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Considerations for electron capture dissociation efficiency in FTICR mass spectrometry

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Abstract

An experimental approach for increasing the efficiency of Electron Capture Dissociation (ECD) with Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) is presented. The approach is based on manipulating the spatial distribution of an ion cloud inside an FTICR trap during electron irradiation, which is realized by using both on-resonance pre-excitation of the ions and sustained off-resonance irradiation (SORI). The achieved fragmentation efficiency is compared with the theoretical prediction. This method may be useful in biological applications of FTICR, such as identification of posttranslational modifications in proteins and de novo sequencing, where the ECD technique is most applicable.

Keywords: FTICR-MS; Electron capture dissociation (ECD); SORI

1. Introduction

Electron Capture Dissociation (ECD) [1] is increasingly used in FTICR mass spectrometry to characterize peptide and protein primary structures [2]. Cleavage of N–C backbone bonds in biomolecule ions during ECD is believed to occur within 10^{-12} s upon energy deposition at the capture site of a low-energy electron, which preserves more distant labile groups and is, thus, more effective for both identification and localization of posttranslational modifications [3–7]. However, the reported efficiency of fragmentation in ECD is lower than other fragmentation methods [2], and signals from fragment ions are often barely noticeable above the noise level (especially for larger molecules), limiting the broader application of this technique in proteomics. Reasons for low fragmentation efficiency include: (1) low electron emission; (2) misalignment between the electron beam and the ion cloud inside the FTICR trap; and (3) magnetron motion of the ions in the trap, which prevents the ions from overlapping with the electron beam [8] (Fig. 1). These problems can be addressed by using indirectly heated wide area dispenser cathodes, which were introduced in FTICR in earlier studies of ion–electron interactions [8] and were later shown to have a dramatic improvement in ECD efficiency [9,10].

In this work, we show that in some cases, such as off-axis ion injection (side-kick trapping, open-ended traps), extensive magnetron expansion of ions (pressure assisted accumulated trapping for an extended time period), and other radial disturbances of the ion cloud (e.g. during pre-selection of precursor ions), the use of a large emission area dispenser cathode does not necessarily lead to efficient fragmentation. To address this issue and further improve ECD fragmentation efficiency, we propose a solution that involves the use of sustained off-resonance irradiation (SORI) of the precursor ions simultaneously with electron emission in the absence of a buffer gas to prevent collisional activation.

2. Experimental

Experiments were performed using a 3.5 T ESI FTICR mass spectrometer with external ion accumulation that

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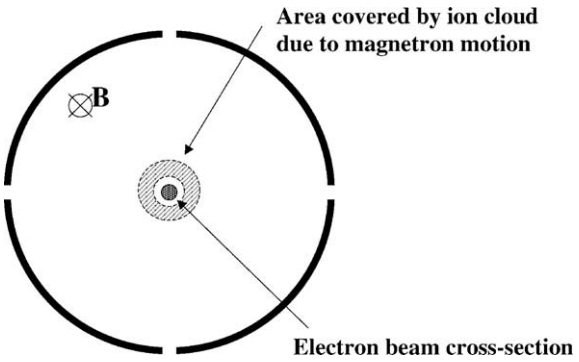

Fig. 1. FTICR ion trap cross-section. Due to magnetron motion of the ion cloud inside the trap and the possible misalignment between geometrical and electrostatic axes of the trap, an electron beam may not overlap with the ions path.

was developed at Pacific Northwest National Laboratory (PNNL). The instrument, coupled to an Odyssey (Finnigan, Madison, WI) data station to control timing and distribution of potentials, is equipped with an electrospray ionization source, and incorporates a 3.2 in. closed cylindrical FTICR trap and external ion accumulation in an rf quadrupole located in the third stage of the differentially pumped vacuum system [11]. Ions were trapped using a variant of the gated trapping technique based on the incorporation of an additional cylindrical electrode into the front (ion injection side) trapping plate of the ICR cell. This electrode was maintained at a DC potential of −10 to −50 V while the trapping plate was kept at a typical potential of +1 to +2 V [12]. The duration of the FTICR experimental sequence was 0.5 s. This sequence included external ion accumulation in an rf linear quadrupole ion trap, the transfer of ions to the ICR cell, a SORI–ECD event (typically, 0.1 s in duration), a broadband frequency sweep excitation, a detection event, and an FTICR trap quench period (Fig. 2). Parameters for SORI were experimentally determined using a frequency offset of 1.0–1.5 kHz above the cyclotron frequency for the precursor ions and an rf amplitude of 15–20 $V_{p\text{-}p}$. For ECD experiments, we used a 1.2 mm diameter dispenser cathode with a current of 0.4 A, and a voltage of 3–4 V applied to a heater and the emission surface. The emission current was in the nA range using a 3–4 eV electron energy source.

Peptide solutions were prepared in a water/methanol/acetic acid solution (49 vol.%:49 vol.%:2 vol.%) at concentrations of 0.01 mg/ml. All peptides were purchased from Sigma Chemicals Co. (St. Louis, MO) and used without further purification. The solutions were infused into the ESI source at a flow rate of 300 nl/min using a syringe pump (Harvard, South Natick, MA). A voltage of +2 kV was applied to the ESI emitter, and charged species were injected through a 500 μm diameter heated metal capillary maintained at 160 °C. At the exit of the metal capillary, the ion beam was focused to the entrance of the ion funnel [13].

3. Results and discussions

3.1. Definition of ECD efficiency and its theoretical limit

In this work we define an efficiency of fragmentation as *a ratio of the sum of relative peak heights for all the fragments to the relative peak height of the precursor ion BEFORE irradiation*. Let us now use this definition to estimate the theoretical limitations of ECD fragmentation.

First, consider the very simple case of doubly charged precursor ions, which covers a good number of current ECD applications. We will assume that the peak heights for these particular ions are proportional to the number of ions for all the peaks in the spectrum. In the most general case, this assumption is inaccurate because different ions may produce time-domain signals of different duration due to,

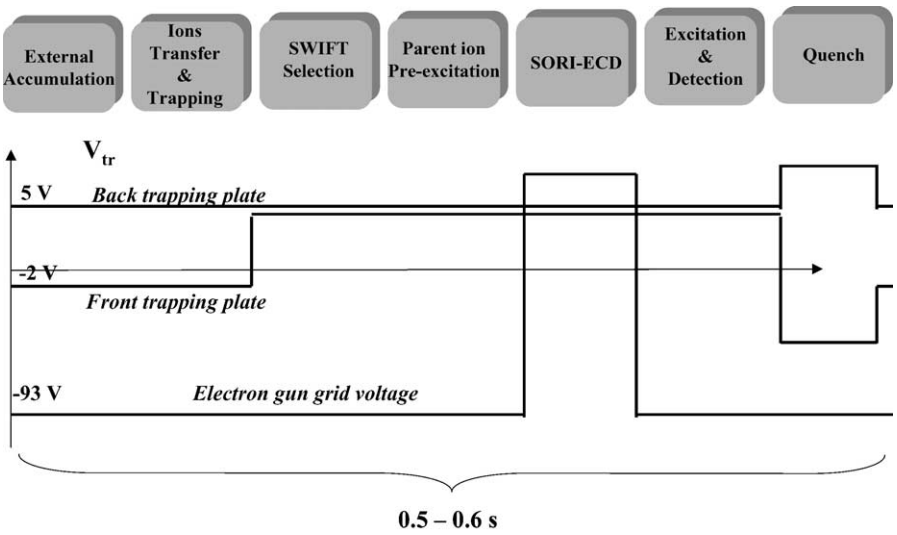

Fig. 2. Typical experimental sequence used in this work.

for example, different axial energy distributions or different space charge effects. However, let us assume that all the ions are produced in the source and trapped in the ICR trap at the same experimental conditions, that all ions are cooled both axially and radially before excitation and detection, and that the total number of ions remain below the level at which the space charge starts affecting the FTICR spectral line shape. In these conditions, our initial assumption is verified. Let P_n be the (undetermined) probability that an ion of $n+$ charge state captures an electron over the electron irradiation period. Because the product ions are formed during irradiation and can, therefore, capture additional electrons, the probability P_{n-1} for a product ion to capture an electron over the same irradiation period will be:

$$P_{n-1} = 0.5 \times \frac{P_n}{N} \times \frac{(n-1)^2}{n^2} \quad (1)$$

where, N is the number of product ions (we adhere to the generally accepted assumption that ECD is non-ergodic, that is, that each product has the same probability to appear) and n is the charge state of the precursor ion ($n = 2$ in our case). We assume that the electron capture cross-section is proportional to the charge state squared as suggested in the literature [2]. A coefficient 0.5 appears as we further assume a uniform rate of dissociation over the irradiation period. This probability represents the probability of loss of the product ion by neutralization ($n = 2$) or further fragmentation ($n > 2$). Therefore, the probability that a product ion will remain in the trap and contribute to the signal, is

$$P_{\text{product}} = \frac{P_n}{N} - P_{n-1} = \frac{P_n}{N} \times \left[1 - 0.5 \times \frac{(n-1)^2}{n^2}\right] \quad (2)$$

Finally, by assuming that the peak height in the FTICR mass spectrum is proportional to the charge state of the ions, we can obtain the following equations for ECD fragmentation efficiency, ξ_{ECD}, (as defined above) measured from the spectrum (where the sum of signal intensities from the fragment ions is normalized on the intensity of the precursor ion before irradiation, I_0):

$$\xi_{\text{ECD}} = \frac{\sum_{j=1}^{N} I_{\text{fr}}(j)}{I_0} = \frac{n-1}{n} \times N \times \frac{P_{\text{product}}}{P_n} = \frac{n-1}{n} \times \left[1 - 0.5 \times \frac{(n-1)^2}{n^2}\right] \quad (3)$$

Eq. (3) gives a maximum obtainable fragmentation efficiency in ECD of 43.75% for doubly charged ions. Similar estimations can be obtained using a system of rate equations. The problem becomes more complex for large precursor ions with increasing n (for example, when working with heavy proteins) because the precursor ions can dissociate into products with different charge states ranging from 1+ to $(n-1)+$, and using the system of rate equations requires extensive computation efforts. Our preliminary estimations based on the above probabilistic approach show that the theoretical limit for ECD fragmentation efficiency approaches 50%. The actual fragmentation efficiency is expected to be even smaller due to charge reduction for the molecular ions occurring without dissociation, which was not taken into account in our estimations.

3.2. SORI–ECD

Misalignment between the ion cloud and electron beam is a common problem that arises for a number of reasons. First, the ion cloud may be located off-axis of the ICR trap. This occurrence happens for both “side-kick” and accumulated trapping (when the ions are trapped in collisions with a buffer gas injected into the trap for this purpose), and ions may experience magnetron motion or expansion to radii larger than the emission area of the cathode that generates the electron beam. Second, an emission cathode may be placed off-axis when it is used together with a laser beam, for example, in IRMPD/ECD experiments employing so-called hollow cathodes [14]. Finally, an ordinary mechanical misalignment of the ion guide, which transmits ions from an external source, or of the ion accumulation device into the FTICR cell can result in ions not being trapped along the cell axis. There are several possible solutions for resolving these problems depending on the origin of misalignment. An obvious solution involves ion manipulations inside the FTICR cell in such a way that the ion cloud moves into or crosses the electron beam during irradiation. These manipulations may be realized either by on-resonance pre-excitation of the ions or by using SORI [15]. A basis for increased ECD efficiency is expected when all the ions from the ion cloud cross the continuous electron beam during their radial oscillations under SORI conditions or during their cyclotron motion after pre-excitation. We usually observed better efficiency with the SORI–ECD method compared to the on-resonance pre-excitation approach. It is worth noting that we suggest using SORI in the absence of a buffer gas to prevent collisions as a channel for ion fragmentation. In Fig. 3 we compare FTICR spectra obtained for doubly charged *Bradykinin* ions when the ECD and SORI events were inactivated (a), when only the ECD event was activated (b), and when both ECD and SORI events were activated (c). In these experiments we were able to achieve fragmentation efficiency comparable to SORI–CAD. In a separate set of experiments, we measured the effect of SORI excitation (using the same experimental parameters) on the precursor ions (without electron irradiation) and expectedly observed no effect on the relative peak heights. Also, we found further improvement in fragmentation efficiency when the precursor ions were synchronized by on-resonance cyclotron excitation (0.8–1.0 $V_{\text{p-p}}$ in our case) before the SORI–ECD event (see Fig. 2) although this option was not always efficient, probably due to the reasons mentioned above. With this technique we were able to achieve fragmentation efficiency closer to the theoretical efficiency for doubly charged peptide ions. For example, Figs. 4 and 5 show ECD spectra of *Substance P* and *Fibrinopeptide B* for which the measured

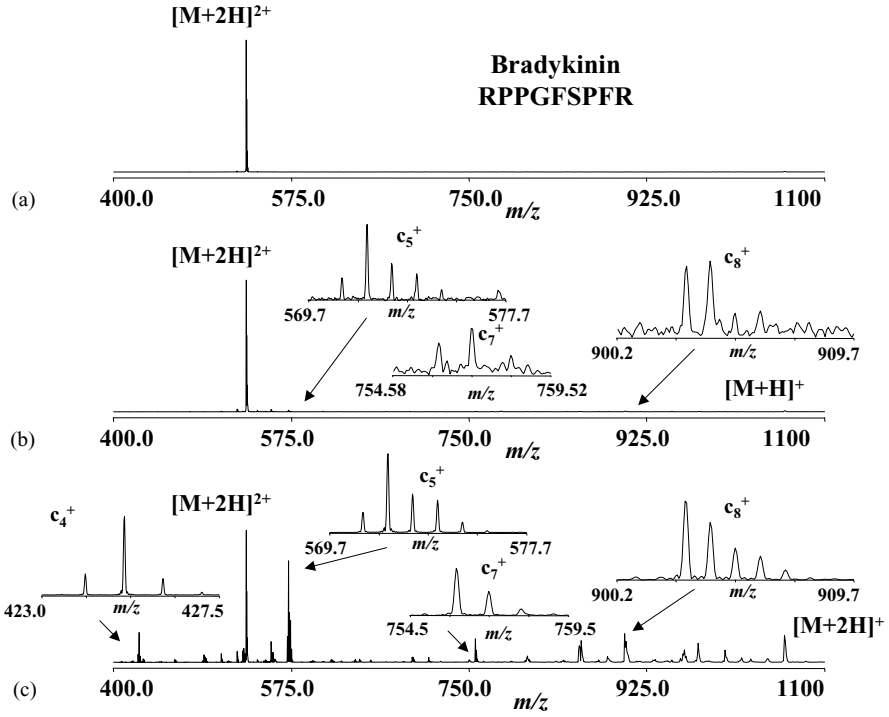

Fig. 3. (a) Spectrum of SWIFT isolated $[M+2H]^{2+}$ *Bradykinin* ions in the absence of electron irradiation; (b) ECD spectrum of $[M+2H]^{2+}$ *Bradykinin* ions, 50 ms irradiation, no SORI, or "pre-excitation"; (c) SORI–ECD spectrum of $[M+2H]^{2+}$ *Bradykinin* ions, 50 ms irradiation.

fragmentation efficiency is 33% (based on peak height measurements of the fragments resultant from ECD and the precursor ion *before* irradiation). This efficiency is not far from the predicted theoretical limit of 43.75% for doubly charged ions. To quantify the misalignment between the ion cloud and electron beam, we used on-resonance pre-excitation of ions before electron irradiation and measured the ratio between the peak heights for the major fragment and precursor ions. Fig. 6 shows the results of these experiments. When the radius of the cyclotron motion of the precursor ions approaches the distance between the ion cloud and electron beam (misalignment factor), the ECD fragmentation

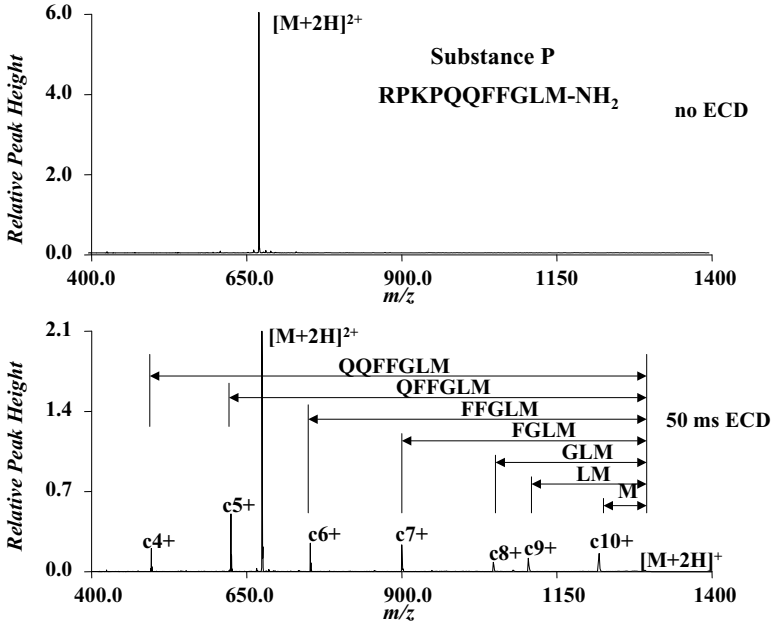

Fig. 4. SORI–ECD spectrum of $[M+2H]^{2+}$ *Substance P* ions. The measured efficiency for fragmentation is 33%.

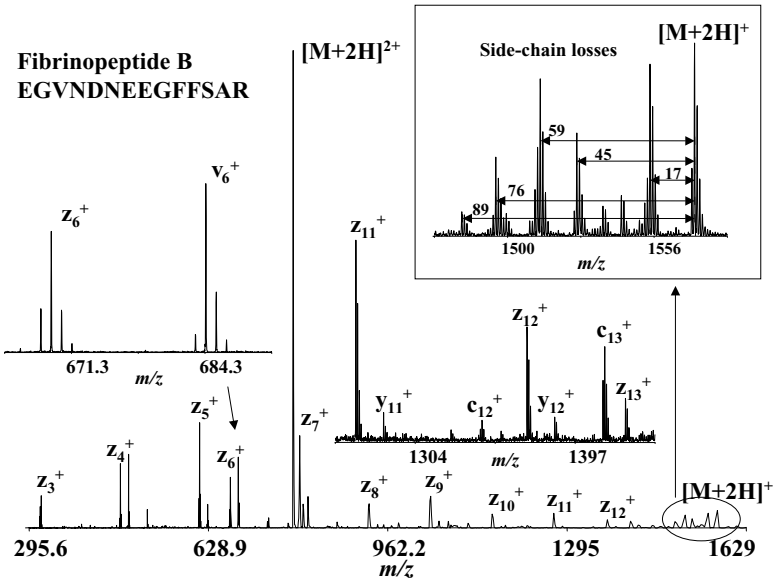

Fig. 5. SORI–ECD spectrum of $[M + 2H]^{2+}$ *Fibrinopeptide B* ions. The spectrum reveals a dominance of z-fragments that can be attributed to the location of an arginine residue at the end of the peptide aminoacid sequence.

achieves maximum efficiency. In our experiments, the misalignment factor was 0.065–0.07 cm. Note, that even such a small misalignment may lead to a large decrease in dissociation efficiency.

3.3. Fragmentation pattern in SORI–ECD

In spite of using SORI for extended periods of time, we did not find any b- and y-fragmentation in the spectra resulting from collisions with the background molecules in the ICR trap, as shown in Figs. 4 and 5 for ECD of *Subtance P* and *Fibrinopeptide B*. In these experiments we clearly see that the ECD fragmentation pattern is characteristic for a particular peptide amino acid sequence, which supports previous observations by Hakansson et al. in experiments performed at lower levels of ECD efficiency [16]. For example, the ECD spectrum for *Substance P* reveals the presence of only N-terminal c-fragments (Fig. 4), although the dissociation spectrum for *Fibrinopeptide B* exhibits dominant C-terminal z-fragments (Fig. 5). While the appearance of c- and z-fragments are accepted characteristics of ECD spectra, we attribute the difference between these two spectra to the location of the arginine residue, which would be the preferential site for any remaining charge after precursor

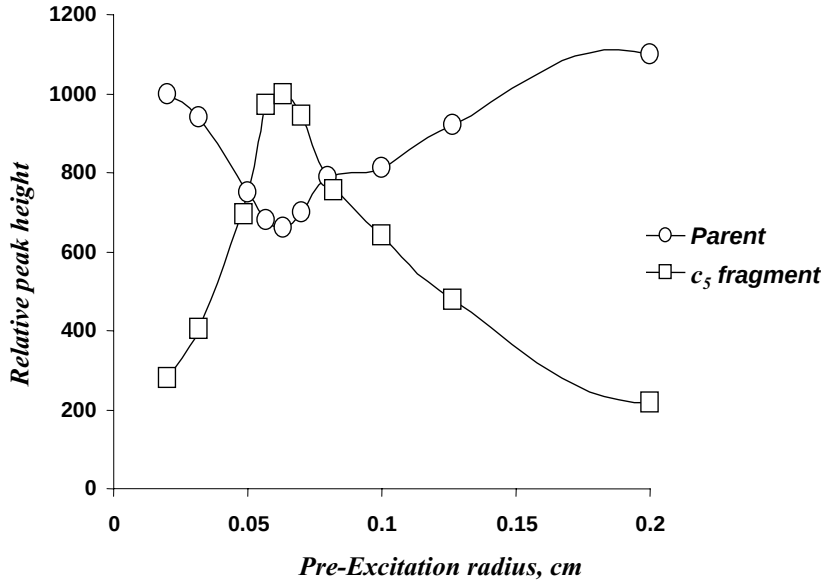

Fig. 6. Pre-excitation of precursor ions before ECD. Precursor ion: *Bradykinin*, $[M + 2H]^{2+}$, 150 ms ECD, electron emission area of 1 mm.

ion fragmentation. If this observation proves correct, then this feature of ECD fragmentation would help in sequencing both peptides and proteins and would also provide insights towards a better understanding of the ECD mechanism.

4. Conclusions

A combination of ECD and manipulation of ions trapped in an FTICR cell before and during electron irradiation results in higher fragmentation efficiency. In this work, we were able to achieve fragmentation efficiencies close to the theoretical limit by using SORI during irradiation without a buffer gas. This approach can be especially useful when the electron source and the ion cloud are not well aligned. This can occur for example, in IRMPD/ECD experiments using hollow cathodes [17] or when placing the filament off-axis [18]. It can also be the case when employing the "side-kick" trapping technique; and generally, when there is significant magnetron expansion of the ion cloud. As a variation to this technique, the authors also suggest a combination of azimuthal quadrupolar excitation/axialization (QE) [19] and ECD for increasing fragmentation efficiency; such efforts are currently under way. Finally, in support of previous observations by other research groups [16], we found that the fragmentation pattern in ECD depends strongly on the location of a particular amino acid residue, for example, arginine, in a sequence that affects the distribution of the remaining charge carriers among the fragments.

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