Electrospray Time-of-Flight Mass Spectrometry of the Intact MS2 Virus Capsid

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Received October 19, 1999

Transmission in the atmosphere is one of the natural vector mechanisms available to viruses for infection. The technique of electrospray closely mimics this mechanism by producing a fine aerosol of multiply charged ions from solution. The fact that viruses are known to survive in droplets and the recent marriage of electrospray (ES) with time-of-flight (ToF) mass spectrometry (MS),[1] with a theoretically infinite mass to charge (*m*/*z*) range, suggests that it should now be possible to detect whole particles such as viruses in a mass spectrometer. Since the earliest observations of noncovalent interactions between peptides and small proteins in the mass spectrometer[2] there has been increasing interest in maintaining larger complexes with molecular masses between 1000 and 8000 kDa.[3−5] In addition highly charged ions of covalently bound polymers have been isolated,[6,7] a virus has been shown to retain infectivity after ES and collection,[8] and the different conformations of individual viral proteins have been demonstrated by MS and proteolysis.[9] Maintaining and detecting an intact viral capsid, however, has hitherto eluded the MS processes of acceleration, focusing, and mass measurement. Here we show that it is possible to obtain a mass spectrum for the intact assembly of the bacteriophage MS2.

Bacteriophage MS2 is an icosahedral virus with 180 copies of a coat protein forming a shell around a single-stranded RNA molecule.[10] The protein shell assembles spontaneously in the absence of the single stranded RNA[11] and is composed of 90 dimers forming hexameric interactions.[10] A solution containing the virus capsid at pH 7.1[12] was introduced into a ToF mass spectrometer using nanoflow ES, under carefully controlled conditions, Figure 1. To maintain the intact capsid we have used collisional cooling with dry nitrogen in the intermediate pressure region of a ToF mass spectrometer to reduce the internal energy of the ions.[5,13] This has the effect of minimizing the explosive forces of the ES process and cooling the ions so that they retain sufficient stability as they traverse the interior of the mass spectrometer enabling them to reach the detector intact. In the absence of collisional cooling no high *m*/*z* ions are detected, presumably because the internal energy of the ions is too great leading to dissociation to monomers. On average three ions were detected in an individual acquisition step with $m/z > 20000$, Figure 1a. Signal averaging across 50 acquisition steps and conventional data processing techniques show that the peaks in the spectrum can be resolved at the onset of the charge state distribution at an intensity of >80%, Figure 1b. Thus despite the large molecular weight of this particle and anticipated heterogeneity of small molecule and cellular RNA binding during preparation, it is possible to define the highest charge states in the mass spectrum.

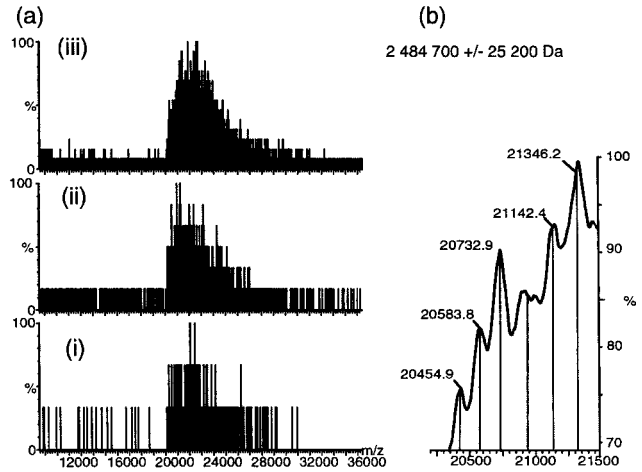

Figure 1. Mass spectrum of the intact MS2 virus capsid showing the histogram produced for the raw data (a) and the onset of the charge state distribution after data processing (b): (i) a single 10 s acquisition step in which single ions are visible in each bin above the level of background noise; (ii and iii) summation of 10 and 50 acquisitions in which a maximum of 13 ions are detected in each bin. The total ion current measured for the charge state distribution was 1.67×10^3. Spectra were recorded on a LCT mass spectrometer (Micromass UK Ltd.) modified to allow increased pressure in the first hexapole. The following voltages were found to be optimal needle 1.3 kV, cone 150 V, RF lens 480 V, extractor 0 V, and MCP 2.7 kV.

From a simulation of the natural abundance isotopes, under the instrument conditions used here, the peaks were predicted to be ∼2 *m*/*z* units wide at 50% intensity and separated from neighboring peaks by ∼200 *m*/*z* units.[14] By comparison, the peaks in the mass spectrum recorded for the capsid are ∼150 *m*/*z* units wide at 80% intensity. This broadening is consistent with previous results that show that protein assemblies with large channel structures and irregular surfaces give rise to heterogeneous binding or small molecules or salt adducts.[3,5,15] One consequence of the broad nature of the peaks is the difficulty in determining a value

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10.1021/ja993740k CCC: $19.00
Published on Web 03/25/2000

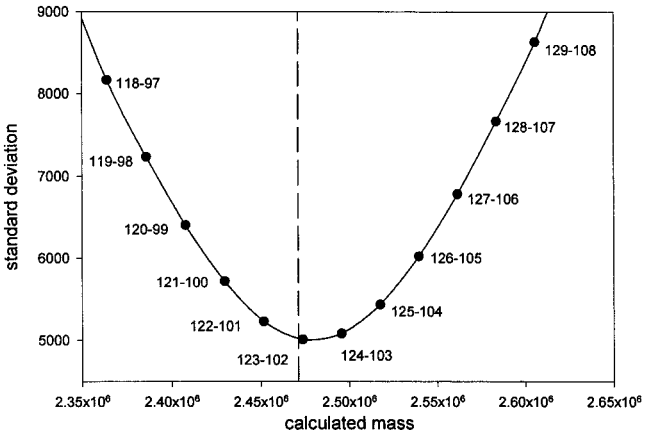

Figure 2. Plot of the mass calculated from the different charge state assignments, labeled at each point, against the standard deviation. The *m*/*z* values for the charge state assignments 122−101, 123−102, 124−103, and 125−104 were used to calculate the experimental mass 2484700 ± 25200 Da from 84 *m*/*z* values. The dotted line represents the mass calculated for 180 copies of the MS2 monomer as 2471130Da.

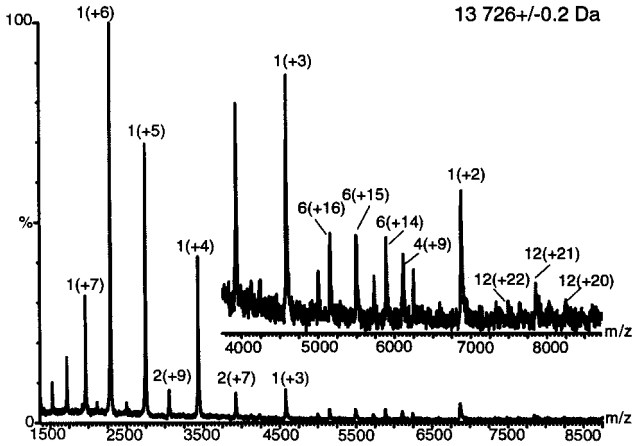

Figure 3. Mass spectrum of the products from collision-induced dissociation of the virus capsid ions with neutral gas molecules with the insert showing a 10-fold expansion of the region from *m*/*z* 3750 to 8750. The spectrum represents the summation of fifty 10-s acquisition steps and has an intensity of over 100 ions in a single bin. To obtain this spectrum the sample used for Figure 1 was diluted 4-fold, the cone and needle voltages raised to 180 V and 1.5 kV, respectively, and the effects of collisional cooling were removed. The peaks are labeled with the number of MS2 subunits and the charge is in parentheses.

for the mass and consequently the charge of the peak. This is in contrast to the ES spectra of individual proteins where the mass accuracy of the charge state series enables the solution of simultaneous equations to determine the mass and charge unambiguously.[16] To overcome this problem we have iterated the assignment of the charge to the 21 measured *m*/*z* values.[17] The results of this process show that the lowest standard deviations occur for four charge-state assignments, Figure 2. Consequently these four charge assignments were computed for each of the 21 peaks leading to a total of 84 *m*/*z* values from which the experimental mass was found to be 2484700 ± 25200 Da, a difference of +0.55% over the calculated mass. This difference arises from the ambiguity in the assignment of the charge and from the propensity of small molecules or counterions to bind to protein assemblies.[15] Although this level of accuracy would not be acceptable for the analysis of individual proteins, the experimentally determined mass is surprisingly close to the calculated mass for a complex of this magnitude.

To induce a controlled dissociation of the intact capsid the solution containing the assembly was diluted and the capsid ions accelerated in the absence of collisional cooling. Under these conditions the interactions in solution are weakened and the internal energy of the gas-phase ions is increased leading to dissociation of the capsid. The series of peaks assigned to the monomeric unit was measured as 13726 ± 0.2 Da, a value in close agreement with that calculated from the amino acid sequence (13728.5 Da). Many oligomers are observed but the fact that 6-mer and a low intensity 12-mer are readily identified suggests that the stable building block of the virus capsid is a hexamer consistent with the trimer of dimers observed in the X-ray crystal structure.[10] The observation that disassembly in the gas phase may mirror the assembly process in solution is testament to the fact that the subunit interactions are retained in the gas phase.

Mass spectrometry has been widely acclaimed as the method of choice for identifying peptides derived from proteins separated by gel electrophoresis.[18] This application is the major incentive behind the design of commercial instrumentation and the drive to reach lower levels of detection and efficiency.[19] A further facet of electrospray ToF mass spectrometry, however, is its unique ability to record spectra from very large noncovalently assembled protein complexes. This demonstration, that the 180 protein subunits can be induced to remain intact during their flight and dissociated to give monomeric units and assemblies containing six and twelve MS2 molecules, will enable the number of subunits and therefore the packing of other viruses to be addressed through mass spectrometry. Moreover the accuracy of the measured mass, obtained without a precise determination of the charge on the ion, suggests that the procedure may be extended to the study of unknown protein assemblies of molecular weights greater than 1 MDa. More generally this example highlights the potential for ES ToF methodology to contribute to the analysis of intact functional particles.

Acknowledgment. We acknowledge helpful discussions with Paula Tito, Helena Hernández, Christopher Dobson, and Lars Liljas. This research is supported by the Oxford Centre for Molecular Sciences, funded by the BBSRC, EPSRC, and MRC, and the Swedish Research Councils, NFR and MFR, and was in part funded by an EU-biotech grant. M.A.T. is grateful for support from CBD Porton Down. C.V.R acknowledges support from the Royal Society.

Supporting Information Available: Listing of measured masses for 21 charge states as well as charge state assignments across the series from +118 to +129 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) To obtain the mass of the virus capsid 50 acquisitions were signal averaged and a mean smoothing algorithm applied (2 × 25) (MassLynx version 3.2). The spectrum was imported into Win-IR (Bio-Rad/Galactic Industries Co., Salem, NH) and fitted with a linear baseline. The charge states over the mass range *m*/*z* 20147−24152.7 were modeled with Gaussian distributions. Masses were calculated from *m*/*z* values obtained for the center of the fitted peak. 21 consecutive Gausssian distributions were fitted with the exception of the peak between *m*/*z* 23809.0 and 24152.7, which could not be determined with confidence. From the two highest intensity consecutive *m*/*z* values in Figure 1b (21346.2 and 21142.4) the charges on the ions were assigned initially as 105 and 104 by the solution of simultaneous equations.[16] Since this method does not take account of the difficulties in determining *m*/*z* values of very large molecular weight species the charge on the peaks was iterated across the series and the results of this process are shown in the Supporting Information. 12 charges were assigned to each *m*/*z* value and each series used to calculate the mass of the neutral molecule. The standard deviations of the 12 series showed that four charge state assignments had the lowest standard deviations, between 5010 and 5434, across the 21 peaks, Figure 2 and Supporting Information. From these four charge state assignments a value for the experimental mass was calculated as 2484700 ± 25200 Da. It is important to note that this mass was calculated from the peak tops of the highest charge states in the series and does not therefore take into account the natural asymmetry of the peaks and the increased counterions that bind to the lower charge states.

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