Cold-Spray Ionization Mass Spectrometry: Applications in Structural Coordination Chemistry

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Electrospray ionization (ESI)-mass spectrometry (MS) is generally used for the characterization of labile supramolecules in which non-covalent bonding interactions are predominant. However, molecular ions are not detected in many cases because of their instability, and even if such ions are detected, thermal decomposition generates fragment ions that also appear in the mass spectrum. Cold-spray ionization (CSI) is designed for the MS detection of labile organic species. It is used to analyze the structures of biomolecular complexes and labile organic species in solution. The method, a variant of ESI-MS, operates at low temperature, allowing simple and precise characterization of labile non-covalent complexes that are difficult or impossible to observe by conventional MS techniques. The CSI method is particularly suitable for elucidating the structures of labile organometallic compounds in solution as it offers a means to investigate the dynamic behavior of unstable molecules and/or labile clusters in solution. Various labile organic compounds are analyzed by using the CSI method in the field of organic chemistry. CSI-MS is also used to investigate the behavior of aggregated steroid compounds, namely, bisguanidinobenzene–benzoic acid complexes, in solution. This method is a powerful tool for analyzing the equilibria of multiply linked self-assembling catenanes in solution. Its application to unstable and complex supramolecules will be shown. We have developed an effective ionization method that uses metal-complex-based ionization probes containing 2,6-bis(oxazolyl)pyridine (pybox) ligands. Using this method, we were able to detect multiply charged ions of target molecules. This method was proven to effectively ionize large complex molecules, including biomolecules and various supramolecules, as well as carbon clusters, such as fullerenes. Moreover, isotope-labeled pybox-La complexes were used to clearly detect isotopic labeling shifts. Their applications to multiply charged ionization, including isotope labeling of biomolecules and carbon clusters using CSI-MS, will be shown.

Keywords: cold-spray ionization, cluster, supramolecule, coordination oligomer, ion probe attachment ionization, isotope labeling

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INTRODUCTION

One major drawback of mass spectrometry (MS) is that it does not allow us to observe the real behavior of labile organic molecules in solution. The nature of the hydrogen bonding interaction has remained unclear even in the case of simple water molecules, to say nothing of the precise behavior of complex biomacromolecules in solution. This is because higher-order structures are linked by weak intermolecular interactions that are readily disrupted. A key aim of modern MS is to shed light upon such structures that are central to the function of life itself.

The development of the soft ionization technique is thought to have sparked the advancement of MS. Since electron ionization (EI), the first practical ionization method used in chemistry, was developed, remarkable progress has been made in this area. The currently available soft ionization techniques have made it possible to analyze such biomacromolecules as proteins. The techniques are based on electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). ESI-MS is used for the characterization of coordination species, including labile supramolecules and biomacromolecules. However, molecular ions are not detected in most cases because of their susceptibility to thermal decomposition. The heat given off by the desolvation chamber, which is necessary for ionization in the gas phase in conventional ESI, may be responsible for destroying these complexes.

We have developed cold-spray or cryospray ionization (CSI), a low-temperature variant of ESI, in order to observe the real behavior of labile organic molecules in solution by MS. We have used this method to investigate the structures of primary biomolecules; labile organic species, including Grignard reagents; asymmetric catalysts; and supramolecules in solution. This method allows for the simple and precise characterization of labile noncovalent complexes that are difficult or impossible to observe by conventional MS techniques, including fast atom bombardment (FAB) and MALDI, as well as ESI. I wish to present here recent progress in CSI-MS observations of several complexes, as our contribution to structural coordination chemistry.

APPARATUS

The cold sprayer shown in Fig. 1 consists of an electrospray or ion spray ionization probe operating at low temperature. It features a dry gas (N$_2$) cooling device to maintain the temperature of the capillary and the spray itself below $-20^\circ$C, in order to promote ionization based on the
increased polarizability of compounds owing to the high dielectric constant at low temperature. Dielectric polarization ($P$) is a function of vacuum permittivity ($\varepsilon_0$), relative permittivity or dielectric constant ($\varepsilon_r$), and electric field ($E$).

$$P = \varepsilon_0(\varepsilon_r - 1)E$$  \hspace{1cm} (1)

Generally, a high dielectric constant of the solvent used is observed at low temperature, as described by Eq. (2) ($T$: temperature, $\Theta$: constant).

$$\varepsilon_r = \varepsilon_0e^{-T/\Theta}$$  \hspace{1cm} (2)

Therefore, it should be possible to ionize thermally unstable ionic species by solvation without decomposition. Although desolvation with a heated capillary and/or drying gas is thought to be an important step for ionization in the conventional ESI process, we confirmed that solvation (promoting electrolytic dissociation to form molecular ions) is a critical step in the CSI process. Further, cooling may favor the aggregation of the target molecules. In consequence, comprehensive structure elucidation may be achieved because highly characteristic molecular clusters are likely to be formed. The nebulizing gas is cooled by liquid nitrogen and its temperature can be controlled precisely from $-80$ to $+15^\circ C$. As a large amount of solvent is introduced into the mass spectrometer in the axial-type system, which interferes with ionization, an orthogonal spray device was developed.

We applied CSI-MS to highly ordered supramolecules derived by the self-assembly of transition metals, i.e., self-assembling nano-sized structures, in solution to confirm the performance of this apparatus compared to that of conventional ESI. MS measurements were performed with a four-sector (BE/BE) tandem mass spectrometer (JMS-700T, JEOL) equipped with a CSI source. Typical measurement conditions are as follows: acceleration voltage, 5.0 kV; needle voltage, 0 to 2.8 kV; needle current, 300 to 700 nA; orifice voltage, 20 to 70 V; resolution (10% valley definition), 1000 to 5000; sample flow, 8 mL/min; solvent, acetonitrile (CH$_3$CN); concentration, 0.01 to 0.1 mmol/L; spray temperature, $-20^\circ C$; ion source temperature, 100°C. The CSI- and conventional ESI-MS spectra of a 0.1 mmol/L acetonitrile solution of cate-type Pt complex ($\text{Ms}$), which is constructed from four rigid tridentate ligands, were compared. Whereas no significant result was obtained by conventional ESI-MS presumably because of the high desolvation plate temperature ($200^\circ C$), multiply charged molecular ions of [Ms$\equiv$(PF$_6$)$_n$ + (CH$_3$CN)$_m$]$^{\text{+}}$ ($n=3–10$, $m=0–21$) were clearly observed without decomposition in the CSI-MS spectra. Interestingly, the number of acetonitrile molecules ($m$) attached to the molecular ion increased with increasing positive charge. This suggests that the acetonitrile molecules solvated the Pt$^{2+}$ ions. Thus, CSI was proved to be an effective method to characterize the structures of labile self-assembling complexes in solution.

Some macrocyclic polyethers, such as crown ethers, exhibit excellent performance as internal calibrants for exact MS measurements in CSI-MS. The reasons are as follows: 1) Crown ethers with alkali metal ions (Mc$^+$, K$^+$, Rb$^+$, and Cs$^+$) are easily ionized as [crown$^+\cdot$Mc$^+$] under CSI conditions. 2) Crown ethers exhibit no interference with the analyte when the sample solution contains various kinds of calibrants and adducts. 3) Essentially quantitative ionizing ability of crown ethers is observed in the positive CSI-MS process.

**ORDERED CLUSTER FORMATION**

We have investigated the formation of large-scale aggregated chain structures or clusters in solution through intermolecular hydrogen bonds by using CSI-MS as well as NMR and X-ray crystallography. In the course of this study, the hydrogen-bonded stoichiometry-controlled complexation of o-phenylenebis(N,N-dimethyl-N,N'-ethylene)-guanidine (BG) and benzoic acid (BA) in the crystalline state was observed and the absolute ordered cluster formation of BG–BA complexes in solution was investigated by CSI-MS. The cluster formation was thought to be the most important step to generate supramolecular systems in solution. 1:1, 1:2, 1:3, and 1:4 BG+BA complexes [BG+BA $n=(1, 2, 3, 4)$] based on hydrogen bonding observed in the crystalline state were obtained from each stoichiometrically identical BG+BA system in solution. Conventional ESI-MS showed no ion peaks corresponding to the complex observed in the solid state. In contrast, the molecular-ratio-dependent complex was clearly observed by CSI-MS measurement, as shown in Fig. 2.

In all systems, the negative ion [BA$^-$H$^-$] was attached to the neutral BG+BA complexes, giving rise to the [BG+BA]+(BA$^-$H$^-$)$^-\text{+}$ signal. The molecular ion peaks of 1:n systems ([BG+BA]+(BA$^-$H$^-$)$^-\text{+}$ ($n=1, m/\text{z}$ 543; 2, m/\text{z} 665; 3, m/\text{z} 787; 4, m/\text{z} 909) were unambiguously observed. In the cases of 1:2, 1:3, and 1:4, other ion peak(s) corresponding to lower stoichiometric systems [BG+BA]+(BA$^-$H$^-$)$^-\text{+}$ ($n=2–4, m=1–n–1$) were observed. Therefore, CSI-MS measurements enable the direct observation of the formation of ordered clusters in solution. These results were supported by diffusion studies by pulsed field gradient (PFG) NMR.

The stoichiometry-controlled absolute ordered cluster formation of BG and BA in solution was confirmed by CSI-MS. The results give experimental evidence that the desired molecular complex in solution as well as in the solid state can be obtained by simply mixing acid and base components.
CSI-MS was adopted to investigate the self-assembly of giant $M_{24}L_{48}$ coordination spheres from 24 palladium ions (M) and 48 curved bonding ligands (L).\(^{20}\)

The $M_{24}L_{48}$ composition of b, a dipyridylthiophene (Fig. 3A), was first established by ultrahigh-resolution CSI time-of-flight MS (CSI-TOF-MS). The molecular weight of sphere b $[\{\text{Pd}_{24}(\text{C}_1\text{H}_9\text{BrN}_2\text{S})_{48}\}]^{48+} \cdot 48(\text{BF}_4^{-})$ was calculated to be 21,946.73 daltons, confirming the $M_{24}L_{48}$ composition, from a series of prominent peaks for $[b-(\text{BF}_4^{-})]_m^m$ ($m=28$ to $\sim 15$). The resolution of each peak was quite high (>45,000 based on the full width at half maximum) and the isotope distribution closely matched the simulated spectrum (error $= <3.5$ ppm) (Fig. 3C). Similar CSI-TOF-MS spectra were observed for $M_{24}L_{48}$ structures a and c. Structural confirmation of the $M_{24}L_{48}$ coordination geometry was also accomplished by X-ray crystallographic analysis. By mapping the 48 ligands to the edges and the 24 metal ions to the vertices, we found that $M_{24}L_{48}$ c forms a rhombicuboctahedron, which is an Archimedean solid with eight triangular and 18 rectangular faces (26 in total), with one triangle and three rectangles meeting at each vertex (Fig. 3B). The rhombicuboctahedral structure of c is highly spherical with the 5.0-nm-diameter circumscribed and 3.6-nm-diameter inscribed spheres defining the molecular shell. We previously reported that a 36-component $M_{12}L_{24}$ coordination sphere was formed when the analogous dipyridylfuran ligand was used.\(^{20}\) The structure was also determined by CSI-MS. Systematic variation of the mean ligand by mixing dipyridylthiophene and dipyridylfuran in various ratios revealed that even a slight change in the mean ligand bend angle critically switched the final structure between $M_{24}L_{48}$ and $M_{12}L_{24}$ coordination spheres. CSI-MS was also applied to this analysis together with DOSY (diffusion-ordered NMR spectroscopy) analysis.

**MOLECULAR SPHERE**

**SUPRAMOLECULAR POLYMER**

We have reported the design and preparation of a new class of back-to-back twin bowls of $D_3$-symmetric tris(spiroborate) cyclophanes that exhibit molecular recognition behavior via $\pi$- and electrostatic interactions at both sides of a symmetry plane.\(^{23}\) Mimicking vertebrate spine, this molecular coupling behavior makes it possible to glue various cationic guest molecules to each other via dual host–guest interaction. Particularly in the case of cationic metal complexes, interactive clathration takes place to form supramolecular metallopolymers. The spiroborate linkage was chosen for spontaneous cyclophane formation because its accessibility and unique thermodynamic behavior.
allowed us to obtain highly symmetric cyclic structures from simple bis(dihydroxyarene) units in a self-organization manner (Fig. 4).

Tris(spiroborate) cyclophanes were readily prepared from 2,2',3,3'-tetrahydroxy-1,1'-binaphthyls (2) and boric acid. Equimolar amounts of rac-2 and boric acid were mixed in DMF at 150°C to afford trimer cyclophane rac-1a (Me₂NH₂). Dimethylamine was generated by hydrolysis of DMF.

To demonstrate the molecular recognition ability of 1⁻ in solution, we chose [Ir(tpy)₂](PF₆)₃(3⁻) as the guest molecule (tpy: 2,2':6',2"-terpyridine) and the complexation of 1⁻ with 3⁻ was monitored by ¹H NMR. The formation of a chain structure was also confirmed by CSI-MS. When a 1 : 1 mixture of 1⁻ and [Fe(tpy)₂](PF₆)₃ in DMF/acetonitrile was sprayed in the positive-ion mode at 25°C, various ion peaks derived from oligomeric structures were observed. Among them, characteristic ion peaks were found at m/z = 2846.7, 3503.3, and 4159.2, which were assigned to [(−)−1b⁻][3⁻]⁺, [(−)−1b⁻][3⁻][2⁻], and [(−)−1b⁻][3⁻][3⁻], respectively.

Twin bowls 1⁻ have two kinds of molecular recognition sites: two bowl-shaped cavities and a crown-ether-like cavity that would bind a suitable guest individually. Therefore, heterobimetallic supramolecular polymers were prepared by utilization of the dual molecular recognition modes of the molecular connection module 1⁻. It was found that the polymerization of 1⁻ and [Fe(tpy)₂](PF₆)₃(4⁻) was enhanced by the addition of a potassium cation, whereas the presence of a barium cation disturbed the association of the monomers. AUC-SV (Analytical ultracentrifugation sedimentation velocity) showed the approximate molecular weight and CSI-MS confirmed the results.

COORDINATION Oligomer

Coordination chemistry, which was originally the study of "inorganic complexes," is one of the largest and most important research fields because of its application to the construction of macromolecules by using bridged organic ligands and host-guest chemistry. Subsequent to the progress made in this field, coordination polymers (CPs) and metal-organic frameworks (MOFs) having nano-sized channels were reasonably formed depending on the shape and size of the organic ligand. However, there is no appropriate procedure to elucidate the structures of CPs and MOFs. Although NMR is used to characterize structures in solution and examine molecular dynamics, including reaction and coordination, it does not yield information of the precise numbers of organic ligands and metals in a complex. CP formation is an unexplored area in weak-interaction chemistry due to the difficulty of monitoring the coordination process. By using CSI-MS, several kinds of coordination compounds were detected when CuI metal and the 4,7-phenanthroline organic bridge ligand were mixed in solution. The observed ion peaks could be reasonably and simply assigned to various combinations of the metal and the ligand without any fragmentation.²⁴

The complexation of organic ligand 1, 4,7-phenanthroline, and Cu(I) iodide in acetonitrile solution by a liquid-liquid diffusion method gave a red prism crystal that appeared within a few minutes (Fig. 5a). Single-crystal X-ray structure analysis clearly revealed two different 1-D chain structures, as shown in Figs. 5b–d. In one chain structure, the nitrogen of ligand 1 is coordinated to the copper of Cu to form structure I, a Cu-I chain [Cu(I)]ₙ (Fig. 5b). The tetrahedral coordination of Cu•••I with a couple of Cu and I atoms
its isotope pattern was identified and its specific ligands were observed as positive ions with high intensity. The observed isotope patterns of the other ion peaks also showed good agreement with their calculated ones in Fig. 5f, and the ion peak at m/z 612.90 was ascribed to \([\text{Cu}((\text{CuI}))_2]\) and the next larger molecular ion peak at m/z 804.73 was assigned to \([\text{Cu}((\text{CuI}))_3]\) as an adduct of CuI, although \([\text{Cu}((\text{CuI}))_1]\) was not observed. From m/z 1700 to 2400, the addition of a specific ligand or metal was clearly observed in the form of a large cluster.

CSI-MS can detect many kinds of coordination oligomers in solution and this feature enables us to discuss oligomer formation trends. In general, polymer solubility is decreased as the number of repeating units is increased. The solubility of CPs is decreased when the numbers of metals and ligands are increased. Therefore, these coordination oligomers observed in solution are important to discuss the formation of CPs. The observed coordination species in CSI-MS could correspond to a part of the structure of CP, or a precursor of crystalline CPs. From this analysis, we suggest that CSI-MS may be used to identify a part of CP in order to detect a soluble coordination oligomer prior to crystallographic analysis.

IONIC PROBE

We have developed very recently a new ionization method that uses metal-complex-based ionization probes containing the 2,6-bis(o xoazolinyl)pyridine (pybox) ligand: NHS-TMpybox, Mal-TMpybox, BrAc-TMpybox, oxime-TMpybox, and Sar-TMpybox. NHS-TMpybox, Mal-TMpybox, BrAc-TMpybox, and oxime-TMpybox were reacted selectively with the functional groups of biomolecules, whereas Sar-TMpybox was reacted with carbon clusters. Multiply charged ions of target molecules were obtained. This method was proven to effectively ionize large complex molecules, including biomolecules and various supramolecules, as well as carbon clusters, such as fullerences. Moreover, isotope-labeled pybox-La complexes were used to clearly detect isotopic labeling shifts. Their applications to multiply charged ionization, including the isotopic labeling of biomolecules and carbon clusters using CSI-MS, were investigated.

We have designed ionic probes called the TMpybox series that can donate multiple charges contained in the metal charged site of the probe molecule to the target compound. The probe comprises three functional parts: a charged site, a linker, and an anchoring site. Pybox-La complex, which enables complex formation under mild conditions, is used as the charged site. An alkyl chain is used as the linker. Five anchoring sites that enable selective reaction with various target compounds were developed: N-hydroxysuccinimide (NHS) for amino groups, maleimide (Mal) for thiols, \(\alpha\)-bromoacetate (BrAc) for carboxylic acids, hydroxylamine for carbonyl groups, and sarcosinate (Sar) for carbon clusters.

Each probe is reacted selectively with the target compound to obtain the corresponding ionic species having multiple charges. The charged site is formed by the pybox-La complex through the five probes. NHS-TMpybox, carbon clusters using CSI-MS, were investigated.

furnished another 1-D chain structure called structure II, \([\text{Cu}I]\) (Fig. 5d). Cu•••I lengths of 2.706, 2.639, and 2.670 Å are in good agreement with the previously reported Cu•••I length, and a \(\pi-\pi\) interaction is noted between two adjacent ligands 1 within 3.4 Å in the crystal (Fig. 5e).

The filtrate of the crystallization solution was subjected to CSI-MS after the crystals were grown for three days. Molecular ion peaks were observed as positive 1+ charge in the range of m/z 400 to over 2500 (Fig. 5f). The results indicated that there were many kinds of coordination complexes in solution. For example, the ion peak at m/z 423.0 was ascribed to \([\text{Cu}((\text{CuI}))_2]\), where two ligands 1 occupied two positions of the copper tetrahedral coordination site. Moreover, its isotope pattern was identical with the calculated one, as shown in the inset of Fig. 5f. A unique pattern was derived from the copper atom, \(^{65}\text{Cu}+^{65}\text{Cu}=100:44.6\). Similarly, the isotope patterns of the other ions also showed good agreement with their calculated ones in Fig. 5f, and the ion peaks were combinations of \((m,n)\) in \([\text{Cu}((\text{CuI}))_n]\) (m and n are integers). The ease of assignment was attributed to the CSI method. In fact, ESI-MS gave more molecular ion peaks than CSI-MS, and only an inorganic compound was observed in the negative ion detection mode. We then focused on the relationships among the observed coordination complexes. Up to m/z 1000, four types of coordination species were formed from various combinations of CuI and ligand 1. The observed coordination species were obtained by adding a specific ligand or metal. For example, the molecular ion peak at m/z 612.90 was ascribed to \([\text{Cu}((\text{CuI}))_2]\) and the next larger molecular ion peak at m/z 804.73 was assigned to \([\text{Cu}((\text{CuI}))_3]\) as an adduct of CuI, although \([\text{Cu}((\text{CuI}))_1]\) was not observed. From m/z 1700 to 2400, the addition of a specific ligand or metal was clearly observed in the form of a large cluster.

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Each probe is reacted selectively with the target compound to obtain the corresponding ionic species having multiple charges. The charged site is formed by the pybox-La complex through the five probes. NHS-TMpybox,
Mal-TMpybox, BrAc-TMpybox, Sar-TMpybox, and oxime-TMpybox are reacted selectively with amines, thiols, carboxylic acids, and fullerenes in the presence of benzaldehyde and ketones, respectively (Fig. 6). Furthermore, isotope-labeled ionic probes were also investigated. The combination of stable isotope labeling and MS using ESI and MALDI was developed. This technique has made it possible to solve various problems in bioanalytical research by means of metabolic tracer experiments.

An effective and reliable ionization method called ionic probe attachment ionization has demonstrated the utility of novel MS probes in the ionization of large complex molecules, including biomolecules and carbon clusters, with CSI-MS. NHS-TMpybox that was reacted with the amino group by simple mixing in acetonitrile clearly produced multiply charged ions of the probe-attached compound ([M+n]⁺) in CSI-MS. TMpybox series could react with correlative molecules under mild conditions and were easily synthesized. In addition, an analyte to which isotope-labeled pybox-La complexes were attached was easily ionized in soft conditions and exhibited reasonable isotopic shifts.

CONCLUSION

Various labile organic compounds have been analyzed by CSI-MS in the field of organic chemistry to date. In this paper, several applications in structural coordination chemistry were shown.

Investigations of the real solution behavior of labile organic molecules by MS are generally difficult. As has been discussed in the Introduction, this is because higher-order structures are linked by weak intermolecular interactions that are readily disrupted. We have shown that CSI-MS is useful for investigating the behavior of bisguanidinobenzene–benzoic acid complexes in solution. In the self-assembly of giant spheres, CSI-TOF-MS made it possible to confirm molecular weights exceeding 20,000 daltons. In the case of supramolecular polymers, CSI-MS also proved polymer formation in solution. The resulted structures from these three examples are identical with those obtained from NMR and X-ray analyses in each compound. In the analysis of coordination polymers, CSI-MS could well detect soluble coordination species that were separated into the ligand-rich type and the CuI-rich type, based on coordination bonding and the halogen–metal interaction. As the polymer grew, its solubility decreased. We believe that structure elucidation of a coordination polymer in solution by CSI-MS is useful to create complicated coordination polymers and metal-organic frameworks with interesting functionalities. Finally, a new method that utilizes ionic probe ionization was shown. This ionization method, which uses various kinds of probes, was proven to be suitable for soft spray ionization, including ESI and CSI. In the case of large molecules, in particular, CSI is the best choice.

In the near future, we foresee that this ionization technique will be widely utilized by many MS users because of its potential importance in a wide variety of structural investigations in natural sciences.

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REFERENCES

2) M. Karas, D. Bachmann, F. Hillenkamp. Influence of the wavelength in high-irradiance ultraviolet laser desorption mass


