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Electron capture induced dissociation of peptide ions: Identification of neutral fragments from secondary collisions with cesium vapor

Preben Hvelplund [a], Bo Liu [b], Steen Brøndsted Nielsen [a,*], Subhasis Panja [a], Jean-Christophe Poully [a,c], Kristian Støchkel [a]

[a] *Department of Physics and Astronomy, University of Aarhus, Ny Munkegade, DK-8000 Aarhus C, Denmark*
[b] *School of Physics and Electronics, Henan University, Kaifeng, 475001, China*
[c] *Laboratoire de Physique des Lasers du CNRS, Institut Galilée, Université Paris-Nord, 93430 Villetaneuse, France*

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Abstract

In high-energy collisions between peptide dications and cesium vapor, cations picked up an electron, which led to N–C_α bond breakage to give the characteristic c^+ and $z^{\bullet +}$ type fragments together with their complementary neutral fragments. Neutrals were converted to anions in secondary collisions with cesium. Peaks corresponding to z^- anions dominate the product ion spectra, whereas $c^{\bullet -}$ anions could not with certainty be identified, instead O^-, OH^-, CN^-, CNH^-, C_2O^-, and OCN^- were formed. A fragment ion is also assigned to $HCOO^-$ formed from dissociation of z^-. We ascribe the outcome of secondary collisions to the fact that a $z^\bullet$ fragment is a radical that when accepts an electron becomes a stable carbanion whereas electron capture to an even-electron c fragment produces a reactive radical anion that dissociates within the timescale of the experiment (few microseconds). Such an experimental scheme in which anions are measured implies that we are not limited to the study of dications; indeed, selective N–C_α bond cleavages of peptide monocations were identified from negative fragment ion spectra that were again dominated by z^- type ions. Neutral y fragments from collision-induced dissociation are also easily detected as y^- ions after electron pickup in secondary collisions.

Keywords: Peptide; Electron transfer; Charge reversal; High-energy collisions; Selective bond cleavage

1. Introduction

Mass spectrometry is used successfully to elucidate the primary structure of peptides and proteins. The standard method is to induce fragmentation by low-energy collisions (collision-induced dissociation, CID), often under multiple collision conditions [1], but also high-energy CID is applied [2]. However, CID produces several fragments that are hard to assign, and secondly, CID often generates much too specific cleavages which results in a few abundant peaks and not much else. These problems have more or less been overcome with the technique electron capture dissociation (ECD), which is based on the capture of low-energy electrons by ions in the cell of a Fourier transform ion cyclotron resonance (FT-ICR) instrument [3,4] or in a quadrupole ion trap [5–7]. Electron capture leads to breakage of specific bonds in the peptide chain with selective formation of the so-called c- and z-ions. Importantly, compared to CID, ECD generates a more even backbone fragmentation pattern that is easier to assign. Nonstatistical (nonergodic) processes seem to be of importance but the mechanism of ECD is still being hotly debated [4,8–15].

We mimic the ECD experiments by transfer of an electron from an alkali metal atom to a peptide of high translational energy (50–100 keV) (Scheme 1) [15,16]. The experiments are based on the combination of an electrospray ion source with an accelerator mass spectrometer [16]: protonated peptides are accelerated to tens of keV kinetic energy, mass-to-charge selected and passed through a heated Na or Cs vapor cell. During the few fs interaction time of the collision, transfer of the valence shell electron of the metal to a peptide ion can occur. The electron transfer is to a good approximation a vertical process in which the nuclei lie still. Alkali metal atoms are special in the sense that the outer electron is loosely bound and therefore easy to transfer to the projectile ion. We have dubbed this technique

* Corresponding author.
E-mail address: sbn@phys.au.dk (S.B. Nielsen).

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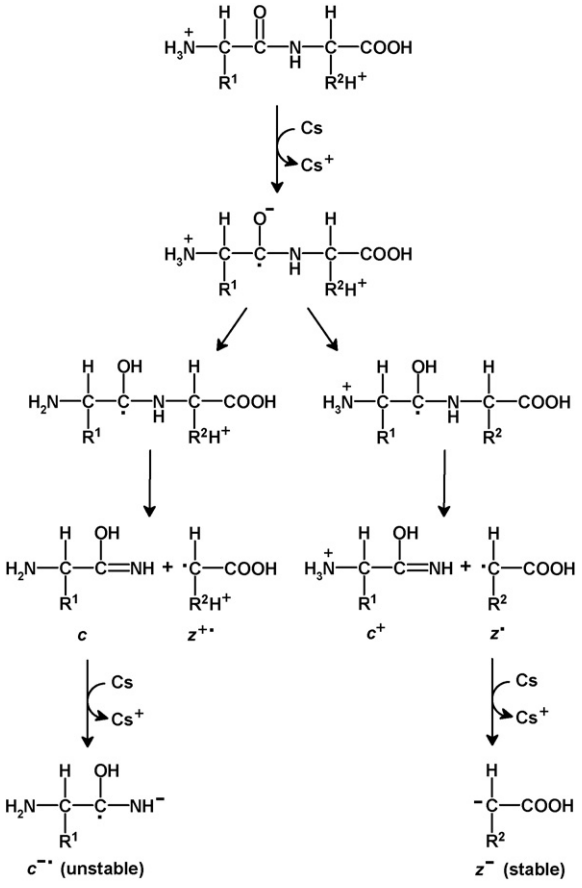

Scheme 1. Electron capture induced dissociation of a doubly protonated dipeptide followed by a second electron transfer from cesium to neutral fragments. R^1 and R^2 are amino acid side chains, e.g., $R^1 = CH_3$ and $R^2 = (CH_2)_4NH_2$ in $[AK + 2H]^{2+}$. The capture of the first electron to the amide group oxygen is based on the model suggested by Syrstad and Tureček [10].

electron capture induced dissociation (ECID). The actual recombination energy is not known since we do not know what state the electron is transferred to. In addition to electron capture, ion activation occurs for collisions with small impact parameters, a process that plays a role for large peptides with significant geometrical cross-sections. ECID is closely related to the technique of electron transfer dissociation (ETD) in which an electron is transferred from singly charged anions to multiply protonated peptides in a quadrupole ion trap [17,18].

Previous results from the Aarhus group have shown that the fragment ions of substance P and bradykinin peptides from ECID are nearly identical to those observed after capture of a free electron [15,16]. The dissociation timescale, corresponding to the flight time from the collision cell to the analyzer, is a few μs, whereas the timescale for FT-ICR mass spectrometry experiments which use free electrons is several milliseconds to seconds. The ECID method makes it possible to study early events in the dissociation process that cannot readily be observed using other methods.

In the present work we report that neutral $z^\bullet$ radicals formed after one-electron transfer from cesium to peptide dications or monocations can be identified from a second collision in which they capture an electron to become stable carbanions. Neutral c fragments on the other hand dissociate after electron capture to give a number of low mass anions.

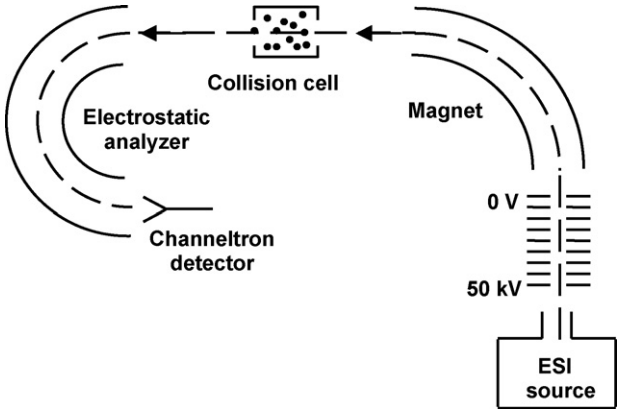

Fig. 1. The accelerator mass spectrometer in Aarhus used for electron capture induced dissociation experiments. See text for details.

2. Experiments

The experimental setup has been described in detail elsewhere [19,20]. Briefly, doubly and singly protonated peptides were generated by electrospray (1:1 water/methanol with 5% acetic acid), introduced into an accelerator mass spectrometer, accelerated to 100 and 50 keV, *m/z* selected and passed through a heated Cs vapor cell (Fig. 1). During the few fs interaction time of the collision, electron transfer of the 6s electron of Cs to a peptide ion can occur. The Cs vapor pressure was kept high to allow for secondary collisions. Both positive and negative fragment ions were analyzed by a hemispherical electrostatic analyzer in separate experiments. We will use the abbreviations $^+ECID^+$ and $^+ECID^-$ when a positive and a negative fragment ion spectrum is recorded, in both cases the parent ion entering the collision cell being positively charged. A linear calibration between analyzer voltage and ion mass was done based on two points: for positive ions zero voltage was taken to be zero mass and a voltage difference between the two analyzer plates of 10.4 kV corresponded to the mass of the parent ion. The same calibration was applied for negative ions, which introduces an uncertainty of about 1 amu.

3. Results and discussion

First we studied the outcome of the collisions between dipeptide cations and cesium vapor. The $^+ECID^+$ spectrum of $[AK + 2H]^{2+}$ is shown in Fig. 2a. It is evident that electron capture to $[AK + 2H]^{2+}$ (A = alanine, K = lysine amino acid residues) led to dominant cleavage of the N–C_α bond to give $z^{\bullet+}$ ions as was reported earlier [15]. A scan of negative ions formed in secondary collisions with cesium showed strong formation of z^- even-electron carbanions (Fig. 2b). They are the result of electron capture to neutral $z^\bullet$ fragments (Scheme 1). The flight time through the collision cell was less than 1 μs, which implies that N–C_α bond breakage occurred on a submicrosecond timescale. The negative ion fragment spectrum of $[AK + H]^+$ is quite similar to that obtained of $[AK + 2H]^{2+}$ (Fig. 2c). For

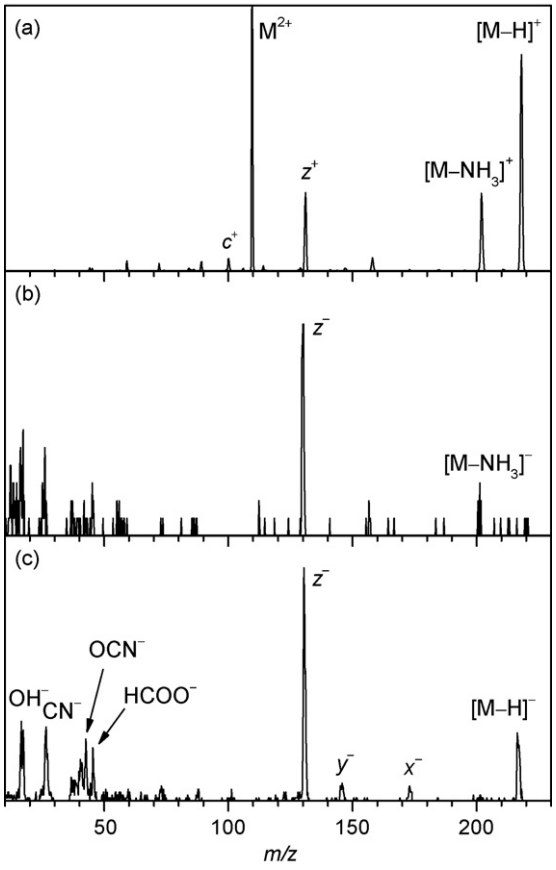

Fig. 2. Product ion spectra obtained from the collisions between $[AK+2H]^{2+}$ ($=M^{2+}$) and Cs. (a) Positive ion fragments. (b) Negative ion fragments. (c) Product ion spectrum obtained from the collisions between $[AK+H]^{+}$ ($=M^{+}$) and Cs. Negative ion fragments.

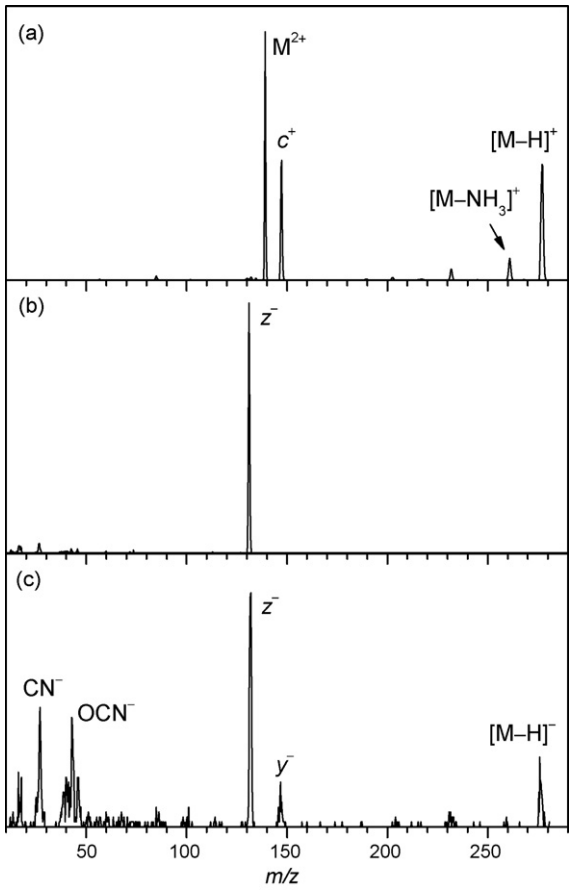

Fig. 3. Product ion spectra obtained from the collisions between $[KK+2H]^{2+}$ ($=M^{2+}$) and Cs. (a) Positive ion fragments. (b) Negative ion fragments. (c) Product ion spectrum obtained from the collisions between $[KK+H]^{+}$ ($=M^{+}$) and Cs. Negative ion fragments.

both precursor ions, peaks corresponding to low-mass fragment anions such as O^-, OH^-, CN^-, CNH^-, C_2O^-, and OCN^- are observed. They likely originate from the fragmentation of unstable $c^{\bullet -}$ anion radicals (Scheme 1). A peak at *m/z* 45 is assigned to $HCOO^-$ formed from z^-. Minor peaks in the $^+ECID^-$ spectrum of $[AK+H]^+$ correspond to y^- and x^- fragment ions; the corresponding neutral x and y fragments arise from collision induced dissociation of $[AK+H]^+$ and are due to the cleavage of the C_α–C(O) bond and the amide bond (C(O)–N), respectively. The corresponding a^+ and b^+ ions were observed in an experiment with neon (CID only). In summary, negatively charged z fragments can be clearly discriminated from $c^{\bullet -}$, and, importantly, singly charged peptides can be subject for study. Finally, we note that Kleinnijenhuis et al. [21] have reported that secondary electron capture to lantibiotics stabilizes the radical in agreement with our results.

In another experiment, $[KK+2H]^{2+}$ and $[KK+H]^+$ peptide ions were collided with cesium. Electron transfer to $[KK+2H]^{2+}$ led dominantly to the production of c^+ ions and neutral $z^\bullet$ fragments (Fig. 3a). The $^+ECID^-$ spectrum of $[KK+2H]^{2+}$ reveals an abundant formation of z fragments (Fig. 3b). This fragment peak is much stronger than in the case of $[AK+2H]^{2+}$ where $z^{\bullet +}$ were formed dominantly together with neutral c fragments. Singly charged precursor ions also resulted in z^- ions being formed after the second collision (Fig. 3c) but the signal is reduced compared to the total signal from the low-mass anions since now two neutral fragments were formed in equal abundance every time a monocation captured an electron and dissociated. The peaks in the low-mass region are the same as those observed for the AK peptide. This particular example demonstrates how z fragments not being abundant in the positive fragment ion spectra can be identified as negative ions instead. Thus, the complementary c^+ and z^- ions are both measured. There is also a peak from $y^{\bullet -}$ in the $^+ECID^-$ spectrum of $[KK+H]^+$.

When the $[GHK+2H]^{2+}$ dication (G = glycine, H = histidine amino acid residues) captured an electron, the dominant ions formed after N–C_α bond cleavage were c_2^+ and $z_2^{\bullet +}$ (Fig. 4a). The complementary $z_1^\bullet$ fragment formed together with c_2^+ was measured as a negative ion after a second collision (Fig. 4b). In collisions between $[GHK+H]^+$ and Cs, both $z_1^\bullet$ and $z_2^\bullet$ were formed as revealed from the peaks corresponding to z_1^- and z_2^- in the $^+ECID^-$ spectrum (Fig. 4c). This experiment shows how the charge state of the peptide dictates the relative yield of $z^\bullet$ fragments, which may be a result of different conformations of the monocation and dication. The monocation is likely more

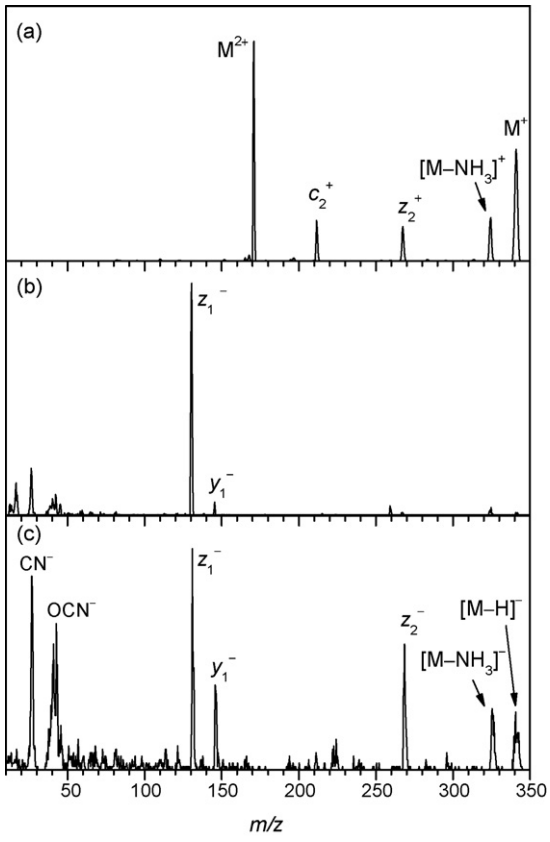

Fig. 4. Product ion spectra obtained from the collisions between $[GHK + 2H]^{2+}$ ($=M^{2+}$) and Cs. (a) Positive ion fragments. (b) Negative ion fragments. (c) Product ion spectrum obtained from the collisions between $[GHK + H]^{+}$ ($=M^{+}$) and Cs. Negative ion fragments.

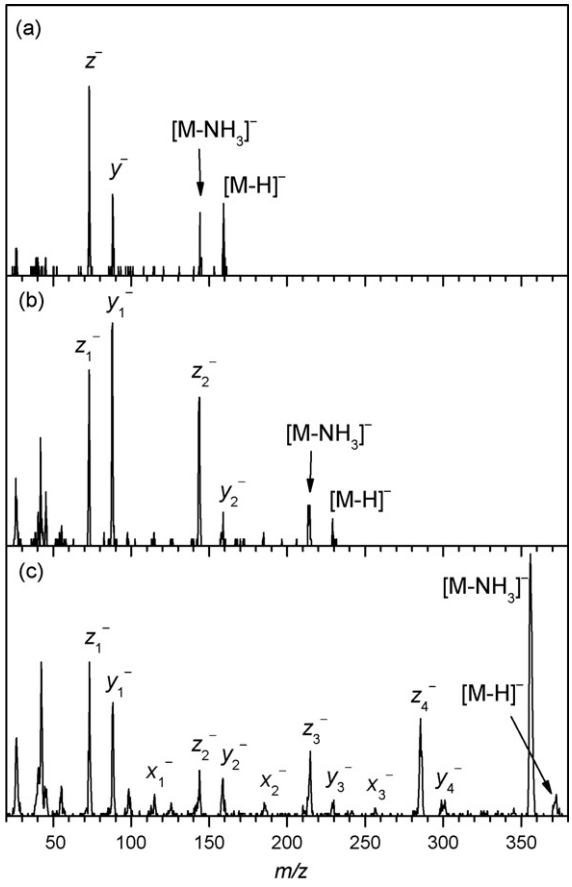

Fig. 5. Negative ion product spectra obtained from the collisions between Cs and (a) $[AA + H]^{+}$, (b) $[AAA + H]^{+}$, and (c) $[AAAAA + H]^{+}$.

folded than the dication since the dication has a larger propensity for a more extended structure due to the Coulomb repulsion between the two positive charges. The influence of conformation and charge state has been discussed in several papers [22–30]. For example, Williams and coworkers [22] found that the charge state of a 16-mer peptide of alanine and lysine residues determined the range of z and c fragment ions; in their case the charge state varied between two and five. Interestingly, they did not see a change in the fragment ion distribution from heating of the peptide to 150 °C and concluded that the limited products are due to backbone cleavages occurring near charges, in agreement with work by McLafferty and coworkers [23,24]. On the other hand, Mihalca et al. [25] reported that the ECD fragments of substance P and gramicidin S provide a snapshot of the conformational heterogeneity since for these two peptides fewer fragments were observed at low temperature.

In the $^{+}ECID^{-}$ spectrum of $[GHK + H]^{+}$ a peak from the $y_1{}^{-}$ ion is also present. The neutral y_1 fragment is formed together with the $b_2{}^{+}$ ion after the first collision process, and this neutral fragment picks up an electron in the second collision to become $y_1{}^{-}$. Interestingly, the abundance of O^{-} and OH^{-} is much lower for $[GHK + H]^{+}$ than those for the other precursor ions.

Finally, we investigated peptides that can only become singly protonated under normal electrospray conditions and therefore not amenable for conventional ECD experiments. It should be mentioned that an alternative strategy to study peptides with no basic side chains is to use divalent metals as charge carriers, which also circumvents the multiple charging issue in ECD [31–33]. Fig. 5 shows the $^{+}ECID^{-}$ spectra of $[AA + H]^{+}$, $[AAA + H]^{+}$, and $[AAAAA + H]^{+}$. The proton is sequestered on the N-terminal amino group. Again peaks corresponding to z^{-} ions are dominant, but y^{-} ions are also formed. In ECD, y^{+} cations are frequently detected [4,34]. However, in this work, the origin of y^{-} anions is most likely a result of electron capture to y neutrals formed after collision-induced dissociation since a large abundance of y^{-} ions correlates quite well with an abundant formation of b^{+} ions (data not shown).

In all of the $^{+}ECID^{-}$ spectra of singly charged peptide cations there are peaks corresponding to the peptide anions formed from two electron transfer collisions without fragmentation in between, except for hydrogen loss. Hence not all peptides that have captured an electron dissociated before the second collision, and it is possible that some of the fragment anions were formed from the dissociation of the peptide anions and not purely from capture to neutral peptide fragments. In agreement with this, O'Connor and co-workers [35] have shown that lifetimes of radical intermediates can be milliseconds to seconds, and we have demonstrated microsecond to millisecond timescale for dissociation of a reduced decapeptide [36]. Furthermore, it was recently shown by Lam and Chu [37] that peptide radi-

cal anions of angiotensin III derivatives, formed from CID of a negatively charged metal-peptide complex, dissociated into z-type ions predominantly and x-type ions to some extent. Also side-chain cleavages occurred [37].

4. Conclusions

Electron capture to peptide dications led to $z^{\bullet +}$ radical cations together with neutral c fragments or neutral $z^{\bullet}$ radicals together with c^+ cations. Neutrals picked up an electron in collisions with cesium, which resulted in the formation of z^- ions that were stable enough to be measured on a microsecond timescale. In contrast, $c^{\bullet -}$ radical anions dissociated before their detection. Hence z^- ions stand out in the negative fragment ion mass spectra and complement the measurement of c^+ ions, especially of importance when peaks from $z^{\bullet +}$ ions are absent in the positive fragment ion mass spectrum. This technique therefore provides additional information on the sequence-specific peaks, and, importantly, it allows for the study of electron-induced dissociation of singly charged peptide cations. ECID could therefore be successfully combined with MALDI which mainly produces singly charged ions. The distribution of z^- ions for tripeptides or larger peptides is strongly dependent on the peptide charge state, a finding that may be useful in future work for shedding light on the mechanism behind ECD. Also the systems under study here are relatively small and may be subject for future theoretical calculations in order to provide information on the connection between the charge state of the peptide and N–C_α bonds dominantly being cleaved.

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