Electrospray Ionization of a Whole Virus: Analyzing Mass, Structure, and Viability

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Introduction

Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) were the result of decades of research effort dedicated toward analyzing intact biomolecules. The practicality of these ionization methods fueled the explosion of mass spectrometry witnessed in the past decade. In fact, the widespread utility of ESI and MALDI led to the awarding of the 2002 Nobel prize in Chemistry to Fenn and Tanaka for their roles in developing these techniques. The focus of this article is largely on ESI, which has the unique ability to gently generate gas-phase ions directly from solution.[1] Initially, the ability to transfer intact proteins and nucleic acids into the gas phase was reason enough for excitement, yet soon after it was shown that even noncovalent interactions could survive the ESI ionization/desolvation process. The first noncovalent interactions observed included a protein heme[2] and receptor ligand complexes,[3] multimeric enzyme inhibitor complexes,[4] and, ultimately, whole-virus analysis was achieved.[5]

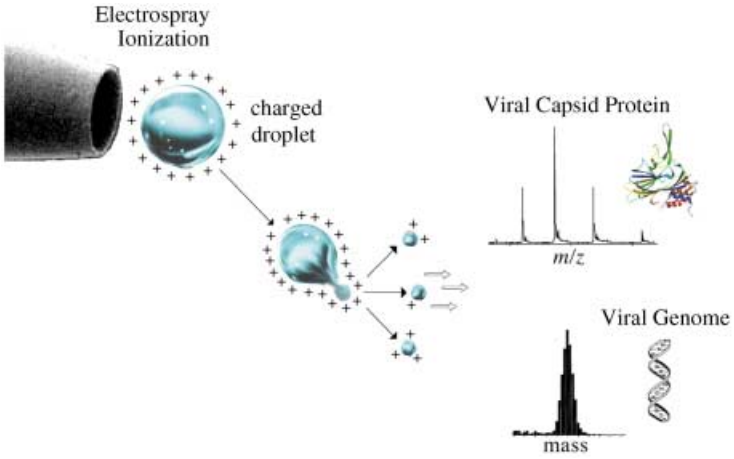

Figure 1. *Electrospray ionization allows for the analysis of viral proteins and genetic material. The ability of ESI to generate intact multiply-charged ions in the gas phase led to the analysis of viral proteins and nucleic acids.*

The Success of Mass Spectrometry for Virus Protein and Genome Analysis

The study of viruses by mass spectrometry began with the mapping of strain variants and post-translational modifications of viral capsid proteins.[6] Early proteomics-based approaches that used mass spectrometry were employed to identify viral structural proteins[7] and map the disulfide bridging pattern in a virus capsid.[8] In addition to viral proteins, the viral genome has also been successfully measured with ESI-MS (Figure 1). Genome size varies tremendously from small viruses with only $\sim$4000 base pairs to Mimivirus with a genome of 800000 base pairs.[9] Mass measurement of the intact genome of Coliphage T4 (1.3 MDa) by ESI Fourier transform ion cyclotron resonance mass spectrometry[10] was an early success in the mass analysis of viral DNA. The typical viral genome is much larger than that of T4 and represents an extreme challenge for conventional mass spectrometry instrumentation. Ultimately, it was the introduction of ESI-MS with charge detection[11] that allowed for the analysis of a 5 MDa DNA molecule.[12]

Early Success: Virus Viability after ESI-MS

The analysis of whole viruses or any megadalton structure by ESI-MS presents a number of technical challenges that have proven difficult to overcome. The first challenge is that the mass range of most ESI instruments is not high enough, even with the multiply-charged species generated by ESI. Second, slow-moving megadalton particles generate a small signal with conventional detectors. Large macromolecular complexes of folded proteins, such as viruses, will have mass-to-charge ratios (m/z) much higher than 20000,[13] which is beyond the upper m/z limit of most commercially available mass analyzers. The other limitation is that standard mass spectrometry detectors operate by amplifying an initial cascade of electrons generated from the colliding ion. For small molecules the amplification is $\sim 10^6$, however, as ion size increases, the amplification decreases significantly; this results in a lower detection efficiency, hindering or making impossible the analysis of megadalton molecules.

In 1993, our laboratory became interested in the prospect of analyzing viruses by using mass spectrometry. We believed that viruses represented an interesting analytical challenge and

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would make a good model system for testing the limits of ESI. The primary goal of these first attempts to analyze an intact virus was to determine if viruses could survive ionization and be transferred into the gas phase intact, and at the same time perform mass selection.[14] Initial attempts to detect Tobacco Mosaic Virus (TMV, 40.5 MDa) and Rice Yellow Mottle Virus (RYMV, 6.5 MDa) were conducted on a standard ESI instrument with a triple quadrupole mass analyzer with a maximum detection of $m/z = 2400$. However, the m/z ratios of the ionized viruses were far above the mass analyzer limit. This limitation was overcome by operating the quadrupole mass analyzer in radio frequency mode, in which only ions of high m/z were allowed to pass. When these simple mass spectrometry experiments were performed, a weak yet reproducible signal approximately 20-fold above background was detected; this demonstrated that large ions were reaching the detector.

The next step in these experiments was to determine whether the viral ions were intact. To accomplish this, we collected the viral ions from a glycerol-coated brass plate placed in front of the detector (Figure 2). The idea behind collecting ions within a mass spectrometer is reminiscent of the Calutron mass spectrometers used to separate uranium isotopes.[15] As illustrated in Figure 2, the ions passed through the mass analyzer and were then captured on a glycerol-coated plate within the instrument. The isolated sample was then directly analyzed by negative-stain transmission electron microscopy. Both RYMV and TMV particles remained intact based on the electron microscopy (EM) images, the native structure had been preserved.

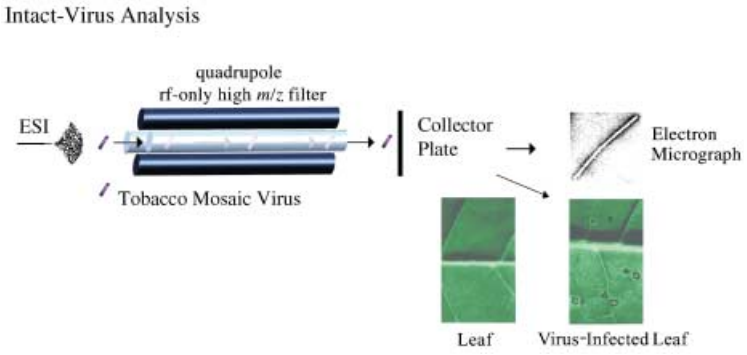

Figure 2. *Original ESI virus selection, capture, and viability experiments. Electrospray-generated viral ions of high* m/z *were selected for with a quadrupole mass analyzer and deposited on a glycerol-coated collector for subsequent visual and biological testing. The native structure of the virus was preserved throughout the experimental behavior as determined by electron microscopy and infectivity.*

Even with the success of these experiments, it was clear that damage to the protein capsid or the packaged RNA could be undetected in the EM images. Therefore, the viability of the collected virus following ESI mass spectrometry was tested to provide definitive evidence of whether the native state was retained. This experiment was conducted by inoculating Tobacco cultivar Xanthi plants with TMV collected in the mass spectrometer. The tobacco plants developed lesions characteristic of infection (Figure 2); this demonstrated that the viruses were viable.

Ultimate Success: Mass Measurement of a Virus

Even though it was demonstrated that viruses remained intact and viable throughout the analysis process, albeit with crude mass selection, the realization of a mass measurement of a complete virus particle required the development of new technology. A critical step along the pathway was the design and use of a novel detector capable of measuring the charge and velocity of an ion simultaneously. By using an ESI/time-of-flight (TOF) mass spectrometer equipped with a charge-detection device designed by Benner,[16, 17] an intact viral genome was mass measured.[18] This represented, at that time; the single largest molecule to be successfully mass analyzed.

Charge-detection mass analysis circumvents the problem of detecting large ions by making a simultaneous measurement of charge (z) and m/z ratio for individual ions. The same instrumental setup that successfully measured the megadalton viral DNA molecule was also used for the first mass measurements of intact viral particles. In this ESI-TOF charge-detection experiment, two viruses were examined, RYMV and TMV.[5] Ions were generated from a 5 pM solution, and the signal from thousands of individual ions was averaged over 30 minutes (Figure 3). RYMV and TMV both possessed a charge distribution of between 300 and 1000 positive charges. The mass spectrum obtained for RYMV had a maximum intensity between 6 and 7 MDa, which agrees well with a calculated mass of 6.5 MDa. The mass spectrum of the TMV sample gave a mass centered between 39 and 42 MDa. Both of these measurements were accurate, yet contain a large error. Much of this error is associated with variation in signals from charge detection and a short linear flight path. The large tailing in the mass spectra is most likely the result of adduct formation and incomplete desolvation.[19–21] A similar distribution was observed in the analysis of a homogeneous DNA molecule.[12]

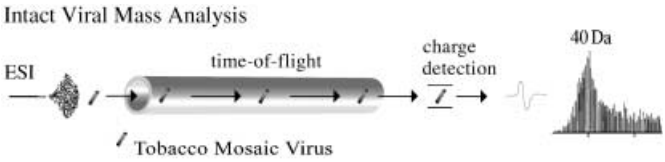

Figure 3. *Intact-virus analysis achieved with a TOF charge-detection mass spectrometer. The successful mass measurement of an intact virus particle ultimately required the use of an instrument combining electrospray ionization, TOF mass analysis, and a novel charge-detection device designed by Benner.*

Recently, the protein complex from an empty virus capsid was successfully mass measured by using a commercially available

mass spectrometer.[13] In these experiments, the observed *m/z* of the particles was approximately 20000. Separate charge-state assignments were made for ions with 101 to 125 charges and were used to determine the mass of the capsid. Even though salt and small-molecule adducts on the capsid surface significantly broadened the peaks representing distinct charge states, the mass of the capsid was calculated to be 2484700 ± 25200 Da, a difference of only +0.55% over the calculated value. The accuracy of this measurement was truly impressive and emphasizes the potential of viral analysis by ESI.

A New Platform: ESI Ion Mobility Spectrometry for Viral Structure Measurements

A recent analytical development was the coupling of ESI to ion mobility spectrometry (IMS). IMS allows the cross-sectional area of an ion to be determined by measuring the time required for ions to cross a diffusion chamber. The measurements are then used to calculate particle diameter, which correlates linearly with protein mass.[21] It has recently been demonstrated that ESI-IMS can detect human rhinovirus[22] and MS2 bacteriophage.[23] Inspired by these initial reports, we have characterized viruses from a range of viral families by using ESI-IMS.[24] ESI-IMS analysis of the icosahedral RYMV virus particle revealed a single charged species with a measured diameter of 28.5 nm (Figure 4). Since IMS measurements are based on the cross-sectional area of the analyte, these experiments provide conclusive evidence that no large-scale disruption of the tertiary or quaternary structure of the capsid occurred during desolvation and ionization.

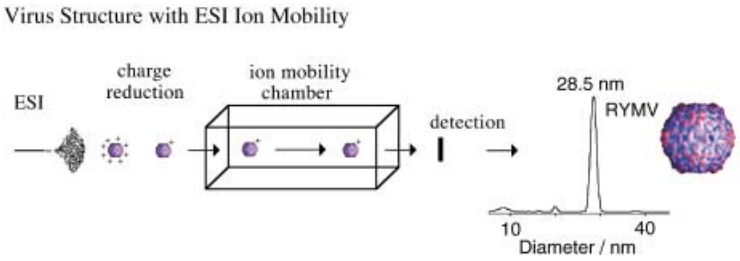

Figure 4. *Electrospray ion mobility spectrometry provides viral-structure measurements. Charge-reduction electrospray ionization coupled with ion mobility spectroscopy measures the gas-phase quaternary structure of particles. The ESI ion mobility spectrum of RYMV demonstrates a monodisperse peak of ions of the expected diameter (based on the virus's crystral structure).*

Summary and Outlook

Mass spectrometry is now a pervasive tool in virology and it is being used for the routine characterization of viral proteins, viral capsid dynamics, and viral structure, even aiding in the identification of new viral receptors.[25] The realization of mass measurements on intact viruses opens an interesting window into their further characterization. For example, if it were possible to characterize viruses with the accuracy and resolution with which proteins are currently analyzed, questions such as the heterogeneity of nucleic acid and protein packaging could potentially be addressed. Even more interesting, mass analysis could be used to study viral populations and rapidly follow evolutionary changes that alter the composition of the particles, ultimately addressing the age-old question as to why viruses in general require a very high multiplicity of infection.

Keywords: electrospray ionization · ion mobility · mass spectrometry · noncovalent interactions · viruses

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