

Ion Spray–Tandem Mass Spectrometry of Supramolecular Coordination Complexes

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Double-helical $[M_2L_2]^{n+}$, triple-helical $[M_2L_3]^{n+}$, and toroidal $[M_3L_3]^{n+}$ ($M = Cu, Co, Fe, Ni, La, Eu, Gd, Tb, \text{ or } Lu$) supramolecular complexes have been fully characterized by ion spray mass spectrometry (IS-MS). The IS-MS spectra from pure acetonitrile solutions reflect the nature of the cations present in solution with conservation of the charge state and allow an efficient qualitative speciation of the compounds. The mass spectrometry results can be correlated with other powerful techniques (nuclear magnetic resonance and electronic spectroscopy) for the characterization of supramolecular complexes in solution. Structural information is obtained by collision-induced dissociation, which strongly depends on the metal ions used in the supramolecular complexes and on the various connectivities and topologies of the ligands. When the ligand contains 3,5-dimethoxybenzyl groups bound to the benzimidazole rings, the partial fragmentation of the complexes is associated with a decrease of the total charge of the complexes and the appearance of the characteristic fragment at m/z 151 that corresponds to the 3,5-dimethoxybenzyl cation. A detailed analysis of the fragmentation pathways of these supramolecular complexes suggests that the metal–nitrogen coordination bonds are very strong in the gas phase. (*J Am Soc Mass Spectrom* 1994, 5, 748–756)

In the field of supramolecular chemistry the spontaneous and selective assembly of metal ions with tailored ligands is a theme of considerable interest [1]. The formation of polynuclear helical complexes with heterocyclic nitrogen ligands is particularly attractive [2], especially for the development of luminescent chemical probes and sensors [3–6], but the characterization of the resulting supermolecules is often difficult. Mass spectrometry is a powerful technique, but for many systems studied, the low volatility and the tendency of the complexes to undergo reduction during the ionization process have limited the application of conventional techniques of mass spectrometry [7, 8]. Fast-atom-bombardment (FAB) has been successfully applied to many cases as exemplified by the observation of molecular peaks for double-helical [2, 9–14]

and triple-helical complexes [6, 15–18], for a trefoil molecular knot [19], and for the first deoxyribonucleohelicates [20]. However, the use of a matrix in FAB mass spectrometry (e.g., nitrobenzyl alcohol) limits the scope of this technique to soluble complexes and often leads to matrix interferences, reduction, or demetalation [21]. As far as supramolecular chemistry is concerned the use of a matrix that is chemically very different from the solvents in which the studies in solution [nuclear magnetic resonance (NMR), UV/vis] are performed is a severe handicap for the correlation of mass spectrometry results with those from other techniques.

Electrospray ionization or ion spray (pneumatically assisted electrospray) mass spectrometry (IS-MS) is widely used for the characterization of nonvolatile high molecular compounds such as proteins [22] at relatively low mass-to-charge ratio by producing multiply charged ions. Electrospray is particularly suitable for the observation of preformed ions in solution and

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has been applied successfully to the characterization of metal complexes [23-26] and to the study of a self-assembled trinuclear complex [27]. In a preliminary communication [28], we reported the use of ion spray mass spectrometry for the characterization of self-assembled helical complexes in acetonitrile solutions. A significant extension of this work is reported here and includes the detailed study of the collision-induced dissociation (CID) spectra of these supramolecular complexes obtained either in the free-jet expansion region of the atmospheric pressure source (up-front CID) or in the collision cell of a triple quadrupole mass spectrometer.

Experimental

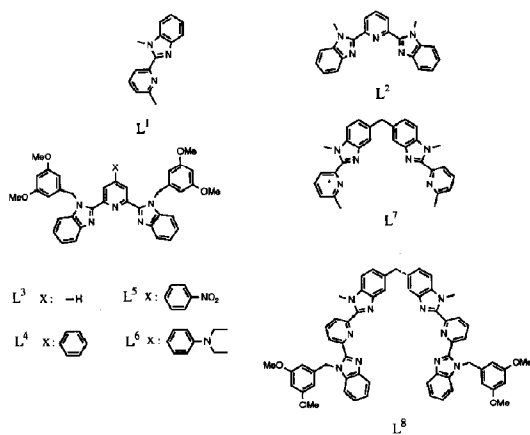
The ligands L^1 [16], L^{2-3} [13], L^{4-6} [17], L^7 [16], and L^8 [6] and their complexes were prepared according to procedures in the literature. The ion spray mass spectra of the complexes (10^{-4} - 10^{-5} M) and of the ligands (10^{-6} - 10^{-7} M + 0.1% HCOOH) were recorded from acetonitrile solutions (Merck LiChrosolv, Darmstadt, Germany). The analyses were performed on three mass spectrometers: a Sciex TAGA 6000E triple quadrupole mass spectrometer (Thornhill, Ontario, Canada) upgraded to an API III with a scan range to m/z 2400, a Sciex API III system (Thornhill, Ontario, Canada) with a scan range to m/z 2400, and an in-house modified [29] Hewlett-Packard (Palo Alto, CA) HP-5985B mass spectrometer with a scan range to m/z 1000. Propylene glycol and quaternary ammonium salts were used to tune and mass calibrate the instruments to achieve unit mass resolution (0.5-0.6 u at half peak height). The isotopic patterns were recorded at higher resolution (0.4-0.2 u at half peak height) with a loss in sensitivity. For ion spray-mass spectrometry [30] and tandem mass spectrometry experiments the samples were introduced at 4-10 μ L/min with an infusion pump (Harvard Apparatus, South Natick, MA). Up-front collision-induced dissociation spectra [29] were obtained by increasing the potential difference (ΔV) between the heated capillary and the skimmer for the HP-5985B mass spectrometer and between the orifice and the first quadrupole (Q_0) of the API III. Argon was used as the collision gas (Q_2) for the tandem mass spectrometry experiments with a collision gas thickness (CGT) in the range of $250-800 \times 10^{12}$ atoms/cm². Single mass spectrometry experiments were carried out on the Sciex by scanning either Q_1 or Q_3 . Typical dwell times were in the range 2-5 ms per step with a step size of 0.2-0.3 u. To determine the isotopic pattern, we used a dwell time of 20 ms and a step size of 0.05 u. As a result of the high concentrations used for the complexes, the sprayer was positioned approximately 1 cm off-axis and 2-3 cm away from the ion-sampling orifice with sprayer potentials in the range of 2-5 kV. A fused silica sprayer was preferred to a stainless steel sprayer to avoid contamination with

iron. For similar reasons we preferred to infuse the sample instead of performing flow injection analysis where metal contamination from the high-performance liquid chromatography pump or from stainless steel tubes can severely affect the mass spectra.

Results

Ligands

As a first step toward the characterization of supramolecular complexes, we recorded the IS-MS spectra of the free ligands L^1 - L^8 from acetonitrile solutions with traces of formic acid as a source of protons (Scheme 1). Dependent on the number of available basic sites on the ligands, the IS-MS spectra under low declustering energy conditions display singly, doubly, and sometimes triply protonated species. The doubly charged ion is the most abundant ion for the heaviest ligands (L^7 , L^8). Structural information can be obtained by increasing the energy of the ions in the free-jet expansion region of the mass spectrometer that leads to up-front collision-induced dissociation or in the collision cell (Q_2) of the tandem mass spectrometer. When the main species is a singly charged ion, we obtain similar mass spectra for both modes. When several cations are present (doubly or triply charged ligands) the up-front CID mass spectra correspond to the sum of the individual product ion mass spectra. The ligands L^1 (223.11-u MW), L^2 (339.15-u MW), and L^7 (458.56-u MW) require rather high potential differences (up-front CID potentials $\sim 80-90$ V) to produce significant fragmentation. L^1 fragments by losing the methyl group (15 u) and gives an ion at m/z 208. Some lower mass ions associated with the aromatic structure of the ligands also are observed at m/z 92, 77, and 65, whereas for L^2 , the fragments at m/z 338, 324, and 208 are observed. The CID mass spectrum of L^7 (up-front CID potentials ~ 90 V) shows two abundant ions at m/z 222 and 236 that correspond to



Scheme 1

cleavage of the carbon-carbon bond between the methylene group and the aromatic rings of the molecule. On the contrary, the ligands L^3 (611.25-u MW), L^4 (687.80-u MW), L^5 (732.80-u MW), L^6 (758.92-u MW) and L^8 (963.11-u MW) show a common abundant product ion at m/z 151 that corresponds to the 3,5-dimethoxybenzyl cation. For the doubly charged $[L^8 + 2H]^{2+}$ (m/z 482) and triply charged $[L^8 + 3H]^{3+}$ (m/z 322) the main fragmentation pathway corresponds to the loss of one or two 3,5-dimethoxybenzyl cations, which is associated with a decrease of the total charge of the residual cation by one (m/z 813) and two charges (m/z 407 and 663), respectively. The product ion spectrum of the singly protonated L^8 ligand ($[L^8 + H]^+$; m/z 963) shows that the loss of the 3,5-dimethoxybenzyl cation is not the main fragment in this case, and other fragments are also observed (m/z 301, 338, 488, 662, and 811).

The ligands may be divided into two categories. The first category includes L^1 , L^2 , and L^7 , which require relatively high energy to produce fragmentation, and the second includes L^3 to L^6 and L^8 , where the fragmentation pathway is mainly controlled by the 3,5-dimethoxybenzyl substituents.

Mononuclear Complexes of Cobalt(II), Europium(III), and Iron(II) with Ligands L^1 to L^3

Because no decomplexation equilibria are involved for the free ligands L^1 to L^8 , the IS-MS spectra can be obtained at rather low concentrations (10^{-6} – 10^{-7} M). The situation is completely different for the coordination complexes of these ligands with metal ions, because the distribution of the various species in solution strongly depends on the stability constants and the concentrations. The bidentate ligand L^1 , an analog of 6-methyl-2,2'-bipyridine [16], reacts in acetonitrile with copper(I) to give a stable pseudotetrahedral $[Cu(L^1)_2](ClO_4)$ complex (609.55-u MW) as confirmed by the IS-MS spectrum, which shows essentially the peak that corresponds to the expected cation $[Cu(L^1)_2]^+$ [28]. However, as a result of the steric hindrance between the methyl groups [31], L^1 reacts with $[Co(H_2O)_6](ClO_4)_2$ to give an unstable pseudo-octahedral $[Co(L^1)_3](ClO_4)_2$ complex (927.67-u MW) [28, 32]. This complex undergoes a partial decomplexation at 10^{-4} M as exemplified by the IS-MS spectrum (Figure 1a) under very low declustering conditions ($\Delta V = 0$ V), which shows the expected cation $[Co(L^1)_3]^{2+}$ (m/z 364.2) together with $[Co(L^1)_2]^{2+}$ (m/z 252.6) and adduct ions of these complexes at m/z 273.2 ($[Co(L^1)_2(CH_3CN)]^{2+}$) and 475.8 ($[Co(L^1)_4]^{2+}$). No protonated ligand ions are observed in the absence of a protonation source in acetonitrile solution. When the energy of the ions is increased ($\Delta V = 20$ V) in the free-jet expansion region, the cluster ions are removed from the spectrum as shown in Figure 1b. Fragmentation of $[Co(L^1)_2]^{2+}$ and

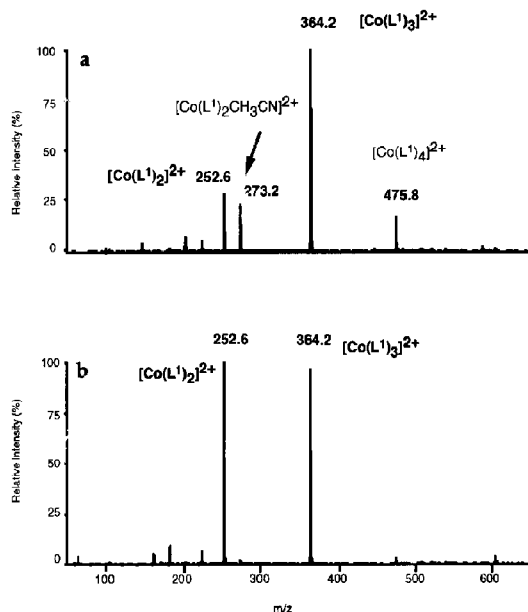


Figure 1. IS-MS spectra of the pseudo-octahedral $[Co(L^1)_3](ClO_4)_2$ complex from pure acetonitrile (927.67-u MW). Single scan spectra (a) $\Delta V = 0$ V; (b) $\Delta V = 20$ V.

$[Co(L^1)_3]^{2+}$ can be obtained under up-front collision-induced dissociation conditions ($\Delta V = 90$ V). The product ion mass spectrum of the doubly charged $[Co(L^1)_3]^{2+}$ at m/z 364 shows the loss of a neutral ligand and the appearance of $[Co(L^1)_2]^{2+}$ at m/z 252.6. An increase of the collision energy produces a spectrum very similar to that obtained by up-front CID, but we do not observe cleavage of the complex into metal and ligands, which strongly suggests that the coordination bonds are strong in the gas phase.

The tridentate ligand L^2 gives a stable pseudotri-capped prismatic complex $[Eu(L^2)_3](ClO_4)_3$ (1468.51-u MW) whose IS-MS spectrum displays (Figure 2a) a major peak corresponding to $[Eu(L^2)_3]^{3+}$ (m/z 390) and a weaker peak at m/z 368 $[Fe(L^2)_2]^{2+}$ that results from contaminations with traces of iron in the infusion system. As expected, the isotopic pattern of $[Eu(L^2)_3]^{3+}$ shows peaks separated by 0.3–0.4 u, and the peaks intensities depend on the isotopic contribution of the metal because europium has two major isotopes (Table 1). For the triply charged cation $[Eu(L^2)_3]^{3+}$, a fragmentation pathway similar to that found for $[Co(L^1)_3]^{2+}$ is observed and the complex loses a neutral ligand as demonstrated by the product ion mass spectrum (m/z 277, $[Eu(L^2)_2]^{3+}$, Figure 2b). However, a second ion at m/z 416 ($[Eu(L^2)_2]^{2+}$) suggests that $[Eu(L^2)_3]^{3+}$ can also lose a ligand with charge separation probably associated with reduction of the metal [33, 34].

The ligand L^3 reacts with iron(II) to form a stable pseudo-octahedral complex (1478.15-u MW). The ion spray mass spectrum of $[Fe(L^3)_2](ClO_4)_2$ (Figure 3a)

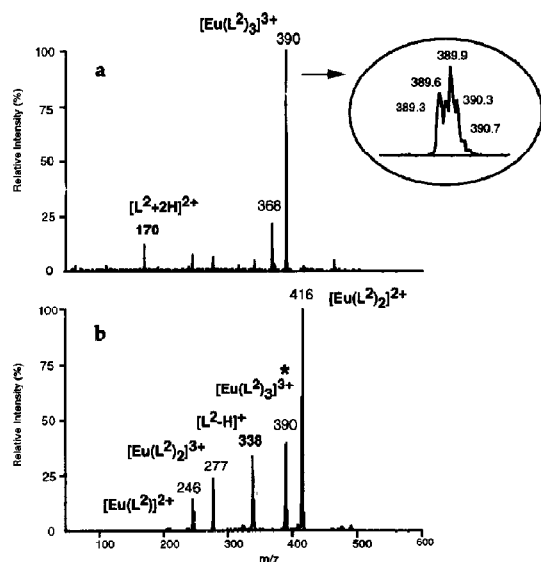


Figure 2. IS-MS spectra of the pseudotrigonal prismatic $[\text{Eu}(\text{L}^2)_3]$ (ClO_4)₃ (1468.51-u MW) complex from pure acetonitrile. (a) Single scan mass spectrum $\Delta V = 90$ V; (b) product ion mass spectrum of $(\text{Eu}(\text{L}^2)_3)^{3+}$ m/z 390 (CGT = 300×10^{12} atoms/cm²; $E = 156$ eV).

displays a major peak at m/z 639 that corresponds to $[\text{Fe}(\text{L}^3)_2]^{2+}$ and partial decomplexation is confirmed by the presence of the singly or doubly charged ligand ($[\text{L}^3 + \text{H}]^+$ m/z 612 and $[\text{L}^3 + 2\text{H}]^{2+}$ m/z 306) in the spectrum. The observation of the ligand in the mass spectrum probably results from acidic contamination of the 100% acetonitrile solution or from hydrolysis of the residual water by free Fe(II). However, because the IS-MS response strongly depends on the compound, peaks that correspond to the protonated ligand can only be used qualitatively. The product ion mass spectrum (Figure 3b) of the doubly charged precursor cation $[\text{Fe}(\text{L}^3)_2]^{2+}$ shows a fragment at m/z 151 as previously observed for the free ligand L^3 and that corresponds to the 3,5-dimethoxybenzyl cation (F).

Table 1. Atomic mass and natural abundance of metal ions

Element	Atomic mass and natural abundance (%)
Cu	62.93 (69.09), 64.93 (30.91)
Co	58.93 (100)
Fe	53.94 (5.82), 55.93 (91.66), 56.94 (2.19), 57.93 (0.33)
Ni	57.94 (68.27), 58.93 (26.10), 60.93 (1.13), 61.94 (3.59), 63.93 (0.90)
La	137.91 (0.09), 138.91 (99.91)
Eu	150.92 (47.82), 152.93 (52.18)
Gd	151.92 (0.2), 153.93 (2.15), 154.92 (14.73), 155.92 (20.47), 156.93 (15.68), 157.9241 (24.87), 159.93 (21.90)
Tb	159.93 (100)
Lu	174.94 (97.41), 175.94 (2.59)

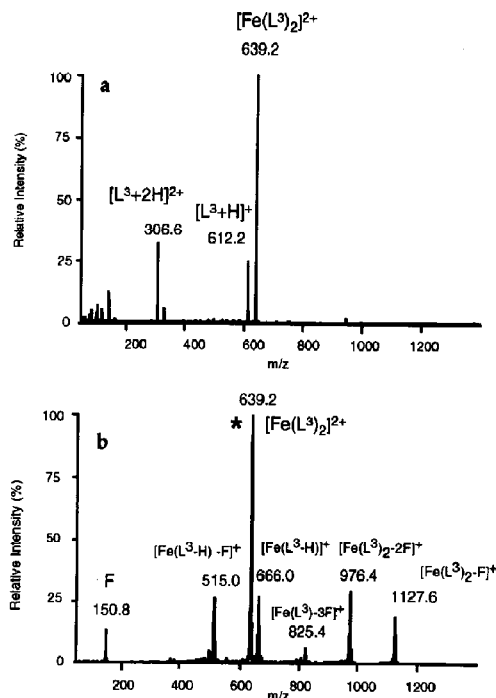


Figure 3. IS-MS spectra of the pseudo-octahedral $[\text{Fe}(\text{L}^3)_2](\text{ClO}_4)_2$ (1478.15-u MW) complex from pure acetonitrile. (a) Single scan mass spectrum, $\Delta V = 10$ V; (b) product ion mass spectrum of $[\text{Fe}(\text{L}^3)_2]^{2+}$ m/z 639.2 (CGT = 464×10^{12} atoms/cm²; $E = 120$ eV).

Cleavage of a part of the ligand (F) results in a decrease of the charge of the complex that corresponds to $[\text{Fe}(\text{L}^3)_2\text{-F}]^+$ at m/z 1127.6. A second fragmentation pathway may be the loss of one ligand followed by cleavage of the 3,5-dimethoxybenzyl substituent at m/z 515 which leads to $[\text{Fe}(\text{L}^3\text{-H})\text{-F}]^+$ (Figure 4b).

All these complexes display the conservation of their charge state in the gas phase, which demonstrates that under low declustering conditions IS-MS spectra of coordination compounds easily can be recorded and interpreted. Electrospray may be considered as an electrolysis cell of a special type [35], but we did not observe any oxidation or reduction products for the analyzed compound. However, the sprayer voltage should be kept as low as possible to prevent possible transformations of the analyte. Under CID conditions various fragmentation pathways are observed, but we did not detect a significant amount of free metal ions in CID spectra, which suggests that the metal-ligand interaction is maintained in the gas phase.

Dinuclear Double-Helical Complexes with Copper(I)

The characterization of the self-assembled dinuclear double-helical complexes $[\text{Cu}_2(\text{L}^n)_2](\text{ClO}_4)_2$ ($n = 3-6$; 1549.39-, 1553.43-, 1701.59-, and 1843.83-u MW, respectively), which represent typical cases of supramolecular complexes, is critical because the singly charged

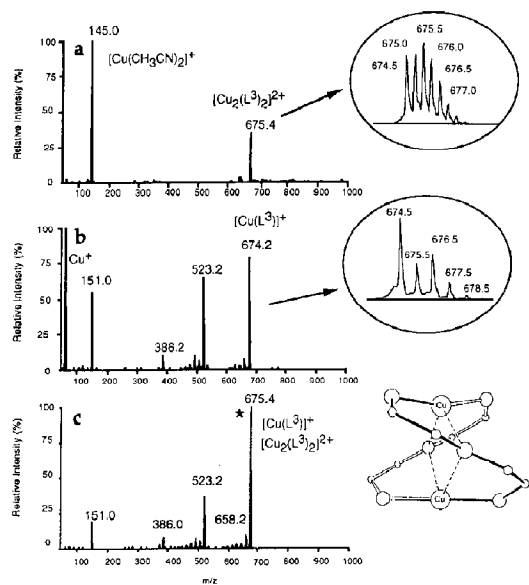


Figure 4. IS-MS spectra from the dinuclear double helical $[\text{Cu}_2(\text{L}^3)_2](\text{ClO}_4)_2$ complex from pure acetonitrile. (a) Single scan mass spectra $\Delta V = 10$ V; (b) $\Delta V = 110$ V; (c) product ion mass spectrum of $[\text{Cu}_2(\text{L}^3)_2]^{2+}$ m/z 675.4 (CGT = 780×10^{12} atoms/cm²; $E = 120$ eV).

monomers $[\text{CuL}^n]^+$ and the doubly charged dimers $[\text{Cu}_2(\text{L}^n)_2]^{2+}$ display the same mass-to-charge ratio [13, 14]. The FAB mass spectra only partially clarify this issue, because the peaks that correspond to the dimer $[(\text{Cu}_2(\text{L}^n)_2)(\text{ClO}_4)]^+$ are weak and are always observed together with intense peaks that correspond to the monomers $[\text{CuL}^n]^+$ [13]. Conductivity measurements have clearly established that $[\text{Cu}_2(\text{L}^3)_2]^{2+}$ is the major species in solution [13], but IS-MS brings the same conclusion with less effort. Under very low declustering conditions ($\Delta V = 0$ V) we observe an abundant peak at m/z 675.4 that corresponds to either $[\text{CuL}^3]^+$ or $[\text{Cu}_2(\text{L}^3)_2]^{2+}$, but the isotopic pattern of this signal shows peaks separated by 0.5 u, which is only compatible with the existence of the doubly charged species $[\text{Cu}_2(\text{L}^3)_2]^{2+}$. The lower mass ion observed at m/z 145 corresponds to solvated copper(I) (Figure 4a).

Under up-front CID conditions an abundant ion ($\Delta V = 90$ V; Figure 4b) at m/z 674 is still present in the spectrum, but the peaks of the isotopic pattern are now separated by 1.0 u, which suggests that the dimer $[\text{Cu}_2(\text{L}^3)_2]^{2+}$ is converted into the monomer $[\text{CuL}^3]^+$ in the gas phase. Further fragmentations produce the ion at m/z 523.2 that corresponds to the loss of the 3,5-dimethoxybenzyl fragment (m/z 151) as confirmed by the product ion spectrum of the doubly charged precursor ion at m/z 675.4 $[\text{Cu}_2(\text{L}^3)_2]^{2+}$ recorded under low declustering conditions (Figure 4c). In the case of multiply charged ions, at least two different charge states are necessary to determine the molecular weight,

but an alternative approach is to use the isotopic pattern as described for these complexes. However, the resolution of isotopic peaks with quadrupole mass analyzers is limited to doubly or triply charged ions and for higher charge state, magnetic sector or ion cyclotron resonance mass spectrometers may be required.

Dinuclear Triple-Helical Complexes with Cobalt(III)

As expected from the stability constant found for the triple-helical complex $[\text{Co}_2(\text{L}^7)_3](\text{ClO}_4)_4$ (1891.37-u MW) in acetonitrile [16], a concentration of 10^{-4} [M] limits the decomplexation to $\sim 5\%$ and leads to observation of the cation $[\text{Co}_2(\text{L}^7)_3]^{4+}$ at m/z 373 with isotopic peaks separated by 0.25 u (Figure 5a). Association with ClO_4^- produces $[\text{Co}_2(\text{L}^7)_3(\text{ClO}_4)]^{3+}$ and $[\text{Co}_2(\text{L}^7)_3(\text{ClO}_4)_2]^{2+}$ at m/z 530 and 846, respectively. The product ion mass spectrum of $[\text{Co}_2(\text{L}^7)_3]^{4+}$ shows the presence of the protonated ligand at m/z 459 (Figure 5b) and the main fragmentation pathway seems to be loss of a ligand with a decrease of the total charge of the complex to give $[\text{Co}_2(\text{L}^7)_2]^{3+}$ (m/z 344.5). The triple-helical $[\text{Co}_2(\text{L}^7)_3(\text{ClO}_4)_4]$ complex also was analyzed with a HP-5885B mass spectrometer equipped with a heated capillary atmospheric pressure ionization (API) interface in single scan mode. The spectrum is almost identical to that obtained with the API III. The most abundant ion at m/z 373 corresponds to $[\text{Co}_2(\text{L}^7)_3]^{4+}$, and association with the perchlorate counterion at m/z 530 and 845 is also observed. The great similarity between the spectra illustrates that

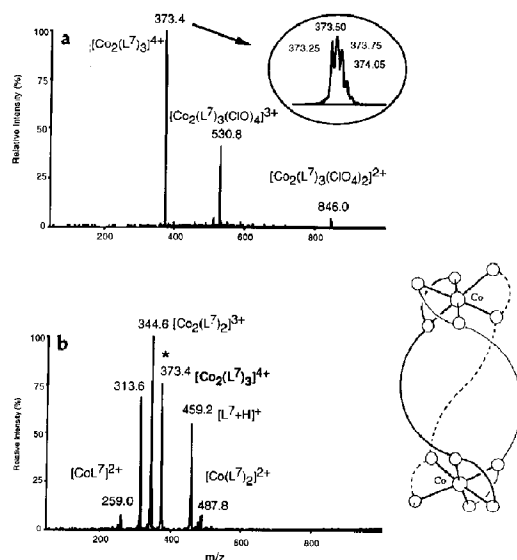


Figure 5. IS-MS spectra of the triple-helical $[\text{Co}_2(\text{L}^7)_3](\text{ClO}_4)_4$ (1891.37-u MW) complex from pure acetonitrile. (a) Single scan mass spectrum $\Delta V = 0$ V; (b) product ion mass spectrum of $[\text{Co}_2(\text{L}^7)_3]^{4+}$ m/z 373.4 (CGT = 348×10^{12} atoms/cm²; $E = 200$ eV).

supramolecular complexes also can be recorded on instruments with different types of API interfaces.

Dinuclear Triple-Helical Complexes with Lanthanide(III)

The dinucleating ligand L^8 reacts with lanthanide perchlorates to give dinuclear complexes $[Ln_2(L^8)_3(ClO_4)_6]$ ($Ln = La, Eu, Gd, Tb, \text{ and } Lu$; 3763.85-, 3789.96-, 3800.54-, 3803.82-, and 3835.97-u MW, respectively) that are produced by strict self-assembly and adopt a triple-helical structure in the solid state and in solution [6, 18]. The IS-MS spectra of these complexes in acetonitrile 5×10^{-5} [M] ($\sim 5\%$ of decomplexation) display abundant peaks that correspond to the multiply charged cations $[Ln_2(L^8)_3]^{6+}$ and the protonated ligand $[L^8 + H]^+$, $[L^8 + 2H]^{2+}$ (Figure 6a). The presence of the protonated ligand is explained by the partial decomplexation and the excellent IS-MS response of the ligand. The intensities of the peak that corresponds to cations $[Ln_2(L^8)_3]^{6+}$ significantly decrease when going from $Ln = La$ to Lu , which is in good agreement with the stability constants of the complexes [6]. Further peaks that result from association with perchlorate anions are observed and correspond to the species $[Ln_2(L^8)_3(ClO_4)_i]^{(6-i)+}$ ($i = 1-3$). A detailed study of the IS-MS spectra of supramolecular complexes that possess different anions and various charge states suggests that this association results from electrostatic interactions, but its observation depends on the sprayer position.

Because of the high charge state (6+) and the limited mass resolution of quadrupole instruments, the isotopic pattern alone cannot be used to clearly establish the charge state of the complexes present in solution. However, the peaks that result from association with the counterions are very useful and can be used to determine the molecular weight of the complex and the charge state according to eq 1, (where M is the molecular mass of the complex, m/z_i is the mass-to-charge ratio $m/z_1 < m/z_2$, C is the mass of the associated anion, and z is the integer number of charges borne by the ion m/z_1).

$$\frac{m}{z_1} = \frac{M}{z} \quad \frac{m}{z_2} = \frac{M + C}{z - 1} \quad z = \frac{m/z_2 + C}{m/z_2 - m/z_1} \quad (1)$$

Such an approach is known for protein chemistry [36], but for cationic supramolecular complexes, the association with a counterion (an anion) results in an increase of the mass and a decrease of the total charge state. Nevertheless, a very precise determination of the molecular weight of the supramolecular complexes at unit mass resolution is difficult due to the relatively low charge state and the significant isotopic contribution of metal ions and counterions (Table 1).

The product ion mass spectrum of $[La_2(L^8)_3]^{6+}$ (Figure 6b) shows a fragment at m/z 151 that corresponds

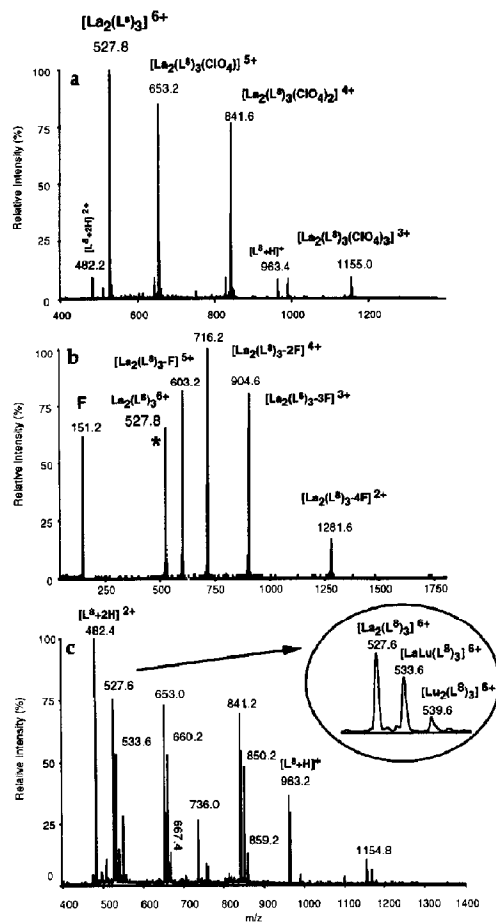


Figure 6. IS-MS spectra of the triple helical $[La_2(L^8)_3]KClO_4$ complex from pure acetonitrile solutions. (a) Single scan mass spectrum $\Delta V = 10$ V; (b) product ion mass spectrum of $[La_2(L^8)_3]^{6+}$ m/z 527.8 (CGT = 369×10^{12} atoms/cm²; $E = 330$ eV); (c) single scan spectrum of the heterodinuclear complex $[LaLu(L^8)_3]KClO_4$, $\Delta V = 0$ V.

to the 3,5-dimethoxybenzyl cation and four ions (m/z 603, 716, 905, and 1282). The ion at m/z 603 corresponds to the loss of the 3,5-dimethoxybenzyl fragment (F) to give $[La_2(L^8)_3-F]^{5+}$, whereas the ions at m/z 716, 905, and 1282 correspond to the loss of two, three, or four charged F fragments, respectively. Qualitatively, the fragmentation pattern of the complex $[La_2(L^8)_3]^{6+}$ is very similar to that obtained for the triply protonated ligand, that is, successive loss of F fragments. This result is in agreement with the fact that a proton may be considered as a small metal ion [37]. To investigate if this fragmentation pathway is controlled by the nature of the ligand or the type of metal coordinated, we performed the same tandem mass spectrometry experiments for $[Ln_2(L^8)_3]^{6+}$ ($Ln = Tb, Lu, \text{ and } Gd$) and we observed that a similar fragmentation pathway occurs for all $[Ln_2(L^8)_3]^{6+}$, which

suggests that the nature of the ligand controls the fragmentation process.

We also used IS-MS to characterize the complexes obtained by mixing stoichiometric quantities of ligand L^8 (3 equiv) with $Ln^1(ClO_4)_3$ (1 equiv) and $Ln^2(ClO_4)_3$ (1 equiv) ($Ln^1 \neq Ln^2$; $Ln = La, Eu, Gd, Tb, Lu$). In all cases, we observe only three different complexes in solution that correspond to the three expected dinuclear cations $[(Ln^1)_2(L^8)_3]^{6+}$, $[(Ln^2)_2(L^8)_3]^{6+}$, and $[Ln^1Ln^2(L^8)_3]^{6+}$ (Figure 6c) together with the ions that correspond to the associations with perchlorate anions. The mass resolution of the quadrupole mass analyzer was sufficient to separate even heterodinuclear $[EuTb(L^8)_3](ClO_4)_6$ (3796.92-u MW) from its homodinuclear analogs $[Eu_2(L^8)_3](ClO_4)_6$ (3789.96-u MW) and $[Tb_2(L^8)_3](ClO_4)_6$ (3803.89-u MW). Because of the partial decomplexation observed in the conditions of these IS-MS measurements (approximately 15–30%, depending on the metal ion), it was not possible to compare the relative concentrations of hetero- and homodinuclear complexes with those obtained by 1H NMR [6].

Trinuclear Toroidal Complexes

Following the general principles that control the assembly of supramolecular helical complexes [1, 2, 6, 16], the bis-terdentate ligand L^8 is not restricted to the preparation of triple-helical complexes $[Ln_2(L^8)_3]^{6+}$, but L^8 is also a good candidate for the self-assembly of dinuclear double-helical complexes with pseudo-octahedral metal ion $[M_2(L^8)_2]^{4+}$ as recently reported by Constable [2] for the analogous bis-terdentate ligand sexipyridine with various metal ions. Spectrophotometric titrations with $M(ClO_4)_2 \cdot 6H_2O$ [$M = Fe(II), Co(II), Ni(II)$] in MeCN clearly indicate the presence of only one absorbing complex for each titration, which corresponds to a metal-to-ligand ratio of 1.0. 1H NMR titrations in CD_3CN of L^8 by $Fe(ClO_4)_2 \cdot 6H_2O$ confirm this statement and also establish that the ligands are symmetrically bound to the metal ion in agreement with point groups D_2 or D_3 [6, 13, 16]. These results are in agreement with the expected double-helical $[M_2(L^8)_2]^{4+}$ of D_2 symmetry, and the IS-MS spectrum of the Ni(II) complex shows a peak at m/z 511 that may correspond to this formulation (Figure 7a). However, the shape of the peak at m/z 511 suggests a charge state higher than $4+$, and no perchlorate adduct at m/z 714 that corresponds to $[Ni_2(L^8)_2](ClO_4)_3]^{3+}$ is observed. Based on the ions at m/z 633, 816, 1121, and 1731 that correspond to perchlorate adducts of a $6+$ charged species, it is possible to clearly establish with a relatively low amount of sample that the species formed in solution is not the expected double-helical complex, but a trinuclear toroidal complex $[Ni_3(L^8)_3](ClO_4)_6$ (3662.11-u MW) of D_3 symmetry. The assignment of the product ion mass spectrum of $[Ni_3(L^8)_3]^{6+}$ is shown in Figure 7b. As previously described for the dimeric double-helical copper complexes, we observe the situation where the trimeric species is converted into the dimer $[Ni_2(L^8)_2]^{4+}$ and

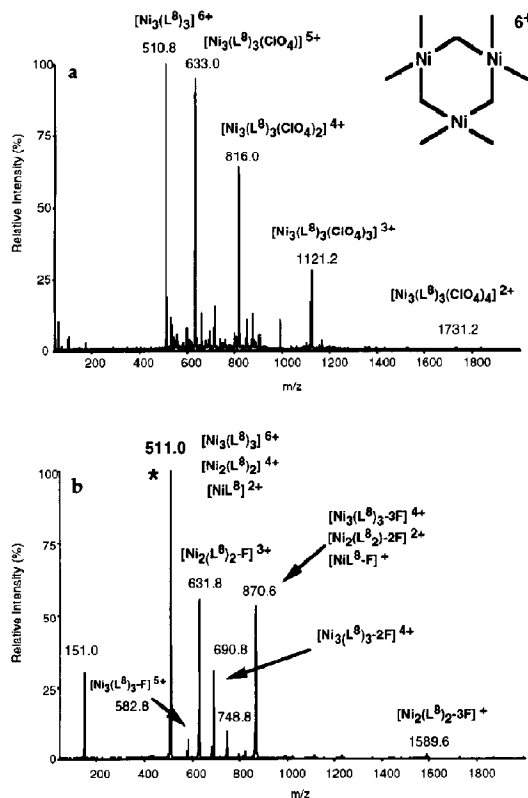


Figure 7. IS-MS spectra of the toroidal $[Ni_3(L^8)_3](ClO_4)_6$ complex from pure acetonitrile solutions. (a) $\Delta V = 0$ V; (b) product ion mass spectrum of $[Ni_3(L^8)_3]^{6+}$ (CGT = 300×10^{12} atoms/cm 2 ; $E = 330$ eV).

the monomer $[Ni(L^8)]^{2+}$ under CID. Evidence for this mechanism is supported by the ions formed by partial cleavage of the ligand L^8 (loss of the 3,5-dimethoxybenzyl cation) in a similar way as previously described for the triple-helical complexes $[Ln_2(L^8)_3]^{6+}$.

Discussion

All the supramolecular cationic complexes studied are characterized easily by IS-MS at relatively low mass-to-charge ratios. Sensitivity is not an issue because, to avoid decomplexation, the spectra are recorded at high concentration for IS-MS. The systematic use of the isotopic patterns for low charge states (singly to triply charged ions) allows the determination of the molecular weight of the supramolecular complexes. In the case of higher charge states, the association between the cation and anions in the present case ClO_4^- is observed and produces a series of ions with increasing mass and decreasing charge that allows the determination of the charge state according to eq 1, but the accuracy is strongly affected by the isotopic contribution of the perchlorate anions. According to our results, we believe that this association is mainly electrostatic and limited to charge states equal to or greater than $3+$ in acetonitrile.

The systematic application of this technique to the study of self-assembled supramolecular complexes shows that IS-MS mass spectra qualitatively reflect the nature and the distribution of the different species in solution. As demonstrated by the observation of various species for different total complex concentrations, the IS-MS results are in good agreement with spectrophotometric measurements [6, 16]. In the gas phase no metal reduction is observed under low CID conditions and the charge state is conserved for all the complexes, which probably results from ligand stabilization.

One of the very attractive features of API interfaces is the ability to perform up-front CID with a single quadrupole. Up-front CID is very useful for the observation of dissociation of dimeric complexes, but is limited to the case where only one or two main ions are present in the spectra. We also observe a good correlation between the up-front CID spectra and tandem CID spectra for the complexes and for the ligands. During collision-induced dissociation experiments, the main fragmentation pathway, in particular for L^3-L^6 and L^8 and their complexes, is a partial cleavage of the ligand with loss of the well stabilized 3,5-dimethoxybenzyl cation that leads to a decrease of the total charge of the residual ligand or of the residual complex. This fragmentation is induced by the coordination of the unsubstituted nitrogen of the benzimidazole to the charged metal ion or to the proton. Extended Hückel calculations with charge iteration [38] of the cisoid form [17] of the ligand 2,6-bis[1'-benzyl-benzimidazole-2'-yl]pyridine show that the substituted nitrogen atoms of the benzimidazole rings bear a negligible negative charge and cannot be protonated or coordinated, but when the unsubstituted nitrogen atom is complexed by a metal ion, the negative charge produced by cleavage of the carbon-nitrogen bond may be efficiently stabilized as previously reported for octahedral Fe(II) [39] and Ru(II) [40] complexes with an analog of L^2 . This effect, together with the stabilization of positive charge by 3,5-dimethoxybenzyl units, may be responsible for the observed fragmentation.

In the case of dimeric $[Cu_2L_n]^{2+}$ ($n = 3-6$) and trimeric $[Ni_3L_3]^{6+}$ complexes, we observe a fragmentation into monomeric and dimeric species, respectively, under CID conditions.

Although the fragmentation pathway under CID conditions strongly depends on the nature of the metal ions and the ligands, the observation of fragmentations without complete decomplexation for many complexes suggests that the metal-ligand interaction is strong in the gas phase.

Conclusions

Ion spray-tandem mass spectrometry is a very attractive tool that may be used in association with 1H NMR and spectrophotometric titrations for the study of self-assembled supramolecular complexes in solution. The

study of noncovalent complexes from biological systems by IS-MS also has been described [41-43] and is a topic of great interest. In addition to the mass spectrometric characterization of these synthetic supramolecular complexes, the study of their behavior in the gas phase may be very useful for mass spectrometric studies of larger noncovalent complexes that involve metal ions.

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References

1. Lehn, J.-M. *Angew. Chem. Int. Ed.* **1990**, *29*, 1304.
2. Constable, E. C. *Tetrahedron* **1992**, *48*, 10013.
3. Tangay, J. F.; Suib, S. L. *Catal. Rev. Sci. Eng.* **1987**, *29*, 1.
4. Leiner, M. J. P. *Anal. Chim. Acta* **1991**, *255*, 201.
5. Piguet, C.; Williams, A. F.; Bernardinelli, G.; Bünzli, J.-C. G. *Inorg. Chem.* **1993**, *32*, 4139.
6. Piguet, C.; Bünzli, J.-C. G.; Bernardinelli, G.; Hopfgartner, G.; Williams, A. F. *J. Am. Chem. Soc.* **1993**, *115*, 8197.
7. Beavis, R.; Lindner, J.; Grotemeyer, J.; Atkinson, I. M.; Keene, F. R.; Knight, A. E. *J. Am. Chem. Soc.* **1988**, *110*, 7534.
8. Cetini, G.; Operti, L.; Vaglio, C. A.; Bandini, A. L.; Bantitelli, G.; Mingbetti, G. *Org. Mass Spectrom.* **1989**, *24*, 479.
9. Lehn, J.-M.; Rigault, A.; Siegel, J.; Harrowfield, J.; Chevri er, B.; Moras, D. *Proc. Nat. Acad. Sci. USA* **1987**, *84*, 2565.
10. Constable, E. C.; Ward, M. D.; Tocher, D. A. *J. Chem. Soc., Dalton Trans.* **1991**, 1675.
11. Lehn, J.-M.; Rigault, A. *Angew. Chem. Int. Ed.* **1988**, *27*, 1095.
12. Zarges, W.; Hall, J.; Lehn, J.-M.; Bolm, C. *Helv. Chim. Acta* **1991**, *74*, 1843.
13. R uttimann, S.; Piguet, C.; Bernardinelli, G.; Bocquet, B.; Williams, A. F. *J. Am. Chem. Soc.* **1992**, *114*, 4230.
14. Piguet, C.; Bernardinelli, G.; Williams, A. F. *Inorg. Chem.* **1989**, *28*, 2920.
15. Williams, A. F.; Piguet, C.; Bernardinelli, G. *Angew. Chem. Int. Ed.* **1991**, *30*, 1490.
16. Piguet, C.; Bernardinelli, G.; Bocquet, B.; Quattropiani, A.; Williams, A. F. *J. Am. Chem. Soc.* **1992**, *114*, 7440.
17. Piguet, C.; Bochet, C. G.; Williams, A. F. *Helv. Chim. Acta* **1993**, *76*, 372.
18. Bernardinelli, G.; Piguet, C.; Williams, A. F. *Angew. Chem. Int. Ed.* **1992**, *31*, 1622.
19. Dietrich-Buchecker, C. O.; Sauvage, J.-P. *Angew. Chem. Int. Ed.* **1989**, *28*, 189.
20. Koert, U.; Harding, M. M.; Lehn, J.-M. *Nature (London)* **1990**, *346*, 339.
21. Naylor, S.; Hunter, C. A.; Cowan, J. A.; Lamb, J. H.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 6507.
22. Fern, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64.
23. Katta, V.; Chowdhury, S. K.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 5348.
24. Colton, R.; Traeger, J. C. *Inorg. Chim. Acta* **1992**, *201*, 153.
25. van den Bergen, A.; Colton, R.; Percy, M.; West, B. O. *Inorg. Chem.* **1993**, *32*, 3408.
26. Curtis, J. M.; Derrick, P. J.; Schnell, A.; Constantin, E.; Gallagher, R. T.; Chapman, J. R. *Org. Mass Spectrom.* **1992**, *27*, 1176.
27. Leize, E.; Vandorssclaer, A.; Kramer, R.; Lehn, J. M. *J. Chem. Soc., Chem. Commun.* **1993**, 990.

28. Hopfgartner, G.; Piguët, C.; Herion, J. D.; Williams, A. F. *Helv. Chim. Acta* **1993**, *76*, 1759.
29. Hopfgartner, G.; Wachs, T.; Bean, K.; Henion, J. *Anal. Chem.* **1993**, *65*, 439.
30. Bruins, A. P.; Covey, T. R.; Henion, J. D. *Anal. Chem.* **1987**, *59*, 2642.
31. Piguët, C.; Bernardinelli, G.; Bocquet, B.; Schaad, O.; Williams, A. F. Unpublished.
32. Onggo, D.; Goodwin, H. A. *Aust. J. Chem.* **1991**, *44*, 1539.
33. Cheng, Z. L.; Siu, K. W. M.; Guevremont, R.; Berman, S. S. *Org. Mass Spectrom.* **1992**, *27*, 1370.
34. Blades, A. T.; Jayaweera, P.; Ikonomou, M. G.; Kebarle, P. *J. Chem. Phys.* **1990**, *92*, 5900.
35. Blades, A. T.; Ikonomou, M. G.; Kebarle, P. *Anal. Chem.* **1991**, *63*, 2109.
36. McLuckey, S. A.; Glish, G. L.; Van Berkel, G. J. *Anal. Chem.* **1991**, *63*, 1971.
37. Constable, E. C.; Elder, S. M.; Walker, J. V.; Wood, P. D.; Tocher, D. A. *J. Chem. Soc., Chem. Commun.* **1992**, *35*, 22937.
38. Hoffmann, R. *J. Chem. Phys.* **1963**, *39*, 1397.
39. Rüttimann, S.; Moreau, C. M.; Williams, A. F.; Bernardinelli, G.; Addison, A. W. *Polyhedron* **1992**, *11*, 636.
40. Xiaoming, X.; Haga, M. A.; Matsumura-Inoue, T.; Ru, Y.; Addison, A. W.; Kano, K. *J. Chem. Soc., Dalton Trans.* **1993**, 2477.
41. Li, Y.-T.; Hsieh, Y.-L.; Henion, J.; Senko, M. W.; McLafferty, F. W.; Ganem, B. *J. Am. Chem. Soc.* **1993**, *115*, 8409.
42. Li, Y.-T.; Hsieh, Y.-L.; Henion, J.; Ganem, B. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 631.
43. Ganem, B.; Henion, J. *Chemtracts* **1993**, *6*, 1.