
Characterization of Self-Assembled Supramolecular $[Ga_4L_6]$ Host–Guest Complexes by Electrospray Ionization Mass Spectrometry

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Self-assembled supramolecular host–guest complexes have been characterized by electrospray ionization mass spectrometry. The spectra obtained by use of a Q-TOF instrument equipped with a Z-spray ion source show primarily the 3- and 4- charge states of the assemblies. The assemblies have the general formula $[guest \subset Ga_4L_6]^{11-}$ where L represents the chelating bidentate catechol ligand 1,5-bis(2',3'-dihydroxy-benzamido)naphthalene and guests are tetramethyl ammonium (Me_4N^+), tetraethyl ammonium (Et_4N^+), tetra-*n*-propyl ammonium (Pr_4N^+) and decamethylcobaltocenium ($Cp^*_2Co^+$) cations. For the first time, the mass spectrum of the empty assembly $[Ga_4L_6]^{12-}$ is reported. This article also reports that provided the electrospray ion source is capable of preserving noncovalent interactions, it is possible to observe host–guest complexes containing both weak binding guests as well as sterically demanding guests in the mass spectra. The present data suggest that electrospray mass spectrometry is a powerful tool for characterization of supramolecular host–guest complexes. (J Am Soc Mass Spectrom 2006, 17, 292–296) © 2006 American Society for Mass Spectrometry

In the area of supramolecular chemistry, metal–ligand interactions have been exploited to design highly symmetric closed shell structures that self-assemble from simple metal and ligand components. The formation of discrete molecular architectures relies on labile coordinative bonds that allow for self-correction and exclusive formation of the thermodynamic product. A great variety of self-assembled structures of different molecular sizes and shapes is present in the literature [1–5]. Here, we focus on contributions to this field made by Raymond and coworkers [2, 6–10]. The common theme in these metal–ligand assemblies is the presence of chelating bidentate catechol amide ligands, which bear certain geometric constraints upon coordination to octahedral metal centers resulting in M_4L_6 coordination tetrahedra (Figure 1). The complexes are highly charged, rendering them soluble in polar solvents such as water and methanol, yet contain hydrophobic cavities of variable sizes. The presence of these hydrophobic cavities affords rich host–guest chemistry,

enabling encapsulation of monocationic guest molecules [2, 6–8]. Several analytical methods are necessary to efficiently characterize these host–guest complexes. 1H -NMR spectroscopy shows remarkable upfield shifts in the proton signals of encapsulated guests within the cavities [2]. However, since the structures exhibit high point symmetry and the metal components are often paramagnetic, complete characterization by NMR is not always possible. Although solid-state structures may, in the best of cases, be determined by single crystal X-ray crystallography, intrinsically labile metal–ligand interactions do not always allow for unambiguous characterization of solution state structures. Additionally, mass spectrometry is more sensitive than NMR spectroscopy and allows analysis of lower concentrations, thus observing species with solubility properties insufficient for NMR measurements. Mass spectral analysis is therefore the method of choice for analysis of host–guest assemblies in the solution state. However, the high charges of the complexes and the presence of metal ions complicate the isotope pattern, requiring high resolving power to obtain full isotopic resolution. Given the symmetric nature of the assemblies, it is important to unambiguously identify the charge state as well as to

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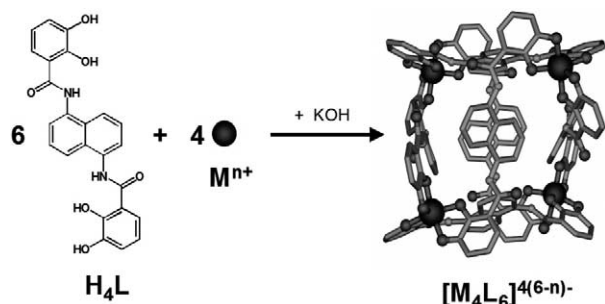


Figure 1. Self-assembly of M_4L_6 coordination tetrahedra from 1,5-bis(2',3'-dihydroxy-benzamido)naphthalene (H_4L) and various metal ions M^{n+} ($M = Al^{III}, Ga^{III}, In^{III}, Fe^{III}, Ti^{IV}, Ge^{IV}, Sn^{IV}$) upon addition of base. Large spheres represent metal ions, medium spheres are oxygen, and small spheres are nitrogen. Carbon is represented by sticks and hydrogen is omitted for clarity.

compare the calculated with the experimentally obtained isotope pattern.

Electrospray ionization has long been the method of choice when analyzing noncovalently bound peptide and protein complexes [11, 12]. It is widely accepted that, under the appropriate conditions, the mass spectra of protein complexes can be used to determine the stoichiometry of interacting subunits [12] as well as the binding of substrates to active sites [13]. Electrospray mass spectrometry has gained increasing popularity in the characterization of self-assembled, highly charged supramolecular structures [3, 6–10, 14–16]. The main obstacle to mass spectral analysis of these complexes has been the high degree of fragmentation during ionization. Recent developments in source design have greatly helped to overcome this issue. For example, using cold-spray ionization (CSI), Yamaguchi, Fujita, and coworkers [15, 17, 18] were able to detect intact cage-type platinum(II) complexes with various guests as well as trimeric box-type palladium(II) complexes with guest encapsulation. The Z-spray source developed by Waters also allows for the observation of intact clusters. The first stage in the Z-spray process is extracting the ions orthogonally from the aerosol spray into the sampling cone aperture. Large droplets, nonvolatile materials and other neutral components are not extracted but collected in the baffle/vent port. A second orthogonal extraction step directs the ions into the mass analyzer. Gas at atmospheric pressure samples through an aperture into a partial vacuum and forms a freely expanding jet, which represents a region of high-pressure compared with the surrounding vacuum. In the Z-spray source, gentler conditions can be obtained by carefully adjusting the cone voltage along with both the desolvation and source temperatures. This reduces the energy of the analyte ion, thus helping to provide maximum sensitivity for thermally labile compounds by preventing in-source fragmentation often observed in conventional electrospray sources. To further prevent fragmentation of noncovalent complexes, the collision energy in the collision cell (Q2—located at the end of the quadrupole section) can be reduced.

In this paper, we report on the electrospray mass spectra of supramolecular assemblies that were unattainable with previous mass spectral techniques. The general formula of the complexes is $[guest \subset Ga_4L_6]^{11-}$ ($guest^{1+}, Ga^{3+}, L^{4-}$) where the guests are tetramethyl ammonium (Me_4N^+), tetraethyl ammonium (Et_4N^+), tetra-*n*-propyl ammonium (Pr_4N^+), and decamethylcobaltocenium ($Cp^*_2Co^+$) ["C" denotes encapsulation of guest molecules into the cavity of the host structure and "Cp*" denotes *bis*-(η^5 -pentamethylcyclopentadienyl) cobaltocenium]. For the first time, the mass spectrum of the empty assembly $[Ga_4L_6]^{12-}$ is reported. Previously, only mass spectra of a few $[Ga_4L_6]^{12-}$ host–guest complexes with encapsulated guest molecules such as Me_4N^+ and Et_4N^+ were attainable [6]. This contribution emphasizes that the Z-spray ion source combined with the Q-TOF instrument is very powerful for these types of coordination assemblies, and independent of the presence of stabilizing encapsulated guest molecules. While NMR spectroscopy has proven very successful in indicating the encapsulation of a guest species [2], electrospray mass spectrometry is used to determine the stoichiometry of these highly symmetrical complexes.

Experimental

The host–guest complexes were prepared according to literature procedures [6, 19] in the presence of one equivalent decamethylcobaltocenium, five equivalents of tetra-*n*-propyl ammonium, and seven equivalents of tetramethyl and tetraethyl ammonium ions. The mass spectra were acquired using a Micromass Q-TOF API US (Micromass, Manchester, UK) equipped with Z-spray ion source operated in V mode (where ions travel in the TOF detector in a "V-shape" path) with a resolving power of over 10,000 and in negative ion mode. To preserve the host–guest interaction, the cone voltage was set to 5–15 V, the collision energy in the collision cell (Argon was used as a collision gas) was set to 3–4 V, the source temperature was set to 90 °C, and the desolvation temperature to 120 °C. The instrument was calibrated using maltooligosaccharide (Sigma, St. Louis, MO) for mass range 500–2000. The samples were dissolved in methanol at a concentration of 200 μM and infused at 5 $\mu L/min$.

Results and Discussion

While labile and reversible metal–ligand interactions favor the formation of the thermodynamic product, they also render the final assembly more susceptible to fragmentation during the ionization process. In our experience, the encapsulation of guest molecules stabilizes the host assemblies, thus enabling ionization without fragmentation. The binding constants for the different guest molecules used in this study were previously determined by NMR spectroscopy: Me_4N^+ ($0.32 M^{-1}$), Pr_4N^+ ($1.11 \times 10^2 M^{-1}$), and Et_4N^+ ($196 \times 10^2 M^{-1}$) [3]

(the binding constant for Cp^*_2Co^+ could not be determined by NMR spectroscopy due to insolubility upon addition of more than one equivalent of guest molecules). The large binding constant of Et_4N^+ and the high stability of these host–guest complexes might explain the greater reliability for obtaining consistent mass spectral data, whereas previous detection of weaker bound host–guest complexes were strongly dependent on experimental conditions [6]. Additionally, regardless of binding constants, neither the Cp^*_2Co^+ host–guest assembly [19] nor the empty cluster have been previously detected. Cp^*_2Co^+ represents the largest guest successfully encapsulated in this type of cluster. The fact that both Cp^*_2Co^+ and Pr_4N^+ are bulkier than Me_4N^+ and Et_4N^+ could also explain the lack of success in detecting these in host–guest complexes in earlier experiments. Previous spectra were obtained using an older source design, where the ESI-plume is focused directly at the sampling cone aperture. In the electrospray process, the bulk solution is dispersed into a stream of droplets [20]. The Rayleigh fission model predicts that Coulombic repulsion between like charges in the droplet lead to dissociation when the repulsive force between like charges exceeds the surface tension of the droplet [20]. In electrospray ionization gas and/or heat are the means of shrinking the droplets. We believe that thermal and collisional decomposition was the main reason for the unsuccessful mass spectral detection of the more labile assemblies. For this, we reduced cone voltage, both desolvation and source temperature, and collision energy settings for optimal ion transfer of intact clusters. In the samples analyzed here, intact cluster signals were primarily observed in the 3- and 4-charge states, presumably indicating a favorable stabilization of the highly charged, 12-cluster assembly by 9 or 8 counter ions, respectively.

While the $^1\text{H-NMR}$ spectrum of the empty $[\text{Ga}_4\text{L}_6]^{12-}$ assembly shows only six aromatic signals indicating a highly symmetric composition, it fails to provide information about the stoichiometry of the assembly. Here we found it possible to characterize the empty $[\text{Ga}_4\text{L}_6]^{12-}$ assembly using the Z-spray ion source combined with the Q-TOF instrument (Figure 2). The peaks of the most abundant isotopes corresponding to the $[\text{Ga}_4\text{L}_6\text{K}_9]^{3-}$, $[\text{Ga}_4\text{L}_6\text{K}_8\text{H}]^{3-}$, and $[\text{Ga}_4\text{L}_6\text{K}_7\text{H}]^{4-}$ species can be observed at m/z 1062.97, 1052.32, and 777.49, respectively. Comparison between the measured and theoretical obtained masses (see insert in Figure 2) suggests that the mass accuracy is better than 10 ppm. This mass accuracy was obtained for all peaks highlighted in Table 1, and along with the ability to mass resolve the isotope peaks greatly enhances the possibility of assigning the individual peaks and consequently use mass spectrometry to confirm the stoichiometry of these assemblies. The remaining peaks shown in Figure 2 result from fragmentation of the cluster, which can be observed throughout the spectrum and could not be assigned. Since no guest is present to stabilize the Ga_4L_6 framework, the assembly is held together solely by non-

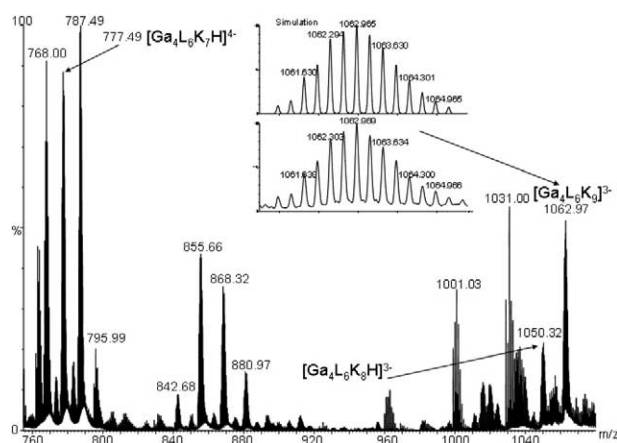


Figure 2. Negative electrospray spectrum of the empty $\text{K}_{12}[\text{Ga}_4\text{L}_6]$ assembly. The insert shows a mass scale expanded segment of $[\text{Ga}_4\text{L}_6\text{K}_9]^{3-}$. The top half shows the theoretical distribution of the 3- charge state.

covalent metal–ligand interactions. This is the first time it has been possible to observe the empty capsule by mass spectrometry, and it opens up new possibilities for using mass spectrometry to study the interactions between the Ga_4L_6 framework and various guests, regardless of their binding affinity or size.

A plethora of species are observed in the mass spectra of the $[\text{Ga}_4\text{L}_6]^{12-}$ tetrahedron with Me_4N^+ , Pr_4N^+ , and Et_4N^+ as guests (G), and an overview of these are listed in Table 1. For each tetrahedron, ions corresponding to 3- and 4-charge states of the intact host–guest assemblies can be observed. In the case of Me_4N^+ , an excess (seven equivalents) of tetramethyl ammonium was added during the synthesis, and Me_4N^+ , K^+ and H^+ all serve as counter ions. Five peaks corresponding to seven, six and five equivalents of Me_4N^+ are observed in the 3-charge state (see Table 1) that result from an exchange series of Me_4N^+ , K^+ , and H^+ counter ions (Figure 3). The $[\text{Et}_4\text{N}^+\text{C}[\text{Ga}_4\text{L}_6]^{11-}]$ assembly was also synthesized with seven equivalents of tetraethyl ammonium, one of which being encapsulated, leaving six equivalents of Et_4N^+ to serve as counter ions. Ions corresponding to six tetraethyl ammonium counter ions can thus be observed in the MG^*_5 series, as well as ions corresponding to various counter ion combinations of Et_4N^+ , K^+ , and H^+ (see Table 1). The $[\text{Pr}_4\text{N}^+\text{C}[\text{Ga}_4\text{L}_6]^{11-}]$ assembly was synthesized in the presence of five equivalents of tetra-*n*-propyl ammonium. Species listed in Table 1 confirm the presence of encapsulated host–guest assemblies with up to four Pr_4N^+ ions serving as counter ions. Both the 3- and the 4-charge state envelopes show exchange reactions between tetrapropyl ammonium, potassium, and protons. Earlier $^1\text{H-NMR}$ studies [3] have confirmed that one equivalent of guest molecules is indeed confined within the cavity of the tetrahedral cluster while additional guest molecules are associated with the exterior surface of the assembly. We attribute the exchange between counter ions to the fact that excess of guest molecules as

Table 1. Observed species of encapsulated host-guest assembly

Guest	Species observed as 4-	Species observed as 3-
Me_4N^+	MG^*_5K_2 MG^*_5KH MG^*_5H_2	MG^*_6K_2 MG^*_5K_3 $\text{MG}^*_5\text{K}_2\text{H}$ $\text{MG}^*_4\text{K}_3\text{H}$ $\text{MG}^*_4\text{K}_2\text{H}_2$
Et_4N^+	MG^*_5K_2 $\text{MG}^*_4\text{K}_2\text{H}$ MG^*_5KH MG^*_4KH_2 MG^*_5H_2	MG^*_6K_2 $\text{MG}^*_5\text{K}_2\text{H}$ MG^*_6KH MG^*_5KH_2 MG^*_6H_2
Pr_4N^+	MG^*_3K_4 $\text{MG}^*_2\text{K}_4\text{H}$ MK_7 $\text{MG}^*_3\text{K}_3\text{H}$ $\text{MG}^*_2\text{K}_3\text{H}_2$ MK_6H $\text{MG}^*_3\text{K}_6\text{H}$ $\text{MG}^*_3\text{K}_2\text{H}_2$ MG^*_3K_6 $\text{MG}^*_3\text{K}_5\text{H}_2$ MG^*_2K_5 $\text{MG}^*_3\text{K}_5\text{H}$	MG^*_4K_4 MG^*_3K_5 MG^*_2K_6 MG^*_2K_7 $\text{MG}^*_4\text{K}_3\text{H}$ $\text{MG}^*_3\text{K}_4\text{H}$ $\text{MG}^*_2\text{K}_5\text{H}$ $\text{MG}^*_4\text{K}_2\text{H}_2$ $\text{MG}^*_3\text{K}_3\text{H}_2$ $\text{MG}^*_2\text{K}_4\text{H}_2$ $\text{MG}^*_4\text{K}_2\text{H}_3$ $\text{MG}^*_3\text{K}_2\text{H}_3$ $\text{MG}^*_2\text{K}_3\text{H}_3$

Observed species of encapsulated host-guest assembly $[\text{guest} \subset \text{Ga}_4\text{L}_6]^{11-}$ (M), where the guest represents tetramethyl ammonium (Me_4N^+), tetraethyl ammonium (Et_4N^+) and tetra-n-propyl ammonium (Pr_4N^+) respectively. G*, K (potassium) and H (proton) represent additional counter ions associated with the exterior surface of the cluster.

well as K^+ and H^+ are readily available in the sample solution. The assignment of the mass spectra is in very good agreement with the equivalent number of guest molecules present during synthesis, contrary to observations with previous, less reliable techniques [6]. This also confirms that it is paramount for the mass spectral analysis of these assemblies to use an ion source that limits source fragmentation and preserves noncovalent interactions in the gas phase.

In the case of $[\text{Cp}^*_2\text{Co} \subset \text{Ga}_4\text{L}_6]^{11-}$, evidence of encapsulation is based on significant NMR-upfield shifts of the Cp^*_2Co^+ guest signals in solution over the course of weeks without the encapsulated Cp^*_2Co^+ guest being released from the cavity [19]. Peaks corresponding to the intact assembly can be observed at m/z 1159.70 (MK_3^{3-}), m/z 860.03 (MK_7^{4-}), and m/z 664.65 ($\text{MK}_4\text{H}_2^{5-}$) for the most abundant isotopes. For both the 3- and 4-charge states, peaks corresponding to K^+/H^+ exchange can be observed. This is in agreement with

similar trends observed for smaller, less bulky guests. The dodecamethylcobaltocenium (Cp^*_2Co^+) guest is particularly interesting since it represents the bulkiest guest thus far encapsulated within the Ga_4L_6 tetrahedron. The spectrum could not be obtained with previous electrospray techniques presumably due to distortion of the Ga_4L_6 framework upon encapsulation of the large guest. This distortion is expected to enhance dissociation of the ligands from the metal vertices, which resulted in cluster fragmentation in more conventional ionization conditions.

Conclusions

In this paper, we have shown that mass spectral characterization of self-assembled supramolecular host-guest complexes is possible, provided the ionization process allows for preservation of non-covalent interactions. The examples shown here demonstrate that host-guest complexes containing both weak binding guests as well as sterically demanding guests can be analyzed using electrospray mass spectrometry. This opens up the possibility of using mass spectrometry to study reactive reaction intermediates stabilized via encapsulation [10, 21, 22], as well as giving insight into catalytic reaction pathways [23], both of which occur inside the cavity. It will also allow for using mass spectrometry as a screening technique to monitor the competitive binding of various guest molecules at lower concentrations than previously observable by NMR spectroscopy.

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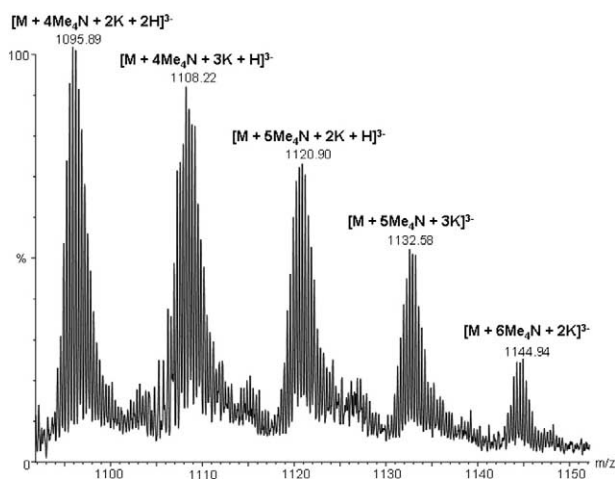


Figure 3. A mass scale expanded segment of the negative electrospray spectrum of the $[\text{Me}_4\text{N}^+ \subset \text{Ga}_4\text{L}_6]^{11-}$ assembly showing that various combinations of Me_4N^+ , K^+ , and H^+ can be observed as counter ions.

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