Mass Spectrometry

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Laser-Induced Acoustic Desorption Mass Spectrometry of Single Bioparticles**

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The pioneering work on electrospray ionization (ESI) by Fenn and co-workers[1] as well as matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) by Tanaka et al.[2] and Karas and Hillenkamp[3] now allows the soft ionization and mass measurement of large biomolecules. With the simplicity of the mass spectra, MALDI-MS has become a major tool for current proteomic research and in the diagnosis of diseases.[4] We previously demonstrated that MALDI is also a useful technique for laser desorption/ionization of intact bacterial particles such as *Escherichia coli*.[5] However, to produce gas-phase ions of viruses and cells that do not have rigid walls, the method is not so applicable because these bioparticles can disintegrate easily when mixed with the matrix and the disintegration proceeds further during the course of matrix crystallization and subsequent laser desorption/ionization processes. Herein, we report a laser-induced acoustic desorption (LIAD) method that allows facile desorption of intact bioparticles (including viruses, bacteria, and mammalian whole cells) for the determination of absolute mass with a quadrupole ion trap (QIT). While LIAD has been demonstrated previously for desorption of intact organic and biomolecular ions,[6–9] the mass range was limited to less than 20 kDa.

The LIAD method developed here involves laser ablation of a silicon wafer at a site opposite to the surface that contains the biological samples. Acoustic waves generated by the laser ablation desorb the analyte. Interestingly, some bioparticles with mass $m > 1 \times 10^9$ Da are desorbed as "precharged" ions and therefore their analysis by mass spectrometric can be performed without the need of external ionization. Although the efficiency of the desorption/ionization process is low, only a single particle is required for each mass measurement. Compared to ESI, which has been applied successfully to the time-of-flight mass measurement of intact MS2 virus capsids[10] and plant viruses,[11] LIAD is advantageous that pathogenic or potentially dangerous bioparticles can be analyzed more safely in an environment without aerosol production.

The experimental setup comprised a quadrupole ion trap that operated in the audiofrequency region for analyzing charged particles with very high masses (Figure 1).[12,13] The biological sample of interest was first loaded on a bare Si(100) wafer (0.5-mm thickness)[14] devoid of any energy-absorbing organic matrix. A frequency-doubled Nd:YAG laser beam (532 nm) was then applied to the backside of the sample plate to desorb the bioparticles, which entered the ion trap through the gap between the ring and end-cap electrodes. Each end-cap was drilled with separate holes for the collection of scattered laser light and for the entrance of an electron beam. An argon ion laser (488 nm) illuminated the trapped particles

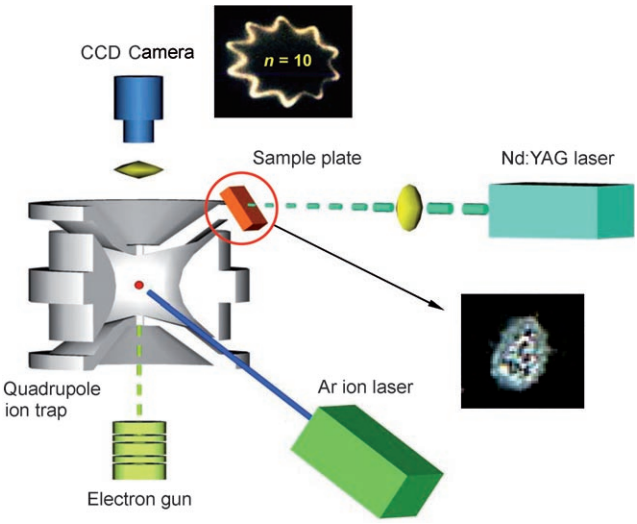

Figure 1. Experimental setup: The charged bioparticles were generated by laser-induced acoustic desorption and introduced to the quadrupole ion trap through the gap between the ring and end-cap electrodes. The typical laser fluence used for desorption was 3 J cm^{-2}, above the ablation threshold of Si.[27] The photo (top) shows a starlike oscillation trajectory of the trapped particle when the radial oscillation frequency is in resonance with the trap-driving frequency (*n* is the number of branches in the star). Observation of the stationary starlike light-scattering pattern ensures confinement of a single bioparticle in the ion trap. The image (right) is an enlarged image of the laser-ablated spot with a size of approximately 1 mm along the long axis on the sample plate (0.5-mm-thick Si wafer).

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for light-scattering measurement. A 55° forward light-scattering path and an electron-multiplier charge-coupling device (CCD) were adopted to assure high collection and detection efficiencies of the scattered photons. Trapping of the bioparticles from the laser desorption was achieved with 100–2000 Hz ac field (depending on the particle size) and approximately 1 mTorr helium buffer gas.[15] By observing the light-scattering pattern (Figure 1) associated with the trap-driving frequency ($\Omega/2\pi$) and voltage amplitude (V_{ac}), highly precise mass-to-charge ratios (m/z) were obtained for a single trapped particle.[16] The typical accuracy of the m/z measurement was on the order of 10^{-3}, primarily limited by the machining errors and mechanical misalignments of the ion-trap electrodes.[15,17] To determine the mass m, accurate measurement of the charge z was made by creating one-electron differentials[18] in the charge states by electron bombardment of the bioparticle.[19] After a few (typically five to seven) measurements for the same particle carrying different numbers of charges, mass information was deduced with a procedure that iteratively tried all possible mass-to-charge ratios for various charge states and then selected the set of the assigned m/z values that produced the lowest standard deviation.[20] The signs of the charges were finally determined from the changes (either increase or decrease) of the charge states upon secondary electron emission[19] owing to the electron bombardment.

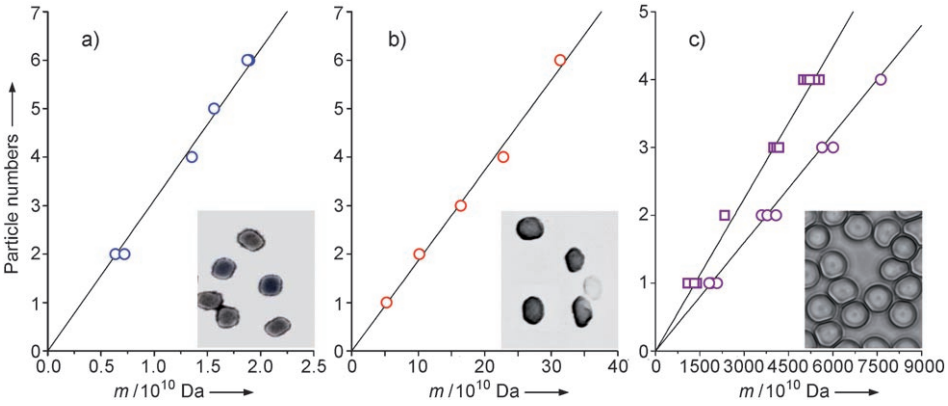

Figure 2. Plots of measured masses versus particle numbers for a) vaccinia viruses, b) *E. coli* K-12 bacteria, and c) human red blood cells. Insets: electron microscopy images of vaccinia viruses (ca. 0.33-μm long and 0.27-μm diameter; a), *E. coli* bacteria (ca. 1-μm long and 0.6-μm diameter; b), and optical microscopy image of normal red blood cells (ca. 1.7-μm thick and 7.3-μm diameter; c). The typical trapping parameters ($\Omega/2\pi$ and V_{ac}, respectively) used in each measurement were: a) 1500 Hz and 300 V, b) 300 Hz and 300 V, and c) 300 Hz and 1000 V. The two data sets shown in (c) are those of normal (○) and anemic (□) red blood cells. Note that the lack of the data point for single viruses in (a) is mainly because the light scattering from these particles is too weak to be detected.

Three different kinds of bioparticles were examined in this study:[21] vaccinia viruses, *E. coli* K-12 bacteria, and human red blood cells (representatives of viruses, bacteria, and mammalian whole cells, respectively). Furthermore, they cover the mass range from 1×10^{9} to 1×10^{14} Da (1.6 fg to 160 pg in weight) or the size range from 200 nm to 10 μm. As reported elsewhere,[20] polystyrene particle size standards (NIST SRM 1690) with a nominal diameter of 1 μm were examined with the same setup to ensure high-accuracy mass measurement. We obtained a mass of $2.38\pm0.03\times10^{11}$ Da,[22] which is in excellent agreement with the value of $2.38\pm0.07\times10^{11}$ Da calculated from the size of 0.895 ± 0.008 μm for SRM 1690[23] and the density of 1.055 ± 0.001 g cm^{-3} [24] for the polystyrene sphere.

The greatest advantage of using LIAD is that the technique can be applied to desorption of viruses and cells without rigid envelopments. As cluster particles are formed during the laser desorption process,[5,8,20] least-squares fitting of measured masses versus particle numbers was used to derive the mass of the monomeric particle (see Figure 2). For vaccinia virus, we determined a mass of $3.26\pm0.15\times10^{9}$ Da, which is equivalent to a dry weight of 5.4 ± 0.2 fg (Figure 2a). This weight is in good agreement with the median weight of 5.26 fg determined by quantitative electron microscopy,[25] suggesting that the desorbed viral particles are intact. Application of the same technique to the mass measurement of *E. coli* K-12 gave a number-average mass of $5.35\pm0.24\times10^{10}$ Da (Figure 2b), which is also consistent with our previous measurement ($5.03\pm0.14\times10^{10}$ Da) for the dehydrated bacterial particle using MALDI as the ion source.[5] Similar successful applications were made for human red blood cells (Figure 2c). Note that because only a single bioparticle and an ion-trap device were used in each measurement, poor mass resolution associated with the position spread and velocity spread in an ensemble measurement with a time-of-flight mass spectrometer is eliminated.

An interesting application of LIAD-QIT-MS is to distinguish normal from abnormal red blood cells. For human red blood cells, an index commonly used to characterize the corpuscles is MCH (mean corpuscular hemoglobin), which is a measure of the average weight of hemoglobin within a cell. The normal result of the MCH index for a healthy adult is 27–31 pg.[26] Shown in Figure 2c are the results of the mass measurements for red blood cells from normal (circles) and anemic (squares) male adults in this work. The former gave a number-average mass of $1.9\pm0.1\times10^{13}$ Da, with a mass variation of about 5%. This measured mass (or 32 ± 2 pg in weight) corresponds to the mean mass of a dehydrated erythrocyte that consists mainly of hemoglobin.[26] Notably, the weight matches closely with the MCH value of a normal red blood cell. In comparison, the mean mass of the anemic red blood cells from a patient with α-thalassemia is substantially lower at $1.3\pm0.1\times10^{13}$ Da (or 22 ± 2 pg in weight). The difference in mass between these two types of dehydrated corpuscles is approximately 30%, beyond the limit of our experimental uncertainty (≈1%) as well as the mass variation of each sample (≈10%). The ability to distinguish these two types of red blood cells substantiates the utility of this method for biomedical applications.

In this experiment, shock waves are generated by laser ablation due to material ejection from the Si surface (Figure 1 and Figure 3a).[27,28] These waves decay rapidly to acoustic waves that propagate through the 0.5-mm-thick substrate to the other surface and desorb the biological cells. We repeated the measurements for the same kind of bioparticles many times. The distribution of the charge number shows that more than 100 charges (up to 2000 charges for red blood cells) can be attached to each bioparticle without external ionization. Both positively and negatively charged particles are observed with nearly equal probability when an undoped Si wafer is used as the substrate in the measurement (Figure 3a). The result stands as an interesting contrast to the observation that predominantly only positively charged particles are produced by MALDI using sinapinic acid as the matrix (Figure 3b).[5,17]

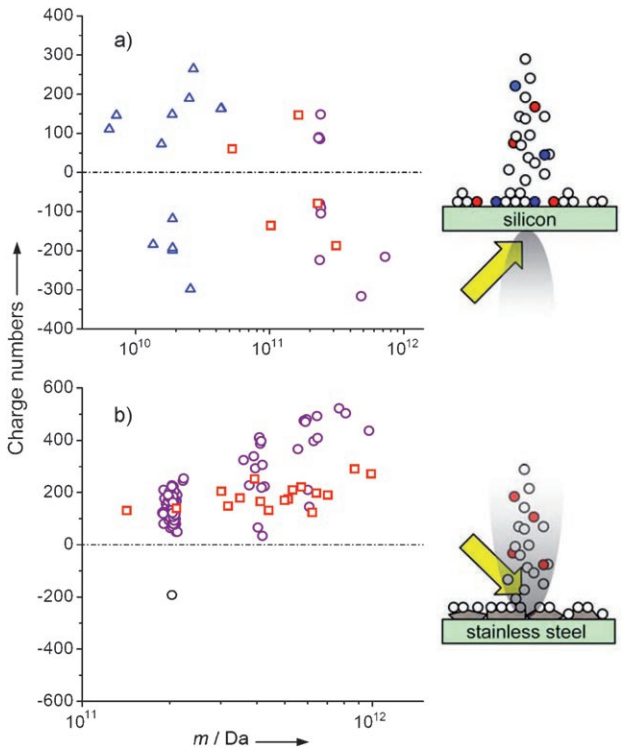

Figure 3. a) Charge-state distribution of particles generated by LIAD using an undoped Si wafer as the substrate. The three samples used were polystyrene size standards (NIST SRM 1690; ○), *E. coli* K-12 bacteria (□), and vaccinia viruses (△). b) Charge-state distribution of aminopolystyrene spheres (○) and *E. coli* K-12 particles (□) generated by MALDI using sinapinic acid as the matrix.[5,17] The corresponding laser desorption processes are illustrated on the right, where neutral, positively charged, and negatively charged particles are denoted by white, red, and blue spheres, respectively.

For LIAD, the process is mostly based on mechanical shaking forces and the desorption/ionization efficiency is usually low. The production of hundreds to thousands of charges with one particle by acoustic desorption, conceivably, is an unlikely process. Also, the ionization during acoustic desorption to produce such a large number of charges on a bioparticle is unlikely to occur. To explain our observations, we consider that a small percentage of bioparticles are present as precharged ions and that they are liberated from the surface by LIAD.[29] These charges originate from either electron-transfer or proton-transfer processes.[30] If the net charge of a sample is neutral, the number of particles with positive charges should be counterbalanced by particles with negative charges and therefore both types of charged bioparticles should be seen. This expectation is indeed in accord with our observation that the number of desorbed particles with negative charges is about equal to the number with positive charges, for three different samples deposited on the undoped Si wafer (Figure 3a). To elucidate this mechanism further, we conducted the same acoustic desorption measurement for a variety of particles (including organic, inorganic, and biological particles) from the surface of an *n*-type Si wafer biased at different dc voltages (up to 500 V). The observed charge-state distribution showed no direct correlation with the voltage applied to the wafer, indicating that acoustic forces dominated the desorption process in all cases.

Mass spectrometry without the need of matrix and external ionization offers major advantages in terms of sensitivity as well as background and fragmentation interference. Micron-sized bioparticles, including cellular organelles (such as chromosomes and mitochondria) and whole cells, are particularly amendable to this type of analysis because they can be easily charged by electron bombardment and detected by light scattering, and their masses can be measured directly and accurately with a quadrupole ion trap on a single-particle basis. The present demonstration of single mammalian cell experiments, in which the normal red blood cells were distinguished from the anemic red blood cells, can lead to possible detection of cancer cells (or even stem cells) with mass spectrometry. If the mass of a cancer cell is distinctly different from that of a normal cell, the spectrometer developed in this work can become a rapid cell sorter in the future.

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[14] Thin foils such as that made of tantalum also worked well as the sample substrates. The 0.5-mm Si wafer was chosen in this study for its rigidity and versatility of surface modification.
[15] The efficiency of the overall process (including ionization and ion trapping) is approximately 10^{-6}; that is, a loading of 10^7 bioparticles on the sample plate allows the analysis of the mass of about 10 charged particles.
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[21] Wild-type vaccinia viruses were prepared as intracellular mature virus stocks in BSC40 cells. They were purified according to published procedures (see: C. S. Chung, J. C. Hsiao, Y. S. Chang, W. Chang, *J. Virol.* **1998**, *72*, 1577) and resuspended in filtered (0.2-μm pore-size filter) deionized water before use. *E. coli* K-12 bacteria were obtained from Sigma and purified by repeated centrifugation in filtered distilled water. Both samples were prepared as suspensions at a concentration of about 1×10^{10} particles mL^{-1}. An aliquot (10 μL) of each solution of sample was deposited on a bare Si wafer and dried in air prior to laser desorption. Peripheral blood samples were taken from a healthy individual and a patient with α-thalassemia (a type of hereditary anemia). Red blood cells were separated by centrifugation and rinsed three times in phosphate-buffered saline at room temperature. A thin layer of red blood cells was smeared on the Si wafer and dried in air. The MCH values of these two blood samples were measured independently with an automated hematology analyzer (Sysmex K-1000) to be 31 and 22 pg, respectively.
[22] As the accuracy of the mass measurement for each particle is better than 1%, the reported variation in mass represents the intrinsic mass distribution of these particles.
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[29] Besides bioparticles, we found that some nano- and microparticles composed of metals, inorganic salts, organic compounds, and polymers were also liberated in charged forms. The masses of sinapinic acid powders, NaCl crystallites, gold nanoparticles, and polystyrene microparticles were all measured with this approach. The result indicates that the setup presently described may become a convenient mass spectrometer for the determination of mass of micro- and nanoparticles without the need of external ionization.
[30] Although the mechanism of the particle-charging process is not exactly known, the surface charge density of these desorbed precharged ions is actually very low. For a red blood cell carrying 1000 charges as an example, the calculated surface charge density is on the order of 10^{-7} $C m^{-2}$, a value which is much lower than that of small molecular ions desorbed/ionized by LIAD (Ref. [8]). Because of this low charge density, electron bombardment of negatively charged particles is possible.

