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Potentiometric and spectral analyses of Cu(II) ternary complexes between GlyGly, GlyVal, GlyLeu or GlyPhe with histidine

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ABSTRACT

The ternary complexes of copper (II) with some dipeptides (GlyGly, GlyVal, GlyLeu or GlyPhe) and histidine were studied in aqueous solution by potentiometric titration at 25 ºC and I = 0.1 mol L^{-1}. The complexes were analysed by ultraviolet-visible, infrared and EPR spectra. The values of formation constants for CuL_1L_2 species decrease in the order: Cu:GlyLeu:His > Cu:GlyPhe:His > Cu:GlyVal:His > Cu:GlyGly:His. The species distribution diagrams showed that the CuL_1L_2 species predominates at physiological pH. The EPR spectra at 77 K suggest that the copper(II) is in a square planar geometry. The infrared data indicated tridentate behavior of dipeptides ($N_{amine}N_{pept}O_{carboxy}$) and monodentate of histidine (N_{amine}). The imidazole ring does not coordinate to Cu(II) in these complexes, suggesting that the heteroaromatic nitrogen can bind to other metals in biological systems.

Keywords: *Alzheimer, copper complexes, dipeptides, histidine, spectroscopy.*

1. INTRODUCTION

All the neurodegenerative diseases have as a characteristic the malformation of proteins in the central nervous system [1, 2]. These proteins have a typical tendency to form solid deposits not only in the brain but also in the skeletal and muscular tissues and in the heart and liver [2].

The Alzheimer disease (AD) is the most common neurodegenerative disease, affecting directly approximately 10 % of human beings with more than 65 and 50 % of those with more than 85 [1]. This disease is an irreversible and progressive one that causes the loss of memory, unusual behavior personality changes and a decline in the thinking ability.

It is accompanied by three main structural changes in the brain: diffuse loss of neurons; intracellular protein deposits containing phosphorylated tau protein and extracellular protein deposits (amyloid (A*β*) or senile plaques) [3-5]. The Aβ-amyloid is a peptide containing 39-43 amino acids that is produced by the digestion of amyloid precursor protein (APP) by β and α secretases [5]. Aβ1-40 and Aβ1-42 are the main proteins in this disease. The Aβ1-40 isoform is the predominant soluble species in biological fluid and the Aβ1-42 is less soluble and is found in the plaque deposits [6-8].

The AD has been associated with the modified copper metabolism [9]. Elevated copper concentration has been found in biological fluids of Alzheimer patients. This contributes directly or indirectly to the increase of oxidative stress, an important factor in the neuronal toxicity [2, 9]. The extracellular Aβ-42 peptide has a high affinity with copper [10]. In addition to this, in the vicinity of the peptide there are a lot of amino acid and dipeptide fragments as potential ligands to the metal.

In this work we explored copper(II) ternary complexes CuL_1L_2 between some dipeptides present in the sequence of Aβ-42 with amino acid histidine (L_1 = GlyGly, GlyVal, GlyLeu or GlyPhe; L_2 = His). The complexes were studied by potentiometric, UV-Vis, Infrared and EPR spectroscopies. The *in vitro* study help us to understand the biological processes that lead to the neurotoxicity and the cellular death.

2. EXPERIMENTAL SECTION

2.1. Materials.

$Cu(NO_3)_2{\cdot}3H_2O$, standard solutions of potassium hydroxide 0.1 mol L^{-1} and the buffer solutions of pH 4.0 and 7.0 were from Merck. Glycylglycine, Glycylvaline, Glycylleucine, Glycylphenylalanine and histidine were purchased from Sigma-Aldrich. Deuterium oxide 99.9% (D_2O) was purchased from Tedia Brasil.

2.2. Apparatus.

The titrimetric data were obtained using an 809 Methrom automatic burette, a B375 Micronal pH meter and a combined glass electrode. All titrations were performed at 25.0 ± 0.1 ºC by coupling the titration cell with a thermostatic bath set at this temperature.

Ultraviolet visible spectra were recorded in a double-beam Perkin/Elmer Lambda 35 spectrophotometer with a quartz cell and 1 cm path length.

Electron paramagnetic resonance (EPR) spectra were obtained in 1 . 10^{-3} mol L^{-1} solution at 298 K and in the liquid nitrogen temperature (77 K) using a Bruker ESP300E equipment with modulation frequency of 100 kHz operating at 10 mW. Spectra simulations were performed using EasySpin [11].

The infrared spectra of free ligands and complexes were collected in 0.2 mol L^{-1} D_2O solution using a FT-IR 2000 Perkin-Elmer spectrophotometer.

2.3. Procedure.

2.3.1. pH Titrations.

For determination of protonation constants of ligands (GlyGly, GlyVal, GlyLeu, GlyPhe or His), aqueous solution (1 . 10^{-3} mol L^{-1}) of protonated ligand was titrated with 0.1 mol L^{-1} KOH at 25 ºC, under ionic strength of 0.1 mol L^{-1} using a 1.2 mol L^{-1} of KNO_3 solution. For determination of formation constants in the binary systems, solutions containing the ligands (GlyGly, GlyVal, GlyLeu, GlyPhe or His) and copper (II) ion were titrated at metal:ligand ratios 1:1 and 1:2. For determination of formation constants in the ternary systems, solutions containing Cu(II), one of the dipeptides and His were titrated at the ratio 1:1:1. All the constants were determined using the HYPERQUAD program and the species distribution diagrams were generated using the HYSS program [12, 13].

2.3.2. Spectral analysis.

Binary and ternary Cu(II) complexes were studied in aqueous solution at 25 ºC by UV–Vis spectroscopy. These solutions were prepared in the same way as previously done by potentiometry. The same metal:ligand ratio solutions were prepared and analyzed in the wide range of pH values, adjusted by adding small increments of 0.1 mol L^{-1} KOH solution. The ionic strength was 0.1 mol L^{-1} using 1.2 mol L^{-1} of KNO_3 solution.

3. RESULT SECTION

3.1. Potentiometric studies.

In the ternary systems, the model of species used in the HYPERQUAD program includes the protonation constants of ligands, the constant values of binary complexes as well as the hydrolysis constants of Cu(II) [14].

The formation constants of the binary systems were refined using the titration data (Table 1). In the binary complex containing histidine, the highest value of the log CuL constant observed, indicates a more basicity of amino acid than the dipeptide ligands. Our results agree with the data found in the literature [15-18].

Table 1. Stability constants for the binary systems CuL (L = GlyGly, GlyVal, GlyLeu, GlyPhe or His) at 25 ºC, μ = 0.1 mol L^{-1} (KNO_3). Standard deviations are given in parenthesis.

Systems	log β Cu: GlyGly	log β Cu: GlyVal	logβ Cu: GlyLeu	logβ Cu: GlyPhe	logβ Cu: His
CuL	5.55 (0.01)	5.80 (0.02)	5.86 (0.01)	5.95 (0.02)	10.35 (0.01)
$CuH_{-1}L$	1.32 (0.01)	0.84 (0.01)	0.81 (0.01)	1.69 (0.01)	1.25 (0.03)
$CuH_{-2}L$	-8.24 (0.01)	-8.25 (0.01)	-9.26 (0.01)	-7.87 (0.01)	-
CuL_2	-	-	-	-	17.95 (0.02)

In the ternary systems, the similarity of potentiometric curves indicates that the same species are formed in aqueous solution. The formation constants for the possible species present in these systems are shown in Table 2. The log CuL_1L_2 constant decrease in the following order: Cu:GlyLeu:His > Cu:GlyPhe:His > Cu:GlyVal:His > Cu:GlyGly:His.

3.2. Validation of the model of the species.

The model of species in the ternary systems was simulated using the HYSS program [13]. The experimental and simulated curves were compared showing good agreements (Fig. 1a-d).

Once calculated the formation constants, the HYSS program was used to generate the species distribution diagrams. For the ternary systems the diagrams are similar, showing the presence of CuL_1L_2 species at physiological pH (Fig. 2), coexisting with hydrolyzed binary species.

Table 2. Stability constants of the species present in ternary systems CuL_1L_2 (L_1 = GlyGly, GlyVal, GlyLeu or GlyPhe; L_2 = Met) at 25 ºC, μ = 0.1 mol L^{-1} (KNO_3). Standard deviations are given in parenthesis.

Systems	log β Cu:GlyGly: His	log β Cu:GlyVal: His	logβ Cu:Gly Leu:His	log β Cu:Gly Phe:His
HL_1L_2	15.79 (0.01)	16.45 (0.01)	16.91 (0.01)	16.7 (0.01)
$H_2L_1L_2$	23.62 (0.02)	23.64 (0.01)	23.89 (0.01)	23.78 (0.01)
CuL_1L_2	14.6 (0.02)	14.98 (0.01)	15.69 (0.01)	15.55 (0.01)
$CuHL_1L_1$	21.9 (0.01)	22.39 (0.01)	23.05 (0.01)	22.65 (0.01)
$CuH_{-1}L_1L_2$	7.74 (0.02)	6.82 (0.02)	7.01 (0.02)	7.55 (0.01)

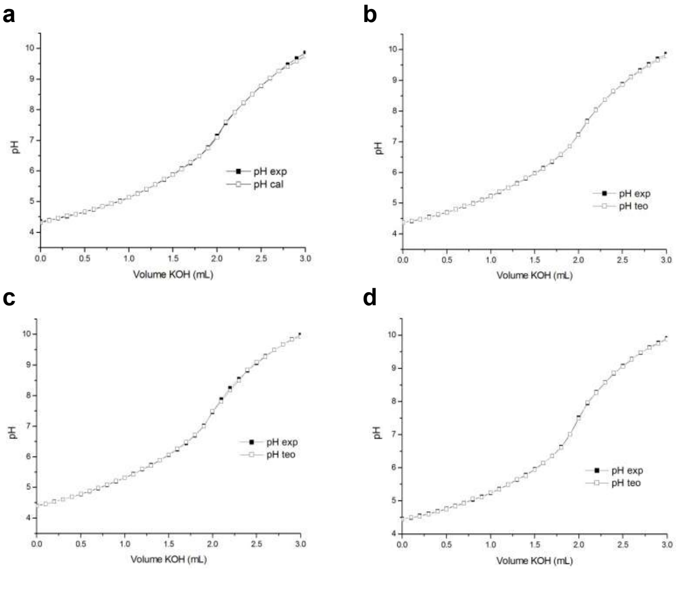

Figure. 1. Experimental and simulated titration curves for the ternary systems: a) Cu:GlyGly:His; b) Cu:GlyVal:His; c) Cu:GlyLeu:His and d) Cu:GlyPhe:His in the ratio metal:dipeptide:histidine (1:1:1) at 25 ºC and μ = 0.1 mol L^{-1} (KNO_3).

3.3. Ultraviolet visible spectral analyses.

The electronic spectra of the ternary complexes presented two absorption bands. The more intense band located in the ultraviolet region (≈ 290 nm) is the ligand to the metal transfer charge (LMTC) [18]. At low pH values, a less intense band observed at the range 697-672 nm was attributed to the $d_{xy}\rightarrow d_{x2-y2}$ transition. With the increase of pH values, this band was changed to lower wavelengths, indicating the entrance of the nitrogen in the Cu (II) coordination sphere [19]. At pH around 7, the values of ε at the range 53-65 L mol^{-1} cm^{-1} (Table 3) are characteristic of the complexes in a square planar geometry [18-21].

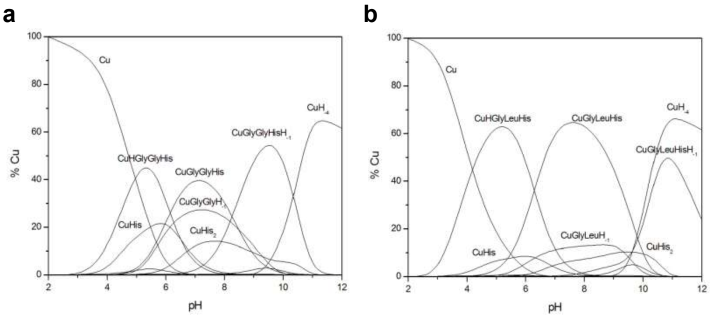

Figure 2. Species distribution diagrams for the ternary systems: a) Cu:GlyGly:His and b) Cu:GlyLeu:His in the ratio metal:dipeptide:histidine (1:1:1) at 25 ºC and μ = 0.1 mol L^{-1} (KNO$_3$).

Table 3. Electronic absorption data, maximum wavelength and molar absorption coefficient for the ternary complexes Cu(II):L$_1$:L$_2$ (L$_1$ = GlyGly, GlyVal, GlyLeu or GlyPhe; L$_2$ = His). Solutions in the ratio metal:dipeptide:histidine (1:1:1) at 25 ºC and μ = 0.1 mol L^{-1} (KNO$_3$).

Complexes	pH	Maximun wavelength (nm)	Molar absortivity (mol^{-1} cm^{-1}L)
Cu:GlyGly:His	7.2	634	65
Cu:GlyVal:His	6.9	623	50
Cu:GlyLeu:His	7.0	613	55
Cu:GlyPhe:His	7.0	615	53

3.4. Infrared Spectra.

In the infrared spectra of free dipeptides and histidine, the ν(NH) band observed at the range 3315-3392 cm^{-1} shifts to 3305-3323 cm^{-1} in those of the complexes, suggesting the coordination of amine nitrogens [22,23]. The dipeptides exhibit three characteristic bands at 1740-1749, 1665-1673 and 1586-1618 cm^{-1}. In the spectra of the ternary complexes, exist only one strong band at 1633-1634 cm^{-1}. This spectral change is interpreted as the deprotonation of peptidic nitrogen and its entrance in the copper(II) coordination sphere [23-26]. The ν_s(COO$^-$) absorption observed at 1448-1453 cm^{-1} in the spectra of dipeptides shifts to 1396-1413 cm^{-1} in spectra of the complexes, indicating the coordination of carboxylic oxygen [20]. In the spectrum of histidine, the ν(NH) band (1635 cm^{-1}) attributed to the nitrogen of the imidazole ring does not suffered changes in the spectra of the ternary complexes, suggesting does not participation of the heteroaromatic nitrogen in coordination to Cu(II) [27].

The infrared data indicated that the dipeptides coordinate in a tridenatate mode through the amine and peptidic nitrogens and the carboxylic oxygen (N$_{amine}$N$_{pept}$O$_{carboxy}$). The histidine ligand binds to copper in a monodentate way through the amine nitrogen (N$_{amine}$).

3.5. EPR Spectra.

The EPR spectra of the ternary complexes, in aqueous solution at room temperature (298 K), are presented in Figure 3a together with their simulation using EasySpin.

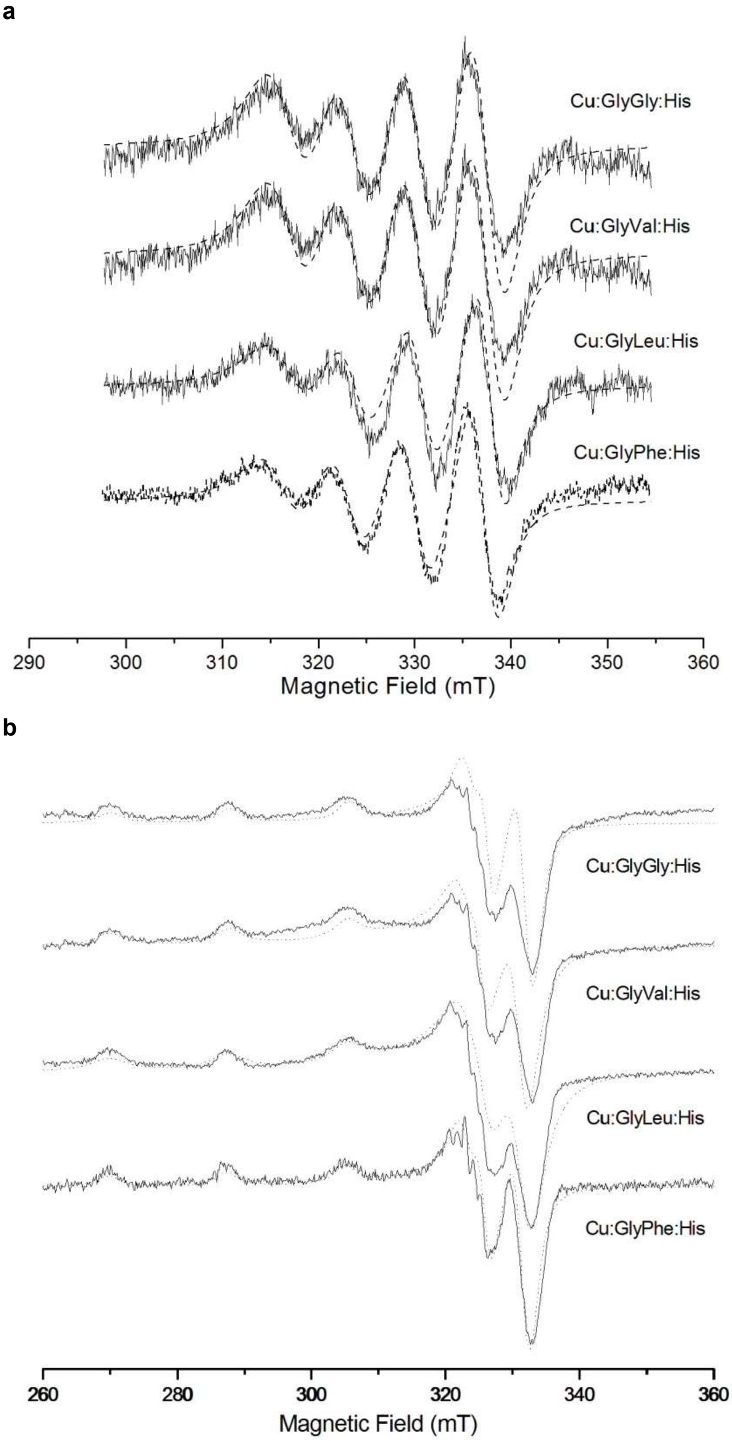

Figure. 3. a) Ambient temperature EPR spectra of the ternary complexes in aqueous solution (line) and the simulations (dotted lines) using EasySpin (S= 1/2, axial symmetry, function garlic, correlation time 2.0 . 10^{-11} s, lorentzian and gaussian line width 1.5 mT); b) EPR spectra of the ternary complexes in aqueous solution at 77 K and the simulations without the nitrogen superhyperfine structure, using EasySpin (function pepper, parameters in Table 4).

Table 4. EPR spectral parameters for the ternary complexes in aqueous solution at room (298 K) and nitrogen liquid (77 K) temperatures obtained by spectral simulations using the software EasySpin (FWHM: full width at half height) [11].

Complexes (298 K)	ΔB$_{FWHM}$ (mT) Gauss; Lorentz	$g_{//}$	$g_{\perp}$	$A_{//}^{a}$	$A_{\perp}$	
Cu:GlyGly:His	1.5; 1.5	2.374	2.002	135	35	
Cu:GlyVal:His	1.5; 1.5	2.362	2.003	156	23	
Cu:GlyLeu:His	1.5; 1.5	2.358	2.000	156	23	
Cu:GlyPhe:His	1.5; 1.5	2.362	2.002	156	23	

Complexes (77 K)	ΔB_{FWHM} (mT) Gauss; Lorentz	$g_{//}$	$g_{\perp}$	$A_{//}^{a}$	$A_{\perp}$	$g_{//}/A_{//}^{a}$
Cu:GlyGly:His	0; 3.2	2.265	2.070	165	15	133
Cu:GlyVal:His	0; 2.5	2.260	2.069	168	15	134
Cu:GlyLeu:His	0; 2.0	2.258	2.063	170	13	125
Cu:GlyPhe:His	1.2; 1.2	2.255	2.059	172	12	133

^{a}All hyperfine coupling constants in units of 10^{-4} cm^{-1}.

The spectra are similar and almost isotropic due to fast rotation and show four lines hyperfine splitting due to the interaction with nuclear spin of Cu(II) (I= 3/2) [28]. The line shapes of the four hyperfine lines are well reproduced using the fast-motion regime with a correlation time of $2 \cdot 10^{-11}$ s.

The spectra in aqueous solution at 77 K are characteristic of axial symmetry (Fig. 3b). The *g* values are consistent with a d_{x2-y2} ground state. The perpendicular region does not exhibits a nitrogen super hyperfine structure.

The empirical factor $f = g_{//}/A_{//}$ (with $A_{//}$ in cm^{-1}) is a convenient empirical index of distortion from planar [29], varies from 105 to 135 for small to extreme distortion. The quotient factor indicated a more distortion of Cu:GlyGly:His and Cu:GlyVal:His than Cu:GlyLeu:His and Cu:GlyPhe:His. The EPR parameters for the ternary complexes are listed in Table 4.

4. CONCLUSIONS

The potentiometric results indicate that the $Cu:L_1:L_2$ ternary species (L_1 = GlyGly, GlyVal, GlyLeu or GlyPhe; L_2 = His) are formed at physiological pH value. The infrared data showed tridentate coordination mode of dipeptides ($N_{amine}N_{pept}O_{carboxy}$) and monodentate behavior of histidine (N_{amine}) in the complexes. The EPR spectra at 77 K were characteristic of copper(II) compounds in a square planar geometry. In these complexes, the non coordination of the imidazole ring to copper suggests that the heteroaromatic nitrogen can participate in the coordination with other metals in biological systems.

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