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SPECIAL FEATURE: TUTORIAL

Probing Viruses with Mass Spectrometry

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Mass spectrometry offers a new perspective on the solution and gas-phase properties of viruses. Its broad application to local and global viral structure provides unique insights into many biological processes, including viral–antibody binding, protein–protein interactions and protein dynamics. Mass measuring viral proteins is now routine and since viruses are typically well characterized, in that the capsid proteins and DNA (or RNA) sequences are known, identifying a virus based on the mass of the protein and enzymatic digestion fragments is relatively straightforward. Using mass spectrometry, this paper describes the identification of viral protein post-translational modifications such as myristoylation, phosphorylation and disulfide bridging. Furthermore, complementary data obtained with mass spectrometry and x-ray crystallography demonstrate that viruses are highly dynamic particles whose viral capsid's mobility could, until recently, be inferred only from inherently static spectroscopic methods. Lastly, mass spectrometry has been applied on a global scale via the mass measurement of entire intact viruses. Given the general utility of mass spectrometry, its continuing development should further its application to viral dynamics, structure, function and identification. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Mass spectrometry (MS) will fundamentally alter the way we look at viruses, offering a new perspective on their solution-phase intermolecular interactions[1,2] as well as their physical properties in the gas phase.[3] Since 1978, when the generation of 2.9 Å crystal structures of tomato bushy stunt virus and the disk protein of tobacco mosaic virus began the field of high-resolution structural virology,[4,5] advances in the field have been largely driven by x-ray crystallography and electron microscopy.[6–10] While these techniques provide a great deal of structural information, substantive obstacles in understanding viral structure–function relationships still exist, namely in the acquisition of quality virus crystals, the large quantity of material needed and the inherently static nature of information that such techniques provide. Mass spectrometry, when examined in combination with crystallographic data, represents a significant advance over such obstacles and has recently been extended to study local and global aspects of the viral organism.

Generally defined, viruses are nucleoprotein particles designed to transport genes between hosts and the cells of a host. Most viruses are composed of two parts: genetic information and packaging material. The nucleic acid stores genetic information encoded in either RNA or DNA and the packaging material is made up of a protein capsid which can be enveloped by a combination of lipids, proteins and carbohydrates (known as an enveloped virus). A non-enveloped virus (Fig. 1) is made up of the nucleic acid and protein capsid which is not enveloped by a lipid-based membrane. In terms of function, the life cycle of a virus can be divided into four events which are regulated by the capsid proteins (Fig. 2): transport to the cell, attachment at the cell surface, endocytosis (or incorporation into the cellular membrane) and nucleic acid release. What is especially interesting is that viruses accomplish these interactions with products from a relatively limited genome.

Mass spectrometry has recently been recognized as a valuable source of information on both local[1,2] and global viral structure.[3,11] For instance, the mass-based techniques used to analyze protein–protein interactions[12,13] are being employed (in combination with chemical and enzymatic probes) to study viral capsid protein dynamics, assembly, autocatalytic proteolysis and disassembly.[1,2] Furthermore, mass spectrometry has been used to mass measure an intact

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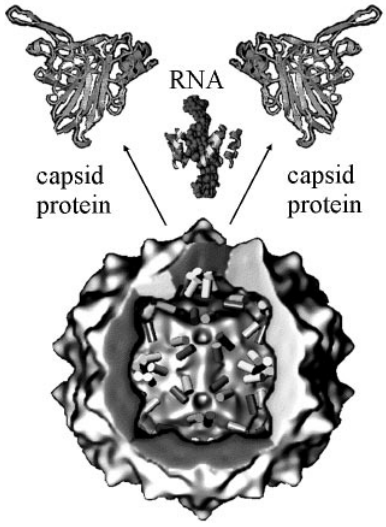


Figure 1. A non-enveloped icosahedral virus with a magnified portion of the capsid proteins and RNA above the virus.[10] These encapsulated RNA viruses are a structural class that includes thousands of viruses responsible for plant and animal diseases such as polio and the common cold.

virus.[11,14] This paper discusses three areas of related mass spectral research: (i) characterizing viral components, (ii) probing the dynamics of the viral structure and (iii) examining intact viruses. The reader will note that many of the references cited are recent or the results have not yet been published; we are only beginning to probe viruses with mass spectrometry and thus far the results have been most intriguing and in some cases surprising.

THE TOOLS: MALDI AND ELECTROSPRAY

Mass spectrometry has become an integral part of biological research primarily owing to the establishment of matrix-assisted laser desorption/ionization (MALDI)[15] and electrospray ionization (ESI).[16] Both MALDI and ESI have greatly advanced our ability to characterize large, thermally labile molecules by providing an efficient means of generating intact gas-phase ions.[17,18] Most significantly, MALDI and ESI have been used to gain molecular mass information on biological samples with unprecedented speed, accuracy and sensitivity. Recent developments in instrumentation, along with new sampling methods, have not only allowed for higher levels of sensitivity, increased mass range and better mass accuracy, but have also led to an increasing number of mass spectrometry-based applications in the study of both covalent and non-covalent biopolymer structure. This paper summarizes these technological advances and discusses their implications in viral research.

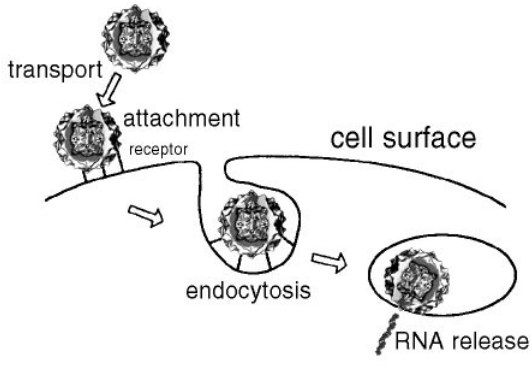


Figure 2. Different stages of viral activity including attachment to the cell wall, an event regulated by the capsid proteins.

MALDI and ESI are fundamentally different ionization techniques, yet they achieve essentially the same end result—non-destructive vaporization and ionization. With electrospray, ions are formed directly from solution (usually an aqueous or aqueous–organic solvent system) by creating a fine spray of highly charged droplets in the presence of a strong electric field. Subsequent vaporization of these charged droplets results in the production of singly or multiply charged gaseous ions. There are a number of advantages associated with using ESI in viral research. Beginning with the ability to interface ESI with liquid chromatography, this makes possible the analysis of viral proteins directly from their natural environment, the solution phase. A second advantage is the ability to perform H–D exchange experiments to examine different protein conformers within a virus. Finally, ESI has the ability to generate intact viruses in the gas phase for whole viral mass measurement.

MALDI mass analysis generates gas-phase ions by the laser vaporization of a solid matrix–analyte mixture in which the matrix (usually a small crystalline organic compound) acts as a receptacle for energy deposition. The relatively small number of charge states generated with MALDI (1–3), along with its high sensitivity and ability to generate simultaneously ions from multicomponent mixtures, make it especially well suited for complex biomolecular samples. Moreover, MALDI/MS offers a reliable way of analyzing viral proteins with little to no preparation. Clearly, both approaches offer unique and complementary capabilities.

CHARACTERIZING VIRAL SUBUNITS

Identifying capsid proteins

The protein-based packaging material of many viruses has a mass of less than 10 000 Da, making its analysis by mass spectrometry relatively straightforward; analyzing the viral proteins often requires nothing more than placing the solubilized virus on a MALDI plate with matrix, placing the plate in the instrument, adjusting the laser intensity and positioning the laser on the plate to optimize the signal intensity.[1] Such mass analysis often generates results on all of the capsid proteins (Fig. 3).[1,2] The primary limitation of this approach can be the surprising robustness of some virus particles which, when exposed to the highly acidic environment of the MALDI matrix, remain intact and thus produce no signal (however, the majority of viruses analyzed in our laboratory have denatured under MALDI conditions). The MALDI analyses are typically successful using traditional matricies such as 3,5-dimethoxy-4-hydroxycinnamic acid or sinapinic acid with 1 μl of a 1.0 mg ml^{-1} solution of virus. An interesting aspect of this analysis is that since viruses have a

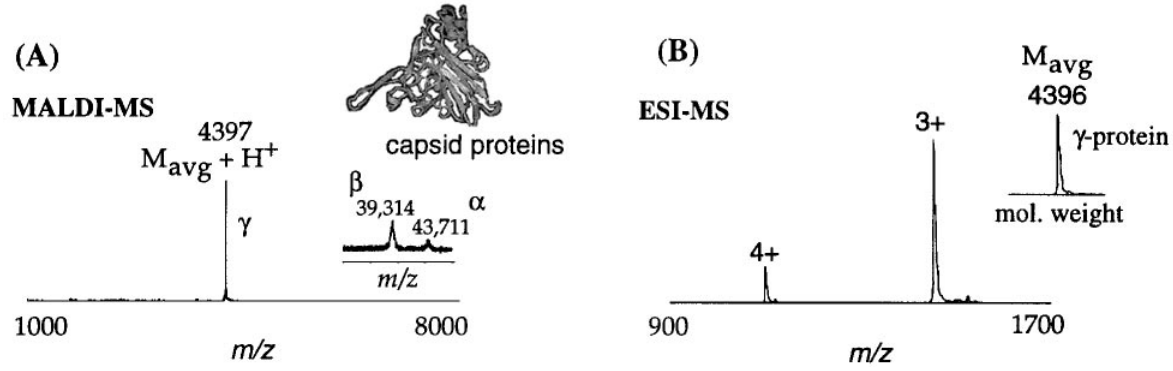


Figure 3. An icosahedral virus: (A) MALDI/MS data (3,5-dimethoxy-4-hydroxycinnamic acid matrix) generated from the Flock House virus showing α, β and γ capsid proteins and (B) ESI/MS data generated from the γ-protein of Flock House virus; only γ, one of the three capsid proteins, was observed upon direct injection of the virus into the mass spectrometer from a 25% $CHCl_3$–50% MeOH–25% H_2O solution. The inset in (B) is a molecular mass spectrum constructed from the *m/z* spectrum.

mass of the order of 10^7 Da (or greater), a 1 mg ml^{-1} solution of virus corresponds to a 100 nM concentration, which is a relatively dilute solution even in the context of mass spectral analysis. However, since there are typically multiple copies of each capsid protein per virus, the actual concentration of each protein corresponds to $\sim$5 μM, well within the routine sensitivity range of most ESI and MALDI mass spectrometers.

Preparing the virus for mass spectral analysis typically involves concentrating the particles by pelleting them through a sucrose cushion using an ultracentrifugation step. During ultracentrifugation the virions move to the bottom of the centrifuge tube and can be collected by resuspension in a buffer at the end of the run. At this point the viral solution can be made ready for MALDI or ESI/MS analysis via dialysis. As described above with MALDI/MS, the viruses may also retain their native structure when exposed to an organic solvent, potentially making it difficult to examine the viral proteins. However, in many cases, exposure to an organic solvent such as acetonitrile facilitates denaturation.

Clearly, mass measuring viral proteins is routine.[1,2,19–21] Since viruses are typically well characterized, in that the capsid proteins and DNA (or RNA) sequences are known, identifying a virus based on the mass-to-charge (*m/z*) value of enzymatic digestion fragments is relatively straightforward using database searching methods.[22] This is especially true since proteases offer high specificity, as seen in Table 1, and can thus be used to generate many different protein-specific fragments.[22] We have employed a variety of proteolytic digests on viruses including trypsin, Lys-C, clostripain (Arg-C), V8 Protease (Glu-C) and Asp-N (Table 1). Enzyme-to-virus ratios (*m/m*) are typically 1:200 or can be adjusted down to 1:3000 to achieve limited proteolysis for time-resolved experiments. Reaction volumes are typically 10–20 μl, 0.5 μl of which is removed from the reaction at each time point and placed directly on the MALDI analysis plate.

However, in cases where more than one type of protein inhabits the capsid, unequivocal identification of the protein fragments requires additional information. Here we applied sequential digestion[1,23] in which proteins were first digested by an endoprotease such as trypsin, followed by exposure to an exoprotease such as carboxypeptidase Y (otherwise known as CPY). Endoproteolytic fragments were generated by exposing the virus to trypsin (Fig. 4). Then, in order to identify unambiguously the tryptic fragments, partial sequence information was generated by exposing the entire digest mixture to the exoprotease CPY. The trypsin/CPY sequential digests were performed by first exposing the virus to trypsin which was then inhibited with N^{α}-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) at 50 μg ml^{-1} and allowed to dry on the MALDI sample plate. Carboxypeptidase Y (CPY) diluted to 1 mg ml^{-1} in water was then added (1–3 μl) to the dried trypsin digest.

In general, MALDI has been the method of choice for the analysis of capsid proteins primarily owing to the ability of the matrix to denature the particle and simultaneously generate mass data on all or most of the protein and its proteolytic fragments. ESI has also shown good utility for capsid protein analysis; however, owing to its milder ionization conditions, we have observed numerous cases where only one (or none) of the capsid proteins can be observed.

Identifying capsid protein post-translational modifications

In addition to identifying viral proteins based on sequence, mass spectral analysis can often lead to the

Table 1. Protease specificity[a]

Protease	Amino acid sequence specificity
Trypsin	X-Lys/-X and X-Arg/-X
Lys-C	X-Lys/-X
Clostripain	X-Arg/-X
V8 Protease	X-Glu/-X and X-Asp/-X
Asp-N	X-/Asn-X

[a] Proteolysis experiments can use any of a number of enzymes to perform digestion. The cleavage specificity of some of the different enzymes is denoted by a slash (/) before or after the amino acid responsible for specificity. Combinations of proteases can be used to reduce specificity and to mimic other proteases. For example, Lys-C and clostripain together are specific for the same sites as trypsin.

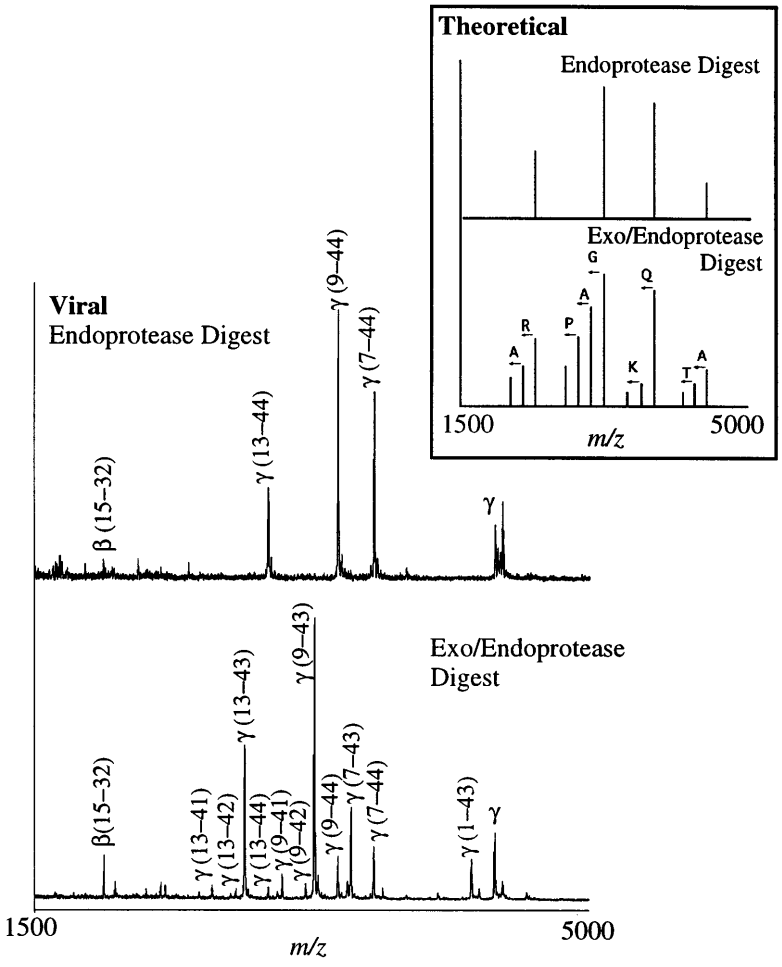


Figure 4. Digestion of Flock House viral capsid proteins, performed directly on the virus. (Top) MALDI/MS data generated from the trypsin digest. (Bottom) data generated from the carboxypeptidase Y digest of the trypsin digest (or *endo/exo* sequential digestion) of the viral capsid protein. The numbers in parentheses corresponds to viral protein fragments. (Inset) theoretical exoprotease digestion and a theoretical *exo/endo*-protease digestion;[1] the single-letter code correspond to amino acids in the peptide sequence.

identification of post-translational modifications[21] such as myristoylation, phosphorylation and disulfide bridging. In recent work[2] we identified a previously uncharacterized site of myristoylation on one of the structural proteins of the common cold virus. Electron density maps of rhinovirus indicated electron density which potentially corresponded to the myristoylation on VP4.[24] The experimentally measured mass of VP4 was 212 Da larger than predicted by the sequence (obtained from the SWISS-PROT protein database). This mass measurement is consistent with VP4 myristoylation; however, the measurement of mass alone did not reveal the exact location of the modification (the modified amino acid). Further digestion and tandem mass spectral studies of VP4 purified from HRV14 localized myristoylation of the *N*-terminus.

The identification of post-translational modifications has also been accomplished on the Sendai virus.[25] The polymerase-associated phosphoprotein (P protein) from this virus is reported in the literature to be highly phosphorylated. *In vitro* studies have detected phosphorylation in different regions of the protein, while a single phosphopeptide (identified as the sole phosphorylation site) was observed using *in vivo* techniques. In this work, two phosphorylation sites of the P protein from the Sendai virus were localized by using MALDI/quadrupole ion trap mass spectrometry and a computer-aided identification approach.

More recently,[26] mass spectrometry was used to identify a disulfide bridge modification in a virus known as human respiratory syncitial virus (RSV). Here the attachment protein of RSV was digested with trypsin and the resultant peptides were separated by reversed-phase high-performance liquid chromatography (HPLC). One tryptic peptide produced a mass by MALDI time-of-flight (TOF) mass spectrometry corresponding to residues 152–187, with the four Cys residues (residues 173, 176, 182 and 186) in a disulfide linkage. Further digestion of this tryptic peptide with pepsin and thermolysin produced peptides consistent with disulfide bonds between Cys 173 and Cys 186 and between Cys 176 and Cys 182.

Identifying viral DNA

A current limitation in analyzing DNA with mass spectrometry is its relatively low mass range, although recently the analysis of viral DNA fragments via MALDI/MS was performed by Jurinke *et al.*[27] Here a nested polymerase chain reaction (PCR) was performed with DNA preparations from serum samples. Hepatitis

B virus related products of nested PCR were first purified, immobilized, denatured and finally analyzed via MALDI/MS. Most interesting, however, has been the development of a new mass spectrometry-based method[28] which uses an ESI/TOF instrument with charge detection for analyzing megadalton DNA particles. While this approach has not yet been widely implemented, it does open up new possibilities for the mass analysis of intact viral DNA.

PROBING VIRAL DYNAMICS

Beyond identifying viral subunits, mass spectrometry is also useful for higher order structural studies, specifically in the analysis of capsid quaternary protein structure (protein–protein interactions) of non-enveloped viruses. Capsid protein subunits which make up the protective outer shell of the virus provide structural stability and play a major role in infectivity. While such protein–protein interactions have typically been mapped through x-ray crystallography, protein mass mapping is gaining more recognition as an effective technique.[12,13] Conventional protein mapping has been used for probing the primary structure (amino acid sequence) of individual proteins, incorporating chromatography and/or gel electrophoresis techniques. However, while proteolytic cleavage can provide indirect information about the domain structure of proteins, the method has not been routinely applied to protein–protein complexes owing to the limitations in resolving and identifying the multiple fragments produced with conventional methods. Here, protein mass mapping shows great promise as mass spectrometry is well suited to the analysis of complex mixtures of biomolecules and viral proteins, offering high sensitivity, resolving power and accuracy. Large proteins, for example, generate many peptide fragments that may be difficult to resolve by chromatographic and electrophoretic separation techniques.

Limited proteolysis combined with MALDI/MS has been used in our laboratory to explore protein–protein interactions,[12,13] specifically to characterize the interface between cell-cycle regulatory proteins, p21 and Cdk2. The results, which were later validated crystallographically with a homologous system,[29] allowed the identification of sites of interaction between the two regulatory proteins. We have also applied this MALDI/MS approach toward identifying viral proteolysis products in order to examine viral surface structure. The limited proteolysis/MALDI/MS experiments were performed on Flock House virus (FHV), a non-enveloped, icosahedral, RNA animal virus (Fig. 1) with dimensions similar to those of the rhino and polio viruses (~300 Å). Its protein coat or capsid is composed of 180 copies of a single gene product, protein α, which is autocatalytically cleaved to peptides, β-protein and γ-peptide, during maturation.[30] The autocatalytic cleavage products can be easily observed through MALDI/MS. By using time-resolved proteolysis (Fig. 5) followed by MALDI/MS analysis, it was expected that the reactivities of virus particles to different proteases would reflect the surface-accessible regions of the viral capsid and offer a new way of mapping the viral surface. When these experiments were performed, cleavages on the surface-accessible regions were observed; however, cleavages internal to the viral capsids (based on the crystal structure) were also generated. Observation of such cleavages was a surprising and initially perplexing result.

The proteolytic time-course experiments clearly demonstrate (Fig. 5) that the kinetics of proteolysis are specific to certain domains of the virus. What was surprising in these experiments was that in addition to observing cleavage on the viral surface, invariably the *first* digestion products were from domains internal in the x-ray model. Regions in the *N*- and *C*-terminal portions of the β-protein subunit and the γ-peptide located within the shell in the x-ray structure were the most susceptible to cleavage. These results, along with the x-ray

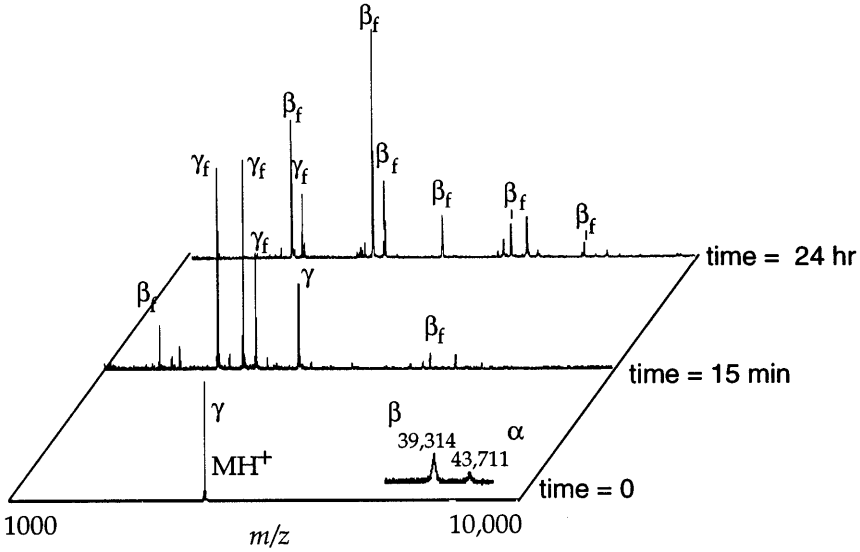


Figure 5. MALDI/MS data generated from the trypsin digest of Flock House virus.[1] Time = 0: native FHV. The capsid protein undergoes an autocatalyzed cleavage event in most of its subunits during maturation. The precursor α-protein and products, β-protein and γ-peptide, were detected. Time = 15 min after the addition of trypsin: the γ-peptide and three fragments were observed along with proteolytic fragments from β-protein. Time after 24 h of exposure: the γ-peptide exists only as the uncleavable fragment of *m/z* 376–407. The ion of highest abundance contains the loop region of the β-protein present on the viral capsid surface. All digests were performed of FHV at 1.0 mg ml^{-1} and at 25 °C.

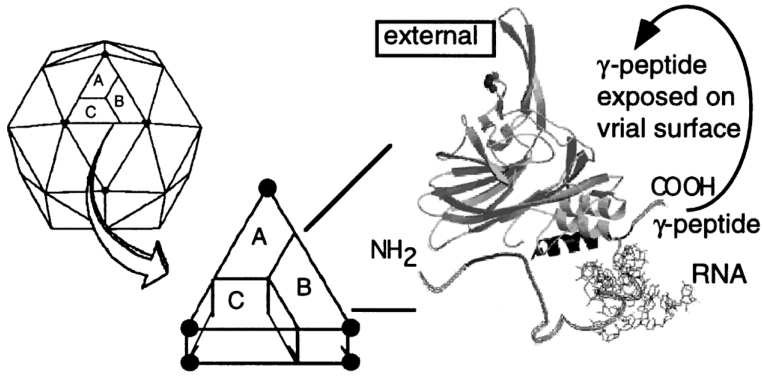


Figure 6. Crystal structure of Flock House virus shows that the γ-peptide and the *N*- and *C*-termini of β-protein are localized internal to the virus. However, proteolytic time-course experiments demonstrated that these domains are transiently exposed on the viral surface.[1]

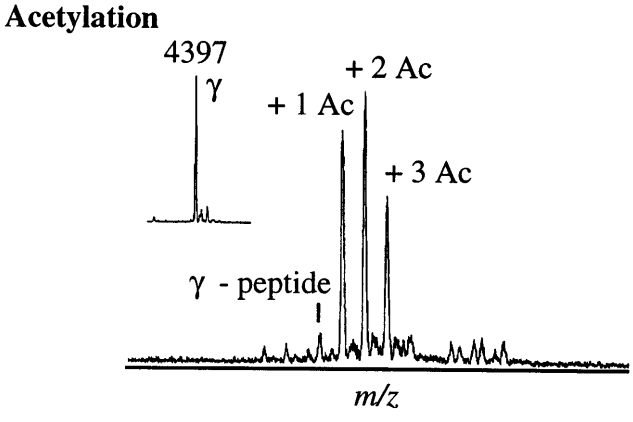
data, indicate that the portions of the β-protein and γ-peptide are transiently exposed on the surface of the virus (Fig. 6). Interestingly, these portions of the virus are implicated in RNA release and delivery.

Our observations are consistent with previous studies[31,32] performed with poliovirus. Monoclonal antibodies specific to internal domains of poliovirus capsid proteins VP1 and VP4 were shown to neutralize the virus reversibly.[31] Such neutralization could only occur if the antibodies were able to bind the epitopes of VP1 and VP4. Considering the size of the antibodies, the most probable explanation of their neutralization is that the epitopes of VP1 and VP4 must have been exposed to the viral surface. Additional experiments[32] on poliovirus with proteases, antiviral monoclonal antibodies and antisera also suggest that externalization may occur in solution. Along with our experiments on FHV, these studies are consistent with a dynamic capsid in which specific protein regions can translocate to the capsid surface. It is noteworthy that in comparison with the mass spectrometric work, the poliovirus experiments[31,32] required the costly and time-consuming generation of monoclonal antibodies and more than 100 times more sample and the need to purify individual peptide fragments. More recently, we have extended our research to the common cold virus (human rhinovirus) and have found our results to be consistent with those of the FHV studies; proteolytic digests suggest a dynamic viral capsid.[2]

In addition to examining the dynamic events of viruses via proteolytic methods, we have also employed chemical modification. In combination with mass analysis, chemical modification has been used further to examine viral surface topology and mobility, and promises to have even greater versatility than proteolysis. In initial studies, exposing the viral proteins to such chemical reagents as acetylation is allowing us to further investigate quasi-equivalent protein subunits and mobility (Fig. 7). In H–D exchange experiments performed with electrospray mass spectrometry, the amount of H–D exchange reflects the different folding populations as reflected in the mass, shape and width of the mass spectral peak. For instance, denatured proteins can rapidly undergo exchange whereas native versions are significantly restricted. Based on these measurements, it is possible to access both the amount of folding and the different folding states. We have also performed exchange experiments (Fig. 7) on whole viruses to examine different protein conformers.

CHARACTERIZING WHOLE VIRUSES

In the early 1990s, a great deal of interest was generated by mass spectrometric experiments which correlated gas-phase biomolecular complexes with those in the



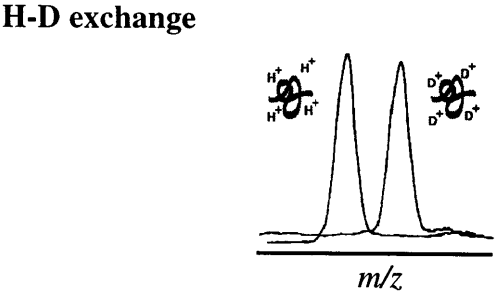


Figure 7. (Top) Mass spectral data of the acetylated γ-peptide of the Flock House virus after exposure of the virus to acetic anhydride. The amount of acetylation provides information about γ-peptide exposure on the virus while the specific location of acetylation (determined by tandem mass spectrometry) distinguishes the region of the peptide that is exposed. (Bottom) mass spectral data for an ESI/MS hydrogen–deuterium exchange experiment on a viral capsid protein. The different populations observed in these experiments provide information about the different capsid proteins conformers observed in the virus.

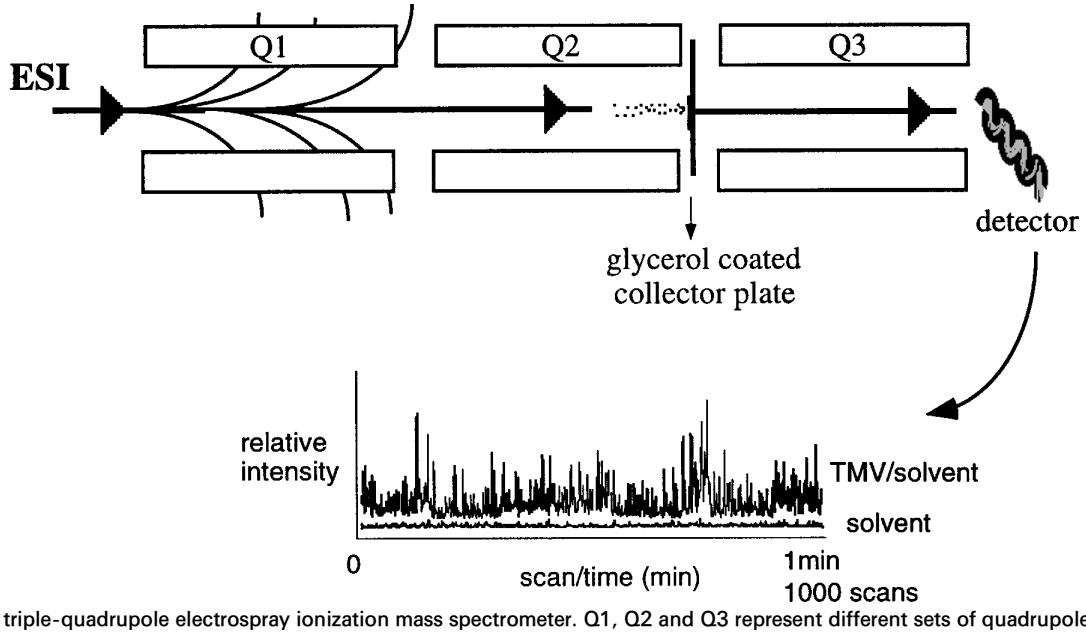


Figure 8. A triple-quadrupole electrospray ionization mass spectrometer. Q1, Q2 and Q3 represent different sets of quadrupoles in the mass analyzer allowing for ion focusing and mass selection. After initial experiments that allowed for the electronic detection of the virus (below), the instrument was modified by placing a brass plate as an ion collector in the flight path of the ions between Q2 and Q3. The collector was coated with a thin layer of methanol–glycerol (50:50). Once the viral ions were formed in the gas phase, the quadrupole mass analyzer electrostatically selected and directed the ions to the collector.[3]

condensed phase.[33,34] Since this original work, numerous other complexes have been observed, including the ternary complex of dimeric HIV-1 protease and an inhibitor,[35] calcium mediated cell-surface carbohydrate association[36] and antibody-inhibitor–substrate interactions.[37,38] Along with those, ESI-MS experiments[3] have been performed with whole viruses with the aim of generating mass spectral data on the entire complex. These preliminary studies, however, did not yield mass data on the intact viral complex. The lack of signal was attributed to the m/z of the viral ions being beyond the range of the quadrupole mass analyzer. Consequently, the same experiments were performed using the radio-frequency (r.f.)-only mode of the quadrupole mass

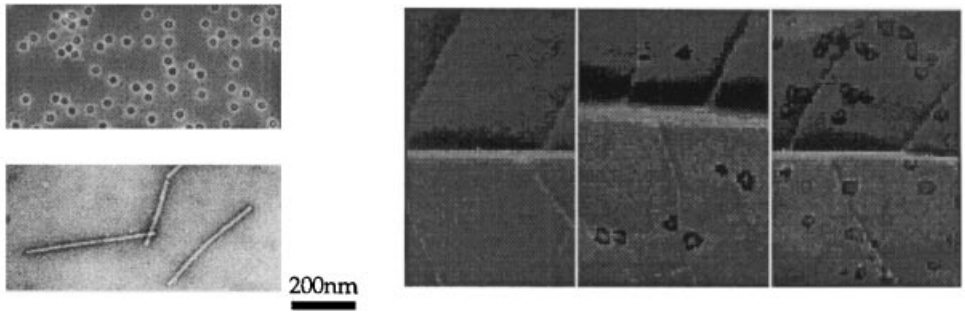


Figure 9. Electron micrographs of rice yellow mottle virus (left, top) and tobacco mosaic virus (left, bottom) detected after electrospray/transfer through a mass spectrometer.[3] Tobacco leaves inoculated with a control glycerol solution (right), inoculated with TMV–glycerol collected inside the spectrometer (right, center) and inoculated with untreated TMV–glycerol (far right).

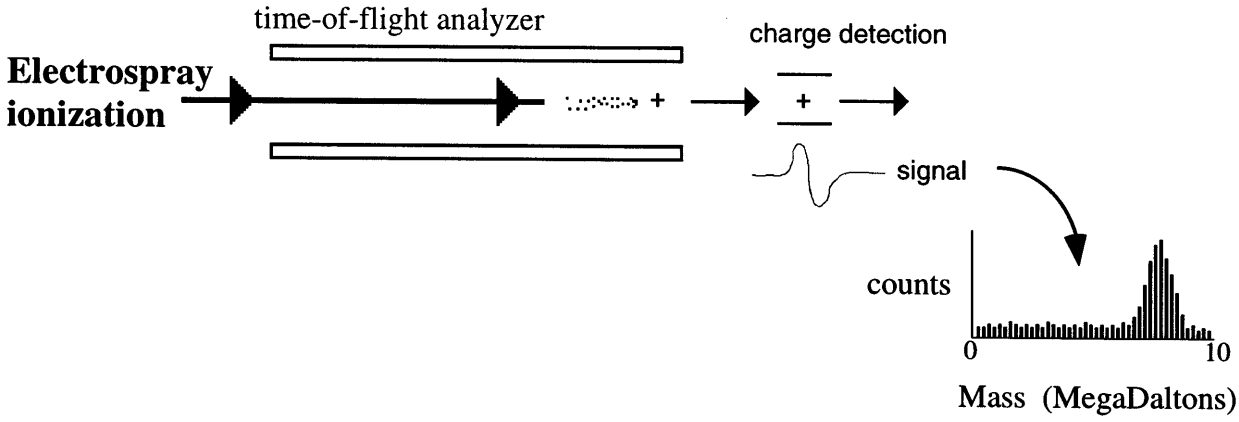


Figure 10. A time-of-flight mass analyzer with charge (inductive) detection was used by Fuerstenau and Benner[28] and Park and Callahan[39] to perform charge detection mass spectrometry. Fuerstenau and Benner used an electrospray ionization source on their instrument to mass measure megadalton DNA and, more recently, to measure whole viruses (unpublished data).

analyzers. The r.f.-only mode did indeed allow for the observation of ion current due to viral ions striking the detector (Fig. 8).

Our laboratory has examined the passage of whole viruses through the mass spectrometer to determine whether they remained intact.[3] Because of the limitations of the mass analyzer, we again compensated for our inability to scan within the m/z range of the viral ions by employing the r.f.-only mode for Q1 and Q2. The instrument was modified by placing a brass plate as an ion collector in the flight path of the ions between Q2 and Q3 (Fig. 8). Using a low accelerating voltage prior to Q1, the free expansion of gas into the vacuum resulted in massive ions gaining much higher translational energies and reaching the detector, whereas low mass ions were rejected by the analyzer before reaching the detector. This approach was validated using a mixture of egg white lysozyme ($M_r = 14\,317$ Da) and a small peptide ($M_r = 709$ Da), where the peptide was filtered out by adjusting the potential to Q1. The accelerating voltage was likewise reduced, thus removing contaminating low-mass ions, allowing all higher mass ions to reach the detector, and thereby facilitating (albeit crudely) selection of the virus.

Transmission electron microscopy of the electrosprayed viral ions collected on the brass plate revealed that rice yellow mottle and tobacco mosaic viruses retained their respective spherical and rod-like ultrastructure. Further, the viability of the isolated, post-experimental tobacco mosaic virus was confirmed by inoculation and infection of tobacco plants (Fig. 9). These experiments offer evidence that native biomolecular structures can be conserved through the ESI/MS process.

While the research reviewed above has demonstrated the utility of mass spectral viral analysis, some of the most exciting experiments (recognizing the author's bias) are those performed on mass measuring whole viruses. The limitations of the mass analyzer, and to some extent the detector, in the experiments described above have recently been addressed using TOF mass analysis with charge (or inductive) detection (Fig. 10). Park and Callahan[39] demonstrated its use to measure small cesium cations, while Fuerstenau and Benner[28] employed a design to examine larger ions (megadalton DNA molecules). The Fuerstenau and Benner instrument[28] used TOF mass analysis with a charge detection system and employed an electrospray ionization source. In initial studies, they observed megadalton DNA particles, while more recently they used the same instrument to measure a whole tobacco mosaic virus at 4×10^7 Da (unpublished work). Their system measured both the charge on individual ions and, from TOF measurements, the m/z. Both measurements were then used to produce a molecular mass spectrum.

CONCLUSION

In its present state, the application of mass spectrometry to viruses is limited only by our imagination. Since mass spectrometry does not require the formation of crystals, consumes only microgram quantities (or less) of material and can unequivocally identify proteolytic cleavage sites, it offers a unique and efficient approach to viral analysis. Most importantly, since the proteolysis is performed in solution, this method when combined with crystallographic data can contribute to an understanding of the dynamic domains which initiate nucleic acid release and translocation (the final phase of the virus life cycle). Mass spectrometry has also taken the first steps toward routinely measuring massive biomolecules and even living organisms. Given the general utility of mass spectrometry, its continued development for viral analysis will surely offer new insights into the fundamental aspects of viral dynamcs, structure, function and identification.

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