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The Contribution of Electrospray Mass Spectrometry to the Study of Metal Complexes: The Case of Copper(II)–Dipeptide Systems

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Following a previous thermodynamic and spectroscopic investigation, 13 copper(II)–dipeptide complexes have been characterized by electrospray ionization mass spectrometry (ESI-MS) and visible optical spectroscopy. The data obtained confirm the presence of the species singled out by the thermodynamic approach, showing the ability of electrospray mass spectrometry to give evidence of the presence of a water molecule in the copper(II) in-plane coordination.

Introduction

The interaction of metal ions with functional groups in the peptide chains of proteins can induce conformation changes that affect their secondary structure and hence their properties.[1] Furthermore, protein complexes found inside and outside of biological cells take part in many biochemical pathways and perform many different functions. Oligopeptides have proved to be the most useful model compounds to elucidate the correlation between structure and biological activity, since they are able to mimic, to a great extent, the metal binding site of much more complicated protein molecules and enzymes.[2] On the other hand, there is a large number of amino acid and oligopeptide metal complexes that present potential therapeutic applications as chemotherapeutic, antiinflammatory and antiulcerous agents.[3] Since the 1970s the complexes of several dipeptides with different metal ions and in particular with copper(II) have been extensively studied by different techniques[4] and hence the principal modes of coordination of the copper(II) ion with simple dipeptides in aqueous solution are well established. Valuable contributions from techniques such as EPR and CD spectroscopy have permitted studies to delve deeper into the structural characteristics of these complexes.

Electrospray ionization mass spectrometry (ESI-MS), widely employed in the last two decades in the characterization of organics and biomolecules,[5] has recently been used for the characterization of inorganic species, like metal-coordinated complexes, which form precharged ions,[6] offering the ability to examine aspects of metal coordination in the gas phase and thus in a solvent-free environment.[7] In this field, mass spectrometry has been used for the differentiation of isomeric peptides, particularly using metal ion complexation, which appears to be very efficient for such purposes.[8] However, a proper picture of the liquid-phase complexation processes can only be obtained if the mass spectroscopic data adequately reflects the metal–ligand complexes formed in solution. It has been demonstrated that in several model systems a good correlation exists between experimental data obtained by ESI-MS and theoretically predicted metal–ligand concentrations.[9]

In a recent paper,[10] some of us, in order to confirm some specific coordination details of copper(II) towards some oligopeptides by ESI-MS, have verified the presence of the in-plane coordinated water molecules in the gas phase in some model complexes. For this kind of information the advantage of the ESI source is invaluable, because of the possibility of directly introducing the sample solution without any preliminary treatment. In this regard, a MALDI source, demanding a preliminary treatment like the addition of a matrix, might yield incorrect information about the investigated system in an aqueous solution.

In this paper, together with a visible optical spectroscopy study, we report a systematic ESI-MS investigation on 13 copper(II)–dipeptide complexes in order to evaluate the ability of this technique to ascertain the species present in solution and their coordinative environment, with particular reference to the ability of ESI-MS to distinguish between the in-plane and the out-of-plane coordinated water molecules.

Results and Discussion

The formation equilibria of the complexes of copper(II) with the studied dipeptides were previously investigated.[4] For all the systems the main species are $[CuL]^+$ and

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[CuLH$_{-1}$], where L indicates the fully deprotonated anionic dipeptide (L^-). In Figure 1, as an example, the species distribution diagram for the Cu^{II}/L-Leu–L-Leu system is reported. The diagram shows that at pH < 4.2 no complex formation is observed and that the [CuLH$_{-1}$] species predominates at pH > 5.3. The $[CuL]^+$ species shows a very low degree of formation owing to the β-conformation of the dipeptides,[11] which makes the weakly basic amide oxygen atom responsible for coordination in this species.[12] Hence, at pH < 5.0 the $[CuL]^+$ species is formed with the dipeptide acting as a bidentate ligand. Around pH 5.0 the amide proton ionizes in the presence of Cu^{2+} ions allowing rearrangement of the donor centres to give a complex with the dipeptide acting as a tridentate ligand.[12]

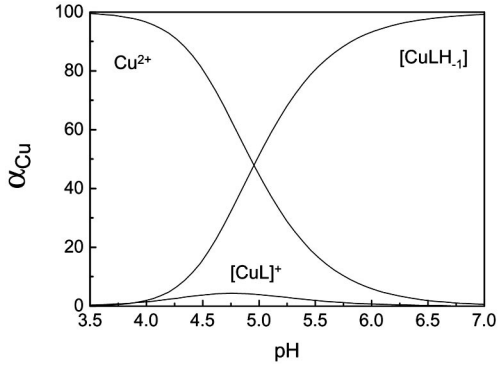

Figure 1. Distribution diagram for the Cu^{II}/L-Leu–L-Leu system. [Cu] = [L] = 1.0×10^{-3} mol dm^{-3}.

The visible optical spectroscopy data confirm these results. For all the investigated systems, in fact, at pH 4.2 no absorption bands were detected on the entire wavelength range investigated owing to the very low ε values of the $[Cu(H_2O)_6]^{2+}$ cation, whereas at pH 5.5 an absorption band in the 595–635 nm range is observed. λ_{max} and ε_{max} values are reported in Table 1. Wavelength values are in good agreement with those calculated according to two empirical equations for estimating λ_{max} for Cu^{II}-dipeptide complexes containing two or three coordinated nitrogen atoms.[12] As expected, the order of increasing ligand field strength is reflected by the ability to effect a blue shift. In fact, whereas the free-histidine dipeptide complexes show λ_{max} values in the range 625–634 nm, the histidine containing dipeptide complexes show a blue shift towards 600 nm, owing to further stabilization due to the Cu–Im bond.[13] The spectra are characteristic of a Cu^{II} ion in a square-bipyramidal environment,[12] allowing assignment of the main absorption band to a $d_{xy} \rightarrow d_{x^2-y^2}$ transition.

Mass spectrometric data of the free dipeptides and of their complexes are shown in Tables 2 and 3, respectively. At the investigated pH (5.5), the free dipeptides are present in the zwitterionic form, that is, with the amino group protonated and the carboxylate group deprotonated (HL). For solutions containing the free dipeptides (HL) only single charged ions are observed, either as protonated species or as sodium or potassium adducts. Furthermore, an aggregation of the peptides occurs in the ion source. Peaks at *m*/*z* values higher than that of the monomeric species, due to the di- and trimeric single charged peptides, are present (Table 2). In particular, for the histidine containing dipeptides and L-Leu–L-Leu the spectra are essentially constituted by peaks from the monomeric species only (Figure 2a), whereas all the other dipeptide spectra, in addition to the peaks relative to the monomer, also show several quite intense signals from the oligomeric species (Figure 2b).

Table 1. Visible optical data (λ_{max} and ε_{max}) for the copper(II)–dipeptide complexes in aqueous solution at 25 °C.

Species	λ_{max} [nm]	ε_{max} [dm^3 mol L^{-1} cm^{-1}]
[Cu(L-Ala–L-Ala)]	629	82
[Cu(L-Ala–D-Ala)]	634	75
[Cu(L-Ala–L-His)]	599	50
[Cu(L-Ala–L-Phe)]	625	82
[Cu(Gly–L-His)]	603	50
[Cu(Gly–L-Leu)]	633	63
[Cu(L-Leu–L-Leu)]	627	72
[Cu(L-Leu–D-Leu)]	625	56
[Cu(D-Leu–L-Tyr)]	628	59
[Cu(L-Met–L-Met)]	628	81
[Cu(L-Met–L-His)]	602	42
[Cu(L-Trp–L-Trp)]	628	69
[Cu(L-Tyr–L-Tyr)]	628	73
[Cu(L-Val–L-Phe)]	628	85

For all the dipeptide/copper(II) mixtures tested, the mass spectra were found to be very informative on the preformed species present in solution. Since mass spectrometry is a gas-phase technique, its applicability for analyzing solution systems is contingent upon being able to reproducibly generate a mixture of ions that accurately reflects the overall solution composition. The ESI-MS technique actually allows the transfer of metal complexes from the solution state into the gas phase, where the mass assignment gives direct information on the metal complex stoichiometry. The mass spectra of solutions containing the ligand and copper(II) ion (1:1 molar ratio) at pH < 4.2 show the same signals of the free ligand spectra (see Table 2), thus indicating no complex formation under this pH value. At pH > 5.5 the spectra show the signals arising from the free ligand and several other signals (Table 3) that give direct evidence of the copper(II)–dipeptide complex formation, according to the thermodynamic[4] and the visible optical spectroscopy data that predicted no complex formation under pH 4.2 and a high degree of formation for the CuLH$_{-1}$ species over pH 5.5 (Figure 1). As expected, the spectra are essentially constituted by peaks arising from mono- and dicharged ions. Peaks resulting from the CuLH$_{-1}$ neutral species are observed, either as protonated species or as sodium or potassium adducts for all the investigated systems, with the only exception of [Cu(L-Met–L-Met)], which will be described later.

The principal modes of coordination of the copper(II) ion with simple dipeptides are well established.[4d] For almost all the investigated systems, spectroscopic data, especially EPR data,[4b,4e,14] clearly indicate that in the

Table 2. Assignment of the ions detected in the ESI-MS mass spectra of free dipeptides $C_L = 1 \times 10^{-3}$ mol dm^{-3}, pH 5.5.

m/z L-Ala–L-Ala	% Rel. intensity	Assignment	*m/z* L-Met–L-Met	% Rel. intensity	Assignment	*m/z* L-Trp–L-Trp	% Rel. intensity	Assignment
161.2	100	[LH] + H^+	281.2	38	[LH] + H^+	391.3	53	[LH] + H^+
183.2	80	[LH] + Na^+	303.2	100	[LH] + Na^+	413.3	100	[LH] + Na^+
199.2	20	[LH] + K^+	319.2	18	[LH] + K^+	781.1	9	2[LH] + H^+
321.2	30	2[LH] + H^+	560.9	29	2[LH] + H^+	803.1	34	2[LH] + Na^+
343.2	30	2[LH] + Na^+	582.9	74	2[LH] + Na^+			
358.9	30	2[LH] + K^+	598.8	18	2[LH] + K^+			
480.6	28	3[LH] + H^+	862.3	20	3[LH] + Na^+			
502.7	50	3[LH] + Na^+						
518.9	15	3[LH] + K^+						
L-Ala–D-Ala			L-Ala–L-Phe			Gly–L-His		
161.2	100	[LH] + H^+	237.2	96	[LH] + H^+	213.2	100	[LH] + H^+
183.4	87	[LH] + Na^+	259.2	100	[LH] + Na^+	235.2	22	[LH] + Na^+
199.2	27	[LH] + K^+	275.2	23	[LH] + K^+	251.2	8	[LH] + K^+
321.1	24	2[LH] + H^+	473.1	29	2[LH] + H^+	424.9	7	2[LH] + H^+
343.2	40	2[LH] + Na^+	495.1	58	2[LH] + Na^+	636.7	14	3[LH] + H^+
359.0	33	2[LH] + K^+	511.0	30	2[LH] + K^+	658.9	6	3[LH] + Na^+
480.7	20	3[LH] + H^+	708.6	6	3[LH] + H^+			
502.6	80	3[LH] + Na^+	730.6	68	3[LH] + Na^+			
518.6	10	3[LH] + K^+						
662.3	28	4[LH] + Na^+						
L-Leu–L-Leu			L-Val–L-Phe			L-Met–L-His		
245.5	100	[LH] + H^+	265.2	42	[LH] + H^+	287.3	100	[LH] + H^+
267.4	18	[LH] + Na^+	287.2	100	[LH] + Na^+	573.0	8	2[LH] + H^+
283.4	4	[LH] + K^+	529.1	37	2[LH] + H^+	858.8	5	3[LH] + H^+
489.3	22	2[LH] + H^+	551.1	85	2[LH] + Na^+			
511.3	8	2[LH] + Na^+	792.6	7	3[LH] + H^+			
732.7	6	3[LH] + H^+	814.5	77	3[LH] + Na^+			
L-Leu–D-Leu			D-Leu–L-Tyr			L-Ala–L-His		
245.3	46	[LH] + H^+	295.4	100	[LH] + H^+	227.2	100	[LH] + H^+
267.3	99	[LH] + Na^+	317.4	54	[LH] + Na^+	249.2	30	[LH] + Na^+
283.3	12	[LH] + K^+	333.4	10	[LH] + K^+	265.1	9	[LH] + K^+
489.2	72	2[LH] + H^+	589.2	92	2[LH] + H^+	452.9	8	2[LH] + H^+
511.2	94	2[LH] + Na^+	611.2	75	2[LH] + Na^+	475.1	5	2[LH] + Na^+
527.0	44	2[LH] + K^+	627.2	35	2[LH] + K^+	678.7	16	3[LH] + H^+
732.8	26	3[LH] + H^+	905.0	97	3[LH] + Na^+			
755.0	100	3[LH] + Na^+	1198.9	26	4[LH] + Na^+			
998.6	96	4[LH] + Na^+						
Gly–L-Leu								
189.2	100	[LH] + H^+						
211.2	64	[LH] + Na^+						
227.3	11	[LH] + K^+						
377.1	27	2[LH] + H^+						
399.1	34	2[LH] + Na^+						
415.0	13	2[LH] + K^+						
564.6	40	3[LH] + H^+						
586.6	49	3[LH] + Na^+						
602.9	32	3[LH] + K^+						

[$CuLH_{-1}$] species, which predominates at pH > 5.5, the copper(II) ion is in a more or less tetragonally-distorted octahedral environment, where the dipeptide is tridentate, with a CuN_2O_2 chromophore, indirectly suggesting that the in-plane coordination of the metal ion is completed by a water molecule.[15] This conclusion appears in perfect agreement with the circular dichroism (CD) data,[12] although this technique permits the observation of the ligand, and not directly the coordination sphere. Among the studied complexes, specific EPR investigations were reported on [Cu(L-Ala–L-Ala)], [Cu(L-Ala–D-Ala)], [Cu(L-Leu–L-Leu)], [Cu(L-Leu–D-Leu)], [Cu(Gly–L-Leu)], [Cu(L-Ala–L-Phe)], [Cu(L-Val–L-Phe)][4e] and [Cu(Gly–L-His)].[14] Thus, at least for these systems, there is further confirmation of the presence of this species.

Thus, the complexes of copper(II) with dipeptides provide a suitable model system to test the response of ESI-MS spectrometry measurements to verify the presence of water molecules in the coordination sphere of metal complexes. Even though there has been a great debate in the literature on the use of this technique directly for this purpose,[16] in a recent paper[10] some of us showed that ESI-

Table 3. Assignment of the ions detected in the ESI-MS mass spectra of the Cu–dipeptide complexes, $C_{Cu} = C_L = 1 \times 10^{-3}$ mol dm^{-3}, pH 5.5. The reported *m*/*z* values refer to the ^{63}Cu species.

m/*z*[a]	% Rel. intensity	Assignment	*m*/*z*[a]	% Rel. intensity	Assignment
L-Ala–L-Ala			L-Val–L-Phe		
244.3	100	$[CuLH_{-1}] + Na^+$	348.3	53	$[CuLH_{-1}] + Na^+$
262.1	45	$[CuLH_{-1}] + Na^+ + H_2O$	366.1	55	$[CuLH_{-1}] + Na^+ + H_2O$
404.4	48	$[CuLH_{-1}] + L^- + H^+ + Na^+$	612.6	57	$[CuLH_{-1}] + L^- + H^+ + Na^+$
465.2	20	$2[CuLH_{-1}] + Na^+$	673.4	91	$2[CuLH_{-1}] + Na^+$
483.0	63	$2[CuLH_{-1}] + Na^+ + H_2O$	937.3	44	$2[CuLH_{-1}] + L^- + H^+ + Na^+$
625.2	40	$2[CuLH_{-1}] + L^- + H^+ + Na^+$			
688.1	40	$3[CuLH_{-1}] + Na^+$			
L-Ala–D-Ala			D-Leu–L-Tyr		
244.3	100	$[CuLH_{-1}] + Na^+$	187.8[b]	45	$[CuLH_{-1}] + 2H^+ + H_2O$
404.4	57	$[CuLH_{-1}] + L^- + H^+ + Na^+$	325.9[b]	19	$[CuLH_{-1}] + L^- + 3H^+$
465.2	7	$2[CuLH_{-1}] + Na^+$	356.3	13	$[CuLH_{-1}] + H^+$
483.1	28	$2[CuLH_{-1}] + Na^+ + H_2O$	377.9	15	$[CuLH_{-1}] + Na^+$
625.2	28	$2[CuLH_{-1}] + L^- + H^+ + Na^+$	650.4	40	$[CuLH_{-1}] + L^- + 2H^+$
664.0	10	$3[CuLH_{-1}] + H^+$	672.4	78	$[CuLH_{-1}] + L^- + H^+ + Na^+$
686.1	13	$3[CuLH_{-1}] + Na^+$			
L-Leu–L-Leu			L-Trp–L-Trp		
324.0	65	$[CuLH_{-1}] + H^+ + H_2O$	266.3[b]	33	$[CuLH_{-1}] + Cu^{++} + H_2O$
328.3	100	$[CuLH_{-1}] + Na^+$	474.1	100	$[CuLH_{-1}] + Na^+$
572.6	74	$[CuLH_{-1}] + L^- + H^+ + Na^+$	925.2	54	$2[CuLH_{-1}] + Na^+$
693.3	87	$2[CuLH_{-1}] + Na^+$			
877.3	67	$2[CuLH_{-1}] + L^- + H^+ + Na^+$			
L-Leu–D-Leu			Gly–L-His		
171.7[b]	60	$[CuLH_{-1}] + 2H^+ + 2H_2O$	195.3[b]	59	$[CuLH_{-1}] + Cu^{++} + 3H_2O$
275.9[b]	46	$[CuLH_{-1}] + L^- + 3H^+$	274.2	78	$[CuLH_{-1}] + H^+$
306.3	38	$[CuLH_{-1}] + H^+$	292.1	26	$[CuLH_{-1}] + H^+ + H_2O$
324.2	11	$[CuLH_{-1}] + H^+ + H_2O$	314.3	86	$[CuLH_{-1}] + Na^+ + H_2O$
328.3	47	$[CuLH_{-1}] + Na^+$	547.1	35	$2[CuLH_{-1}] + H^+$
572.5	66	$[CuLH_{-1}] + L^- + H^+ + Na^+$	569.2	75	$2[CuLH_{-1}] + Na^+$
855.4	66	$2[CuLH_{-1}] + L^- + 2H^+$			
877.3	39	$2[CuLH_{-1}] + L^- + H^+ + Na^+$			
Gly–L-Leu			L-Ala–L-His		
143.7[b]	98	$[CuLH_{-1}] + 2H^+ + 2H_2O$	202.2[b]	56	$[CuLH_{-1}] + Cu^{++} + 3H_2O$
219.8[b]	27	$[CuLH_{-1}] + L^- + 3H^+$	288.3	100	$[CuLH_{-1}] + H^+$
250.3[b]	32	$2[CuLH_{-1}] + 2H^+$	306.1	31	$[CuLH_{-1}] + H^+ + H_2O$
272.3	100	$[CuLH_{-1}] + Na^+$	575.1	65	$2[CuLH_{-1}] + H^+$
289.9	45	$[CuLH_{-1}] + Na^+ + H_2O$	597.2	69	$2[CuLH_{-1}] + Na^+$
L-Met–L-Met			L-Met–L-His		
320.3	88	$[CuLH_{-1}] - CO_2 + Na^+$	348.3	100	$[CuLH_{-1}] + H^+$
705.1	75	$2[CuLH_{-1}] + Na^+$	404.1	42	$[CuLH_{-1}] + K^+ + H_2O$
1046.0	24	$3[CuLH_{-1}] + Na^+$	695.1	22	$2[CuLH_{-1}] + H^+$
L-Ala–L-Phe					
320.2	18	$[CuLH_{-1}] + Na^+$			
338.0	92	$[CuLH_{-1}] + Na^+ + H_2O$			
534.3	20	$[CuLH_{-1}] + L^- + 2H^+$			
556.4	65	$[CuLH_{-1}] + L^- + H^+ + Na^+$			
617.3	96	$2[CuLH_{-1}] + Na^+$			

[a] $z = 1$, except if indicated differently. [b] $z = 2$.

MS spectra of copper complexes, all having oxygen and nitrogen as donor atoms, give evidence of equatorial water molecules.

The ESI-MS spectra show the presence of water molecules in the coordination sphere of metal complexes for all the systems investigated here with the exception of L-Met–L-Met (Table 3), which, as mentioned above, shows a peculiar fragmentation pattern. The species with a water molecule were detected either as $[CuLH_{-1}] + H^+ + H_2O$ and $[CuLH_{-1}] + Na^+ + H_2O$ or $[CuLH_{-1}] + K^+ + H_2O$, or as doubly charged ions $[CuLH_{-1}] + 2H^+ + H_2O$. For the diastereoisomeric couple L-Ala–L-Ala, L-Ala–D-Ala (Figure 3), a dimeric species $2[CuLH_{-1}] + Na^+ + H_2O$ was also detected, whereas L-Trp–L-Trp, Gly–L-His and L-Ala–L-His also show the presence of peaks resulting from the double charged ion $[CuLH_{-1}] + Cu^{++} + nH_2O$, where $n = 1$ for the

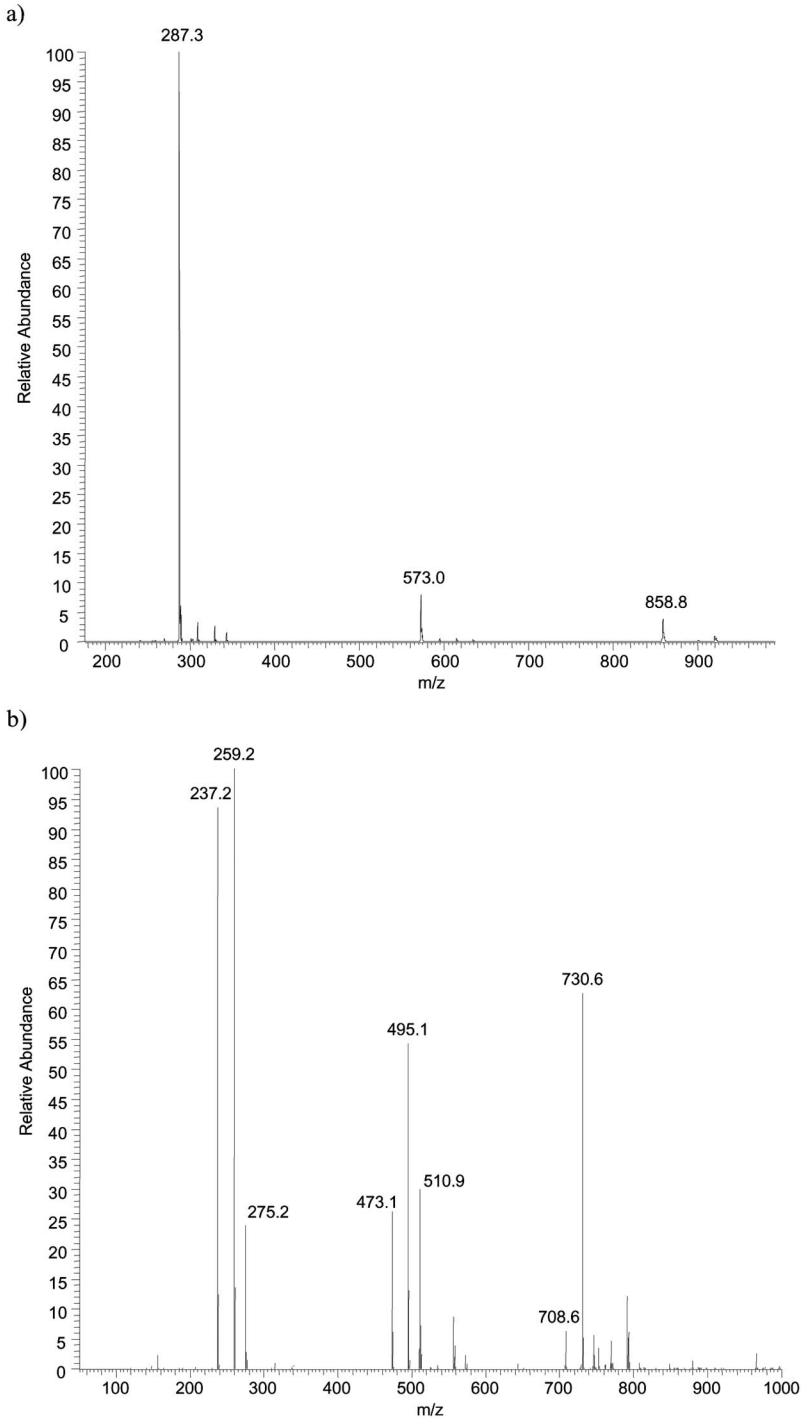

Figure 2. (a) ESI mass spectra of the L-Met–L-His free dipeptide in aqueous solution; (b) ESI mass spectra of the L-Ala–L-Phe free dipeptide in aqueous solution.

first dipeptide and $n = 3$ for the remaining two. If we consider that the classical coordination geometry of copper(II) complexes is a tetragonally-elongated octahedron, as a result of the Jahn–Teller effect, it appears that only water molecules coordinated to copper in the equatorial plane will be detected.[10] The ESI-MS data reported here confirm this behaviour. In fact, only a water molecule is detected by ESI-MS spectra in all the complexes investigated here with the exception of $[Cu(Gly–L\text{-}His)H_{-1}] + Cu^{++} + 3H_2O$ (m/z = 195.3) and $[Cu(L\text{-}Ala–L\text{-}His)H_{-1}] + Cu^{++} + 3H_2O$ (m/z = 202.2), because of the presence of a second copper(II) ion, which can bond other water molecules in its in-plane coordination sphere.

With regard to L-Met–L-Met, while the mass spectrum of the free dipepide shows the typical signals from the monomeric species (either as protonated species or as a sodium

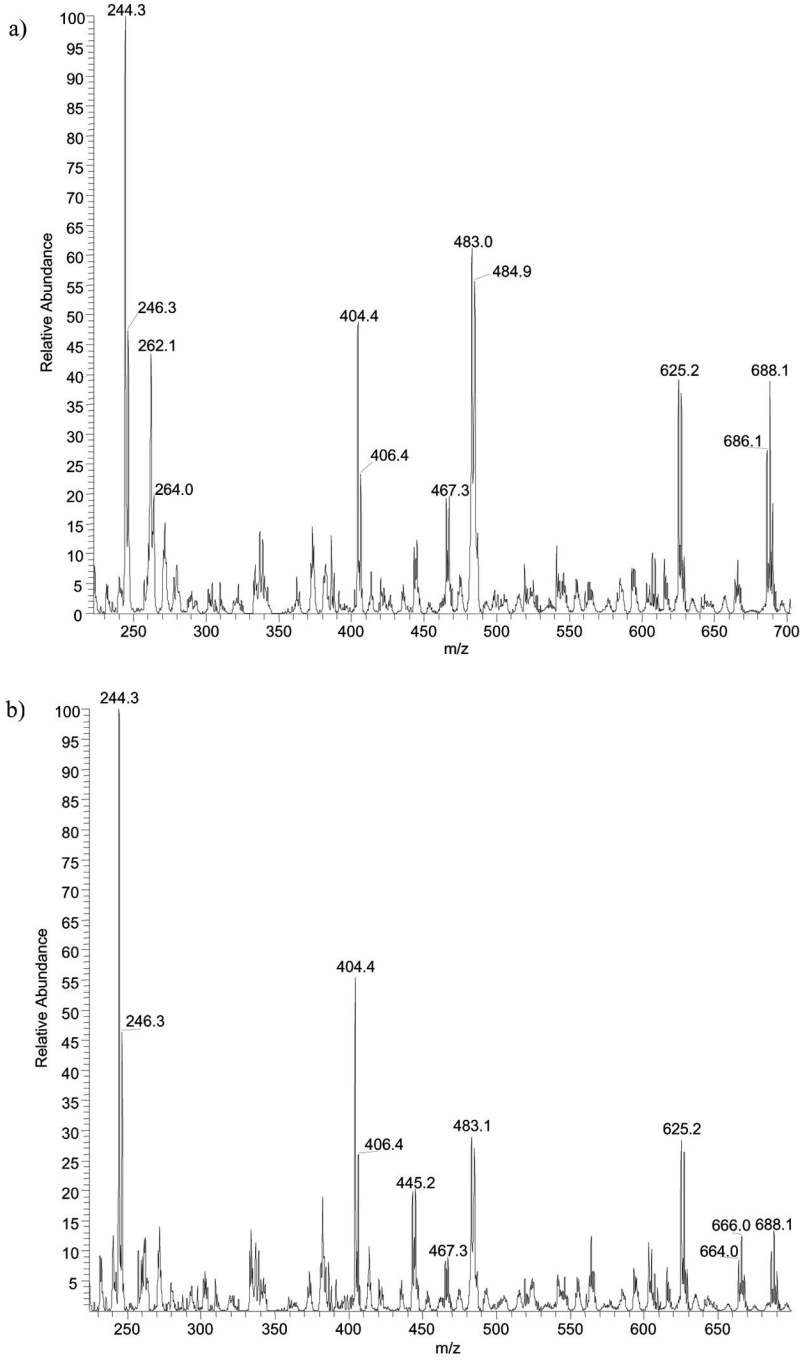

Figure 3. (a) ESI mass spectra of the [Cu(L-Ala–L-Ala)] complex; (b) ESI mass spectra of the [Cu(L-Ala–D-Ala)] complex.

or potassium adduct), as well as the signals from the corresponding dimeric and trimeric monocationic ions (Table 2), the spectrum of the copper(II) complexes (Figure 4) provides three main intense peaks at m/z = 320.3, 705.1 and 1046.0 (see Table 3) from the $[CuLH_{-1}] + Na^+ - CO_2$, $2[CuLH_{-1}] + Na^+$ and $3[CuLH_{-1}] + Na^+$ species, respectively. It has been reported, in fact, that copper(II) promotes radical losses and decarboxylation depending on stereochemical side chain effects.[8] As can be seen in Figure 4, in addition to the main peaks at m/z = 320.3, 705.1 and 1046.0 there are other peaks at m/z = 322.3, 707.1 708.1, 1048.0 and 1050.0. These clusters of signals arise from the ^{63}Cu–^{65}Cu isotopic distribution, as demonstrated for the couple at m/z = 320.3, 322.3 by the sequential MS^2 experiment reported in Figure 5. Both species, in fact, lose the same fragment at m/z = 61 corresponding to $^{\cdot}CH_2$–S–CH_3, thus demonstrating that these signals belong to the $[CuLH_{-1}] + Na^+ - CO_2$ complex, as also confirmed by a simulation process that reproduces a spectrum strictly superimposable with the experimental one. Analogously, the other clusters of peaks (i.e. 705.1, 707.1, 708.1 and 1046.0, 1048.0, 1050.0) are also typical of the ^{63}Cu–^{65}Cu iso-

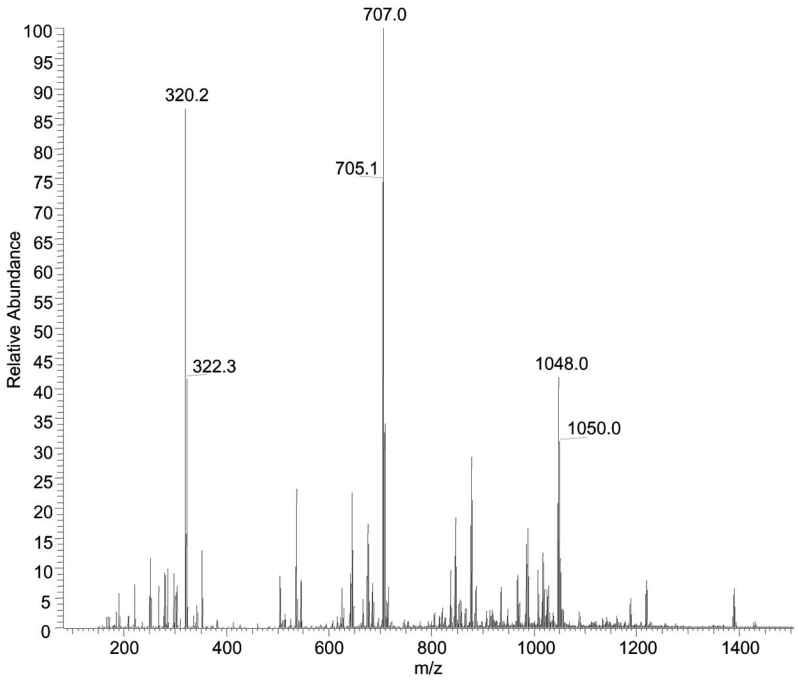

Figure 4. ESI mass spectra of the [Cu(L-Met–L-Met)] complex.

topic distribution, according to the presence of two and three copper ions in the species $2[CuLH_{-1}] + Na^+$ and $3[CuLH_{-1}] + Na^+$, respectively.

Conclusions

The structures of the species formed in solution by copper(II) and some dipeptides have been elucidated by combining information from different techniques such as potentiometry, UV/Vis spectroscopy and ESI-MS. Although mass spectrometry data indicate the formation of more species than that reported in the thermodynamic investigations, the ESI-MS technique actually allows the transfer of the main species from the solution state into the gas phase, where the mass assignment, together with MS^2 experiments and simulation processes, give direct information on the metal complex stoichiometry. Furthermore, even though complexes of transition metal ions are known to undergo redox reactions during electrospray ionization (ESI),[17] also verified by some of us in a previous investigation,[18] for the systems studied here, probably owing to the low temperature in the ion transfer tube of the mass spectrometer and to the low capillary voltage potential values used, no redox reactions and unusual reduced neutral metal loss occurred.

A peculiar behaviour was observed for the copper(II) complex with L-Met–L-Met, showing once more how an otherwise very soft technique such as ESI-MS can, however, for very specific systems, induce an unpredictable fragmentation.

Finally, ESI-MS data confirm and directly ascertain that in the copper(II)–dipeptide complexes the ligands behave in a tridentate manner and the central ion completes the in-plane coordination by a water molecule, as already pointed out by the thermodynamic and spectroscopic studies. Hence, the ESI-MS technique appears to be a suitable tool to investigate the coordination sphere of metal complexes and to evidence water molecules coordinated to the central metal ion. This study, in fact, demonstrates that, for several model systems, good correlations are found between experimental data obtained by ESI-MS and other techniques, such as thermodynamic investigations and UV/Vis and EPR spectroscopy. We hope that this paper helps to encourage inorganic chemists to use this technique more frequently, also considering that this fundamental approach may aid in the study of more complex systems, such as complexes of copper(II) with biological ligands that cannot easily be characterized by other techniques.

Experimental Section

Materials: L-Ala–L-Ala, L-Ala–D-Ala, L-Ala–L-His, L-Ala–L-Phe, Gly–L-Leu, L-Leu–L-Leu, L-Leu–D-Leu, D-Leu–L-Tyr, L-Met–L-Met, L-Met–L-His, L-Trp–L-Trp and L-Val–L-Phe were purchased from BACHEM, Heidelberg, Germany, whereas Gly–L-His was obtained from Sigma–Aldrich, St.Louis, MO and their solutions were prepared by dissolving weighted amounts in water. Copper(II) sulfate stock solutions were standardized as recommended by Flaschka.[19] Cu^{II} dipeptide solutions were obtained by mixing the appropriate volumes of metal and ligand stock solutions and adjusting the pH of the resulting solutions to the values of 4.2 or 5.5 by adding the appropriate volume of a KOH stock solution. The concentrations of copper(II) and of the selected dipeptide were 1.0×10^{-3} mol dm^{-3} in all cases. All solutions were prepared with Milli-Q water.

Visible Optical Spectroscopy: Visible absorption spectra were recorded using a 1 cm quartz cell at 25 °C by using a Varian UV/Vis near IR Cary 500 Scan spectrophotometer in single-beam mode.

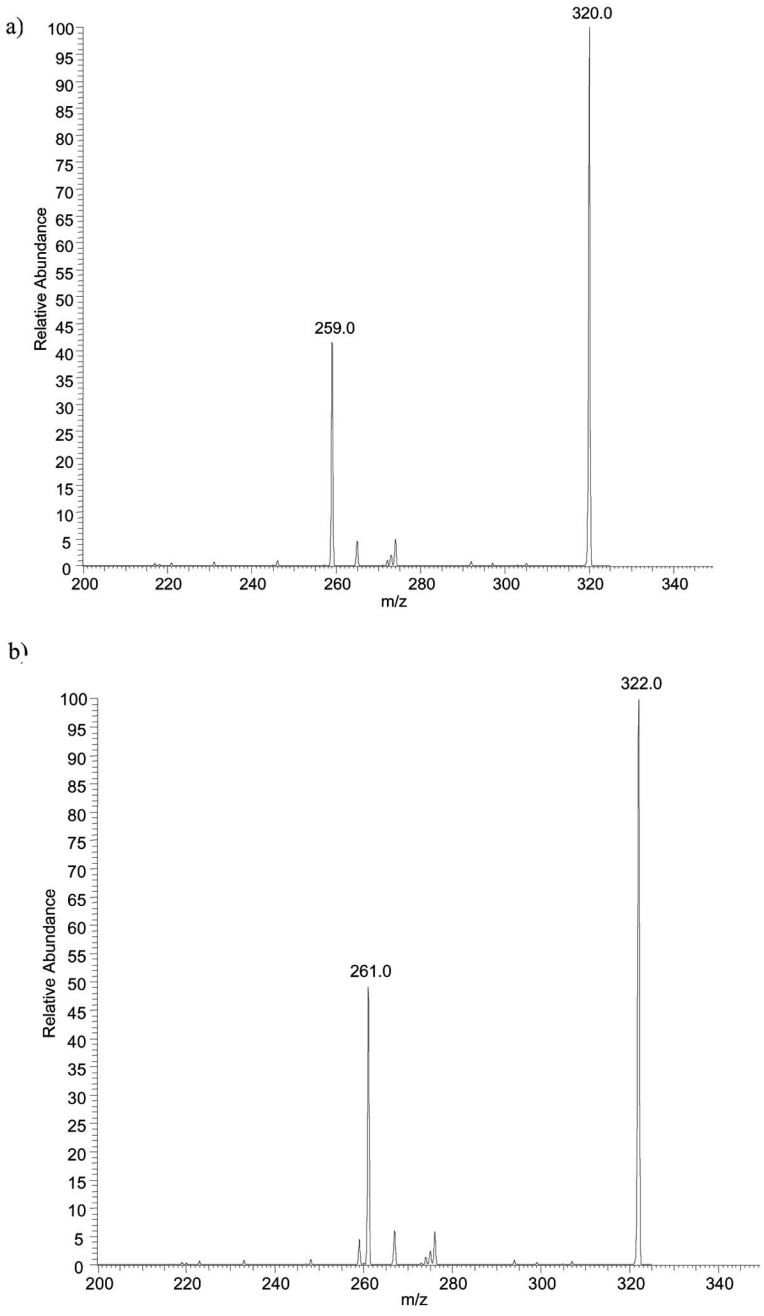

Figure 5. (a) MS^2 experiment on the 320.3 peak of the [Cu(L-Met–L-Met)] complex; (b) MS^2 experiment on the 322.3 peak of the [Cu(L-Met–L-Met)] complex.

Electrospray Mass Spectrometry Spectra: Mass spectra were recorded with a Thermo Finnigan LXQ linear ion trap electrospray mass spectrometer (San Jose, CA) equipped with a 45 probe-degrees ESI source. Solutions of free dipeptides and copper–dipeptide complexes were introduced into the ESI source through a fused-silica filter (100-μm i.d.) from a Unimetrics 500 μL syringe. The experimental conditions for all the spectra, acquired in positive mode, were optimized as follows: needle source voltage 3.95 kV, flow rate 5 $\mu L\,min^{-1}$, nitrogen sheath gas flow rate 35.0 (arbitrary units), capillary voltage 11.5 V, capillary temperature 70 °C, tube lens voltage 74.92 V. For all MS/MS experiments, the source potentials and cone position were optimized for maximum ion abundance.

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