Application of a multi-turn time-of-flight mass spectrometer, MULTUM II, to organic compounds ionized by matrix-assisted laser desorption/ionization

Daisuke Okumura,* Michisato Toyoda, Morio Ishihara and Itsuo Katakuse

Department of Physics, Graduate School of Science, Osaka University 1-16 Machikaneyama, Toyonaka, Osaka 560-0043, Japan

Received 6 October 2003; Accepted 16 October 2003

The circuit shape of the ion path, or the multi-turn, provides a solution for achieving unrestricted mass resolution from time-of-flight mass analyzers. The potential of a multi-turn type mass spectrometer, the MULTUM II, with a 1.308 m circuit controlled by four toroidal electric sector fields in biological applications was examined. With matrix-assisted laser desorption/ionization, the ion flight of 18 cycles gave a mass resolution of 10 000 for MH⁺ of protophororphin IX. This resolution was correlated with the flight length, and a resolution of 61 000 was achieved for MH⁺ of angiotensin I after 75 cycles or a 98.75 m total flight. The results demonstrate that the multi-turn mass spectrometer allows not only high resolution but also very high separation of the ions of molecular species from organic compounds. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: multi-turn time-of-flight mass spectrometer; high resolution; liquid matrix; matrix-assisted laser desorption/ionization; organic compounds

INTRODUCTION

Soft laser desorption represented by matrix-assisted laser desorption/ionization (MALDI) is currently a powerful tool for analyzing both synthetic polymers and biopolymers.1,2 Because laser desorption generates pulsed ion signals, simultaneous detection analyzers or time-of-flight (TOF) analyzers are required. The TOF analyzer is not a scanning device and thus permits the transmission of all ions. In addition, it has, in principle, an unlimited mass range. Given these advantages, the combined use of TOF mass spectrometers and MALDI allows the molecular mass measurement of large molecules with good sensitivity.

Mass resolution is affected by the factors that create a distribution in flight times among ions with the same mass-to-charge ratio. These factors include the length of the ion formation pulse (time distribution), the size of the volume in which the ions are formed (space distribution), and variation in the initial kinetic energy of the ions (kinetic energy distribution). In order to minimize the effects of space and time distributions, space focusing and time-lag focusing methods were developed.3 For the same purpose and to improve the time distribution, orthogonal acceleration ion sources were developed.4 The ion mirror was devised to improve the kinetic energy distribution.5 Finally, electric sectors were proposed by Pochenrieder to achieve isochronous focusing and space focusing.6 Obviously, however, the simple and clear way to increase the resolution of TOF analyzers is to elongate the flight tube, because the mass resolution is proportional to the flight time.

With conventional TOF instruments, the length of the flight path is fixed, and the maximum flight path is limited to a few meters for size reasons. As a result, the mass resolution of these mass spectrometers is up to 50 000. An elegant means of overcoming this limitation is to store the ions in a closed orbit and pass them through this same orbit repeatedly. In this way, the pathlength can be extended ad infinitum. Pochenrieder first proposed this type of geometry, but could not find a way to inject and pick off the ions. We have overcome this problem by passing the ion beam through a small hole in each of the outer electrodes of two cylindrical electric sectors, and thereby developed multi-turn TOF mass spectrometers, the MULTUM Linear plus7,8 and the MULTUM II9,10 Both have flight paths within a frame with dimensions of 0.4 m².

In our previous studies, the performances of these instruments were evaluated using the CO−N₂1 doublet with an electron ionization source. The mass resolution increased linearly according to the number of cycles and, in fact, a mass resolution of 350 000 (FWHM) was achieved at m/z 28 after 501.5 cycles.8 In this work, we evaluated the feasibility of our multi-turn instrument for separating biomolecule ions using MALDI.
**Application of a Multi-Turn Time-of-Flight Mass Spectrometer, MULTUM II, to Organic Compounds Ionized by Matrix-Assisted Laser Desorption/Ionization**

**OKUMURA Daisuke and TOYODA Michisato**

(Graduate School of Science)

**INTRODUCTION**

Time-of-flight (TOF) mass spectrometers are relatively simple, inexpensive instruments with high sensitivity and virtually unlimited mass range. There has been great interest in exploiting this type of mass analyzer for the structural analysis of biological macromolecules such as proteins, carbohydrates, and oligonucleotides since the introduction of matrix-assisted laser desorption/ionization (MALDI) [1].

The mass resolution of a TOF mass spectrometer is directly proportional to its total flight path length. If we want to achieve high mass resolution, it will be necessary to maximize the flight. In most TOF instruments, the length of the flight path is fixed, and the parameter is proportional to the size of the instrument whether it be linear or reflectron. Thus, the maximum flight path that can be achieved in a typical laboratory instrument is of the order of a few meters. It is difficult to achieve very high resolution by simply extending the length of the flight tube. In order to circumvent this fundamental limit, it is necessary to place the ions in a closed orbit and to pass the ions around the same orbit many times. In this way, the path length can be easily extended ad infinitum.

If we decide to pursue multi-turn ion optical geometries, there are other issues that also need to be addressed. If the ion beam diverges in both time and space, we will be in a situation where both the mass resolution and the ion transmission (sensitivity) are compromised as the number of cycles around the instrument increases. To avoid this, we need to design an ion optical system that gives "perfect focusing" [2], where the ions return to their point of origin in the system, in both time and space.

We investigated ion optics for a multi-turn TOF mass spectrometer with electrostatic sectors. One of these proposed TOF systems, the “MULTUM Linear plus,” was developed [3]. It consists of four cylindrical electrostatic sectors and 28 electrostatic quadrupole lenses, and has a vacuum chamber 60 cm x 70 cm x 20 cm in size. Mass resolution has been demonstrated to increase according to the number of ion cycles. A mass resolution of 350,000 (mass to charge ratio (m/z) 28, full width at half maximum (FWHM)) [3], which is the highest resolution of a TOF mass spectrometer in the world, was achieved after 501.5 cycles (flight length: 644 m). The TOF spectra of a CO-N₂ doublet are shown in Fig. 1.

The MULTUM Linear plus analyzer is not simple, however, as 28 electrostatic quadrupole lenses are used. In order to reduce the number of ion optical parts, an improved multi-turn TOF mass spectrometer, “MULTUM II,” consisting of only four toroidal electrostatic sectors, was also developed [4]. A mass resolution of 250,000 (m/z 28, FWHM) was achieved with the MULTUM II. In the present study, we evaluated the feasibility of using our multi-turn instrument for separating biomolecule ions using MALDI. Here we describe the improved instrument and the results obtained. The possibility of tandem mass spectrometric applications using multi-turn TOF mass spectrometers is also discussed.
Detector

The combination of a conversion dynode and a dual-stage microchannel plate (MCP) (F4655-10, Hamamatsu Photonics, Hamamatsu, Japan) was used to detect ions. The output signals from the detector were accumulated by a digital oscilloscope (LC564DL, LeCroy Japan, Osaka, Japan) with a sampling rate of 2 GS/s, bandwidth of 1 GHz, and memory of 1 Mpts.
Ion flight control program

A block diagram of the timing control system is shown in Fig. 5. A digital pattern generator (Model 555 Pulse Generator, Berkeley Nucleonics, CA, USA) supplied the timing signals to the ion source, sector electrodes I and IV, and the digital oscilloscope. The ions were extracted from the ion source by applying the pulsed voltage to the sample plate at $T_1$ 1 to 3 µs after laser irradiation ($T_1$); the delay provided space-velocity focusing [7]. The time of flight from time $T_2$ was measured by digital oscilloscope. The voltage at Sector IV was turned off when ions were injected into the multi-turn system. After the ions had been injected into the circuit, the voltage at Sector IV was turned on ($T_3$). Finally, the voltage at Sector I was turned off at $T_4$ to allow the ions to pass into the detector, after the preset number of cycles.

RESULTS AND DISCUSSION

Angiotensin I was measured as a sample. In this case, the resolution of the MH$^+$ ions ($m/z$ 1296.7) after various cycles of flight was examined. As shown in Fig. 6, the peak width was nearly constant in a range of 8.5 to 12.0 ns during cycles 4 through 40, with flight times of 180 µs to 1600 µs, respectively. The resolution increased as the ions passed through the circuit, and finally reached > 60,000 after 30 cycles, or a 40.0 m total flight. It was also noteworthy that the signal intensity, or the abundance of detected ions, was not significantly reduced during cycles 11 through 40. This was consistent with our previous studies [3] carried out under similar conditions including vacuum level, where the signal intensity of CO or N$_2^+$ ions was only minimally diminished after the initial several cycles of flight.

The present study indicates that this type of mass spectrometer is capable of separating the ions of molecular species from organic compounds to an extremely high degree. It is possible to devise a high-performance tandem TOF mass spectrometer by introducing the technology of multi-turn TOF mass spectrometers. A new tandem TOF mass spectrometer for structural analysis of peptides or proteins was designed and constructed in 2004 at the University of Warwick, U.K. [8]. Fig. 7 shows a photograph of the instrument. A multi-turn TOF mass spectrometer having the same dimensions as the MULTUM II was adopted as the first mass spectrometer (MS1), to achieve the high mass resolution necessary for monoisotopic precursor ion selection. A TOF mass spectrometer developed at the University of Warwick with a quadratic-field ion mirror was adopted as the second mass spectrometer (MS2), to achieve time focusing independent of ion energy. The combination of a multi-turn TOF mass spectrometer and a quadratic-field ion mirror constitute a powerful instrument for structural analysis of peptides and proteins.

References