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Electron capture dissociation of multiply charged peptide cations

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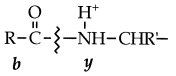
Abstract

For five 12- to 17-mer multiply charged peptide cations, capture of low energy electrons yields unique products, mainly *c* and $z^{\cdot}$ ions from amine bond cleavage. Their mass values for $m > 400$ define the complete sequence for two peptides, all but the ordering of a doublet in another, and all but the partial ordering of a triplet in the other two. The mass values from collisionally activated dissociation (CAD), on the other hand, indicate cleavages of 33 amide bonds (*b* and *y* ion products) of the 68 possible bonds between the amino acids of these peptides. Because the other common methods for ion dissociation yield products similar to those from CAD, electron capture dissociation (ECD) should provide a valuable complementary technique for sequencing of multiply charged peptide cations. (Int J Mass Spectrom 185/186/187 (1999) 787–793) © 1999 Elsevier Science B.V.

Keywords: Electron capture dissociation; Peptides; Fourier transform ion cyclotron resonance; Electroscopy

1. Introduction

Although tandem mass spectrometry (MS/MS) has been used for decades to sequence peptides [2,3], MS-II dissociations of the MS-I separated ions have almost exclusively employed methods that increase the ions' internal energy. These include collisionally activated dissociation (CAD) [3–7], photodissociation (UV [8], IR [9–11]), surface induced dissociation [12–14], and energetic electron impact excitation (EIEIO) [15,16]. For these methods the dominant dissociation is that of the amide bond to yield *b*, *y* [17] products [Eq. (1)]. Further the *b*, *y*



(1)

ion abundances strongly reflect the nature of the neighboring amino acids [18]. Higher energies can increase the number of amide bonds cleaved, but this also increases dissociation to form smaller *b*, *y*, and internal ions. For masses $> \sim 3$ kDa, these methods are only effective for multiply charged ions; the preferred method for their formation is electrospray ionization [19].

As recently reported [20], a new method for MS/MS of multiply charged protein cations, electron capture dissociation (ECD), instead cleaves the amine bond to produce *c*, $z^{\cdot}$ products [Eq. (2)] and minor quantities of $a^{\cdot}$, *y* products [Eq. (3)]. The Eq. (2) cleavage occurs between almost any combination of amino acids, except for that to the cyclic N of Pro

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Dedicated to Professor Michael T. Bowers on the occasion of his 60th birthday.

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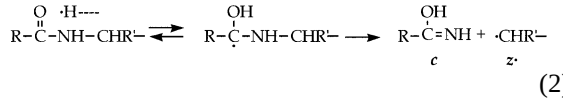

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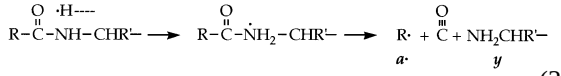

(3)

(two imino bonds) so that ECD was shown to dissociate a far higher proportion of backbone bonds than CAD for 2.8–17 kDa proteins [20]. As reported here, ECD also provides complementary and far more extensive sequence information for peptides.

2. Experimental

HPLC grade MeOH and 99.99% HOAc were obtained from Sigma. A Millipore water purification system supplied 18 MΩ H_2O that was stored in Teflon containers. Lyophilized peptides, graciously provided by Dr. Ted Thannhauser of the Cornell DNA/Peptide Synthesis Facility, were dissolved in 49:49:2 (v/v) H_2O/MeOH/HOAc to make 1–5 μM solutions. Samples were electrosprayed without further purification using a syringe needle biased at 2.7–3.0 KV and a Harvard Apparatus syringe pump at 1 μL/min. The spray was sampled by a heated metal capillary (110 °C) and ions were guided by three rf-only quadrupoles through five stages of differential pumping into a modified open-ended trapped ion cell (10^{-9} Torr) of a modified 6T Finnigan FT/MS-2000 [20,21].

Data were collected and stored using Odyssey v4.0 software, and spectra were externally mass calibrated using bovine ubiquitin from Sigma. Analyses were performed using Mass Spectrometry Visual Data System software written in PV-Wave [22]. Molecular ions of desired charge states were isolated (trapping electrodes at + 4 V, source side, and + 3 V) with stored wave form inverse Fourier transform (SWIFT) [23] and subjected to fragmentation by sustained off-resonance irradiation (SORI) [6,7] or by ECD using three separate events. In each ECD event (trapping electrodes + 1 V), a 10^{-5} Torr pulse of Ar gas precedes the production of electrons from a conventional heated-filament electron gun (0.75–1.0 μA current). The electrons are trapped (− 1 V) between extra outside electrodes at both ends of the cell, with the gas pulse presumably cooling the electrons further (without the outside electrode trapping, the ECD efficiency is reduced by ~ 25%). To allow ions to cool, a delay is effected after each event and before excitation and detection. Both ECD and CAD spectra represent single scans, but only above m/z 400, so that smaller ion products were not measured.

3. Results and discussion

Structural information from CAD and ECD spectra is compared here for 12- (2+), 12- (2+, 3+), 15- (2+, 3+), 17- (3+), and 17-mer (4+, 5+) peptides (Figs. 1–5). The most obvious limitation of ECD is that the cation dissociated must have at least two charges. However, for peptides too small for efficient multiple charging (10–12 residues), CAD methods usually provide extensive sequence information. Note that the sequence information from the CAD data would have been far more complete if masses below m/z 400 had been scanned.

3.1. Nondissociative electron capture

One of the most abundant ECD products arises from reduction, $(M + nH)^{n+} + e^- \rightarrow (M + nH)^{(n-1)+\cdot}$, without subsequent dissociation (Figs. 1–5). These correspond in mass to odd-electron ions, and their subsequent CAD dissociation also yields c, $z^\cdot$ products [20]. Although more highly charged precursors can show multiple reduction products [20], $(M + 4H)^{2+}$ is at the noise level in Fig. 5.

3.2. Amine bond cleavage, c, z·

The dominant ECD cleavage of proteins, Eq. 2 [20], also accounts for the most abundant peptide ECD products, c and $z^\cdot$ ions (Figs. 1–5). Smaller yields of these products that have gained or lost a hydrogen atom, such as $c^\cdot$ and z, cause deviations in

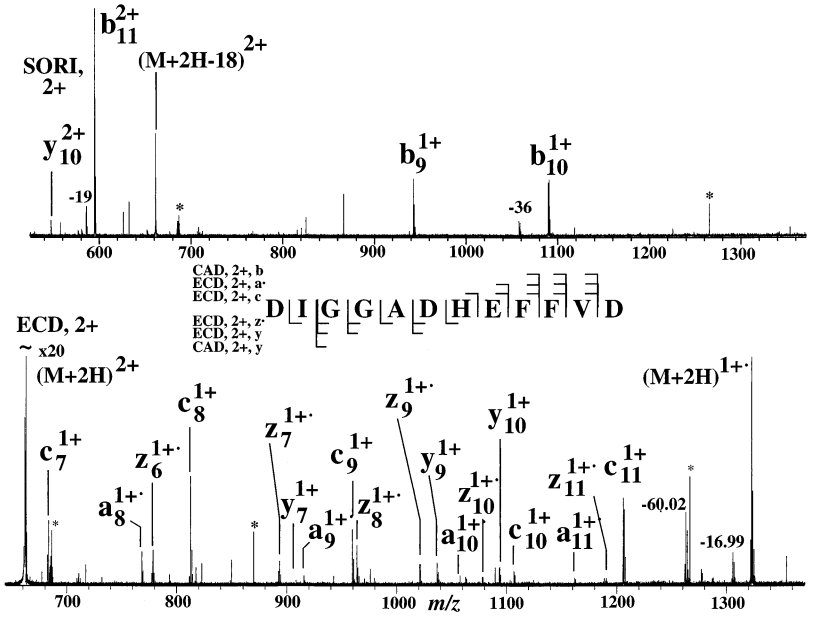

Fig. 1. SORI and ECD spectra of DIGGADHEFFVD. Cleavages indicated by vertical lines between amino acids form (above) N-terminal and (below) C-terminal fragment ions. The asterisks represent artifact peaks.

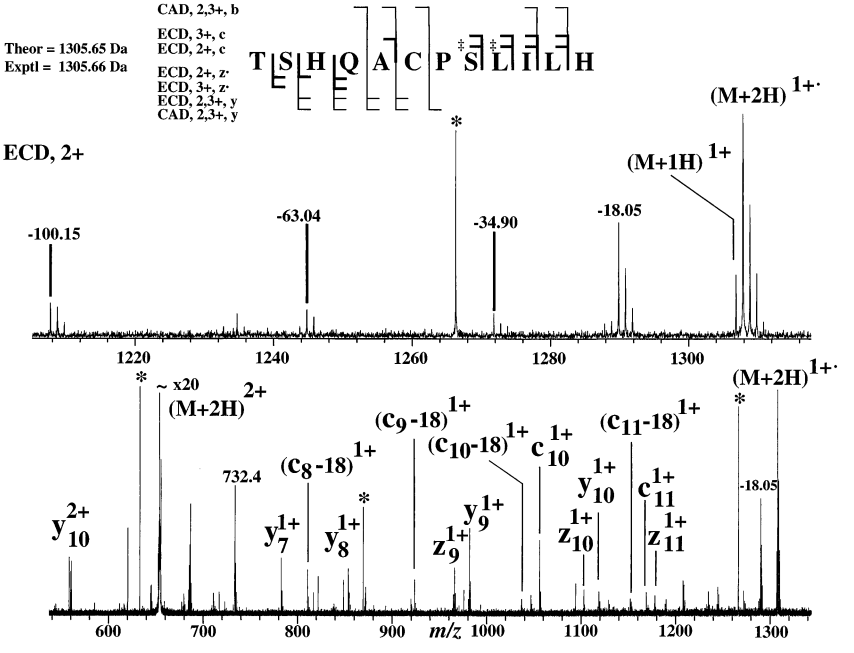

Fig. 2. ECD spectrum of TSHQACPSLILH. The double dagger represents a $(c - H_2O)^+$ ion.

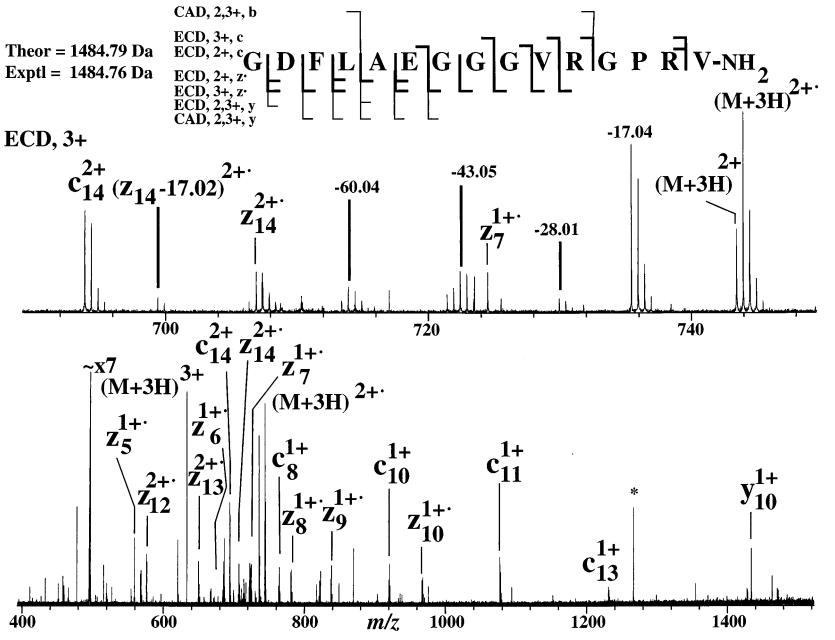

Fig. 3. ECD spectrum of GDFLAEGGGBRGPRV-NH_2.

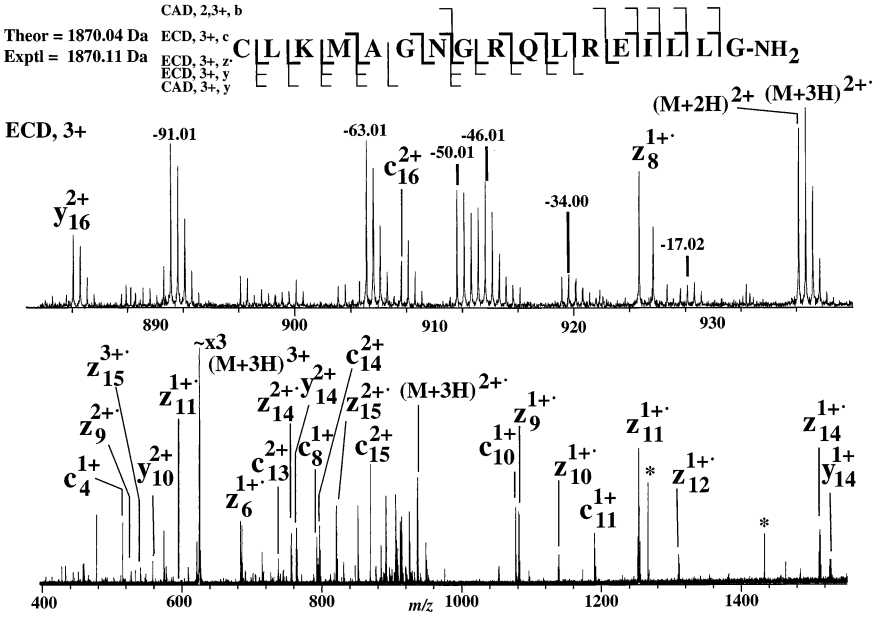

Fig. 4. ECD spectrum of CLKMAGNGRQLREILLG-NH_2.

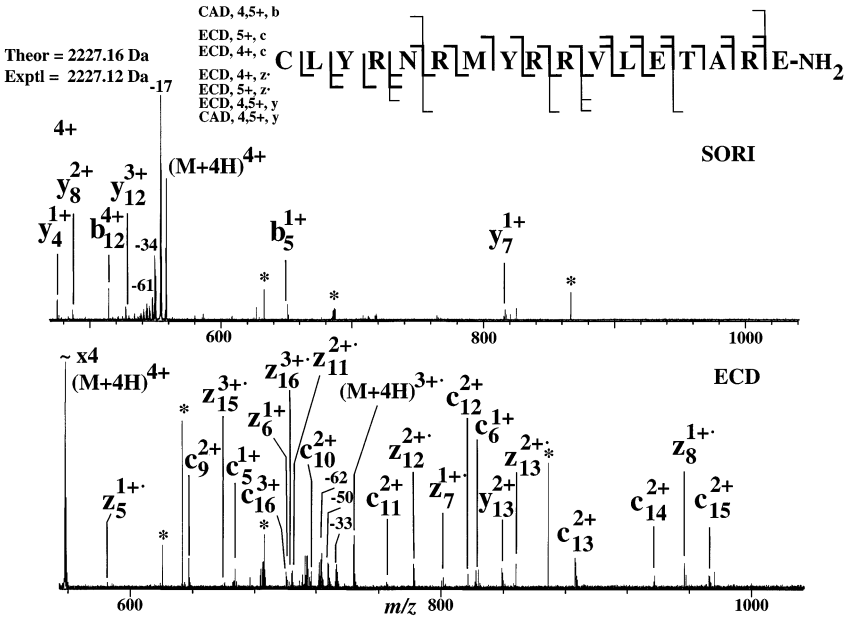

Fig. 5. SORI and ECD spectra of CLYRNRMYRRVLETARE-NH_2.

the expected isotopic peak abundances. A few $a\cdot$ and y products [Eq. (3)] are also observed. Thus ECD dissociations of peptides are not as specific as those of proteins, as already observed for the corresponding CAD dissociations. Presumably, the far greater number of degrees of freedom in proteins leads to a higher proportion of lowest energy backbone dissociations, Eq. (1) for CAD [7,9,17] and Eq. (2) for ECD. (The even gentler method, blackbody infrared dissociation, increases the loss of H_2O and other small neutrals in addition to Eq. (1) [10,11].)

Basically, MS/MS sequence ordering of two adjacent amino acids requires cleavage of a backbone bond between them. These ECD spectra represent cleavages of a far higher proportion of such backbone bonds than those from CAD (SORI) [6,7]. The most obvious exception is the N-terminal side of the Pro nitrogen, whose c, $z\cdot$ products would require the cleavage of its two imino bonds to N. Fortunately, this resistance to cleavage also provides sequence information, and Pro cleavage is favored in CAD spectra [4,6,7,18]. For the peptide containing multiple side chain–OH groups (2 Ser, 1 Thr, Fig. 2) the c_8 and c_{11} ions show substantial loss of water, with only these H_2O-loss ions measurable for c_8 and c_9. For the $a\cdot$, y [Eq. (3)] products, only y_8 in Fig. 2 indicates an ECD cleavage not shown by c, $z\cdot$ products. The five underivatized peptides contain a total of 68 connected pairs of amino acids; all but five of these are ECD cleaved, with two of these at the imino N of Pro. Smaller ions ($m < 400$) were not scanned; these could include additional products of cleavages near the two termini. The five ECD spectra show no internal ions from secondary fragmentations, as also observed for proteins [20].

In contrast, CAD of these five peptides has caused cleavage of only 33 of the 68 amide bonds. Note, however, the complementary nature of the ECD and CAD cleavages that allows N-terminal fragment ions to be distinguished from C-terminal; an ECD $z\cdot$ will be 16.02 Da lighter than the CAD y ion from cleavage between the same amino acids, whereas the ECD c ion will be 17.03 Da heavier than the corresponding CAD b ion (the extra 1.008 Da is due to H· from e^- reduction of H^+). The CAD spectra show a few internal ions from secondary fragmentation of b, y ions, but these do not involve cleavage of bonds in addition to those represented by the b, y ions.

3.3. ECD loss of small neutrals

In CAD spectra, ions formed by the loss of H_2O, NH_3, and other small neutrals provide little specific structural information. Such ions are less abundant in ECD spectra. Adjacent to the isotopic cluster of the reduced $(M + nH)^{(n-1)+}$ ions, ECD spectra show a variable amount of $[M + (n - 1)H]^{(n-1)+}$ from loss of a hydrogen atom, H· (Figs. 1–5). As pointed out [20], this should occur readily by e^- neutralization of the protonated basic amino acids His [24], Lys, and Arg, especially from hypervalent species such as $R - NH_3$. However, the relatively low abundance of H· loss found is postulated to result from subsequent H· capture at other sites [Eqs. (2), (3)], an explanation for the surprisingly facile ECD cleavage at S–S bonds [25].

Product ions formed by the loss of other small neutrals from $(M + nH)^{(n-1)+\cdot}$ are more common for these peptides than for proteins (e.g. H_2O loss, Fig. 2, vide supra). In addition to the losses of H·, NH_3, $(H_2N)_2C\cdot$, and $(H_2N)_2C{=}{=}NH$ (44 and 59, from Arg) found for proteins [20,25], small additional peaks corresponding to losses within 1 or 2 Da of these values and of 28 Da (Figs. 1 and 3) could be attributed to a variety of C, H, N, and O losses. In addition, the peptides that contain Cys (Figs. 2, 4, and 5) show losses that probably involve its $-CH_2SH$ group: 32–35 (H_nS), 46–50 (H_nCS, H_nNS), 61–64 (H_nCNS?), and even larger mass losses. This could result from initial H· capture at the −SH, reflecting the postulated favorability of H· capture at −S–S− [25]. This type of loss appears to be much less important for the $-CH_2OH$ groups of Ser and Thr, with H_2O loss substantial only for the two Ser, one Thr peptide of Fig. 2 (ECD of 2+ ions; far less 18 loss for the 3+ ions) and not for the Thr−peptide of Fig. 5. Peaks for H_2O loss (also NH_3 loss if C-terminal $-NH_2$) are generally much larger in the SORI spectra. Identifying these several losses by isotopic labeling or exact mass measurement is not critical for sequencing, as none is coincident with the mass losses expected for a small N− or C−terminal amino acid. These losses correspond [Eq. (2)] to $H_2NCHRC(OH){=}{=}NH$ and $\cdot CHRCOOH$, losses of 74 (Fig. 3) and 59 (− 58 in Fig. 4, C-terminal NH_2) for Gly (R = H) and of 88 and 73 for Ala ($R = CH_3$).

4. Conclusions

The most obvious advantage of ECD for peptides is that ECD spectra show more extensive sequence information than do CAD spectra, as well as the complementarity of the information from both types of spectra. ECD is far more effective than CAD in cleaving S–S bonds [25]. The efficiency of producing fragment ions (summed ion currents) by ECD is only one-third of that by CAD [20], a problem for very limited sample sizes. Implementation of an ECD capability should be relatively straightforward for FTMS (the extra trapping electrodes used here are not critical, giving a relatively small efficiency increase) and, presumably, for ion trap instruments; this could even be achieved in magnetic sector and time-of-flight instruments by matching the velocities of the electron and ion. Hopefully, instrument manufacturers will respond promptly to this need.

Acknowledgements

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References

[1] This milestone birthday for Mike Bowers sounded like a publication error to many of us—he still acts like a vigorous, young scientist of wonderfully broad interests. However, there is also highly convincing evidence for his published birth date; how could his exceptionally impressive and diverse scientific contributions have been achieved in a shorter time? Mike, on an even more personal note, the stimulation and fun you have brought to my favorite field also deserves my (FWM) enthusiastic “Thank You.”

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