Mass Determination

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Microscopy-Based Mass Measurement of a Single Whole Virus in a Cylindrical Ion Trap**

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Measuring the mass of a single whole virus is a technological challenge in the field of mass spectrometry.[1] The measurement is also important because knowing the masses of individual virus particles and the mass variability of each population is essential for the development of concepts about the structure and nature of these genetically diverse agents. Recent studies show that viruses are the most abundant biological entities in the world's oceans and are second to prokaryotes in terms of biomass on the planet.[2] It is thus timely and of interest to develop mass spectrometry of whole viruses to expand our understanding of the simplest "life forms".

With the development of soft ionization techniques such as electrospray ionization (ESI)[3] and laser-induced acoustic desorption (LIAD),[4] it has become possible to measure both masses[5–7] and sizes[8–10] of intact viruses (and viral capsids) in the gas phase with high accuracy. One of the most successful methods developed to date is charge detection mass spectrometry, which measures masses of the individual viral ions generated by ESI with a charge-sensitive detector.[6] Thousands of virus particles can be analyzed in a reasonably short period of time ($\approx$30 min). However, the typical error involved in the measurement was $\pm$15% as derived from the image current detection as well as the time of flight of the viral ions through the charge-sensitive tube. To achieve higher mass resolution and measurement accuracy, we have previously proposed an alternative scheme to determine the absolute mass of a single virus particle in a quadrupole ion trap (QIT) by using a scattered light detection method.[11] The technique, reminiscent of Millikan oil drop experiments, has permitted mass measurement of a single *E. coli* whole cell with an accuracy better than 1%.[7,12]

Although a QIT provides an ideal environment for long-term interrogation of a single trapped ion, the hyperbolic shape of the electrodes makes collection of scattered laser light difficult. For example, when the light was collected through a small hole drilled in one of the end-cap electrodes, virus particles with a size of less than 300 nm could not be readily detected.[7] In contrast to the QIT, a cylindrical ion trap (CIT) has a much simpler geometry although the equation of motion of the trapped ion is complex and cannot be solved analytically.[13] Cooks and co-workers[14] recently demonstrated that after geometry optimization to minimize higher-order field contributions, the CIT can achieve a performance comparable to that of a standard QIT with hyperbolic electrodes. It holds the advantage that one of its end-cap electrodes can be replaced by a flat, electrically conductive glass plate that enables collection of more than 10% of the light radiating from the trapped particle. Furthermore, the dimension of this trap can be varied at the user's convenience without complicated machine operations. Such a technological advancement allows integration of the transparent CIT with a variety of highly sensitive optical methods, such as the interferometric technique,[15] to detect particles of approximately 10 nm in diameter. Herein, we present a demonstration of this approach by using a CIT equipped with transparent end-cap electrodes to determine the absolute mass of a single whole virus in the gas phase.

Three viruses were examined in this study: recombinant human adenovirus type 5 (Ad5), grouper iridovirus (GIV), and vaccinia virus (VV). Their sizes range from 80 to 300 nm with the corresponding masses varying by two orders of magnitude. Figure 1a shows a schematic diagram of the experimental setup. Similar to our previous work,[7] LIAD was used as the ion source to generate multiply charged viral ions. Three major modifications have been made to improve the performance as well as detection sensitivity of the apparatus designed for investigation of monomeric virus particles: 1) a miniature transparent CIT was employed as the mass analyzer, 2) a small AC voltage (10 V) was applied between two end-cap electrodes to select ions with smaller mass-to-charge ratios (m/z), and 3) a fast Fourier transform (FFT) method was adopted to determine the oscillation frequencies of the trapped particle from its modulated scattered light signal (Figure 1b).[16] To calibrate this home-built mass spectrometer, we used the NIST polystyrene size standard SRM 1963, which has a nominal diameter of

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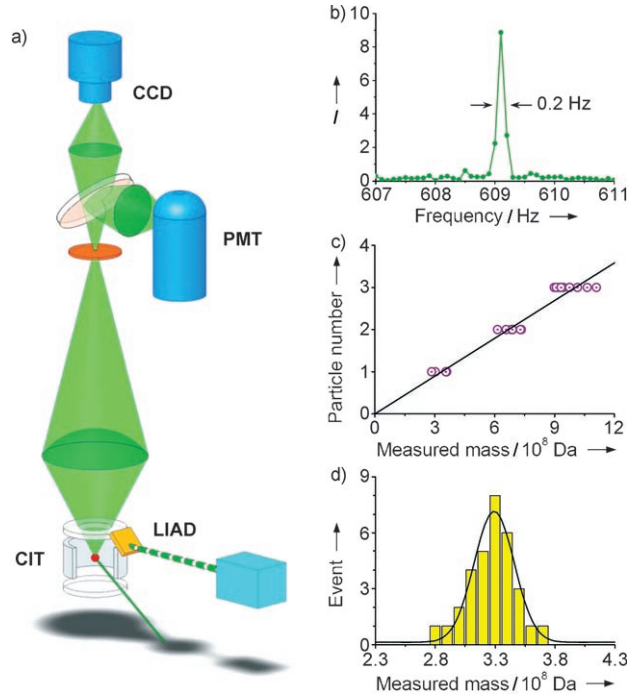

Figure 1. a) Schematic of the experimental setup consisting of a CIT made of a stainless steel barrel and two flat indium tin oxide (ITO)-coated glass plates. The scattered laser light was collected with a long working distance objective (numerical aperture of 0.42) arranged in a confocal configuration. One-half of the collected light was directed toward an electron-multiplier charge-coupling device (CCD) for trajectory imaging to ensure trapping of a single particle.[7,12] The other half was detected by a photomultiplier tube (PMT) for frequency spectrum analysis.[16] b) Typical frequency spectrum obtained by FFT of the time-domain signal acquired by PMT for 10 s. c) Plot of measured masses versus assigned particle numbers for the polystyrene size standard, SRM 1963. d) Mass distribution of the monomeric 100-nm polystyrene spheres.

100 nm.[17] Figure 1c shows the result of the mass measurement for this sample. In accord with previous observations,[7,12] both monomeric and cluster particles were detected. From 40 independent measurements (Figure 1d), we obtained a number-average mass of $3.32 \pm 0.12 \times 10^8$ Da or a mean size of 100.0 ± 1.2 nm calculated from the density of $1.055\ g\,cm^{-3}$ for the polystyrene spheres.[18] This measured size is in excellent agreement with the diameter of 99.7 ± 1.7 nm certified by NIST.

Vaccinia viruses (VVs) are large, brick-shaped particles with dimensions of approximately $360 \times 270 \times 250\ nm^3$.[19] They have a linear double-stranded DNA genome of 192 kbp in length and can potentially express more than 200 proteins.[20] An application of the microscopy-based CIT technique to this virus yielded a mean mass of 3.26×10^9 Da, which corresponds to the dry mass of the whole VV particle. Note that this measured mass is identical to our previous measurement that used a QIT as the mass analyzer for the same virus within experimental errors.[7] The major improvement made here is that we can detect monomeric VV particles quite easily with this new setup owing to the approximately 10^3-fold increase in detection sensitivity. Applying the same technique to smaller viruses such as GIV, we obtained a mean mass of 4.48×10^8 Da, accompanied with a mass variation of $\pm 0.26 \times 10^8$ Da. The result was obtained by measuring more than 40 monomeric and cluster particles (Figure 2a), requiring approximately 10 min for each successful measurement. As the accuracy of the mass measurement for each particle is better than 1%, the observed mass variation should represent the intrinsic mass distribution of these virus particles or, more likely, is a combined result of this mass distribution, degradation of the virions upon desorption/ionization, incomplete dehydration, and formation of adducts.

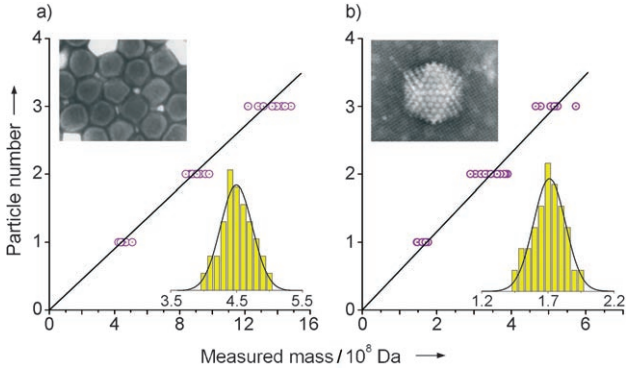

Figure 2. Plots of measured masses versus particle numbers for a) grouper iridoviruses and b) recombinant human adenoviruses. Upper insets: electron micrographs of negatively stained iridoviruses (≈170 nm in diameter)[21a] and adenoviruses (≈80 nm in diameter).[24] Lower insets: mass distributions of the respective viruses. The typical trapping parameters ($\Omega/2\pi$ and V_{ac}) used in each measurement were 5 kHz and 1000 V (a) and 7 kHz and 1000 V (b). Comparison of the mass and size of these two viruses suggests that about 70% of the total weight of the iridovirus in its native form is contributed by water.

The grouper iridovirus studied herein is an icosahedral virus with a diameter of about 170 nm.[21a] The virus is composed of a proteinaceous capsid, a lipid membrane, and a central deoxyribonucleoprotein core holding a single linear molecule of double-stranded DNA. The genome contains approximately 140 kbp (49% G + C in content)[21b] and has a calculated mass of 9.1×10^7 Da. Compared with our measured mass of 4.48×10^8 Da for the whole virus, it suggests that the iridoviruses extracted from diseased yellow groupers contain 20% nucleic acids by weight. Such a dry mass measurement also allows estimation of the number of copies of major capsid proteins in GIV. According to sequence analysis of the GIV gene, the capsid protein consists of 463 amino acids with a predicted mass of 50272 Da.[21c] Although the exact surface structure of this virus particle is not yet known, the major capsid protein was determined to account for about 40% of the total virion protein mass. Based upon this mass ratio, the genome mass, and assuming a lipid content of approximately 10% by weight,[22] we estimate that the capsid of GIV comprises about 2500 copies of the proteins self-assembled to form an icosahedral cage.

Human adenovirus Ad5 is the smallest and simplest virus examined in this study. The virus (≈80 nm in diameter) is non-enveloped, icosahedral, and has a double-stranded DNA of 36 kbp and 55% G + C in content.[23] The capsid of this

virus is built of 252 capsomers, of which 240 are hexavalent and 12 (situated at the apices) are pentavalent.[24] The total mass estimated for the adenovirus is in the range of 1.5–1.8 × 10^8 Da.[25] Figure 2b shows the result of our measurements for 40 recombinant Ad5 particles. We obtained a number-average mass of 1.72×10^8 Da, which falls nicely into the calculated mass range. The observed mass variation is $\pm 0.13 \times 10^8$ Da, or $\pm 7\%$, which translates to a size distribution of about 2% for spheroidal particles like adenoviruses. One may compare this size distribution directly with the distribution measured by Thomas et al.,[9] who used charge-reduced ESI ion mobility spectrometry to characterize the supermolecular structure of human pathogen adenovirus in the gas phase. They determined a modal diameter of 79.1 nm with a size distribution (full width at half maximum) of 72–88 nm. Our measured size distribution is about fivefold narrower than their measurement and expected to be further reduced after refinement of the experimental conditions.

Light scattering is an attractive technique for detection of nanoscale particles because of its non-destructive nature, high sensitivity, and the potential for real-time analysis.[15] Compared with conventional techniques such as quantitative electron microscopy and sedimentation field-flow fractionation that have been used to measure the dry mass of the vaccinia virus[26] and chlorovirus,[27] respectively, the ion-trap method as presently illustrated can clearly provide more direct and precise mass information. Furthermore, the design of this CIT mass spectrometer is simpler and its size can be reduced to that of an optical fluorescence microscope. However, an inherent disadvantage of the light scattering technique is that the observed light intensity (I) decreases very rapidly with the particle diameter (d) as $I \propto d^6$. This sets a lower detection limit of $d \approx 50$ nm for monomeric virus particles by using the present setup. An ultimate approach to overcome this size limitation is to measure the electromagnetic field amplitude (instead of the intensity) of the scattered light interferometrically, which gives a d^3 dependence and has been shown to be capable of detecting a single 15-nm polystyrene sphere in water.[15] An integration of this method with the CIT is expected to extend the present detection limit to the 20 nm region, where the experimentally determined masses can be compared directly with their calculated values for model virus particles such as MS2 bacteriophage[5] and rice yellow mottle virus.[6]

To conclude, we have illustrated a technique capable of determining the absolute mass of a single whole virus with a size as small as 80 nm. The typical mass-to-charge ratio determined for these medium-sized virus particles was in the range of 1×10^7, which overlaps closely with the highest m/z region covered by cryodetection time-of-flight mass spectrometry as recently demonstrated for single megadalton protein ions.[28] This microscopy-based technique is complementary to such existing commercial instruments and can be applied to mass measurement of nanoscale particles in general.

Experimental Section

Recombinant human adenoviruses were prepared by cotransfection of 293 cells with appropriate plasmids (pJM17 and pAdvPGK) and isolated according to the procedures described in reference [29]. Iridoviruses were isolated from spleen tissues of diseased yellow groupers (*Epinephelus awoara*), propagated in grouper kidney cells, and purified with ultracentrifugation in CsCl gradients.[21a] Wild-type vaccinia viruses were prepared as intracellular mature virus stocks in BSC40 cells and isolated and purified as described in reference [30]. All these virus particles, after additional purification by dialysis, were resuspended in filtered (0.2 μm pore size filter) deionized water at a concentration of about 1×10^9 particles mL^{-1} before use. To prepare the sample for LIAD,[7] an aliquot (10 μL) of the virus suspension was deposited on a bare Si wafer and dried in a dust-free desiccator. After loading into the vacuum chamber for further dehydration, intact viral ions were vaporized from the Si substrate and introduced into the CIT by laser-induced acoustic desorption. The miniature CIT has a dimension of $r_0 = z_0 = 5.0$ nm, where r_0 is the radius of the ring electrode and z_0 is half the separation between the two end-cap electrodes. Further experimental details about the mass determination of these viruses by using the CIT can be found in reference [7] and also in the Supporting Information.

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