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Accelerated Articles

Electron Capture Dissociation in a Radio Frequency Ion Trap

Takashi Baba,* Yuichiro Hashimoto, Hideki Hasegawa, Atsumu Hirabayashi, and Izumi Waki

Central Research Laboratory, Hitachi, Ltd., Kokubunji, Tokyo 185-8601, Japan

We report on the first evidence of electron capture dissociation (ECD) in a radio frequency (rf) ion trap. Peptide ions, [substance P]$^{2+}$, trapped in a two-dimensional, linear rf ion trap were cleaved by electrons injected along the central axis of the trap. Along the axis, the rf field component was zero and a magnetic field of 50 mT was applied. This electron injection scheme keeps the energy of the electrons below 1 eV, preventing them from heating by the rf field. The present ECD efficiency is ∼4% by irradiation of electron current of 0.2 μA for 80 ms. ECD in rf traps may open high-throughput and low-cost ECD applications to obtain molecular structure information complementary to collision-induced dissociation.

Electron capture dissociation (ECD) is a powerful tool for structure analysis of biomolecules, such as peptides, proteins, and their posttranslationally modified products,[1−3] because it allows adiabatic, nonergodic fragmentation at c sites and z sites of peptides. It is complementary to collision-induced dissociation and infrared multiphoton dissociation, which contain adiabatic b site and y site fragmentation. Because ECD reaction cross sections are small (typically 10^{-15} m^2) and they have the maximum at low electron kinetic energy of sub 1 eV[3], Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry has been the only technique that has enabled practical ECD to date. Long interaction time and low-energy electrons are achieved by FT-ICR because it traps precursor ions by a static electromagnetic field so that heating of electrons by time-varying electromagnetic field is avoided.[1]

ECD in radio frequency (rf) traps, which are small systems without superconducting magnets, has been a challenging target in order to develop low cost and easily operated ECD devices.[1,4−6] Vachet et al. reported that ECD was not observed when electrons were injected into a three-dimensional radio frequency-quadrupole (RFQ) ion trap, or Paul trap, in 1995, because the electrons were energized, or heated, by the rf field.[4] Recently, Ivonin and Zubarev showed, by computer simulation,[5] the feasibility of ECD using a Paul trap with a weak magnetic field. Baba et al. proposed and tested an ECD device that used a three-dimensional static electromagnetic trap (Penning trap) without success.[6] This paper reports the first evidence of ECD in an rf ion trap, which was enabled by a newly designed rf-ECD cell that avoids heating of electrons.

Concept of rf-ECD Cell. Our ECD mass spectrometer shown in Figure 1 contains the following two items; (1) an ECD reaction device, i.e., an ECD cell composed of a linear rf ion trap combined with a magnetic field, and (2) a mass analyzer separated from the ECD cell.

A linear rf ion trap in the ECD cell for ion confinement consists of a two-dimensional radio frequency electric field in the radial direction, or *x* and *y* direction, and a static electric field in the axial direction, or *z* direction. To avoid perturbation by the ion trap rf field, or rf heating, electrons are injected along the *z* axis, in which direction the rf field is zero. In addition, a magnetic field is applied in the trap parallel to the *z* axis of the linear rf trap to confine electrons along the *z* axis. The magnetic field of ∼0.1 T is used to obtain high transmission efficiency of electrons and to avoid the heating of electrons by the ion trap rf field. The rf

* To whom correspondence should be addressed. E-mail: baba@harl.hitachi.co.jp. Telephone: +81-42-323-1111 ext 3911. Fax: +81-42-327-7807.

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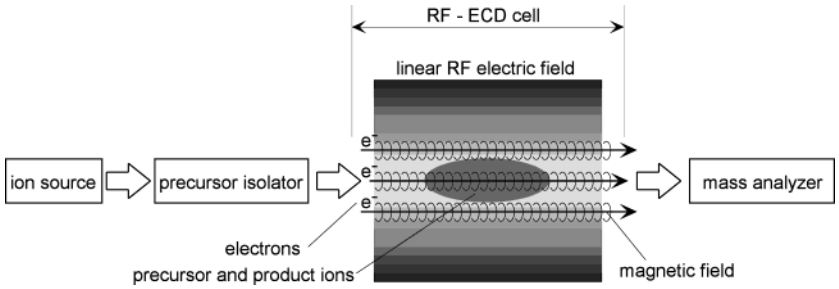

Figure 1. Schematic of rf-ECD mass spectrometer. Isolated precursor ions generated by the ion source are injected into the rf-ECD cell composed of a linear rf field and a magnetic field. Low-energy electrons are injected along the center axis of the linear rf electric field and magnetic field to avoid rf heating. Product ions are mass analyzed by a mass spectrometer.

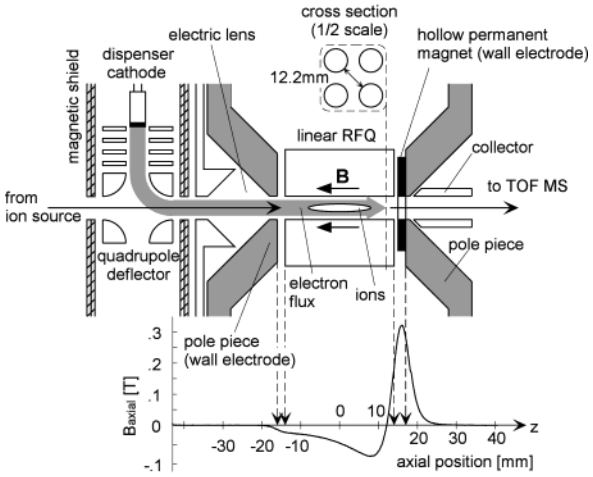

Figure 2. ECD reaction cell with a linear RFQ ion trap and an electron source. Magnetic field of 0.05 T is generated by a hollow permanent magnet attached on a wall electrode. Return yoke for magnetic field confinement outside the reaction cell is not shown in the figure. Inset graph shows axial magnetic field density along the central axis of the reaction cell. $Z = 0$ represents the center of the linear RFQ ion trap electrode in axial direction.

trapping stability condition of ions is modified a little but not critically by the weak magnetic field when mass-to-charge ratios are over 100 Da.

EXPERIMENTAL SECTION

Figure 2 shows details of the RFQ-ECD cell. The linear RFQ ion trap consists of a quadrupole rod set with a trap volume diameter of 12 mm and a length of 27 mm. We applied an rf voltage of 150 V_{pp} (peak-to-peak) and 1.00 MHz to the quadrupole rods. Two wall electrodes for the axial confinement were placed beside the quadrupole rods. These electrodes are made of magnetic iron and also work as pole pieces of a magnetic circuit. To generate the magnetic field along the axial direction, a hollow permanent magnet disk (neodymium−iron−boron magnet, Neomax Co., Ltd.) was attached on the outlet wall electrode of the ECD cell.

Electrons were produced by a commercial dispenser cathode (Heat Wave Labs). For efficient operation of the dispenser cathode, high vacuum quality is essential because cathode activation is sensitive to residual gas components such as H_2O and O_2, where their critical partial pressures are 10^{-4} Pa.[7] Since our ECD cell was placed in a vacuum chamber with O_2 partial pressure of 2×10^{-4} Pa when the aperture of the ESI source was opened, the heater current was kept at 1100 °C. Typical electron current passing through the ECD cell was 0.2 μA, whereas typical electron currents in FT-ICR ECD experiments are 1nA−1 μA[2]

Thermal electrons emitted from the dispenser cathode were injected into a quadrupole deflector after focusing by a set of hollow electrodes as an electric lens. The dispenser cathode, the electric lens, and the quadrupole deflector were placed in a magnetic shield housing to avoid a residual magnetic field from the ECD magnetic circuit. Before injecting the electrons into the ECD cell, they are focused by an electric lens placed between the shield housing and the ECD cell. A bias voltage of the ion trap controlled the electron kinetic energy at the center of the ion trap. The ECD cell was filled with helium gas of 2×10^{-3} Pa.

Axial magnetic field density along the central axis is shown in the inset figure. This asymmetric magnetic field configuration decreased a residual magnetic field along the electron inlet trajectory into the ECD cell with a high transmission efficiency of electrons in the quadrupole deflector. The overall electron transmission efficiency passing through the ECD cell was 5% of the dispenser cathode emission.

The precursor ion for this experiment is doubly charged substance P (MW 1347.6). The ions were produced by infused electrospray ionization of 10 μM substance P (by Sigma-Aldrich) in 100% methanol solution. The ions were introduced into a linear RFQ ion guide in order to isolate the precursor ions from other ions. We rejected ions whose mass-to-charge ratios were lower than 660 Da by applying high rf amplitude to the linear RFQ ion guide. The precursor ions were injected into the ECD cell and trapped. After electrons were injected through the ECD cell as a reaction period, product ions were ejected from the outlet port of the cell and injected into a TOF mass spectrometer to obtain mass spectra of the product ions.

EXPERIMENT AND RESULTS

We measured the kinetic energy distribution of electrons passing through the ECD cell because it is the most essential parameter for ECD achievement in RFQ, as shown in Figure 3. The electron current passing through the ECD cell was measured at a collector electrode that was placed between the ECD cell and

(7) Operation tips of dispenser cathodes are available on the Web cite of Heat Wave Labs.

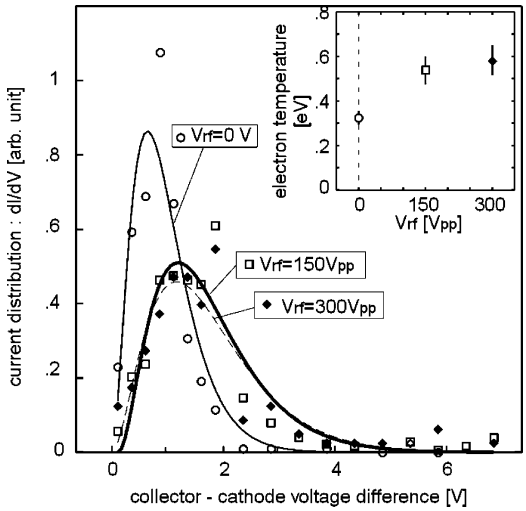

Figure 3. Electron kinetic energy distribution by measuring electron current at the collector electrode. V_{rf} shows the ion trap rf voltage amplitude. Each line shows a fitting of Maxwell distribution. Inset shows best-fit electron temperature vs rf amplitude. Electron temperature elevated by 0.2 eV when the rf field of V_{rf} = 150 V was applied.

the TOF mass spectrometer in Figure 2. The electron current on the outlet wall electrode of the ECD cell was less than 0.1% of the collector current. This means adequate electron confinement along the central axis was successfully performed by the magnetic field. Measured collector electron current differentiated by the collector voltage represents a thermal Maxwell distribution, where the width of the distribution was related to the temperature of the electrons. The electron temperature shown in the inset of Figure 3 was obtained by fitting of Maxwell distribution to the data. The electron temperature was raised by 0.2 eV when we applied the rf voltage (V_{rf}) of 150 V_{pp} to the quadrupole rods, which was a condition during the ECD period. The rf voltage had no obvious effect on the total intensity of electron current passing through the ECD cell.

Using this ECD cell, we successfully cleaved substance P^{2+} and obtained c site and z site fragment spectrum as shown in Figure 4a. First, ions isolated by the linear RFQ ion guide were transferred to the ECD cell through the quadrupole deflector by setting the quadrupole dc voltage to introduce ions straight into the ECD cell. Then, electrons were injected into the ECD cell by setting the quadrupole deflector voltage to deflect electrons toward the ECD cell. The dc bias voltage of the ECD cell was set 1.7 V above the cathode potential so that the electron kinetic energy is ~1 eV, for which the ECD cross section is the maximum. The electron current was 0.2 μA, and the duration of the electron injection in an ECD sequence was 80 ms. The spectrum in the figure is a summation of 6000 ECD sequences for 10 min. c_4^+, c_5^+, c_6^+, c_7^+, and z_3^+ fragments were observed clearly. In addition, b_7^+ and y_3^+ fragments were also observed. When the trap bias was set to −2.3 V lower than the cathode potential, these peaks were not observed because electrons did not reach the center of the ECD cell, as shown in Figure 4b. Other peaks of $m/z = 680-750$ were nonisolated residual ions from the ion source.

The relative intensity of the c_4^+, c_5^+, c_6^+, and c_7^+ fragments depended on the electron kinetic energy as shown in Figure 5. The relative fragment intensity was defined as a ratio of a fragment

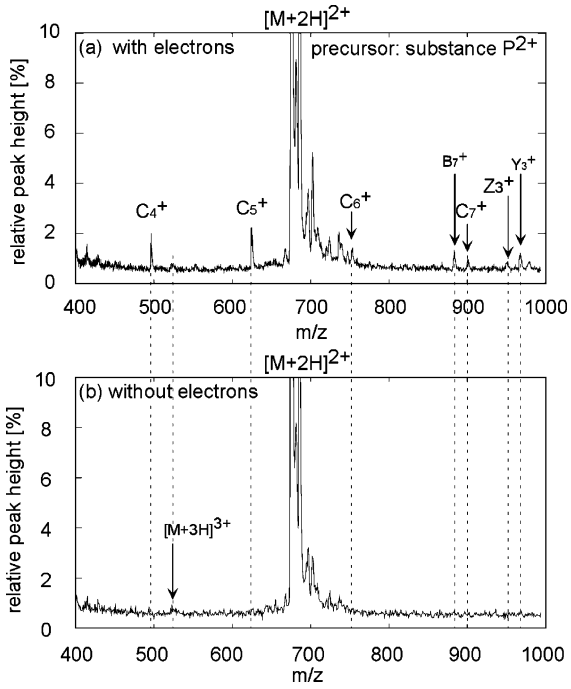

Figure 4. (a) ECD fragment spectrum of substance P^{2+} (shown by $[M + 2H]^{2+}$) in the RF-ECD cell. The trap bias was set 1.7 V above the cathode potential. (b) Spectrum without electron current at the center of ECD cell. Electrons did not reach the trap center because trap bias was set 2.3 V lower than the cathode voltage.

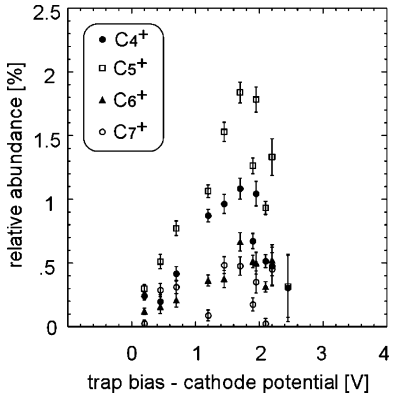

Figure 5. c_4^+, c_5^+, c_6^+, and c_7^+ fragment abundance vs trap bias.

intensity to the precursor intensity, where the peak intensity is the area of each peak subtracted by background noise. Error bars show Poissonian statistical errors. The sharp dependence on the electron kinetic energy is consistent with conventional ECD in FT-ICR,[2]

Observed fragments and their kinetic energy dependence are strong evidence of a successful ECD reaction in an RFQ system. The present dissociation efficiency is 4% for a total of c_4^+, c_5^+, c_6^+, and c_7^+ fragments, i.e., 0.05%/ms, which is comparable to a typical FT-ICR case, by applying electron current of 0.2 μA.

DISCUSSION

The ECD in our rf trap seems to proceed in largely the same manner as in FT-ICR, since the ECD fragment pattern shown in Figure 4a is similar to an ECD fragmentation obtained by FT-ICR[2]. In addition, b and y fragments were observed. A mechanism for y and b fragment generation should be studied further, such as contamination by hot electron capture dissociation.[8]

An advantage of this RF-ECD system is that high-throughput ECD is feasible for proteomics application, because the data acquisition speed of the TOF mass spectrometer (typically 100 μs.) is much faster than that of FT-ICR (typically 0.1−1 s). Other instrumental advantage over FT-ICR is fabrication of a low-cost and compact ECD device, which replaces the superconducting magnet with inexpensive permanent magnets and eliminates the ultrahigh vacuum system.

To enable a faster reaction, we plan to optimize the electron transport system because we currently lose 95% of emitted electrons from the dispenser cathode. This optimization also should save the dispenser cathode lifetime by lowering the dispenser temperature.

Present transmission efficiency of the ions from the ion source into the ECD cell was poor (0.25%) during the ECD experiment. Since the trapping efficiency of the ECD cell is ∼40% when the dispenser cathode was turned off, we estimated that this was caused by electrical charge-up of the electrodes and that the transmission efficiency can be improved by optimizing the quadrupole deflector optics.

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CONCLUSION

We demonstrated electron capture dissociation in a radio frequency ion trap. A linear radio frequency-quadrupole with a weak magnetic field along the central axis allows for the introduction of low-energy electrons when the electron is injected along the axis. The present reaction rate is 0.05%/ms by irradiation of 0.2 μA electrons, which is comparable to the FT-ICR ECD system.

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