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Copper(II) complexes with L-lysine and L-ornithine: is the side-chain involved in the coordination? A thermodynamic and spectroscopic study

Chiara Conato[a], Annalinda Contino[b], Giuseppe Maccarrone[b,*], Antonio Magrì[b], Maurizio Remelli[a], Giovanni Tabbì[c]

[a]*Dipartimento di Chimica, Università di Ferrara, Via L. Borsari 46, 44100 Ferrara, Italy*
[b]*Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy*
[c]*Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico, CNR Sezione per lo Studio dei Metalloenzimi, Viale A. Doria 6, 95125 Catania, Italy*

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Abstract

The involvement of the ω-NH_2 terminal group of L-lysine and L-ornithine in the complexation of Cu(II) was investigated by means of potentiometry, calorimetry, UV–Vis and ESR. The thermodynamic parameters (ΔG^0, ΔH^0 and ΔS^0) obtained by combining the potentiometric and calorimetric data show that the two ligands complex copper(II) in a different manner, indicating that the ω-NH_2 terminal group is involved in ornithine complexes only. UV–Vis and ESR spectra further support the involvement of ornithine ω-NH_2 and provide details on the complexation geometries of the complexes of the two amino acids.

Keywords: Calorimetry; Copper; Aminoacids; ESR

1. Introduction

The complexes of amino acids with biologically active metal ions, and in particular with copper(II), has attracted the attention of several researchers; the goal was not only to shed new light on the coordination characteristics of the complexes but also to use these complexes as models for metalloproteins [1].

Since the early 1950s, quite a few authors have focused their attention on lysine and ornithine complexes with copper(II) in aqueous solution, as testified by the large number of papers present in the literature on this specific subject [2–11].[1] In particular, a great effort was devoted to find experimental evidences to establish if the side-chain ω-amino group is able to coordinate copper(II).

Lysine and ornithine contain a side-chain ω-amino group, which is potentially able to coordinate a metal ion in addition to the α-amino and the carboxylate groups that are typically involved in the complex of copper(II) by amino acids having no side chains. These two amino acids differ from one another for the length of the spacer between the ω-NH_2 terminal and α-NH_2/carboxylate group; the ω-NH_2 and α-NH_2

* Corresponding author. Tel.: +39-095-7385046; fax: +39-095-337678.
E-mail address: gmacca@dipchi.unict.it (G. Maccarrone).

[1] For a short review about copper(II) complexes with lysine and ornithine, see Ref. [12].

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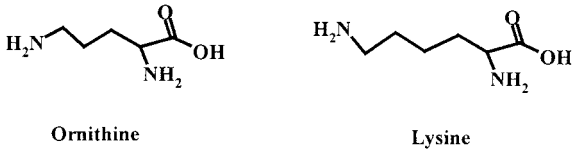

Scheme 1.

groups are separated by three and four methylene residues in ornithine (Orn) and lysine (Lys), respectively (Scheme 1).

At physiological pH value, the ω-nitrogen starts deprotonating and in theory both lysine and ornithine become potential tridentate ligands. Brookes and Pettit [9] reported the first potentiometric study on lysine–copper(II) and ornithine–copper(II) systems. These authors carried out titrations up to pH = 7.5, because above this pH value, precipitation was reported to take place in the copper(II)–ornithine system even in the presence of a large (5:1) ligand-to-metal ratio. It was concluded that the two amino acids coordinate in all the complex species in a ''glycine-like'' fashion, i.e. without any involvement of the ω-amino group. Previously, Brubacker and Busch [3] and Wilson et al. [7] had come to the same conclusion on the basis of spectroscopic measurements. Gergely et al. [10] studied the same systems extending their investigation up to pH = 11 using a 2:1 ligand-to-metal ratio to avoid precipitation. Interestingly, these authors indicated that the two ligands do not coordinate copper(II) in the same manner.

However, the results so far obtained have not come to a firm conclusion; therefore, we have carried out an investigation using several techniques such as potentiometry, direct calorimetry, electron spin resonance (ESR), UV–Vis and molecular mechanics, in order to establish whether the ω-amino group is involved in the coordination. This study was carried out over the pH range 2.5–11.0 with a ligand-to-metal ratios ranging from 2:1 to 3:1, focusing especially on the physiological pH region. The stability constants for the complex species between lysine and ornithine with proton and copper(II) were determined by potentiometric titrations. The ΔG^0 values were then dissected into ΔH^0 and ΔS^0 contributions. ESR spectra and UV–Vis measurements were carried out in pH range 7–11 to obtain further information on the coordination environment of the copper(II) species. All the possible structures hypothesised on the basis of the spectroscopic data were computed by molecular modelling techniques to have indications on the most stable structures.

2. Experimental

2.1. Materials

L-lysine and L-ornithine were obtained as commercial reagents by Merck and were used as received. Their purity, checked by alkalimetric titrations, was always greater than 99%. Copper(II) nitrate stock solutions was standardised by EDTA titrations [13]. Carbonate-free KOH and HNO_3 solutions were standardised with potassium hydrogenphthalate and tris(hydroxymethyl)-aminomethane, respectively. The ionic strength was adjusted to 0.1 mol dm^{-3} with KNO_3.

2.2. Potentiometric measurements

Potentiometric measurements were performed at 25.0 ± 0.2°C using a home-made experimental apparatus that was described in a previous work [14,15]. The electrode couples (Metrohm) were calibrated on the pH = $-\log[H^+]$ scale by titrating HNO_3 with CO_2 free base. The E^0, E_j and K_w were determined in separate experiments. Usually a solution containing 4.0–10.0 mmol dm^{-3} of copper(II) and 10.0–16.0 mmol dm^{-3} of the ligand was titrated with standard KOH (0.1 mol dm^{-3}), recording 50–70 points for each of the 10–12 independent titrations. Metal-to-ligand ratios ranging from 1:1 to 1:3 were employed; initial pH was adjusted to 2.5 with standard HNO_3 and titrations were performed until precipitation occurred.

2.3. Calorimetric measurements

Calorimetric measurements were carried out at 25.000 ± 0.001°C using a Tronac 450, an isoperibolic calorimeter, equipped with a 25 ml titration vessel. Blank experiments were carried out in all cases to account for heat of dilution and heat of friction effects resulting from the addition of the titrant to the solution contained in the calorimetric vessel. The titration

data, corrected for all non-chemical terms, determined in separate experiments were refined to obtain the final ΔH^0 for each system. Usually a solution containing 3.0–4.0 mmol dm^{-3} of copper(II) and 6.0–9.0 mmol dm^{-3} of the ligand at pH 10.5 was titrated with standard HNO_3 (0.4 mol dm^{-3}), recording 20–30 points for each of the 4–6 independent titrations.

2.4. Spectroscopic ESR measurements

Frozen solution ESR spectra have been recorded on a Bruker ER 200 D spectrometer equipped with the 3220 data system at the temperature of 150 K. Copper(II) complex solutions of 3 mM were prepared in situ by mixing a standard solution of $^{63}Cu(NO_3)_2$ with a solution of the pertinent ligand in the metal-to-ligand ratio 1:2, and adjusting the pH of the resulting solution to the values of 7, 9.5 and 11 by adding KOH of 10 mM. Methanol not exceeding 10% was added to the aqueous copper(II) complex solutions to increase resolution. Room temperature ESR spectra were obtained by using a Bruker quartz aqueous solution flat cell. Frozen solution spectra did not reveal overlap of signals coming from the various species which could be present in these systems. In fact, the pH of the aqueous solution was chosen so as to maximise the percentage of the formation of the selected complex species, even if it is not possible to exclude that low temperatures could change the relative formation ratios of the complex species. Parallel spin hamiltonian parameters from frozen solution spectra have been obtained directly from the experimental spectra, recorded in an enlarged scale, calculating them from the second and third lines in order to get rid of second order effects [16]. Perpendicular magnetic parameters were calculated by using the field of an overshoot in all the spectra as previously reported [17]. Room temperature spectra have been analysed by generating computed spectra employing a computer program, substantially devised by Pilbrow [18].

2.5. Calculations

Calculations concerning the calibration of the electrode system (E^0, E_j, K_w) were performed using the program ACBA [19]. SUPERQUAD [20] was used to handle all other potentiometric data. Reaction enthalpies were obtained using the program DOEC [21].

2.6. Optical measurements

Visible optical spectra were carried out at 25°C over the pH range 7–11 by using a Hewlett-Packard 8452A spectrophotometer equipped with a Crioterm 10–80-circulation thermostat. The pH was increased step by step by adding a measured volume of KOH to the starting solution. The concentrations of copper(II) and the ligand (L-lysine or L-ornithine) were 1×10^{-2} and 2×10^{-2} mol dm^{-3}, respectively. All the measurements were performed at constant ionic strength (0.1 mol dm^{-3} KNO_3) to reproduce the experimental conditions of both potentiometric and calorimetric measurements. These experiments were replicated at least three times for each ligand. All the spectra, collected over the investigated pH range, were processed with SPECFIT [22] that uses a non-linear least-squares method to generate the calculated spectrum for each individual complex species, $[Cu(AA)_2H_2]^{2+}$, $[Cu(AA)_2H]^{+}$, and $[Cu(AA)_2]$, where AA = L-lysine or L-ornithine.

2.7. Molecular mechanics calculations

Molecular mechanics calculations were performed by making use of Hyperchem [23] and MOMEC 97 [24,25] packages. In the first step, the atomic charges of each complex were calculated by a semi-empirical method (ZINDO/1). The complex geometries were then refined by MOMEC 97 using the standard force field included in the package. The refined structures were screened on the basis of the lowest strain energies. The lowest strain energy structures, in order to have a cross-check, were also refined by using Hyperchem, inserting the complex in a cubic box having 350 water molecules, and the energy of the resulting system was calculated using the MM+ force field. No fundamental differences in the complex structures and in the trend of their energies were found.

3. Results and discussion

3.1. Potentiometric and calorimetric results

The stability constants and the thermodynamic parameters for L-Orn and L-Lys proton complexes are reported in Table 1.

Table 1
log β values and thermodynamic parameters for proton and copper(II) complex formation of L-Lys and L-Orn at 25°C and $I = 0.1$ mol dm^{-3} (KNO_3)[a]

Reaction	log β	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} deg^{-1})
$H^+ + L\text{-}LysO^- \rightleftarrows [H(L\text{-}LysO)]$	10.63 (1)	−60.64 (6)	−52.6 (6)	27 (2)
$2H^+ + L\text{-}LysO^- \rightleftarrows [H_2(L\text{-}LysO)]^+$	19.73 (1)	−112.58 (6)	−97.0 (5)	52 (2)
$3H^+ + L\text{-}LysO^- \rightleftarrows [H_3(L\text{-}LysO)]^{2+}$	21.82 (2)	−124.5 (1)	−98.1 (7)	88 (2)
$Cu^{2+} + L\text{-}LysO^- + H^+ \rightleftarrows [Cu(L\text{-}LysO)H]^{2+}$	18.17 (1)	−103.65 (6)	−75.8 (3)	93 (1)
$Cu^{2+} + 2L\text{-}LysO^- + 2H^+ \rightleftarrows [Cu(L\text{-}LysO)_2H_2]^{2+}$	35.18 (1)	−200.69 (6)	−154.1 (3)	156 (1)
$Cu^{2+} + 2L\text{-}LysO^- + H^+ \rightleftarrows [Cu(L\text{-}LysO)_2H]^+$	25.27 (2)	−144.1 (1)	−100.7 (7)	146 (2)
$Cu^{2+} + 2L\text{-}LysO^- \rightleftarrows [Cu(L\text{-}LysO)_2]$	14.83 (1)	−84.61 (6)	−44.0 (1)	135 (5)
$H^+ + L\text{-}OrnO^- \rightleftarrows [H(L\text{-}OrnO)]$	10.49 (1)	−59.86 (6)	−49.9 (5)	33 (2)
$2H^+ + L\text{-}OrnO^- \rightleftarrows [H_2(L\text{-}OrnO)]^+$	19.23 (2)	−109.7 (1)	−94.0 (4)	53 (1)
$3H^+ + L\text{-}Orn^- \rightleftarrows [H_3(L\text{-}OrnO)]^{2+}$	21.21 (2)	−121.0 (1)	−95.8 (6)	85 (2)
$Cu^{2+} + L\text{-}OrnO^- + H^+ \rightleftarrows [Cu(L\text{-}OrnO)H]^{2+}$	17.81 (1)	−101.60 (6)	−73.6 (2)	94 (1)
$Cu^{2+} + 2L\text{-}OrnO^- + 2H^+ \rightleftarrows [Cu(L\text{-}OrnO)_2H_2]^{2+}$	34.48 (1)	−196.68 (6)	−150.7 (2)	154 (1)
$Cu^{2+} + 2L\text{-}OrnO^- + H^+ \rightleftarrows [Cu(L\text{-}OrnO)_2H]^+$	25.50 (2)	−145.5 (1)	−107.2 (3)	128 (1)
$Cu^{2+} + 2L\text{-}OrnO^- \rightleftarrows [Cu(L\text{-}OrnO)_2]$	15.53 (3)	−88.6 (2)	−55.3 (4)	112 (1)

[a] σ are given in parentheses.

The proton complex-formation constants shown in Table 1 represent a protonation sequence that, on the basis of the thermodynamic parameters (ΔH^0 and ΔS^0), seems to be the same for both ligands, namely ω-NH_2, α-NH_2, –COO^-, as previously hypothesised by Brookes and Pettit [9] solely on the basis of free energy values. In fact, the larger protonation constant value of the ω-NH_2 results from an enthalpic contribution that is more favourable than that of the α-NH_2 group. The lower basicity of the α-NH_2 group is to be ascribed to the electron withdrawing effect of the carboxylate group which is more effective on the α-NH_2 than on the ω-NH_2 group. Furthermore, the constants of the second protonation step for both L-Orn and L-Lys are lower than those for non-basic amino acids and, in particular, than those for aliphatic amino acids of comparable length (e.g. norvaline and norleucine) [26]. This indicates that the ω-NH_3^+ group exerts a sizeable electron withdrawing effect on the α-NH_2 group; such an effect is more pronounced for ornithine which has a spacer between the α-NH_2 and the ω-NH_3^+ shorter than lysine. This parallels the behaviour observed for L-asparagine and L-glutamine; also in these amino acids the shorter spacer (L-asparagine) determines the lower basicity of the amino group [27].

The enthalpic and entropic contributions concerning the protonation of the carboxylate residues of the two amino acids (i.e. a markedly favourable ΔS^0 and a slightly favourable ΔH^0 value) are very similar to those found for analogous amino acids [15,27–31]; this indicates that no additional interactions are involved in these steps.

The potentiometric measurements for the binary complexes were performed within the pH range 2.5–11.0. Unlike Brookes and Pettit [9], we detected no precipitate formation at this pH value as also reported by Gergely et al. [10]; very likely this accounts for the different speciation obtained for ornithine by Brookes and Pettit. The hydrolytic species of Cu^{2+} and their thermodynamic parameters were determined previously [32]. The formation constants as well as the enthalpy and entropy values for L-Orn and L-Lys Cu(II) complexes are reported in Table 1. As indicated in Section 2, metal-to-ligand ratios ranging from 1:1 to 1:3 were investigated; for simplicity distribution diagrams for both Cu–L-Lys and Cu–L-Orn systems are reported for metal-to-ligand ratios equal to 1:2 (Figs. 1 and 2).

Unlike aliphatic amino acids such as alanine, valine, etc. [15,28–31], L-ornithine and L-lysine have a protonatable terminal group (the ω-NH_2) which is potentially able to coordinate copper(II). Thus, L-ornithine and L-lysine form with this metal ion species that are not detected with other amino acids. The species $[Cu(AA)H]^{2+}$ and $[Cu(AA)_2H_2]^{2+}$ (AA = lysine or ornithine) predominate at acidic and neutral pH, respectively. In both these species, the ω-

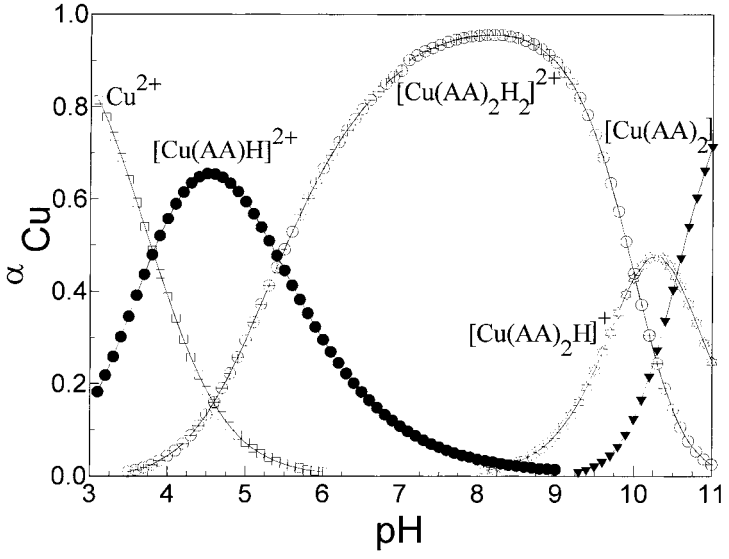

Fig. 1. Distribution diagram for the Cu(II)–L-Lys system. $[Cu] = 1 \times 10^{-3}$, Cu : L = 1 : 2.

NH_2 group is protonated and L-ornithine and L-lysine can coordinate the metal ion via the carboxylate/α-NH_2 chromophore in a glycine-like mode only. As the pH raises, the ω-NH_2 groups begin to deprotonate and the species $[Cu(AA)_2H]^+$ and $[Cu(AA)_2]$ are formed; the involvement of the ω-NH_2 group has been debated for long, both in the case of the single amino acids [9,10] and of oligopeptides containing the Lys residue [33]. The thermodynamic parameters for Cu–L-Orn and Cu–L-Lys systems (Table 1) are in good agreement with those reported by Gergely et al. [10] at $I = 0.2\,\text{mol}\,\text{dm}^{-3}$ (KCl) and show that the complex formation is enthalpically and entropically favoured for both systems. The favourable enthalpic and entropic contributions clearly indicate the coordination of both the α-NH_2 nitrogen and the carboxylate to the

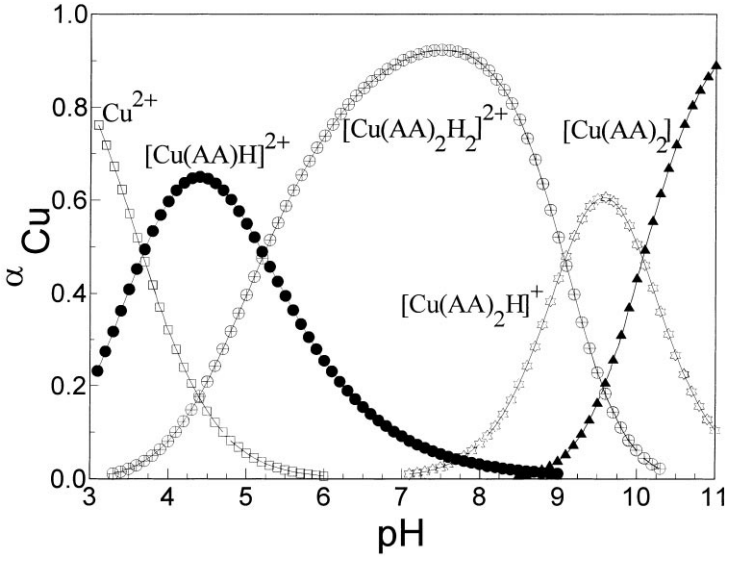

Fig. 2. Distribution diagram for the Cu(II)–L-Orn system. $[Cu] = 1 \times 10^{-3}$, Cu : L = 1 : 2.

Table 2
log K values and thermodynamic parameters for complexation steps of copper(II) ion with L-Lys and L-Orn at 25°C and $I = 0.1$ mol dm^{-3} (KNO_3)

Reaction	log K	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} deg^{-1})
$Cu^{2+} + H(L\text{-}LysO) \rightleftarrows [Cu(L\text{-}LysO)H]^{2+}$	7.54	−43.01	−23.1	67
$[Cu(L\text{-}LysO)H]^{2+} + H(L\text{-}LysO) \rightleftarrows [Cu(L\text{-}LysO)_2H_2]^{2+}$	6.38	−36.41	−25.5	36
$[Cu(L\text{-}LysO)H]^{2+} + (L\text{-}LysO)^- \rightleftarrows [Cu(L\text{-}LysO)_2H]^{+}$	7.10	−40.50	−25.1	52
$[Cu(L\text{-}LysO)_2] + H^+ \rightleftarrows [Cu(L\text{-}LysO)_2H]^{+}$	10.44	−59.54	−56.5	12
$[Cu(L\text{-}LysO)_2H]^{+} + H^+ \rightleftarrows [Cu(L\text{-}LysO)_2H_2]^{2+}$	9.91	−56.57	−53.3	11
$Cu^{2+} + H(L\text{-}OrnO) \rightleftarrows [Cu(L\text{-}OrnO)H]^{2+}$	7.32	−41.76	−23.8	60
$[Cu(L\text{-}OrnO)H]^{2+} + H(L\text{-}OrnO) \rightleftarrows [Cu(L\text{-}OrnO)_2H_2]^{2+}$	6.18	−35.23	−27.2	27
$[Cu(L\text{-}OrnO)H]^{2+} + (L\text{-}OrnO)^- \rightleftarrows [Cu(L\text{-}OrnO)_2H]^{+}$	7.69	−43.89	−33.9	35
$[Cu(L\text{-}OrnO)_2] + H^+ \rightleftarrows [Cu(L\text{-}OrnO)_2H]^{+}$	9.97	−56.82	−51.9	17
$[Cu(L\text{-}OrnO)_2H]^{+} + H^+ \rightleftarrows [Cu(L\text{-}OrnO)_2H_2]^{2+}$	8.98	−51.21	−43.5	26

copper(II), as reported for non-basic amino acids [15,28–31]. The stepwise formation equilibria (Table 2) give more detailed information.

In particular, the coordination of both H(L-OrnO) and H(L-LysO) in the species $[Cu(AA)H]^{2+}$ and $[Cu(AA)_2H_2]^{2+}$ results in an enthalpic contribution that is roughly equivalent to that associated with the formation of $[Cu(GlyO)]^+$, $[Cu(L\text{-}AlaO)]^+$, $[Cu(L\text{-}LeuO)]^+$, etc. (−23.1 to −23.8 kJ mol^{-1} vs. −26.4 kJ mol^{-1}) [15,28–31], in which the amino acid can coordinate in a glycine-like mode only. Thus, as predictable, we have to conclude that in $[Cu(AA)H]^{2+}$ and $[Cu(AA)_2H_2]^{2+}$ the amino acid molecule coordinates in a glycine-like mode.

Unlike in $[Cu(AA)H]^{2+}$ and $[Cu(AA)_2H_2]^{2+}$, in $[Cu(AA)_2H]^+$ and $[Cu(AA)_2]$ one or both the ω-NH_2 terminal groups, respectively, are not protonated and consequently are potentially coordinating groups. There are some interesting differences between the $[Cu(L\text{-}LysO)_2H]^+$ and the $[Cu(L\text{-}OrnO)_2H]^+$ value. The ΔH^0 value for the formation step of $[Cu(L\text{-}LysO)_2H]^+$ (−25.1 kJ mol^{-1}) (Table 2) indicates that the coordination of a second unprotonated L-LysO$^-$ molecule takes place in a glycine-like way, thus ruling out the coordination of the ω-NH_2 nitrogen. On the contrary, the formation of the analogous species with ornithine shows a ΔH^0 value that is more exothermic than that of Cu–L-Lys (−33.9 kJ mol^{-1} vs. −25.1 kJ mol^{-1}). This difference is an indication of the involvement of the nitrogen of the ornithine ω-NH_2 terminal group in $[Cu(AA)_2H]^+$; the ω-NH_2 nitrogen could coordinate in the apical position only and this would also explain the relatively low ΔH^0 contribution.

The enthalpy data concerning the equilibria $[Cu(AA)_2H]^+ + H^+ \rightleftarrows [Cu(AA)_2H_2]^{2+}$, which refer to the protonation of the coordinated ligands, provide further details as to the involvement of ornithine ω-NH_2 terminal group in $[Cu(AA)_2H]^+$. In fact, while the ΔH^0 value (Table 2) for the protonation step of $[Cu(L\text{-}Lys)_2H]^+$ is almost identical to the protonation value of the free ligand (−53.3 kJ mol^{-1} vs.−52.6 kJ mol^{-1}), the analogous value for $[Cu(L\text{-}Orn)_2H]^+$ is less exothermic than the free ligand protonation value (−43.5 kJ mol^{-1} vs. −49.9 kJ mol^{-1}); this indicates that in $[Cu(L\text{-}Orn)_2H]^+$ part of the energy associated with the protonation of the ω-NH_2 terminal group is spent for the detachment of the same group from the copper(II) coordination sphere.

3.2. ESR and UV–Vis spectra

The ESR frozen solution spectra are characterised by the presence of signals due to one complex species only. For the copper–L-lysine system, a broad signal centred at $g = 2.109$ overlaps with the well-defined peaks associated with the complex species at pH 7; perhaps, owing to low temperature, precipitation of copper hydroxide occurs. In fact, the magnetic parameters (see Table 3) do not change as the pH of the aqueous solution raises from 7 to 11.

However for Cu–L-ornithine small, significant differences are observed as the pH is raised from 7 to 11;

Table 3
Magnetic parameters obtained from ESR frozen solution spectra and room temperature spectra for the copper(II) bis-amino acidate complexes with L-lysine and L-ornithine on changing the pH of the aqueous solution

pH	$g_{\parallel}$	$A_{\parallel}$ (cm^{-1})	$g_{\perp}$	$A_{\perp}$ (cm^{-1})	g_{iso}	A_{iso} (cm^{-1})	A^{N}_{iso} (cm^{-1})
Copper(II)–L-lysine system 1:2							
7–9	2.246 (2)	0.0178 (2)	2.043 (3)	0.0012 (3)	2.123 (1)	0.0066 (1)	0.0009 (1)
11	2.248 (2)	0.0180 (2)	2.048 (3)	0.0013 (3)	2.124 (1)	0.0070 (1)	–
Copper(II)–L-ornithine system 1:2							
7–9	2.245 (2)	0.0182 (2)	2.045 (3)	0.0012 (3)	2.121 (1)	0.0068 (1)	0.0009 (1)
11	2.238 (2)	0.0179 (2)	2.042 (3)	0.0012 (3)	2.122 (1)	0.0065 (1)	–

in particular, both $g_{\parallel}$ and $A_{\parallel}$ values decrease slightly. Fig. 3 shows the parallel parts of frozen solution spectra of the copper(II) bis complex with L-ornithine at pH 7 and 11.

The isotropic magnetic parameters for the two systems at room temperature (Figs. 4 and 5) also show some differences.

The usual five-line shf structure, due to the presence of two nitrogen atoms in the equatorial plane of the copper(II) complexes, was detected on the high field feature at pH 7; such a structure is more evident in the second derivative mode. However, the shf structure is not well resolved at higher pH values. Since the speciation study indicates that the side-chain amino groups of both ligands deprotonate within the pH range 7–11, one might expect that both nitrogen atoms belonging to the ligand side chains may be involved in the coordination. Both low temperature as well as room temperature ESR data seem to rule out this possibility for the Cu–L-lysine system; the slight increase in the $A_{\parallel}$ and A_{iso} values is probably the result of the decrease of charge of the bis-complex as a whole. However, for the Cu–L-ornithine system, these differences could also be consistent with a pseudo-octahedral geometry, in which one or both apical water molecules are replaced by nitrogen atoms.

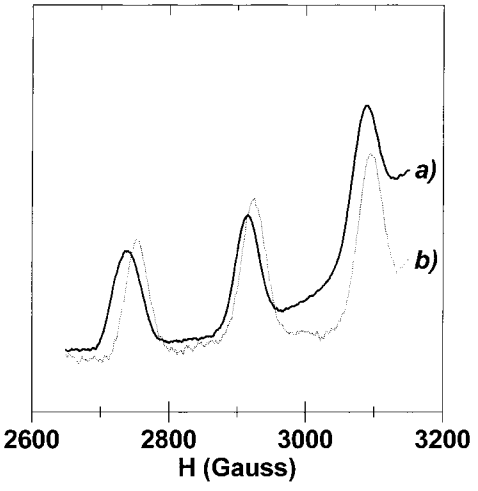

Fig. 3. Parallel parts of frozen solution ESR spectra recorded on copper(II) bis complex with L-ornithine at (a) pH 7–9 (heavy line), and (b) pH 11 (soft line). Instrumental settings: modulation frequency = 100 kHz; modulation amplitude = 2.11 G; time constant = 164 ms; sweep time = 1.5 min; receiver gain = 2×10^3; microwave power = 20 mW; microwave frequency = 9.428 Hz.

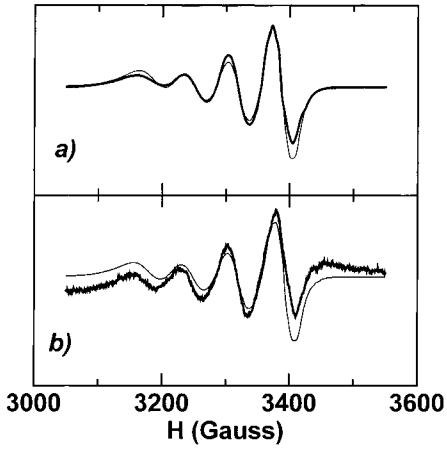

Fig. 4. Room temperature ESR spectra (heavy line) recorded on aqueous solution of copper(II) bis complex with L-lysine at (a) pH 7, and (b) pH 11 (computed spectra soft line). Instrumental settings: modulation frequency = 100 kHz; modulation amplitude = 1.94 G; time constant = 328 ms; sweep time = 3.0 min; receiver gain = 5×10^4; microwave power = 60 mW; microwave frequency = 9.777 Hz.

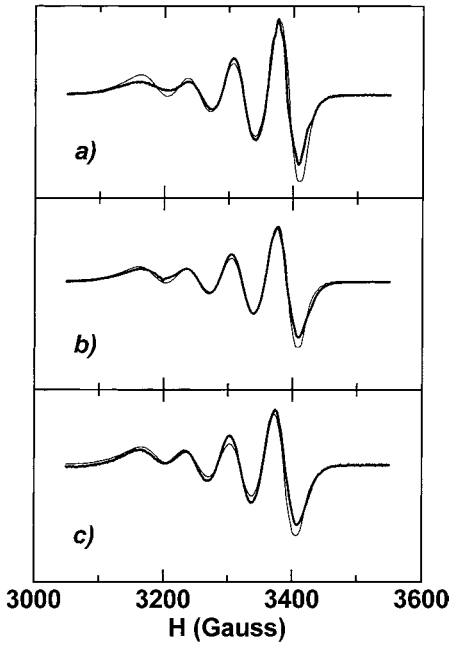

Fig. 5. Room temperature ESR spectra (heavy line) recorded on aqueous solution of copper(II) bis complex with L-ornithine at (a) pH 7, (b) pH 9, and (c) pH 11 (computed spectra soft line). Instrumental settings: the same as Fig. 4.

The substitution of two apical oxygen atoms (of two coordinated water molecules) with one or two nitrogen atoms would not result in a large modification of the copper(II) complex geometry; in fact, the equatorial field would still be characterised by the coordination of the two carboxylate oxygen atoms and the two nitrogen atoms of the α-amino groups. However, this picture is an approximation since it does not take into account that the copper(II) bis-amino acidate complex can exist in both the *cis* and *trans* isomer forms; in the *cis* and *trans* isomers, the amino acid side chains do not point to the same direction. In particular, as stated by Pettit and Hefford [34], in the *cis* isomer one side chain lies above and the other one under the coordination equatorial plane, whilst in the case of the *trans* isomer, both side chains lie on the same side.

In order to obtain a more detailed picture on the coordination sphere of copper(II) we carried out UV–Vis measurements on Cu–L-Lys and Cu–L-Orn systems as a function of the pH. The UV–Vis spectra were handled by the computer program SPECFIT [22] to obtain the λ_{max} and ε_{max} values of each single species (Table 4).

Table 4
Visible optical data (λ_{max} and ε_{max}) for copper(II) bis amino acidate complexes with L-lysine and L-ornithine at room temperature[a]

Ligand	$[Cu(L)_2H_2]^{2+}$	$[Cu(L)_2H]^{+}$	$[Cu(L)_2]$
L-lysine	615 nm (58)	614 nm (61)	618 nm (59)
L-ornithine	618 nm (55)	629 nm (63)	637 nm (63)

[a] Molar absorption coefficients ($dm^3\ mol^{-1}\ cm^{-1}$) are given in parentheses. Presumed errors on λ_{max} were evaluated to be ±3 nm, and on ε_{max} about 10%.

The λ_{max} values obtained for the two $[Cu(AA)_2H_2]^{2+}$ species are coincident within the experimental error. Since in these deprotonated species both L-Lys and L-Orn can only coordinate copper(II) ion as bidentate ligands, the λ_{max} and ε_{max} values obtained for the $[Cu(AA)_2H_2]^{2+}$ species may be taken as the reference values. The λ_{max} values of $[Cu(L\text{-}Lys)_2]$ and $[Cu(L\text{-}Lys)_2H]^{+}$ are practically identical to that of $[Cu(L\text{-}Lys)_2H_2]^{2+}$ and it is also close to the "theoretical" value of 619 nm for a Cu(II) complex with two glycine-like-coordinated ligands, computed according to Sigel and Martin [35]. This result rules out the involvement of lysine ω-NH_2 group in the coordination sphere of copper(II). The slight variation detected for the ε_{max} values of these species can be ascribed to the progressive charge neutralization of the whole complex species; this may exert some strain in the equatorial plane of the complex species and thus could have a slight influence on their geometry. On the contrary, the λ_{max} and ε_{max} values of the Cu(II)–L-Orn complexes considerably change on going from $[Cu(L\text{-}Orn)_2H_2]^{2+}$ to $[Cu(L\text{-}Orn)_2]$ species. Such variations are a clear indication of the involvement of the ω-NH_2 group nitrogen atoms in the apical coordination sites of copper(II) [27]. Interestingly, the visible optical data provide more evidence than room temperature ESR spectra as to the involvement of the ω-NH_2 groups of the amino acid side chains (Table 4). In fact, whereas for the L-lysine species, both λ_{max} and ε_{max} remain practically the same as the pH is raised, for L-ornithine λ_{max} increases with the pH, thus indicating that at least one more nitrogen is involved in the coordination.

However, for the copper(II)–L-ornithine system, the apical nitrogen bonds should be at distances greater

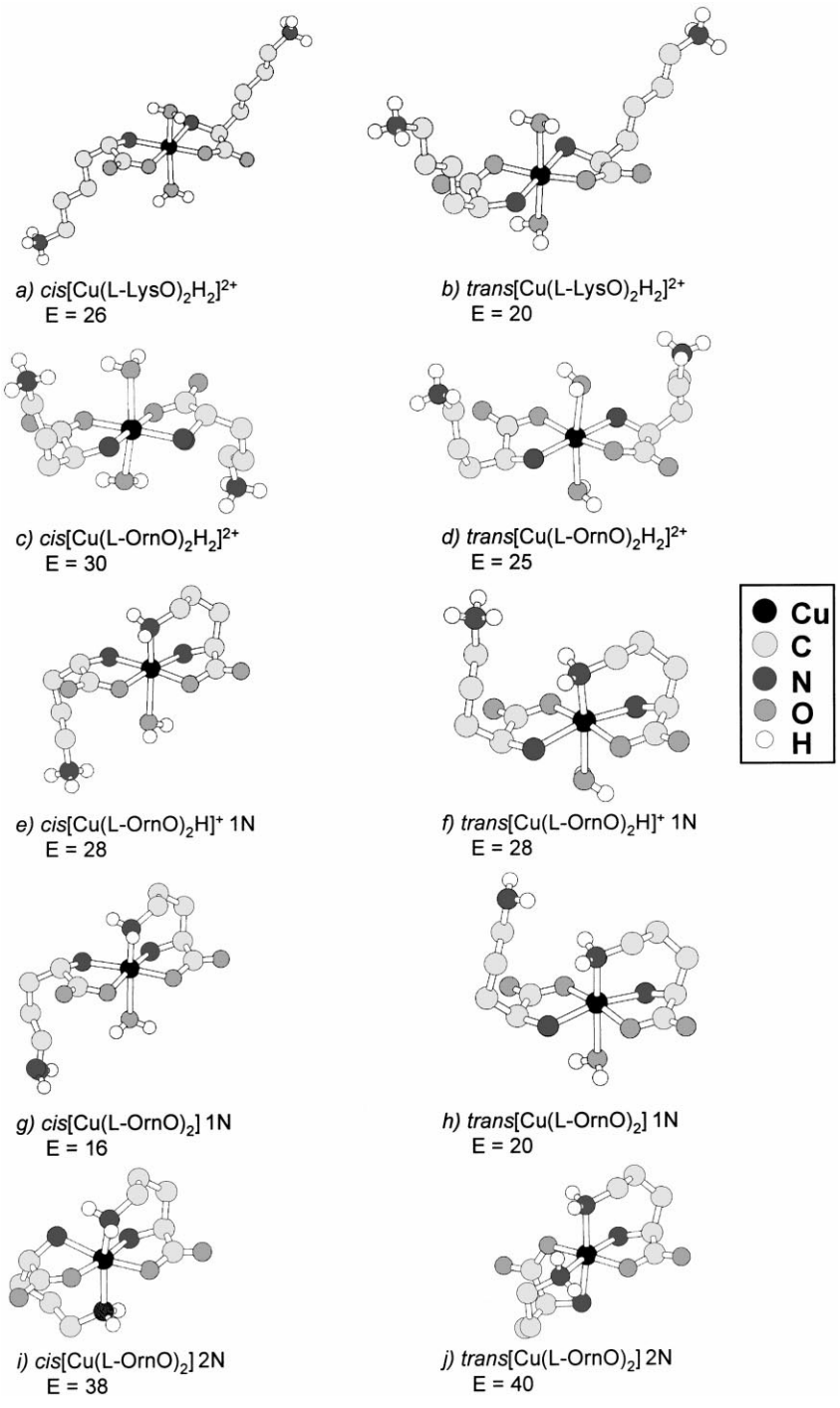

Fig. 6. Optimised structures for some of the species present in the copper(II)–L-Lys and copper(II)–L-Orn systems. The figures given for each structure are the energy minima (kJ mol^{-1}). 1N and 2N, where reported, indicate the coordination of one or two terminal nitrogen atoms, respectively.

than the copper–oxygen and copper–nitrogen distances of the equatorial plane. Unfortunately, the analysis of shf structure in the room temperature ESR spectra does not provide further experimental evidence supporting also an apical nitrogen coordination. In fact, the axis perpendicular to the equatorial plane, i.e. the principal symmetry axis, is the same for all complex species. As the pH is raised (i.e. as we go from $[Cu(AA)_2H_2]^{2+}$ to $[Cu(AA)_2]$), only the oxygen atoms of apical water molecules may be replaced by nitrogen atoms of the amino acid ω-NH_2 terminal groups. It is well known that apically bound nitrogen atoms give very little contribution to shf structure, since only a dipolar interaction mechanism comes into play [36].

In other words, the oxygen and nitrogen atoms of the equatorial plane essentially determine the ligand field in these complexes. The substitution of water apical oxygen atoms with deprotonated amino nitrogen atoms in the copper(II) bis-L-ornithine system causes a slight but still detectable ligand field modification. Based on these data, we can conclude that in the case of the copper–L-ornithine system, experimental evidences tend to support the involvement of one or two side-chain nitrogen atoms. On the contrary, for the copper(II)–L-lysine system, the deprotonation of the side-chain nitrogen atoms does not seem to give any appreciable contribution to the ligand field and the little increase in A_{iso} value has to be regarded as the result of charge neutralization of the whole complex molecule [37].

3.3. Molecular mechanics

Molecular mechanics calculations shed new light on the coordination characteristics of the species present in both systems. These calculations were carried out for all the species detected potentiometrically. For simplicity, optimised geometries and their energy minima are reported for $[Cu(AA)_2H_2]^{2+}$ (AA = L-lysine or L-ornithine), $[Cu(L\text{-}OrnO)_2H]^{+}$ and $[Cu(L\text{-}OrnO)_2]$ only (Fig. 6).

For each one of the above species, the geometries were optimised for both the *trans* and the *cis* forms; in addition, for $[Cu(L\text{-}Orn)_2]$ two possible geometries were taken into consideration, namely those resulting from the coordination of one or two ω-NH_2 nitrogen atoms. The energy values obtained for the $[Cu(AA)_2H_2]^{2+}$ species support what is asserted by Brookes and Pettit [30]; in fact for both lysine and ornithine the *trans* isomer is more stable than the *cis* form. However, the energy differences between the *cis* and the *trans* forms tend to level off as the complexes are deprotonated; the same values are obtained for the *trans* and *cis* form of both $[Cu(L\text{-}OrnO)_2H]^{+}$ and $[Cu(L\text{-}OrnO)_2]$. This shows that in these species, (i) the trans isomer is no longer favoured, and consequently (ii) the spectra obtained are even more to be considered as an average of the contributions of both isomers. Incidentally, the high energy values obtained for the analogous lysine species further rule out the involvement of the ω-NH_2 in the coordination. Interestingly, for $[Cu(L\text{-}OrnO)_2]$ the energy difference between the structures in which only one nitrogen is assumed to be coordinated and the structures in which both ω-NH_2 nitrogen atoms are forced to coordinate is remarkably high. Such a difference is in favour of the coordination of one nitrogen only, which is in line with the relatively small enthalpic gain associated with the formation of both $[Cu(L\text{-}Orn)_2H]^{+}$ and $[Cu(L\text{-}Orn)_2]$.

On the basis of these results, the shifts on the magnetic parameters as well as the visible optical spectra are consistent with the coordination of one of the side-chain nitrogen atoms, the sixth coordination being occupied by an apical water molecule.

4. Conclusions

We have studied the complexation of L-lysine and L-ornithine with copper(II) by several techniques. The combination of potentiometric, calorimetric, ESR, UV–Vis and molecular mechanics data permits to accurately describe the species formed in solution and their structural characteristics. The two amino acids complex copper(II) in a different manner. The thermodynamic parameters indicate that L-Lys coordinates in a glycine-like manner even in the pH range where the ω-NH_2 group is deprotonated. In contrast, the ΔH^0 values for the analogous species of Cu–L-Orn system provide evidence for the involvement of ω-NH_2 terminal group of ornithine in the copper(II) coordination sphere. UV–Vis and ESR spectra also provide additional evidence that allow to definitively rule out the involvement of the ω-NH_2 group of

L-Lysine in the copper(II)-complex species, very likely owing to the different length of the side chain bearing the ω-NH_2 group. Furthermore, spectroscopic data strongly support the involvement of ornithine ω-NH_2 group and provide details on the geometries of the complexes of the two amino acids.

Acknowledgements

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