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ARTICLE *in* ANALYTICAL CHEMISTRY · NOVEMBER 2010

Impact Factor: 5.64 · DOI: 10.1021/ac102217e · Source: PubMed

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Anal. Chem. **2010,** *82,* 10125–10128

MALDI Ion Trap Mass Spectrometer with Charge Detector for Large Biomolecule Detection

Chien-Hsun Chen,[†] Jung-Lee Lin,[†] Ming-Lee Chu,[‡] and Chung-Hsuan Chen[†,*]

Genomics Research Center, and Institute of Physics, Academia Sinica, Taipei, Taiwan

Up to now, all commercial matrix-assisted laser desorption/ionization (MALDI) mass spectrometers still can not efficiently analyze very large biomolecules. In this work, we report the development of a novel MALDI ion trap mass spectrometer which can enrich biomolecular ions to enhance the detection sensitivity. A charge detector was installed to measure the large ions directly. With this design, we report the first measurement of IgM with the mass-to-charge ratio (*m/z*) at 980 000. In addition, quantitative measurements of the number of ions can be obtained. A step function frequency scan was first developed to get a clear signal in the *m/z* range from 200 000 to 1 000 000.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MS) has evolved into one of the most powerful tools in analysis of large biomolecules. However, the detections have been achieved mostly by a charge amplification detector such as an electromultiplier or a channeltron or a microchannel plate (MCP). The efficiencies of these detectors are a strong function of the velocity of the ion.[1] Since the velocity of an ion is inversely proportional to the root square of the mass at a fixed energy, the detection sensitivity is very poor when molecular weight is above 100 kDa and the molecule is with a single charge. ESI has also been used to detect quite large molecular ions since there can be many charges on one very large molecules.[2,3] Nevertheless, ESI is not easy to be used to detect a complete mixture of several large biomolecular ions due to the complexity of mass spectra from the broad charge distributions. Cryogenic detector based on the measurements of the thermal energy deposited by the particle impact was developed to overcome the difficulty of detection of ions with very large mass-to-charge ratio (*m/z*). The sensitivity of a cryodetector is largely independent of the mass. Cryogenic detector has been used successfully to detect very large molecules and even viral capsid.[4−6] Nevertheless, the collection area of a typical cryogenic detector is smaller than that of a typical MCP. Therefore, the overall detection efficiency is lower. In general, the cryogenic facility is bulky and the cost is high.

In order to solve the problem of low detection efficiency of large biomolecular ions, a planar electrode to directly measure the induction current of the ions was used. Although there is no charge amplification for the charge detector, the detection efficiency is still higher than that from a typical charge amplification detector for large biomolecular ions due to the very low secondary electron ejection efficiencies for very heavy biomolecular ions (M/Z > 100 000). Charge detectors were first used by Hillenkamp and his co-workers[7] for MALDI- TOF. Later, Imrie et al.[8] extended the mass range to 300 kDa with a poor mass resolution and low detection sensitivity by means of lowering the electronic noise.[9] In this work, we provide a new approach to combine our homemade charge detector with an ion trap to accumulate ions from the MALDI source.[10−13] After the large biomolecular ions are accumulated to enough quantity in the ion trap, the ion signal can be easily detected by the charge detector.

EXPERIMENTAL SECTION

Instrumentation. The experimental schematic is shown in Figure 1. The sample probe was directly inserted into the center hole (3.8 mm in diameter) on the ring electrode of the ion trap. Since all ions are produced inside of the trap, it can provide better trapping efficiency for ions from MALDI due to no ion loss through the transmission.[14] A laser beam (355 nm, Nd: YAG laser, LOTIS TII, LS-2137U) was passed through the opposite hole and was focused onto the sample probe. A charge detector was mounted on one of the end-caps. The back side of the charge detector was sealed up with a piece of copper tape and the front

* To whom correspondences should be addressed. Fax: (882)2-2789-9931. E-mail: winschen@gate.sinica.edu.tw.

† Genomics Research Center, Academia Sinica.

‡ Institute of Physics, Academia Sinica.

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10.1021/ac102217e
Published on Web 11/12/2010

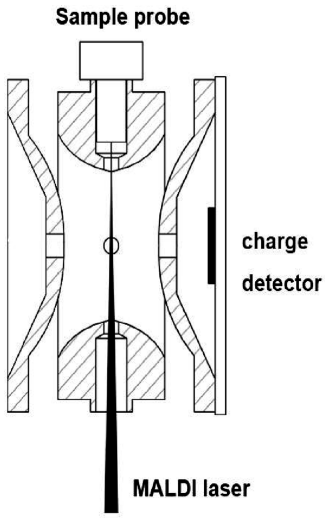

Figure 1. Experimental setup of MALDI ion trap charge detection mass spectrometer.

side was protected with the grids welded on the hole of the end-cap to prevent the radiofrequency (rf) interference.

Nearly all commercial quadrupole ion trap (QIT) mass spectrometers are operated with radiofrequency at about 1 MHz. Lower frequency (1 kHz − 100 kHz) was used for our homemade QIT to trap ions with large m/z. Trapping frequency can be changed to cover the different m/z regions. However, trapping frequencies of most commercial ion trap mass spectrometers are fixed and mass spectra are obtained by scanning the voltage amplitude. For our design, a broadband power amplifier (TREK, PZD2000A) was used to sustain 2000Vp-p RF voltage between 1 kHz and 100 kHz and frequency scan was used to identify the m/z values of the ions. RF voltage was applied to the ring electrode and the two end-caps were both connected to the ground. Our homemade QIT was operated in the mass selective axial instability mode. The ion trap was designed to be able to ramp down the trapping frequency at a constant voltage amplitude.[15]

Helium was directly applied to the inside of the ion tap through a tube, and a pressure gauge was installed to get the real helium pressure inside the ion trap. Bovine serum albumin (BSA, molecular weight at 66 kDa) was tested with 10−80 mtorr of helium pressure inside the ion trap and the rf voltage was kept at 1600 $V_{p\text{-}p}$. Ions were accumulated in ion trap with ten laser shots for all experiments reported in this work.

Phase lock between the laser and the rf trapping voltage was used to choose the polarity of ions from MALDI. It was achieved by controlling the firing time of the laser. The laser beam was fired at the selecting phase angle of the RF voltage. BSA (5 pmol/μL) was used for this test. The phase angles to optimize the trapping efficiency were searched for both positive and negative ions.

BSA was also used to test whether accumulation is helpful in signal enhancement. Each mass spectrum reported in this work was obtained from just one scan without averaging of more than one spectrum. The numbers of accumulation we tested were 2−12 shots.

Sample Preparation. In our experiment, biomolecules from 1 kDa to 1000 kDa such as angiotensin (1296 Da), insulin (5733 Da), cytochrome C (12.4 kDa), BSA (66.4 kDa), Immunoglobulin G (IgG, ~150 kDa), Secretory Immunoglobulin A (sIgA, ~385 kDa), and Immunoglobulin M (IgM, ~980 kDa)[18,19] were measured. Samples with low molecular weight such as angiotensin and insulin were mixed with 2,5-dihydroxybenzoic acid (DHB) as matrix. Sample with medium molecular weight such as cytochrome c, BSA, and IgG mixed with Sinapinic acid (SA) as matrix. Sample with high molecular weight such as sIgA and IgM were also mixed with SA and the molar ratio for sIgA to matrix was 1:200 000, and the molar ratio for IgM to matrix was 1:500 000. All the matrix solution was added 0.1% trifluoroacetic acid (TFA). After mixing with the sample solution, 2 μL sample solution was air-dried on the sample probe.

Charge Detector. A circular metal disk in the center of the detector board was used to act as a Faraday disk to collect the image charge induced by ions. The diameter of the Faraday disk was 1 cm which should be sufficient to cover all the particles that come out of the ion trap. The first stage of the charge detector circuit was charge integrator which included a low-noise JFET and a low-noise operational amplifier. The 1 pF capacitor was used to determine the charge to voltage conversion ratio. Two resistors provided a voltage gain of 4. The discharge time constant was determined by RC value and found as 10 ms. The second stage voltage amplifier provided a gain of 40 and with a simple band-pass filter for filtering out both high frequency and low-frequency noise. In general, the output of the second stage amplifier provided a pulse which is sufficient to drive the analog to digital converter (ADC) located at the outside of the vacuum chamber.

The gain calibration of the charge detector was performed in two different levels. They include (1) the relative gain calibrated with an electronic pulse and (2) the absolute gain with particle of a known mass. Electronic calibration was carried out by applying an electronic pulse of known amplitude and shape to the "test pulse input" connector. The test pulse voltage was attenuated by a factor of 100 with two resistors, then fed to a 1pF capacitor. The other terminal capacitor was virtually grounded to the amplifier input. The test pulse calibration gave the charge conversion gain of about 1 mV/50e. The measured noise of the charge amplifier, 10 mV(rms), then corresponded to about 500e rms noise.[16,17]

Step Frequency Scan. In general, a function generator can be used to sweep down in frequency using linear sweeping mode. Nevertheless, a concern is raised that the change of frequency can occur before the completion of one full cycle. Therefore, the ejected ions can not clearly be attributed to a selected frequency. To solve the concern especially for ions with m/z above 300 kDa, a new approach, step frequency scan, was introduced. For step scan, the frequency profile is defined by the start frequency (F_{start}), the frequency increment (Δf) and the number of increments (N_{inc}) per scan. The step scan between start and end is from F_{start} incrementally increased to $F_{start} + N_{inc} \times \Delta f$. Each

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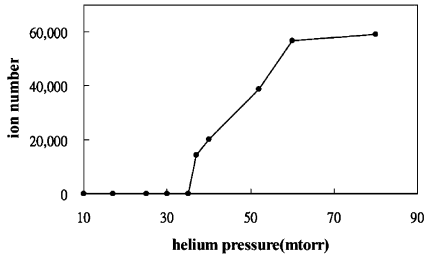

Figure 2. Dependence of the numbers of BSA^+ trapped for the various helium pressure inside the trap.

frequency could be run several cycles and detector will collect and integrate signal at the same time. Complete ion ejection can be assured at specific frequency before it changes to next frequency. The advantage of step scan is the relationship between ion signal and ejection frequency is well-defined. It also can be ramped down the trapping frequency at a constant voltage. It meant frequency changed point by point from high to low frequency instead of changing continuously. The number of ions at every ejecting frequency was measured by the charge detector and the corresponding molecular weight was determined.

RESULTS AND DISCUSSIONS

Collision Cooling. For ions from MALDI to be trapped in the ion trap,[20] the equation below must be satisfied.

$$1/2mv^2 < E_{cooling} + D_z$$

where m is the mass of the ion, v is the velocity of the ions from MALDI, $E_{cooling}$ is the energy from collision cooling, and D_z is the potential well depth of the ion trap.[21] Since the velocity of biomolecular ions from MALDI is almost the same as the velocity of the matrix ions,[22,23] the kinetic energy of biomolecular ions are proportional to the their molecular weights. In other words, larger biomolecular ions are more difficult to be trapped because of larger kinetic energy. Since the tuning range of rf voltage is narrow, increasing the $E_{cooling}$ by increasing cooling gas pressure is a feasible approach. The numbers of BSA ions trapped at various helium pressures are shown in Figure 2. The lowest helium pressure for trapping BSA^+ ion from MALDI was about 37 mtorr, and the number of trapped ions was increased with increasing helium gas pressure.

Phase Lock. The numbers of BSA ions ejected at different phase angles are shown in Figure 3. The best trapping angle of the positive ions is ~90° which is the same as the result published earlier.[12] We give the first report on the best negative ion trapping angle as ~140°. The number of positive ions trapped is about twice of the number of negative ions. If phase lock was not applied, positive and negative ions might neutralize during the trapping process and signals were reduced dramatically.

Accumulation. Accumulation of large biomolecules from MALDI was demonstrated in Figure 4. The BSA^+ signal was too low to be observed when ions were accumulated with the

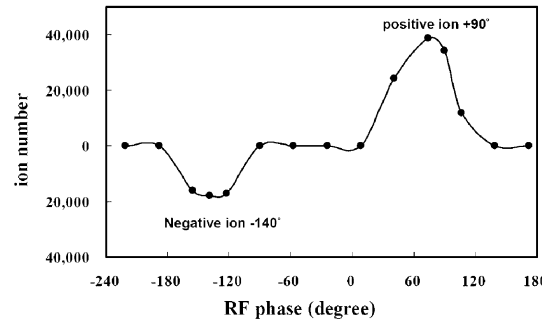

Figure 3. Dependence of the number of BSA^+ on the phase angle of rf at the time of the laser firing.

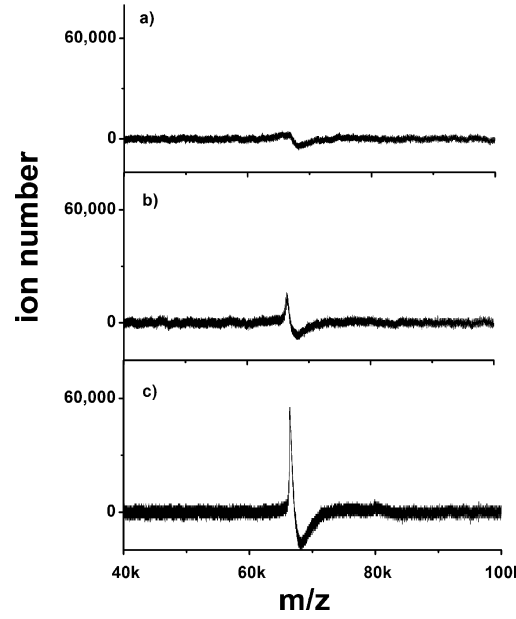

Figure 4. Accumulation of BSA^+ from MALDI. (a) 2 laser shots (b) 4 laser shots (c) 12 laser shots.

accumulation of only two laser shots. The signal started to appear with accumulation of four laser shots. The signal with much better signal-to-noise ratio was obtained with accumulation of 12 laser shots. Accumulation of enough ions before detection improved the detection sensitivity.

With a charge detector, the number of ions can be measured. Therefore the MALDI ionization efficiency can be estimated. When a charge amplification detector is used, quantitative determination of the number of ions produced is nearly impossible since the gains of the charge amplification detectors are difficult to be kept constant. MALDI ionization efficiency of BSA was estimated from Figure 4c. Total amount of sample (10 pmole) was air-dried (d = 1 mm) on the probe. About 1% of BSA sample was depleted with 12 laser shots (laser diameter: 0.1 mm, laser power: 3 μJ, phase: 90°, helium pressure: 60 mtorr) and the number of ions produced was estimated as 60 000 BSA^+ ions. The MALDI ionization efficiency of BSA can be estimated ~10^{-6}.

Mass Range. A broad mass range from 1−1000 KDa has been demonstrated with this frequency scan mass spectrometer. Results are shown in Figure 5. The asymmetry in the peak shapes is from the characteristic of the charge detection. The left side to the pulse height of peak is charge collection time and the other side is discharge time. The discharge time constant of 10 ms is much

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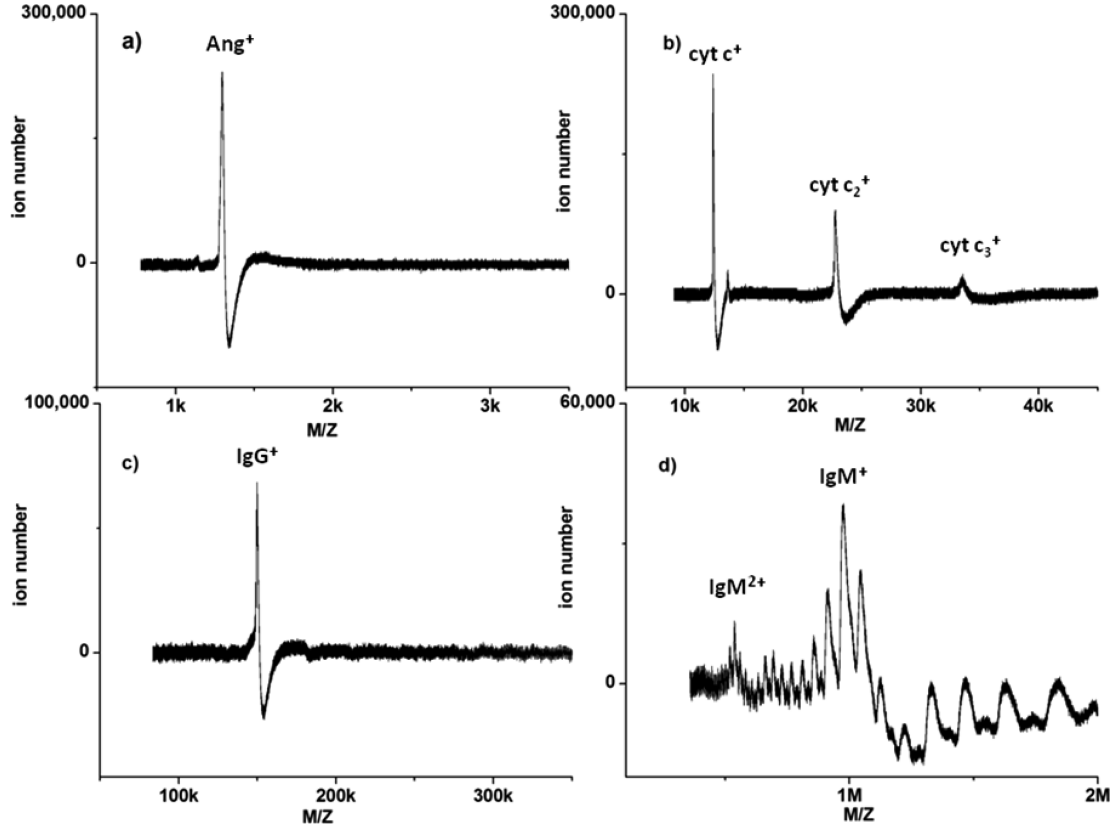

Figure 5. Mass Spectra with *m*/*z* from 1 k to 1000 k. (a) Angiotensin (b) cytochrome C (c) Immunoglobulin G (d) Immunoglobulin M.

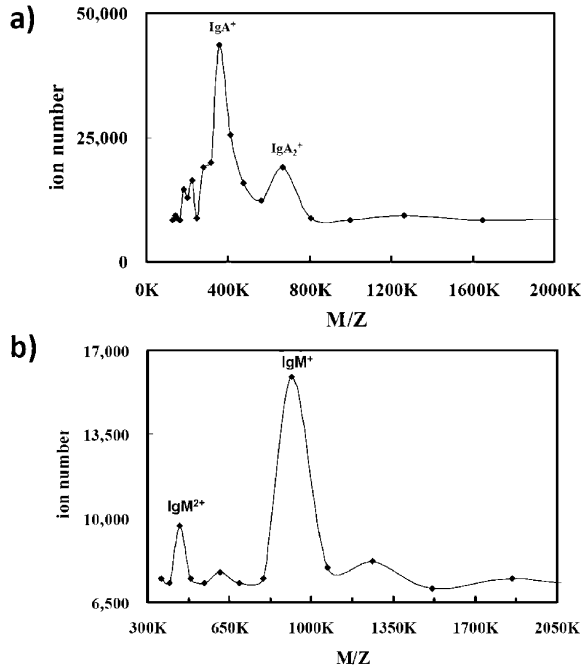

Figure 6. Step frequency scan. (a) In the sIgA experiment, frequency scan is divided into 20 steps from 25 to 5 kHz. (b) In the IgM experiment, frequency is divided into 20 steps from 15 to 1 kHz.

longer than the charge collection time which is about a few hundreds μs. In order to compensate discharge signal, the opposite polarity signal was induced in tail of each peak. Therefore, the asymmetry peak shape was observed in the figures. The m/z value of a commercial ion trap mass spectrometer is typically less than 6000. In this work, we demonstrate m/z can be extended to ~980 000 with collision cooling, phase lock, and ion accumulation.

Step Frequency Scan. When step frequency scan was applied to IgA sample, both IgA^+, and IgA_2^+ were observed (Figure 6a) When IgM sample was tested, IgM^{2+} and IgM^+ were observed (Figure 6b). The mass spectrum of IgM with step frequency scan successfully solved the splitting problem of continuous frequency scan of IgM (Figure 5d).

ACKNOWLEDGMENT

We acknowledge Genomics Research Center, Academia Sinica, the National Science Council of Taiwan and National Health Research Institute of Taiwan for financial support. Valuable discussions with Professor Yuan T. Lee are acknowledged. The help on the submission by K. J. Cherry Lin is also acknowledged.

Received for review August 24, 2010. Accepted October 29, 2010.

AC102217E