Tandem Mass Spectrometry Using an Axially Resonant Excitation Linear Ion Trap

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We performed collision-induced dissociation (CID) and electron capture dissociation (ECD) analyses using a new ion trap: an axially resonant excitation linear ion trap (AREX LIT). Unlike a conventional linear ion trap, the AREX LIT can trap and detect low m/z fragment ions, such as immonium ions and iTRAQ^{TM} reporter ions, that are produced by CID. This capability to detect low m/z fragments is realized by CID excitation in a nearly harmonic direct current potential along the linear axis. In contrast, CID excitation in a conventional linear ion trap is in a pseudo-harmonic radio frequency potential that destabilizes fragment ions whose m/z is less than 1/4 that of the precursor ions. ECD was achieved in an axial magnetic field of about 0.2 Tesla superimposed along the AREX LIT. We observed that sequential ECD/CID inside the trap improved the sequence coverage of a peptide.

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1. Introduction

Tandem mass spectrometry (MS/MS) using a two-dimensional quadrupole ion trap (or linear ion trap, LIT) is a powerful tool for analyzing proteins,[1] peptides,[2] carbohydrates,[3] metabolites,[4] and other compounds. LITs are widely used as mass selective ejection devices in low-resolution mass spectrometers[5)–7)] or as fragmentation and precursor selection devices in higher-resolution hybrid mass spectrometers.[8)–10)] However, the conventional LIT has difficulties as a fragmentation device; it has low m/z fragment loss and an isolation shift caused by magnetic field perturbations as described below.

Collision-induced dissociation (CID) is the most common dissociation method for MS/MS analysis of LITs,[2)–6), 8), 9)] because it is inexpensive and has a simple configuration. However, the CID inside the conventional LIT that is performed by resonant excitation cannot detect low m/z ions, because the detectable mass range and the dissociation efficiency are in a "trade-off," as described below. Effective dissociation requires a high potential where ions are oscillated and activated by collisions with the bath gas.[11] The minimum m/z of product ions, M_{min}, that can be stabilized inside quadrupole ion trap (QIT), which includes LIT, is

$$M_{min} = \frac{q}{q_{ej}} M_p , \qquad (1)$$

where M_p is the m/z of the precursor ion, q is the stability parameter for the precursor ion, and q_{ej} is a constant value of 0.908. Because the oscillation potential of a QIT, which corresponds to the pseudo potential formed by the trapping radio frequency (RF) field, is proportional to the square of the stability parameter q for the precursor ion, dissociation usually requires a high q of over 0.20–0.25.[9] For $q = 0.23$, Eq. (1) becomes

$$M_{min} = 0.25 M_p . \qquad (2)$$

Therefore, product ions, whose m/z is less than about 1/4 that of the parent ion, are neither trapped nor detected. To overcome this problem, two CID methods, high amplitude short time excitation (HASTE)[12] and pulsed q collision-induced dissociation (PQD),[13] were reported. In HASTE, after precursor ions are excited at a high q of 0.25 for a short duration of 1–2 ms, q is decreased to 0.10 by decreasing the trapping RF voltage over a short period of 100 μs. Low m/z product ions, which are produced after the q decrease, can be trapped inside the trap. PQD obtains a relatively high dissociation efficiency of over 60% for some ion species. However, because the optimum excitation strength and duration are dependent on the lifetimes of the ion species after activation, low dissociation efficiencies of less than 20% have been also reported for some species.[13]

Electron capture dissociation (ECD) in an LIT was also achieved by perturbation of an axial magnetic field of 50–200 mTesla.[14)–16)] The axial magnetic field affects the ion's motion as,

$$\boldsymbol{F} = e\boldsymbol{v} \times \boldsymbol{B} \qquad (3)$$

where the magnetic field

$$B \approx \begin{pmatrix} 0 \\ 0 \\ B_z(x, y, z) \end{pmatrix}$$

is variable in space. Because the precursor ion selection and CID of the conventional LIT increase the

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radial velocity (*x, y*-directions), axial magnetic field will cause mass shifts. We estimated that the mass shift caused by an axial magnetic field of 200 mTesla is 10 Th for an m/z of 1,000 at $q=0.10$.[16] Because the magnetic field made by a cylindrical magnet is not uniform inside the LIT; mass shifts may vary in space, which is not favorable for mass spectrometric performance.

Our previously reported axially resonant excitation linear ion trap (AREX LIT) is capable of high efficiency mass selective ejection (over 60%) from an LIT.[7] The LIT has a set of electrodes between the quadrupole rods that make the direct current (DC) potential approximately harmonic along the central z-axis. Inside the DC quasi-harmonic potential, ions with a specific m/z range can be resonantly oscillated and ejected in the axial direction by applying a supplemental alternating current (AC) field. In this paper, we report on solving the above two problems affecting CID and ECD in RF ion traps, by using this new trap.

2. Experimental

Samples were ionized using a gas-assisted electrospray ion source (ESI source) at a sample flow rate of 5 μL/min. Ions were introduced from the ESI source through high-pressure multipole rods into a first linear ion trap (hereafter pre-LIT). Ions that were mass-selected in the pre-trap are put into the AREX LIT.[9] The details of the AREX LIT experimental setup (Fig. 1) were described in previous reports.[7] The differences between the devices of the previous reports and this one are the addition of a cylindrical magnet and an electron source in front of the inlet lens. The vane potential (D_0), which is a DC potential of the vane electrodes relative to the quadrupole rods' DC offset, makes a potential approximately harmonic along the central axis of the quadrupole field. A supplemental AC voltage ($<\pm 20.0$ V (0-peak), 5–100 kHz) applied between the front and rear vanes oscillates ions with a specific m/z in the axial direction, which enables precursor ion selection and CID. The resonant frequency in the axial direction, f, is

$$f=\frac{1}{2\pi}\sqrt{\frac{2neA_2D_0}{mL^2}} \qquad (4)$$

where e is the elementary charge, A_2 is a coefficient of 0.833, D_0 is vane DC potential, m is ion mass, and L is vane length. The cylindrical magnet forms a magnetic field of about 200 mTesla along the quadrupole axis. The electron source in front of the inlet lens introduces electrons with kinetic energies of 0–3 electron volts; the kinetic energy and the electron current are controlled by the potential difference between the electron source and the linear trap offset.

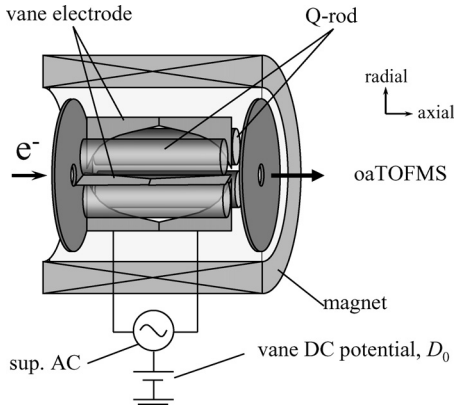

Fig. 1. Structure of AREX LIT with an ECD magnet.

The MS/MS analysis measurement sequence (ECD followed by precursor selection and CID) is depicted in Fig. 2. ECD and precursor ion selection processes are omitted for CID. The measurement sequence consists of accumulation, ECD, precursor ion selection, CID, and ejection. During the accumulation period, ions that were mass-selected in the pre-trap are put into the AREX LIT, where the vane electrodes make an axial trapping field and quadrupole rods make a radial trapping field. During the ECD period, the vane potential (D_0) of 1–5 V is applied, so that ions are weakly focused near a central point O. During the precursor ion selection and CID periods, the vane potential rises to 20–40 V, and the supplemental AC voltage is applied. Oscillated ions are ejected or collisionally activated with mass selectivity. Helium is used as a 5 mTorr bath gas in the AREX LIT; the base pressure of the chamber is about 3×10^{-5} Torr. After the MS/MS analysis, ions are detected by using an orthogonal acceleration time-of-flight mass spectrometer (oaTOFMS) with a mass resolving power of over 5,000.

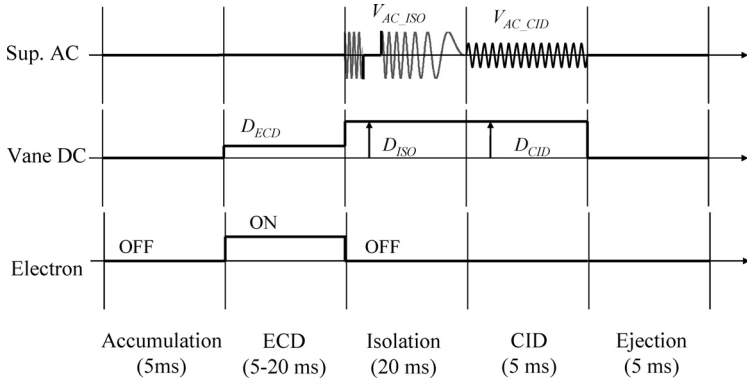

Fig. 2. Measurement sequence for ECD followed by CID.

3. Results and Discussion

3.1 CID experiment

Figure 3 shows the CID efficiencies of AREX LIT and conventional LIT *versus* the stability parameter q. CID efficiency is defined by,

$$E_{\mathrm{CID}} = \frac{\sum \mathrm{Fragment}}{\mathrm{Precursor}}. \qquad (5)$$

where Precursor means the intensity of precursor ions before CID and $\sum$Fragment means the sum of the intensities of fragment ions after CID. Because the detection efficiency of lower m/z range is higher than that of higher m/z range in the oaTOFMS, CID efficiency became over 1.0. As the basis for comparison in the experiment, we used a conventional LIT/oaTOFMS mass spectrometer, whose configuration and measurement sequence were described previously.[9] This result supports the conclusion that dissociation of the conventional LIT only proceeded with a high q of over 0.20, because the oscillation potential was not sufficient for dissociation with a lower q. As described in the introduction, the fragment ions whose m/z is less than about 1/4 that of the precursor ions will be neither trapped nor detected by the conventional LIT. On the other hand, effective dissociation occurred with low q values of less than 0.10 in the AREX LIT, because the oscillation potential formed by the vane DC potential did not affect the trap's stability.

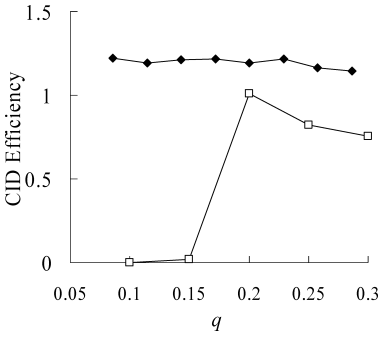

Fig. 3. CID efficiencies *versus* q for AREX LIT (◆) and a conventional LIT (□). Sample: reserpine (m/z 609.3), D_0: 40 V, CID period: 5 ms.

Figure 4 shows CID results for a peptide that is labeled with iTRAQ™ reagent.[17] In the AREX LIT, low m/z fragment ions such as iTRAQ™ reporter ions (m/z 114.1 and m/z 117.1) and iTRAQ™ intact ions (m/z 145.1) were detected as strong peaks in the spectra. This CID spectrum is similar with the other CID spectra of iTRAQ™-labeled sample that were obtained by quadrupole time-of-flight mass spectrometer (QqTOFMS) or tandem time-of-flight mass spectrometer (TOF/TOF).[18] On the other hand, we chose $q=0.23$ for the effective CID in the conventional LIT experiment, because CID efficiency decreases with lower q, as shown in Fig. 3. Equation (2) indicates that no fragment peaks with m/z of less than 130 would be observed. No iTRAQ™ reporter ions (m/z 114.1 and m/z 117.1) were observed in Fig. 4(B), which is consistent with the above discussion.

3.2 Sequential ECD/CID

Figure 5 shows the mass spectra gotten by ECD (A) and sequential ECD/CID (B). The ECD target was

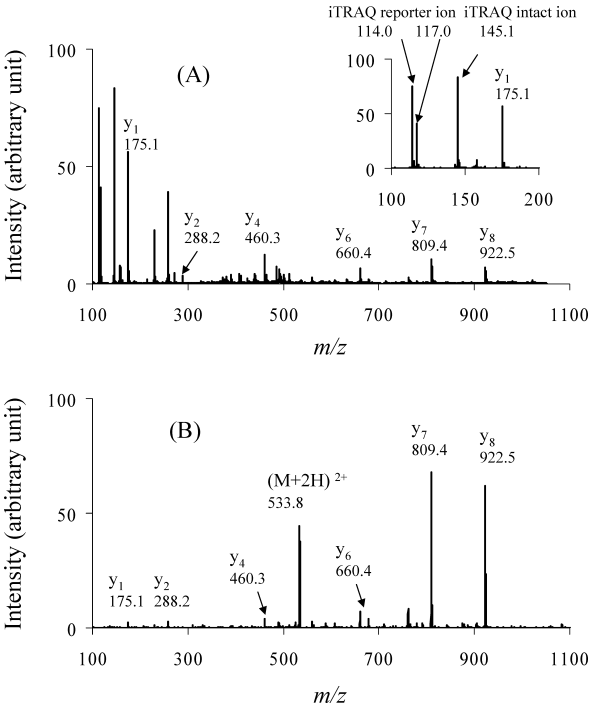

Fig. 4. CID mass spectra by AREX LIT (A) and conventional LIT (B). Sample: iTRAQ™-LCTVATLR, m/z 533.8, D_0: 40 V, CID period: 5 ms, $q=0.10$ (AREX LIT), $q=0.23$ (conventional CID).

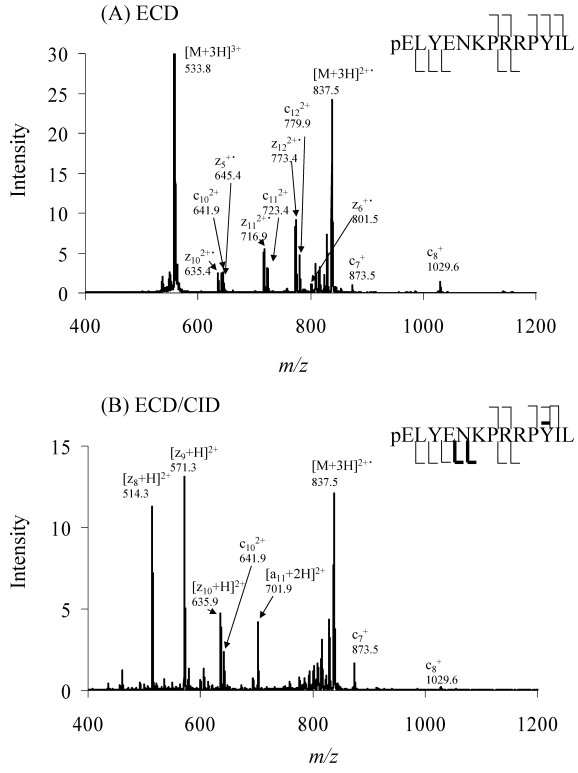

Fig. 5. Mass spectra by ECD (A) and sequential ECD/CID (B). Sample: neurotensin, m/z 553.8, ECD period: 15 ms, CID period: 5 ms.

triply-charged ions of $[M+3H]^{3+}$. Dissociation sites identified by ECD and sequential ECD/CID are depicted in the upper right. Not only fragment ions but also charge-reduced radical ions of $[M+3H]^{2+\cdot}$ were observed in the ECD spectra. After ECD, we selected the charge-reduced radical ions of $[M+3H]^{2+\cdot}$ as the precursor ions of the following CID. By axial resonant excitation, the charge-reduced ions were collisionally dissociated into *c*, *z* fragment ions that are characteristic of ECD/CID fragments. Sequential ECD/CID improved the sequence coverage of the peptide compared with ECD alone (bold-line cleavages in Fig. 5(B) are dissociation sites only observed by sequential ECD/CID). In the AREX LIT, ions can be oscillated mass-selectively by a resonant excitation within the axial DC harmonic potential. The ion motions during isolation and CID periods are little affected by the magnetic field (Eq. (3)), because both the direction of the magnetic field and the ion velocity are axial.

4. Conclusion

We performed ECD/CID in a linear ion trap, called an axially resonant excitation linear ion trap (AREX LIT), with an axial magnetic field. AREX LIT can detect low *m/z* fragment ions that cannot be mass analyzed with conventional linear ion traps. Precursor ion selection and CID can be achieved by axially resonant excitation because the ion's axial motions are not affected by the axial magnetic field. These superior properties of the AREX LIT will help to improve proteome analyses.

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