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Electron-capture dissociation (ECD), collision-induced dissociation (CID) and ECD/CID in a linear radio-frequency-free magnetic cell

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Almost immediately after the advent of electron-capture dissociation (ECD), it was recognized that complementary fragmentation information (i.e. 'golden complementary pairs') obtained from tandem mass spectrometric (MS/MS) analysis of a given sample of peptides via both ECD and collision-induced dissociation (CID) could substantially increase the accuracy of peptide and, ultimately, protein identifications;¹ consequently, several groups began seeking ways to combine ECD with CID. Recently, consecutive ECD/CID MS/MS in a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer was demonstrated for the first time.^{2–6} Although this was a noteworthy accomplishment, the time scale for ECD in the FTICR experiment is so much longer than that for CID⁷ that it is difficult to reconcile its use in conjunction with high-performance liquid chromatography (HPLC). Hence, a number of attempts to record 'golden complementary pairs' by combining electron-transfer dissociation (ETD) and CID in linear and three-dimensional (3D) quadrupolar ion traps have been undertaken.^{8–13} One of the most recent of these approaches¹³ is potentially compatible with HPLC; however, this will be difficult to achieve because, ultimately, ETD and CID must be performed consecutively. All of the aforementioned approaches to complementary dissociation have been either tandem-in-time (e.g. as in a FTICR ECD/CID experiment^{8–11,13}) or tandem-in-space (e.g. the linear/3D quadrupole ETD/CID experiment¹²) because, as of yet, no one has been able to conceive of a radio-frequency (RF)-based cell that would allow both dissociation reactions to occur simultaneously in the same space.

Magnetostatic lenses, which have exceedingly high transmission efficiencies and are routinely employed in electron microscopes, linear accelerators, and traveling wave tubes,^{14–16} are not currently used in commercial mass spectrometers. This is probably due in large part to the historical fact that permanent magnets small and strong enough for the design and fabrication of practical, mass spectrometric, ion-optical components were unavailable. Recent advances in fabricating low-cost permanent magnets in a range of sizes, shapes, strengths (0.1–5 T), and polarizations now makes their incorporation into mass spectrometers practical.^{17,18}

Placing soft iron pole pieces on either side of a hole bored through a permanent magnet disc creates a powerful magnetostatic-focusing lens for charged particles; the focusing action of this element can be set in various manners, depending on how the magnet is polarized.^{14–16}

A traveling wave tube (TWT) is formed when two or more magnetostatic lenses are arranged so that the polarity of the lenses alternates periodically. If the lens elements are focused axially, charged particles transiting a TWT are forced toward the cavity's axis. The iron pole pieces are electrically insulated from that of the magnets to create a hybrid device that permits separate magnetostatic focusing and electro-static focusing, enabling electrons and ions to be simultaneously transported with kinetic energies commonly found in mass spectrometers.^{19,20}

Previously, we described a five-magnet electromagneto-static (EMS) cell and demonstrated its use in recording ECD spectra of substance P,¹⁹ gramicidin S,²⁰ and neurotensin.²⁰ The source of electrons in this cell was a circular tungstenrhenium filament placed very near the ion entrance;²⁰ the initial experiments conducted with this cell clearly established that the ions' flight times through the cell were on the order of 10 μ s and, further, that ECD was occurring in the segment closest to the filament. As a result of this observation, the size of the original cell was reduced to only two magnets (Fig. 1). The initial set of experiments with the two-magnet cell showed that the simpler cell indeed has the same ECD efficiency as the original five-magnet cell.

The two-magnet cell was tested in the CID mode by using Ar as the collision gas, setting the cell's potential so that the ion energy (laboratory frame of reference) was 200 eV, and recording a CID product-ion spectrum of doubly protonated Glu-fibrinopeptide (Fig. 2(A)). Comparison of this spectrum with a published spectrum²¹ (Fig. 2(B)) shows that both spectra exhibit the same series of γ -type ions. The distinctly different distributions of peak intensities in these two spectra is probably due for the most part to the different collision energies, *viz.* 200 eV for the electromagnetostatic cell versus <30 eV for the cell used in the Q-STAR XL.

After having demonstrated that the single-segment EMS cell could be operated in both ECD and CID modes independently, simultaneous ECD and CID was attempted in the cell on doubly protonated substance P. This was done by first recording a CID product-ion spectrum of the peptide (Fig. 3(A)) and subsequently turning on the electron filament to record its combined ECD/CID spectrum (Fig. 3(B)). In the CID product-ion spectrum of substance P (Fig. 3(A)), a relatively complete series of b -type fragment ions accompanied by a less intense series of a -type fragment ions is observed, as is generally the case for a peptide that has an arginine on its N-terminus. In the combined CID/ECD product-ion spectrum, the CID series of b -type and a -type ions is virtually unchanged; however, superimposed on this CID series of fragment-ion peaks is a series of the same six c -type ions (*i.e.*, c_4 – c_{10}) typically observed^{22–25} in an ECD product-ion spectrum of substance P. This result is striking. While others have produced 'golden complementary pairs' by means that were either tandem-in-time or -space,^{8–13} this is, to the best of the authors' knowledge, the first ever demonstration of 'golden complementary pairs' (actually, the presence of a -, b -, and c -type ion signals in a single product-ion mass spectrum constitutes triplets in this particular example) being simultaneously (*i.e.* non-tandemly in time or space) produced while in-flight through a single dissociation cell.^a

If the filament was left on to produce electrons for ECD and the gas valve was left open to provide collision gas for CID, any combination of ECD, CID, or ECD/CID experiments could be interchangeably carried out in the EMS cell. Reducing the filament's potential would stop the ECD process and increasing the cell's potential (*i.e.* making it less negative) would stop the CID process. Since voltages can easily be switched in nanoseconds, changing

^aAfter this paper was submitted, a quasi-simultaneous (actually tandem-in-time) ECD/CID that was realized in a single RF cell was reported.²⁶

from one dissociation mode to another can easily be done on a time-scale commensurate with the ions' flight times through the mass spectrometer (i.e. microseconds). Rapidly switching the ECD mode off and on while recording a product-ion spectrum could, for example, be used to confirm the presence of golden complementary pair or triplets. Use of a fast, automated, alternating dissociation mode with an EMS cell also might, when used in conjunction with Walsh-Hadamard transforms,²⁷ be a means for increasing signal-to-noise ratio or for decreasing the duty cycle in time-of-flight measurements of product ions.

In summary, *a*-, *b*-, and *c*-type ion signals have been recorded in a single product-ion mass spectrum by simultaneously performing ECD and CID in an EMS cell. Use of this technique in MS/MS analyses of peptides could significantly increase the number of peptides (and ergo proteins) that can be accurately matched to sequence entries in genomic and proteomic databases and improve the reliability of *de novo* sequencing.

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Figure 1.
Tandem quadrupole mass spectrometer with RF-free magnetic ECD/CID cell.

Figure 2.
CID spectra of doubly protonated Glu-fibrinopeptide: (A) CID in electro-magnetostatic cell. (B) CID in Applied Biosystems Q-STAR XL hybrid quadrupole-TOF mass spectrometer. Mass spectrum (B) reproduced from Wang *et al.*²¹ Copyright 2007, reprinted with permission from Elsevier.

Figure 3.
(A) EMS CID product-ion spectrum of doubly protonated substance P. (B) Combined ECD/
CID product-ion spectrum of doubly protonated substance P.