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Study on guanidino–carboxylate interactions in copper(II) ternary complexes of guanidinoacetic acid with glutamic and aspartic acids

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Abstract

The possibility of occurrence of biological relevant guanidino–carboxylate interactions was investigated in ternary systems involving guanidinoacetic (Gaa) and glutamic acid (Glu), aspartic acid (Asp) or glycine (Gly). The study was done in solution using potentiometry, ultraviolet visible and EPR spectroscopy. The stability constants of the ternary complexes as well as the binary ones were potentiometrically determined ($T = 25$ °C, $\mu = 0.1$ mol l^{-1}, KNO_3). The order of the stability constants of ternary complexes was CuAspGaa > CuGluGaa > CuGlyGaa. $\Delta\log K(\log \beta$ CuLGaa $-(\log \beta$ CuL $+\log \beta$ CuGaa) L = Glu, Asp or Gly) showed that Asp and Glu favored more stable ternary complexes while Gly formed a less stable ternary complex than its binary ones. Comparison between statistical and experimental log β CuLGaa suggested guanidino–carboxylate interactions in CuAspGaa and CuGluGaa, involving the guanidino group from Gaa and the non-coordinated-carboxylate group from Glu or Asp, possibly through hydrogen bonds.

Keywords: Guanidinoacetic acid; Guanidino–carboxylate interactions; Ternary complexes; Stability constants; Potentiometry; Copper(II)

1. Introduction

Guanidinoacetic acid (Gaa) is an amino acid characterized by the presence of a guanidino group, which is an important group in many biological processes. Gaa is mainly synthesized in the kidneys [1,2] involving the transamidination of glycine (Gly) via arginine, catalyzed by glycineamidinotransferase. One part of Gaa can be excreted in urine and the other is methylated in the liver for the creatine production, catalyzed by guanidinoacetate methyltransferase [3]. The main site of Gaa synthesis explains its usage as a more sensitive marker of the integrity of the renal tubules, signalizing many chronic renal failures [4–6]. In addition, Gaa is also involved in cholesterol production [7], thyroid dysfunction [8], epileptic seizures [9], hepatic encephalopathy [10] and insulin regulation [6]. Besides, Gaa may behave as an endogenous chemoconvulsant [11] and its accumulation in the nervous system has been detected in a inborn error caused by the guanidinoacetate methyltransferase deficiency, which was first reported in 1994 [12–14]. Recently, the determination of Gaa in dried blood spots using an automated HPLC method has been proposed as an early diagnosis of this disease [15]. It has also been detected a significant decrease of urinary excretion of Gaa after administration of cisplatin in patients with urinary tract neoplasm [16].

The presence of the guanidino group in Gaa may be the key of the diversity of its functions due to the fact that this group is an important biological recognition site because of its wide protonation range of pH and the possibility of making double hydrogen bonds. One of the most interesting cases of molecular recognition involving this group is the guanidino–carboxylate interaction made by hydrogen bonds, which are present in enzymatic reactions and protein stabilization. These have already been mentioned between the carboxylate groups of aspartic acid (Asp) or glutamic acid (Glu) and

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the guanidino group of arginine, the precursor of Gaa and a very similar ligand. The substrate fixation through these interactions occurs, for example, in Cu, Zn-superoxide dismutase and in carboxypeptidase-A [17]. More recently, a guanidino–carboxylate bond was identified on the complex formation between a human immunodeficiency virus and the receptor on the cell wall [18]. These interactions have also been observed in a chiral–double-helical complex, [Cu(L-arginine)$_2$](*m*-phthalate) [19], where the hydrogen bonds formed between the guanidino group of arginine (Arg) and the carboxylate group of *m*-phthalate are the principal factor in the maintenance of the handedness and self-assembly of molecules. A recent study on Cu(II) dipeptides complexes containing Arg–Asp, Arg–Glu dipeptides has also reported the occurrence of these interactions in solution and in solid state [20].

In this study, these guanidino–carboxylate interactions were investigated in copper(II) aqueous solution ternary complexes involving the Gaa (Fig. 1(a)), as the main ligand, and one of the following amino acids: Glu (Fig. 1(b)), Asp (Fig. 1(c)) and Gly (Fig. 1(d)). From previous studies [21,22], we already know that Gaa preferentially behaves as an α-nitrogen–oxygen bidentate ligand towards Cu(II) (both in solution and in solid complexes), presenting the terminal nitrogen atoms of guanidino group uncoordinated. Gaa may, however, behave as a bidentate ligand using only oxygen atoms from a bridge coordinated-carboxylate group as in the dimeric complex synthesized in lower pH: tetrakis[μ-(Gaa–O–O′)dinitrato–O]dicopper(II) [23]. On the other hand, Asp and Glu could behave both as tridentate or bidentate ligands towards Cu(II), depending on the pH [24]. As this latter ion has a preference for the square-planar geometry, it is expected that its ternary complexes should present both Gaa and Glu or Asp as bidentate ligands, leaving the terminal-nitrogen-guanidino atoms from Gaa and one carboxylate group from Glu or Asp free from coordination, and thus allowing their interaction. Cu(II)–Gly–Gaa system was studied just for comparison with a situation where this interaction is quite improbable to be present due the fact that Gly only has one already coordinated carboxylate group.

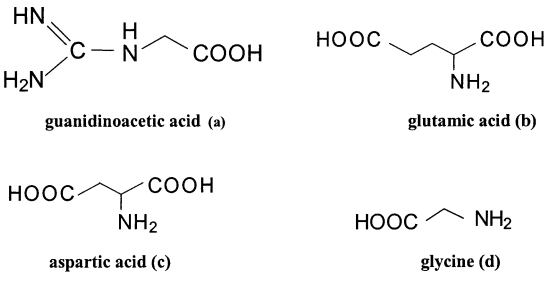

Fig. 1. Structures of the amino acids used as ligands: Gaa (a), Glu (b), Asp (c) and Gly (d).

2. Experimental

2.1. Materials

All the starting materials were of reagent grade and used without further purification. Gaa was purchased from Aldrich and Glu, Asp and Gly from Sigma Chemical. Copper(II) nitrate trihydrated, potassium nitrate, potassium biphthalate and the standard solutions of potassium hydroxide 0.1 mol l^{-1}, nitric acid 0.1 mol l^{-1}, EDTA 0.1 mol l^{-1} and of the buffer solutions of pH 4.0, 7.0 and 9.0 were from Merck. Water was purified by Milli-Q water purification system, deionized and distilled.

2.2. pH titrations

2.2.1. Reagents

Carbonate-free potassium hydroxide 0.1 mol l^{-1} was prepared and standardized against potassium biphthalate and a standard solution of nitric acid 0.1 mol l^{-1}. Copper (II) nitrate trihydrate solution (0.01 mol l^{-1}) was prepared by dissolving copper(II) nitrate trihydrate in water and was standardized with standard solution of EDTA 0.1 mol l^{-1} (Titriplex).

2.2.2. Apparatus

All pH titrations were performed using a Mettler DL25 automatic titrator, coupled with a thermostating bath at 25 °C (±0.1 °C) and a Metrohm combined glass electrode (Ag/AgCl). The pH meter was calibrated with Merck standard buffer solutions (4.00, 7.00 and 9.00).

2.2.3. Procedure

For the determination of the acid dissociation constants of the ligands (Gaa, Glu, Asp and Gly) an aqueous solution (1×10^{-3} mol l^{-1}) of the protonated ligand was titrated with 0.1 mol l^{-1} KOH at 25 °C under nitrogen atmosphere and ionic strength of 0.1 mol l^{-1} (KNO_3). For the determination of binary (one ligand and Cu(II)) and ternary systems (Cu(II), one of the other L ligands and Gaa), the ratios used were 1:1, 1:2 Cu(II):ligand and 1:1:1, 1:1:2 Cu(II):L:Gaa, L = Glu, Asp or Gly. The concentrations were Cu(II), 30 mM; Glu/Asp/Gly, 30–60 mM and Gaa, 30–60 mM. These solutions were titrated with 0.1 mol l^{-1} KOH under the same conditions mentioned above. Each titration was repeated three or four times in order to check the reproducibility of the data.

2.3. Spectral analyses

Cu(II) complexes were spectrophotometrically studied in solution at 25 °C. These were prepared in the same way previously done in potentiometry: the same ratio ligand:metal solutions were prepared and analyzed in a wide range of pH values, adjusted by adding small increments of 0.1 mol l^{-1} KOH. The ionic strength was 0.1 using 1.2 mol l^{-1} KNO_3. A double-beam Perkin–Elmer Lambda 19 spectrophotometer was used with a quartz cell and 1 cm path length.

EPR analyses were done with aqueous solutions of Cu(II) (1×10^{-3} mol l^{-1}), Gaa and Asp and were examined as a function of pH. The ionic strength was adjusted to 0.1 (KNO_3). X-Band EPR spectra were recorded at 100 kHz and 10 mW with a 300-E Bruker spectrophotometer at 77 K (liquid nitrogen). Ethylene glycol was added to all solutions to ensure good glass formation.

3. Results and discussion

The stability of ternary complexes may be evaluated by the following equilibrium:

$$pM + qH + rA + sB \rightleftharpoons M_pH_qA_rB_s \quad (1)$$

where M is the metal ion, H is the proton, A and B are the ligands.

The global stability constant for the ternary complexes [25] may be represented as following:

$$\log \beta_{pqrs} = [M_pH_qA_rB_s]/[M]^p[H]^q[A]^r[B]^s \quad (2)$$

It is also possible to define the stability constants for ternary complexes in relation to their binary ones [25], represented by the equilibria (3) and (4):

$$M + B \rightleftharpoons MB \qquad K^{M}_{MB} = [MB]/[M][B] \quad (3)$$

$$MA + B \rightleftharpoons MAB \qquad K^{MA}_{MAB} = [MAB]/[MA][B] \quad (4)$$

The difference between the stability of the ternary and binary complexes shows the tendency of the formation of ternary species [25]. This could be expressed by Eq. (5):

$$\Delta\log K = \log K^{MA}_{MAB} - \log K^{M}_{MB} = \log K^{MB}_{MBA} - \log K^{M}_{MA} \quad (5)$$

or Eq. (6):

$$\Delta \log K = \log \beta_{1011} - (\log \beta_{1010} + \log \beta_{1001}) \quad (6)$$

A statistical evaluation of ternary complex formation was also done using Eqs. (7) and (8) [26]:

$$\beta_{1011} = 2\beta^{1/2}_{1020}\beta^{1/2}_{1002} \quad (7)$$

$$\beta_{1020\text{stat.}} = \log 2 + 1/2 \log \beta_{1020} + 1/2\ \beta_{1002} \quad (8)$$

The difference between the constant refined from experimental data and those calculated statistically using Eq. (8) indicates the possibility of ligand–ligand interaction.

The constants were refined using the SUPERQUAD program [27] and the speciation as a function of pH, using Hyss [28] program. The correction factor used for calculations of hydrogen ion concentration was $10^{-pH}/[H^+] = 0.855$ and the ionization constant of water used were 13.98 [29].

3.1. Solution equilibria of copper(II), glutamic acid and guanidinoacetic acid

3.1.1. Potentiometric analyses

The model of species for this ternary system that was used in SUPERQUAD program includes all the species of Table 1 as well as the hydrolysis constants of Cu(II) [21,30]. The stability constants of the binary complexes (01–13) were refined separately using the titration data of these systems in a 1:1 and 2:1 ligand:Cu(II) ratio in the same conditions of temperature and ionic strength. As they were in good agreement with reported value [29], they were fixed and, consequently, only ternary species (14–18) were refined in the ternary model of the species. The titration data from 1:1:1 Cu(II):Glu:Gaa metal:ligands ratio were not considered for the calculations because they were quite similar to those from 1:1 Cu(II):Glu system, indicating the difficulty of ternary complexes analysis or potentiometric detection in solution when the two ligands are present in the same concentration. In addition, even though there has been tried to refine the species using these data, the calcula-

Table 1
Logarithms of the stability constants of binary and ternary complexes of copper(II), Glu and Gaa at 25 °C, $I = 0.1$ mol l^{-1} (KNO_3)

Number of species	Species	log β this work	log β	Reference
1	HGaa	10.65 (0.03)	10.91	[21]
2	H_2Gaa	13.47 (0.06)	13.81	[21]
3	CuGaa	7.69 (0.01)	7.69	[21]
4	$Cu(Gaa)_2$	15.25 (0.05)	15.20	[21]
5	CuH_2Gaa	20.90 (0.03)	20.96	[21]
6	$CuGaaH_{-1}$	−0.27 (0.09)	−0.27	[21]
7	$Cu_2(Gaa)_2H_{-2}$	3.77 (0.09)	3.77	[21]
8	HGlu	9.41 (0.02)	9.60	[30]
9	H_2Glu	13.56 (0.01)	13.74	[30]
10	H_3Glu	16.21 (0.04)	15.92	[30]
11	CuGlu	8.60 (0.03)	8.54	[30]
12	$Cu(Glu)_2$	15.19 (0.06)	15.22	[30]
13	CuHGlu	13.27 (0.02)	12.73	[30]
14	H_2GluGaa	23.44 (0.02)		
15	HGluGaa	14.82 (0.02)		
16	CuGluGaa	16.39 (0.02)		
17	CuHGluGaa	25.85 (0.03)		
18	CuH_2GluGaa	32.42 (0.06)		

The standard deviation are given in parenthesis.

tion has not converged. For these reasons, the 1:1:2 Cu(II):Glu:Gaa ratio titration has been done.

From the data of Table 1, it can be observed that the ternary complex CuGluGaa is very stable, with strong indications of ligand–ligand interactions reinforcing this stability.

The protonated ternary complexes, CuHGluGaa and $CuH_2GluGaa$, were also very stable. Analyzing Eq. (9), it can be concluded that the proton of CuHGluGaa complex should be bound to the α-nitrogen atom of Gaa, because the difference between log β CuHGluGaa and log β CuGluGaa is 9.46, very close to log β of HGlu (9.41). In relation to $CuH_2GluGaa$, however, the second proton should be bound to α-nitrogen of Glu inasmuch as log K of Eq. (10) is 6.57, thus, much smaller than its deprotonation constant.

$$CuHGluGaa \rightleftharpoons CuGluGaa + H^+ \qquad \log K = 9.46 \quad (9)$$

$$CuH_2GluGaa \rightleftharpoons CuHGluGaa + H^+ \qquad \log K = 6.57 \quad (10)$$

The speciation diagram as a function of pH of this ternary system (Fig. 2) shows the predominance of protonated complexes until pH ≈ 9, namely, one binary complex, CuH_2Glu, up to pH 4, and two ternary ones, $CuH_2GluGaa$ (pH 4–6.5) and CuHGluGaa (pH 6.5–9.5). CuGluGaa has begun to be formed close to pH 7, but only predominated at pH ≈ 9.5, where Glu was totally deprotonated and Gaa was loosing the proton attached to its α-nitrogen. It should be regarded that although log β of HGaa (deprotonation of α-nitrogen) is 10.65 (Table 1), multiple equilibria reactions could occur and cause a slight difference in the value of pH in which this proton was lost. From Fig. 2, it also can be observed that the ternary complex CuGluGaa has predominated in a great extent in relation to the binary $Cu(Gaa)_2$ complex reinforcing the presence of guanidino–carboxylate interactions.

3.1.2. Spectral analyses: absorption

The spectral analyses were performed using the solutions of 1:1:2 Cu:Glu:Gaa system in the same conditions of temperature and ionic strength of the potentiometric analyses, in a pH range from 4 to 10.6 and [Cu] varying from 5.55×10^{-4} to 5.43×10^{-4} mol l^{-1}. It was observed a LMCT band, which has shifted from 301 to 272 nm as pH values increased. The other band, relative to d–d transition between the d_{xy}, d_{z^2} and the pair d_{xz}, d_{yz} to the σ anti-ligand-half-filled d^2_{x-y} orbital [32], has shifted from 763 to 634 nm from pH 4 to 10.6.

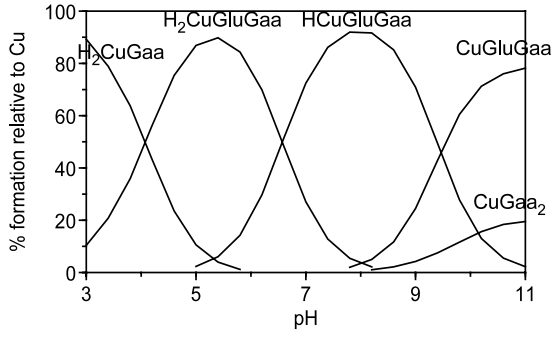

Fig. 2. Species distribution as a function of pH of 1:1:2 Cu:Glu:Gaa. Calculated from the stability constants listed in Table 1. Concentrations: Cu(II), 30 mM; Glu, 30 mM and Gaa, 60 mM.

Table 2 shows the relation among pH, the maximum wavelength of the d–d band and the molar absortivity. The observed shift to lower values of maximum wavelength as pH increased agreed with the deprotonation of amino groups, in addition of the already deprotonated carboxylate groups, as a result of the formation of more stable nitrogen–oxygen chelated complexes.

3.2. Solution equilibria of copper(II), aspartic acid and guanidinoacetic acid

3.2.1. Potentiometric analyses

The species used for refinement in SUPERQUAD program for this system are listed in Table 3. For the same reason of the Cu(II):Glu:Gaa system, only the titration data from of 1:1:2 Cu:Asp:Gaa metal:ligands ratio converged during the refinements of the species. The binary constants previously determined in this work (Table 3, species no. 1–13) in the same conditions of temperature and ionic strength agreed with literature [21,30] and were fixed in the ternary system.

The ternary species (Table 3, species no. 14–18) presented greater stability than their correspondent binary ones and those from Cu:Glu:Gaa system. Similarly to Cu:Glu:Gaa system, the protonated complex

Table 2
Relation among pH, maximum wavelength and molar absortivity of each solution of Cu(II):Glu:Gaa in a 1:1:2 ratio with Cu(II) concentration varying from 5.55×10^{-4} to 5.43×10^{-4} mol l^{-1}, at 25 °C and $\mu = 0.1$ mol l^{-1} (KNO_3)

pH	Maximum wavelength (nm)	Molar absortivity ($mol^{-1}cm^{-1}$ $l/10^{-2}$)
4.02	763	89.1
4.21	755	93.6
4.55	748	122.5
4.88	748	131.5
5.35	744	153.1
6.25	700	178.4
7.06	700/688 [a]	189
7.80	673	206
8.98	642	265.7
9.96	642/639 [a]	271
10.36	634/631 [a]	282
10.55	634	284

[a] Spectra where it was observed more than one maximum wavelength of the same molar absortivity.

Table 3
Logarithms of the stability constants of binary and ternary complexes of copper(II), Gaa and Asp at 25 °C, $I = 0.1$ mol l^{-1} (KNO_3)

Number of species	Species	log β this work	log β	Reference
1	HGaa	10.65 (0.03)	10.91	[21]
2	H_2Gaa	13.47 (0.06)	13.81	[21]
3	CuGaa	7.69 (0.01)	7.69	[21]
4	$Cu(Gaa)_2$	15.25 (0.05)	15.20	[21]
5	CuH_2Gaa	20.90 (0.03)	20.96	[21]
6	$CuGaaH_{-1}$	−0.27 (0.09)	−0.27	[21]
7	$Cu_2(Gaa)_2H_{-2}$	3.77 (0.09)	3.77	[21]
8	HAsp	9.48 (0.02)	9.56	[30]
9	H_2Asp	13.35 (0.01)	13.71	[30]
10	H_3Asp	15.83 (0.03)	15.96	[30]
11	CuAsp	8.46 (0.03)	8.30	[30]
12	$Cu(Asp)_2$	15.08 (0.05)	15.03	[30]
13	CuHAsp	12.48 (0.03)	12.52	[30]
14	$H_2AspGaa$	23.15 (0.01)		
15	HAspGaa	20.04 (0.02)		
16	CuAspGaa	17.82 (0.01)		
17	CuHAspGaa	25.46 (0.01)		
18	$CuAspGaaH_{-1}$	7.24 (0.01)		

The standand deviations are given in parenthesis.

$CuH_2AspGaa$ should have two protons bound to the α-nitrogen from Gaa and from Asp.

The speciation as a function of pH (Fig. 3) shows the predominance of the ternary complex CuAspGaa in relation to the binary ones in a large pH range (≈6.6–10.5). The protonated ternary complex CuHAspGaa coexisted with the binary CuAsp and CuGaa (not shown in Fig. 3 because of their low percentages) in the 5–7 pH range with less than 10% of formation. The hydrolyzed species $CuAspGaaH_{-1}$ has been formed above pH 9 and had 30% relative concentration at pH 11.0. This speciation corroborated the occurrence of guanidino–carboxylate interactions because of the great predominance and hence, the great stability of CuAspGaa complex.

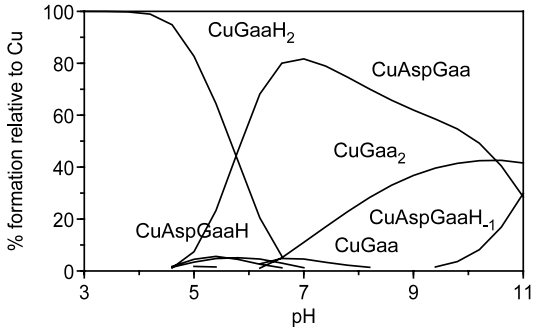

Fig. 3. Species distribution as a function of pH of 1:1:2 Cu:Asp:Gaa. Calculated from the stability constants listed in Table 3. Concentrations: Cu(II), 30 mM; Asp, 30 mM and Gaa, 60 mM.

3.2.2. Spectral analyses: absorption and EPR

The absorption spectra for the 1:1:2 Cu(II): Asp: Gaa system showed a great shift in the band related to d–d transition, from 782 to 630 nm in a 4–10 pH range and a little shift in the LCMT band, from 301 to 299 nm. The values of maxima wavelength and molar absortivity are shown in Table 4. This shift could be explained by the formation of more stable complexes coordinated by the oxygen atoms of carboxylate groups and the nitrogen ones of amino groups of both Gaa and Asp ligands. The greater blue shift relative to d–d transition compared with the Cu(II):Glu:Gaa system confirmed the greater stabilization of the ternary species of Cu(II):Asp:Gaa system compared with Cu(II):Glu:Gaa system.

The EPR spectra are shown in Fig. 4 for different values of pH, from 3 to 11. The aliquots were taken from the solution of a potentiometric titration of the Cu:Asp:Gaa 1:1:1 system, which have been done simultaneously. Table 5 shows some EPR parameters: $A_{||}$ (G), $g_{||}$ and $g_{\perp}$. It was observed an increase in $A_{||}$ values and a decrease in both $g_{||}$ and $g_{\perp}$ values as pH augments, characteristic of square-planar Cu(II) complexes [33]. In addition, the values of $g_{||} > g_{\perp} > 2$ observed in Table 5 also indicate the square-planar symmetry [33]. The tendency of increase of $A_{||}$ as $g_{||}$ and $g_{\perp}$ values diminish should be explained by the increase of covalent interactions with the equatorial ligands [34].

Analyzing the spectra from pH 4 to 6 in Fig. 4 and their correspondent $A_{||}$ values in Table 5, it was observed only signals relative to Cu(II) coordinated to oxygen atoms, which should be of carboxylate groups in a agreement with the predominance of CuH_2Gaa complex (Fig. 3). From pH 8 to 10, it could be also observed the signals (five lines) relative to a coupling of two nitrogen atoms (Table 6), which agrees with the presence of the ternary complex CuAspGaa at this pH range (Fig. 3), where two α-nitrogen atoms should be

Table 4
Relation among pH, maximum wavelength and molar absortivity of each solution of Cu(II):Asp:Gaa, in a 1:1:2 ratio, with Cu(II) concentration varying from 5.25×10^{-4} to 5.14×10^{-4} mol l^{-1}, at 25 °C and $\mu = 0.1$ mol l^{-1} (KNO_3)

pH	Maxima wavelength (nm)	Molar absortivity (mol^{-1} cm^{-1} $l/10^{-2}$)
4.01	782	136
4.22	752	151
4.52	748	155
4.95	728	171
5.82	725	187
6.88	710	204
7.50	679	216
8.07	648	237
9.04	636	293
9.81	631	327
10.22	630	331

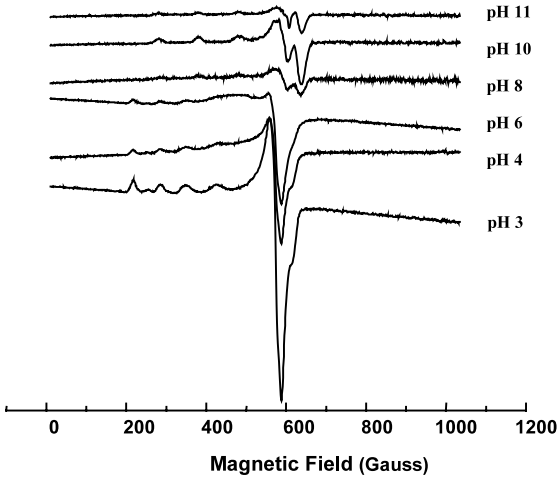

Fig. 4. EPR spectra of Cu(II):Asp:Gaa 1:1:1 in a 3–11 pH range. X-band EPR, 100 kHz, 10 mW, 77 K. Concentration: Cu(II), 1 mM.

Table 5
EPR parameters: $A_{||}$ (Cu (II), $g_{||}$ and $g_{\perp}$ for 1:1:1Cu(II): Asp:Gaa

pH	$A_{||}$ (G)	$g_{||}$	$g_{\perp}$
3	121	2.399	2.088
4	121	2.399	2.088
6	124	2.392	2.090
8	166/176		2.065
10	173	2.217	2.060
11	173	2.214	2.058

Table 6
Values of a_N for the 1:1:1 Cu(II):Asp:Gaa system

pH	Number of lines	a_N
8	5	12.794/13.721/12.979/12.979
10	5	11.182/11.182/11.182/10.844
11	5	14.501/13.785/13.785/13.427

coordinated to Cu(II). At pH 11, the EPR spectra presented a_N values very distinct from the previous ones, which could be explained by the presence of three different species with proximate values of percentage of formation: CuAspGaa, $Cu(Gaa)_2$ and $CuAspGaaH_{-1}$.

3.3. Solution equilibria of copper(II), guanidinoacetic acid and glycine

3.3.1. Potentiometric analyses

Similarly to the previous systems, the data used in the refinements of the constants were from 1:1:2 Cu:Gly:-Gaa titration data and the model of the species used includes all of those related in Table 7. Again the binary species (Table 7, species 1–12) were calculated separately under the same conditions of temperature and ionic strength and were fixed in the ternary model, after which the ternary species (Table 7, species 14–17) were actually refined. The great value of log β of CuH_2Gly-Gaa (30.46) suggested that both α-nitrogen atoms from Gaa and from Gly were protonated. However, the great difference observed in relation to the other systems is the smallest stability of the ternary complex CuGlyGaa compared with CuGluGaa and CuAspGaa and even to $Cu(Gaa)_2$. The hydrolyzed ternary complex $CuGlyGaaH_{-1}$ has begun to compete with CuGlyGaa at pH ≈ 8.5, above which has predominated until pH 11.

Table 7
Logarithms of the stability constants of binary and ternary complexes of copper(II), Gaa and Gly at 25 °C, $I = 0.1$ mol l^{-1} (KNO_3)

Number of species	Species	log β this work	log β	Reference
1	HGaa	10.65 (0.03)	10.91	[21]
2	H_2Gaa	13.47 (0.06)	13.81	[21]
3	CuGaa	7.69 (0.01)	7.69	[21]
4	$Cu(Gaa)_2$	15.20 (0.05)	15.20	[21]
5	CuH_2Gaa	20.96 (0.03)	20.96	[21]
6	$CuGaaH_{-1}$	−0.27 (0.09)	−0.27	[21]
7	$Cu_2(Gaa)_2H_{-2}$	3.77 (0.09)	3.77	[21]
8	HGly	9.68 (0.02)	9.60	[30]
9	H_2Gly	12.39 (0.01)	12.29	[30]
10	CuGly	8.27 (0.05)	8.11	[30]
11	$Cu(Gly)_2$	14.96 (0.03)	14.96	[30]
12	CuHGly	13.47 (0.02)		
13	$H_2GlyGaa$	23.43 (0.01)		
14	HGlyGaa	14.66 (0.02)		
15	CuGlyGaa	14.88 (0.01)		
16	$CuH_2GlyGaa$	30.46 (0.01)		
17	$CuGlyGaaH_{-1}$	6.44 (0.03)		

The standard deviations are given in parenthesis.

In the speciation as a function of pH of Cu:Gly:Gaa system (Fig. 5), the lack of stability of CuGlyGaa complex was confirmed. It can be seen that this species was less predominant than $Cu(Gaa)_2$ and presented almost in the same percentage of formation as $Cu(Gly)_2$. The protonated ternary complex $CuH_2GlyGaa$ was the major species until pH ≈ 7 above which $Cu(Gaa)_2$

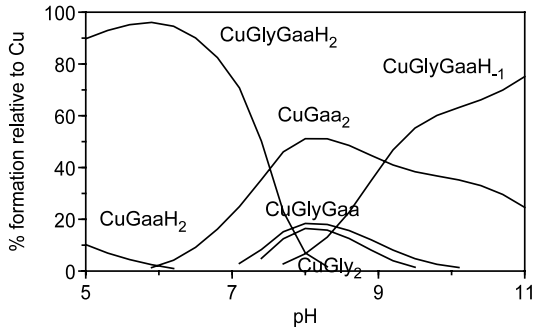

Fig. 5. Species distribution as a function of pH of 1:1:2 Cu:Gly:Gaa. Calculated from the stability constants listed in Table 7. Concentrations: Cu(II), 30 mM; Gly, 30 mM and Gaa, 60 mM.

became more predominant. The hydrolyzed complex $CuGlyGaaH_{-1}$ began to be formed at pH ≈ 7.5 and became predominant above pH 9. It should be noticed the greater percentage of formation of the hydrolyzed complex $CuGlyGaaH_{-1}$ in this system compared with the Cu(II):Asp:Gaa one.

3.3.2. Spectral analyses: absorption

In this case, the absorption spectra of Cu:Gly:Gaa 1:1:2 showed the smallest shift in the band related to d–d transition, from 775 to 637 nm in a 4.1–10.5 pH range and a little shift in the LCMT band, from 301 to 299 nm. The values of maxima wavelength and molar absortivity are shown in Table 8. For this system, this blue shift could be explained by the formation of binary N–O– bidentate complexes, which were predominant in relation to the less stable ternary ones.

3.4. Validation of the model of the species

The models of the species were validated by the titration simulation with the stability constants of all the species proposed, using Hyss program [28]. These titration curves are shown in Fig. 6 together with the correspondent experimental curves. It can be observed only a little difference between the simulated and experimental curve. It can be concluded that the models of the species used for the three systems should be regarded suitable even though in the presence of a complexity chemistry involving ligand–ligand interactions.

Table 8
Relation among pH, maximum wavelength and molar absortivity of each solution of Cu(II): Gly: Gaa, in a 1:1:2 ratio, with Cu(II) concentration varying from 9.25×10^{-4} to 9.01×10^{-4} mol l^{-1}, at 25 °C and $\mu = 0.1$ mol l^{-1} (KNO_3)

pH	Maximum wavelength (nm)	Molar absortivity (mol^{-1} cm^{-1} $l/10^{-2}$)
4.10	775	154
4.37	768	170
4.70	738	202
5.05	731	225
5.58	721	255
6.26	716	277
6.85	696	290
7.28	680	319
7.61	672	353
8.00	650	418
8.90	640	502
9.83	642	510
10.19	642	517
10.38	635	539
10.51	637	557

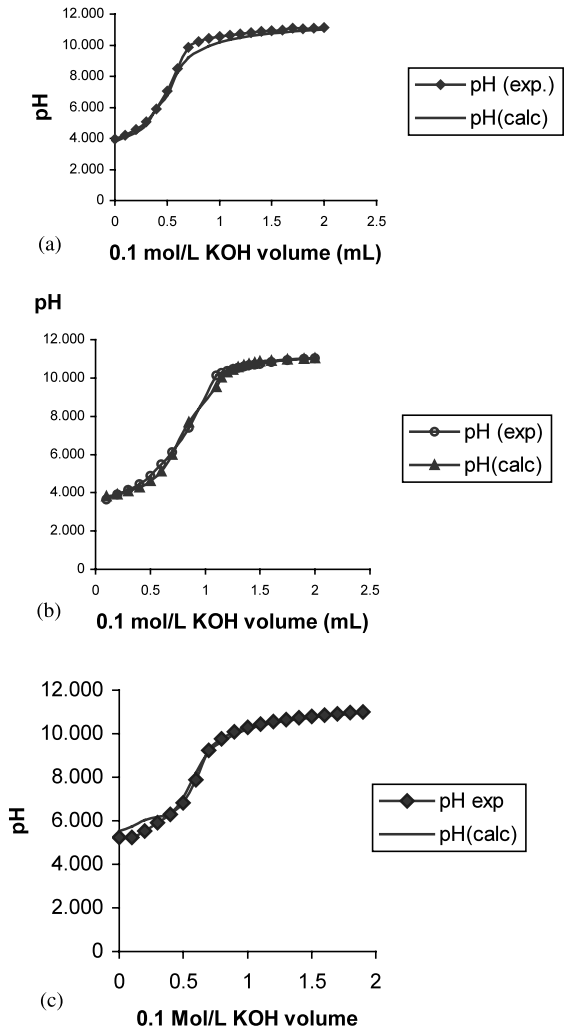

Fig. 6. Simulation of titration curves of (a) Cu(II):Glu:Gaa, (b) Cu(II):Asp:Gaa and (c) Cu(II):Gly:Gaa systems.

3.5. Evaluation of guanidino–carboxylate interactions

The presence of ligand–ligand interaction can be evaluated by $\Delta\log K$ (Eq. (6)) and the comparison between $\log \beta_{exp.}$ and $\log \beta_{stat.}$ Values of $\Delta\log K$ greater than zero indicates ligand–ligand interactions, thus, there are some other factors, besides those already expected, stabilizing this complex. The value of $\Delta\log K$ estimated for a Jahn–Teller distorted coordination sphere of Cu(II) to two bidentate ligands was approximately −0.9 [25,31]. From Table 9, it can be presumed that there were ligand–ligand interactions in CuGluGaa and CuAspGaa, which should involve the terminal nitrogen atoms of guanidino group from Gaa and the oxygen atoms of the non-coordinated-carboxylate group from Glu–Asp. Due probably to carbon chain size and steric effects, Asp could favor the occurrence of these interactions in a greater extent.

However, both ($\log \beta_{exp.} - \log \beta_{stat.}$) and $\Delta\log K$ have negative values for CuGlyGaa. This could be explained

Table 9
Evaluation of guanidino–carboxylate interactions: comparison between log β experimental and statistical and calculation of $\Delta\log K$

Species	log β exp.	log β stat.	log β exp $-$log β stat.	$\Delta\log K$ [a]
CuGluGaa	16.39	15.49	0.9	0.1
CuAspGaa	17.82	15.44	2.38	1.67
CuGlyGaa	14.88	15.37	−0.49	−1.08

[a] $\Delta\log K = \log \beta_{CuLGaa} - \log \beta_{CuGaa} - \log \beta_{CuL}$ (L = Glu, Asp or Gly).

by the non-existence of guanidino–carboxylate interactions for this species, easily understood by the presence of only one carboxylate group in Gly, which is already coordinated to Cu(II), preventing any interaction with the guanidino group from Gaa.

4. Conclusions

The guanidino carboxylate interactions have been proposed in two ternary systems: Cu:Glu:Gaa and Cu:Asp:Gaa. The order of the stability of the studied ternary complexes in solution was: CuAspGaa > CuGluGaa > CuGlyGaa. The formation of CuGluGaa and CuAspGaa was stabilized more than expected because of these interactions, which should probably be intramolecular involving the terminal nitrogen atoms of guanidino group from Gaa and oxygen atoms of the non-coordinated carboxylate group from Asp–Glu. These interactions were indicated by the potentiometric data with which we calculated the stability constants and $\Delta\log K$ ($\log \beta_{CuLGaa} - \log \beta_{CuGaa} - \log \beta_{CuL}$, L = Glu, Asp or Gly). These values were greater than zero in CuGluGaa and CuAspGaa, which indicates ligand–ligand interactions: the guanidino–carboxylate ones. In CuAspGaa complex these interactions should be stronger, probably because of its shorter alkyl chain that enables a greater proximity between the neighbor groups and a more stable four-membered chelate ring. We have also statistically estimated log β values and compared them with the experimental ones. Again, CuGluGaa and CuAspGaa experimental log β values exceed those statistical calculated ones. In addition, the electronic spectra had shown greater blue shifts for Cu(II):Asp:Gaa system. The EPR spectra of Cu(II):Asp:Gaa system agreed very well with its speciation diagram.

The systems involving Gly and Gaa have also been studied. As it was previously expected, there were not guanidino–carboxylate interactions in the complexes with Gly and Gaa because Gly has only one carboxylate group already involved in coordination towards copper (II). The stability of CuGlyGaa was less than its binary complex, $Cu(Gaa)_2$.

The speciation diagrams showed the predominance of ternary complexes at the main physiological pH (≈ 7.0) for the systems involving Glu and Asp, species CuHGluGaa and CuAspGaa, respectively.

Finally, it is important to emphasize the biological role of these interactions between the amino acids involved. Gaa is the precursor of creatine and play diverse functions in our organism. Some of these functions may involve these interactions. For example, the way Gaa interacts with guanidinomethyltransferase, the enzyme that catalyses the methylation of Gaa, may be explained through these interactions. The order that substrates bind to this enzyme is well established: *S*-adenosylmethionine is the first to be bound, followed by Gaa [35]. As guanidinomethyltransferase presents Glu and Asp as important residues of recognition to *S*-adenosylmethionine [36], this work may help in future studies on this subject, especially because a very recent proposed mechanism for Gaa binding site [37] contemplated a pair of hydrogen bonds between Asp and Gaa.

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