Communication

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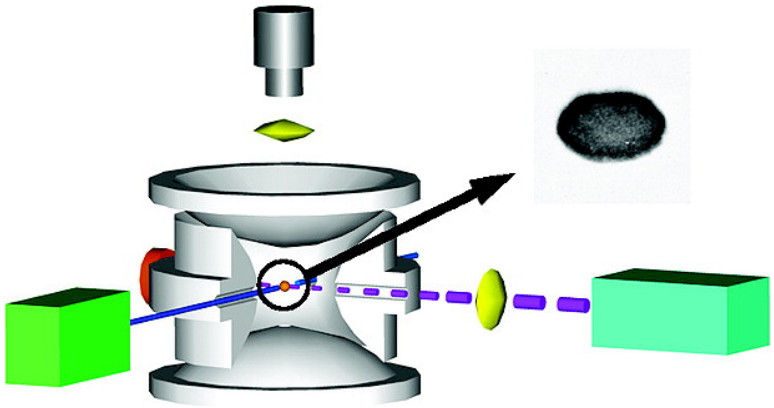

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Measuring Masses of Single Bacterial Whole Cells with a Quadrupole Ion Trap

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Measuring masses of intact microorganisms holds much interest in the scientific and mass spectrometric communities. One of the motivations for such measurement is to provide an alternative means for rapid identification of potentially dangerous viruses and bacteria.[1,2] The conventional ways of quantifying biomass involve both flow cytometry and gravimetric determination, which can be sufficiently accurate but are laborious and time-consuming.[3] Recently, new technologies such as quartz crystal microbalances,[4] superconducting quantum interference devices,[5] and micromechanical oscillators[6,7] have been developed to detect single bacterial and viral particles to achieve highest sensitivity. However, the errors involved in these measurements are relatively large, typically more than 10%. Part of the reason for this inaccuracy is that the mass measurements were all conducted in fairly complicated environments.

With the development of soft ionization methods, both electrospray ionization (ESI)[8] and matrix-assisted laser desorption/ionization (MALDI),[9] it has become possible to directly measure the masses of single bioparticles in the gas phase. Multiply charged bioparticles such as ESI-generated viruses have been detected successfully by ionization-based[10] and charge-sensitive[11] detectors (and potentially by cryodetectors[12]). Mass measurement accuracy approaching 1% was achieved for intact MS2 capsids with $m = 2.485 \pm 0.025 \times 10^6$ Da in a time-of-flight instrument.[10] In applying the same technique to mass determination of bacteria (typically 1 μm in size and 5×10^{10} Da in mass), the main difficulty one faces is the low detection sensitivity. We have previously explored[13] the feasibility of using elastic light scattering (ELS) to detect single submicron-sized particles ejected from a quadrupole ion trap (QIT) for mass/charge analysis. This work demonstrates that it is possible to measure the absolute masses of single *Escherichia coli* macro-ions within the QIT using MALDI as the ion source[14] and ELS for particle detection. Partial success of the measurement has been previously reported for bacillus spore clusters,[15] and complete measurements have been accomplished for SiO_2 particles in the same mass range.[16]

The *E. coli* K-12 whole cells were obtained from Sigma and used without further purification. Scanning electron microscopy (SEM) revealed that all the bacteria are nonflagellated and similar in size. They formed aggregates and accumulated on the surfaces of the energy-absorbing materials when the matrix crystallized.[17] Three matrixes have been adopted and tested to ensure the cellular integrity of the gaseous bacterial ions upon laser desorption and ionization: sinapic acid (SA), 4-hydroxy-α-cyanocinnamic acid (4HCCA), and 2-(4-hydroxy-phenylazo)-benzoic acid (HABA). To minimize damage of the bacterial whole cells during MALDI, the laser power density was kept low ($\sim 10^6$ MW/cm^2) and exposure of the same sample spot to repetitive laser irradiation was avoided. In all cases, no scattered laser light was detected with the bare matrix, confirming that the observed signals are indeed derived from the trapped bacterial particles, rather than from clusters composed of the matrix molecules. Further confirmation of the signals came from careful statistical analysis of the masses determined for the bacterial particles and a control experiment using polystyrene microspheres of known diameters as the sample (see Supporting Information).

The experimental setup consists of a QIT as an electrodynamic balance operating in the audio frequency region.[13,14] Intact bacterial particles were introduced into the ion trap by MALDI from a sample probe inserted in one of the holes on the ring electrode with a frequency-tripled Nd:YAG laser. Spatial confinement of the evaporated macro-ions was achieved with 300 Hz ac fields and 1 mTorr He buffer gas for ion deceleration. Although both the ion formation and trapping may not be very efficient, only a single particle is needed for our measurements. When more than one particle was confined in the QIT, the trap driving frequency (Ω) was decreased slowly to eject axially until there was only one left for examination. Light scattering from the single isolated particle revealed the trajectory of its oscillatory motion in the radial plane. A stationary star pattern emerged after fine-tuning of Ω to resonate with the radial secular frequency (ω_r), i.e., $\Omega = n\omega_r$. From the number (n) of the branch of the stationary star (inset in Figure 1a), the mass-to-charge ratio (m/Ze) of the particle was deduced from fitting of the experimental data to the equation[15,18]

$$\frac{2}{n} = \frac{q_r}{\sqrt{2}}\left(1 + \frac{25}{128}q_r^{\,2} + \frac{34\,951}{294\,912}q_r^{\,4} - \frac{7925}{294\,912}q_r^{\,6} - \frac{100\,489}{10\,616\,832}q_r^{\,8}\right)$$

where $q_r = 4ZeV_{ac}/mr_0^2\Omega^2$, Z is the charge number, e is the elementary charge, V_{ac} is the trap driving voltage amplitude, and r_0 is the radius of the ring electrode. For the particular practice shown in Figure 1a, we obtained $m/Ze = 1.3027 \pm 0.0013 \times 10^9$ prior to charge state change of the *E. coli* K-12 particle.

The reproducibility of the m/Ze measurement constantly achieved with this method is ~0.1%. It alternatively means that the resolution of this single-particle quadrupole ion trap mass spectrometer is ~1000 at $m/Ze > 1 \times 10^9$. To determine m, Z was measured independently using an electron gun to change the charge state of the particle randomly by producing one-electron differentials.[16,19] The stationary star (inset in Figure 1a) abruptly lost its stability upon secondary emission of the electrons,[16] an event that can be easily detected by the CCD camera. A new set of data was then collected after this detection to attain a new m/Ze value. In assigning the absolute charge number, we iterated the assignment for five independently measured m/Ze values of the same particle (i.e., the same mass) until the standard deviation in the least-squares linear fitting of the data reached its minimum (Figure 1b). An assignment of the charge states at the minimum ($Z = 38$, 41, 42, 44, and 45) leads to $m = 4.957 \pm 0.020 \times 10^{10}$ Da or a weight of 82.3 ± 0.3 fg per *E. coli* dry cell.

In the course of this measurement, clusters of bacterial whole cells as originally observed in the matrix were also found in the gas phase. These cluster macro-ions can be readily identified from

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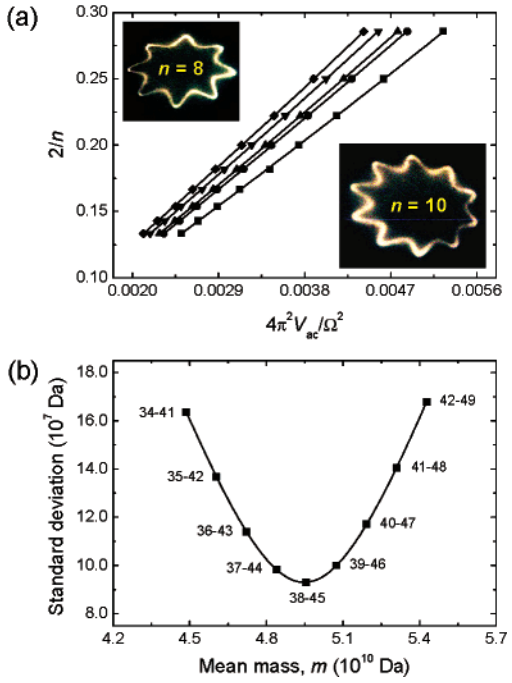

Figure 1. (a) Plot of the star branch number (n) as a function of the trap driving frequency ($\Omega/2\pi$) and voltage (V_{ac}). The bacterial particles were trapped at $q_z \approx 0.5$, and the m/Ze values were determined by fitting the experimental data to the equation described in text with $n = 7-15$. Consecutive changing of the charge state by electron bombardment (from right to left) of the same particle yields five distinct m/Ze values. Inset: Photos showing the starlike trajectories of the particle's oscillatory motions projected on the radial plane. (b) Plot of the mean mass calculated from different charge state assignments (labeled at each point on the curve) versus the resulting standard deviation.

the brightness of their star patterns displayed on the CCD camera. Shown in Figure 2 is the result of the mass analysis for cluster particles captured by the QIT for 60 independent measurements. Following the same iteration procedure for the charge state determination described earlier and elsewhere,[10] accurate cell number (N) assignments of the bacterial clusters can be made from a standard deviation analysis in the least-squares fitting of the experimental data plotted in terms of N versus m. Increasing or decreasing N by one unit results in a substantial (~10-fold) increase in the standard deviation, confirming the uniqueness of this cell number assignment. We determined a statistically averaged mass of $m/N = 5.03 \pm 0.14 \times 10^{10}$ Da for a single *E. coli* K-12 whole cell in a vacuum or a dry weight of 83.5 ± 2.3 fg per particle (inset in Figure 2). To the best of our knowledge, this is the first high-precision mass measurement for intact microorganisms with masses greater than 1×10^{10} Da.

The mass distribution of *E. coli* K-12 examined in this work for more than 60 particles is ±3%, which can derive from the intrinsic mass variation of the bacteria, fragmentation of the bacterial cells, and/or formation of the bacterium−matrix adducts during MALDI. However, this deviation is unlikely to arise from the mass loss due to electron bombardment or further dehydration of the bacterial particle in a vacuum over a long period of time. As a stability test, a mass measurement was conducted for the same particle over 18 h, during which the charge state was changed consecutively 11 times. The measured masses differ by less than 0.1%, well within the limit of our experimental uncertainty. To further assess the degree to which the cells ruptured during the ion formation, we employed 4HCCA and HABA as the matrixes for comparison. Masses of $5.02 \pm 0.17 \times 10^{10}$ Da and $5.02 \pm 0.16 \times 10^{10}$ Da were determined for 4HCCA and HABA, respectively. Together

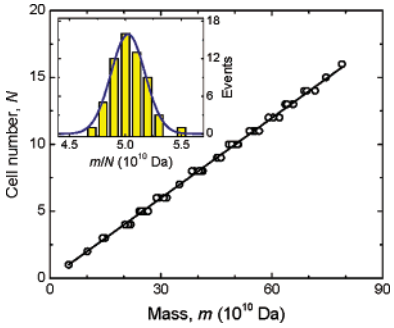

Figure 2. Plot of the assigned cell number (N) versus the measured mass (m) from 60 independent measurements, showing the linear correlation between these two parameters. Inset: Mass distribution of single *E. coli* monomers after proper scaling of the masses of the clusters with the cell number (i.e., m/N).

with the result of SA, the measured masses differ by only 0.2%, strongly suggesting that the bacterial cells remain intact during the laser desorption/ionization. We attribute this remarkable feature to the fact that the bacterial particles are only loosely attached to the surfaces of the energy-absorbing crystals, rather than being incorporated into the matrix. Conceivably, they evaporate through a process resembling surface-enhanced neat desorption,[20] and multiply charged macro-ions may form in the plume via proton-transfer reactions.[21]

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Supporting Information Available: Materials, methods, procedures, and figures showing the experimental setup and SEM images of the bacteria (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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