Two-Fold Efficiency Increase by Selective Excitation of Ions for Consecutive Activation by Ion−Electron Reactions and Vibrational Excitation in Tandem Fourier Transform Ion Cyclotr…

ARTICLE *in* ANALYTICAL CHEMISTRY · JUNE 2005

Impact Factor: 5.64 · DOI: 10.1021/ac048531j · Source: PubMed

3 AUTHORS:

Roman Zubarev
Karolinska Institutet
251 PUBLICATIONS **10,936** CITATIONS
SEE PROFILE

Matthias Witt
Bruker Corporation
107 PUBLICATIONS **2,701** CITATIONS
SEE PROFILE

Goekhan Baykut
Bruker Corporation
43 PUBLICATIONS **903** CITATIONS
SEE PROFILE

Anal. Chem. **2005,** *77,* 2992−2996

Two-Fold Efficiency Increase by Selective Excitation of Ions for Consecutive Activation by Ion−Electron Reactions and Vibrational Excitation in Tandem Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Roman A. Zubarev,*,† Matthias Witt,‡ and Gökhan Baykut‡

Laboratory for Biological and Medical Mass Spectrometry, Uppsala University, Sweden, and Bruker Daltonik GmbH, Bremen, Germany

A new technique called selective excitation of ions for consecutive activation (SEICA) is proposed for obtaining complementary fragmentation mass spectra from the same precursor ion population. SEICA utilizes precursor ions remaining intact after electron capture dissociation or another ion−electron reaction for efficient MS/MS based on a vibrational excitation (VE) technique, such as infrared multiphoton dissociation. SEICA uses the ability of ion-trapping instruments to detect product ions while retaining inside the trap intact precursor ions, making the latter available for consecutive activation by a VE technique. The possibility of practical implementation of SEICA by software-only modification of a commercial instrument is demonstrated. A 2-fold increase in the efficiency is achieved for both "single-scan" and "multiple-scan" experiments. This improvement can be particularly important for high-sensitivity applications in, for example, proteomics, where limited ECD efficiency poses an obstacle for broad implementation of this technique.

To fragment polypeptide ions in tandem mass spectrometry (MS/MS), collision-induced dissociation (CID; also called collisionally activated dissociation, CAD) or infrared multiphoton dissociation (IRMPD) are most commonly employed. Both of these techniques produce vibrational excitation (VE) of precursor ions above their threshold for dissociation. The efficiency (ratio of the detected fragment abundances and the precursor ion abundance before fragmentation) of VE techniques can at optimal conditions reach nearly 100%. The drawback of the VE techniques is often incomplete sequence coverage, abundant small-molecule losses, and detachment from backbone fragments of labile groups, which renders difficult the analysis of post-translational modifications (PTMs). Ion−electron reactions,[1] on the contrary, provide a means for PTM analysis and yield more abundant sequence coverage and complementary types of backbone fragmentation. For instance, electron capture dissociation (ECD)[2] cleaves the $N-C_\alpha$ backbone bonds, while VE cleaves the amide C−N backbone bonds (peptide bonds). Moreover, disulfide bonds inside the peptides, which usually remain intact in collisional and infrared multiphoton excitations, fragment specifically upon electron capture.[3] Finally, some easily detachable groups remain attached to the fragments upon ECD, which allows the determination of their positions. This feature is especially important in the analysis of labile post-translational modifications in proteins and peptides, such as phosphorylation, glycosylation, and γ-carboxylation.[4−7]

Other ion−electron fragmentation reactions also provide analytical benefits. Increasing the electron energy to 3−13 eV leads to hot-electron capture dissociation (HECD),[8] in which electron excitation precedes electron capture. The resulting fragment ions undergo secondary side-chain fragmentation, which distinguishes between the isomeric leucine and isoleucine residues.[8,9] In electron detachment dissociation (EDD),[10] 20 eV electrons ionize peptide dianions, which produces an effect similar to ECD. EDD is advantageous for acidic peptides and peptides with acidic modifications, such as sulfation.

The efficiency of fragmentation in ion−electron reactions is generally lower than in VE techniques.[11] Partially this is because of the charge reduction of precursor ions before dissociation.

* Corresponding author telephone/fax: +46 18 471 72 09; e-mail: Roman.Zubarev@angstrom.uu.se.

† Uppsala University.

‡ Bruker Daltonik GmbH.

(1) Zubarev, R. A. *Mass Spectrom. Rev.* **2003**, *22,* 57−77.

(2) Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *J. Am. Chem. Soc.* **1998**, *120,* 3265−3266.

(3) Zubarev, R. A.; Kruger, N. A.; Fridriksson, E. K.; Lewis, M. A.; Horn, D. M.; Carpenter, B. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **1999**, *121*, 1, 2857−2862.

(4) Kelleher, N. L.; Zubarev, R. A.; Bush, K.; Furie, B.; Furie, B. C.; McLafferty, F. W.; Walsh, C. T. *Anal. Chem.* **1999**, *71*, 4250−4253.

(5) Mirgorodskaya, E.; Roepstorff, P.; Zubarev, R. A. *Anal. Chem.* **1999**, *71*, 4431−4436.

(6) Shi, S. D.-H.; Hemling, M. E.; Carr, S. A.; Horn, D. M.; Lindh, I.; McLafferty, F. W. *Anal. Chem.* **2000**, *73*, 19−22.

(7) Stensballe, A.;Jensen, O. N.; Olsen, J. V.; Haselmann, K. F.; Zubarev, R. A. *Rapid Commun. Mass Spectrom.* **2000**, *14,* 1793−1800.

(8) Kjeldsen, F. Budnik, B. A.; Haselmann, K. F.; Jensen, F.; Zubarev, R. A. *Chem. Phys. Lett.* **2002**, *356*, 201−206.

(9) Kjeldsen, F.; Sørensen, E.; Zubarev, R. A. *Anal. Chem.* **2003**, *75*, 1267−1274.

(10) Budnik, B. A.; Haselmann, K. F.; Zubarev, R. A. *Chem. Phys. Lett. 342*, **2001**, 299−302.

(11) Zubarev, R. A.; Fridriksson, E. K.; Horn, D. M.; Kelleher, N. L.; Kruger, N. A.; Carpenter, B. K.; McLafferty, F. W. *Anal. Chem.* **2000**, *72,* 563−573.

10.1021/ac048531j CCC: $30.25
Published on Web 03/25/2005

Another reason is that, under the conditions of the maximum fragment yield, a certain fraction of precursor ions does not react with electrons and as a consequence remains intact. This fraction can be significant: for dications, it is nearly 30%.[12] Currently these remaining ions do not serve any analytical purpose.

Combined use of ion−electron fragmentation reactions with VE techniques provides additional sequence information. First, comparison between the two types of cleavage allows one to determine the fragment types. For example, the mass difference between the N-terminal c′ and b ions is 17 Da, while that between the C-terminal y′ and z ions is 16 Da. Second, the cleavage sites are often complementary. For instance, VE techniques cleave preferentially at the N-terminal side of the proline residues, while this site is immune to ECD. Although ion−electron reactions can be used simultaneously with VE techniques, the complementary character of the analytical information obtained in these techniques favors independent consecutive use of them.[11,13,14] For instance, the detection of characteristic losses by IRMPD or CAD with subsequent ECD of enzymatic products could provide an efficient means for the automated PTM mapping in highly modified proteins.[15]

Unfortunately, consecutive use of these reactions demands at least twice as long for the analysis as required by the fastest of these techniques. A short analysis time is especially critical while analyzing low-concentration samples, which is the case when the sample quantity is limited. Low-concentration samples require either long (several seconds) accumulation of the precursor ions in the ion trapping device or integration of many individual MS/MS spectra. In both cases, the time loss due to the consecutive use of ion−electron reactions and VE techniques can be on the order of several seconds. This limits the analytical utility of tandem mass spectrometry when it is combined with the separation techniques, such as liquid chromatography (HPLC) or capillary electrophoresis (CE), where the entire signal from an individual compound often lasts for just a few seconds. Therefore, while on-line use of VE and ion−electron reactions together with both HPLC and CE has been demonstrated,[16−18] consecutive use of these fragmentation techniques on-line with separation techniques requires a larger amount of sample.

Here we propose a technique for a 2-fold sensitivity improvement for fixed analysis time while consecutively using an ion−electron reaction and a vibrational excitation technique. Alternatively, the time of analysis shortens by a factor of 2 at a fixed detection limit. This technique, which we call selective excitation of ions for consecutive activation (SEICA) utilizes the precursor ions remaining intact after ECD or another ion−electron reaction for efficient VE-based MS/MS. SEICA uses the fact that in ion-trapping instruments, such as Fourier transform ion cyclotron resonance (FTICR) mass spectrometers, fragment ions can be detected and ejected from the trap while remaining intact precursor ions continue residing inside the trap, being available for consecutive activation by a complementary MS/MS technique. The unreacted ions in ECD remain intact in terms of the primary and secondary structures, because the energy of the electrons used in ECD is too low to excite electronic or vibrational degrees of freedom in the ion that did not capture an electron. Nondetection of the precursor ions in such a scheme is not a drawback, since their mass-to-charge values are usually known from the overview mass spectrum. Besides, as we will show below, imperfect implementation of this technique still gives some signal from the precursor ions, which is sufficiently abundant for accurate mass measurements. We demonstrate the possibility of practical implementation of this technique in a pilot experiment realizing all steps of the suggested approach and leading to two independent fragmentation mass spectra from one and the same ion population accumulated once in the FTMS trap, thus affording the efficiency doubling. We show also that a slightly modified SEICA approach increases the efficiency, even when only one fragmentation technique is employed, by means of integration of multiple spectra.

Modern FTICR mass spectrometers can select for analysis a range of m/z values of interest, while ions with other m/z values can remain in the trap for further reactions. Another feature of modern FTICR instrumentation is the ability to accumulate precursor ions in a storage device (usually a linear multipole) while performing fragmentation and m/z analysis of the previously accumulated ion population.[19,20] Although previous works have utilized IRMPD and ECD consecutively as well as simultaneously, the reduction of the time of analysis while acquiring separate mass spectra of both VE and ion−electron reactions is reported for the first time.

EXPERIMENTAL SECTION

Mass Spectrometry. The 7 T Apex-Q (Bruker Daltonics, Billerica, MA) mass spectrometer was used in the experiments. Figure 1a presents its schematic diagram. The ions produced by the electrospray ionization (ESI) source enter the atmosphere−vacuum interface through the electrospray capillary. After the capillary, the ions pass through two skimmers and enter a linear radio frequency (rf) multipole ion trap used as an ion accumulation device. Here, the ions are trapped radially by the rf field and axially by the reflective potentials of the second skimmer and the trap/extract electrode. Ions can be accumulated in this linear multipole ion trap and then extracted at a predetermined time by changing the polarity of the trap/extract electrode. After ejection, the ions enter through the ion transfer optics into the ICR trap, which is placed in a strong magnetic field generated by a 7 T superconducting magnet. The ion transfer optics was based on a quadrupole m/z filter followed by electrostatic ion lenses. The complete system was differentially pumped, which allowed for a pressure drop from atmospheric at the ion source gradually down to approximately 10^{-10} mbar near the ICR trap.

(12) Zubarev, R. A.; Haselmann, K. F.; Budnik, B. A.; Kjeldsen, K.; Jensen, F. *Eur. J. Mass Spectrom.* **2002**, *8,* 337−349.
(13) Horn, D. M.; Zubarev, R. A.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97,* 10313−10317.
(14) Tsybin, Y. O.; Witt, M.; Baykut, G.; Kjeldsen, F.; Hakansson, P. *Rapid Commun. Mass Spectrom.* **2003**, *17,* 1759−1768.
(15) Kjeldsen, F.; Haselmann, K. F.; Budnik, B. A.; Sørensen, E. S.; Zubarev, R. A. *Anal. Chem.* **2003**, *75*, 2355−2361.
(16) Palmblad, M.; Tsybin, Y. O.; Ramstrom, M.; Bergquist, J.; Hakansson, P. *Rapid Commun. Mass Spectrom.* **2002**, *16,* 988−992.
(17) Davidson, W.; Frego, L. *Rapid Commun. Mass Spectrom.* **2002**, *16,* 993−998.
(18) Tsybin, Yu. O.; Wetterhall, M.; Markides, K. E.; Håkansson, P.; Bergquist, J. *Eur. J. Mass Spectrom.* **2002**, *8,* 389−396.
(19) Senko, M. W.; Hendrickson, C. L.; Emmet, M. R.; Shi, S. D.-H.; Marshall, A. G. *J. Am. Soc. Mass Spectrom.* **1997**, *8,* 970−976.
(20) Haselmann, K. F.; Budnik, B. A.; Olsen, J. V.; Nielsen, M. L.; Reis, C. A.; Clausen, H.; Johnson, A. H.; Zubarev, R. A. *Anal. Chem.* **2001**, *73*, 2998−3005.

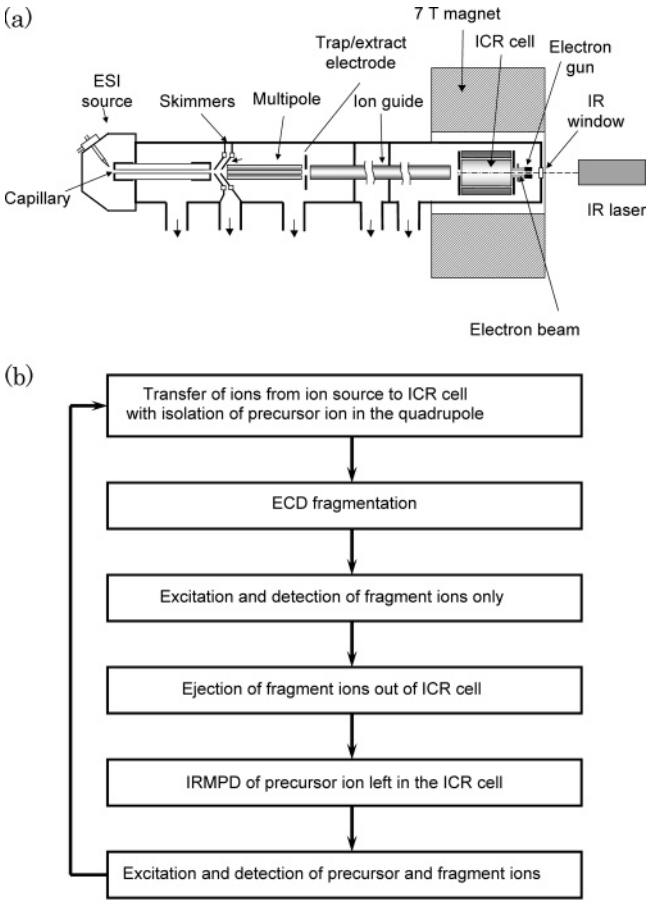

Figure 1. (a) Diagram of a Fourier transform ion cyclotron resonance mass spectrometer used in this work. (b) Flowchart of the sequence of events.

The script for the controlling software XMASS 6.0.2 was modified as follows (see the flowchart of the sequence of events in Figure 1b). Positive ions produced continuously by the ESI source were accumulated in the linear rf multipole trap (Figure 1a), being prevented from entering the ion guide by the positive potential of +25 V on the trap/extract electrode. At the end of the accumulation period lasting 100 ms, this potential was made negative (−10 V) so that the trapped ions were ejected from the multipole through the trap/extract electrode and the ion transfer optics, reaching the ICR trap after 1200 μs upon ejection. The ions were captured and trapped in the ICR trap by the sidekick trapping. Immediately upon the ion ejection, the polarity of the potential on the trap/extract electrode was made again blocking for cations, to start a new storage period. The mass-to-charge ratio (m/z) of the precursor ions for dissociation was selected by the quadrupole of the transfer optics during the ion transfer. After ion capture in the ICR cell, the hollow-cathode electron source[14] based on an indirectly heated dispenser cathode[20,21] (HeatWave) produced an electron beam of <1 eV energy, which passed through the ICR trap and interacted with the trapped ions, producing ECD. After 50−100 ms of irradiation, when the majority of the precursor polycations captured electrons, the cyclotron motion of the ions in the ICR trap was excited by a frequency chirp.[22] The following were the chirp parameters: correlation pulse length, 100 μs; correlation sweep attenuation, 10 dB (about 50 V Vp−p). The excitation frequencies corresponded to the m/z range from 300 to 1800 but excluded the ±10 m/z units wide window around the m/z value of the precursor multiply charged species (isolated ion, m/z 674.37; ejection safety belt, 3 kHz). The precursor ions that did not capture electrons remained therefore unexcited and continued to reside near the ICR trap center. After that, the frequencies of ion motion were detected by induced image currents, as is customary in the FTICR mass spectrometry.[23] The ECD product ions were then ejected from the ICR trap by applying the same cyclotron orbit excitation. Again, the intact precursor ions remained unexcited. Now the IR laser was allowed to emit a beam of photons for 50−500 ms, which was sufficient to produce IRMPD of the remaining precursor ions.[24] Another cyclotron orbit excitation event was then produced followed by the frequency detection event. This event was now a frequency chirp covering the whole mass range. Again, a frequency spectrum was acquired. Thus two subsequent tandem mass spectra, one with ECD and another with IRMPD, were acquired from the same population of precursor ions. In the end of the measurement cycle, a "quench pulse" on the trapping plates of the ICR cell was initiated that purged the remaining ions from the cell.

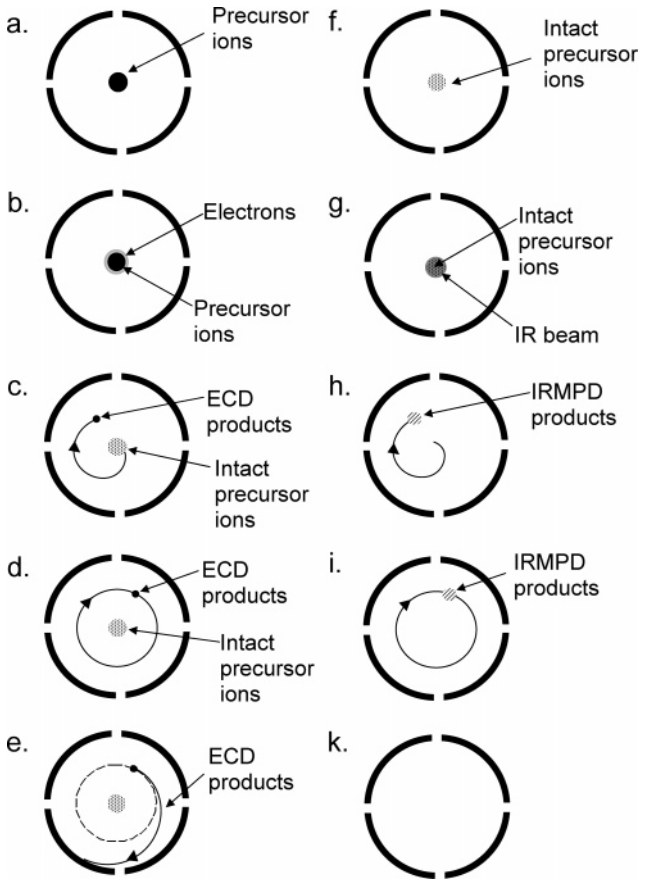

Figure 2. The cross section of the ICR trap at different moments.

To illustrate the ion behavior in the FTICR trap, Figure 2 shows schematically the trap cross section at different moments of the

(21) Tsybin, Yu. O.; Håkansson, P.; Budnik, B. A.; Haselmann, K. F.; Kjeldsen, F.; Gorshkov, M.; Zubarev, R. A. *Rapid Commun. Mass Spectrom.* **2001**, *15,* 1849−1854.

(22) Comisarow, M. B.; Marshall, A. G. *Chem. Phys. Lett.* **1974**, *26,* 489−490.
(23) Comisarow, M. B.; Marshall, A. G. *Chem. Phys. Lett.* **1974**, *25,* 282−283.
(24) Little, D. P.; Speir, J. P.; Senko, M. W.; O'Connor, P. B.; McLafferty, F. W. *Anal. Chem.* **1994**, *66,* 2809−2815.

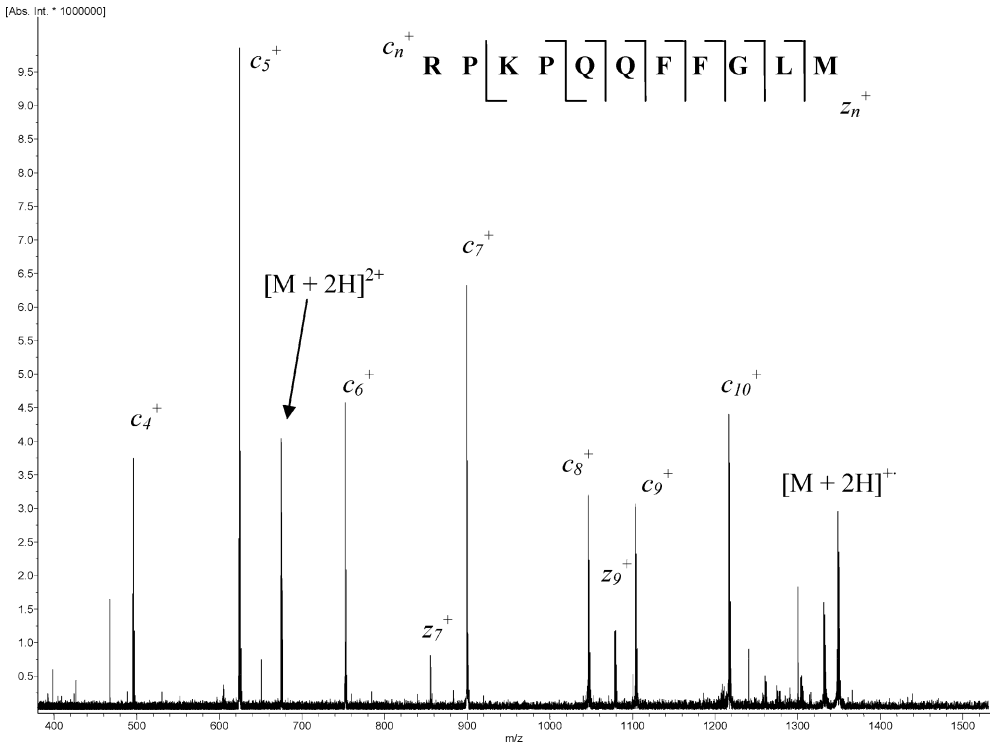

Figure 3. An experimentally obtained ECD mass spectrum (50 ms electron irradiation) of 2+ ions of substance P after selective excitation excluding the precursor ions (the small signal of the latter species is due to parasitic sideband excitation).

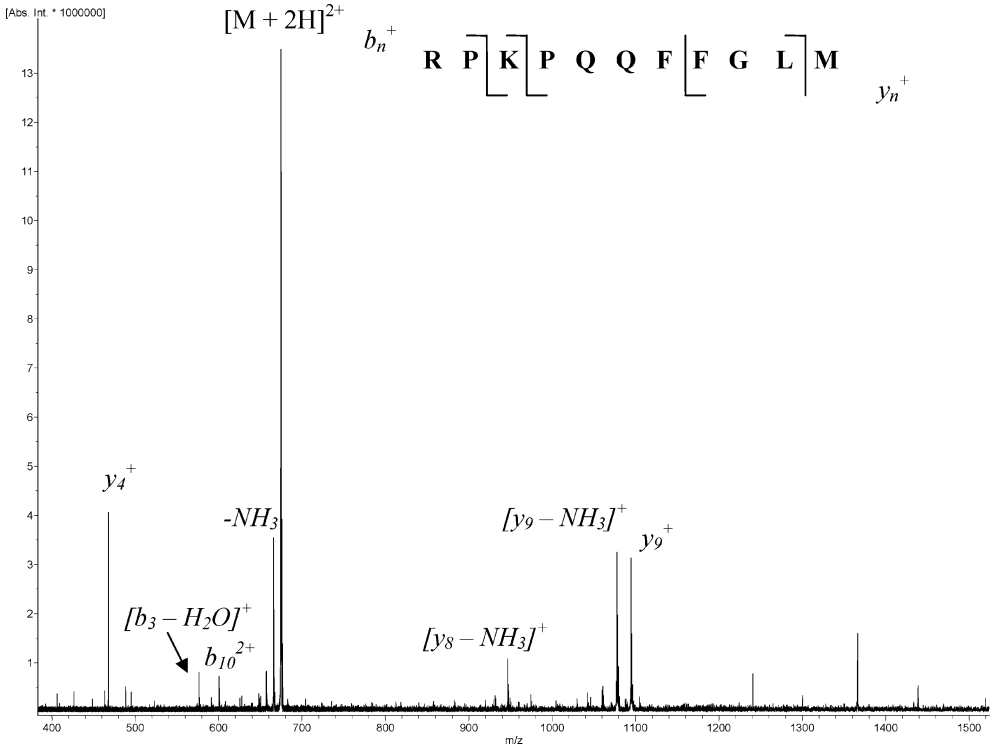

Figure 4. An experimentally obtained IRMPD mass spectrum (500 ms irradiation) of 2+ ions of substance P remaining intact after 50 ms of electron irradiation.

SEICA cycle. The cycle starts with multiply charged polypeptide ions being trapped in a cylindrical ICR trap (cell) (Figure 2a). Upon electron irradiation (Figure 2b), the cyclotron orbits of the product ions are excited (Figure 2c), while the intact precursor ions remain residing in the center of the cell. Upon excitation followed by detection (Figure 2d), the detected product ions are further excited and eliminated from the cell through collisions with the excitation/detection electrodes (Figure 2e). The elimination process does not affect the remaining parent ions that are still circling in small orbits near the center of the trap (Figure 2f). In the next stage of the experiment, the remaining parent ions are exposed to the infrared laser beam and dissociate (Figure

2g). Upon IRMPD, the ion ensemble consisting of the IRMPD products and the parent ions are nonselectively broadband-excited (Figure 2h) and detected (Figure 2i). Finally, the detected ions are eliminated by quenching the ICR trap using a dc voltage pulse at one of the trapping electrodes (Figure 2k). The ICR trap is now ready for the introduction of new ions.

A modified SEICA procedure was applied to build up the population of the precursor ions in the ICR trap and thus effectively improve the ECD efficiency in the "multiscan" experiment. In the modified procedure, the IRMPD event was canceled, the last chirp of excitation excluded the window around the m/z value of the precursor ions, and the same excitation was applied instead of the "quench pulse". The trapping conditions for cations were constantly maintained in the ICR cell, even during the ion transfer from the accumulating multipole and ion capture in the ICR cell. By those means, the population of the precursor ions in the ICR cell was growing from cycle to cycle, reaching a steady level within several cycles. At the steady level, the influx of new ions from the storage multipole at the beginning of each cycle was compensated by the precursor ion loss from the trap due to different reasons.

Sample Preparation. Substance P was purchased from Sigma (St Louis, MO) and used without further purification. This peptide was dissolved in methanol/water (1:1 v/v) and diluted in methanol/water (1:1 v/v) with 0.2% formic acid to a final concentration of 2 pmol/μL.

RESULTS AND DISCUSSION

Figure 3 shows the ECD mass spectrum of doubly charged positive ions of substance P acquired after 50 ms of electron capture dissociation (ECD) with selective excitation of cyclotron frequencies of all ions within the m/z range from 300 to 1800 except a 3 kHz window around the cyclotron frequency of the precursor doubly protonated molecule $[M + 2H]^{2+}$ with m/z 674. A low-intensity signal of the precursor ions was still detected due to the parasitic sideband excitation. The ECD spectrum contains seven c_n^+ and two z_n^+ ions, which together with the accurately measured molecular mass are sufficient to identify uniquely this molecule by a database search. Compared to an ECD spectrum acquired in the conventional mode with excitation of all ions, the fragment ion abundances in the spectrum in Figure 3 were the same.

Figure 4 shows the IRMPD mass spectrum obtained after 500 ms of IRMPD of those substance P dications that did not undergo ECD during the 50 ms long interaction period (Figure 3). The spectrum was acquired with a broadband excitation of cyclotron frequencies of all ions. The spectrum contains three y_n^+ and two b_n^+ ions. Note that IRMPD produced complementary cleavages to ECD, with only one inter-residue bond remaining intact after application of both excitation techniques. Together, spectra in Figures 3 and 4 represent the complementary information obtained by the SEICA approach from a single population of precursor ions. The 500 ms IRMPD irradiation time was by a factor of 4 longer than was required for obtaining a similar degree of fragmentation of the same ions without ECD. This effect is attributed to the increase of the average cyclotron and magnetron radii of unreacted ions after the ECD event (ions with small radii had a higher chance to react with electrons).

The effective improvement in the ECD efficiency by the modified SEICA procedure was monitored via the intensity of the c_5^+ ion of substance P (m/z 624). After integration of 16 consecutive spectra, the average intensity was 9.5 ± 0.2 arbitrary units. In the control experiment, integration of 16 conventional ECD spectra with ICR-cell quenching between individual spectra gave the intensity of only 5.2 ± 0.2 arbitrary units. Thus >80% improvement in the effective ECD efficiency of dications was obtained. The term "effective efficiency" is used here to underline that the ECD efficiency in individual spectra (measured as the ratio of the abundances of ECD products and precursor ions injected into the ICR cell in a *single* "scan") remained the same, but an overall improvement in the intensity of the detected fragment was achieved. This improvement was due to the buildup inside the ICR cell of the population of precursor ions remaining undissociated in the previous "scans".

CONCLUSIONS

The SEICA technique provides 2-fold improvement in the overall efficiency of ion trapping tandem mass spectrometry in at least two situations. When both ECD and IRMPD are desired, SEICA obtains from the same ion population complementary fragmentation spectra in the single-acquisition ("single-scan") regime. If only ECD or IRMPD is required and the precursor ion abundance is low, SEICA improves the effective efficiency by reusing the precursor ions remaining intact in the previous tandem MS "scans". In the latter case, the gain should be higher in ECD, where the efficiency is usually lower than in IRMPD. For the same reason, the greatest improvement is expected for lower charge states of the precursor ions. Although increased gas pressure or quadrupolar axialization were not used in this work, one can expect these features to further improve the efficiency via better confinement and centering of the ion cloud. Combined use of both regimes, i.e., consecutive use of ECD and VE with "multiple-scan" accumulation, should also result in increased efficiency.

The proposed approach is not limited to ECD or to positive ions and can be applied to other ion−electron reactions, such as HECD and EDD, as well as ion−ion reactions, such as electron-transfer dissociation (ETD).[25] Similarly, CID/CAD can be used (e.g. SORI CAD)[26] instead of IRMPD. The inverse use of the techniques, first VE and then an ion−electron reaction, is also possible, although less advantageous.

The implementation of SEICA in other ion-trapping instruments than FTICR mass spectrometers, e.g. quadrupolar three-dimensional or linear ion traps, will require selective ion excitation and detection. Finally, we note that SEICA implementation does not require any hardware modification and is relatively easy on a flexible instrument allowing for user-defined experiments.

ACKNOWLEDGMENT

This work was supported by the Swedish Research Council (grant 621-2002-5025 to RZ) and Wallenberg Consortium North (grant WCN2003-UU/SLU-009).

Received for review October 3, 2004. Accepted February 23, 2005.

AC048531J

(25) Syka, J. E. P.; Coon, J. J.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9528−9533.

(26) Gauthier, J. W.; Trautman, T. R.; Jacobson, D. B. *Anal. Chim. Acta* **1991**, *246*, 211−225.