Mass spectrometry and viral analysis

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Background: Electrospray ionization (ESI) mass spectrometry is a powerful new approach for analyzing biomolecules and biomolecular complexes. Previous studies have provided evidence that non-covalent biomolecular complexes can be observed by ESI mass spectrometry; it is not clear, however, whether the native conformation of the biomolecules is maintained throughout the ionization and analysis process. We set out to address this question using live viruses.

Results: Viral ions have been generated in the gas phase using electrospray ionization mass spectrometry. These ions have been collected, following ion filtering through the mass analyzer, and then analyzed by transmission electron microscopy. Transmission electron microscopy revealed that rice yellow mottle virus and tobacco mosaic virus retained their respective spherical and rod-like ultrastructure. The viability of the isolated tobacco mosaic virus was confirmed by inoculation and infection of tobacco plants.

Conclusions: These results demonstrate the utility of electrospray for supramolecular complexes with molecular weights of over 40 million Da and offer conclusive evidence that native biomolecular structures can be conserved through the electrospray process.

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Introduction

In the past 10 years mass spectrometry has emerged as a powerful tool for the analysis of biomolecules [1]. In particular, electrospray ionization (ESI) mass spectrometry has generated a great deal of interest; using this technique, gas-phase biomolecular interactions [2,3] can be correlated with those in the condensed phase. Several biomolecular interactions have been observed using ESI mass spectrometry, including the ternary complex of dimeric HIV-1 protease and an inhibitor [4], a duplex DNA–drug complex [5], calcium-mediated cell-surface carbohydrate association [6] and catalytic antibody–hapten/substrate interactions [7,8]. It is thus apparent that ESI mass spectrometry offers great promise as a tool for chemists and biologists alike; here, we demonstrate the potential of this technique for the analysis of viruses.

Advances in viral research have been largely associated with the development of physical techniques such as X-ray crystallography and electron microscopy [9–12]. Developing mass spectrometry to mass measure viral ions and other supramolecular complexes could significantly improve on the speed and accuracy of existing methods. Viral analysis could also help answer some basic questions about ESI mass spectrometry, particularly concerning non-covalent interactions and the potential use of this technique as a preparative tool.

While convincing evidence exists that non-covalent interactions can be observed with ESI mass spectrometry [2–9], it remains to be shown that native conformations of biomolecules are preserved throughout the ionization/vaporization process and in the vacuum of the mass spectrometer [13]. A second question, reminiscent of the Manhattan Project where Calutron mass spectrometers were used to separate uranium isotopes [14], is whether this technology can be used as a separation and collection device for biomolecules. Our work addresses these issues with the first direct observation that the biological activity of a supramolecular (4×10^7 Da) complex can be preserved following ESI mass spectrometry. Our ability to non-destructively generate, electrostatically focus and collect supramolecular complexes also demonstrates the feasibility of using mass spectrometry as a high resolution separation/purification technique. This work suggests that there is potential for mass spectrometry to be used in a preparative manner.

Results and discussion

It is well established that large ions can be generated in the gas phase using ESI [15,16], but the ability to mass analyze and detect these ions has been limited by the analyzing and detection devices available. Detectors typically generate secondary electrons from colliding ions, which then cascade to generate more electrons, resulting in amplification on the order of $\sim 10^6$ electrons per striking ion. Large ions (>1 000 000 Da), unless very highly charged, do not produce significant ion signals, because low energy collisions produce little or no secondary electron emission.

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Another consideration in performing these experiments on viral ions is that the m/z range of their charge states is higher than the m/z range of most mass analyzers. For instance, it has been demonstrated that native proteins typically produce a charge state distribution at higher m/z when compared to their denatured conformer [17–19]. Since viruses are highly folded, extremely large non-covalent complexes, it was expected that the charge state distribution would be beyond the scanning range of our instrumentation (m/z 2300). Indeed, when initial viral electrospray experiments with rice yellow mottle virus (RYMV) and tobacco mosaic virus (TMV) were performed by scanning the quadrupoles up to m/z 2300, there was no detectable accumulation of viral ions at the collector. The results of these experiments were consistent with the idea that the m/z of the viral ions exceeded the range of the quadrupole mass analyzer. These experiments also demonstrated that neutral viruses were not drifting onto the collector.

To compensate for our inability to scan within the m/z range of the viral ions, we used the radio frequency (RF) only mode for quadrupoles Q0, Q1 and Q2. The basic design of the mass analysis instrumentation we have used is illustrated in Figure 1. The RF-only mode can allow ions of high m/z to pass through the quadrupoles. When the viral experiment was performed in RF-only mode, viral ions were physically collected on a glycerol coated brass plate. The isolated specimen was then directly examined by transmission electron microscopy (Fig. 2). In addition to identifying the viruses following selection, the transmission electron micrographs also showed that the structure of the icosahedral RYMV capsid and the rod shape of the helical TMV capsid had been preserved (Fig. 2). The high molecular weight and characteristic rod-like structure of TMV provided a striking example of the applicability of ESI mass spectrometry for viruses. It is important to note that the capsids of RYMV and TMV are stabilized by non-covalent interactions between protein subunits.

We also demonstrated here that mass selection can be achieved with these large ions. Mass selection was achieved by lowering the accelerating voltage between Q0 and Q1. Under these conditions, the free expansion of gas into the vacuum results in massive ions gaining much higher translational energies and reaching the

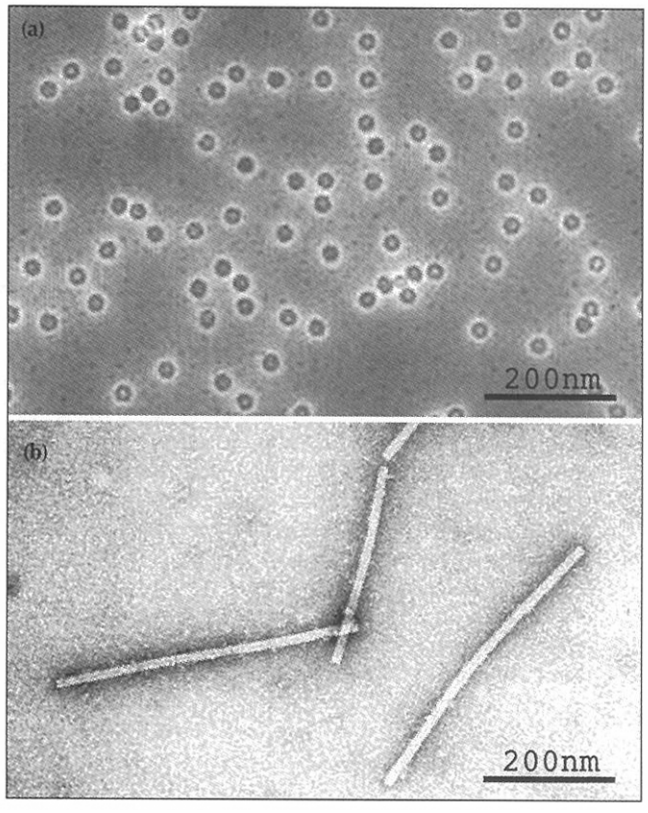

Fig. 2. RYMV and TMVretain their quarternary structure following electrospray ionization. Transmission electron micrographs of **(a)** RYMV and **(b)** TMV collected after ESI and mass selection.

detector, whereas low mass ions drift out of the analyzer before reaching the detector. This approach was validated using a mixture of egg white lysozyme (molecular weight = 14 317 Da) and a small peptide (molecular weight = 709 Da), where the peptide was filtered out by adjusting the potential between Q0 and Q1. In the analysis of the viruses, the accelerating voltage was similarly reduced, thus removing contaminating low mass ions and allowing all higher mass ions to reach the detector. This procedure facilitated (albeit crudely) selection of the virus.

To further demonstrate the charged nature of the collected viral ions, a collector was placed directly behind the orifice and before the quadrupoles. Ionization was then performed under normal operating conditions for positive ions and virus was collected. When the voltage

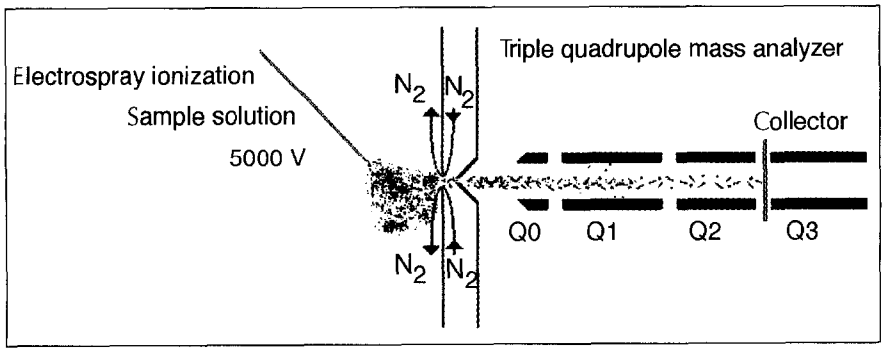

Fig. 1. An electrospray ionization quadrupole mass spectrometer modified for these experiments. Virus is ionized and electrostatically directed through four sets of quadrupoles, Q0, Q1, Q2 and Q3, which allow for ion focusing and mass selection. The glycerol coated brass plate placed at Q3 serves as an ion collector. Nitrogen serves as a curtain gas that restricts the entrance of neutral particles into the mass spectrometer.

Fig. 3. TMV remains viable following electrospray ionization. Tobacco cultivar Xanthi NN leaves inoculated with a control solution of glycerol (left), TMV/glycerol collected inside the mass spectrometer from the ion collector (Fig. 1) following electrospray ionization (center) or untreated TMV/glycerol (right) are shown. Leaves were photographed 4 days after inoculation.

on the electrostatic lenses was adjusted such that ions would be deflected from the orifice, allowing only neutral species to pass, no virus was collected. These results provided additional confirmation that we were collecting charged viral ions and not neutral viruses.

The viability of the virus particles collected following ESI mass spectrometry was determined to provide the ultimate test of whether the viral ions truly retained their native conformation throughout the ionization/analysis procedure. This procedure exposed the virus to a number of conditions that could potentially damage tertiary or quaternary protein structure or the nucleic acid structure, resulting in a non-viable specimen. Damage could be caused by ionization, vacuum conditions, glycerol, electromagnetic fields and impact with the collector. Since electron microscopy is limited in its ability to determine whether damage has occured, specimen viability was also tested by infecting the tobacco cultivar Xanthi NN plants with the post-analysis TMV. The production of characteristic local lesions on the leaves of plants inoculated with post-analysis TMV demonstrated that the virus retained infectivity (Fig. 3).

Significance

Viral ions have been generated in the gas phase using electrospray ionization mass spectrometry and were found to retain their ultrastructure and viability following isolation. This is the first direct evidence that gas-phase non-covalent complexes can reflect the solution structure of supramolecular complexes that have molecular weights of over 40 million Da. The retention of virus ultrastructure and biological activity provide evidence that the quaternary structure of non-covalent complexes can be conserved throughout the analysis process. Given its nondestructive nature, ESI mass spectrometry could be developed as a purification technique for biomolecules and biomolecular complexes offering the ability to differentiate compounds that vary in mass by 5% or more. The ability to select or mass measure a subgroup within a population would allow virologists to better understand the diversity of viruses and to select members of a population that have significant mass differences, for example, due to alteration of a viral protein or binding to an antibody.

Materials and methods

Electrospray MS

A Perkin Elmer SCIEX API-3 triple quadrupole mass spectrometer was modified by placing a brass plate as an ion collector in the flight path of the ions between the second (Q2) and third quadrupoles (Q3) (Fig. 1). Additional experiments were also performed with the collector placed directly behind the orifice within the vacuum of the mass spectrometer. In both cases the collector was coated with a thin layer of methanol/glycerol (50:50) and dried for 3 min, leaving a thin, gelatinous surface. The sample solution (1.35 mg ml^{-1}) was then directly infused at a rate of 3 $\mu l\ min^{-1}$, with the declustering potential typically maintained at 100 V and the potential on the emitter at 5000 V. In each experiment ~0.1–0.5 mg was used. The viability experiments required the use of ~2.0 mg of TMV. The electrospray experiments were performed either by scanning up to m/z 2300 or by maintaining the quadrupoles in R.F-only mode. These experiments were performed predominantly in positive ionization mode; initial experiments performed in negative ion mode did not result in the detectable collection of viral ions.

Electron microscopy

The viruses deposited in glycerol were transferred from the plate by dissolving the solution in distilled water. The samples, deposited on a parlodion-carbon grid, fixed in glutaraldehyde and negatively stained in aqueous uranyl acetate, were viewed with a Hitachi H600 electron microscope at 60 000 times original magnification.

Viruses

Two viruses were analyzed in this study, RYMV and TMV, belonging to the *Sobemovirus* and *Tobamovirus* genera, respectively [9]. RYMV is a single-stranded RNA virus with a non-enveloped icosahedral capsid, 28.8 nm in diameter with a molecular weight of ~6.2×10^6 Da [20]. TMV is a single-stranded RNA virus with a non-enveloped helical capsid. The rod-like structure of TMV has a diameter of 17.5 nm, a length of 300 nm and a molecular weight of 40.5×10^6 Da. Prior to analysis, the samples were desalted by centrifugal filtration

(100 000 m.w. cut-off, Millipore) and resuspended in distilled water. Purified TMV, common strain, was purchased directly from ATCC, and RYMV (*Oryza sativa*) was obtained from a rice field on the Ivory Coast. RYMV extraction and purification were previously described [20]. These viruses were selected because they represent contrasting viral structures, and could thus test the generality of the experimental method.

Viability experiments

The virus inoculation experiments were performed with one-month-old *Nicotiana tabacum* L. 'Xanthi NN'. Following the mass spectrometry experiments the TMV virus was collected from the ion collector, diluted 1:2 with 20 mM sodium phosphate buffer pH 7.0 and directly placed onto Carborandum-dusted (330 grit, Fisher Scientific) tobacco Xanthi NN leaves. Control plants were also inoculated with phosphate buffer alone and phosphate buffer with virus. Immediately after inoculation, the plants were rinsed with water and placed in growth chambers.

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