Multi-stage mass spectrometry in an electron activated dissociation device

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ABSTRACT

Multi-stage tandem mass spectrometry, or MS^n, was installed in a dissociation device induced by electron irradiation, including electron capture dissociation (ECD) and electron impact excitation of ions from organics (EIEIO) as well collision induced dissociation (CID). For product isolation purpose, linear radio-frequency quadrupole (RFQ) portion is used with quadrupole DC voltage, or resolving DC voltage. An abnormal stability region out of the standard linear RFQ stability region was observed, which caused interference between the linear RFQ and the central octapole portion. Using this device, sialic acid linkage analysis in N glycans in glycopeptides was demonstrated.

INTRODUCTION

Electron activated dissociation (EAD), which is including electron capture dissociation (ECD), hot ECD and electron impact excitation of ions from organics (EIEIO) and electron induced dissociation (EID) shows various potential to inform detailed molecular structures in mass spectrometry, which may not be obtained by conventional collision induced dissociation (CID). The applications of these electron-based dissociation techniques range from conventional peptide and protein analysis to singly charged metabolites including small molecules, (ref. 1), various classes of complex lipids (ref. 2), and fatty acids (ref. 3). We had previously reported ECD devices in a branched RF ion trap configuration since 2015 (ref. 4), which is installed between Q1 and Q2 in a research grade quadrupole-TOF MS system (Fig. 1).

Multiple tandem mass analysis: MS^n is often applied to molecular ions to analyze the detailed molecular structures that are not allowed by single application from various dissociation methods. By modification of the ECD device, we have enabled an ion isolation functionality which allows MS^n workflows. With access to MS^n workflows, multiple iterations of fragmentation can be applied to a single sample.

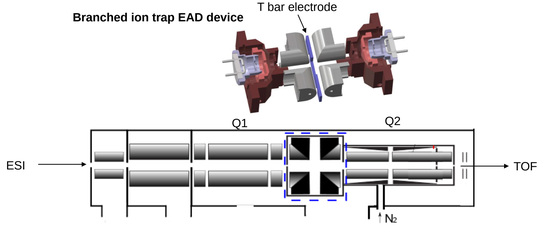

Figure 1. An advanced research grade EAD-enabled QTOF mass spectrometer, based on X500B system (Sciex). The EAD device is inserted between Q1 filter and Q2-CID cell.

Method

The ECD device has a geometry of a quadrupole in the four branches, which was used for isolation in this work. The ECD RF was operated at a frequency of 600 kHz. The power supply was modified to enable the application of resolving DC to the branched trap electrodes (quadrupolar voltage to the branches). The device was installed in a research-grade quadrupole Time-of-Flight mass spectrometer. The egg yolk glycopeptide was extracted in-house. For the standard of the egg yolk glycopeptide with α(2,6) sialic acid linkage was purchased from QA bio. Glycopeptides in tryptic digest of bovine fetuin was used as the α(2,3) sialic acid linked standard after LC separation. T bar bias is used to avoid ion loss to the top/bottom direction in regular operation, but high T bar bias (typically +20V to the trap electrodes) was applied to push the trapped ions toward the electron beam branches.

RESULTS: Isolation of ions in the branched EAD device

Conventionally, quadrupolar field is used for isolation in ion traps in both the resonant excitation and the Mathieu instability approaches. Our strategy in this work is to use the apex of the stability diagram. But, our trap is not a pure quadrupole ion trap, which has four branches and it has an octapole field at the trap center. The stability diagram was obtained when reserpine ions (m/Z = +609) were trapped at the center of the device (Figure 2(a)) (T bar bias = +5V relative to the trap electrodes). The obtained stability diagram was distorted from the standard linear quadrupole case, and the apex was not clear.

When the ions were pushed toward the more quadrupolar portion in the electron beam branches by applying high T bar voltage (+20V), the stability diagram became similar to the standard quadrupolar stability diagram (Figure 2(b)). We used the apex for isolation by applying high T bar voltage. The resolution of isolation was m/Δm ~20.

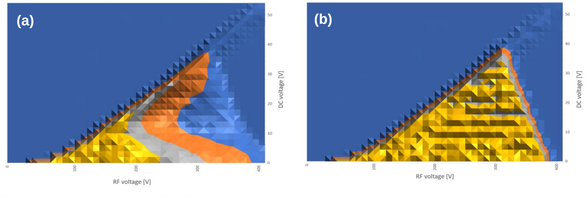

Figure 2. Experimental stability diagram in the EAD device. (a) In the case that ions are trapped at the center (T bar bias = 5V). (b) In the case that ions are trapped in the electron beam branches (T bar bias = +20V).

Interestingly, the stability diagrams have an "appendix" from the apex, which is the a0 line in Mathieu equation theory. This means that the excitation of ions outside of b1 line is not strong enough to eject ions. Ion motion simulation by SIMION reproduced this abnormal stability (Fig. 3). The simulation tells that dodecapole field is overlapped to the quadrupolar field and the contamination of the higher polar field creates new potential minima near the EAD electrode (Fig. 3(b)).

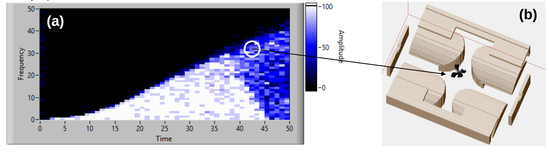

Figure 3. (a) Stability diagram in the EAD device simulated by SIMION. (b) A contamination of the dodecapole field pushes the ions toward the electrode from the trap center, which reduces the excitation of ions by the trap RF field.

RESULTS: Study of sialic acid linkage in glycopeptides by CID → EIEIO mass spectrometry

Determination of sialic acid linkage in glycopeptides were demonstrated in this work using MS^3 (CID → EIEIO) (Fig.4 and 5). The antenna portions with three sugars in N glycans including a sialic acid were detached from isolated glycopeptides by CID (Fig5(c)), where CID was induced by the kinetic energy when the isolated precursor ions were introduced into the EAD device. The tri-sugars were isolated in the EAD device by applying high T bar bias and resolving quadrupolar DC voltage (Fig.5(d)). Electron beam with a kinetic energy of 10 eV (i.e., EIEIO) was applied to the isolated singly charged species to induce cross ring cleavage to distinguish sialic acid linkage between α(2,3) or α(2,6) (Fig5(e)).

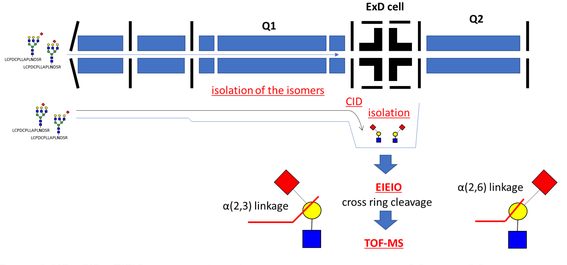

Figure 4. MS^3 (CID→EIEIO) workflow to distinguish sialic acid linkage: α(2,3) and α(2,6)

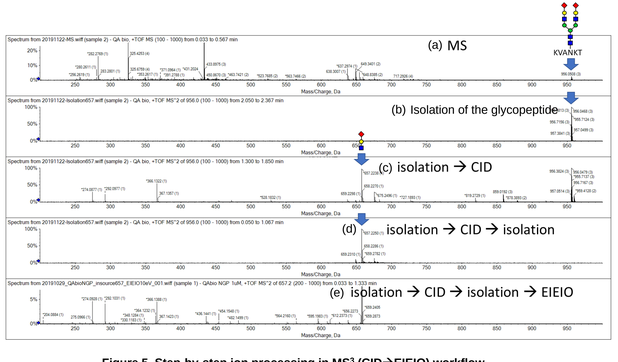

Figure 5. Step-by-step ion processing in MS^3 (CID→EIEIO) workflow

Figure 6 (a) and (b) show the EIEIO spectra of different sialic acid linkages using standard samples. Significant difference were observed by the different dissociation patterns shown in Fig.6(d) and (e). A natural glycopeptide extracted from egg yolk shown the diagnostic peak of α(2,6) linkage as well as similar intensity profiles of other peaks related to the dissociation of the tri-sugar.

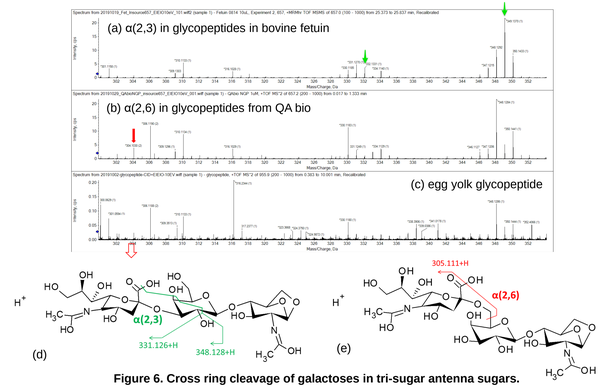

Figure 6. Cross ring cleavage of galactoses in tri-sugar antenna sugars.

CONCLUSIONS

By applying a high DC voltage bias to T bar electrodes, ion isolation using the apex of the quadrupolar stability diagram can be implemented. Using this isolation functionality, MS^n including electron activation dissociation is demonstrated.

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TRADEMARKS/LICENSING