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

Abstract / This paper reports the first mass determination of whole viruses with sizes in the range of 80 - 300 nm using a miniature cylindrical ion trap. The trap is unique in that its endcap electrodes were made of transparent, electrically conducting glass plates, allowing collection of more than 10% of scattered laser light from a single trapped viral ion produced by laser-induced acoustic desorption. Three viruses have been successfully examined in this study: vaccinia virus, grouper iridovirus, and recombinant human adenovirus. Our results suggest a broad and promising application of this new technology to viral systems.

Viruses are the simplest life forms on our planet, consisting of only DNA (or RNA) and a protein shell. After the prokaryotes, viruses are the second most common type of organism. In our oceans, they are the most common life form. In order to gain a better understanding of the structure and characteristics of these genetically varied little organisms, it would be highly useful to be able to determine their masses and how much these vary within a given population. This work demonstrates that it is possible to use very gentle ionization techniques and a miniaturized ion trap of our own devising to very accurately analyze the masses of individual, intact viruses.

Previous methods for determining the masses of viruses had an error rate of ±15%, which made them too inaccurate to ensure the resolution of small differences in mass. This work presents a new concept to attain higher precision. In order to determine their mass, viruses must first be converted to the gas phase, given an electric charge, and accelerated in an electric field. However, this process must leave the viruses intact. We chose to use a very gentle method known as LIAD (laser-induced acoustic desorption). In this method, the virus particles are released from the sample by laser-induced sound waves. They are then caught in an ion trap, which holds charged particles by means of its special geometry and the superposition of a direct and alternating electric field. Once trapped, the virus particles are ready for mass determination (Figure 1). Laser light is beamed into the ion trap. If a particle is present, it scatters the light. The scattered light can be detected through the transparent surfaces of the ion trap. A portion of the light is sent to a CCD camera, which records the flight path of the trapped particle. The rest of the light goes to a measuring device that precisely analyzes the scattering signal. The scattered light is different from the initial light beam because the virus particle in the
The electric field of the ion trap begins to oscillate. This oscillation depends on the mass-to-charge ratio of the virus. Final mass determination is made by changing the charge state of the particle with electron bombardment ("electron stepping"), a technique reminiscent of Millikan oil drop experiments.

By using this device, we have been able to determine the masses of three different types of viruses with diameters varying between 80 and 300 nm: human adenovirus type 5, grouper iridovirus, and vaccinia virus. Their masses were determined, respectively, to be $3.26 \times 10^9$ Da, $4.48 \times 10^8$ Da and $1.72 \times 10^8$ Da (Figure 2). The deviation in each mass measurement is low, only about $\pm 1\%$. The masses of the viruses, in combination with other analytical processes, can be used to infer how many building blocks are used to make up the shell of the virus or how many copies of genetic material it contains.

These highly precise measurements were made possible by the special structure of the ion trap. Instead of a classic quadrupole ion trap, we chose to use a cylindrical ion trap (CIT). In this type of trap, the movement of the trapped ions is considerably more complex and not mathematically ascertainable. However, it has the advantage of a much simpler geometry. We constructed a CIT with smaller dimensions than usual, optimized the geometry, and exchanged the usual terminal electrodes of the cylinder with transparent, electrically conducting plates. This special construction allowed collection of more than 10% of scattered laser light from a single trapped viral ion, which is what made application of the precise light-scattering technique possible.

To summarize, we have developed 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 is in the range of $1 \times 10^7$, which overlaps closely with the highest $m/z$ region covered by conventional time-of-flight mass spectrometry. This microscopy-based technique is complementary to such existing commercial instruments and can be applied to mass measurement of nanoscale particles in general.

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