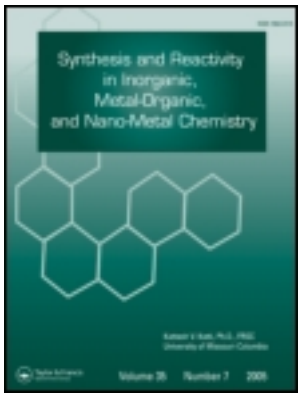

Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsrt19

STUDY OF NEW COMPLEXES OF CHROMIUM(III), COBALT(II), NICKEL(II), COPPER(II), AND ZINC(II) WITH GUANIDINOACETIC ACID, THE PRECURSOR OF CREATINE

Jussara Lopes de Miranda [a] & Judith Felcman [b]

[a] Department of Chemistry, Pontificia Universidade Cato´lica do Rio de Janeiro, Rua Marquês de São Vicente, 225, Gávea, Rio de Janeiro, 22453-900, Brazil

[b] Department of Chemistry, Pontificia Universidade Cato´lica do Rio de Janeiro, Rua Marquês de São Vicente, 225, Gávea, Rio de Janeiro, 22453-900, Brazil

Version of record first published: 15 Feb 2007.

To cite this article: Jussara Lopes de Miranda & Judith Felcman (2001): STUDY OF NEW COMPLEXES OF CHROMIUM(III), COBALT(II), NICKEL(II), COPPER(II), AND ZINC(II) WITH GUANIDINOACETIC ACID, THE PRECURSOR OF CREATINE, Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry, 31:5, 873-894

To link to this article: http://dx.doi.org/10.1081/SIM-100104857

SYNTH. REACT. INORG. MET.-ORG. CHEM., 31(5), 873–894 (2001)

STUDY OF NEW COMPLEXES OF CHROMIUM(III), COBALT(II), NICKEL(II), COPPER(II), AND ZINC(II) WITH GUANIDINOACETIC ACID, THE PRECURSOR OF CREATINE

Jussara Lopes de Miranda[a,b] and Judith Felcman[a,*]

[a]Pontificia Universidade Católica do Rio de Janeiro, Department of Chemistry, Rua Marquês de São Vicente, 225, Gávea, 22453-900, Rio de Janeiro, Brazil
[b]Department of Inorganic Chemistry, Universidade Federal do Rio de Janeiro, Brazil
E-mail: jussara@ufrj.br

ABSTRACT

Guanidinoacetic acid (H_2Gaa), the essential precursor of creatine, plays an important role in our organism. However, H_2Gaa interaction with metal ions has not yet been studied. We have previously studied H_2Gaa complexation with some metallic ions in solution. In this study, Cr(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes of H_2Gaa were synthesized and characterized (C, H, N, thermogravimetric analyses, infrared spectroscopy). Electron paramagnetic resonance and ultraviolet spectroscopy were also used but in aqueous

*Corresponding author. E-mail: felcman@rdc.puc-rio.br

solutions at 25 °C. In all of the cases the coordinating atoms were the alpha-nitrogen and oxygen atoms. The most probable geometry of each of the complexes is *cis*-square-planar in $Cu(HGaa)_2$, tetrahedral in $Zn(H_2Gaa)NO_3Cl$ and octahedral in $Cr(HGaa)_3$, $CoH_2Gaa(HGaa)_2$ and $NiH_2Gaa(HGaa)_2$.

INTRODUCTION

Guanidinoacetic acid belongs to the class of guanidino compounds which are characterized by the presence of a basic guanidino group, $HN{=}C(NH_2)$-NH-. This amino acid has two ionizable protons at the studied pH range and for this reason it will be represented as H_2Gaa in the neutral form, as $HGaa^-$ and Gaa^{-2} when it loses one and two protons, respectively. H_2Gaa has already been quantified in different mammalian tissues such as kidney, brain, liver and muscle[1], explaining the diversity of metabolic pathways that it takes part in[2–10]. H_2Gaa (Figure 1) is mainly synthesized in the kidney[11,12] as the result of the transamidination of glycine *via* arginine, catalyzed by glycineamidinotransferase[13,14]. Then it is transported to the liver, where it is methylated by S-adenosylmethionine, catalyzed by guanidinomethyltransferase, for further creatine production[15–18]. Therefore, H_2Gaa is involved in many important biological processes, such as renal metabolism[19–21], cholesterol production[22], thyroid dysfunction[23], creatine deficiency[24], epileptic seizures[25], hepatic encephalopathy[26] and insulin regulation[27]. Levels of the H_2Gaa urinary/creatine urinary ratio can be used as more sensitive and useful indicators of early stages of nephropathy[28]. H_2Gaa can induce severe convulsions after intracisternal administration in rabbit, suggesting that it is an endogenous convulsant[29]. Its neurotoxicity may be due to the generation of radicals, which affect the neurotransmitter system, and peroxidation of polyunsaturated fatty acids, which are important components of neuronal membranes[30]. An accumulation of H_2Gaa was reported in hepatic guanidinomethyltransferase deficiency, a newly recognized inborn error of creatine biosynthesis[31]. Also, it is known that the levels of H_2Gaa were increased in the cerebral cortex after hyperbaric oxygenation, probably due

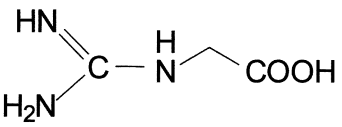

Figure 1. Structure of guanidinoacetic acid.

to a decrease in the arginase activity and as a consequence, it causes an increase of both Mn and Cu-Zn superoxide dismutase activities[32]. The complexation of H_2Gaa was previously studied in aqueous solution, in which the stability constants of several metal ion complexes were potentiometrically determined[33]. Here, the complexation of H_2Gaa was studied with the following metal ions: Cr(III), for its involvement in insulin regulation and participation in glucose tolerance factor (GTF)[34]; Co(II), for its presence in cobalamin (vitamin B_{12}), as a good methyl acceptor as H_2Gaa[35], Cu(II), for its role in superoxide dismutase[36]; Zn(II), for its participation in carboxypeptidase A[37] (where there is a guanidino-carboxylate interaction), in insulin metabolism[38] and in superoxide dismutase[36], and Ni(II), for its participation, with the urease enzyme, in the catalysis of urea hydrolysis in plants and bacteria[39].

RESULTS AND DISCUSSION

Synthesis

The formation of the complexes may be represented by the following equations:

$$M^{X+} + xH_2Gaa \rightarrow M(HGaa)_x + xH^+$$

M = Cr(III), Cu(II) and $x = 2$ for Cu(II) and 3 for Cr(III).

$$M^{z+} + 3\,H_2Gaa \rightarrow MH_2Gaa(HGaa)_2 + 2H^+$$

M = Co(II) and Ni(II)

$$M^{z+} + H_2Gaa + NO_3^- + Cl^- \rightarrow MH_2GaaNO_3Cl$$

M = Zn(II).

Table I shows the elemental analyses, formula weights, colors, yields and decomposition temperatures of the complexes.

Thermogravimetric Analyses

The decomposition temperatures determined for all complexes are listed in Table I. The less stable complexes were those with one or two moles of the ligand, which were $Zn(H_2Gaa)NO_3Cl$ and $Cu(HGaa)_2$, while the others with three moles of guanidinoacetic acid (Ni(II), Co(II) and Cr(III)) began to decompose only at higher temperatures.

Thermogravimetric analyses were done for the complexes of Cr(III), Ni(II), Cu(II) and Zn(II), which decomposed in one stage. The presence of

Table I. Elemental Analyses, Formula Weights (F.W.), Colors, Yields, and Decomposition Temperature (D.T.) of the Complexes

	Calc. (Found)						
Compound	C	H	N	F.W.	Color	Yield %	D.T. °C
$Cr(HGaa)_3$	27.00	4.50	31.50	400	Violet	60	235
$C_9H_{18}N_9O_6Cr$	(27.06)	(4.46)	(31.51)				
$CoH_2Gaa(HGaa)_2$	26.48	4.66	30.89	407.9	Pink	56	258
$C_9H_{19}N_9O_6Co$	(26.55)	(4.60)	(30.73)				
$NiH_2Gaa(HGaa)_2$	26.49	4.66	30.90	407.7	Green	70	277
$C_9H_{19}N_9O_6Ni$	(26.45)	(4.58)	(30.75)				
$Cu(HGaa)_2$	24.36	4.06	28.43	295.5	Blue	63	215
$C_6H_{12}N_6O_4Cu$	(24.46)	(4.07)	(28.37)				
$Zn(H_2Gaa)NO_3Cl$	12.86	2.50	20.01	279.9	White	50	200
$C_3H_7ClN_4O_5Zn$	(12.83)	(2.54)	(20.03)				

water was not observed, neither of crystallization, which is usually lost in the 70–110 °C-temperature range, nor of coordination, which is lost at higher temperatures of 200–250 °C. The weight loss of the complexes was in the order of 20–30% on heating to 310–375 °C, which could correspond to the CN_2H_4 guanidium residue. After heating to 600 °C the residue weights were 40–50% of the original value.

Infrared Analyses

Table II shows the important infrared spectral data of the ligand H_2Gaa and its complexes. The characteristic bands observed in the H_2Gaa spectrum were the following: those relative to ν_s and ν_{as} of primary amines in the region of 3000 to 3200 cm^{-1}, the ν(N-H) of the guanidino group at 3386 cm^{-1}, the ν(C=N) of the guanidino group at 1670–1672 cm^{-1} and ν_{as} and ν_s of the carboxylate group at 1584 and 1411 cm^{-1}, respectively. All of these data are in good agreement with literature[40] values. It was observed that some of the ν(N-H) bands shifted to higher wave numbers in the spectra of the complexes indicating that not all of the nitrogen atoms present in H_2Gaa have coordinated to the metal ion. These nitrogen atoms could possibly be involved in hydrogen bonds and most probably be those of the guanidino group which have the highest pK_a[33]. In addition, there were bands that could be attributed to metal-oxygen and metal-nitrogen bonds in all of the complexes. These

Table II. Characteristic Infrared Bands of H_2Gaa and Its Complexes (Wave Numbers in cm^{-1})

Compound	ν(N-H)	ν(C=N) Guanidino	ν_a C=O	ν_s C-O	ν M-N	ν M-O
H_2Gaa	3386 vs 3303 vs 3174 vs	1672 s 1668 s	1584 vs	1411 s	–	–
$Cr(HGaa)_3$	3386 vs 3286 vs 3180 vs	1671 s	1583 vs	1412 s	507 w	319 w
$CoH_2Gaa(HGaa)_2$	3384 vs 3293 vs 3173 vs	1671 s	1579 vs	1410 s	360 w	254 w
$NiH_2Gaa(HGaa)_2$	3386 vs 3300 vs 3173 vs	1671 s	1583 vs	1410 s	346 w	254 w
$Cu(HGaa)_2$	3394 vs 3386 vs 3336 vs 3229 vs	1669 s	1566 vs	1405 s	446 m 410 m	351m 328 m
$Zn(H_2Gaa)NO_3Cl$	3386 vs 3293 vs 3172 vs	1669 s	1581 vs	1412 s	445 w	345 w

vs = very strong; s = strong, m = medium and w = weak.

observations lead to the assumption that the coordination site should be the nitrogen of the α-amino group and the oxygen of the carboxylate group in all complexes. This is reinforced by the following facts: 1) the ν(C=N) band of the guanidino group has not shifted considerably, 2) C=O stretching bands have been shifted for all complexes and appear in the range of coordinated carboxylates[41] and 3) the metal-oxygen and metal-nitrogen stretching modes appeared in a region close to that characteristic of amino acid complexes with α-N and O coordination, such as glycine[41], methylglycine and phenylglycine[42] and alanine[41]. Therefore, H_2Gaa behaves as a bidentate ligand with all of the metal ions studied. This coordination was previously proposed for H_2Gaa complex formation in solution at 25 °C and $\mu = 0.1$ mol/L (KNO_3)[33] which was studied potentiometrically. Metal-nitrogen stretching modes increased in the order $Cr(III) > Cu(II) > Zn(II) > Ni(II) \cong Co(II)$, which is consistent with the stability constants of their similar aqueous complexes with one mole of the ligand coordinated. The values of the formation constants of these aqueous complexes were: $CuGaa(H_2O)_2$, $\log \beta_{ML} = 7.69 \pm 0.01$; $ZnGaa(H_2O)_2$, $\log \beta_{ML} = 7.38 \pm 0.01$; $NiGaa(H_2O)_4$, $\log \beta_{ML} = 6.01 \pm 0.01$ and $CoGaa(H_2O)_4$, $\log \beta_{ML} = 5.59 \pm 0.01$[33]. Although the formation constant of the chromium complex has not yet been determined, it can be presumed to be greater than that of $[CuGaa(H_2O)_2]^+$, due to the greater nuclear charge of the former metal ion.

The infrared spectra of the solid complexes showed similar coordination behaviour for H_2Gaa. In the complexes below, H_2Gaa corresponds to the neutral form, but with the deprotonated carboxylate group and protonated guanidino group, while HGaa represents the guanidinoacetate ion, when the carboxylate proton has been lost.

In the infrared spectrum of the $Cu(HGaa)_2$ complex, two metal-nitrogen and two metal-oxygen stretching bands were observed, which is typical of the *cis*-square-planar isomer, while the *trans*-isomer shows only one band for each of these modes[41].

Considering the infrared spectrum of the tetrahedral $Zn(H_2Gaa)NO_3Cl$, there are two possibilities for the coordination relative to nitrate and chloride ions. Either both nitrate and chloride ions are coordinated to the metal ion, assuming that nitrate behaves as an unidentate ligand, or, only nitrate is coordinated in a bidentate mode. The observed band at 1450 cm^{-1} [43] is ascribed to nitrogen-oxygen while the band at 287 cm^{-1} [44] to metal-chloride.

The $CoH_2Gaa(HGaa)_2$, $Cr(HGaa)_3$ and $NiH_2Gaa(HGaa)_2$ spectra present two bands corresponding to metal-oxygen and metal-nitrogen stretching modes (Table II). These complexes should be octahedral; fac or mer isomers are possible.

Electronic Spectra

The electronic spectral study of the complexes has been done in aqueous solutions with the metal ions which present d-d transition in the visible spectrum, that is, Co(II), Ni(II) and Cu(II), in different metal:ligand ratios. Although the complexes formed in aqueous solution were different (with H_2O molecules coordinated) from those in the solid phase, interesting comparisons on H_2Gaa coordination were possible to be done.

Co(II) Complexes

1:1 and 2:1 H_2Gaa:Co(II) solutions were studied in the pH range 6–10. In all spectra (Figures 2 and 3) at least two bands were observed in addition to the LMCT (ligand to metal charge transfer) band. These bands were shifted to lower wave lengths as the pH was increased. This can be attributed to changes in coordination with only oxygen (carboxylate groups and water molecules) to coordination with both nitrogen (amino groups)

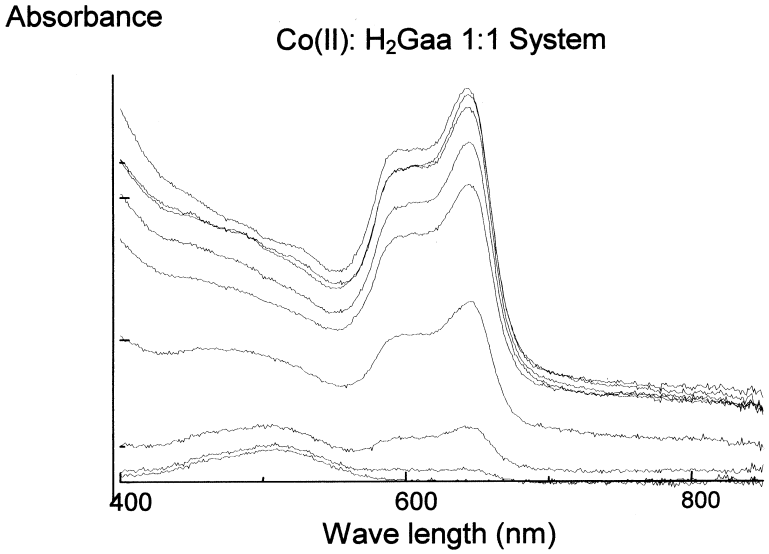

Figure 2. Electronic Spectra of Co(II):H_2Gaa 1:1 System. The range concentration of Co(II) solution was 8.33×10^{-4} mol/L to 8.18×10^{-4} mol/L. Aliquots of 0.1 mol/L KOH were added before the spectra were recorded. From bottom to top p[H] values were: 6.0, 8.6, 8.8, 8.9, 9.1, 9.3, 9.5, 9.9 and 10.2.

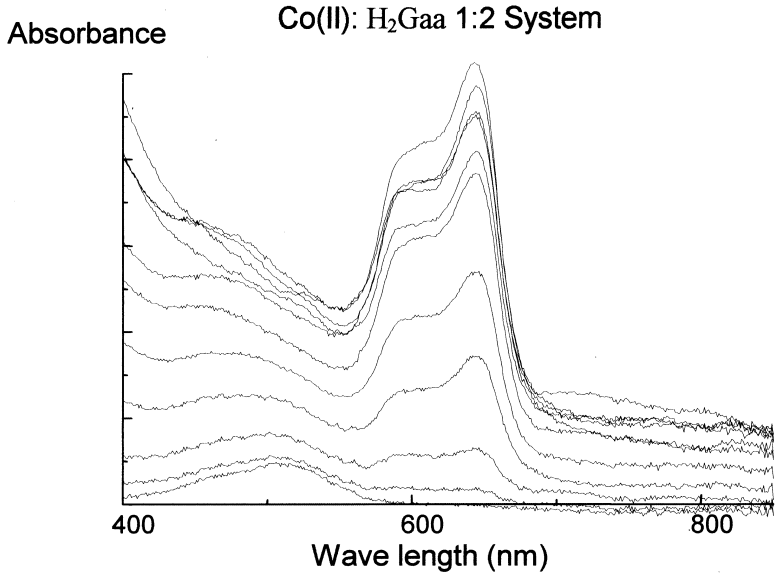

Figure 3. Electronic Spectra of Co(II):H_2Gaa 1:2 System. The concentration range of Co(II) solution was 8.33×10^{-4} mol/L to 8.18×10^{-4} mol/L. Aliquots of 0.1 mol/L KOH were added before the spectra were recorded. From bottom to top p[H] values were: 5.9, 8.7, 8.8, 8.9, 9.1, 9.2, 9.4, 9.7, 10.0, 10.3 and 10.4.

and oxygen (carboxylate groups or possibly hydroxide). The asymmetric bands with maxima at 478–508 nm in 1:1 complexes and 473–506 nm in 2:1 systems correspond to the $^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$ transition[45]. Another band, which only becomes visible above pH 8.5, occurs at 640–643 nm in 1:1 complexes and 642–644 nm in 2:1 systems, is due to the $^4A_{2g}(F) \leftarrow {}^4T_{1g}(F)$ transition[45]. The spectra analyzed were characteristic of hexa-coordinated octahedral or pseudo-octahedral Co(II) nitrogen and oxygen coordinated complexes.

Ni(II) Complexes

The 1:1 H_2Gaa:Ni (II) solution spectra were analyzed in the pH range 6–10.5 (Figs. 4 and 5). Besides the LMCT band with a maximum at 301–302 nm, there were two other bands: one that shifts from 396 to 382 nm relative to the $^3T_{1g}(P) \leftarrow {}^3A_{2g}$ (F) transition and the other, occurring at 650–800 nm, which corresponds to the $^3T_{1g}(F) \leftarrow {}^3A_{2g}$ (F) and to the spin-prohibited $^1E_g(D) \leftarrow {}^3A_{2g}(F)$ transitions[46]. In the 2:1 H_2Gaa:Ni(II)

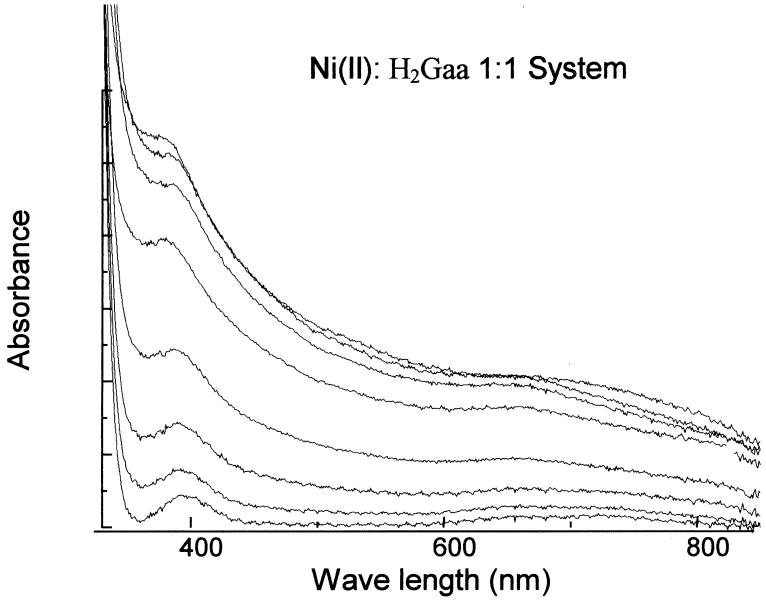

Figure 4. Electronic Spectra of Ni(II):H_2Gaa 1:1 System. The concentration range of Ni(II) solution was 8.33×10^{-4} mol/L to 8.16×10^{-4} mol/L. Aliquots of 0.1 mol/L KOH were added before the spectra were recorded. From bottom to top p[H] values were: 6.1, 8.6, 8.8, 8.9, 9.2, 9.4, 9.9 and 10.3.

solution spectra, the first band shifts from 400 to 378 nm and the second, from 716 to 638 nm. Therefore, pseudo-octahedral structures are attributed to the complexes $Ni(Gaa)(H_2O)_4$ and $[Ni(Gaa)_2(H_2O)_2]^{-2}$ considering their electronic spectra in the ranges pH = 6–8 and 8–9.5, which were, respectively, the pH region where these two species exist (Figure 6).

Cu(II) Complexes

The electronic spectra of the solutions of the Cu:H_2Gaa system were studied in the 6 to 7.4 pH range (Figure 7). It was observed the LMCT band at 301 to 209 nm and a broad band which has shifted from 720 to 666 nm that may be due to d-d transitions: from d_{xy}, d_z^2 orbitals and the pair of orbitals d_{xy}, d_{yz} to the half-filled anti-ligand $d_{x^2-y^2}$ orbital. The wave length shift to lower values indicated coordination with nitrogen together with the oxygen atoms from carboxylate group[47].

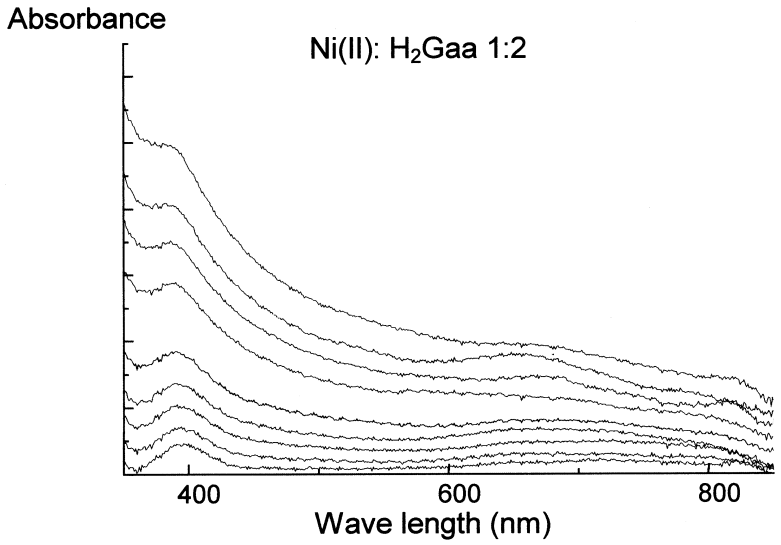

Figure 5. Electronic Spectra of Ni(II):H_2Gaa 1:2 System. The concentration range of Co(II) solution was 8.33×10^{-4} mol/L to 8.16×10^{-4} mol/L. Aliquots of 0.1 mol/L KOH were added before the spectra were recorded. From bottom to top p[H] values were: 6.0, 8.6, 8.8, 8.9, 9.0, 9.1, 9.3 and 9.5.

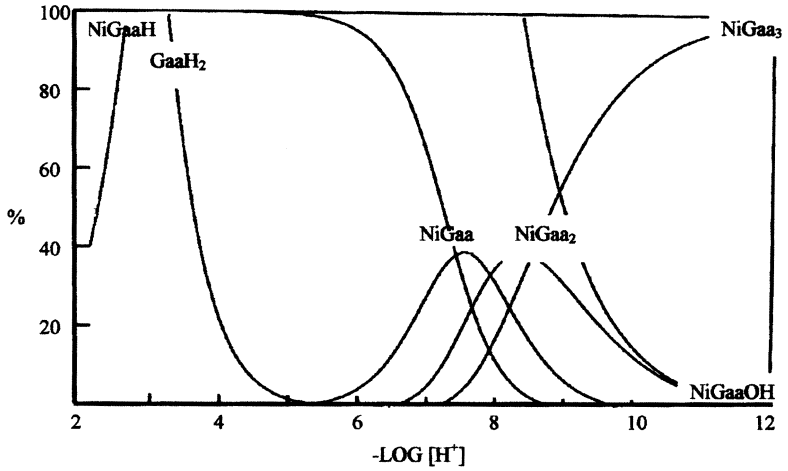

Figure 6. Speciation as a function of pH for Ni(II):H_2Gaa 1:2 system. This speciation was done by using SPE program with the potentiometric data refined by SuperQuad program. Charges were omitted for simplicity.

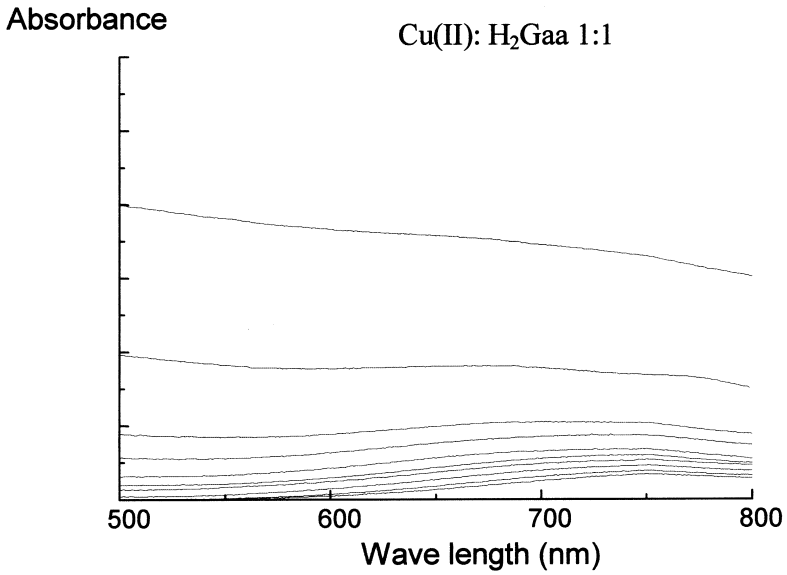

Figure 7. Electronic Spectra of Cu(II):H_2Gaa 1:1 System. The concentration range of Cu(II) solution was 8.33×10^{-4} mol/L to 8.16×10^{-4} mol/L. Aliquots of 0.1 mol/L KOH were added before the spectra were recorded. From bottom to top p[H] values were: 6.0, 6.23, 6.35, 6.54, 6.69, 6.78, 6.86, 6.94, 7.09 and 7.37.

EPR Analysis

According to EPR theory, in copper complexes there is a correlation between the g values and the energy of the d-d transitions, as well as the covalency of the metal-ligand bond and the symmetry of the complex[48,49]. In our study, 1:1 ligand:metal ratio systems were analyzed at different values of pH, at 4.6, 5, 6, 7, 9, 10 and 11 (Fig. 8). The symmetry of all the cómplexes analyzed is square-planar which is strongly indicated by the relation $g_{//} > g_{\perp} > 2$[48]. An increase in the values of $A_{//}$ and a decrease in the values of $g_{//}$ and $g_{\perp}$, with increasing pH has been observed (Table III). This behavior can be explained as covalent interactions with the equatorial ligands in a square-planar complex, suggesting a gradual substitution of only the oxygen-coordinated species by oxygen-nitrogen coordinated systems. The spectra at pH 4.6, 5 and 6 (Figure 8) show the presence of complexes in which the Cu(II) ion is coordinated to oxygen atoms from carboxylate groups and from water. In Figure 9 it can be seen that the predominant species below pH 6 is $[CuH_2Gaa]^{2+}$, which is in agreement

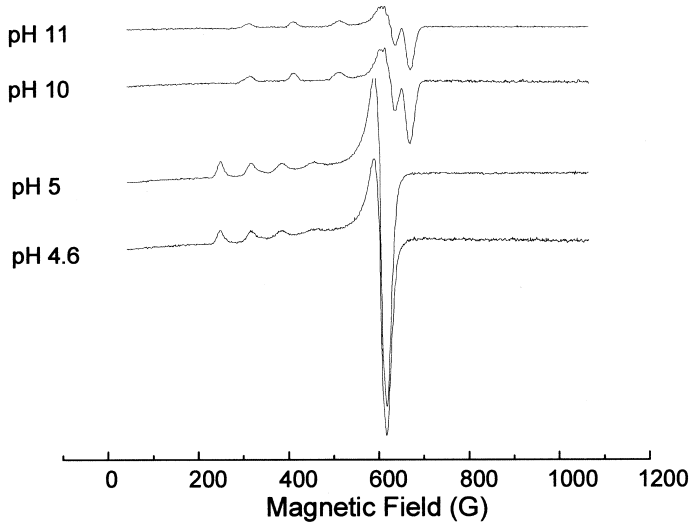

Figure 8. Electronic Paramagnetic Resonance Spectra for Cu(II):H_2Gaa 1:1 System. Solutions with different values of pH were recorded after adding aliquots of KOH solution. The initial concentration of Cu(II) solution was 1 mmol/L. EPR conditions: microwave frequencty at 9.46 GHz, microwave power, 10 mW, temperature, 77 K and 100 kHz field modulation.

with the EPR data[50]. At pH 6.7 and 7 the spectra present six lines for Cu(II) instead of four, indicating the presence of more than one species. Figure 9 also shows that there are three species, CuGaa, $[CuGaaOH]^-$ and $[Cu_2(Gaa)_2(OH)_2]^{-2}$ in this pH range. At pH 9 (Figure 10) the EPR

Table III. EPR Parameters: $A_{//}$ (Cu(II), $g_{//}$ and $g_\perp$ for the 1:1 Cu(II): H_2Gaa System

pH	$A_{//}$(G)	$g_{//}$	$g_\perp$
4.6	121	2.402	2.088
5.0	124	2.402	2.088
6.0	121	2.399	2.088
6.7	153.7/173.3	—	2.074
7.0	156.9/179.9	—	2.074
9.0	173.3	2.222	2.063
10.0	173.3	2.217	2.059
11.0	173.3	2.217	2.059

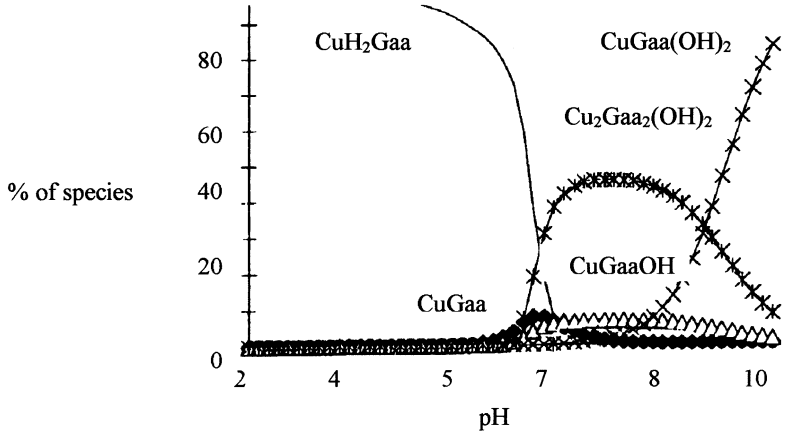

Figure 9. Speciation as a function of pH for Cu(II):H_2Gaa 1:1 system. This speciation was done by using SPE program with the potentiometric data refined by SuperQuad program. Charges were omitted for simplicity.

spectrum is in agreement with the coordination of one nitrogen atom (three hyperfine lines), which is the α-nitrogen of Gaa.

Conclusions

It is concluded here that H_2Gaa behaves as a bidentate ligand, with the α-nitrogen and oxygen carboxylate atoms coordinating to the metal ions Cr(III), Co(II), Ni(II), Cu(II) and Zn(II). The experimental data suggest that the geometry of the synthesized compounds is *cis*-square-planar for $Cu(HGaa)_2$, tetrahedral for $Zn(H_2Gaa)NO_3Cl$ and octahedral for $Cr(HGaa)_3$, $CoH_2Gaa(HGaa)_2$ and $NiH_2Gaa\ (HGaa)_2$ as shown in Fig. 11. There was good agreement between the infrared data of the solid complexes and the electronic and EPR data of the complexes in solution.

This kind of coordination of H_2Gaa leaves the guanidino group free for possible interactions with other biologically important molecules. This explains one of the reasons for the methylation of the α-nitrogen in creatine formation. This study of H_2Gaa complexation can help us to understand the variety of its functions in our body, especially in relation to its interactions with metal ions, which has been rarely studied before. It has also shown the preference that H_2Gaa has for metal ions with greater nuclear charge, a behavior that was expected.

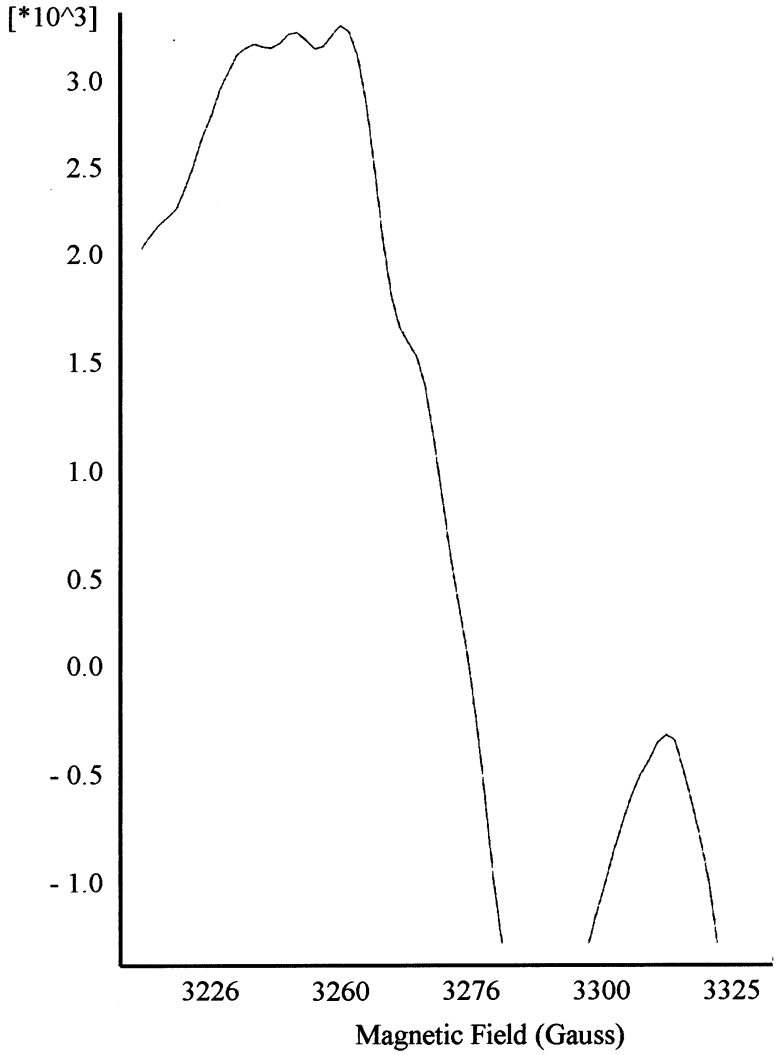

Figure 10. Electronic Paramagnetic Spectra of 1:1 Cu(II):H_2Gaa system at pH = 9.0, showing the nitrogen lines. The pH 9.0 was recorded after adding aliquots of KOH solution. The initial concentration of Cu(II) solution was 1 mmol/L. EPR conditions: microwave frequency at 9.46 GHz, microwave power, 10 mW, temperature, 77 K and 100 kHz field modulation.

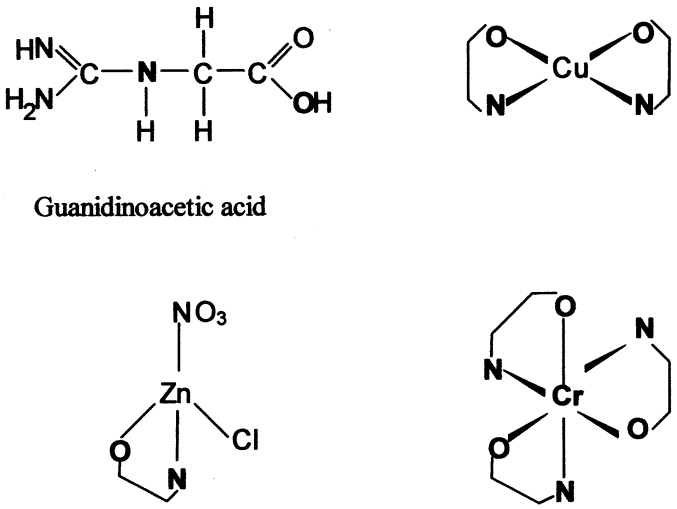

Figure 11. Guanidinoacetic Acid Structure (with bolded donor atoms) and the Suggested Structures of the Cu(II), Zn(II) and Cr(III) Complexes (For M = Co(II) and Ni(II), one ligand is protonated in the guanidino group, but the structure is similar to that of Cr(III) complex).

EXPERIMENTAL

General

All chemicals used, the metal salts $CrCl_3 \cdot 6H_2O$, $CoCl_2 \cdot 7H_2O$, $NiCl_2 \cdot 6H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, $ZnCl_2$ (all from Merck), guanidinoacetic acid (Aldrich), KOH, HNO_3, ethanol, methanol and acetone (all from Merck) were of analytical grade and were used without further purification. Elemental analyses of the complexes were performed by a CHNS/O Carlo Erba EA 1110 analyzer. Thermogravimetric analyses were done in a Perkin-Elmer TGA 7 thermogravimetric balance, using nitrogen flux and a 25 to 600 °C temperature range. Infrared analyses were done using KBr pellets for the 4000–370 cm^{-1} range and polyethylene pellets for the 700–30 cm^{-1} range for all complexes in a Perkin-Elmer FT-IR 2000 spectrometer.

Spectrophotometric Analyses

The Co(II), Ni(II) and Cu(II) H_2Gaa complexes were spectrophotometrically studied in solution at 25 °C. These were prepared in the

same way that was done previously for the potentiometry[33]; 1:1 and 2:1 ligand:metal ratio solutions were prepared and analyzed at a wide range of pH values, adjusted by adding small increments of 0.1 mol/L aqueous KOH solution. The ionic strength was kept constant at 0.1 using 1.2 mol/L aqueous KNO_3 solution. A double-beam Perkin-Elmer Lambda 19 spectrophotometer was used with a 1 cm path length and water as background.

EPR Analyses

Aqueous solutions of $CuSO_4$ (1×10^{-3} mol/L) and H_2Gaa (1×10^{-3} mol/L) 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 spectrometer at 77 K (liquid nitrogen). Ethylene glycol was added to all solutions to ensure good glass formation.

Preparation of the Complexes

The complexation of H_2Gaa with the mentioned ions were previously studied in aqueous solution (25 °C and $\mu = 0.1$ (KNO_3)), using the potentiometric method[33]. The syntheses of H_2Gaa binary complexes followed a general procedure. First, H_2Gaa was dissolved in distilled water and acidified with nitric acid or hydrochloric acid. Metal ion salt solutions were added with stirring and the temperature was kept constant, not above 60 °C. The ligand/metal ratio was chosen based on the maximum coordination number of each ion studied in solution. Cu(II) ion formed only ML_2 species and thus a 2:1 ligand/metal ratio was used for the synthesis of its complex. For the other ions, a different ratio was used: 3:1 for Cr(III), Co(II) and Ni(II); as Zn(II) formed a ML_2 complex in solution, a 2:1 ratio was first tried for the Zn(II) ion. However, only a 1:1 complex was precipitated. Higher ratios were also used for the Zn(II) ion. However, even at a 10:1 ligand/metal ratio only the 1:1 complex was synthesized.

After 1 hour of stirring H_2Gaa and the metal salt solution, KOH 0.1 mol/L was added very slowly, with stirring and the temperature controlled up to 60 °C, until a color change was observed. The resulting solution was stirred for another 2 or 3 hours, concentrated to half of the initial volume and left in the hood for 24 hours. The formed precipitates were then washed three or four times with absolute ethanol, acetone and distilled water, respectively. Recrystalization was not successful due to the very poor solubility of these complexes in the majority of inorganic and organic solvents.

Copper(II) Complex

An H_2Gaa (10 mmols, 1.1711 g) aqueous solution was prepared and acidified with 0.1 mol/L nitric acid (6 mL) until the pH was 3.5. A solution of $Cu(NO)_3 \cdot 3H_2O$ (5 mmols, 1.2308 g) was added with stirring (1 hour) at 60 °C. 0.1 mol/L KOH was slowly added with stirring at 60 °C during 2 hours until a color change was observed. The resulting solution was concentrated to half of this volume and left in the hood with absolute ethanol (1–2 mL) for 24 hours. A blue precipitate was formed and three times washed with absolute ethanol and distilled water, respectively.

Zinc(II) Complex

An H_2Gaa (10 mmols, 1.1711 g) aqueous solution was prepared and acidified with 0.1 mol/L nitric acid (3 mL) until complete dissolution. A solution of $ZnCl_2$ (5 mmols, 0.6820 g) was added with stirring (1 hour) at 60 °C. 0.1 mol/L KOH (9 mL) was slowly added with stirring at 60 °C during 2 hours until it was slightly turbid. The resulting solution was concentrated to half of this volume and left in the hood with absolute ethanol (1–2 mL) for 24 hours. A white precipitate was then formed which was washed three times with absolute ethanol and distilled water, respectively.

Cobalt(II) and Nickel(II) Complexes

An H_2Gaa (15 mmols, 1.7610 g) aqueous solution was prepared and acidified with 0.1 mol/L nitric acid (6 mL) until complete dissolution. A solution of $CoCl_2 \cdot 7H_2O$ (5 mmols, 1.2805 g) or $NiCl_2 \cdot 6H_2O$ (5 milimols, 1.1900 g) was added with stirring (1 hour) at 60 °C. 0.1 mol/L KOH was very slowly added with stirring at 60 °C during 6 hours until a color change was observed. The resulting solution was concentrated to half of this volume and left in the hood with absolute ethanol (1–2 mL) for 24 hours. A precipitate was then formed which was washed three times with absolute ethanol and distilled water, respectively. The Co(II) precipitate was pink and the Ni(II) one was green.

Chromium(III) Complex

An H_2Gaa (15 mmols, 1.7610 g) aqueous solution was prepared and acidified with 0.1 mol/L nitric acid (6 mL) until complete dissolution.

A solution of $CrCl_3 \cdot 6H_2O$ (5 mmols, 1.3325 g) was added with stirring (1 hour) at 60 °C. 0.1 mol/L KOH was very slowly added with stirring at 60 °C during 7 hours until a color change was observed. The resulting solution was concentrated to half of this volume and left in the hood with absolute ethanol (1–2 mL) for 48 hours. A violet precipitate was then formed and washed three times with absolute ethanol and distilled water, respectively.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Dr. Douglas Wagner Franco and Mario Sergio Schultz of the Chemistry Department, Universidade de São Paulo (USP), São Carlos, Brazil, for measuring the X-band EPR spectra (FAPESP process n° 92/3515-3).

REFERENCES

1. Levillain, O.; Marescau, B.; De Deyn, P.P. Renal Handling of Guanidino Compounds in Rat and Rabbit. J. Physiol. **1997**, *499* (2), 561–570.
2. Boeckxstaens, G.E.; Pelckmans, P.A.; Bult, H.; De Man, J.G.; Herman, A.G.; Van Maercke, Y.M. The Arginine-Nitric Oxide Pathway Mediates Non-adrenergic Non-cholinergic Neurotransmission in Gastrointestinal Tissue. In *Guanidino Compounds in Biology and Medicine*; De Deyn, P.P., Marescau, B., Stalon, V., Qureshi, I.A., Eds.; John Libbey & Company Ltd.: London, 1992; 89–96.
3. Thomas, G.; Heim, K.F.; Ramwell, P.W. Effect of Guanidino Compounds on Endothelium-dependent Relaxation and Superoxide Production. In *Guanidino Compounds in Biology and Medicine*; De Deyn, P.P.; Marescau, B., Stalon, V., Qureshi, I.A., Eds.; John Libbey & Company Ltd.: London, 1992; 103–108.
4. Yasuda, T.; Fukasawa, M.; Ohminato, M.; Maeba, T.; Ozawa, S.; Ishida, M. The Effect of Guanidino Compounds on the Membrane Fluidity of the Cultured Rat Mesangial Cell. In *Guanidino Compounds in Biology and Medicine*; De Deyn, P.P., Marescau, B., Stalon, V., Qureshi, I.A., Eds.; John Libbey & Company Ltd.: London, 1992; 293–299.
5. Shimizu, Y.; Morimoto, K.; Edamatsu, R.; Otsuki, S.; Mori, A. Changes of Guanidino Compounds in the Hippocampal-kindled Rat Brain. Jpn. J. Psych. Neur. **1993**, *47* (2), 390–391.

6. Anfinsen, C.B. Principles that Govern Folding of Protein Chains. Science **1973**, *181*, 223–230.
7. Kellogg, M.S.; Klade C.A.; Madigan, D.; Mazul, R.H.; Muller, G.W. Tetrazoles as Carboxylic-acid Surrogates-high-potency Sweetners. Ac. Symp. Ser. **1991**, *450*, 100–112.
8. Meglasson, M.D.; Wilson, J.M.; Yu, J.H.; Robinson, D.D.; Wysee, B.M.; Souza, C.J. Antihyperglycemic Action of Guanidino Alkanoic Acids-3-Guanidinopropionic Acid Ameliorates Hyperglycemic in Diabetic KKA(Y) and C57BL6JOB/OB Mice and Increases Glucose Disappearance in *Rhesus* Monkeys. J. Pharmacol. Exp. Ther. **1993**, *2661* (3), 1454–1462.
9. Sorenson, R.L.; Stout, L.E; Brelje, T.C.; Van-Pilsum, J.R.; Mcguire, D.M. Evidence for the Role of Pancreatic Acinar Cells in the Production of Ornithine and Guanidinoacetic Acid by L-Arginine: Glycine Amidinotransferase. Pancreas **1995**, *10* (4), 389–394.
10. Natelson, S. Biosynthesis of Guanidino Compounds in Health and Disease: The Guanidine Cycle. In *Guanidino Compounds in Biology and Medicine: 2*, De Deyn; P.P., Marescau, B., Qureshi, I.A., Mori, A. Eds.; John Libbey & Company Ltd.: London, 1997; 217–241.
11. Van Pilsum, J.F.; Martin, R.P.; Kito, E. Determination of Creatine, Creatinine, Arginine, Guanidinoacetic Acid, Guanidine and Methylguanidine in Biological Fluids. J. Biol. Chem. **1956**, *222* (1), 225–236.
12. Mcguire, D.M.; Gross, M.D.; Van Pilsum, J.F.; Towle, H.C. Repression of Rat-Kidney-L-Arginine-Glycine-Amidinotransferase Synthesis by Creatine at Pretranslational Level. J. Biol. Chem. **1984**, *259* (19), 2034–2038.
13. Takeda, M.; Koide, H.; Jung, K.Y.; Endou, H. Intranephron Distribution of Glycine-Amidinotransferase Activity in Rats. Ren. Physiol. Bioch. **1992**, *15* (3-4), 113–118.
14. Takeda, M.; Kiyatake, I.; Koide, H.; Jung, K.Y.; Endou, H. Biosynthesis of Guanidinoacetic Acid in Isolated Renal Tubules. Eur. J. Clin. Chem. Clin. Biochem. **1992**, *30* (6), 325–331.
15. Cantoni, G.L.; Vignos, P.J. Jr. Enzymatic Mechanism of Creatine Synthesis. J. Biol. Chem. **1954**, *209*, 647–659.
16. Hamahata, A.; Takata, Y.; Gomi, T.; Fujioka, M. Biochem. Probing the S-Adenosylmethionine-Binding Site of Rat Guanidinoacetate Methyltransferase Effect of Site-directed Mutagenesis of Residues that Are Conserved Across Mammalian Non-nucleic Acid Methyltransferases. Biochem. J. **1996**, *317*, 141–145.
17. Takata, Y.; Fujioka, M. Identification of Tyrosine Residue in Rat Guanidinoacetate Methyltransferase that is Photolabeled with S-Adenosyl-L-Methionine. Biochem. **1992**, *31* (17), 4369–4374.

18. Im, Y.S.; Chiang, P.K.; Cantoni, G.L. Guanidinoacetate Methyltransferase Purification and Molecular Properties. J. Biol. Chem. **1979**, *21*, 11047–11050.
19. Koller, A.; Gomes, T.D.; Natelson, S. Evidence Supporting a Proposed Mechanism Explaining Inverse Relationship Between Guanidinoacetate and Guanidinosuccinate in Human Urine. Clin. Chem. **1975**, *21*, 235–242.
20. Sasaki, M.; Takahara, K.; Natelson, S. Urinary Guanidinoacetate Succinate Ratio-Indicator of Kidney Dysfunction. Clin. Chem. **1973**, *19*, 315–321.
21. Kuroda, M. Study On Impaired Metabolism of Guanidinoacetic Acid in Chronic Renal Failure Rabbits with Special Reference to Impaired Conversion of Arginine to Guanidinoacetic Acid. Nephron. **1993**, *65* (4), 605–611.
22. Sugiyama, K.; Ohishi, A.; Syiu, H.; Takeuchi, H. Effects of Methylgroup Acceptors On the Regulation of Plasma-Cholesterol Level in Rats Fed High Cholesterol Diets. J. Nutr. Sci. Vitamin. **1989**, *35* (6), 613–626.
23. Verhelst, J.; Berwaerts, J.; Marescau, B.; Abs, R.; Neels, H.; Mahler, C. Serum Creatine, Creatinine, and Other Guanidino Compounds in Patients with Thyroid Dysfunction. Metabolism: Clin. & Exper. **1997**, *46* (9), 1063–1067.
24. Ilas, J.; Muhl, A.; Stockler-Ipsiroglu, S. Guanidinoacetate Methyltransferase Deficiency: Non-invasive Enzymatic Diagnosis of a Methyl Recognized Inborn Error of Metabolism. Clin. Chim. Acta **2000**, *290* (2), 179–188.
25. Mori, A.; Kohno, M.; Masumizu, T.; Noda, Y.; Packer, L. Guanidino Compounds Generate Reactive Oxygen Species. Biochem. & Mol. Biol. Int. **1996**, *40* (1), 135–143.
26. De Deyn, P.P.; Marescau, B.; Ď Hooge, R.; Possemiers, I.; Nagler, J.; Mahler, C.H. Guanidino Compound Levels in Brain Regions of Non-Dialyzed Uremic Patients. Neurochem. Int. **1995**, *27* (3), 227–237.
27. Kuroda, M. Study of Impaired Metabolism of Guanidinoacetic Acid in Chronic Renal Failure Rabbits with Special Reference to Impaired Conversion of Arginine to Guanidinoacetic Acid. Nephron. **1993**, *65* (4), 605–611.
28. Ishizaki, M.; Kitamura, H.; Taguna, Y.; Aoyagi, K.; Narita, M. Urinary Excretion Rate of Guanidinoacetic Acid as a Sensitive Indicator of Early-stage Nephropathy. In *Guanidino Compounds in Biology and Medicine*; De Deyn, P.P., Marescau, B., Stalon, V., Qureshi, I.A. Eds.; John Libbey & Company Ltd.: London, 1992; 275–280.

29. Hiramatsu, M.; Ohba, S.; Edamatsu, R.; Kadowaki, D.; Mori, A. Effect of Guanidine Compounds On Membrane Fluidity of Rat Synaptosomes. In *Guanidino Compounds in Biology and Medicine*; De Deyn, P.P., Marescau, B.; Stalon, V., Qureshi, I.A. Eds.; John Libbey & Company Ltd.: London, 1992; 387–393.
30. Hirayasu, Y.; Morimoto, K.; Otsuki, S.; Mori, A. Effect of Guanidino Compounds on Membrane Fluidity of Rat Synaptosomes. In *Guanidino Compounds in Biology and Medicine*, De Deyn, P.P.; Marescau, B.; Stalon, V.; Qureshi, I.A. Eds.; John Libbey & Company Ltd.: London, 1992; 387–393.
31. Stockler, S.; Hanefeld, F.; Frahm, J. Creatine Replacement Therapy in Guanidinoacetate Methyltransferase Deficiency, a Novel Inborn Error of Metabolism. The Lancet **1996**, *348*, 789–790.
32. Ito, T.; Yufu, K.; Mori, A.; Packer, L. Oxidative Stress Alters Arginine Metabolism in Rat Brain: Effect of Sub-Convulsive Hyperbaric Oxygen Exposure. Neurochem. Int. **1996**, *29* (2), 187–195.
33. Felcman, J.; Miranda, J.L. A Potentiometric Study of Guanidinoacetic Acid Complexation with the Ions Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II). J. Braz. Chem. Soc. **1997**, *8*, 575–580.
34. Kaim, W.; Schwederski, B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life* – An Introduction Guide; John Wiley & Sons: New York, 1994; 238.
35. Wilkins, P.C.; Wilkins, R.G. *Inorganic Chemistry in Biology*; Oxford University Press: New York, 1997; 60–62.
36. Osman, R.; Bash, H. On the Mechanism of Action of Superoxide-Dismutase a Theoretical Study. J. Am. Chem. Soc. **1984**, *106*, 5710–5714.
37. Kaim, W.; Schwederski, B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life* – An Introduction Guide; John Wiley & Sons: England, 1994; 254.
38. Kaim, W.; Scwederski, B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry Life: An Introduction and Guide*; John Wiley & Sons: England, 1994; 262–263.
39. Fenton, D.E. *Biocoordination Chemistry*; Oxford University Press: New York, 1995; 78–79.
40. Goto, T.; Nakanish, K.; Ohashi, M. An Account on the Infrared Absorption of Guanidiniums. J. Chem. Soc. **1957**, *30*(7), 723–725.
41. Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds Part B: Applications in Coordination, Organometallic, and Bioinorganic Chemistry*; 5th Edn, John Wiley & Sons: USA, 1997; 62–66.

42. Inomata, Y.; Shibata, A.; Yukawa, Y.; Takeuchi, T.; Moriwaki, T. The Metal Complexes of Amino Acids and Their Derivatives: The IR Spectra and Normal Coordinate Analysis of Bivalent-metal Complexes with N-Methylglycine and Phenylglycine. Spetrochim. Acta **1988**, *44A* (1), 97–107.
43. Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds Part B: Applications in Coordination, Organometallic, and Bioinorganic Chemistry*; 5th Edn, John Wiley & Sons: USA, 1997; 87–89.
44. Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*; 4th Edn, John Wiley & Sons: USA, 1986; 111.
45. Lever, A.B.P. *Inorganic Electronic Spectroscopy*; Elsevier Publishing Company: Amsterdam, 1968; 317–323.
46. Lever, A.B.P. *Inorganic Electronic Spectroscopy*; Elsevier Publishing Company: Amsterdam, 1968; 333–339.
47. Lever, A.B.P. *Inorganic Electronic Spectroscopy*; Elsevier Publishing Company: Amsterdam, 1968; 355–361.
48. Sportelli, L.; Neubacher, H.; Lohmann, W. ESR and Optical Absorption On Copper(II) Interaction with Small Peptides Containing Aromatic Amino Acids. Biophys. Struct. Mech. **1977**, *3*, 317–326.
49. Kruck, T.P.A.; Sarkar, B. Equilibria and Stuctures of Species in Ternary Systems of L-Histidine, Copper(II) and Diglycil-L-histidine, a Peptide Mimicking Copper(II)-Transport Site of Human Serum Albumin. Inorg. Chem. **1975**, *14*, 2383–2388.
50. Micera, G.; Sanna, D.; Dallocchio, R.; Dessi, A. Coordination of Copper(II) to Polyaminopolycarboxylic. J. Coord. Chem. **1992**, *25*, 265–27.

Received : August 17, 2000
Accepted : March 24, 2001

Referee I: A. O. Adeyemo
Referee II: R. Holwerda