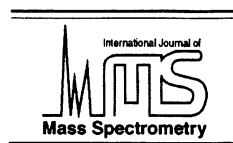




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Complexes of L-histidine with Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} studied by electrospray ionization mass spectrometry

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Abstract

As a first building block towards the study of the active site of numerous metalloproteins, 1:1 complexes of L-histidine with Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , were studied by electrospray ionization MS/MS. It was found that either solvated or nonsolvated complexes could be formed depending upon source conditions. Low cone voltage conditions enabled the formation of four- to six-coordinated complexes that were a direct reflection of the species in solution. Using precursor ion scans, it was shown that these ions were the major precursors of the 1:1 L-histidine/metal complexes. The greater number of collisions existing with higher cone voltages resulted in the loss of labile ligands, and in the case of Cu^{2+} in gas phase redox reactions to give MCu^+ adducts. The fragmentations of 1:1 L-histidine/metal(II) complexes (namely, $[(\text{M} - \text{H})^- \text{Cat}^{2+}]$, using an ionic notation), were then studied by collisionally induced dissociation. All metal complexes were found to undergo decarboxylation. Although for Cu(II) and Zn(II), it is thought that the decarboxylated complexes, unstable, immediately dissociate by losing a reduced cation Cat^0 , giving an immonium ion I_{His} (m/z 110). For Fe(II), Co(II), and Ni(II), the next fragmentation reactions of the stable $[(\text{M} - \text{H})^- \text{Cat}^{2+} - \text{CO}_2]$ complexes could be either loss of NH_3 , $\text{CH}_2 = \text{NH}$, HCN, or Cat^0 in various proportions. Loss of the neutral metal was observed eventually in the fragmentation sequence. This was interpreted in terms of a gradual reduction of the transition metal cation, through ligand-to-metal electron transfers, related to the diminution of the coordination sphere brought on by the fragmentations. For copper, which was the only case when a reduction to MCat^+ was observed, comparison of the fragmentations of Cu(II) and Cu(I) complexes showed the oxidation state influences the reactivity. Several mechanisms, consistent with deuterium labeling, are suggested and discussed. (Int J Mass Spectrom 185/186/187 (1999) 11–23) © 1999 Elsevier Science B.V.

Keywords: Amino acids; Transition metal complexes; Gas phase; Electron transfer; Collisionally induced dissociation

1. Introduction

Recently, there has been a growing interest in the study of interactions of transition metal cations with organic and bioorganic compounds in the gas phase

[1–3]. As in earlier research on other metals (alkali and alkali-earth) [4–10], the original idea behind these studies is twofold. First, transition metal cations constitute a fascinating field of investigation for gas-phase chemistry as they appear to have peculiar reactivities among which C–C and C–H bond activation and remote functionalization are two of the most striking characteristics [1]. The second motive arises from biology. In fact, complexes of transition metals

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Dedicated to Professor Michael T. Bowers on the occasion of his 60th birthday.

and amino acid residues are at the core of numerous biologic processes, such as dioxygen transport, electron transfer, and oxidation or hydrogenation of target compounds [11]. In these processes, the enzymes' active sites are highly specific and often involve one or several histidine residues interacting with divalent transition metal cations, most notably Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} .

Aqueous-phase binding interactions of amino acids and peptides with metals have been the object of numerous studies [12,13]. In fact, several principles are frequently used to refer to and predict stabilities and binding preferences: the Irving-Williams series [14] and the hard-soft acid-base concept. The Irving-Williams series state the complex stabilities should typically follow the order $\text{Ca}^{2+} < \text{Mg}^{2+} < \text{Mn}^{2+} < \text{Fe}^{2+} < \text{Co}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} > \text{Zn}^{2+}$. The metals in our study are therefore the ones that should form the most stable complexes. Concerning ligand preferences, in the hard-soft acid-base concept, Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} can neither be classified as soft nor hard but are rather referred to as "borderline." They can therefore interact with a variety of ligands with a predilection for nitrogen binding sites, like the one provided by the disubstituted, pyridinellike, nitrogen of the imidazole of the side chain of L-histidine. By combining both solution phase and gas phase in its process, electrospray ionization mass spectrometry (ESMS) offers a unique opportunity to study both the intrinsic properties and the effect of the solvent for these metallic complexes. Several studies [15] have shown that, with careful sample preparation, a strong correlation exists between mass spectrometric results and the species present in solution. For example, Yu et al. [16] used ESMS to assess the complexation of zinc and cadmium of metallothioneins. Similarly, Hu et al. [17] studied the complexation of calcium binding proteins. Both studies, however, insisted on the necessary adjustment of solution and source parameters to enhance specific protein–ligand interactions.

Combining both solution-phase properties and gas-phase chemistry should also be interesting for structure elucidation. This has been investigated recently by several groups [18–20]. Gatlin and Turecek have used the stabilizing properties of diimine ligand 2,2'-

bipyridyl to obtain stable complexes of Cu(II) and other late transition-metal cations with amino acids [21–24] and have extended their studies to dipeptides [25], and gas-phase redox reactions [26,27]. Hu and Loo [28], have investigated the influence of Zn^{2+} , Cu^{2+} , Ni^{2+} , and Co^{2+} coordination on the collisionally induced dissociations (CIDs) of histidine-containing peptides. The CID spectra of the complexes with zinc, nickel and cobalt, showed, nearly exclusively, dissociations correlated with the histidine sites, thereby suggesting a metal coordination on histidine residues. The complexes with copper, by contrast, showed a different fragmentation pattern, suggesting an interaction with the C-terminus. This latter observation is surprising considering that according to the Irving-Williams series, Cu^{2+} should have the strongest binding. This might arise from a difference between gas-phase and aqueous-phase chemistry. On that matter, Xu et al. [29], in a study of copper-glycine solutions by electrospray ionization, have argued that although some ions result from a direct desolvation process, others are rather a result of gas-phase reactions.

We present here a study of 1:1 complexes of L-histidine with the divalent transition metal cations Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} . Particular attention was paid to their formation in relationship with the ions preformed in solution. We then examined the CIDs of these complexes. Their different fragmentation patterns are compared in order to possibly correlate them with the cations' electronic structures and binding properties. For copper, a gas-phase redox reaction was observed that produced a reduced complex of Cu(I), MCu^+ . The gas-phase reactivity of Cu(I) and Cu(II) coordinated with histidine is therefore also studied and discussed. Some fragmentation mechanisms consistent with deuterium labeling are discussed.

2. Experimental

2.1. Materials

L-Histidine, as well as all the metallic salts used ($\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, $\text{CoSO}_4 \cdot n\text{H}_2\text{O}$, $\text{NiSO}_4 \cdot 7 \text{H}_2\text{O}$,

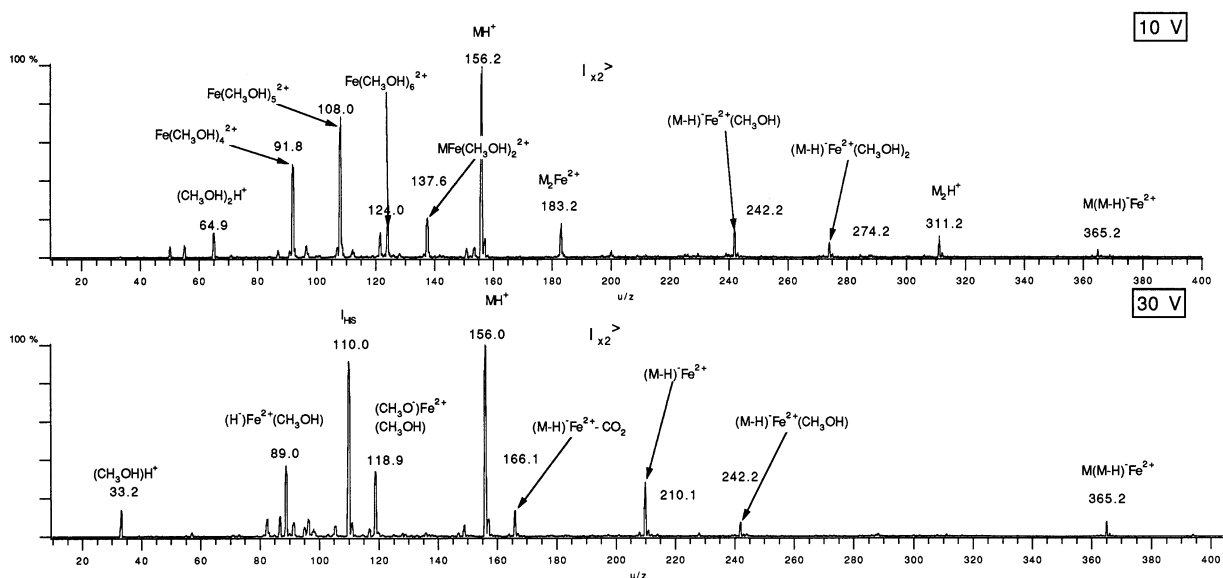


Fig. 1. Direct mass spectrometry spectra of L-histidine and FeSO_4 in $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ 1/1 v/v solvent, at cone voltage 10 V and 30 V.

$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, and $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$), were purchased from Aldrich Chemical (St Quentin Fallavier, France) and used as received without any further purification. Sample solutions were prepared by dissolving one equivalent of amino acid and one equivalent of metallic salt in a 50/50 v/v aqueous methanol solvent to achieve a 500 μM concentration in each component. Concentrations in this order of magnitude are typical for amino acid/metal complexes studies [21–27,29]; measurements done at $\sim 100 \mu\text{M}$ yielded similar results with lower intensities and signal/noise ratio. All solvent used were high-performance liquid chromatography (HPLC) grade. H/D exchanges were carried out by using a 50/50 v/v D_2O and MeOD mixture as solvent. All solutions were infused at a flow rate of 5 $\mu\text{L}/\text{min}$ by a Harvard Apparatus (Southnatic, MA) syringe pump.

2.2. Mass spectrometry

All mass spectra were acquired on a Quattro II (Micromass, Manchester, UK) triple quadrupole electrospray mass spectrometer. Typical optimized values for the source parameters were capillary 2–3 kV, counter electrode 0.3–0.5 kV, temperature 80 $^\circ\text{C}$, RF

lens 0.7 V, skimmer 1.5 V, skimmer lens offset 5 V. Direct mass spectrometry experiments were carried out at several cone voltages ranging from 10 to 150 V. CID was performed with argon as target gas. For all MS/MS experiments, the collision energy was set at 20 eV, and the gas pressure optimized to achieve a 30–50% transmittance of the precursor ion. Resolution and cone voltage were adjusted so as to optimize the intensity of the resulting signal and precursor ion selection. Precursor ion spectra were recorded using a pressure in the same order of magnitude as for the fragment ion scans, and with several cone voltages.

3. Results and discussion

3.1. Formation of unsolvated L-histidine-metal 1:1 complexes ($[(M-H)^- \text{Cat}^{2+}]$ or MCat^+)

For all the histidine/metallic salt mixtures tested, the mass spectra recorded at very low cone voltages were found to be very informative on the preformed ions present in solution. Fig. 1 illustrates the mass spectra of histidine in mixture with FeSO_4 at a cone voltage of 10 and 30 V. Several peaks, particularly in

the low mass region, rapidly disappear as cone voltage is increased. These peaks could be assigned as complexes of the metallic ion solvated by methanol. Namely, the ions observed with FeSO_4 were $[\text{Fe}(\text{CH}_3\text{OH})_4]^{2+}$ (m/z 92), $[\text{Fe}(\text{CH}_3\text{OH})_5]^{2+}$ (m/z 108), and $[\text{Fe}(\text{CH}_3\text{OH})_6]^{2+}$ (m/z 124). These ions, whose masses were shifted by the expected value when MeOD was used instead of ordinary methanol, are in agreement with an octahedral structure for the solvated metallic ions; usual distortions from the O_h symmetry make either one or two ligands more labile, making possible the presence of five- and four-coordinated complexes. Similar complexes $[\text{Cat}(\text{CH}_3\text{OH})_n]^{2+}$ were observed for cobalt and nickel. For copper and zinc different types of ions of the form $[\text{Cat}(\text{CH}_3\text{O})(\text{CH}_3\text{OH})_n]^+$ ($n_{\text{Cu}} = 1-3$; $n_{\text{Zn}} = 1-4$) and $[\text{Cat}(\text{OH})(\text{CH}_3\text{OH})_n]^+$ ($n_{\text{Zn}} = 1-4$) were observed. This has been reported before by Blades et al. [30]. In their early electrospray study of ion-molecule clusters involving doubly charged ions, they report on water clusters $[\text{Cat}(\text{H}_2\text{O})_n]^{2+}$. They observed that as n decreases the ions undergo charge reduction, and a second type of clusters appears, where, formally, a proton is substituted by the metal cation: $[\text{CatOH}(\text{H}_2\text{O})_n]^+$. Below a value $n = 4$ for iron, cobalt and nickel, $n \approx 5$ for zinc, only this second type of cluster ions is observed (for copper they observe reduced Cu(I) species such as $[\text{Cu}(\text{H}_2\text{O})_n]^+$). This is consistent with our observations, except that, here, the minimum n value for zinc and methanol would be around 6, because only the second type of cluster ions were observed. Could this “charge reduction” phenomenon have occurred in the bulk phase? It is not unlikely, because metal ions in solution are known to increase the acidity of labile hydrogens. This effect varies according to the metal, but is greater for zinc and copper than for any of the other metals tested. Consequently, the deprotonation related to the charge reduction observed for copper and zinc could very well have occurred in solution. Apart from this charge reduction issue, the mass spectra at low cone voltage appeared to give a direct account of the ions present in solution, and certainly indicated that coordination numbers from 6 to 4 were stable in these conditions.

Several complexes with histidine also distinctively appeared in the spectra at low cone voltages. These ions are listed in Table 1. Note that we have used an ionic notation $[(\text{M} - \text{H})^- \text{Cat}^{2+}]$, this is a formal notation that does not imply any interpretation by us. Although it emphasizes the formal oxidation state of the cation, it does not give any information as to a covalent or noncovalent nature of the complex or if it is an even electron ion or a radical cation (which should be the case for Co^{2+} and Cu^{2+}). Histidine is potentially a tridentate ligand, with two binding sites on the amino acid chain (amine and carboxylic groups) and one site on the side chain (the imino nitrogen, in δ or ϵ , depending upon the tautomer). An ion such as $[(\text{M} - \text{H})^- \text{Cat}^{2+}(\text{CH}_3\text{OH})_3]$ could therefore very well be six-coordinated or five-coordinated. This hypothesis was supported by the fact that the metal/solvent clusters observed in the same conditions (low cone voltage) were four- to six-coordinated, which corresponds to a filled coordination sphere. A structure similar to the one in aqueous phase [31–33] is therefore probable (Scheme 1).

As cone voltage was increased, labile ligands were lost and lower coordinated complexes remained, both for the metal/solvent clusters and the histidine complexes (Fig. 1). Typically, this change of spectra must have resulted from collisions in the nozzle-skimmer region. The metal/solvent clusters were observed to undergo a charge reduction similar to the one evidenced by Blades et al. [30]. As for the L-histidine-metal 1:1 complexes, in order to verify that the lower-coordinated species really resulted from fragmentations of higher-coordinated ones, precursor ion scans were recorded at three different cone voltages (10, 30 and 45 V). The right-hand column of Table 1 indicates the cone voltage at which each ion could be detected in the precursor ion scans of $[(\text{M} - \text{H})^- \text{Cat}^{2+}]$. Fig. 2 illustrates the breakdown curves of the relative intensities of the most significant parent ions detected in the precursor ion scan of $[(\text{M} - \text{H})^- \text{Fe}^{2+}]$, depending on the cone voltage used (10–60 V). At low cone voltages, $[(\text{M} - \text{H})^- \text{Fe}^{2+}(\text{CH}_3\text{OH})_2]$ (m/z 274) and $[(\text{M} - \text{H})^- \text{Fe}^{2+}(\text{CH}_3\text{OH})]$ (m/z 242) distinctively appeared along with lower intensity $[(\text{M} - \text{H})^- \text{Fe}^{2+}(\text{CH}_3\text{OH})_3]$

Table 1
Metallic complexes with histidine (here noted as M) detected in the electrospray ionization spectra at low cone voltage (10 V)

Metal	Direct mass spectra at a cone voltage of 10 V			Presence in precursor ion scans (cone voltage) (V)
	<i>m/z</i>	Relative intensity ^a	Ionic notation	
Fe ²⁺	242.1	18	(M – H) [–] Fe ²⁺ (CH ₃ OH)	30, 10
	274.2	15	(M – H) [–] Fe ²⁺ (CH ₃ OH) ₂	10
	228.0	1	(M – H) [–] Fe ²⁺ (H ₂ O)	45 (weak)
	183.2	18	M ₂ Fe ²⁺	10
	137.6	12	MFe ²⁺ (CH ₃ OH) ₂	10 (weak)
Co ²⁺	245.1	15	(M – H) [–] Co ²⁺ (CH ₃ OH)	30, 10
	277.2	15	(M – H) [–] Co ²⁺ (CH ₃ OH) ₂	10
	368.2	5	(M – H) [–] Co ²⁺ M	45 (weak)
	184.7	22	M ₂ Co ²⁺	—
	138.9	5	MCo ²⁺ (CH ₃ OH) ₂	—
Ni ²⁺	244.0	19	(M – H) [–] Ni ²⁺ (CH ₃ OH)	30, 10
	276.0	25	(M – H) [–] Ni ²⁺ (CH ₃ OH) ₂	10
	308.1	3	(M – H) [–] Ni ²⁺ (CH ₃ OH) ₃	10 (weak)
	367.2	2	(M – H) [–] Ni ²⁺ M	—
	184.0	14	M ₂ Ni ²⁺	10 (weak)
Cu ²⁺	249.0	42	(M – H) [–] Cu ²⁺ (CH ₃ OH)	^b
	281.3	4	(M – H) [–] Cu ²⁺ (CH ₃ OH) ₂	
	315.1	3	(M – H) [–] Cu ²⁺ (CH ₃ OH) ₃	
	372.2	7	(M – H) [–] Cu ²⁺ M	
	186.6	60	M ₂ Cu ²⁺	
Zn ²⁺	141.1	75	MCu ²⁺ (CH ₃ OH) ₂	
	250.0	10	(M – H) [–] Zn ²⁺ (CH ₃ OH)	10, 30
	282.1	2	(M – H) [–] Zn ²⁺ (CH ₃ OH) ₂	10
	373.1	2	(M – H) [–] Zn ²⁺ M	45 (weak)
	141.5	8	MZn(CH ₃ OH) ₂ ⁺	—
187.0	5	M ₂ Zn ²⁺	—	

Precursor ions scans were recorded for the (M – H)[–] Cat²⁺ complexes (not present in significant quantities at low cone voltage), and gave different results depending upon the cone voltage applied (10, 30, or 45 V). The right hand column lists the cone voltage values for which the corresponding ions were present.

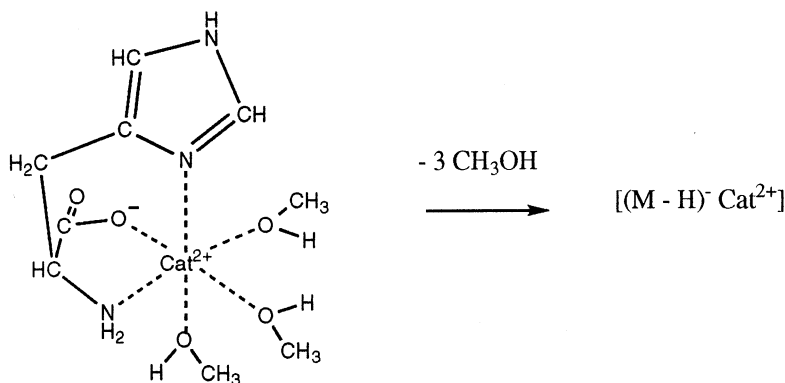
^aThe base peak of the spectra is the protonated molecule of histidine *m/z* 156, except for nickel and zinc for which the solvent ion (CH₃OH)₂H⁺ *m/z* 65 is slightly higher.

^bThe (M – H)[–] Cu²⁺ ion was accompanied by MCu⁺. Precursor ions scans were recorded on MCu⁺.

(*m/z* 306) and [MFe(CH₃OH)₃]²⁺ (*m/z* 153.5) complexes. At higher cone voltages (30–60 V), the relative intensity of the precursor ions dropped and only low intensity [(M – H)[–] Fe²⁺ (CH₃OH)] and [(M – H)[–] Fe²⁺ (H₂O)] could be detected. Cobalt and nickel ions showed very similar behavior to that of iron (Table 1). Only very low intensity signals could be recorded for the 2:1 L-histidine-metal complexes, the major process of formation of [(M – H)[–] Cat²⁺] complexes could therefore be attributed to a decoordination of labile solvent ligands.

For copper, slightly different phenomena were observed. The mass spectra at low cone voltages showed metallic complexes of analogous structure as

before (Table 1). As the cone voltage was increased, however, an MCu⁺ ion was detected in addition to the expected [(M – H)[–] Cu²⁺]. Fragment ion scans of the solvated complex [(M – H)[–] Cu²⁺ (CH₃OH)] yielded [(M – H)[–] Cu²⁺] and MCu⁺ in relatively similar relative intensity (Table 2). A gas-phase reduction, which might involve an innersphere electron transfer from the methanol to Cu²⁺, must therefore have taken place to produce CH₃O[•] and MCu⁺. Similar radical losses have been observed before from bis(peptide)-metal complexes in the negative mode [34]. A precursor ion scan of MCu⁺, recorded at 20 V, yielded essentially three ions: [(M – H)[–] Cu²⁺ (CH₃OH)] (*m/z* 249), [(M – H)[–] Cu²⁺M] (*m/z*



Scheme 1.

372), and M_2Cu^+ (m/z 373) (data not shown). In addition, the fragment ion spectrum of M_2Cu^{2+} (Fig. 3) showed MCu^+ as fragment ion. The expected complementary ion M^+ was not observed, but all other fragment ions were radical cations: $(M - CO_2)^+$ and RH^+ (where R is the side chain).

In solution, the nature and geometry of the ligands around copper are known to influence Cu(I)/Cu(II) reduction potential [35]. The Cu(I) oxidation state favors soft ligands, and being a closed shell d^{10} ion, prefers tetrahedral four-coordinate or three-coordinated geometries. By contrast, the Cu(II) oxidation state are rather square-planar or octahedral. Gas-phase reduction reactions involving metal cations have been reported before, in particular for copper by Turecek and co-workers in their studies on ternary peptide-

metal complexes $[Cu(II)(M - H)(bpy)]^+$ [22,23,25–27]. The reduction, however, appears later in the fragmentation process as the 2,2'-bipyridyl stabilizes the Cu(II) oxidation state. Here, the early loss of labile ligands might induce a geometry more adapted to the Cu(I) oxidation state, thus making an electron transfer reaction favorable.

3.2. Fragmentation of *L*-histidine-metal 1:1 complexes in the gas phase

The $[(M - H)^- Cat^{2+}]$ ions showed two different fragmentation behaviors (Table 3). On one hand, CID spectra of the group VIII metal complexes showed loss of CO_2 as the major fragment ion. This decarboxylation is not new because it has been frequently

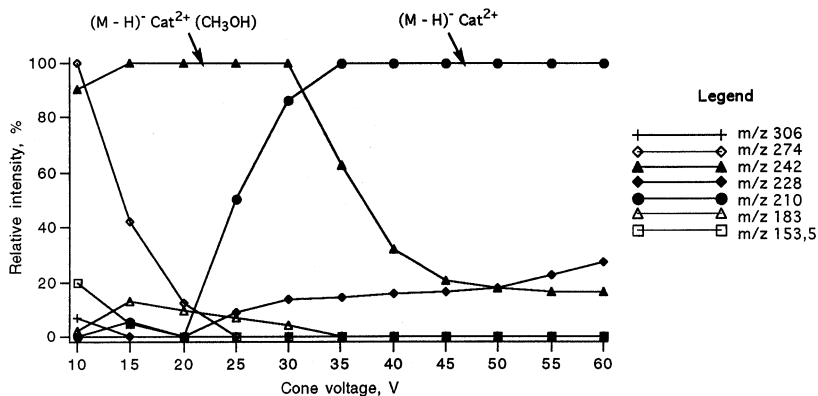


Fig. 2. Relative intensities of the precursor ions of the $(M - H)^- Fe^{2+}$ depending on the cone voltage (precursor ions scans recorded with a 20 eV collision energy, with argon as collision gas $P = 3.9 \cdot 10^{-2}$ Pa).

Table 2

List of the fragment ions observed in the collisionally induced decomposition spectra of the solvated metallic complexes of histidine

Fragment ion scans		m/z (u)	Relative Intensity (%)	H/D exchange
Precursor ion	$(M - H)^- Fe^{2+} (CH_3OH)$	242	67	+4
Fragment ions	$(M - H)^- Fe^{2+}$	210	100	+3
	$(M - H - CO_2)^- Fe^{2+}$	166	55	+3
Precursor ion	$(M - H)^- Co^{2+} (CH_3OH)$	245.2	100	+4
Fragment ions	$(M - H)^- Co^{2+}$	213.1	52	+3
	$(M - H - CO_2)^- Co^{2+}$	169.1	100	+3
Precursor ion	$(M - H)^- Ni^{2+} (CH_3OH)$	243.8	100	+4
Fragment ions	$(M - H)^- Ni^{2+}$	212.0	92	+3
	$(M - H - CO_2)^- Ni^{2+}$	168.0	59	+3
	$(M - H)^- Cu^{2+} (CH_3OH)$	249.0	100	+4
Fragment ions	$(M - H)^- Cu^{2+}$	217.1	47	+3
	MCu^+	218.1	45	+4
	$MH^- Cu^{2+a}$	219.1	19	+4
	$(M - H - CO_2)^- Cu^+$	173.1	73	+3
	$(M - H - CO_2)^- Cu^+ - 29$	144.2	10	^b
	I_{HIS}	110	19	^b
	$(M - H)^- Zn^{2+} (CH_3OH)$	250.0	100	+4
Fragment ions	$(M - H)^- Zn^{2+}$	217.9	82	+3
	I_{HIS}	110	17	+3

^aIon resulting from loss of methanol from the solvated complex.^bThese ions did not appear in the spectra of the deuterated compound.

observed before in mass spectrometric studies of various amino acid- or peptide-metal complexes both in the positive [22,23,25,26,28] and in the negative [19,36] modes. Accompanying this dominant fragmentation, some other fragment ions, most probably resulting from multiple-collision-induced secondary fragmentations, could be detected. These further fragmentations will be described below.

On the other hand, 1:1 metal complexes of L-

histidine and Cu(II) and Zn(II) only, yielded the immonium ion I_{HIS} (m/z 110) as fragment ion. The $[(M - H)^- Cu^{2+}]$ ion fragmented into two additional ions: m/z 144 ($[(M - H)^- Cu^{2+} - CO_2 - CH_2NH]$) and m/z 172 ($[(M - H)^- Cu^{2+} - HCOO^-]$) (Table 3). Formation of an immonium ion from $[(M - H)^- Cat^{2+}]$ corresponds formally to a loss of (Cat, 2O, C), which may either be interpreted as successive fragmentations of CatO and CO or CO_2 and Cat^0 . In the

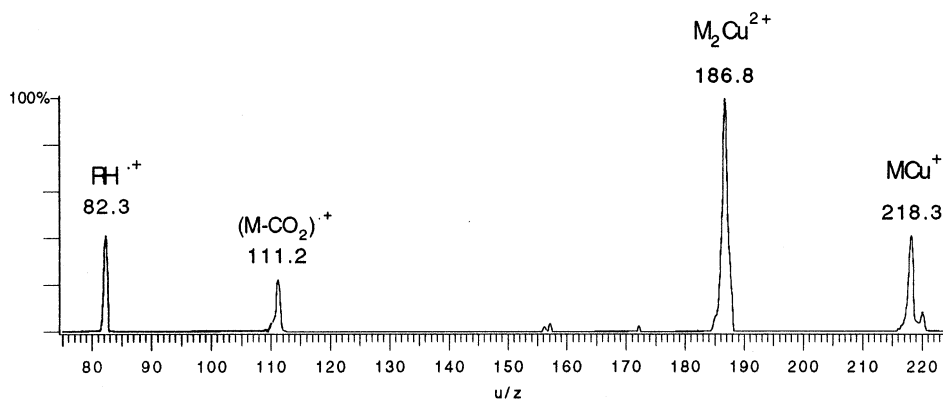
Fig. 3. CID spectrum (fragment ion scan) of the M_2Cu^{2+} (m/z 186.8) ion ($E_{lab} = 20$ eV, $P(Ar) = 4.9 \cdot 10^{-2}$ Pa).

Table 3
Collisionally induced decomposition mass spectra of L-histidine-metal ion 1:1 complexes

Fragment ion scans	Ionic notation	m/z	Relative intensity (%)	Observed mass shift for H/D exchanges
Precursor ion	$(M - H)^- Fe^{2+}$	210.1	100	+3 ^a
Fragment ions	$(M - H)^- Fe^{2+} - CO_2$	166.1	75	+3
	$(M - H)^- Fe^{2+} - CO_2 - NH_3$	149.1	30	+1
	$(M - H)^- Co^{2+}$	213.1	40	+3 ^a
Precursor ion	$(M - H)^- Co^{2+}$	169.1	100	+3
Fragment ions	$(M - H)^- Co^{2+} - CO_2$	152.0	12	+1
	$(M - H)^- Co^{2+} - CO_2 - CH_2NH$	140.2	10	+1 ^d
	$(M - H)^- Co^{2+} - 9^b$	204.0	2	+2,5 ^c
	$(M - H)^- Co^{2+} - H_2O^b$	194.9	5	+2
	$(M - H)^- Co^{2+} - H_2O - CO^b$	167.0	20	+2
	$(M - H)^- Ni^{2+}$	211.9	100	+3 ^a
Precursor ion	$(M - H)^- Ni^{2+}$	168.0	100	+3
Fragment ions	$(M - H)^- Ni^{2+} - CO_2$	150.8	12	+1 ^d
	$(M - H)^- Ni^{2+} - CO_2 - NH_3$	141.0	5	+2 ^d
	$(M - H)^- Ni^{2+} - CO_2 - HCN$	139.2	3	+1 ^d
	$(M - H)^- Ni^{2+} - CO_2 - CH_2NH$	83	5	+2
	RH_2^+	217.1	100	+3 ^a
Precursor ion	$(M - H)^- Cu^{2+}$	217.1	100	+3 ^a
Fragment ions	$(M - H)^- Cu^+ - CO_2 - CH_2NH$	144.3	65	+1
	$(M - H)^- Cu^+ - COOH$	172.0	10	+2
	I_{His}	110.3	10	+3
	MCu^+	218.1	100	+4
Precursor ion	MCu^+	172.2	38	+2
Fragment ions	$MCu^+ - H_2CO_2$	157.2	29	+1
	$MCu^+ - CO_2 - NH_3$	217.9	100	+3 ^a
Precursor ion	$(M - H)^- Zn^{2+}$	217.9	100	+3 ^a
Fragment ion	I_{His}	109.9	89	+3

^aThe number of exchangeable hydrogens for histidine is 4. This ion therefore corresponds to $(M - D)^- Cat^{2+}$.

^bThese fragment ions, which accompany the peak at m/z 204, might involve some doubly charged bimetallic complex of the type $[(M - H)^- Co^{2+} - Co^{2+} (M - H)^-]^{2+}$. This would not be surprising if one considers that Co^{2+} is a cation with an odd number of electron (d^7), a metal-metal bond should thus enable an additional electron pairing.

^cThis ion results from water loss from a doubly charged dimetallic complex $[(M - H)^- Co^{2+}]_2$, which has 6 deuterii, a loss of HDO corresponds to an ion with 5 deuterii.

^dPredominantly.

first hypothesis, the metal cation remains in the +II oxidation state and the second proposition implies a reduction of the metal. Thermochemical calculations giving enthalpies of formation of both proposed final states ($CatO + CO$ and $CO_2 + Cat^0$) are listed in Table 4. From this data, it is apparent (1) that the successive decarboxylation and metal reduction leads to much more stable final states, and (2) that direct formation of I_{His} happens for the metals with the lowest enthalpy of formation values. These arguments point out at the second hypothesis, loss of CO_2 and Cat^0 , as the most probable process. Decarboxylation might therefore be considered as a general fragmentation for all the metals tested but for Cu^{2+} and Zn^{2+} ,

$[(M - H)^- Cat^{2+} - CO_2]$ would be an instable intermediate. Ion m/z 144 ($[(M - H)^- Cat^{2+} - CO_2 - CH_2NH]$) corroborated this conclusion for copper.

In the literature on copper [22,23,28], this decarboxylation has been attributed to a radical-like mechanism involving a reduction of the metal. A similar mechanism could very well be applied here (Scheme 2) as a general process for all metals. In the study on ternary $[Cu(M - H)bpy]^+$ complexes, the radical-like hypothesis was supported by the fact that Cu^{2+} has an odd number of electrons and by some ab initio calculations using the STO-3G* level of theory. No attempt at any calculations was made here, but we note that the first argument would not work for Fe^{2+}

Table 4

Thermodynamical data for the losses of CatO and CO or CO₂ and Cat⁰ (reference 45) in kJ × mol⁻¹

Cat	ΔH_f^0 (Cat ⁰)	ΔH_f^0 (Cat ⁰) + ΔH_f^0 (CO ₂) ^a	ΔH_f^0 (CatO)	ΔH_f^0 (CatO) + ΔH_f^0 (CO) ^b
Fe	416	23	141	251
Co	425	32	191	301
Ni	430	37	187	297
Cu	338	-55	196	306
Zn	130	-263	^c	^c

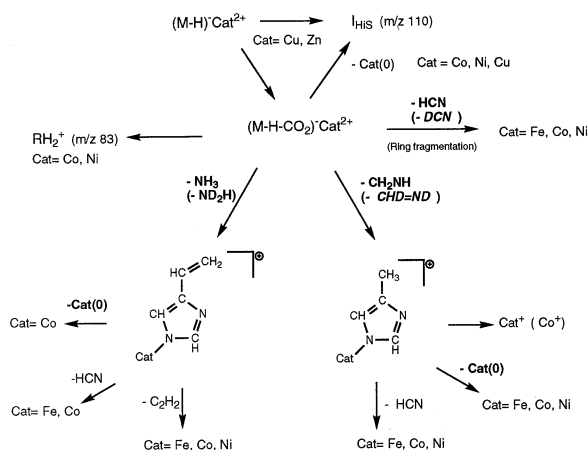
^a ΔH_f^0 (CO₂) = -393 kJ × mol⁻¹.

^b ΔH_f^0 (CO) = -11045 kJ × mol⁻¹.

^cValues not available

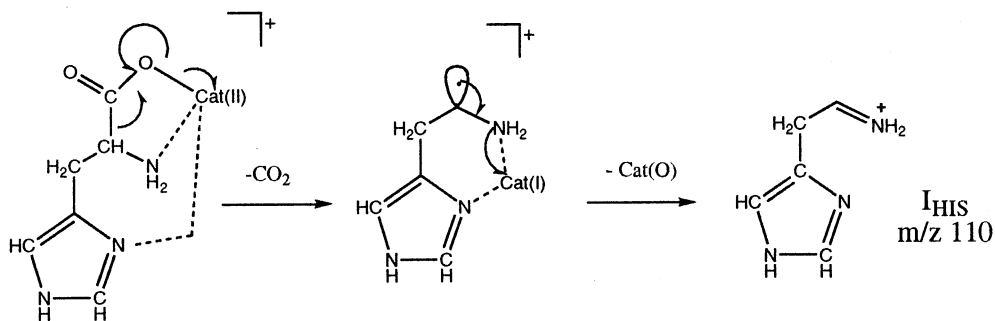
and Ni²⁺, which have an even number of electrons. Here, however, the hypothesis of a reduction taking place was supported by the fact that a loss of a Cat⁰ neutral eventually happened in further fragmentations (Scheme 3).

Table 5 summarises the results of the CID spectra of three fragments observed in the MS/MS experiment on the [(M - H)⁻ Cat²⁺] ion. Namely, [(M - H)⁻ Cat²⁺ - CO₂], [(M - H)⁻ Cat²⁺ - CO₂ - NH₃], and [(M - H)⁻ Cat²⁺ - CO₂ - CH₂NH] could be formed in the source with higher cone voltages (45–60 V), a simulation of MS/MS/MS without mass selection of the precursor ion, but with mass selection of the intermediate ion, was thus possible. The [(M - H)⁻ Cat²⁺ - CO₂] decomposed in five different fragment ions depending on the metal: (1) loss of NH₃ (17 u), shifted to ND₂H (19 u) in deuterated solvent, (2) loss of CH₂ = NH (29 u predominantly shifted to 31 u), (3) loss of HCN (27 u mostly shifted to 28 u), and (4) two purely organic



Scheme 3.

ions I_{His} and RH₂⁺ (R is the side chain). Formation of I_{His} from [(M - H)⁻ Cat²⁺ - CO₂], as discussed before, corresponds to the departure of a reduced metal cation Cat⁰. A similar loss of Cat⁰ was also observed in the CID experiments on [(M - H)⁻ Cat²⁺ - CO₂ - NH₃] giving m/z 93, and [(M - H)⁻ Cat²⁺ - CO₂ - CH₂NH] giving m/z 81. For all metallic complexes, a sequence of fragmentation reactions resulting in a gradual reduction of the metal, involving electron transfers to the ligand, could be inferred (Scheme 3). This sequence of fragmentation reactions also results in a gradual diminution of the coordination sphere around the cation: when neutral molecules are lost, corresponding coordination sites disappear. As these two parallel processes occur (reduction of the metal and diminution of the coordination sphere), it seemed that the second electron



Scheme 2.

Table 5

Collisionally induced decomposition spectra of the main fragments of $(M - H)^- \text{Cat}^{2+}$ (Cat = Fe, Co, Ni, Cu)^a

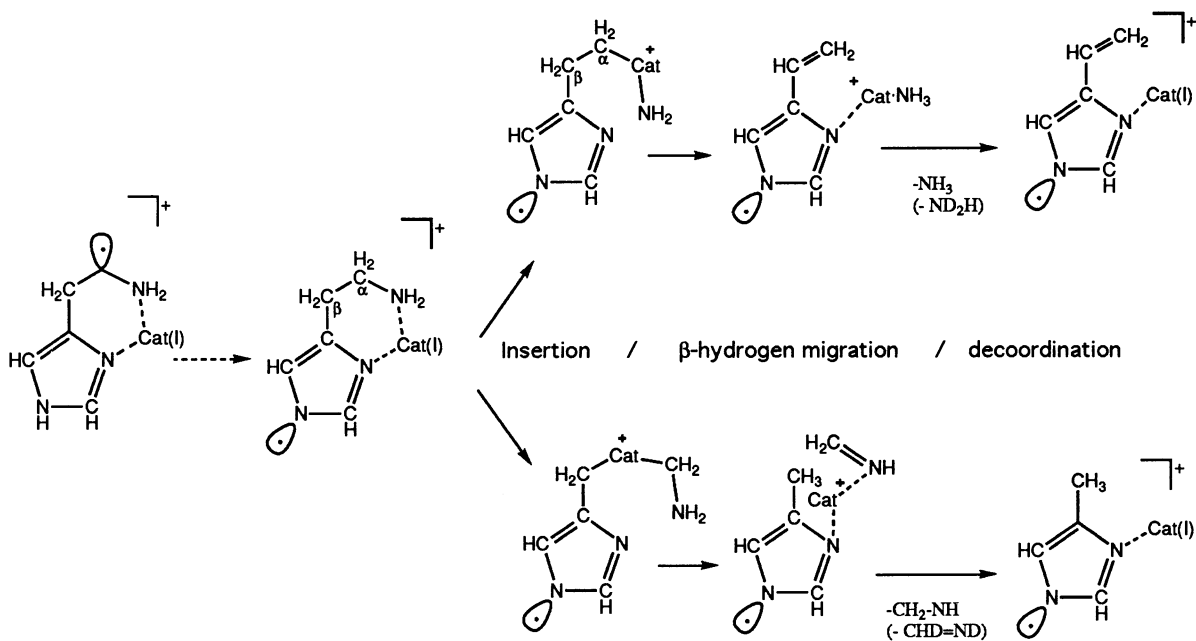
Precursor ions	Fragment ions	Fe^{2+}		Co^{2+}		Ni^{2+}		Cu^{2+}	
		<i>m/z</i>	%	<i>m/z</i>	%	<i>m/z</i>	%	<i>m/z</i>	%
$(M - H)^- \text{Cat}^{2+} - \text{CO}_2$		166.1 (+3)	100	169.1 (+3)	100	167.9 (+3)	100	173.2 (+3)	100
	-NH ₃	149.1 (+1)	63	152.0 (+1)	24	150.9 (+1)	20		
	-HCN	139.1 (+2)	3	—	—	140.8 (+2 ^b)	13		
	-CH ₂ NH	137.1 (+1)	6	140.1 (+1 ^b)	34	138.8 (+1 ^b)	15		
	I _{HIS} ⁺	—	—	110 (+3)	8	109.9 (+3)	10	110.3 (+3)	79
$(M - H)^- \text{Cat}^{2+} - \text{CO}_2 - \text{NH}_3$	RH ₂ ⁺	—	—	83 (+2)	9	82.8 (+2)	19		
		149.1 (+1)	100	152.0 (+1)	100	151 (+1)	100		
	-C ₂ H ₂	123.1 (+1)	61	126.0 (+1)	25	—	—		
	-HCN	122.1 (+1)	29	125.0 (+1)	38	124 (+1)	23		
$(M - H)^- \text{Cat}^{2+} - \text{CO}_2 - \text{CH}_2\text{NH}$	-Cat(0)	—	—	93.3 (+1)	49	92.8 (+1)	50		
		137.1 (+1)	100	140.1 (+1)	100	138.8 (+1)	100		
	-HCN	110.1 (+1)	53	113 (+1)	42	111.8 (+1)	38		
	-Cat(0)	81.1 (+1)	42	81.2 (+1)	21	80.9	50		
	Cat ⁺	—	—	59 (0)	13	—	—		

^aNo ions with zinc appear in this table because the fragmentation of $(M - H)^- \text{Zn}^{2+}$ only yields the immonium ion I_{HIS} at *m/z* 110.^bPredominantly (there are also small amounts of *m/z* 140 and *m/z* 144 in the deuterated experiment, that is loss of HCN and CNDH₂).

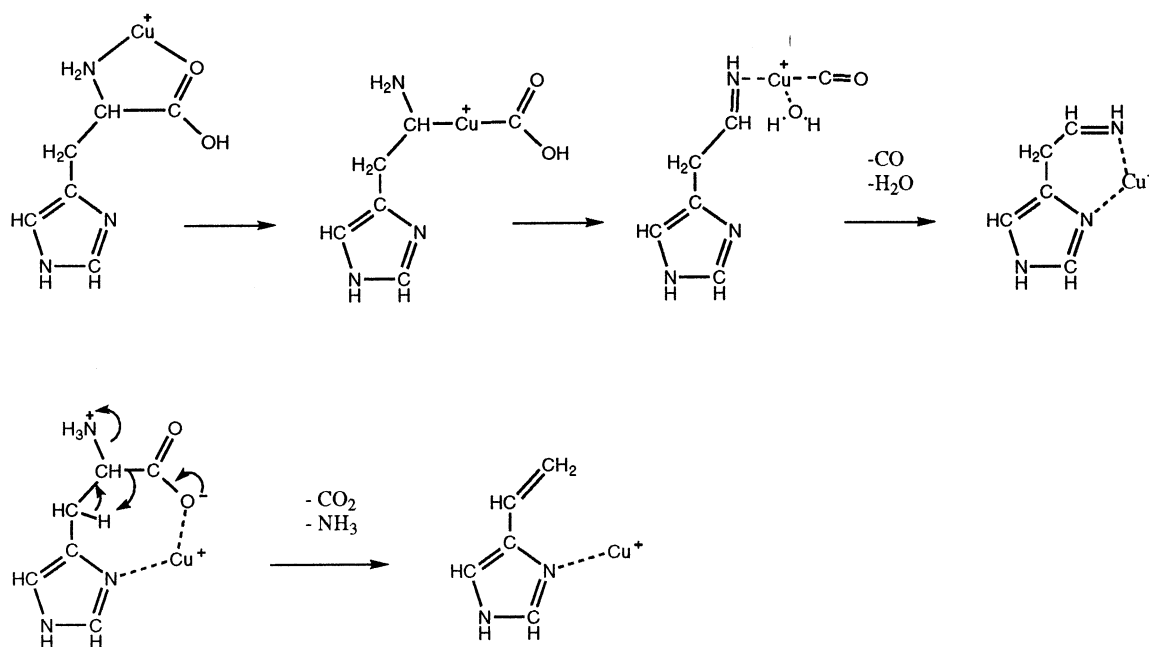
transfer happened earlier or later depending on the metal. From the CID experiments the following order could be deduced: $\text{Fe}^{2+} < \text{Co}^{2+} \leq \text{Ni}^{2+} < \text{Cu}^{2+} < \text{Zn}^{2+}$ (where $<$ means “later electron transfer”).

Deuterium labeling and characteristic fragmenta-

tions (such as loss of C₂H₂; see Table 5) permitted us to suggest some structures for $[(M - H)^- \text{Cat}^{2+} - \text{CO}_2 - \text{NH}_3]$ and $[(M - H)^- \text{Cat}^{2+} - \text{CO}_2 - \text{CH}_2\text{NH}]$, depicted in Scheme 4. When considering possible mechanisms of formation, we noted that loss



Scheme 4.



Scheme 5.

of ND_2H implied a migration of a hydrogen from the C_β . In order to reach a stable final state, it is then necessary to imagine a primary rearrangement of the radical. We therefore suggest a migration of the labile pyrrole hydrogen to the radical site. This migration would be of a 1,5 type if the pyridinelike nitrogen is in δ (Scheme 4) or 1,4 if it is in ϵ . From the rearranged structure, the two main neutral losses could then be formally explained by a classical insertion/ β -hydrogen migration/decoordination mechanism, with an insertion in the $\text{C}_\alpha\text{-N}$ bond for the ammonia loss, and an insertion in the $\text{C}_\alpha\text{-C}_\beta$ bond for the imine loss (Scheme 4). Lastly, loss of HCN (or DCN) might attributed to a fragmentation of the imidazole ring [37].

It is not surprising that MCu^+ and $[(\text{M} - \text{H})^- \text{Cu}^{2+}]$ should have different gas-phase reactivities (Table 3). The CID spectrum of MCu^+ showed two ions m/z 172 and m/z 157 for which some formation suggestions are depicted in Scheme 5. The ion at m/z 172 corresponds to a loss of 46 u, which is similar to the fragmentation usually observed for protonated molecules [38,39]. This fragmentation has been fre-

quently observed before in the studies where MCu^+ was formed using desorption/ionization techniques [40–42]. Several mechanisms had therefore been suggested that involve an insertion of the metal in the C–C bond of the amino acid chain. This was directly inspired by the metal insertion/ β -hydrogen migration/decoordination sequence of reactions usually attributed to bare monovalent transition metal cations interacting with organic molecules in the gas phase. We have chosen to apply it here as well, although Cu^+ , which has a complete shell electronic configuration (d^{10}), has been said to react via a different mechanism, named “dissociative attachment” [43], that excludes a priori any insertion. We have shown in a previous study that the hydrogens involved in the migrations that precede the neutral losses are mainly the ones linked to heteratoms [40].

The second fragment ion, with loss of NH_3 and CO_2 , might result from a zwitterionic conformation of the histidine in the complex, although it has been shown that, for glycine, the complex involving a neutral form of the amino acid was more stable in the gas phase [44].

4. Conclusions

Electrospray ionization is shown to be an efficient method for engendering 1:1 complexes of L-histidine and divalent transition metal cations in the gas phase. At low cone voltages, the species desorbed were still partially solvated by methanol. For Fe^{2+} , Ni^{2+} , and Co^{2+} some octahedral, methanol solvated, $[\text{Cat}^{2+}(\text{CH}_3\text{OH})_6]$ could also be observed. For copper and zinc only, charge-reduced complexes like $[\text{Cat}^{2+}(\text{CH}_3\text{O}^-)(\text{CH}_3\text{OH})_3]$ were observed, consistent with previous results.

As the cone voltage was increased, the greater number of collisions caused the loss of the labile solvent ligands from $[(\text{M} - \text{H})^- \text{Cat}^{2+}(\text{MeOH})_n]$ complexes, thus leaving some lower-coordinated L-histidine-metal 1:1 complexes that probably exist only in the gas phase. For Cu^{2+} , this ligand loss reaction was accompanied by a metal/ligand electron transfer reaction, resulting in a reduced MCu^+ complex.

These gas-phase low-coordinated 1:1 complexes of L-histidine and Cat^{2+} were then studied by MS/MS. All complexes were found to undergo a decarboxylation, although for Zn(II) complexes, and partially for Cu(II) complexes, the resulting $[(\text{M} - \text{H})^- \text{Cat}^{2+} - \text{CO}_2]$ ions were interpreted to be unstable. For these two metal complexes, an immonium ion I_{His} (m/z 110) was formed directly from the CID of $[(\text{M} - \text{H})^- \text{Cat}^{2+}]$, that is to say loss of CO_2 was immediately followed by loss of a neutral metal cation Cat^0 . This loss of a reduced metal could also be observed for other metal complexes, but from some smaller fragments like $[(\text{M} - \text{H})^- \text{Cat}^{2+} - \text{CO}_2]$, $[(\text{M} - \text{H})^- \text{Cat}^{2+} - \text{CO}_2 - \text{NH}_3]$, or $[(\text{M} - \text{H})^- \text{Cat}^{2+} - \text{CO}_2]$. This was interpreted in terms of a gradual reduction of the metal induced by a sequence of fragmentation reactions, that cause a diminution of the coordination sphere. The Cu(I) oxidation state complex MCu^+ exhibited a very different unimolecular reactivity, as the main fragmentations were loss of 46 u (CO and H_2O), which is similar to the process usually observed for protonated molecules, and loss of 61 u (CO_2 and NH_3).

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