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Trap of Biomolecular Ions in the Gas Phase Produced by IR-laser Ablation of Droplet Beam

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We describe a novel method to trap biomolecular ions in the gas phase produced by an IR-laser ablation of a droplet beam. The IR-laser ablation is performed in an ion trap so that the product ions are readily trapped with high efficiency. The ions are thermalized in collisions with the ambient water molecules which are simultaneously produced with the ions by the IR-laser ablation.

Molecules and molecular ions exhibit their intrinsic properties by isolation in the gas phase, because they are free from perturbation by ambient molecules. Therefore, studies of isolated biomolecules provide an important basis for elucidation of their complex functions in solutions. Such studies can be achieved by isolating the molecules directly from the solution into the gas phase and trapping them for a time required by an experimental probe employed.

Direct isolation of biomolecular ions from solution has been attempted by irradiation of an IR laser onto the solution in vacuum by groups of Brutschy,[1] Abel,[2] and ourselves.[3,4] The biomolecules are successfully isolated directly from the solutions into the gas phase without suffering from significant fragmentation and keep the ionic state in the solutions. We apply the isolation method to study of a laser-induced proton-transfer reaction in the gas phase.[4] On the other hand, further studies require maintaining the isolated ions in the gas phase for a time necessary for the probe employed in the study. In this report, we present a method to prepare biomolecular ions isolated from a solution and trapped in the gas phase by use of a droplet beam (a train of liquid droplet in vacuum)[3] in combination with laser ablation-mass spectrometry with an ion trap.

Figure 1 shows a schematic view of droplet-beam laser-ablation mass spectrometry with an ion trap (DB-LAMS-IT). The apparatus consists of a piezo-driven liquid droplet nozzle, a cylindrical quadrupole trap, and a reflectron time-of-flight (TOF) mass spectrometer. The ion trap and the TOF mass spectrometer are housed in a three-stage differentially pumped vacuum chamber. A droplet (ca. 70 μm in diameter) of a 10 μM aqueous solution of lysozyme (Lys) was injected into air from the nozzle. Commercially available Lys (Sigma) and deionized and distilled water were used without further purification. Air flow from the inlet aperture to the first vacuum chamber carries the droplet through the second chamber to the third, where the droplet was admitted into the ion-trap/acceleration region of the apparatus.

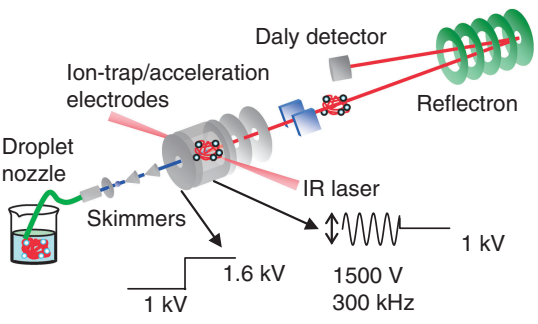

Figure 1. Schematic view of an DB-LAMS-IT apparatus.

The ion-trap/acceleration electrodes were constructed by consulting references.[5,6] It consists of a cylindrical ring electrode (inner diameter: 20 mm, outer diameter: 50 mm, and thickness: 10 mm) and two endcap electrodes (thickness: 1 mm). The three electrodes were mounted with 2 mm spacings. The central axis of the electrodes was concentric with the droplet-beam trajectory. One endcap electrode at the upstream of the trajectory has an aperture of 6 mm in diameter, through which the droplet reaches inside the ion trap. The ring electrode had two holes facing each other at the sides in order to focus the ablation IR laser onto the droplet beam. The droplets and the IR-laser pulses were triggered by pulses generated from the same source to make the droplet beam be hit by the IR laser (2850 nm, 18 mJ pulse^{-1}) when it reached the right position inside the ion trap. Protonated Lys ions were produced by the IR-laser ablation of the droplet beam. A radio frequency (RF) voltage (1.5 kV_{pp}, 150–300 kHz) had been applied to the ring electrode, which trapped the ions produced inside the ion trap. After a prescribed period (trapping time, 0–50 ms), the trapped ions were accelerated by a pulsed electric field applied to the endcap electrodes and analyzed by TOF mass spectrometry. The RF field was turned off during the acceleration pulse in order to avoid deflection of the ions by the RF field which worsen the mass resolution. A Daly-type detector was employed to detect ions with large mass-to-charge ratio (m/z). High detection efficiency and fast response (<100 ns) were achieved by use of a cup-shaped target.[7]

The IR-laser beam was mildly focused to ca. 0.5 mm in diameter. Only about half of the IR-laser pulses aimed at the incoming droplets hit them fluctuating in position.[3] Evidently the ions were intensified when the IR laser hit the incoming droplet, so that we were able to recognize whether or not the IR laser actually hit the droplet. By taking advantage of this observation, true signals were distinguished from false ones. In the present experiment, signals of 50 count/shot were ascribed to the droplet hit by the IR laser. The mass spectra were obtained by collecting such intense signals regarding as those of the ions produced from the droplet irradiated by the IR laser.

Figure 2 shows mass spectra of ions produced by the IR-laser irradiation onto the droplet beam of 10 μM aqueous solution of Lys with trapping times from 0 to 10 ms. Peaks in the mass spectra are assigned to $[Lys + nH]^{n+}\cdot(H_2O)_m$ ($1 \leq n \leq 5$). The peak tails to the higher mass cannot be simply assigned to

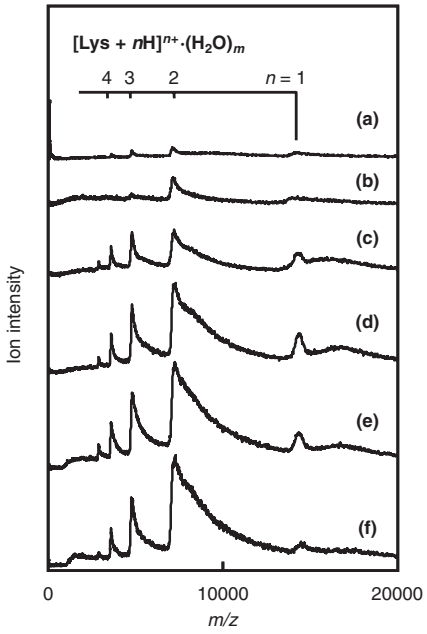

Figure 2. Mass spectra of trapped ions produced by IR-laser irradiation onto the droplet beam of 10 μM Lys with trapping times of (a) 0, (b) 0.2, (c) 0.3, (d) 0.5, (e) 1, and (f) 10 ms.

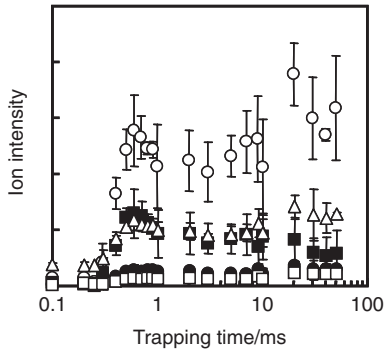

Figure 3. Trapping time dependence of ion intensities of $[Lys + nH]^{n+}\cdot(H_2O)_m$ for $n = 1$ (■), 2 (○), 3 (△), 4 (●), and 5 (□).

the distribution of m, because the peak shape is dependent on detector conditions, such as a post-acceleration voltage of the Daly-type detector. Higher mass resolution is necessary in order to apply this method to studies on hydrated protein ions in the gas phase, which can be achieved by an improvement of the present setup as well as a combination with other high-resolution mass spectrometers. On the other hand, the distributions of n in the mass spectra are not those of the product ions, because the ion-trap efficiency depends upon the m/z of the ions.

Figure 3 shows the ion intensities of $[Lys + nH]^{n+}\cdot(H_2O)_m$ ($1 \leq n \leq 5$) as a function of the trapping time. The ion intensities increase with increase in the trapping time of 0–0.6 ms and level off until 50 ms.

An ion cooling explains the increase of the ion intensity with increase in the trapping time from 0 to 0.6 ms. Initial kinetic energy of the product ions is large, because the ions are produced in the course of the explosion of the droplet beam by the IR-laser ablation. When such hot ions are immediately accelerated by the electric field for the TOF mass analysis, the ions spread in space both parallel and perpendicular to the acceleration axis. The parallel spread result in a broadening of the flight-time distribution and hence decrease of mass resolution. And the perpendicular spread result in the decrease of the number of the ions that reach the detector, and hence decrease of ion intensity. On the other hand, the ions in the ion trap are cooled down by collisions with ambient molecules during the trapping time. Here, the ambient molecules are likely to be the water molecules which are produced simultaneously with the ions by the IR-laser ablation of the droplet beam as shown below. The collision frequency, f, is given as

$$f = \sigma n \sqrt{\frac{8RT}{\pi\mu}} \quad (1)$$

where σ is cross section, n is number density, R is the gas constant, T is temperature, and μ is reduced mass of the colliding molecules. Consider the collisions between Lys ions and the water molecules: σ and μ are approximately the cross section of Lys molecule ($\approx 10\,nm^2$)[8] and the mass of water molecule, respectively. Assume that all the water molecules in the droplet beam evaporate and are confined within the ion trap. The ion trap has a cylindrical shape with a diameter of 20 mm and the length of 14 mm. Then, the evaporation of a single droplet (70 μm in diameter) results in the number density, n, to be ca. 6×10^{15} molecules cm^{-3}. Temperature is also assumed to be room temperature (300 K). By substituting these values to eq 1, the collision frequency is calculated to be ca. 8×10^6 Hz. It follows that the Lys ions undergo ca. 4800 collisions proceed in the trapping time of 0.6 ms. An ion trajectory simulation study indicates that 6000 collisions of an ion with He atoms thermalize translational energy of the ion.[9] The calculated value, 4800, in the present study roughly coincides with the reported number of collisions required for the ion cooling.

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†† Prof. Tamotsu Kondow passed away unexpectedly on May 25, 2009.

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