Ultrahigh-Mass Mass Spectrometry of Single Biomolecules and Bioparticles

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Key Words

single macroions, proteins, DNA, viruses, cells

Abstract

Since the advent of soft ionization methods, mass spectrometry (MS) has found widespread application in the life sciences. Mass is now known to be a critical parameter for characterization of biomolecules and their complexes; it is also a useful parameter to characterize bioparticles such as viruses and cells. However, because of the genetic diversity of these entities, it is necessary to measure their masses individually and to obtain the corresponding mean masses and mass distributions. Here, I review recent technological developments that enable mass measurement of ultrahigh-mass biomolecules and bioparticles at the single-ion level. Some representative examples include cryodetection time-of-flight MS of single-megadalton protein ions, Millikan-type mass measurements of single viruses in a cylindrical ion trap, and charge-detection quadrupole ion trap MS of single whole cells. I also discuss the promises and challenges of these new technologies in real-world applications.

1. INTRODUCTION

Since its invention by J.J. Thompson (1, 2) in the early twentieth century, mass spectrometry (MS) has been recognized as one of the most precise physical methods. The technique has progressed rapidly within the past few decades, and it has become an indispensable analytical tool in many areas of science and technology. Some milestones along the way include the discovery of isotopes, the development of ion trap techniques, and the invention of soft ionization methods (3). The latter invention, which encompasses the development of electrospray ionization (ESI) by Fenn et al. (4) and of matrix-assisted laser desorption/ionization (MALDI) by Tanaka et al. (5) and Karas & Hillenkamp (6), has revolutionized the role of MS in biological research. Both methods allow not only high-precision MS analysis but also high-resolution analysis of biomolecules of very high molecular masses (up to 1 MDa), and they have helped solve a wide range of problems in the life sciences (7).

Although MS has become a routine analytical tool in modern biological research, application of the technique is still hindered by the difficulty of analyzing biomolecules and biomolecular complexes with masses greater than 5 MDa (8). This barrier is seemingly insurmountable because singly charged ions of these ultrahigh-mass biomolecules are technically difficult to detect with good sensitivity and to analyze with good resolution. Nearly all currently available commercial MS instruments, including quadrupole ion trap (QIT) mass spectrometers, time-of-flight (TOF) mass spectrometers, and Fourier transform–ion cyclotron resonance (FT-ICR) mass spectrometers, have a mass-to-charge ratio (m/z) less than 10^6, at which the resolution is typically less than 100. These two factors together severely limit the scope of biological MS applications (7). Such a limitation is undesirable because beyond $m = 5$ MDa, there are a variety of biomolecules and bioparticles such as viruses, bacteria, cellular organelles, and whole cells that are amenable to MS analysis. The study of these entities is not only of fundamental interest, it is also of high biomedical significance. To date, however, the masses (or dry weights) of these bioparticles have not been accurately determined.

The mass of a virus or a cell is a fundamental physiological property regulated by genetic and various environmental factors. The conventional means of quantifying biomass include flow cytometry and gravimetric determination (9); these procedures are sufficiently accurate but are also laborious and time consuming. The recent development of cantilever transducers as a platform for chemical and biological sensors has aroused much interest in using micromechanical oscillators to weigh viruses and cells individually either in water or in air (10–13). Although these methods can achieve high-sensitivity detection of even a single virus particle (11), the precision of these mass measurements is limited, with a margin of error typically greater than 10%. The origin of this error is due in part to the fact that cantilever transducers measure "wet" masses and that slight variations of the water content in the particles can cause large uncertainties in the mass measurement. MS, on the other hand, analyzes a sample based on its "dry" mass and thus can provide a more critical parameter for characterizing and identifying the sample. Furthermore, measuring the mass of a single virus or cell using modern MS technology is within reach.

A notable experiment that successfully used conventional mass spectrometers to analyze megadalton-protein complexes and virus capsids is that of Robinson and coworkers (14). The authors employed an ESI-TOF mass spectrometer to measure the masses of MS2 capsids ($m = 2.49$ MDa) with an uncertainty of only 1% (15). The MS2 virus particle has a diameter of ~20 nm and can carry more than 100 charges when vaporized and ionized by ESI. As a result, the peaks arising from these intact virus capsid ions in the ESI-TOF spectrum were only partially resolved, and they were identified only through careful charge-state analysis. More recent experiments by Uetrecht et al. (16) for hepatitis B virus capsids demonstrated that the mass

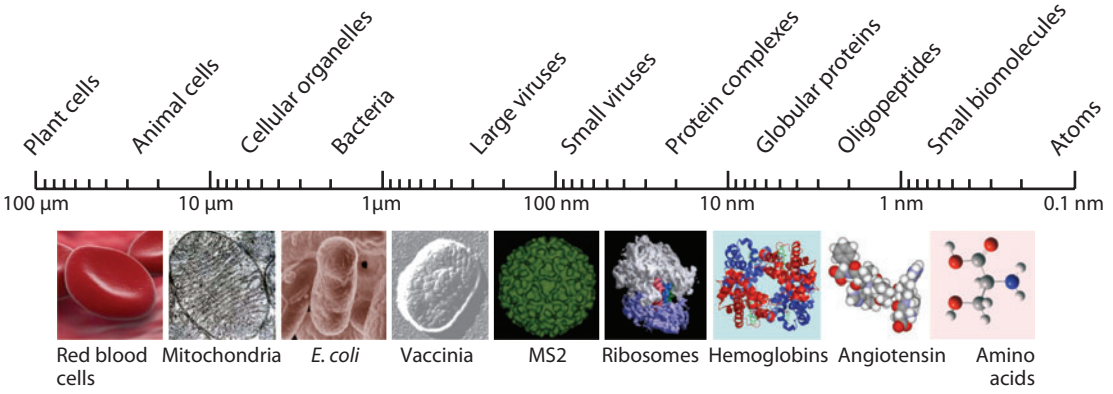

Figure 1

Size comparison of some representative biomolecules and bioparticles. Major categories are listed above the scale, and specific examples from many are shown below.

measurement uncertainty can be significantly reduced to 0.1%. To date, however, no successful application of this technique to mass analysis of whole viruses has been reported.

The main reason conventional mass spectrometers are incapable of measuring the masses of ultrahigh-mass biomolecules and bioparticles is that these instruments are always equipped with electron multiplier detectors. These devices detect ions based on secondary electron emission and have low detection efficiency for slow-moving, heavy ions (17, 18). Using microchannel plates as an example, the detection efficiency for ions with $m/z = 1 \times 10^4$ is approximately 80% at an impact energy of 20 KeV. However, the efficiency drops rapidly with increasing m/z, and a voltage as high as 1 MeV must be applied to accelerate ions with $m/z = 1 \times 10^5$ to a velocity above the detection threshold ($\sim 1 \times 10^4$ m s^{-1}) (17). Several new detection schemes have been developed to circumvent this problem, and they can be separated into three general categories: charge sensitive, energy sensitive, and photon sensitive. A detailed discussion of the advantages and disadvantages of each detection scheme can be found in Reference 19.

This review focuses on single-ion MS of proteins, DNA, viruses, and cells with diameters (d) ranging from 10 nm to 20 μm (**Figure 1**). Because m scales approximately with d as d^3, the masses of these bioparticles can span up to nine orders of magnitude (10^5–10^{14} Da). Although mass measurements conducted at the single-particle level are technically challenging, they have been made possible via the aforementioned three types of nonconventional detection methods. The significance of single-macroion MS, as well as some representative examples of these measurements, is discussed and illustrated in the following sections.

2. WHY STUDY SINGLE-MACROION MASS SPECTROMETRY?

The history of MS began with a study of canal rays composed of small positive ions in a gas discharge tube (1). Following a century's development, we can now perform routine MS analysis of biological macromolecules with masses of up to 1 MDa. With higher masses, it becomes more and more difficult to determine the value of m with good resolution and sensitivity. There are several reasons for this difficulty. First, mass spectra of biomolecules are always complicated by the presence of isotope peaks arising from the abundance of 1.1% ^{13}C in nature. For ions with $m > 1$ MDa, peaks with widths that convolute both the isotope peak distribution and the

instrument resolution often appear if the mass analyzer used does not have a resolving power greater than 10^6. Second, large biomolecules can easily carry a high number of charges, particularly when they are vaporized and ionized by ESI. With increasing mass, the separation of peaks in the isotopic envelopes of the individual multiply charged ion signals decreases, and a higher resolving power of the mass analyzer is required to separate the individual charge states. Third, posttranslational modification is an important step in the biosynthesis of many proteins. The extent of the modification, such as glycosylation, may vary depending upon the nature of the host and on the environment in which the protein is produced. These modifications, all of which cause changes in mass, can result in a higher degree of molecular mass heterogeneity as the protein size increases.

Single-ion studies permit much more accurate MS analysis for biological macromolecules than had previously been possible. The analysis is performed for individual ions and therefore is devoid of any complexity arising from an ion ensemble. Furthermore, single-ion detection circumvents the conventional limitations of achievable resolving power and precision for ultrahigh-mass biomolecules due to coulombic constraints. Such a single-ion detection technique is particularly useful for MS analysis of viruses and cells, both of which are genetically diverse agents. Measuring the masses of these bioparticles individually, obtaining their molar mean masses and mass distributions, and observing how they change with time and environment are expected to expand our understanding of these simple life forms.

3. HISTORY AND BACKGROUND

The origin of single-macroion MS began with the pioneering work of Millikan in 1910 (20). In the famous oil-drop experiments he conducted in 1909, Millikan measured the electric charge of an electron by carefully balancing the gravitational and electric forces on charged oil droplets suspended between two electrostatic metal plates. From repeated measurements for many droplets with known masses and electric fields, he found that the measured values were always multiples of the same number. Subsequently, he determined the charge of a single electron (e) to be 1.602×10^{-19} C. Based on this well-determined fundamental constant, many later experiments were set up to measure the absolute mass of a single suspended particle using the Millikan condenser as an electrostatic picobalance (21). The Millikan condenser, however, is not ideal for long-term levitation of particles in free space. A better device is the radio-frequency (RF) QIT invented in the late 1950s (22).

The capabilities of trapping and detecting a single charged particle in a QIT were first demonstrated by Wuerker et al. in 1959 (23). By adjusting the trap parameters (RF and voltage amplitude), the authors isolated a single aluminum particle (~20 μm in diameter) and observed its oscillatory motions in three dimensions within the QIT. From the observed 2:1 Lissajous curve (**Figure 2**), they (*a*) determined the axial eigenfrequency (ω_z) of the single charged particle by applying a small ac voltage across the end-cap electrodes and (*b*) visually observed when the particle's secular motion was in resonance with the frequency of the applied signal. The m/z of the particle was then calculated from the expression (24)

$$\frac{m}{ze} = \frac{\sqrt{2}V_{ac}}{\omega_z r_0^2 \Omega}, \tag{1}$$

which is valid at the trap parameter $q_z \leq 0.4$, defined as

$$q_z = \frac{4zeV_{ac}}{mr_0^2\Omega^2}, \tag{2}$$

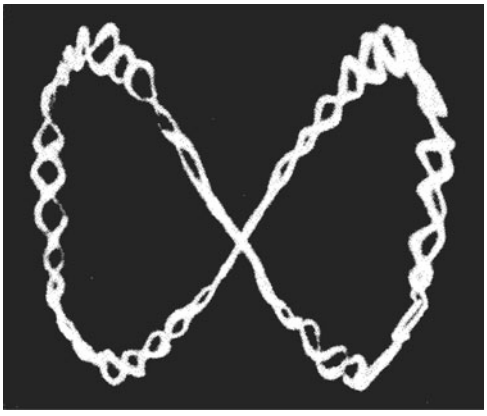

Figure 2

Photograph of a 2:1 Lissajous orbit in the *r*-*z* plane of a single charged aluminum microparticle confined in a quadrupole ion trap. Figure adapted with permission from Reference 23.

where z is the charge number, e is the elementary charge, Ω is the angular frequency, V_{ac} is the voltage amplitude, and r_0 is the radius of the ring electrode. The precision achieved in their measurements was on the order of 10^{-3}.

Using the three-dimensional QIT as a picobalance, Philip et al. (25) performed Millikan-type experiments and determined the absolute number of the charge, and thus the mass, of a single polyvinyltoluene aerosol particle (2.35 μm in diameter). They determined a weight of 6.84 pg with an uncertainty of ±1.5%. Further refinement of the technique by Schlemmer et al. (26) led to the development of a scheme for nondestructive, high-resolution mass measurement for submicrometer-sized particles. The authors recorded and analyzed in detail the amplitude modulation of the laser light scattered from a single trapped particle, from which the secular frequencies (and hence m/ze) of the particle's motions were deduced with high precision using a fast Fourier transform method. A mass measurement accuracy on the order of 10^{-4} was achieved in a 10-s data-collection period. The success of the authors' measurements was demonstrated with SiO_2 particles measuring approximately 500 nm.

Importantly, at the time of the invention of QIT, Shelton et al. (27) developed a nondestructive charge-induction tube to detect multiply charged microparticles generated by a high-voltage dc pulse. The detector consisted of an insulated drift tube mounted coaxially within a grounded shield with grids on both ends. The passage of a charged particle through the detector induced a voltage proportional to the capacity of the system on the tube. Because the duration of this induced signal is equal to the TOF of the particle through the detector, knowing the measured velocity and charges, together with measuring the voltage through which it has been accelerated, allows one to compute the mass of the particle. The device has had a major impact on mass measurement of ultrahigh-mass biomolecules, intact viruses, and nanometer-sized polymeric particles.

4. MASS SPECTROMETRY OF SINGLE PROTEIN AND DNA IONS

The ion source that enables MS analysis of single-protein and single-DNA-fragment ions is ESI. With this soft ionization method, more than 1000 charges can be attached to a megadalton polymeric molecule (28, 29). This high charge number makes it possible to detect a single macroion by FT-ICR-MS. In the ICR cell, imaging currents are induced and detected when

an ion package is in coherent orbiting motion between two parallel conductive plates. After passing a differential amplifier, the currents are digitized, and the digitized time-domain signals are Fourier-transformed to yield a full-range mass spectrum. The ultimate detection limit of this method is ~100 charges in a 1-s data-acquisition period (30). Smith and coworkers (31, 32) pioneered the application of this technique to measure the masses of single-protein ions such as bovine serum albumin (BSA) in an ICR cell. The authors showed that single ions of BSA dimers carrying as few as 60 charges can be detected with a signal-to-noise ratio of ~4. The absolute number of the charges on the individual ions was determined by charge-state shifting through proton-transfer reactions, similar to the Millikan-type experiments. Summing the sequential spectra of the individual multiply charged ions yielded statistically averaged mass spectra (33).

The same research team also applied the ESI-FT-ICR-MS technique to analyze individual ions of DNA ($m \sim 110$ MDa) extracted from T4 coliphages (34) and circular double-stranded plasmids ($m = 1.946$ MDa) cultured in transformed *Escherichia coli* (35). Trapping the plasmid ions in the ICR cell for up to several hours allowed the ions to react with acetic acid and to induce charge-state shifts. Thanks to both the high number of charges ($z > 1000$) carried by the polymeric macromolecules and the superb *m/ze* measurement accuracy, measurements of the multiple peaks arising from the charge-state shifting gave masses of the individual ions with an average accuracy of 99.8%. The results show the potential utility of FT-ICR-MS for mass measurement of very large biomolecules and indicate that it is possible to transfer substantially intact megadalton DNA ions to the gas phase via ESI.

Stimulated by Smith's work, Fuerstenau, Benner, and their colleagues (36, 37) employed a charge-induction tube, similar to the tube described in the previous section, to simultaneously measure the *m/ze* and *ze* of the individual ions generated by ESI. The tube, acting both as a TOF mass analyzer and as a detector, was made of thin-walled brass with a length of 36.1 mm and a bore of 6.35 mm. It was carefully aligned with the ion beam axis, allowing ions to pass through the tube one at a time. Single ions of DNA with masses greater than 1 MDa and charge numbers in excess of 400 were readily detected with this TOF mass spectrometer. Although the charge-induction tube is well suited for MS analysis of megadalton DNA and protein ions, the accuracy of the mass measurements was limited, primarily because of the lack of a good control of the particle velocity due to turbulent gas flow in the ion source. Further improvement of the technique with use of a gated linear electrostatic ion trap operating in a multiple reflection mode has enabled the detection of a single 4.3-kb DNA ion ($m \approx 2.88$ MDa) carrying only 75 charges (38).

For biomolecular ions carrying a low number of charges, e.g., those generated by MALDI, single-macroion MS can also be performed with an energy-sensitive cryogenic detector (39–41). The detector (for example, a liquid-He-cooled Sn- or Nb-based semiconducting tunnel junction detector) responds to the total energies carried by the incoming charged particles and hence is more sensitive to larger molecules. A demonstration of this concept was performed by Frank et al. (42), who obtained mass spectra of single-protein ions ($m \approx 750$ kDa) using a MALDI-TOF instrument. A more recent cryodetection MS experiment confirmed the feasibility of this method by detecting singly charged ions of proteins such as immunoglobulin M and von Willebrand factor with masses in the megadalton range (43). Furthermore, because the cryogenic detector responded to the total energy of the impacting ions, the charge state and/or clustering of the ultrahigh-mass biomolecules were discernible from the measured energy distribution. **Figure 3*a*** shows a typical scatter plot of the individual ion-impact events (43). The corresponding TOF mass spectrum is displayed in **Figure 3*b***.

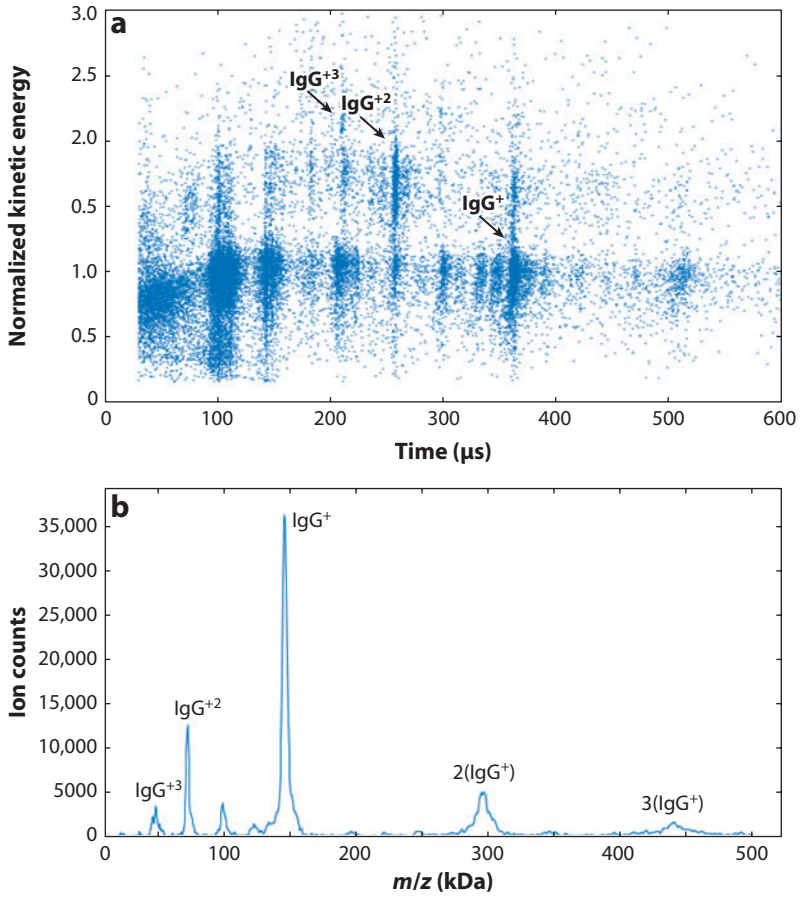

Figure 3

(*a*) Scatter plot of individual ion events of immunoglobin G (IgG) showing the ion-impact time versus the kinetic energy of each impact. (*b*) Mass spectrum of IgG calculated from the scatter plot by binning points over time and converting the time of flight to the mass-to-charge ratio. Figure adapted with permission from Reference 43.

5. MASS SPECTROMETRY OF SINGLE VIRUSES

The use of the charge-induction tube as a mass analyzer succeeds in taking MS to the megadalton scale. This is made possible by simultaneous measurements of the *m/ze* and *ze* of the individual ions. In principle, the technique permits mass analysis of ESI-generated ions with virtually unlimited mass. Using this special type of mass spectrometer, Fuerstenau et al. (44) became the first to determine the absolute masses of intact viruses. Two plant viruses were examined: rice yellow mottle virus (RYMV) and tobacco mosaic virus (TMV), which have known molar masses of 6.5 MDa and 40.5 MDa, respectively. In this experiment, an ESI source produced an aerosol of highly charged virus particles in the ion-evaporation region at atmospheric pressure. Virus ions passed one at a time through a charge-induction tube attached to a charge-sensitive preamplifier. Thousands of virus particles were analyzed in a time period of 30 min. For TMV, which is ~300 nm long and 17 nm in diameter, the typical number of the charges carried by the ESI-generated virus particles was approximately 500. The error involved in the mass measurement

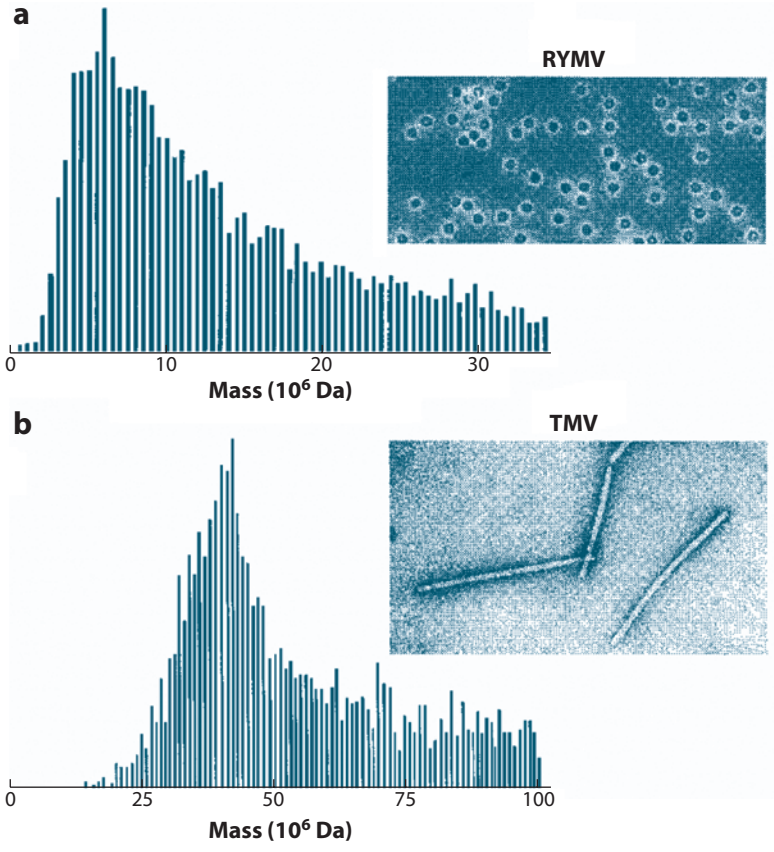

Figure 4

Mass spectra of (*a*) rice yellow mottle virus (RYMV) and (*b*) tobacco mosaic virus (TMV) analyzed with a charge-detection electrospray ionization–time-of-flight (ESI-TOF) mass spectrometer. (*Insets*) Corresponding electron micrographs of icosahedral RYMV (diameter of 28.8 nm) and cylindrical TMV (length of ~300 nm and diameter of 17 nm). The known molecular masses of RYMV and TMV are 6.5 and 40.5 MDa, respectively. Figure adapted with permission from Reference 44.

was ±15%, derived primarily from the errors in image-current detection as well as in TOF measurement of the ions as they passed through the charge-induction tube. **Figure 4** shows a typical result of the authors' mass measurements.

Viruses are submicroscopic infectious agents with sizes typically ranging from 20 to 300 nm in diameter. They are the most abundant biological entities in the world's oceans and are second to prokaryotes in terms of biomass on the planet (45). Because of the infectious nature of these nanoscale particles, producing an aerosol of viruses by ESI in ambient conditions clearly requires caution. A number of studies have addressed the viability of viruses, including MS2, T4, and TMV, after they have been vaporized and ionized by ESI (46, 47). Specifically, Siuzdak et al. (46) demonstrated that TMV maintained its infectivity even after vaporization by ESI and exposure to high vacuum. Such remarkable viability inevitably poses safety concerns and prevents wide application of ESI-MS to mass analysis of intact whole viruses. In this regard, laser-induced acoustic desorption (LIAD) (48–52) represents a feasible alternative to ESI as the ion source. This method uses a pulsed laser to ablate a thin metal foil or a silicon wafer at sites opposite the surface containing the sample. Acoustic waves generated by the laser ablation desorb and ionize the analyte

for MS analysis. Because the entire process occurs in vacuum, it offers a distinct advantage over ESI in that pathogenic or potentially dangerous bioparticles can be analyzed safely in an environment without aerosol production.

In LIAD, no matrix is used as the energy-absorbing material, and the laser beam is not in direct contact with the analyte. Therefore, degradation of the integrity of the analyte either chemically or physically is minimized during the sample deposition and subsequent laser desorption/ionization processes. This unique characteristic makes LIAD an ideal ion source for MS measurements of viruses and cells without rigid walls. Compared to MALDI (53), LIAD has a much lower ionization efficiency, typically less than 10^{-4}. However, this efficiency is sufficient for the performance of single-macroion MS, which analyzes only one particle at a time. With this new ion source, Peng et al. (51) performed Millikan-type experiments in a QIT and successfully determined the absolute mass of a single vaccinia virus with a size of approximately 300 nm. Smaller virus particles, however, could not be detected because of the low collection efficiency (<0.1%) of the scattered light through a small hole (3.1 mm in diameter) on one of the hyperbolic end-cap electrodes.

Light scattering is a useful technique for detection of nanoscale particles owing to its nondestructive nature, high sensitivity, and potential for real-time analysis. However, an inherent disadvantage of the light scattering method is that the observed light intensity scales with the sixth power of the particle size (54). This means that from vaccinia virus to RYMV, the intensity of the light scattered from these particles drops by six orders of magnitude. In order to overcome this hurdle, Nie et al. (55) developed a cylindrical ion trap (CIT) equipped with two end-cap electrodes made of flat, electrically conductive glass plates. Their CIT allowed collection of more than 10% of the light radiating from the trapped particle. **Figure 5** displays a schematic of the authors' experimental setup, which consisted of a CIT made of a stainless steel barrel and two flat glass plates coated with thin layers of indium tin oxide. Virus particles desorbed from a Si substrate by LIAD entered the trap through the gap between the ring and the end-cap electrodes. The oscillation frequencies of the single trapped particle were determined from fast Fourier transform of the recorded light scattering signal, which was modulated as the particle moved in and out of the Gaussian laser beam. While in the CIT, a trapped ion moves in a complex fashion, and the equation of motion cannot be solved analytically; however, the CIT still can achieve a performance comparable to that of a standard QIT at the trap center after proper geometry optimization to minimize higher-order field contributions (56, 57). Use of the transparent CIT enabled Nie et al. to determine the absolute masses of whole viruses ranging from 80 to 300 nm with a low margin of error of ±1%. **Figure 6** shows a result of the mass measurement for two viruses, grouper iridovirus and recombinant human adenovirus (55).

6. MASS SPECTROMETRY OF SINGLE CELLS

With the development of LIAD, it has, in principle, become possible to determine the mass of numerous kinds of bioparticles (e.g., viruses, bacteria, cellular organelles, whole cells) as long as they are large enough for light scattering detection. However, in practice, particles measuring more than a few micrometers are difficult to analyze with this method. This is because micrometer-sized particles captured by the ion trap always carry a high number of charges, typically more than 1000. Determining the number of charges using the charge-state shifting method, as first illustrated by Millikan, becomes impractical. Moreover, the charge-state determination process is quite time consuming. On average, it takes more than 10 min to determine the mass of one trapped microparticle; therefore, the method is unsuitable for mass distribution measurement, which requires sampling a large number of ions. Charge-detection MS (59, 60) is a more promising technique for high-speed analysis of these microparticles. The spectrometer integrating

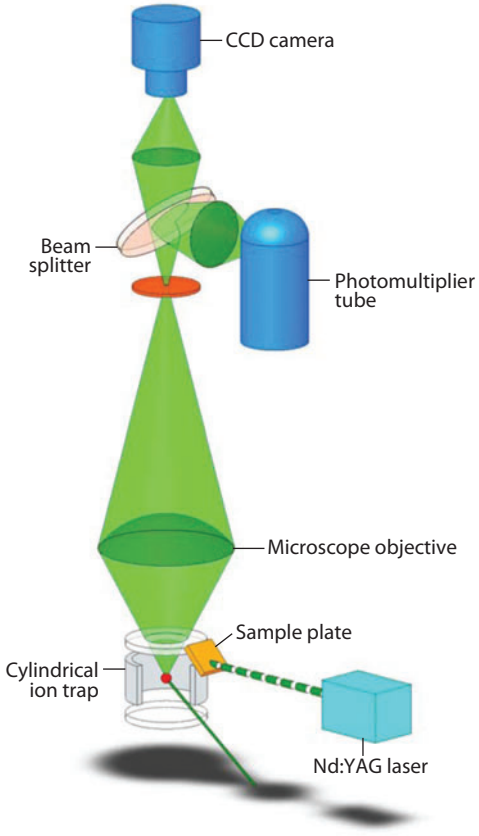

Figure 5

Experimental setup for single-virus mass measurement. The device consists of a cylindrical ion trap made of a stainless steel barrel and two flat, electrically conductive glass plates. The single trapped virus particle is illuminated by a laser beam, and the scattered laser light is collected with optics arranged in a confocal configuration. Abbreviation: CCD, charge-coupled device. Figure adapted with permission from Reference 55.

the techniques of LIAD, charge detection, and QIT operating in the mass-selective axial instability mode is most useful.

Figure 7 displays a schematic diagram of the cell mass spectrometer constructed by Nie et al. (61). This spectrometer consisted of a LIAD ion source, a QIT mass analyzer, and a charge-sensitive detector. The QIT was driven by ac fields at a frequency of approximately 500 Hz. It captured charged particles introduced into the ion trap by LIAD through a hole on the ring electrode in the presence of 20-mTorr He buffer gas. The use of buffer gas at this high pressure substantially helped the ion trapping process. Mass spectra of the cells were obtained by sweeping the trap driving frequency at a constant voltage (62, 63), rather than sweeping the trap driving voltage at a constant frequency, as is done conventionally (64). A charge-sensitive detection plate (10 mm in diameter with an electronic background of ~500 charges) monitored the particle-ejection events and simultaneously measured the number of charges carried by each ejected particle. An important component that enabled operation of the QIT in the frequency scan mode is the high-voltage current-feedback power amplifier, which has a variation of less than 1% in V_{ac} over the frequency scan range of $\Omega/2\pi = 100$–1000 Hz (65). A high-precision peak-to-peak voltage detector precisely measured (better than 100 ppm) the values of V_{ac} throughout the experiment (66).

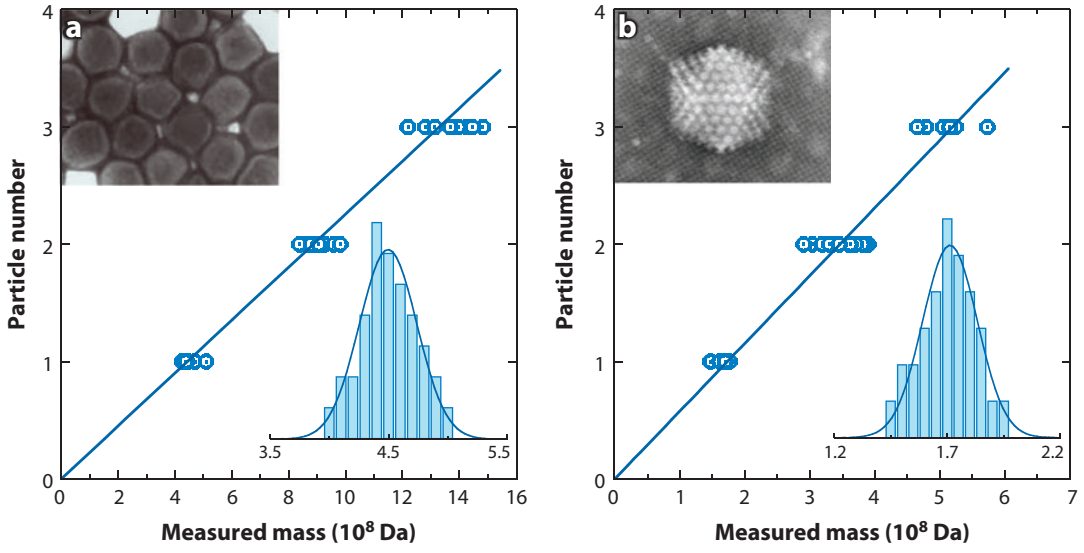

Figure 6

Plots of measured masses versus particle numbers for (*a*) grouper iridovirus and (*b*) recombinant human adenovirus. The adenovirus, which is nonenveloped and has a well-defined icosahedral structure, has a measured average mass of 1.72×10^8 Da. This value agrees satisfactorily with $(1.5–1.8) \times 10^8$ Da, which was calculated from the known DNA content and capsid protein compositions of this virus (58). (*Inset*, *a*) Electron micrograph of negatively stained iridovirus (~170 nm in diameter). (*Inset*, *b*) Electron micrograph of negatively stained adenovirus (~80 nm in diameter). The bar graphs represent mass distributions of the respective viruses. Figure adapted with permission from Reference 55.

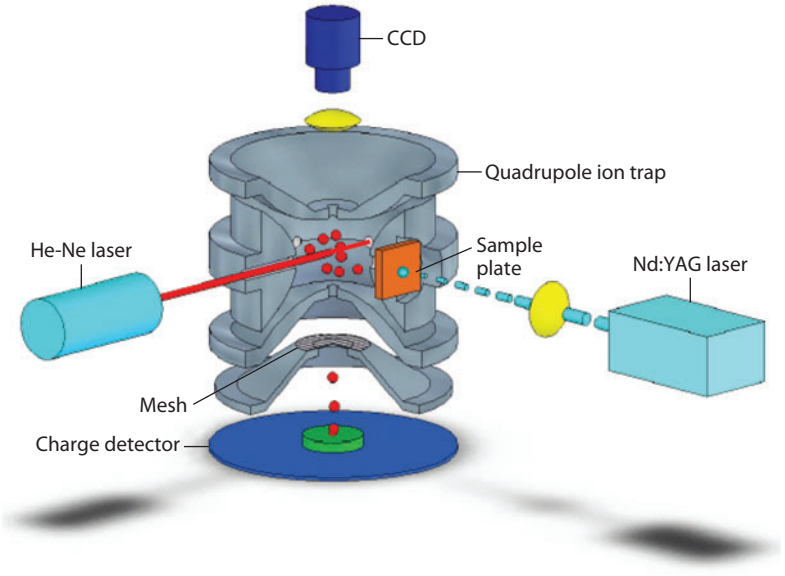

Figure 7

A whole-cell mass spectrometer consisting of a laser-induced acoustic desorption ion source, a quadrupole ion trap mass analyzer, and a charge-sensitive detector. Abbreviation: CCD, charge-coupled device. Figure adapted with permission from Reference 61.

A challenge of ultrahigh-mass MS of single ions with $m/ze > 10^6$ is how to precisely calibrate the mass spectrometer (67–69). This appears to be an impediment to the measurement in such a high m/ze region, as no standard references are available. However, Chang and coworkers (67, 69) have developed a method to calibrate the QIT mass spectrometer based on a single-particle approach. The authors determined the point of particle ejection, q_{eject} (ideally, $q_{eject} = 0.908$), for the QIT operating in the frequency scan mode. This figure was determined by measuring the secular frequencies of a single isolated microparticle inside the trap by light scattering, followed by monitoring the action of the particle ejection outside the trap by charge detection. An m/ze accuracy of 99% was achieved after calibration of the audio-frequency QIT mass spectrometer. Further calibration for the sensitivity of the charge-sensitive detector was made with National Institute of Standards and Technology polystyrene size standards, which vary less than 1% in size and can serve as a mass standard in the ultrahigh-mass region (70).

Red blood cells (RBCs) are ideal samples with which to test the feasibility and utility of the charge-detection LIAD-QIT-MS (61). These cells have well-defined shapes and are very similar in size. Due to their lack of nuclei, typical mammalian RBCs are shaped as biconcave discs and consist mainly of hemoglobin (~90% in weight). Their measured total dry masses can therefore be compared directly with the RBC indices (such as mean corpuscular hemoglobin and distribution width) that are commonly provided by the automated hematology analyzers found in most clinical laboratories (71). **Figure 8** shows the results of the mass measurements for RBCs from human, cow, and goat. In this experiment, RBCs were fixed by glutaraldehyde, loaded on a Si wafer,

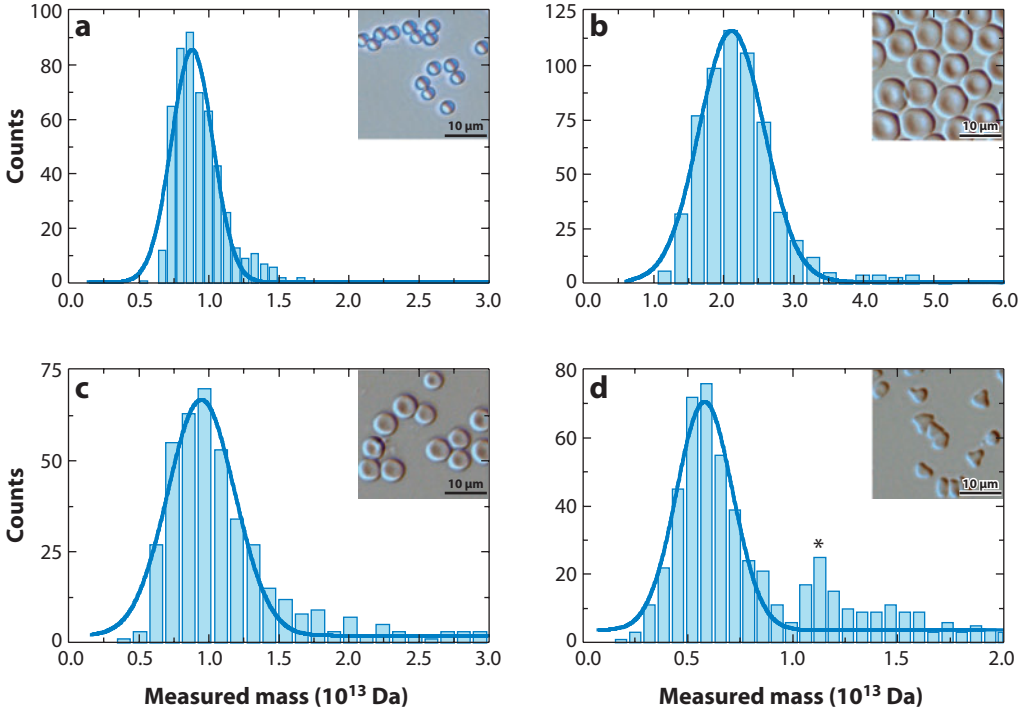

Figure 8

Mass histograms of (*a*) polystyrene size standards (SRM 1692), (*b*) normal human erythrocytes, (*c*) cow erythrocytes, and (*d*) goat erythrocytes. The asterisk in panel *d* indicates dimer formation. (*Insets*) Photographs of the corresponding polystyrene spheres and glutaraldehyde-fixed cells. Figure adapted with permission from Reference 61.

and thoroughly dehydrated in vacuum prior to LIAD. For each sample, more than 600 cells were analyzed individually in 1 h; from this analysis, both the mean mass and the mass distribution width of these dried cells were obtained. This simple method has been successfully used to analyze the RBCs of goat and chicken, whose mean masses (weights per corpuscle) range from 0.58×10^{13} Da (9.6 pg) to 2.80×10^{13} Da (46.5 pg), respectively. In addition, this MS-based technique has allowed differential analysis of normal and anemic human RBCs, which differ in dry masses by only ~30%.

Interestingly, the mass measurement for RBCs also offers an opportunity to determine the dry weight of a cell nucleus without having to isolate it. For example, chicken RBCs contain nuclei, unlike their mammalian counterparts. Assuming that the hemoglobin in a chicken RBC contributes 90% of the cell's total weight, excluding that of the nucleus, Nie et al. (61) estimated a mass of 5.6×10^{12} Da (9.3 pg) in weight for the chicken cell nucleus. In another application, the charge-detection LIAD-QIT-MS has also been used to measure the molar masses of normal and cancer cells, which have diameters of tens of micrometers. Chen and coworkers (59) showed that the technique can distinguish different types of mononuclear cells (e.g., CD3 lymphocytes and CD14 monocytes). Moreover, they obtained various mass distributions to distinguish normal lymphocytes from cancer cells derived from lymphocytes.

7. CONCLUSIONS

Over the past two decades, we have witnessed the remarkable advancement in MS of ultrahigh-mass biomolecule and bioparticle characterization at the single-ion level. As there is no need for ensemble averaging, single-ion MS opens up new opportunities to measure the masses of nanometer-sized and even micrometer-sized particles with unprecedented accuracy. These advances have been greatly assisted by the development of new detection methods (including charge-sensitive, energy-sensitive, and photon-sensitive detections) as well as new types of mass spectrometers (such as charge-induction tubes and transparent CITs as mass analyzers). Several technological issues, however, remain to be solved in order for MS to find practical applications in the life sciences.

FUTURE ISSUES

1. Detection of single-protein ions has been demonstrated with cryodetection TOF-MS. The technique can also be applied to mass measurement of single viruses. Virus particles, which have masses 10 times (or more) greater than those of protein ions, should be easier to detect. Equipping the cryodetection TOF mass spectrometer with a LIAD ion source is expected to lead to single-virus detection with high resolution and accuracy.
2. Performing Millikan-type experiments in a transparent CIT has allowed achievement of the highest mass measurement accuracy possible for single viruses with sizes less than 300 nm. However, the ultimate detection limit of this light scattering–based method is ~50 nm for a single monomeric virus particle (55). Extending the limit to the 20-nm region will rely on integration of advanced optical detection methods such as detecting the scattered light interferometrically (72) with the CIT.
3. The size of the smallest particles that can be measured to date with charge-detection QIT-MS is ~1 μm due to the high electronic background (~500 charges) of the detector. Reduction of the background noise to a level equivalent to 100 electrons (73) is required in order to detect submicrometer-sized particles such as viruses. Employing a multiple-stage charge-induction detector (74) may be a feasible approach and deserves further investigation.

DISCLOSURE STATEMENT

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