} and carboxylate O_{COO} groups. Lately, several works have been devoted to the study of normal and abnormal interactions that might take place between transition metals and proteins, which eventually could be at the heart of some neurodegenerative diseases, such as Parkinson's, Alzheimer's, or Creutzfeldt–Jacob's disease.^{3,4} Copper is the third most abundant transition-metal element in the human body, following iron and zinc, mainly occurring in the divalent oxidation state. It has a relevant role in redox processes and is present in brain and liver tissues. In the interaction with proteins, in addition to the

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Scheme 1. Equilibrium Structures Proposed for Cu(His)₂ Complexes at Neutral pH^{9a}

coordination with N_{am} and O_{COO} , Cu(II) shows a remarkable affinity for nitrogen atoms of imidazole rings, N_{im} , probably related with the high stability of the complexes this metal forms with N-coordinating ligands.^{1,5} Due to the presence of the imidazole ring, histidine is the amino acid showing the highest affinity for Cu(II) ions ($\log \beta_2 = 18.3$), and consequently, knowledge of the structure of the complexes that it forms with Cu(II) ions is of relevance in understanding Cu(II)–protein interactions. However, although these complexes have been extensively studied, they are far from being fully understood.

Through these three coordination sites, N_{am} , N_{im} , and O_{COO} , histidine might act either as a monodentate or bidentate ligand, and some authors have proposed simultaneous coordination to the same cation through the three binding sites.⁶ The type of coordination is determined by several factors, the most important being the ligand-to-metal ratio, which determines the stoichiometry of the complex, and the pH of the solution, because it changes the protonation degree of the coordinating atoms N_{am} , N_{im} , and O_{COO} .^{1,5} The relevance of the knowledge of the metal binding sites in these complexes cannot be overemphasized, and thus, this subject has attracted the attention of many research groups who have used a wealth of techniques to determine their structures.^{6–12} To name but one example, indirect evidence exists attributing the native cellular prion protein, PrP^C, a role in copper metabolism. This protein is proposed to play a role in bovine spongiform encephalopathy and Creutzfeldt–Jacob disease in humans.¹³ Cu(II) is said to bind the N-terminus region particularly rich in histidine and glycine residues with several

modes of coordination being proposed, and in all of them Cu(II) is bound to histidine residues.¹⁴

The first fully solved structure of a Cu–His complex, reported by Evertsson in 1969 from an XRD study of a crystal of the nitrate of bis-histidinecopper(II) dihydrate prepared at pH = 3.7,⁷ shows that each histidine molecule behaves as a bidentate ligand coordinating Cu(II) ions through the N_{am} atom (at 1.98 and 1.99 Å for each molecule) and through the O_{COO} atom (at 1.93 and 1.99 Å). This coordination mode is called “glycine-type”, since it is the most stable one in Cu(II)–glycine complexes. Further studies of 1:2 Cu(II):histidine complexes⁸ showed that at neutral/basic pH each histidine molecule coordinated through the N_{im} and N_{am} atoms in the so-called “histamine-histamine” mode, and at pH = 8.1 there was an equilibrium between this and the mixed “glycine-histamine” mode, the last coordination mode being predominant (see Scheme 1).^{9a} This coordination mode is shown by the [Cu(His)(Asn)] complex, as solved by XRD by Ono et al.^{9b} Further studies of the Cu–(His)₂ complex using CD,^{6,10,11a} UV–vis, EPR and ENDOR spectroscopies^{11b} reached similar conclusions. In contrast, Ozutsumi et al.,¹² who studied a series of complexes formed between Cu(II) and one or two amino acid molecules with EXAFS, including histidine, propose the formation of aminocarboxylate complexes, i.e., “glycine-glycine” coordination type, although the complex was prepared at neutral pH, with coordination distances equal to 1.98 Å for Cu–N and 2.02 Å for Cu–O.

Another key point to understand Cu(II)–metalloprotein interactions is knowledge of the complexes it forms with the amino acid glycine, which has a high affinity for Cu(II) ions as well, forming stable complexes with metal:glycine ratios of 1:1, 1:2, or 1:3. Each glycine molecule can act as a monodentate or bidentate ligand, coordinating through the N_{am} atom and/or O_{COO} atoms. In the 1:2 complexes, glycine usually acts as a bidentate ligand, coordinating through both atoms in the so-called “glycine-type” mode, referred to above.¹⁵ The most stable isomer is the one in which the two

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carboxylic groups occupy contiguous positions (cis coordination), so that the first coordination shell is formed by two oxygen and two nitrogen atoms. The crystal structure of this complex has been solved,¹⁶ yielding 1.98 and 2.02 Å for Cu–N coordination distances and 1.95 and 1.96 for Cu–O coordination distances. Very similar values have been obtained from an EXAFS study of this complex in aqueous solution.¹⁷ From the point of view of the technique, this complex might serve to check if EXAFS spectroscopy is able to solve two contributions at similar distances where the backscatters are consecutive atoms in the periodic table, i.e., N and O atoms.

Although all these studies provide relevant information concerning Cu(II)–histidine/glycine interactions, only two provide values for the metal–ligand coordination distances in solution. The other works are for crystalline compounds, where compact packing is said to be governed by minimum van der Waals plus hydrogen-bond interaction energy, which might not be the same in solution than in the solid state. Moreover, it is of relevance to obtain structural information in a medium as similar as possible to that of the physiological fluids, i.e., low concentrations and neutral pH since, as explained above, these factors determine metal–ligand binding sites. To fill this gap we have undertaken a study aimed at obtaining direct structural information about the complexes formed by Cu(II) cations and amino acids histidine and glycine for 1:2 metal to amino acid ratio, bis-histidinecopper(II), [Cu(His)₂], and bis-glycinatocopper(II), [Cu(Gly)₂], in aqueous solutions at physiological pH = 7.3 and low concentrations (0.01–0.05 M). As a first step we studied Cu(II) model complexes with mono-, bis-, and tetradentate N-coordinating ligands of known structures which are closely related with those proposed for [Cu(His)₂] and [Cu(Gly)₂]. The selected complexes are tetramminecopper(II), [Cu(NH₃)₄]²⁺, bis-ethylenediaminecopper(II), [Cu(C₂N₂H₈)₂]²⁺, hereafter [Cu(en)₂]²⁺, and phthalocyaninecopper (II), [Cu(C₃₂N₈H₁₆)], hereafter CuPc.

In the model complexes the local coordination around copper can be considered to be square planar, the first coordination shell being formed by four N atoms, at ca. 2 Å.¹⁸ For instance, Cu–N coordination distances for these complexes from XRD studies of crystalline salts with several anions, such as chloride, bromide, sulfate, and selenate,^{19–22} range from 1.97 to 2.15 Å. These parameters range from 1.93 to 2.03 Å in the data obtained from EXAFS for the dissolved species.^{23–25} While a shorter distance, 1.935 Å, is

proposed for CuPc, from electron diffraction and molecular dynamic studies,^{26,27} similar values, 1.97–2.02 Å, were obtained for complexes formed with imidazole and histidine.^{14,28–30}

The requirements concerning concentration and media drastically reduce the number of experimental techniques that might be of application to the study of these systems. Extended X-ray absorption fine structure (EXAFS) is one of the most appropriate ones since it is element sensitive and can be applied in a wide range of concentrations to species showing either short- or long-range order.³¹ For this reason it has been applied successfully to the study of biomolecules containing a metal center, such as metalloproteins.³² It has been successfully applied as well to the structural determination of several other Cu(I) and Cu(II) complexes in aqueous and nonaqueous solutions.³³ Given the available structural information, the general aim of this study is determination of the *in-solution* structure of the selected complexes, paying special attention to shells beyond the first one. Moreover, it is of general interest to study the effect of chelation on coordination distances and Debye–Waller (DW) factors of these species in media similar to those formed by the physiological fluids. The detection of labile species such as coordinated water molecules is of relevance as well since they might play important roles in the conformational behavior of the protein.^{1,2}

Experimental and Computational Methods

Sample Preparation. [Cu(NH₃)₄]²⁺ was prepared by dissolving the required amount of Cu(II) perchlorate hexahydrate in 30% commercial ammonia. Under these conditions ammonia molecules, as tested by UV–vis spectroscopy, substitute water molecules.¹⁸ To prepare the [Cu(en)₂]²⁺ complex in aqueous solution, the required amount of Cu(ClO₄)₂ was dissolved in distilled water, and a 25% ethylenediamine solution was added with shaking. The complex is stable and highly soluble.³⁴ The CuPc in solution was obtained by dissolving the corresponding amount of copper(II)

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3,4',4'',4'''-tetrasodiumtetrasulfonate phthalocyanine in distilled water.²⁷ This derivative of phthalocyanine was used due to its higher solubility.

To prepare the *cis*-bis(glycinate)copper(II) monohydrate, a 3.0 g sample of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was dissolved in 17 mL of HCl, then 1.5 g of glycine was added, and the mixture was heated at 40 °C. NaHCO_3 was added to increase the pH and induce complex precipitation. The solid was suction filtered for 5 min to dry it.¹⁵ To obtain $[\text{Cu}(\text{His})_2]$ complex, the required amounts of Cu(II) perchlorate hexahydrate and histidine were dissolved in water in a molar ratio of 1:5. The solution was shaken for 1 h, and afterward the pH was adjusted to 7.3 by adding sodium hydroxide.⁸

With the exception of the bis(glycinate)copper(II), the complexes are moderately soluble in water, so medium (0.1 M in $[\text{Cu}(\text{NH}_3)_4]^{2+}$, $[\text{Cu}(\text{en})_2]^{2+}$, and CuPc, 0.05 M in $[\text{Cu}(\text{His})_2]$) and low (0.01 M) concentration solutions were measured to search for possible effects of concentration on the complex structure. The reported solubility of the $[\text{Cu}(\text{Gly})_2]$ is 0.025 M.¹⁵ Thus, only a saturated solution was measured. In addition to the spectra of the complexes in aqueous solution, the spectrum of the solid compound CuPc was measured to check for changes induced by the solvation process. The amount of solid complex required to obtain the optimum absorption was mixed with BN to obtain a self-supported wafer.

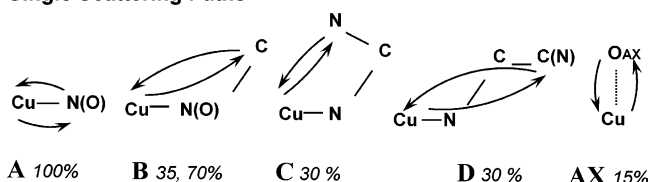
X-ray Absorption Measurements and Data Analysis. XAS spectra of the copper K-edge (8979 eV) were measured at the synchrotron radiation source ESRF (Grenoble, France) at beamline BM29. Ring energy was 6 GeV, and ring current was 200 mA. Energy calibration was carried out with a copper foil. A double-crystal Si (311) monochromator was used. For this energy and using 0.3 mm vertical slits its estimated resolution is better than 0.5 eV, which is confirmed by the resolution of a sharp preedge feature in the XANES spectrum of CuPc. Higher harmonic rejection was carried out by detuning both crystals 50%. The solid and liquid sample spectra were recorded at room temperature in transmission mode. Ion chambers, filled with a He/Ar gas mixture and optimized to absorb 20% of the beam intensity in I_0 and 80% in I_t , were used as detectors. Counting time was evenly increased through the recording of each scan, and several scans were averaged to improve the signal-to-noise ratio.

The EXAFS functions $\chi(k)$ were obtained from the X-ray absorption spectra by subtracting a Victoreen curve followed by a cubic spline background removal using the program AUTOBKX³⁵ and normalizing to the height of the measured absorption edge. E_0 was defined as the energy where the absorption coefficient is one-half of the height of the atomic absorption jump. The EXAFS spectra thus obtained were analyzed using FEFF 8.10³⁶ and FEFFIT 2.54,³⁷ as detailed below.

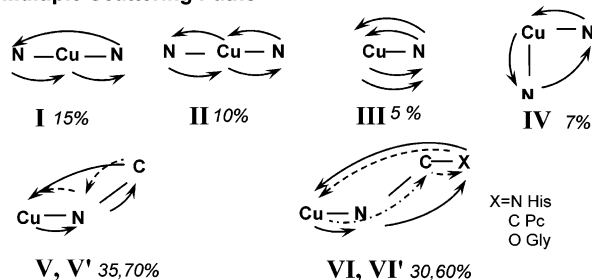
In a first step, *ab initio* calculations (with FEFF code) of the contributions to the EXAFS signal of each scattering path were performed, either single scattering (SS) or multiple scattering (MS), which includes relevant backscatterers, i.e., C and N and excludes hydrogen atoms. A curved wave amplitude filter was used, according to which only paths with amplitude equal to or higher than 5% of the most intense path, obtained by integrating through

Scheme 2. Single Scattering Paths and Most Significant Multiple Scattering Paths Included in the Fits of the EXAFS Spectra of the Cu(II) Complexes^a

Single Scattering Paths



Multiple Scattering Paths



^a The number and type of legs are indicated as well as the amplitudes compared to that of the first shell for zero Debye–Waller factors, $\sigma^2 = 0.0$.

the fitting range, were taken into account. Only those paths whose maximum effective distance is equal to or less than 5.0 Å were considered.

The first coordination shell, formed in all complexes by four N atoms in a square-planar arrangement (except in $[\text{Cu}(\text{Gly})_2]$, where this shell is formed by two N atoms and two O atoms), gives rise to the most intense contribution to the EXAFS spectra, which corresponds to the SS path labeled A in Scheme 2. In all complexes except in $[\text{Cu}(\text{NH}_3)_4]^{2+}$ there is a second coordination shell formed by carbon atoms (path B in Scheme 2). Due to the high symmetry and stability of the CuPc chelate complex, higher shell contributions cannot be neglected. The SS paths corresponding to these shells are paths C and D in Scheme 2. Similar paths had to be taken into account in $[\text{Cu}(\text{His})_2]$ and $[\text{Cu}(\text{Gly})_2]$. It has been assumed that all atoms are coplanar. This is strictly true for the CuPc complex in the gas phase and solid state, as observed from ED and XRD.^{26,27} In $[\text{Cu}(\text{Gly})_2]$ some deviations from planarity have been observed by XRD in the crystalline compound, from 0.02 to 0.04 Å,¹⁶ which would induce changes in Cu(II)–ligand coordination distances far beyond the detection limits of the technique (0.015–0.05 Å for first and higher shells, respectively).

Since octahedral Jahn–Teller-distorted symmetry is the one most frequently found in Cu(II) ions and the existence of axial water molecules is normal in this type of complex,^{5,18} the spectrum of each complex was analyzed with and without an additional single-scattering contribution from two axial water molecules (path AX in Scheme 2).

MS paths within these arrangements have to be taken into account as well. Common contributions to all complexes are the ones within the first coordination shell, paths including aligned atoms labeled I–III as well as triangular paths labeled IV. Inclusion of these MS paths in the analysis procedure is crucial since they are relevant only in a square-planar geometry, where the absorbing atom and those forming the first shell are coplanar. In contrast, in a tetrahedral arrangement paths I–IV are negligible. Thus, their inclusion might serve to discriminate between both coordination environments. Paths of type V have a high amplitude in complexes with a second coordination shell. Paths of type VI, derived from SS path D, have to be considered in the CuPc, $\text{Cu}(\text{Gly})_2$, and $\text{Cu}(\text{His})_2$ complexes.

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MS paths involving second and higher shells (type V and VI) are crucial to discriminate between the different types of ligands. Note that except glycine, the first coordination shell is formed by four N atoms; thus, higher shells are the key feature to distinguish between them.

Between 6 and 13 paths (depending on the complex) have to be taken into account. The number of free parameters could become quite high in some fits, thus exceeding the number of independent points according to the Nyquist theorem.³⁸ Therefore, some strategies were undertaken to reduce the number of fitting parameters. First, assuming the initial structures, coordination numbers are fixed for each shell. Moreover, in complexes with higher coordination shells, which have chelate ligands, bond distances within the ligand have to be coherent. Concerning Debye–Waller factors of MS contributions, those corresponding to paths I–IV were not considered free parameters, rather they were defined as functions of the Debye–Waller factors of SS path A; then according to the independent vibration approximation model³⁹

$$\sigma_{\text{I}}^2 = \sigma_{\text{II}}^2 = \sigma_{\text{IV}}^2 = 2\sigma_{\text{A}}^2$$

$$\sigma_{\text{III}} = 4\sigma_{\text{A}}^2$$

This model has shown to be useful in systems with similar structure⁴⁰ as well as in other systems with octahedral coordination geometry.⁴¹

The EXAFS signals calculated for the paths thus obtained were used to fit the experimental spectra using FEFFIT program. The free parameters in each fit were coordination distances and Debye–Waller (DW) factors for each SS path and DW factors for MS paths type V and VI. Additionally, a unique value of the inner potential correction, ΔE_0 , was taken as a free parameter in the analysis of each complex. Thus, the number of free parameters was kept between 5 and 11, below the number of independent points in experimental spectra. According to the Nyquist theorem, this number was between 22 and 24, depending on the fitting range. Moreover, among the possible results of the fit, we took into account only those having physical meaning, both concerning coordination distances and DW factors. First, in the chelate complexes the only distance left completely free for the fit is the first-shell one, since bond distances within the ligand are fixed, which constrains the values of the distances between Cu(II) and second and higher shells. Second, concerning DW factors of SS paths, they have to increase with coordination distances.

The spectra of the $[\text{Cu}(\text{Gly})_2]$ complex could not be fitted with the above-described procedure. The most probable reason is the split that, according to XRD¹⁶ data, appears in the first shell, formed by two O and two N atoms. In fact, Cu–N and Cu–O contributions are too close in distance to be fitted like independent shells, but since they have different backscatterer atoms they cannot be fitted as a single shell. In addition, there are two pairs of C atoms in the second coordination shell 0.2 Å apart, so they cannot be fitted as

a single shell either. The Cu–O third shell is formed by two O atoms at a well-defined distance, but it is not easy to fit either because the adjacent MS paths of type VI in Scheme 2 are not well defined due to the poor resolution of the first and second coordination shells. Thus, the number of SS and MS contributions is roughly multiplied by two and concomitantly the number of free parameters. A fit of the EXAFS function cannot be performed, but rather a calculation using the structural parameters from XRD studies has to be done.

The amplitude reduction factor, S_0^2 , was set equal to 0.81 in all complexes. This was the value heuristically obtained in the analysis of the EXAFS spectra of another first transition series cation, Cr(III), in a similar environment and measured in similar conditions.⁴² This value is similar to that obtained in the fit of the spectra of a Cu metal foil, $S_0^2 = 0.85$.³⁷

Results and Discussion

The raw EXAFS spectra of the five complexes dissolved in aqueous solutions have been included as Supporting Information in Figure 1aSI, and the magnitude of the Cu–N phase-corrected Fourier transform (FT) of the spectra included in Figure 1aSI is given in Figure 1bSI. The EXAFS function becomes increasingly complex when the number of atoms in the ligand becomes larger: it is a quasi simple wave in the tetrammine, it shows a beating at around 5.5 Å⁻¹ in the $[\text{Cu}(\text{en})_2]^{2+}$ spectra, and in the complex formed with the tetradentate ligand phthalocyanine the function is rather complex. A peak centered at around 2.00 Å, which should be ascribed to the Cu–N first coordination shell, is the main contribution in all spectra. Additional contributions appear beyond 2.8 Å, thus allowing determination of the structural properties of higher coordination shells. The plotted spectra are those corresponding to the higher concentration in each case: 0.1 M in $[\text{Cu}(\text{NH}_3)_4]^{2+}$, $[\text{Cu}(\text{en})_2]^{2+}$, and CuPc, 0.05 M in $[\text{Cu}(\text{His})_2]$, 0.025 M in $[\text{Cu}(\text{Gly})_2]$. Nevertheless, the fit of the 0.01 M solutions was carried out as well for the first three complexes, finding exactly the same results, since the only difference between both series of spectra was the signal-to-noise ratio, which was better in the more concentrated solutions.

All these spectra were analyzed following the procedure described above. Fit results have been included in Table 1 and fitting ranges and goodness of fit as Supporting Information in Table 1SI. The latter table also includes the number and type of free parameters used during the fitting procedure in each case. Fit weight was 2 in all cases.

$[\text{Cu}(\text{NH}_3)_4]^{2+}$. $[\text{Cu}(\text{NH}_3)_4]^{2+}$ is the most simple Cu(II) complex with monodentate N-coordinating ligands. Considering the restrictions described above for path lengths and amplitude filters, the computation with FEFF yielded the single-scattering path corresponding to the Cu–N coordination shell (path A in Scheme 2) and the associated four multiple scattering ones (paths I–IV in Scheme 2).

Inclusion of one additional SS contribution, formed by two axial water molecules, improves the fit slightly. For each SS contribution there are two free parameters: interatomic

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Table 1. Structural Parameters Obtained from the EXAFS Fits^a (see Scheme 2 for path definition)

shell	<i>R</i> (Å)	CN	σ^2 (Å ²)	SS paths	MS paths
[Cu(NH₃)₄]²⁺					
Cu–N	2.02	4	0.004	A	I, II, III, IV
Cu–O _{ax}	3.3	2	0.011	AX	
[Cu(en)₂]²⁺					
Cu–N	2.01	4	0.0036	A	I, II, III
Cu–C	2.84	4	0.006	B	V
Cu–O _{ax}	3.4	2	0.01	AX	
CuPc					
Cu–N	1.95	4	0.0008	A	I, II, III
Cu–C	2.97	8	0.002	B	V, V'
Cu–N	3.37	4	0.004	C	
Cu–C	4.1	8	0.007	D	VI, VI'
[Cu(His)₂]					
Cu–N	1.99	4	0.0034	A	I, II, III
Cu–C	2.97	2	0.007	B	V, V'
Cu–C	3.32	4	0.007	B	V, V'
Cu–N	4.1	2	0.008	D	VI, VI'
[Cu(Gly)₂]					
Cu–N/O	1.95	2+2	0.003	A × 2	(I, II) × 2
Cu–C/C	2.74/2.8	2+2	0.004/0.008	B × 2	V × 2
Cu–O	4.0	2	0.015	D	VI × 2

^a *R*: Coordination distances; CN: coordination numbers, σ^2 : Debye–Waller factors or mean square displacements, SS: Single Scattering, MS: Multiple Scattering. Uncertainties (except for Cu(Gly)₂, see text): *R* in first coordination shell: 0.01 Å, *R* second and higher coordination shells: 0.015–0.05 Å Debye–Waller factors 0.002 Å².

distances *R*(Cu–X) and Debye–Waller factors, σ^2_{X} . The best fit was obtained for a square-planar structure with four molecules of ammonia at 2.02 Å. These values compare well with those previously obtained by XRD or EXAFS.^{19,21–25} DW factors are similar to those found in stable square-planar⁴⁰ complexes and smaller than those of hexahydrates.^{42,43} Concerning axial molecular water, the optimized values for coordination distance *R*(Cu–O_{ax}) and DW factor σ^2_{AX} were 3.3 and 0.011 Å². This distance is much longer than that found in tetrammine and ethylenediamine in crystalline square-planar complexes, 2.5–2.8 Å.⁵ Likewise, axial water molecules in [Cu(H₂O)₆]²⁺ complexes appear around 2.3 Å.^{5,44} Nevertheless, a similar value has been found in crystalline [Cu(NH₃)₄]SO₄·H₂O.^{19,23}

Comparative plots for experimental spectra and best fit, both in *k* and *R* spaces, taking into account all these contributions, are included in Figure 1. As can be seen in the figure, the fit quality, both in *R* and *k* spaces, is good and no well-defined contribution that would improve the fit appears to be missing.

Since the amplitude of the path from axial water molecules is rather small, an additional comparative plot without this shell has been included in the Supporting Information (Figure 2SI). Fit quality is slightly worse in the EXAFS spectra for low *k* values, 2.5–6 Å^{−1}, and around 3 Å in the magnitude of the Fourier transform. The parameters of the Cu–N coordination shell are not affected by this suppression.

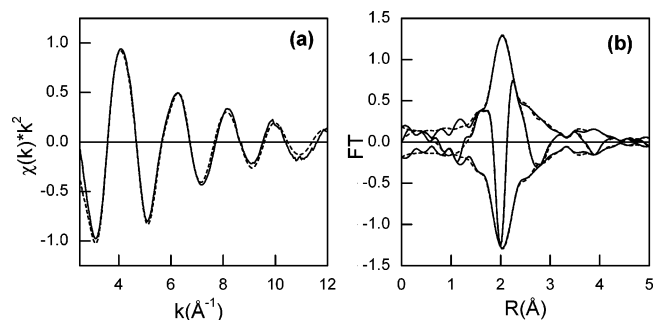


Figure 1. (a) Raw EXAFS data, *k*²-weighted, of [Cu(NH₃)₄]²⁺: experimental function (solid line) and fit obtained by using the parameters included in Table 1 (dashed line). (b) Cu–N phase-corrected FT of the EXAFS spectra included in Figure 1a. Magnitude and imaginary part: experimental (solid line); best fit (dashed line).

[Cu(en)₂]²⁺. Bis(ethylenediamine)copper(II) is a complex closely related with tetrammine copper(II) from the point of view of both reactivity and structure. In [Cu(en)₂]²⁺ each ethylenediamine molecule acts as a bidentate ligand coordinating through the N atom of each –NH₂ group. Its chelating character makes the resulting complex more stable (log *K*₁ = 9.7) than the tetrammine complex (log *K*₁ = 4.1).¹⁸ It forms five-membered chelate rings similar to the ones found in the glycine-type coordination mode described above. The beat appearing at around 5.5 Å^{−1} and a new peak in the FT at 3 Å (see Figure 1SI) are clear indications of a second coordination shell, formed by the two C atoms of each ethylenediamine ligand, corresponding to path B in Scheme 2. As expected, the values obtained for coordination distances and DW factors for the first shell, formed by four N atoms at 2.01 Å, are similar to those found for tetrammine copper(II), Table 1. The chelate effect does not induce significant changes in Cu–N coordination distances compared to those of tetrammine complex, as already observed by Koniya²⁰ and Mazzi¹⁹ in crystalline compounds. These results do not confirm those reported by Fujita et al.,²³ who from an XRD study of an aqueous solution report a Cu–N coordination distance of 1.93 Å. The second shell is formed by four carbon atoms at 2.84 Å from Cu(II) ions, a value consistent with bond distances within the ligand and similar to that obtained in the crystalline complex²⁰ and in solution.²³ The low values of the DW of this shell, 0.006 Å², can be attributed to the chelate effect. As observed in the previous complex, the inclusion of two axial water molecules improves the fit, goodness-of-fit decreases from 0.03 to 0.01, and although two new free parameters are included, the value of χ^2_{red} decreases from 26 to 14. The values obtained for the coordination distance, 3.4 Å, and DW factor, 0.01 Å², are very close to those of [Cu(NH₃)₄]²⁺ as well. As seen in Figure 2, including comparative plots in *k* and *R* spaces and in the parameters included in Table 1SI, fit quality is quite good.

To show the relative amplitudes of the contributions taken into account to fit this spectrum, comparative plots of the amplitudes of their FT's have been included in Figure 3SI. As seen there, the dominant contributions are those corresponding to the first-shell SS Cu–N and to the second-shell SS Cu–C. The two following contributions correspond to MS path type V involving Cu–N–C atoms and to SS Cu–

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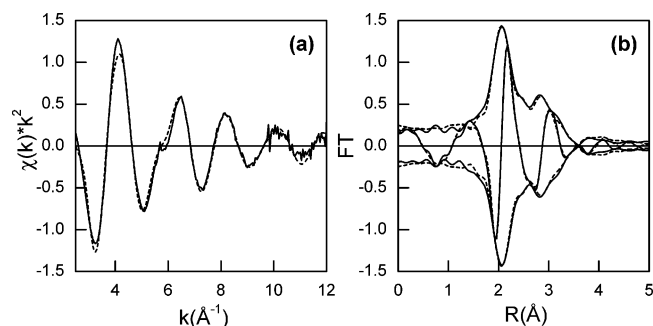


Figure 2. (a) Raw EXAFS data, k^2 -weighted, of $[\text{Cu}(\text{en})_2]^{2+}$: experimental function (solid line) and fit obtained by using the parameters included in Table 1 (dashed line). (b) Cu–N phase-corrected Fourier transform of the EXAFS spectra included in Figure 2a. Magnitude and imaginary part: experimental (solid line); best fit (dashed line).

O_{AX} . The EXAFS functions in k space corresponding to these contributions as well as the sum of three main MS paths within the first coordination shell have been included in Figure 4SI. As shown there some of these contributions are opposite in phase, so they partially cancel each other.

An additional figure has been made including the fit without axially coordinated water molecules (Figure 5SI) to show the relevance of this contribution. Reproduction of the EXAFS spectra at low k values is slightly worse, see Figure 5SIa, at 3, 5, and 7 \AA^{-1} . The imaginary part of the FT worsens between 2 and 4 \AA , and the magnitude is worse at ca. 3.5 \AA , see Figure 5SIb.

CuPc. Phthalocyanine is a macrocyclic conjugated planar molecule formed by four isoindole units, joined by four nitrogen atoms, thus rendering it a unique fully symmetric tetradentate ligand. More than 70 different ions can be incorporated into the central cavity,^{26,27} making the resulting complexes very stable. Although CuPc is a rather large complex, formed by 41 atoms excluding H, this tetradentate ligand is very rigid, probably due to the so-called *macrocyclic effect*, the greater thermodynamic stability of a complex with a cyclic polydentate ligand compared to a complex formed by a comparable noncyclic ligand.¹⁸ This higher stability together with its high symmetry makes the analysis of distant shells ($R > 4.0$ \AA) feasible. Phthalocyanine forms six-membered chelate rings similar to the ones found in the “histamine” coordination mode.

As shown in Figure 1SI, the EXAFS function of this compound is rather complex, and three clear new peaks appear in the amplitude of its FT. A good fit of this spectrum was attained including the first four shells (four N, eight C, four N, eight C) with the corresponding SS (A–D in Scheme 2) and MS paths. MS contributions are particularly relevant in this case due to the high symmetry of this complex as well as its rigidity. The relevant MS paths are those involving quasi aligned atoms: the ones within the four N atoms of the first shell, paths I, II, and III in Scheme 2; those involving atoms from the first and second shell, similar to that appearing in $[\text{Cu}(\text{en})_2]$, path V in Scheme 2; and overall, those involving atoms from the first, second, and fourth shells, paths V, V', VI, VI'. Note that N atoms of the third shell are not aligned with any other and thus do not contribute with significant MS paths. The obtained Cu–N first-shell

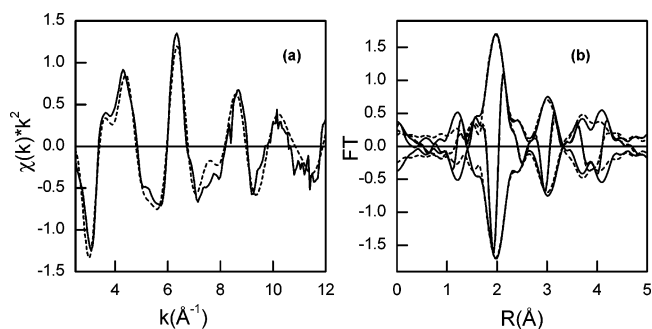


Figure 3. (a) Raw EXAFS data, k^2 -weighted, of CuPc: experimental function (solid line) and fit obtained by using the parameters included in Table 1 (dashed line). (b) Cu–N phase-corrected Fourier transform of the EXAFS spectra included in Figure 3a. Magnitude and imaginary part: experimental (solid line); best fit (dashed line).

coordination distance, 1.95 \AA , is close to that obtained from XRD of the crystal structure,²⁶ from ED of gas-phase molecules, and from DFT calculations.^{27b} Inclusion of axial water molecules did not improve the fit, probably due to the existence of intense single- and multiple-scattering contributions at similar distances. To show the relative amplitude of each individual single- and multiple-scattering path taken into account in this fit, the magnitude of the Fourier transforms of the SS paths and of some of the MS ones has been plotted in Figure 6SI. The main contributions are those corresponding to SS first, second and third shells, while that of the fourth shell is negligible. In contrast, MS associated to the fourth shell are particularly intense, which is due to the focusing effect of the quasi-aligned Cu–C–N atoms. Figure 7SI includes comparative plots in k space of MS and SS contributions. Note again the high amplitude of MS paths associated with the fourth shell (paths V, V', VI, VI'). It is remarkable as well that MS within the first shell keep a high amplitude for high k values, rather unusual in MS contributions, which can be attributed to the extremely low DW factor within the first Cu–N shell.

Figure 3 shows comparative plots of the experimental and best fit of the EXAFS spectra of CuPc in k and R spaces including the SS and MS paths described above, the quality of the fit being rather good. Given the relevance of MS paths shown in Figures 6SI and 7SI, their exclusion yields a poor reproduction of the spectrum both in k and R spaces, as seen in the comparative plots included as Supporting Information (Figure 8SI).

Although fit quality is good, it can be seen in Figure 3 that the intensity of the signal beyond 4 \AA is still high enough to allow for analysis of the fifth and sixth shells, which are formed by eight C atoms at 5.5 and 6.5 \AA , respectively.²⁶ However, inclusion of these additional two coordination shells adds a high number of relevant MS paths, 42 paths with intensity higher than 10%, for which there are not simple ways of computing DW factors. Consequently, a too high number of parameters has to be adjusted, and thus, analysis of the EXAFS spectra could not resolve these shells. Note that exclusion of these shells from the fit affects the quality of it around 4 \AA (see Figure 3b).

Analysis of the solid compound yielded the same results with a significant difference: the DW factor for the first shell

was 0.002 \AA^2 instead of 0.0008 \AA^2 . The difference is already visible in the magnitude of the FT (not plotted), smaller in the spectrum of the solid than in that of the dissolved species. This value indicates that smaller mean-square deviations appear in the dissolved species as compared to the solid-state compound. In principle, the opposite effect was expected, since the disorder is usually higher in liquids than in solid systems. Nevertheless, the lack of packing restrictions in solution might produce a symmetrically averaged structure. Thus, although up to now the structure of the crystalline compounds has been considered as a good approximation to the structure of the dissolved species, there might be significant differences between the structures of the same species in different aggregation states, as we have already observed in $[\text{Ni}(\text{CN})_4]^{2-}$.^{40b}

[Cu(Gly)₂]. The previous analyses are a test of the accuracy of phase shift and backscattering amplitude functions calculated theoretically for this type of complex. They also show the capability of the technique to reproduce previously reported values of first-shell metal–ligand coordination distances within 0.015 \AA . For chelate complexes, such as CuPc, with well-defined symmetric structure, up to four coordination shells can be analyzed using a number of free parameters small enough to make the obtained parameters statistically significant.

Analysis of the spectra of $[\text{Cu}(\text{Gly})_2]$ was carried out with a similar strategy. Nevertheless, although this complex looks simple, especially when compared with CuPc, the splitting of the first shell, formed by two N atoms plus two O atoms, renders the fit unfeasible as explained before. As it happens with ED,²⁷ EXAFS cannot solve adjacent contributions very close in distance, such as those formed by the first (two N plus two O) or second (two C plus two C) coordination shells, although some authors do not take into account the obvious limitations of the technique and have overanalyzed the EXAFS data of this complex.¹⁷ In an alternative approach, we introduced as fixed parameters coordination distances, averaged from those obtained from XRD,¹⁶ and DW factors, similar to those obtained for the complexes already analyzed. They were changed step by step until the EXAFS spectrum was reproduced. It required consideration of the 13 paths indicated in Table 1 (five SS + eight MS) with the parameters included in this table as well. Although, according to XRD, the average value of the Cu–N coordination distance is 2.00 \AA and that of Cu–O is 1.955 \AA , the best reproduction of the spectrum was obtained with a common value of coordination distance, 1.95 \AA , and the same DW factor, 0.003 \AA^2 . Since these values were not yielded by the fitting procedure, goodness of fit values are not provided nor indeterminations in the parameters. Figure 4 includes the comparative plots of the calculated and experimental spectra.

Analysis of this spectrum confirms that the technique is sensitive to small changes in the structure: note that although $[\text{Cu}(\text{en})_2]^{2+}$ and $[\text{Cu}(\text{Gly})_2]$ have rather similar structures, the spectrum of the first was fitted straightforwardly and that of the second was not. The difficulties undergone to analyze this spectrum suggest that a different strategy is required to gain insight into its structural elucidation, for instance, one

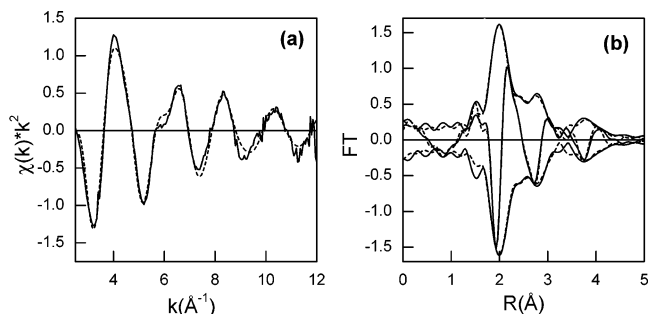


Figure 4. (a) Raw EXAFS data, k^2 -weighted, of $\text{Cu}(\text{Gly})_2$: experimental function (solid line) and spectrum obtained by using the parameters included in Table 1 (dashed line). (b) Cu–N phase-corrected Fourier transform of the EXAFS spectra included in Figure 4a. Magnitude and imaginary part: experimental (solid line); best fit (dashed line).

procedure could be based on the optimization of a few key parameters belonging to a structure of the complex by XAS-independent techniques, such as other spectroscopies or theoretical computations.^{42,45}

[Cu(His)₂]. As explained above, proposed coordination modes for $[\text{Cu}(\text{His})_2]$ include *histamine-histamine*, *histamine-glycine*, and *glycine-glycine*. In all of them histidine acts as a bidentate ligand coordinating through N_{am} and N_{im} or O_{COO} , whereas there is only one set of values of M–L coordination distances in solution obtained from EXAFS,¹² which, on the other hand, correspond to a structure in conflict with that proposed from NMR studies.¹¹ Under these premises, in our first approach we carried out a fit of the spectrum of the $\text{Cu}(\text{His})_2$ complex considering the symmetric structure *histamine-histamine* like. This is sound taking into account the studies by Ramonelli,¹⁰ Grommen,^{11a} and Manikandan,^{11b} who point out that for a neutral pH the most stable coordination is *histamine-histamine* like, since the spectrum was measured at physiological pH. For this type of coordination both the square-planar and tetrahedral arrangements, a coordination found in some copper(II) complexes,⁵ were considered. Since both structures were rather symmetric, they have the minimum number of shells and free parameters, as compared to the other structures already proposed.

No good fit was attained with the tetrahedral arrangement. As outlined before, a distinction between those arrangements was possible due to MS contributions within the first shell because paths I–III are not relevant for tetrahedral geometry. In contrast, a rather good fit was obtained for the square-planar arrangement, taking into account only four coordination shells, with the corresponding four SS paths and nine MS paths. Only nine free parameters (four coordination distances, four DW factors, and ΔE_0) were used, obtaining the values included in Table 1. The first shell is formed by four N atoms at 1.99 \AA . This value is between that obtained for CuPc and $[\text{Cu}(\text{NH}_3)_4]^{2+}$.

The coordination distance is the same as that obtained by Oztsumi for related Cu(II) complexes,¹² similar to that found by Boswell et al. in the amidating enzyme for Cu– N_{im} ,²⁹ 1.97 \AA , where the imidazole N atom comes from histidine, and to that obtained by Strange et al.²⁸ for Cu– N_{im} , 2.00 \AA ,

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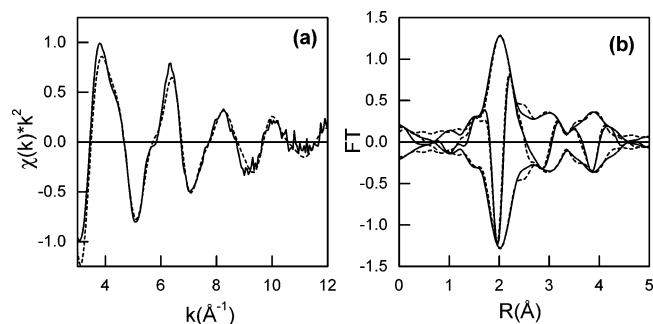


Figure 5. (a) Raw EXAFS data, k^2 -weighted, of $\text{Cu}(\text{His})_2$: experimental function (solid line) and fit obtained by using the parameters included in Table 1 (dashed line) in the *histamine-histamine* coordination mode. (b) Cu–N phase-corrected Fourier transform of the EXAFS spectra included in Figure 5a. Magnitude and imaginary part: experimental (solid line); best fit (dashed line).

in the complex $\text{Cu}(\text{im})_4$. The last paper is the first EXAFS study taking into account MS contributions in this type of system. In fact, due to the presence of four imidazole rings, MS contributions are very relevant in these systems, and their EXAFS spectra are more complex than the spectrum here presented, where there are only two imidazole rings, as can be seen by comparing Figure 5 in this paper with Figures 2–6 in ref 28. Although the coordination proposed here, bidentate with two histidine molecules coordinating through N_{im} and N_{am} , is different from that proposed by Strange et al., monodentate with four imidazole rings coordinating Cu(II), in both cases the existence of imidazole rings coplanar with Cu(II) ions yields the amplitude of MS paths very high.

Note the fit presented here, both in k and R spaces, imaginary part and magnitude, is as good as that obtained in the previous complexes studied for which the already known structures were reproduced. Moreover, all the reported values for coordination distances and Debye–Waller factors are coherent within the complex and consistent with the values found for the other complexes.

Taking into account that other structures were proposed for this complex, e.g., the *glycine-glycine* found by Evertsson using XRD⁷ at pH = 3.7 and by Ozutsumi¹² by EXAFS at neutral pH, an additional calculation of the spectra was carried out using as a starting structure that of the $\text{Cu}(\text{Gly})_2$ complex, with the difficulties already described. No good fit could be obtained with this approach. Similarly, we tried the other structure appearing in Scheme 1, i.e., *histamine-glycine*. Although it implies a higher number of paths and thus of free parameters, reproduction of the spectrum is slightly worse than that attained for the *histamine-histamine* coordination mode (cf. plots in Figure 6a, showing some discrepancies between 4 and 7 \AA^{-1} and Figure 6b around 4 \AA with the similar plots in Figure 5). It is quite clear that there are only minor differences between the two coordination modes.

As outlined before, the key to discriminate between the proposed structures is the number of imidazole rings within the plane of the complex, from which there are two in the first coordination mode and only one in the second. This geometry enhances the amplitude of MS paths V and VI, as already pointed out by Strange et al.,²⁸ thus allowing the

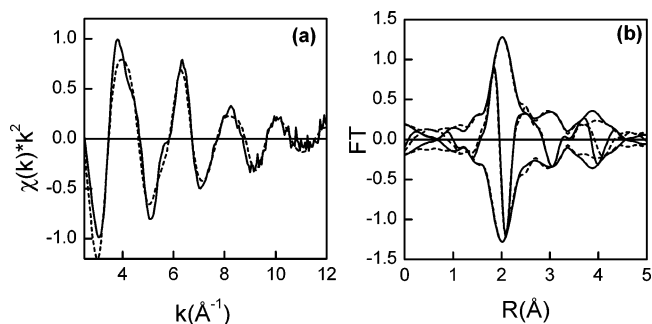


Figure 6. (a) Raw EXAFS data, k^2 -weighted, of $\text{Cu}(\text{His})_2$: experimental function (solid line) and fit obtained for *histamine-glycine* (dashed line). (b) Cu–N phase-corrected Fourier transform of the EXAFS spectra included in Figure 6a. Magnitude and imaginary part: experimental (solid line); best fit (dashed line).

analysis of higher shells which eventually could allow the discrimination among the different coordination modes.

By comparing Figures 5 and 6, we propose that the predominant structure is the so-called *histamine-histamine*, with the coordination parameters included in Table 1. Nevertheless, since an X-ray absorption spectrum provides average values of all the coordination sites in which the absorbing atom is involved, coming back to Scheme 1, we cannot discard the existence of small amounts of *histamine-glycine* coordination mode. This model is consistent with the recently published studies by Grommen and Manikandan using EPR and ENDOR spectroscopies¹¹ and does not confirm those of the early work by Ozutsumi obtained by EXAFS.¹² The fact that the latter authors did not take into account MS contributions might be the reason for the disagreement. EPR and ENDOR studies do not provide M–ligand coordination distances, although it can be inferred from the values of H–H coordination distances provided by the ENDOR technique. We complete these results by providing coordination distances for the first four coordination shells, which are formed by four N atoms at 1.99 \AA , two C atoms at 2.97 \AA , four C atoms at 3.32 \AA , and two N atoms at 4.1 \AA , values very similar to those found for the CuPc complex.

Conclusions

The structures of $[\text{Cu}(\text{NH}_3)_4]^{2+}$, $[\text{Cu}(\text{en})_2]^{2+}$ and CuPc have been determined by fitting their EXAFS spectra using a reduced number of SS and MS contributions (6–13) and free parameters (5–10), and results compare well with the available information. The short coordination distance found for the Cu–N first shell in CuPc, 1.95 \AA , might be attributed to the macrocycle effect. Higher values are found in the other two complexes, 2.01 and 2.02 \AA . Both sets of distances can be solved with EXAFS in these symmetric complexes. The existence of chelating ligands with strong bonds allows the rather precise determination of distant shells, Cu–X ($\text{X} = \text{C}, \text{N}, \text{O}$) with bond distances longer than 4.0 \AA . Concerning the labile axial water molecules, the weakness of the interaction renders the amplitude of the contribution rather small, so their existence can be assessed only in simple complexes where no other significant contribution appears

at similar distances, as happens for $[\text{Cu}(\text{NH}_3)_4]^{2+}$ or $[\text{Cu}(\text{en})_2]^{2+}$.

In the case of the $[\text{Cu}(\text{His})_2]$ complex ($\text{pH} = 7.3$), several coordination models have been tested, obtaining the best results for the symmetric *histamine-histamine* coordination mode in which both histidine molecules coordinate Cu(II) cations through N atoms from the amino group and from the imidazole ring, forming two six-membered chelate rings. The obtained coordination distance for the first Cu–N shell is 1.99 Å. In this coordination mode two imidazole rings are coplanar with the atoms forming the first coordination shell of the complex. For this reason multiple scattering contributions become relevant, thus allowing determination of higher shells. Two C atoms at 2.97 Å, four C atoms at 3.37 Å, and two N atoms at 4.1 Å form the successive shells. Only a slightly worse fit was obtained for the related coordination mode *histamine-glycine*, where only one imidazole ring is coplanar with Cu(II) ions. Nevertheless, the

parent structure *glycine-glycine* can be fully discarded as well as the tetrahedral arrangement.

Through these analyses we observed that the inclusion of MS paths provides indirect information about coordination angles (i.e., discriminating between tetrahedral and square-planar arrangements) since three- and four-body correlations have a significant effect on the spectra.

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Supporting Information Available: Additional figures and tables. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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