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Mass Spectrometry in Viral Proteomics

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ABSTRACT

Mass spectrometry is a valuable tool in structural and functional viral proteomics, where it has been used to identify viral capsid proteins, viral mutants, and posttranslational modifications. Further, mass-based approaches combined with time-resolved proteolysis (mass mapping) have revealed the dynamic nature of viral particles in solution; this method is contributing to an understanding of the dynamic domains of the viral capsid which may have significant value in developing new approaches for viral inactivation. As a result of these experiments, and by comparison with complementary data from X-ray crystallography, a new dimension to viral protein structure and function is emerging.

Introduction

Mass spectrometry is now a mature technology that is being widely applied in biochemical esearch.[1,2] It has found unique applications in such diverse areas as protein characterization,[3−6] structural virology,[7−12] and drug discovery.[12,13] The reason for its relatively rapid maturation (∼10 years) lies in the invention of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI)[14,15] for biomolecule analysis. Interestingly, the two ionization techniques are very different, yet both can generate intact molecular ions from a wide range of biomolecules.[2]

The utility of ESI lies in its ability to produce singly or multiply charged gaseous ions directly from an aqueous or aqueous/organic solvent system by creating a fine spray of highly charged droplets in the presence of a strong electric field (Figure 1). The sample solution is typically sprayed from the tip of a metal nozzle maintained at approximately 4000 V. Dry gas, heat, or both are applied to the highly charged droplets, causing the solvent to evaporate. Evaporation causes the droplet size to decrease, while the surface charge density increases. Ions are transferred to the gas phase as a result of their expulsion from the droplet and are then directed into a mass analyzer through a series of lenses. ESI-MS is useful for probing a wide range of biological problems as a detector for HPLC and capillary zone electrophoresis, in the study of noncovalent complexes, and for obtaining structural information. ESI does have limitations in that it is not very tolerant of the presence of salts (>1.0 mM), nor is it practical for the analysis of multicomponent samples. Fortunately, in several aspects of mass analysis where ESI is not very useful, MALDI-MS has proven to be very effective.

MALDI-MS has also emerged as an effective bioanalytical tool having unique capabilities in handling complex mixtures (such as proteolytic digests) and in high-sensitivity (femtomole or even subfemtomole) measurements. The MALDI ionization technique typically uses a pulsed UV laser beam to desorb and ionize cocrystalized sample/matrix from a metal surface. The matrix (e.g., 2,5-dihydroxybenzoic acid) serves to minimize sample damage from the laser beam by absorbing the incident laser energy, resulting in the sample and matrix molecules being ejected into the gas phase and ionized. Once ions are formed in the gas phase, they can be electrostatically directed to a mass analyzer.

ESI and MALDI mass spectrometers have been widely used for protein and peptide analyses because of their ability to provide highly accurate molecular weight and structural information. Protein analysis with mass spectrometry in combination with chemical and proteolytic digestion has direct applications to protein identification, and its importance increases as the human genome nears completion. Equally important is the value of mass spectrometry in structural and functional proteomics, where it has proven to be a valuable tool for viral characterization[7−11,16] based on its ability to identify viral capsid proteins, viral mutants, and posttranslational modifications. Further, mass-based approaches such as time-resolved proteolysis have even revealed the dynamic nature of viral particles,[8−10] as well as providing insight to viral−drug interactions.[11,12] As a result of these experiments, and by comparison with complementary data from X-ray crystallography, a new dimension to viral protein structure is emerging.

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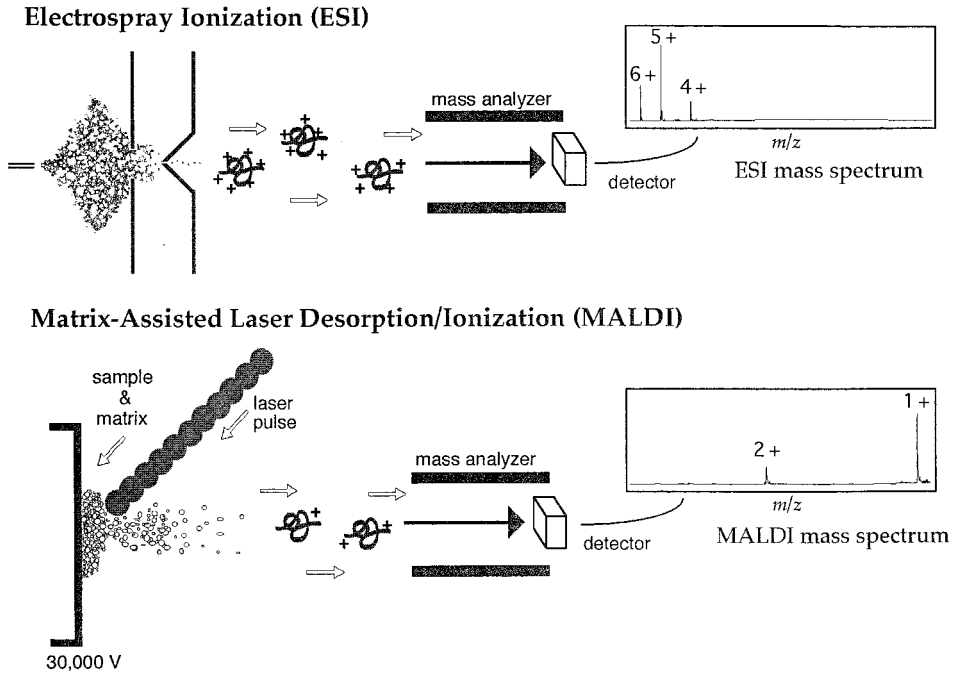

FIGURE 1. Schematic representation of electrospray ionization (ESI) and matrix-assisted laser desportion/ionization (MALDI). ESI occurs through charged droplet evaporation and MALDI via pulsed laser desorption/ionization of a sample from a UV-absorbing matrix. In each case, the ionized species are directed through a mass analyzer, which allows for differentiation and detection of the ions according to their mass-to-charge ratio (*m*/*z*).

Mass Mapping for Viral Protein Characterization

The characterization of genomic proteins for proteomics is arguably the most important application of modern mass spectrometry. In our studies we have chosen to focus on proteins from viral systems, potentially to gain structural information that cannot be obtained using traditional microscopy or crystallography techniques. The primary tools in these mass mapping experiments are mass spectrometry, proteases, and computer-facilitated data analysis. Sequence-specific proteases provide product fragments that can be associated with proteins within a database by correlating observed and predicted fragment masses. Since the occurrence of Arg and Lys residues in proteins is statistically high and these residues are often protease accessible, trypsin cleavage (specific for Arg and Lys) generally produces a large number of fragments which in turn offer a reasonable probability for unambiguously identifying the target protein. The success of this strategy, however, relies on the existence of the protein sequence within the database. With the availability of most viral genomic sequences, identification of the virus can be quickly determined simply by measuring the mass of proteolytic fragments.

Traditional DNA sequencing methods are well-established for identifying viral mutants; however, localizing a mutation prior to genomic sequencing has value in that it narrows the region of the genome that must be sequenced. Recently, mass mapping has been used[10] to localize the region of mutation by comparing differences in the mass of proteolytic fragments from wild-type virus and viral mutants (Figure 2). Mass mapping defines the region containing a mutation and, in cases when nucleotide sequencing is required, significantly narrows the region of the genome that must be sequenced. Additional sequence information on the proteolytic fragments also helps to further refine database identification. The wealth of protein information in current databases can help determine the origin of a protein and thereby identify the particular virus.

The mass mapping scheme shown in Figure 2 was first demonstrated[10] for the identification of naturally occurring mutants of human rhinovirus (HRV) and tobacco mosaic virus (TMV). A mutant of HRV (Cys1199Tyr) which contains the Cys → Tyr amino acid change at residue 199 in VP1 was identified by examining the MALDI mass spectra generated from the trypsin digestion of wild-type HRV14 and the HRV14−Cys1199Tyr mutant. All the fragments were common to both spectra with the exception of the ion signal at *m*/*z* 4700. This signal corresponds to residues 187−227 of the VP1 in the wild-type sequence. The corresponding ion signal in the spectrum of the mutant is observed at *m*/*z* 4783.5 (Figure 3 inset), a difference in mass of 83 Da, accordant with a Cys → Tyr amino acid change (the only possible mutation with a mass difference of 83 Da).

Similarily, comparison of the MALDI mass spectrum resulting from the trypsin digestion of wild-type tobacco mosaic virus (TMV) with that of the mutant, TMV (Asp77Arg) (Figure 3), shows an obvious difference in mass between the two proteins at *m*/*z* 2051.4 in the wild-type spectrum and at *m*/*z* 2091.8 in the spectrum of the mutant. Both ion signals correspond to amino acids 72−90; however, due to the Asp → Arg mutation at residue

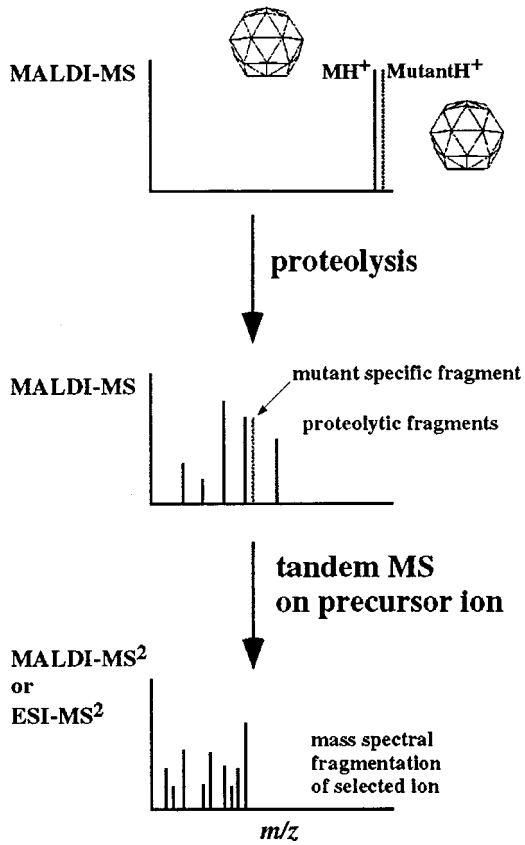

FIGURE 2. Schematic representation of the method for identifying virus mutants by mass spectrometry. (Top) The observation of differences between wild-type and mutant viruses indicates amino acid changes (i.e., mutations). (Middle) Proteolysis of the viral variants generates a set of digestion fragments (one or more of which may contain the mutated amino acid) which appear at different mass-to-charge ratio (*m*/*z*) values as a consequence of an amino acid change(s). This observation provides valuable information about the location of the mutation, augmenting its identification either by further mass tandem spectral analysis (bottom spectra) or by nucleotide sequencing.

77, the fragment at *m*/*z* 2091.8 is higher in mass than that of the corresponding wild-type peptide. The identification of the exact amino acid change required tandem mass spectrometric experiments.

Mass spectrometry combined with proteolysis is also well-suited for detecting other forms of diversity, such as posttranslational modifications (e.g., fatty acylation and glycosylation) and amino acid insertions/deletions.[17−19] In fact, a growing body of literature has been devoted to the application of ESI and MALDI for identification of protein modifications, largely because of their biochemical and physiological importance. For instance, MALDI and ESI have been employed to characterize a modification on human rhinovirus capsid protein.[11] Originally, speculation as to the posttranslational modification of HRV14 structural proteins existed due to the observation of electron density in crystallographic data from HRV14 that corresponded to the myristoylation of VP4. Since mass analysis indicated a 212 Da difference (consistent with myristoylation of VP4), further digestion and tandem mass

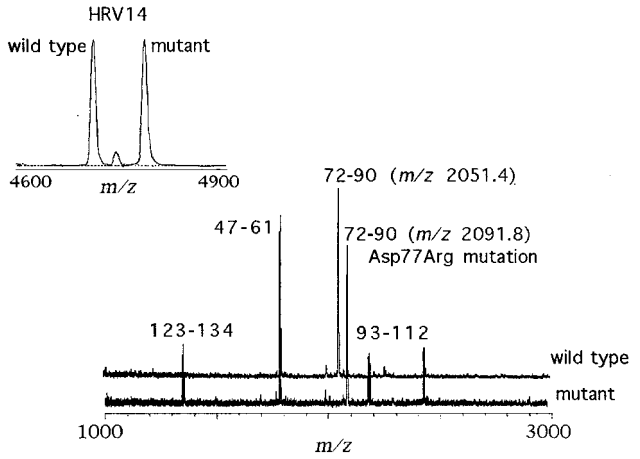

FIGURE 3. Comparison of the MALDI time-of-flight mass spectra resulting from the trypsin digestion of human rhinovirus (HRV) and tobacco mosaic virus (TMV) and their mutants. Most ion signals are common to both spectra, with the obvious exception of the signals observed at *m*/*z* 2050.5 in the wild-type spectrum and *m*/*z* 2091.8 in the mutant spectrum. Both signals represent residues 72−90 with the incorporation of the Asp to Arg amino acid change in the mutant. (Inset) Spectrum resulting from the trypsin digests of wild-type HRV14 and its naturally occurring mutant HRV14−Cys1199Tyr. Similarly, most fragments are common to both spectra, with the exception of the ion signals at *m*/*z* 4700.5 and 4783.8 in the HRV14 and HRV14−Cys1199Tyr spectra, respectively. Both ion signals correspond to residues 187−227 of the VP1 capsid protein, and the mass difference of 83 Da between the two variants is consistent with a Cys → Tyr mutation.

spectrometry structural studies of VP4 localized the myristoylation to the N terminus.

Another example of posttranslational modification identification has been achieved with structurally complex enveloped viruses which often contain glycosylation on proteins located in the lipid membrane of the virus (transmembrane proteins). The most common method for determining the glycosylation site or the glycan structure involves a proteolytic digest followed by glycan removal with exo- and/or endoglycosidases (e.g., mannosidase and PNGase F). With soluble glycoproteins, these steps can be performed directly on the MALDI sample holder. In some cases, determination of the intact protein's molecular weight is useful; however, the difficulty of the whole protein approach lies in the ability to solubilize the amphiphilic glycoproteins while maintaining the glycosidase activity. In addition, the heterogeneity associated with glycoproteins adds another level of difficulty to their characterization. As an example, the molecular mass of the entire Sindbis virus glycoprotein (E2) was determined to ±0.1% accuracy by MALDI analysis.[20] To obtain structural information, gel electrophoresis was performed on the glycosidase digest, the gel band was excised, and the deglycosylated protein was eluted. From this method, MALDI analysis yielded information on the protein as well as the amount and type of glycosylation.

Mass Mapping for Protein Structure

In addition to assisting in protein characterization, the most intriguing potential of mass spectrometry lies in its

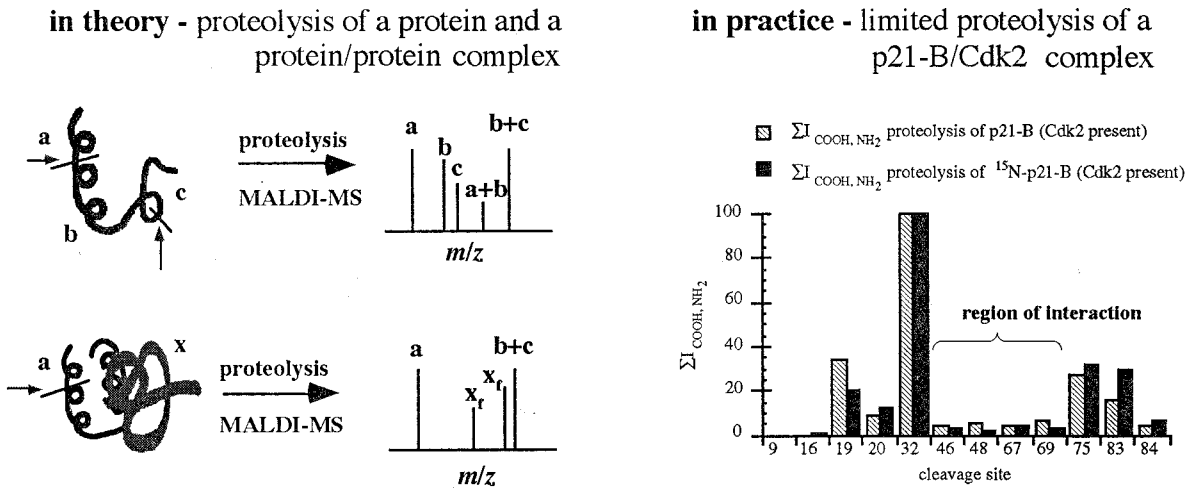

FIGURE 4. Probing protein structure and protein/protein interactions using proteolysis and MALDI-MS. Schematic view (left) of key concepts. Two cleavage sites are accessible for the protein of interest alone (top, left), yielding fragments after limited digestion. In the complex with protein X, one site is protected (below, left), yielding fewer fragments. However, fragments from protein X are also produced. (right) A bar graph expressing trypsin accessibility, where the center region represents the region of interaction on the p21-B.

ability to provide higher-order structural information. The basis for studying tertiary structure using protein mass mapping is the fact that enzymes are initially limited to cleaving surface-accessible regions of the native protein[4,21−23] Another factor that governs the selectivity of cleavage is the sequence specificity of the enzyme (which reduces the number of fragments that are produced). Ideally, only a subset of all possible cleavages are observed due to the structural inaccessibility. An example of this can be seen in Figure 4, where arrows mark potential cleavage sites within a hypothetical protein; these sites are surface-exposed and located in flexible loop regions. The distribution of amino acids in a protein guides the choice of protease to be used as a structural probe. Since amino acids with hydrophilic side chains are found in greater abundance on the surface of proteins (at the solvent interface), proteases that cleave at hydrophilic sites, such as trypsin and V8 protease (which cleave basic (K, R) and acidic sites (D, E), respectively), are preferred in structural analysis.

Protein mass mapping can also be used to probe the quaternary structure of multicomponent assemblies, including protein−protein complexes[4,21] and protein−DNA complexes.[24] A common feature of these applications is that the protease is used to provide contrast between the associated and unassociated states of the system. The formation of an interface between a protein and another macromolecule will exclude both solvent molecules and macromolecules such as proteases and will also protect otherwise accessible sites from protease cleavage. Methods developed for primary sequence elucidation using MS are particularly well-suited for the analysis of higher-order, native protein structure since they are directly transferable to the analysis of native structure. Analysis methods, however, must be modified to take into account the added spectral complexity due to incomplete proteolysis under limiting conditions.

The approach outlined in Figure 4 has been applied to probe the quaternary structure of cell-cycle regulatory proteins, Cdk2 and p21$^{Waf1/Cip1/Sdi}$.[14,21] Tryptic fragments of p21-B were generated in the presence and absence of Cdk2. However, given the limited level of accuracy offered by some MALDI mass spectrometers, there is a finite probability that fragments from the different subunits will have similar masses. Therefore, separate p21-B samples were prepared with natural isotopic abundance and ^{15}N-labeled in order to allow for unambiguous differentiation of p21-B and Cdk2 fragments. The results obtained from the protein mass mapping experiments on the p21-B/Cdk2 complex are summarized in the two bar graphs shown in Figure 4. MALDI analysis of the tryptic fragments of p21-B generated in the absence of Cdk2 was performed. The right bar graph shows data after proteolysis of the p21/Cdk2 complex, with p21 at natural abundance (hashed) and ^{15}N-labeled (black). Cdk2 is unlabeled in both cases. The MALDI data of p21 alone and of the p21/Cdk2 complex were acquired after proteolysis, revealing a segment of 24 amino acids in p21-B that is protected from trypsin cleavage, thus identifying the segment as the Cdk2 binding site on p21-B. A distinct and key advantage of this approach is that the masses of the fragments are obtained together in a *single* mass spectrum without the need for individual purification using, for example, HPLC or SDS−PAGE. The mapping of protein−protein complexes in situ can be complicated by peptide fragments produced from all the subunits within a complex; however, MALDI instruments are now available with part per million (ppm) mass accuracy, which allows for unambiguous identification of proteolytic fragments.

For proteins of known sequence, protein mass mapping offers access to detailed information with only modest investments of time and material. The accuracy and speed of this approach surpass those of traditional methods based on HPLC/SDS−PAGE analysis and Edman degradation. Furthermore, due to the high resolution and sensitivity of MALDI mass analysis, a much greater number of protein fragments can be identified than was previously possible, offering more detailed "maps" of protein/protein

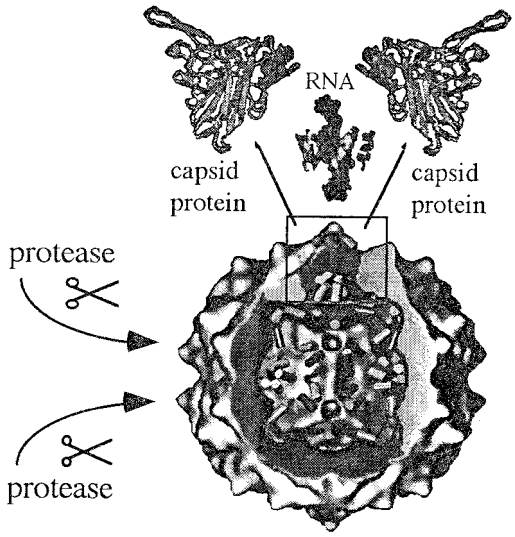

FIGURE 5. Nonenveloped icosahedral virus (Flock House virus), with a magnified portion of the capsid proteins (β-protein and γ-peptide) and RNA shown above the virus. The experiments performed involved exposing viruses to limited proteolysis followed by mass analysis of the proteolytic fragments. Time-resolved proteolysis allowed for the study of protein capsid mobility. This virus belongs to a structural class that includes thousands of viruses, such as polio virus and that which causes the common cold.

structure. Based on the success of protein mass mapping on the DNA/protein[24] and protein/protein complexes[21] described above, this technique has also been demonstrated on highly complex protein viral assemblies.[8,9,11,25,26]

Mass Mapping for Virus Structure

In addition to its utility for proteins and protein/protein complexes, mass spectrometry has recently been recognized as a valuable source of information on both local and global viral structures.[7−11,16] Since the viral capsids represent an association of noncovalent protein subunits, virus structural analysis has been a logical step in the development of protein mass mapping. For instance, cleavage sites which reside on the exterior of the virus are the most accessible to the enzyme and, therefore, are among the first digestion fragments observed. Since proteolysis is performed in solution and can detect different conformers, this method can also contribute to an understanding of the dynamic domains within the virus structure.

Limited proteolysis/MALDI-MS experiments have been performed on human rhinovirus 14 (HRV14) and Flock House virus (FHV). Based on crystal structure data,[27] the four proteins that make up the viral coat (or capsid) of HRV are VP1, VP2, and VP3, which compose the viral surface, and VP4, which lies on the interior at the capsid/RNA interface. Like the human rhinovirus 14, FHV also has a protein coat which is made up of only two proteins with regions (based on crystal structure) that are distinctly internal and external to the viral surface (Figure 5).

Using time-resolved proteolysis followed by MALDI-MS analysis, it was expected that the reactivities of virus particles to different proteases would reveal the surface-accessible regions of the viral capsid and offer a new way of mapping the viral surface. In these studies, identification of the viral capsid protein fragments was facilitated by sequential digestion, in which proteins were first digested by an endoprotease such as trypsin and then exposed to an exoprotease such as carboxypeptidase Y. When these experiments were performed on both HRV and FHV, cleavages on the surface-accessible regions were observed; however, cleavages internal to the viral capsids (based on the crystal structures) were also generated. Observations of digestion fragments resulting from "internal" protein regions were initially perplexing. Examination of these results along with the X-ray data suggests that the portions of the internalized proteins are transiently exposed on the surface of the virus (Figure 6). Additional proteolytic and chemical modification experiments[8] on these viruses further suggest that the viral capsid is, in fact, highly mobile.

Monitoring Virus/Drug Interactions

Because of the greater role mass spectrometry is playing in structural and functional proteomics, it is naturally becoming an important tool in drug discovery. For instance, ESI and MALDI mass spectrometry have found utility in monitoring protein/drug activity as a quantitative assay for the identification of effective ligand−receptor binding, as well as for monitoring new chemical catalysts[28] and screening enzyme inhibitors. In addition, because mass spectrometry does not involve analyte chromophores or radiolabeling, it provides a viable alternative to existing analytical techniques, which typically require extensive sample preparation and optimization time, disposal of biohazardous waste, or a significant amount of sample.

Using the advantages of ESI-MS detailed above, we have investigated inhibition of enzymatic reactions for viruses[11] as well as glycosylation reactions[13] (Figure 7). In this approach, the entire enzymatic mixture (substrate, inhibitor, product, and internal standard) is introduced into the ESI mass spectrometer, while analysis for product formation as a function of the presence of inhibitor is done. Since only product formation is quantitatively monitored, the effectiveness of the inhibitor can be readily determined. As a test of this approach, a galactosyltransferase-catalyzed reaction was examined in the presence of potential inhibitors (Figures 7 and 8). The 20 inhibitor candidates and two control reactions of the initial inhibitor library were assayed individually in 22 parallel reactions, quenched by addition of methanol (followed by the addition of an internal standard), and then injected directly into the electrospray mass spectrometer. Three compounds were found to be potent inhibitors of the galactosyltransferase[13] (Figure 8B). Additional libraries were generated in which the concentrations of the inhibitors were varied to determine the IC_{50} (inhibitor concentration at 50% inhibition). By monitoring one inhibitor at a time, it was possible to determine their effectiveness every 2 min. In an effort to further increase the screening

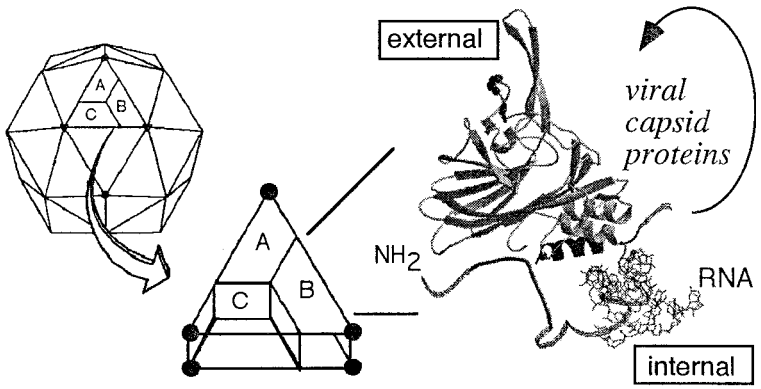

FIGURE 6. Crystal structure of Flock House virus, showing that the γ-peptide, the N-terminus, and the C-terminus of the β-protein are localized internal to the virus. Yet, proteolytic time course experiments demonstrated that internal domains are transiently exposed on the viral surface.

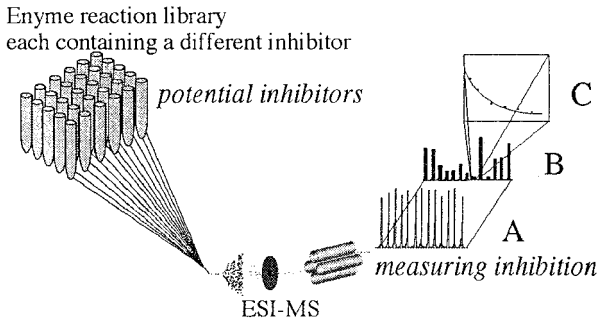

FIGURE 7. Quantitative determination of enzyme inhibition. Enzyme reactions can be monitored in series by automated electrospray ionization mass spectrometry. Each solution contains the enzyme, reactants, an inhibitor, and an internal standard. (A) The total ion current is recorded after each injection into the mass spectrometer. (B) Product formation is then monitored with respect to the internal standard and plotted as a bar graph. (C) If an inhibitor is found to be effective, the degree of inhibition can be generated using ESI-MS.

capacity, multiple inhibitors were also screened against one enzyme simultaneously.

While we have shown the effectiveness of this method in the screening of inhibitors for enzymes involved in carbohydrate processing, one can envision how this approach can be extended to screening libraries of other classes of enzymes (such as enzymes with peptide-based substrates) or to screening metal-catalyzed chemical reactions.[28] Indeed, this method can be applied toward screening any reaction whose product molecule is amenable to mass spectrometric analysis. In the previous example, ESI-MS was the mass spectrometric method of choice mainly because of its ability to analyze low-mass compounds and its amenability to the direct injection of the liquid reaction sample. As the following section will show, MALDI-MS, although it requires the use of a matrix, offers many of the same advantages (automation and soft ionization) and, in addition, is able to analyze high-mass compounds and relatively heterogeneous mixtures.

As a consequence of observing the viral capsid mobility, MALDI-based experiments were designed to explore the effect that antiviral agents (known to bind to the capsid proteins) would have on capsid dynamics. Specifically, the antiviral agent WIN 52084 was examined. WIN 52084 is a

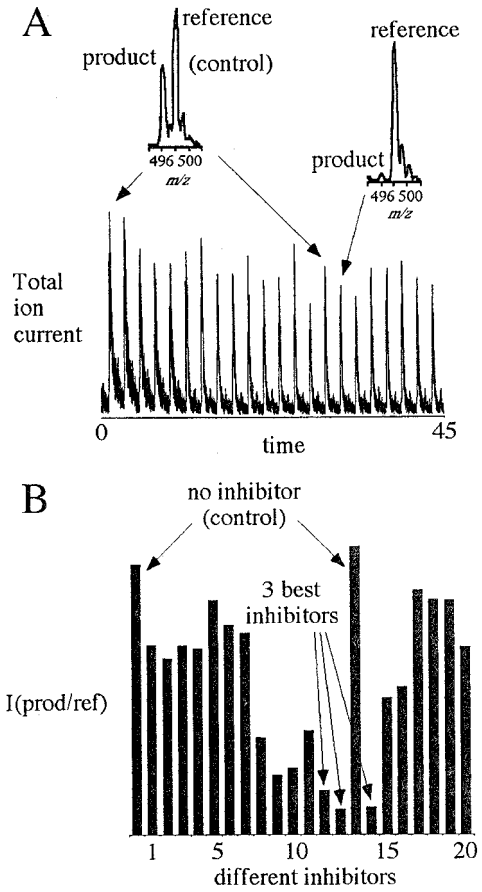

FIGURE 8. (A) Electrospray ionization total ion current observed for the 22 reactions (each having a different inhibitor). Insets show the mass spectra for the reactions with no inhibitor (left) and with an inhibitor found to be effective (right). (B) Bar graph of product ion/reference ion intensity ratio generated from the mass spectrum of each reaction containing potential inhibitors.

member of a family of experimental drugs designed to inhibit the lifecycle of picornaviruses[27,29,30] through binding hydrophobic pockets which lie beneath the canyon floor. Previously, the X-ray crystal structure of HRV14 revealed a 25-Å-deep canyon on the surface of the virion at each 5-fold axis of symmetry. This canyon has since been identified as the site of cell surface receptor attachment (Figure 9), as well as a site for binding WIN 52084. Previous

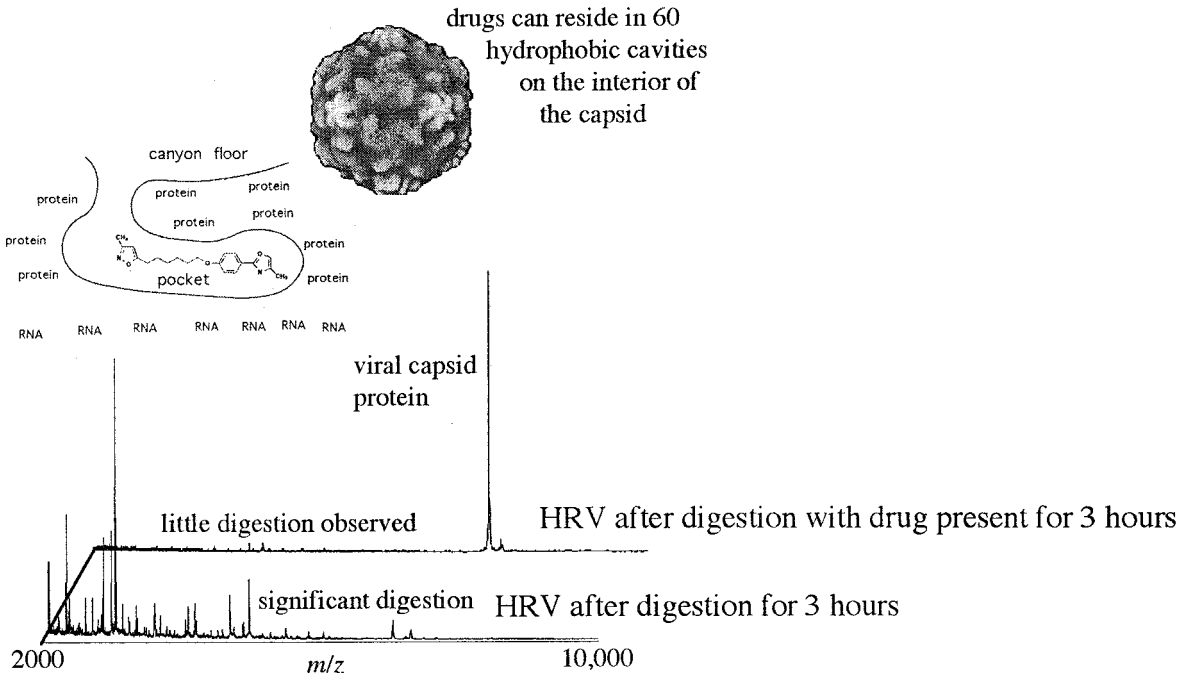

FIGURE 9. Inhibition of HRV capsid dynamics with drug present. MALDI-MS analyses were performed on HRV following proteolysis with and without drug present. The drug (WIN 52084) is bound to a pocket within the viral surface and is believed to inhibit capsid mobility.

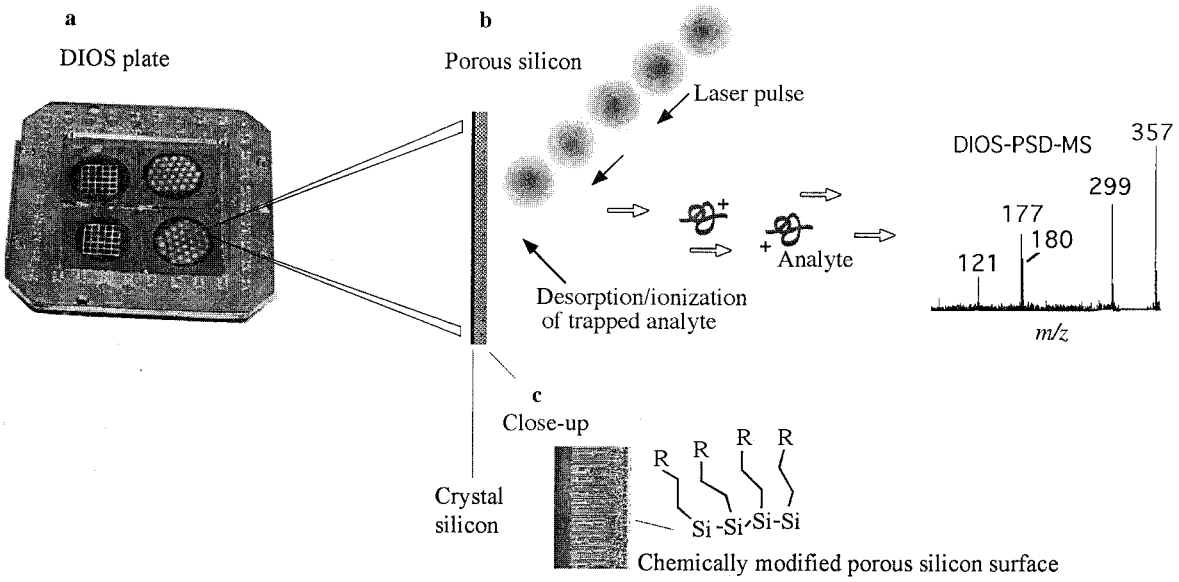

FIGURE 10. Configuration for the desorption/ionization on porous silicon (DIOS)-MS. (a) Photopatterned plates placed on a MALDI plate. (b) A schematic of the laser desorption/ionization process. (c) A schematic cross section of porous silicon, as well as an illustration of the surface functionalities (R = phenethyl).

studies[31−33] have shown that the binding of WIN compounds blocks cell attachment of some rhinovirus serotypes, inhibits the uncoating process, and stabilizes the viral capsid to thermal and acid inactivation.

In what was a very interesting result, HRV digestion was found to be dramatically inhibited by the presence of the antiviral agent WIN 52084. Digestions of other viral systems were not inhibited (indicating that interaction of the inhibitor with the virus HRV14 was specific for the virus and not the enzyme). Based on these results and additional evidence,[30] it is believed that binding of WIN 52084 to HRV14 inhibits capsid dynamics by binding the pocket (Figure 9),[14,29,34] which may act as a sort of hinge for protein movement. By the addition of the drug, movement is inhibited.

As an assay for monitoring viral inhibitors, mass mapping provides a rapid and sensitive mass-based screen for potential antiviral agents. Compounds which show anti-viral potential (i.e., very little to no viral−protein digestion) can be further tested for activity or modified to improve their activity. The conventional means of screening for antiviral drug activity is the plaque assay, a controlled growth procedure that requires significantly more time (days) and is less quantitative than the mass

mapping approach.[12] Ultimately, plaque assays must be performed to confirm antiviral activity, yet the protein mass mapping method acts as a preliminary screening procedure which can significantly reduce the number of drugs that must undergo the plaque assay. Further, the automation of this method was demonstrated with three 96-well plates, with each well containing an analytical solution of 10 drug candidates (for a total of ~2880 unique drug candidates). HRV14 and the digestion buffer were loaded onto trypsin-activated MALDI sample plates and were allowed to digest for 1 h prior to mass analysis. As a control, each sample plate also contained wells for the digestion of HRV14 in the presence and absence of WIN 52084. In a typical screen, digests in the presence of the WIN compound were inhibited, while the digests in the presence of other drug candidates were not, indicating no antiviral activity for this particular candidate library. Similar to WIN 52084, all other WIN drugs assayed were found to inhibit HRV14 digestion. Although they are not as clinically effective as WIN 52084, these other WIN drugs are also known to bind to the virus particles and exhibit antiviral drug activity. Accordingly, the inhibited digestion observed in their presence further confirms the utility of this approach as an accurate preliminary screen for antiviral drug activity.

Currently, the use of MALDI protein mass mapping as a screening tool requires that a protein undergo changes (with respect to proteolytic cleavage) upon interaction with a potential drug candidate. The ability to compare the effectiveness of drug candidates with known drugs provides an ideal scenario; however, without known inhibitors available, it is still possible to screen. With WIN 52084, the inhibition of digestion represents one way a positive "hit" could be recognized; however, with other protein systems, a "hit" may be indicated by the generation of a new set of fragments occurring or even by a significant increase in the rate of digestion. It should also be noted that MALDI can be programmed for automated sample analyses. Analyses are driven by a computer-controlled procedure to monitor for the ion signal as a function of laser position and laser intensity.[12,35] To accomplish this, the computer automatically adjusts the laser intensity and searches the sample well until a signal within the specified mass range and intensity threshold is obtained. Such automated MALDI approaches, as well as automated ESI methods, are already facilitating proteomic and drug discovery research.

As these techniques are applied, it is hoped that the new information about viral drug interactions will aid in the design of novel viral inactivants. Already, combinatorial chemistry combined with mass-based assays have been used to correlate viral−drug structure−function relationships, and, more recently, we are using rational design approaches to design drugs that exploit the dynamics of the proteome to inactivate the viral genome.

Future Prospects and Conclusions

MALDI and ESI enable the analysis of a wide variety of biomolecules and even macromolecular complexes, yet despite numerous existing benefits, new approaches can provide a different and often interesting perspective. For instance, desorption/ionization on porous silicon (DIOS) was recently shown[36] as an alternative means of generating ions in the gas phase. DIOS uses porous silicon to trap analytes deposited on the surface and laser radiation to vaporize and ionize these molecules without the addition of matrix. DIOS surfaces have proven suitable for mass analysis of a wide range of biomolecules, and the surfaces also can be chemically modified to further enhance sensitivity or act as a biocapture device. These features have already been successfully applied to antiviral agents (Figure 10) and are being further developed for viral protein analysis.

Overall, mass spectrometry is making new inroads toward characterizing virus structure and function. For example, protein/virus identification and characterization[10,11] is rapidly maturing with the advent of new high-resolution mass analyzers such as Fourier transform ion cyclotron resonance. Furthermore, its application to enveloped viruses and their corresponding glycoproteins is now providing previously unattainable information. Perhaps most interesting is that, since the mass mapping is derived from experiments performed in solution, this method is contributing to an understanding of the dynamic domains of the viral capsid which we believe will have significant value in developing new approaches for viral inactivation.

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