

# Detection of Hydrogen-bonded Supramolecular Complexes Using Electrospray Ionization from Chloroform

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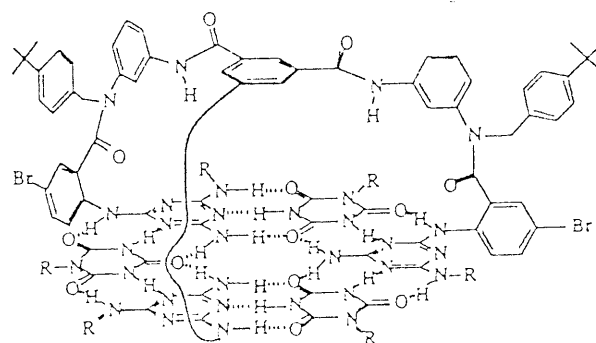
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The stoichiometry of a noncovalent, hydrogen-bonded supramolecular complex,  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$ , was characterized using electrospray ionization from chloroform. The intact (1:3) complex was observed in the negative-ion mode as a  $\text{Cl}^-$ -bound species using  $\text{Ph}_4\text{PCl}$  as the source of the charge donor. Collisionally and thermally induced dissociation of the (1:3) complex resulted in the simultaneous loss of all the three RCA units, indicating a cooperative binding of RCA units in the (1:3) complex. These results suggest that the attachment of small, organic-soluble ions may be a useful technique for mass spectrometric characterization of neutral supramolecular complexes that are stable or soluble only in non-polar organic solvents.

Characterization of noncovalent complexes from solution using electrospray ionization (ESI)<sup>1-4</sup> is a new capability of mass spectrometry. To date, detection of many types of biomolecular complexes have been reported, reflecting the great interest in the use of ESI-MS for the analysis of systems of biological and pharmaceutical significance.<sup>5-19</sup> As a sensitive and structurally informative analytical technique, mass spectrometry of noncovalent complexes should also be important in the study of molecular recognition and molecular self-assembly.<sup>20-27</sup> Accurate mass measurement to determine/verify stoichiometry, and dissociation experiments to probe structural features of noncovalent bonding are readily accomplished using mass spectrometry. It may also be possible to determine or infer compositions in solution from mass spectrometry. The success of ESI in the analysis of biomolecular complexes is due to the ease with which this class of molecules accepts charges in aqueous or mixed solvent systems containing water+methanol or acetonitrile through protonation/deprotonation.<sup>3</sup> Similarly, complexes containing charged species, such as metal cations, can be ionized readily from suitable solvent systems.<sup>28-34</sup> There are, however, many situations where these charging mechanisms are not available, and the detection of neutral, noncovalent complexes becomes difficult using ESI-MS. Such situations might arise because the molecules (complexes) do not have ionizable groups or because they are insoluble/unstable in the more typically used solvents (e.g., aqueous or mixed solvent systems). Chemical modification and electron-transfer reactions have been suggested to overcome some of these problems.<sup>35-37</sup> In this Communication, we show that the use of organic-soluble charge carriers such as tetraphenylphosphonium chloride ( $\text{Ph}_4\text{PCl}$ ) presents another viable strategy to facilitate ionization for these systems. Specifically, the neutral hydrogen-bonded supramolecular complex,  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$ , which is stable only

in non-polar organic solvents, can be detected intact using ESI-MS as  $\text{Cl}^-$ -bound species from chloroform.

The  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  complex (Scheme 1) is an important member of a family of self-assembled aggregates that have been designed based on the cyanuric acid-melamine (CA-M) hydrogen-bonded lattice. It is a model aggregate for the development of supramolecular complexes that are held together by networks of noncovalent interactions (here, hydrogen bonds).<sup>38</sup> The  $\text{hub}(\text{M})_3$  molecule was designed so that the three melamine (M) units were covalently linked to a central 'hub' using 'spokes' that were compatible with the geometry required for the formation of a hydrogen-bonded aggregate with three cyanuric acid (CA) units (Scheme 1). Both the cyanuric acid and melamine units are derivatized to increase the solubility in organic solvents and to prevent the complex from further aggregation through hydrogen-bonding. The complex has been characterized using a variety of techniques.<sup>38</sup> Solubility, <sup>1</sup>H- and <sup>13</sup>C-NMR results indicated the formation of tight complexes in  $\text{CHCl}_3$  and  $\text{CH}_2\text{Cl}_2$ . Gel permeation chromatography and UV spectroscopy results suggested that the complex had 1:3 stoichiometry. A study by vapor pressure osmometry suggested, however, that the molecular weight was 50-80% higher than that expected for a 1:3 stoichiometry, and fast-atom bom-



Scheme 1. Structure of the  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  complex:  $\text{R} = \text{CH}_2\text{CH}_2\text{C}(\text{CH}_3)_3$ .

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bardment (FAB) mass spectrometry gave only ions corresponding to individual components.<sup>38</sup>

## EXPERIMENTAL

A Finnigan MAT (San Jose, CA, USA) TSQ 7000 triple-quadrupole mass spectrometer was used in this work. The instrument was equipped with an ESI interface and has an  $m/z$  range of 4100. The standard ESI emitter provided by Finnigan MAT was replaced with a home-built microspray emitter optimized for very low sample infusion rate (0.2–0.5  $\mu\text{L}/\text{min}$ ).<sup>39</sup> For the ionization from chloroform in the negative-ion mode,  $\text{SF}_6$  gas flow was unnecessary and not used. This is in contrast to typical operational protocols used with biomolecules in aqueous or mixed solvent systems, for which the use of  $\text{SF}_6$  or  $\text{O}_2$  as discharge-suppressing agents is necessary in negative-ion mode, and can be attributed to the electron-scavenging effect of chloroform.<sup>40</sup> The mass spectrometer parameters were set by an autotune procedure and were typically not changed during day-to-day operation. The instrument was operated at unit  $m/z$  resolution throughout the  $m/z$  range, and the high- $m/z$ -range mode (up to  $m/z$  4100) was used for all the experiments. The  $\text{hub}(\text{M})_3$  and RCA compounds were synthesized and purified using the published procedures.<sup>38</sup> The  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  complex was prepared by mixing the components in chloroform with brief heating.<sup>38</sup> Reagent tetraphenylphosphonium chloride ( $\text{Ph}_4\text{PCl}$ ) (98% purity) and solvents were obtained from commercial sources and were used as received. Unless otherwise indicated, a solution containing 1.0 mM  $\text{Ph}_4\text{PCl}$  and 1.0 mM analyte was prepared for the electrospray ionization by mixing appropriate amounts of each component in a 10 mM stock solution with chloroform. The solution was directly infused into the ion source of the mass spectrometer. In the collision-induced dissociation (CID) experiment, the precursor ion at  $m/z$  2768.1 was selected using the first quadrupole with a window of  $\pm 5$   $m/z$  units, accelerated and collided with added argon gas in the second (RF-only) quadrupole ( $E_{\text{lab}} = 35$  eV,  $P_{\text{Ar}} = 1.58$  mTorr). The product ions were detected using the third quadrupole with unit  $m/z$  resolution.

## RESULTS AND DISCUSSION

When  $\text{CHCl}_3$  solutions containing  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  or a mixture of  $\text{hub}(\text{M})_3$  and RCA were electrosprayed, no ions could be observed using either positive or negative polarity. Addition of oxidation/reduction agents such as tetracyanoethylene (TCE), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and  $N,N,N',N'$ -tetramethyl-1,4-phenylenediamine (TMPD), with or without  $\text{CF}_3\text{CO}_2\text{H}$  (0.1%), also failed to give a stable ion current or any useful mass spectra. These reagents have been reported to facilitate ionization in organic solution through electron-transfer for compounds that do not contain acid/base groups.<sup>36, 37, 41</sup> Addition of 5%  $\text{CH}_3\text{OH}/0.5\%$   $\text{CF}_3\text{CO}_2\text{H}$  to the  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  solution produced ions in the positive-ion mode corresponding to protonated species of only the individual components. This result is consistent with the reported instability of the hydrogen-bonded complex in the presence of  $\text{CH}_3\text{OH}$ ; the  $\text{CH}_3\text{OH}$  competes with the individual components for hydrogen-bonding.<sup>38</sup> Addition of

1.0 mM of  $\text{Ph}_4\text{PCl}$ , however, gave an intense signal, in the negative-ion mode, corresponding to  $\text{Cl}^-$  attachment to both the  $\text{hub}(\text{M})_3$  molecules and the 1:3 hydrogen-bonded complex  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  (denoted as 1:3, Fig. 1(a)). The observed ion at  $m/z$  2768.1 is consistent with the  $\text{Cl}^-$ -bound (1:3) complex (calculated  $m/z = 2768.2$ ). Increasing the concentration of  $\text{Ph}_4\text{PCl}$  resulted in the appearance of cluster ions corresponding to the general formula  $\text{Cl}^-(\text{L})_n$  ( $\text{L} = \text{Ph}_4\text{PCl}$ ) (Fig. 1(b)) which could be used as internal standards for calibration of  $m/z$ . The observed species are listed in Table 1 with their values of  $m/z$ , the assignment, the calculated values of  $m/z$ , and the relative intensities. The excellent agreement between the observed and calculated values of  $m/z$  confirmed the detection of intact complex with a 1:3 stoichiometry, and supported the earlier conclusions based on different techniques.<sup>38</sup>

In addition to the  $\text{Cl}^-$ -bound (1:3) complex and the  $\text{hub}(\text{M})_3$ , a small contribution (<10%) corresponding to a  $\text{Cl}^-$ -associated (1:2) complex was observed. There were also small peaks corresponding to adducts of L to doubly charged dimers of the (1:3) complex,  $[(2:6) + \text{L}]\text{Cl}_2^-$  and other doubly and triply charged species (Fig. 1 and Table 1). These multimetric species may be related to the apparent high molecular weight obtained from the vapor pressure osmometry study.<sup>38</sup> The observation of  $[(2:6) + \text{L}]\text{Cl}_2^-$  ion indicates that there might be contributions of  $(2:6)\text{Cl}_2^-$  to the peak at  $m/z$  2768.1 assigned as  $(1:3)\text{Cl}^-$ . The doubly charged species will have an isotopic spacing of  $1/2$   $m/z$  unit which should be readily distinguished from the singly charged species using an instrument with a higher resolving power.

In the positive-ion mode,  $\text{Ph}_4\text{P}^+$  ion was observed as the predominant species (>99%), along with small amounts of cluster species of a general formula  $\text{Ph}_4\text{P}^+(\text{L})_n$ . No ions corresponding to aggregates of the intact (1:3) complex with attached cations were observed. The results from different ionization polarities reflect the much higher stability of  $\text{Ph}_4\text{P}^+$  than  $\text{Cl}^-$  in  $\text{CHCl}_3$  and the different affinity of these ions towards the analyte.

Collision-induced dissociation (CID) experiments on the  $m/z$ -selected  $\text{Cl}^-$ -bound (1:3) complex yielded the  $\text{Cl}^-$ -bound  $\text{hub}(\text{M})_3$  as the predominant product with no detectable ions corresponding to  $\text{Cl}^-$ -bound (1:2) and (1:1) complexes (Fig. 2). This result confirmed the noncovalent nature of the (1:3) complex. The cooperative loss of the three RCA units suggests that the (1:2) and (1:1) complexes are less stable than the (1:3) complex. This suggestion is consistent with the proposed structure of the (1:3) complex in which the three RCA units are bound to  $\text{hub}(\text{M})_3$  cooperatively (Scheme 1).<sup>38</sup>

In another experiment, the temperature of the interface capillary was varied and the relative intensities of the  $\text{Cl}^-$ -bound (1:3), (1:2), (1:1) complexes and  $\text{hub}(\text{M})_3$  were recorded. There were minor amounts of other ions observed, similar to those in Fig. 1. Figure 3 is a plot of the normalized intensity of selected ions vs. the temperature of the interface capillary. This result is similar to that obtained by CID: that is, an increase of the capillary temperature induced dissociation of the (1:3) complex and gave  $\text{hub}(\text{M})_3$  ion as the predominant product. This latter result confirmed the relative

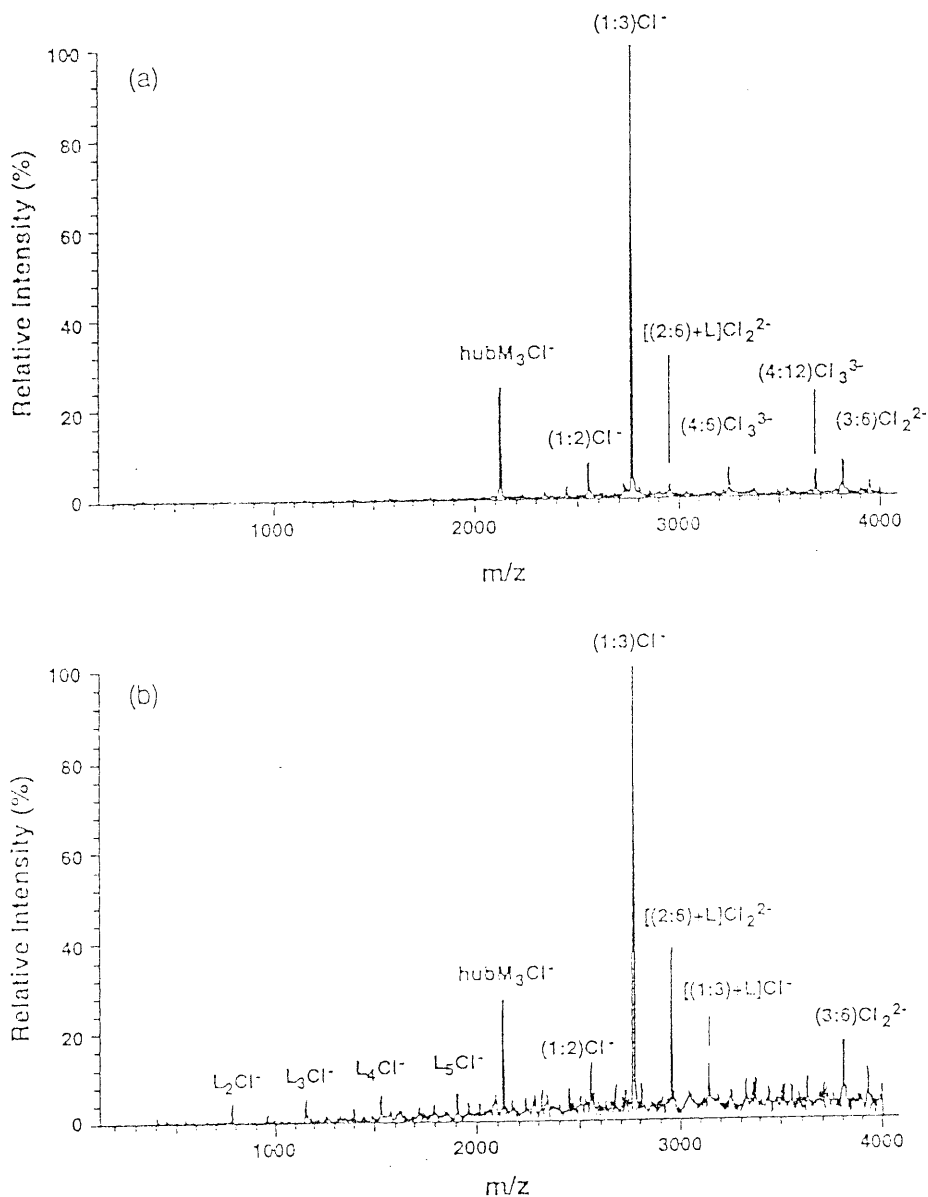


Figure 1. Negative-ion electrospray ionization mass spectra of  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  (1.0 mM) from  $\text{CHCl}_3$  with (a) 1.0 mM  $\text{Ph}_3\text{PCl}$  and an interface capillary temperature of 90 °C and (b) 1.5 mM  $\text{Ph}_3\text{PCl}$  and an interface capillary temperature of 70 °C. See Table 1 for nomenclature.

instability of (1:2) and (1:1) complexes compared to the (1:3) complex. This conclusion should not be affected by possible differential detachment of  $\text{Cl}^-$  from the (1: $n$ ) complexes ( $n = 0-3$ ) because all of these

compounds should have similar  $\text{Cl}^-$  affinity (due to their similar masses). In addition, we expect the  $\text{Cl}^-$  affinity of the (1:2) and (1:1) complexes to lie between those of the (1:3) complex and  $\text{hub}(\text{M})_3$ . The results of

Table 1. Observed ionic species from electrospray ionization of  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  in  $\text{CHCl}_3$  using  $\text{Ph}_3\text{PCl}$

$m/z$ : (observed)	Assignment <sup>a</sup>	$m/z$ : (calculated) <sup>b</sup>	Intensity (%) <sup>c</sup>
2128.9	$\{\text{hub}(\text{M})_3\}\text{Cl}^-$	2128.5	25
2555.0	(1:2)Cl <sup>-</sup>	2555.1	10
2768.1	(1:3)Cl <sup>-</sup>	2768.2	100
2955.3	$\{(2:6) + \text{L}\}\text{Cl}_2^{2-}$	2955.6	20 <sup>d</sup>
3145.0	$\{(2:6) + 2\text{L}\}\text{Cl}_2^{2-}$ or $\{(1:3) + \text{L}\}\text{Cl}^-$	3143.1	5 <sup>d</sup>
3253.2	(4:6)Cl <sub>3</sub> <sup>3-</sup>	3252.7	4
3697.7	(4:12)Cl <sub>3</sub> <sup>3-</sup>	3679.1	3
3813.5	(3:6)Cl <sub>2</sub> <sup>2-</sup>	3814.7	5

<sup>a</sup> The nomenclature ( $n:m$ ) denotes the complex with a stoichiometry of  $\{\text{hub}(\text{M})_n\}_m \cdot \text{RCA}_m$  and  $\text{L} = \text{Ph}_3\text{PCl}$ .

<sup>b</sup> Based on average molecule weights.

<sup>c</sup> The average value from Fig. 1(a) and (b).

<sup>d</sup> Intensity varies with the concentration of L.

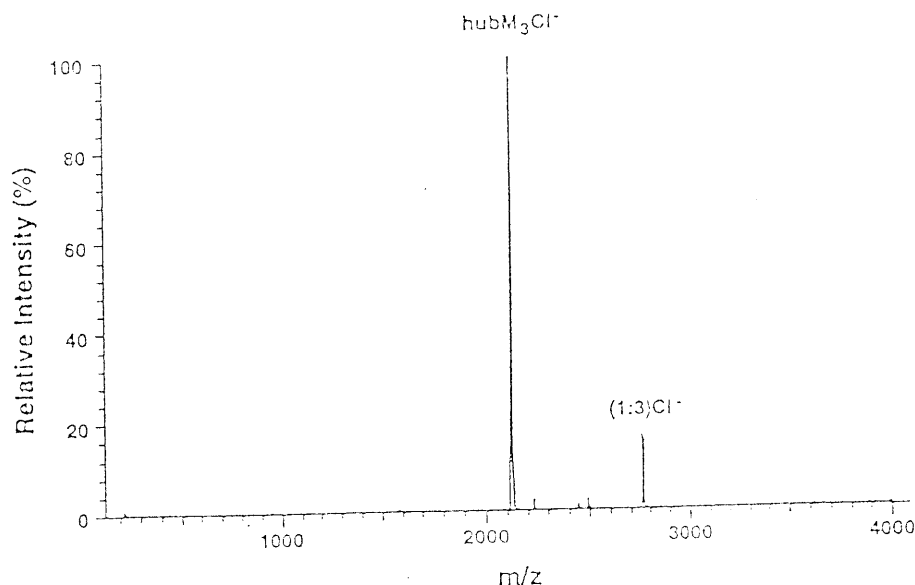


Figure 2. Collision-induced dissociation (CID) product-ion spectrum of  $m/z$ -selected  $\text{Cl}^-$ -bound (1:3) complex from a solution of  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  (1.0 mM) and  $\text{Ph}_3\text{PCl}$  (1.0 mM) in  $\text{CHCl}_3$  with an interface capillary temperature of  $90^\circ\text{C}$ . CID condition:  $E_{\text{lab}} = 33$  eV, target gas (Ar) partial pressure = 1.58 mTorr.

the temperature-dependence studies also indicated that the (1:3) complex was the dominant species in solution and that an accurate assessment of the solution composition requires very mild ESI interface conditions for such noncovalent systems.

## CONCLUSION

We have successfully demonstrated the detection of intact hydrogen-bonded supramolecular complexes that are neutral and are only stable in non-polar organic solvents by ESI-MS. The key element of this success is the attachment of a small, organic-soluble ion to the aggregate. For the first time, the  $\text{hub}(\text{M})_3 \cdot \text{RCA}_3$  complex (and other related compounds) has been observed using mass spectrometry. The accurate molecular weight measurement possible by mass spectrometry confirmed the 1:3 stoichiometry inferred in solution.

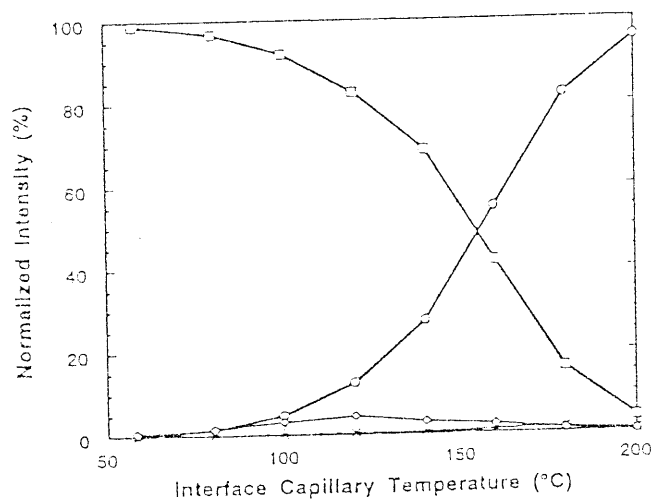


Figure 3. Plot of normalized intensity of  $\text{Cl}^-$ -bound (1:3), (1:2), (1:1) complexes and  $\text{hub}(\text{M})_3$ , vs. interface capillary temperature. The normalized intensity was calculated by dividing each ion intensity by the sum of the intensities of the four ions.  $\circ$ ,  $\text{hub}(\text{M})_3\text{Cl}^-$ ;  $\times$ , (1:1)  $\text{Cl}^-$ ;  $\square$ , (1:2)  $\text{Cl}^-$ ;  $\diamond$ , (1:3)  $\text{Cl}^-$ .

We expect that similar methods of attaching small, organic-soluble ions to noncovalent aggregates will find wide use in the analysis of neutral species that are stable or soluble only in non-polar organic solvents.

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