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Electron Capture Dissociation of Hydrogen-Deficient Peptide Radical Cations

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Abstract

Hydrogen-deficient peptide radical cations exhibit fascinating gas phase chemistry, which is governed by radical driven dissociation and, in many cases, by a combination of radical and charge driven fragmentation. Here we examine electron capture dissociation (ECD) of doubly, $[M + H]^{2+\bullet}$, and triply, $[M + 2H]^{3+\bullet}$, charged hydrogen-deficient species, aiming to investigate the effect of a hydrogen-deficient radical site on the ECD outcome and characterize the dissociation pathways of hydrogen-deficient species in ECD. ECD of $[M + H]^{2+\bullet}$ and $[M + 2H]^{3+\bullet}$ precursor ions resulted in efficient electron capture by the hydrogen-deficient species. However, the intensities of c- and z-type product ions were reduced, compared with those observed for the even electron species, indicating suppression of $N–C_\alpha$ backbone bond cleavages. We postulate that radical recombination occurs after the initial electron capture event leading to a stable even electron intermediate, which does not trigger $N–C_\alpha$ bond dissociations. Although the intensities of c- and z-type product ions were reduced, the number of backbone bond cleavages remained largely unaffected between the ECD spectra of the even electron and hydrogen-deficient species. We hypothesize that a small ion population exist as a biradical, which can trigger $N–C_\alpha$ bond cleavages. Alternatively, radical recombination and $N–C_\alpha$ bond cleavages can be in competition, with radical recombination being the dominant pathway and $N–C_\alpha$ cleavages occurring to a lesser degree. Formation of b- and y-type ions observed for two of the hydrogen-deficient peptides examined is also discussed.

Key words: Electron capture dissociation, Hydrogen-deficient peptide radical cations, Radical recombination, Suppressed $N–C_\alpha$ Bond Cleavages

Introduction

Formation and dissociation of hydrogen-deficient species has attracted considerable attention as these species exhibit exciting gas phase chemistry that is distinct from that of the even electron species. Hydrogen-deficient radical cations can be produced in the gas phase by collision-induced dissociation (CID) of ternary metal complexes, $[Metal(L)M]^{n+}$, containing the peptide (M) and an auxiliary ligand (L) [1–4]. Following dissociation of the metal-ligand-peptide complex, $[Metal(L)M]^{n+}$, an electron is removed from the peptide by the metal-auxiliary ligand system resulting in the generation of a radical species. In addition to this approach several other methods have been employed for the formation of hydrogen-deficient peptide radical cations in the gas phase [5–8]. Regardless of which of these methods [1–8] is used, the resulting peptide radical cations contain one less hydrogen, $[M + nH]^{(n+1)+\bullet}$, relative to the protonated molecular ion and, as such, they are distinct from the radical cations formed in electron capture dissociation (ECD) [9]. The latter contain an extra

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hydrogen, $[M + nH]^{(n-1)+\bullet}$, relative to their protonated counterparts and, therefore, are hydrogen-surplus species.

Dissociation of hydrogen-deficient species is determined by the competition between radical and charge driven processes [10–15]. For singly charged arginine containing radical cations, $M^{+\bullet}$, dissociation is governed by radical driven processes resulting mainly in C_a–C bond cleavages and side chain losses [12, 13, 16]. When arginine is located at the N-terminus, formation of a-type product ions is favorable, whereas x- and z-ions are formed when the arginine is located at the C-terminus [13]. It has been suggested that the highly basic arginine side chain sequesters the proton and, therefore, fragmentations proceed through radical driven processes [11–13, 15]. It has recently been demonstrated that by replacing arginine with the less basic lysine residue, charge-induced dissociation becomes dominant resulting in the formation of b- and y-type product ions [12].

These observations [12] are in agreement with previous reports in which hydrogen-deficient peptides containing lysine or histidine [12, 13, 15, 16] or lacking a basic residue [1, 17–19] dissociated via both charge and radical driven processes. Similar trends were observed for singly charged radical cations containing two arginine residues, such as bradykinin derivatives [16, 20]. Dissociation of doubly, $[M + H]^{2+\bullet}$, and triply, $[M + 2H]^{3+\bullet}$, charged species has also been examined [5, 16, 20–22]. Formation of a-, b-, c-, y-, x-, and z-type product ions along with side chain losses were observed [5, 16, 20–22].

It is mainly believed that radical-directed dissociation leads to the formation of a-, x-, c-, and z-type product ions and side chain losses [13, 16, 19, 20], whereas formation of b- and y-ions is due to charge driven pathways [13, 15]. In general, depending on the availability of the mobile proton, radical and charge directed fragmentation can contribute to the dissociation outcome. Another important factor influencing dissociation of hydrogen-deficient species is radical migration, which leads to cleavages at positions remote from the initial radical site [11, 23, 24].

In contrast to hydrogen-deficient species, a different type of radical cations, classified as hydrogen-rich radicals, can be produced in the gas phase by ECD [9]. In ECD molecular ions, $[M + nH]^{n+}$, are irradiated with low energy electrons (<1 eV) resulting in electron capture and formation of a charge-reduced species $[M + nH]^{(n-1)+\bullet}$. The charge-reduced species dissociates through N–C_a bond cleavages to produce c- and z-type product ions.

The mechanism(s) by which the N–C_a bond cleavages occur following the initial electron capture event has been the focus of several investigations [9, 25–33]. It is believed that following electron capture, a hydrogen atom is transferred to an amide carbonyl [9, 27, 28, 33] or a proton is transferred to an amide anion radical [25, 26, 29, 32], both resulting in the formation of an aminoketyl radical intermediate, which dissociates through N–C_a bond cleavages. Electron capture can occur at a positively charged site [9, 27, 28, 33]. Alternatively, it has been shown that electron capture can occur either directly in an amide π^* orbital that is stabilized by Coulomb interactions or into a Rydberg orbital on a positively charged site with subsequent electron transfer to a Coulomb stabilized amide π^*-orbital [25, 26, 29, 32]. The latter mechanism, referred to as the Utah-Washington (UW) mechanism [25, 26, 29, 32], does not require hydrogen atom transfer, and it was introduced to explain formation of c- and z-type product ions in peptides, in which the charge carriers are arginine residues or metal ions.

ECD on peptides carrying fixed charge tags [34, 35] and electron traps [36] has been performed to examine the effect of these groups on the dissociation outcome. For instance, 2,4,6-trimethylpyridinium (TMP), which was postulated to act as a radical trap and also inhibits hydrogen atom formation, was used as a fixed charge tag for two amyloid β peptides [34]. It was shown that the extent of backbone fragmentation and the abundance of backbone product ions decreased with the addition of fixed charge tag(s), whereas the extent of side-chain cleavages increased. Despite the fact that doubly tagged peptides do not contain a protonated site, several c- and z-type product ions were still detected. The authors stated that formation of c- and z-type product ions can be explained by non-H atom mediated pathways, which may be radical driven or through an amide superbase mechanism [26, 32]. For the latter, it was postulated that the methyl group of the pyridinium ring could provide the required proton for dissociation [34].

Inhibition or suppression of N–C_α backbone bond cleavages was also observed in ECD of a series of doubly protonated nitrated peptides [37] and for peptides containing a high number of glutamic acid and asparagine residues [38]. In the former case, the inhibition of backbone bond cleavages was explained by the electron predator mechanism [36]. In the latter case, the authors suggested that hydrogen bonding between the carbonyl oxygen of the glutamic acids side chains and the backbone amide hydrogen results in stabilization of the charge-reduced species with subsequent inhibition of the ECD fragmentation [38].

Here we focus on ECD of doubly, $[M + H]^{2+\bullet}$, and triply, $[M + 2H]^{3+\bullet}$, charged hydrogen-deficient peptide radical cations. These experiments aim to address whether the presence of a hydrogen-deficient radical site influences the ECD outcome. In addition, dissociation pathways of hydrogen-deficient species have been characterized in CID [5, 11, 13, 15, 16, 18, 20, 22–24], infrared multiphoton dissociation (IRMPD) [21], and electron-induced dissociation (EID) [21]. However, they have not been so far investigated in ECD. Therefore, the experiments presented here also seek to characterize the fragmentation pathways of $[M + nH]^{(n+1)+\bullet}$ species in ECD. Doubly, $[M + H]^{2+\bullet}$, and triply, $[M + 2H]^{3+\bullet}$, charged radical cations were generated by collision-induced dissociation (CID) of the $[Cu^{(II)}(terpy)(M + H)]^{3+\bullet}$ and $[Cu^{(II)}(terpy)(M + 2H)]^{4+\bullet}$ complexes, respectively. The resulting peptide radical cations were isolated and subjected to ECD.

Experimental

Sample Preparation

The peptides angiotensin I, (DRVYIHPFHL-OH), [Ile[7]]-angiotensin III, (RVYIHPI-OH), adrenocorticotropic hormone fragment 1-10 (abbreviated as ACTH 1-10), (SYSMEHFRWG-OH), and neurotensin, (pELYENKPRRPYIL-OH), were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Peptides were electrosprayed from a spraying solvent containing 1:1 water:ACN (Sigma) at a final concentration of 1 μM. Copper(II) nitrate trihydrate and 2,2':6',2"-terpyridine were obtained from Sigma; (2,2':6',2"-terpyridine)copper(II) nitrate monohydrate, ([Cu(II)(terpy)(NO$_3$)$_2$] × H$_2$O), was synthesized as previously described [39], and dissolved in methanol (Sigma) before use. [Cu(II)(terpy)M]$^{n+}$ complexes were formed by mixing the peptide (5–20 μM) and [Cu(II)(terpy)(NO$_3$)$_2$] × H$_2$O (8.5–19 μM) in 50:50 methanol:water as previously described [21].

Mass Spectrometry

Mass spectrometry experiments were performed in positive ion mode on a 7T LTQ FT-ICR Ultra mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Samples were directly infused through a fused silica transfer tubing connected to a stainless steel spraying needle (New Objective, Woburn, MA, USA) at a flow rate of 1 μL/min. Electrospray ionization was achieved by applying 2.50–3.00 kV to a liquid junction. To maximize the signal of the [Cu(II)(terpy)(M + nH)]$^{(n+2)+\bullet}$ complexes, the ESI source parameters were carefully tuned. Typical values were: spray voltage, 2.50–3.00 kV; capillary temperature, 200–240 °C; capillary voltage, 80–140 V and tube lens offset voltage, 95–150 V.

Peptide radical cations were formed from the corresponding [Cu(II)(terpy)(M + nH)]$^{(n+2)+\bullet}$ complexes after isolation and collisional activation of the latter in the linear ion trap. CID was performed with helium as collision gas. To maximize the abundance of the radical precursor ions, collision energies were optimized. The resulting radical precursor ions were then isolated in the linear ion trap, with an isolation width of 5 Da, and transferred into the ICR cell for ECD experiments. The automatic gain control (AGC) target value was set at 5×10^5 or 1×10^6 ions and maximum ion injection times were 1000–2000 ms. ECD was carried out with an indirectly heated dispenser cathode (Heatwave, Watsonville, CA, USA) at a cathode potential of –1.2 V or –2.2 V and irradiation time of 70–100 ms. ECD spectra were acquired with Tune Plus (ver. 2.2; Thermo Fisher Scientific) using a resolution of 25,000 at m/z 400.

Data Analysis

Data were analyzed using the Qual Browser software (ver. 2.0.7, Thermo Fisher Scientific). Product ion spectra were manually interpreted with the aid of Fragmentor 1.2.2 (http://www.faculty.ucr.edu/~ryanj/fragmentor.html) and the MS Product function (http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msproduct) in Protein Prospector. Only peak assignments with a mass accuracy better than 10 ppm were accepted. Product ion abundances were calculated by summing the abundance of each isotopic peak and dividing by the charge, since in FT-ICR MS the ion signal is proportional to charge. The relative product ion intensities normalized to the total ion abundances were obtained by dividing the abundance of each individual product ion by the sum of the abundances of all product ions (backbone fragments, side chain and small neutral losses, charge-reduced species). When a product ion was detected in both singly and doubly protonated form, the relative abundances of each charge state were summed and presented as a single value.

Results and Discussion

The formation of the [M + nH]$^{(n+1)+\bullet}$ species is facilitated by the presence of both a basic and an aromatic residue [1–4]; therefore, the peptide radical cations examined here contain both a basic (Arg, His, or Lys) and an aromatic (Trp or Tyr) amino acid. Product ions were assigned based on the nomenclature proposed by Zubarev and coworkers [40]. N-terminal product ions are assigned as c_n' or $c_n^\bullet$ and C-terminal product ions as $z_n^\bullet$ or z_n', in which "'" denotes a hydrogen and "$^\bullet$" an unpaired electron. For the hydrogen-deficient precursor ions we used the assignment $z_n^\bullet - H$ to indicate z-type product ions, which are one hydrogen atom (1.0078 Da) lighter than those observed in ECD of the even electron counterpart. Product ions detected for each examined peptide are given in Supplemental data (Tables S1A–S6B).

ECD of Doubly Charged Radical Cations [M + H]$^{2+\bullet}$

ECD of the even, [M + 2H]$^{2+}$, and odd, [M + H]$^{2+\bullet}$, electron species of ACTH 1-10 resulted in the formation of c- and z-type product ions (Figure 1), but a noticeable difference between the two spectra is the considerably lower intensity of the product ions following ECD of the hydrogen-deficient species. In contrast to the suppressed backbone bond cleavages, the formation of the charge-reduced species was abundant, indicating efficient electron capture by the hydrogen-deficient species. It should be noted that the non-dissociative charged reduced species at $m/z = 1299.5575$, which is one hydrogen atom heavier (1.00782 Da) than the protonated radical precursor ions, corresponds to an even electron species. In contrast, in ECD of even electron precursor ions the non-dissociative charge-reduced species is an odd electron species or a mixture of radical and even electron hydrogen ejected species [41].
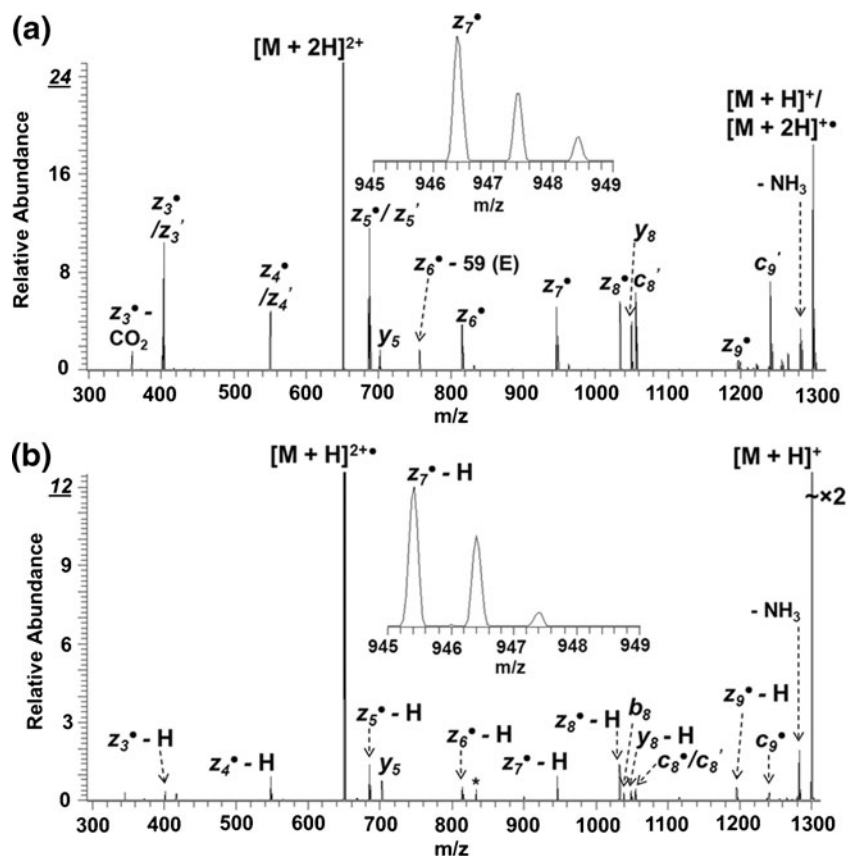
Figure 1. ECD spectra of the even electron **(a)** and hydrogen-deficient **(b)** precursors ions of ACTH 1-10, SYSMEHFRWG-OH. Although the number and type of product ions detected are almost identical for the two species, the product ion abundances are significantly reduced in the spectrum of the hydrogen-deficient species. Product ions of the hydrogen-deficient species are 1 Da lighter than those of the even electron species, as shown in the inset for the z_7 product ion. * = noise peak

It is also worth pointing out that the product ions in the ECD spectrum of the $[M + H]^{2+\bullet}$ precursor ions were 1 Da lighter (assigned as $z_n^\bullet - H$ or $c^\bullet$) compared with those of the even electron precursor ions, reflecting the hydrogen-deficient nature of the precursor ions. The exact location of the unpaired electron of the hydrogen-deficient species cannot be unambiguously determined because radical migration has been shown to commonly occur in large hydrogen-deficient peptides [13, 20, 24]. However, based on the observation that all product ions containing a Trp were detected with a mass decrement of 1 Da, we can postulate that through radical rearrangements, the unpaired electron of the hydrogen-deficient species migrated to the Trp residue. Alternatively, the hydrogen atom loss observed from the $z^\bullet$-type product ions can be attributed to hydrogen atom rearrangements, which have been previously reported in ECD [42]. Loss of hydrogen atom from $z^\bullet$-type product ions is observed when the amino acids Thr and Ser, and to a lesser extent Trp, Phe, and Tyr are adjacent to the radical site [42]. Consequently, some of the $z_n^\bullet - H$ product ions observed in our experiments can also be explained by the presence of these amino acids at the radical site. The alternative explanation that these product ions originate from the unpaired electron being located at the Trp residue

can account for the observation of all $z_n^\bullet - H$ and $c^\bullet$ ions. The c_8 product ion corresponding to cleavage at the N-terminal side of tryptophan was detected as a mixture of the odd and even electron species.

Following ECD of the doubly charged hydrogen-deficient species of neurotensin (Figure S1, Supplemental Data) successful electron capture to produce the charge-reduced species were observed. However, backbone bond cleavages were suppressed compared with those obtained for the $[M + 2H]^{2+}$ precursor ions, as indicated by the reduced intensities of the c- and z-type product ions. These results are similar to those obtained for ACTH 1-10. It should be noted that only the intensities of the product ions were reduced, whereas the number of product ions detected in the ECD spectra of the odd and even electron species were almost identical. Similar to the results obtained for ACTH 1-10, we observed a 1 Da mass decrement for the majority of the product ions of the hydrogen-deficient species compared with the product ions detected for the even electron precursor ions. While the exact location of the unpaired electron cannot be easily determined, based on the formation of $z_n^\bullet - H$ ($n=9$–12) and $c_n^\bullet$ ($n=11$–12), we can postulate that the radical is located at Tyr 11. Although neurotensin contains a second Tyr at position 3, which could also be the radical site, the c_8 and

c_{10} product ions containing the Tyr at position 3 but not at position 11, did not exhibit the 1 Da mass shift. Therefore, it is more likely that the unpaired electron is located at Tyr 11. Furthermore, hydrogen atom rearrangements, which are commonly observed in ECD [42–44], can also account for the observed mass shifts.

The results obtained following MS^3 ECD of the radical precursor ions of angiotensin I, $[M + H]^{2+\bullet}$ (Figure S2, Supplemental Data), are in agreement with those obtained for ACTH 1-10 and neurotensin in that abundant formation of the charge-reduced species and suppressed backbone dissociation were observed. For this peptide, the number of backbone product ions was also reduced following ECD of the hydrogen-deficient species (i.e., several c- and z-type product ions detected in the ECD spectrum of the even electron species were absent from the spectrum of the odd electron precursor ions). The formation of $c_7^\bullet$, $c_8^\bullet$, and $c_9^\bullet$ in the ECD spectrum of the hydrogen-deficient species can be attributed to localization of the unpaired electron at the Tyr at position 9 or to hydrogen atom rearrangements, as discussed above. In fact, for this peptide, hydrogen atom rearrangements were also common for the even electron species, resulting in the formation of $c_3^\bullet$, $c_4^\bullet$, and $c_5^\bullet$ product ions.

In accordance with the results obtained for the other peptides examined, ECD of $[M + H]^{2+\bullet}$ precursor ions of [Ile^7]-angiotensin III (Figure 2) resulted in suppressed N–C$_\alpha$ backbone bond cleavages. The c-type product ions, with the exception of c_1, exhibited a 1 Da shift compared with those obtained for the $[M + 2H]^{2+}$ species, and this might indicate localization of the unpaired electron at the Tyr residue. The c_2 product ion corresponding to cleavage next to the N-terminal side of tyrosine was detected as a mixture of the odd and even electron species. This behavior is analogous to that obtained for the c_8 product ion of ACTH 1-10. For [Ile^7]-angiotensin III several a-, b-, and y-type product ions were detected. The b- and y-type product ions were absent in the ECD of the even electron species (Figure 2a). Furthermore, the charge-reduced species were observed as a mixture of odd and even electron species with the latter being the most dominant. For the other peptides examined, the non-dissociative charge-reduced species were even electron species.

Examination of the relative product ion abundances normalized to the total abundance of all product ions (Figure 3) further indicates that formation of the charge-reduced species is the major pathway for hydrogen-deficient precursor ions. In addition, it is clearly illustrated that formation of c- and z-type product ions is significantly suppressed compared with the even electron species. Therefore, electron capture by a hydrogen-deficient species results in the formation of an abundant low reactivity intermediate, which does not efficiently trigger N–C$_\alpha$ backbone bond cleavages. For the most part, the number of N–C$_\alpha$ backbone cleavages remained unchanged between the ECD spectra of the $[M + 2H]^{2+}$ and $[M + H]^{2+\bullet}$ precursors ions (Figure 3).

Formation of the non-dissociative charge-reduced species indicates successful electron capture by the hydrogen-deficient precursor ions. Due to charge neutralization of one of the protons this singly charge-reduced species is one hydrogen atom heavier (1.00782 Da) relative to the doubly protonated, $[M + H]^{2+\bullet}$, precursor ions and represents an even electron species. It is unclear if the charge-reduced species is a biradical, featuring two separate radical sites (one originating from the hydrogen-deficient precursor ions and one formed following the electron capture event), or the two radical sites recombine to form a new chemical bond. In the former case, the polypeptide will contain one hydrogen-deficient radical site and one hydrogen-surplus radical site.

Formation of biradicals has been previously discussed in electron capture dissociation of the lantibiotic lacticin 481 [45]. Formation of abundant non-dissociative species formed by single and double electron capture was observed. The authors suggested that species resulting from double electron capture can either exhibit a structure containing two separate radical sites or the two unpaired electrons recombine to form a new bond. SORI-CID of these double electron capture species revealed that recombination of the two radical sites is the dominant pathway, whereas a small part of the population of these double electron capture species exist as biradical ions containing two separate radical sites [45].

Recombination of double radicals produced by loss of two iodines from doubly iodinated species was also observed by Liu and Julian [46]. The peptide ion resulting from the loss of two iodines was isolated and collisionally activated. Formation of product ions similar to those obtained in CID of the even electron protonated peptide was observed. Based on these observations, the authors concluded that radical recombination, which resulted in the formation of a new bond, occurred [46].

Based on these reports [45, 46], we reason that radical recombination, leading to a formation of a new bond, is very likely to occur following electron capture by the hydrogen-deficient species. The Cornell mechanism [9, 27, 28, 33] proposed electron capture to a protonation site to form a hypervalent radical from which a hydrogen atom can be released. The hydrogen atom can be transferred to a nearby backbone carbonyl oxygen leading to the formation of an aminoketyl radical, which dissociates to c'- (or $c^\bullet$) and $z^\bullet$ (or z')-type product ions. We can postulate that the hydrogen-deficient radical site can act as a hydrogen atom trap to abstract the hydrogen radical, which is ejected from a hypervalent (or Rydberg) radical formed after the initial electron capture at a protonated site. For this process to occur, we have to assume that the ejected hydrogen radical is properly positioned to be able to access and attach to the hydrogen-deficient radical site. Once the hydrogen atom is captured at the hydrogen-deficient radical site is not any longer available for transfer to the carbonyl oxygen to promote N–C$_\alpha$ dissociation.

Based on the UW mechanism [25, 26, 29, 32], electron capture by the precursor ions leads to the formation of an amide superbase (anion radical). Proton transfer to the amide
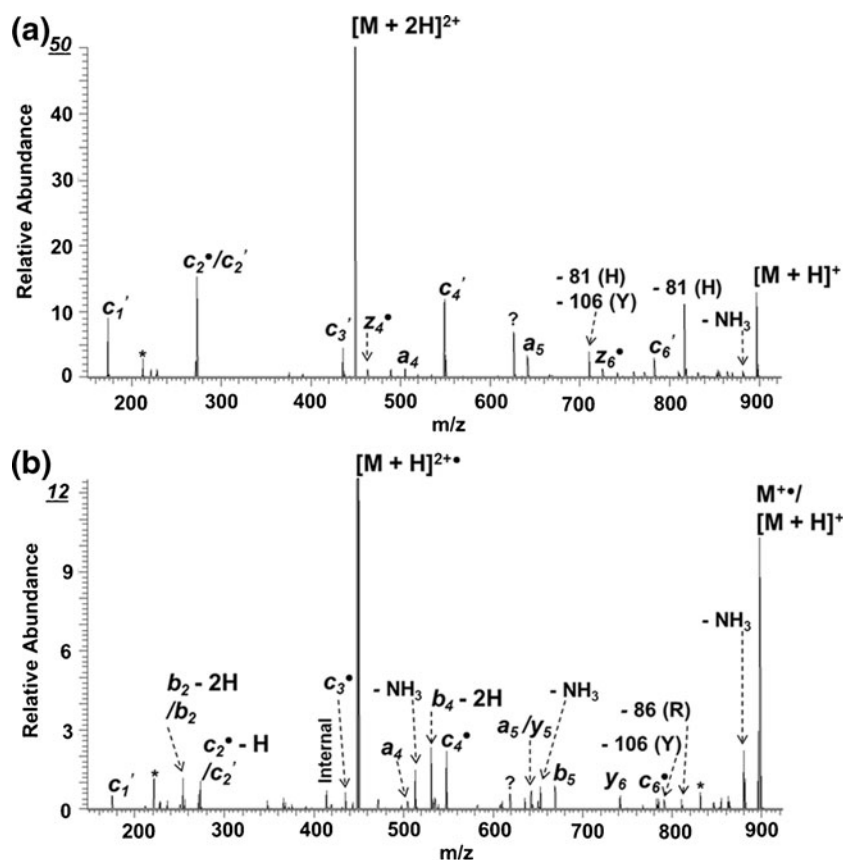
Figure 2. ECD spectra of the even electron **(a)** and hydrogen-deficient **(b)** precursors ions of [Ile[7]]-angiotensin III, (RVYIHPI-OH). N–C_α backbone bond cleavages were suppressed and several b- and y-type product ions were only detected following ECD of the $[M + H]^{2+\bullet}$ species. * = noise peak, ? = unidentified product ions

superbase, from a proximate proton donor group, results in the formation of an aminoketyl radical, which undergoes facile N–C_α backbone dissociations [25, 26, 29, 32]. If we consider the UW mechanism [25, 26, 29, 32], we can postulate that after electron capture by the hydrogen-deficient species, and before proton transfer, radical recombination occurs, leading to the formation of a stable intermediate. Regardless of the precise mechanism(s) by which ECD proceeds, electron capture by the hydrogen-deficient precursor ions will result in two radical sites on the polypeptide. If we further take into consideration that radical migration easily occurs in peptide radicals [20, 24] we can hypothesize that after the initial electron capture event, radical recombination occurs. Such a process will create an even electron species, instead of a radical intermediate, and quenches or suppresses N–C_α bond cleavages. This postulation is in agreement with our experimental results, which revealed formation of an abundant even electron charge-reduced species and generation of low abundance c- and z-type product ions.

Although the abundances of c- and z-type product ions were reduced in ECD of the $[M + H]^{2+\bullet}$ precursor ions, their formation was not completely eliminated (Figure 3). This observation suggests that in addition to radical recombination, other pathways should exist to account for the observed N–C_α bond cleavages, albeit in low abundances. We hypothesize that

in ECD of hydrogen-deficient species, even though radical recombination is the dominant pathway, a small ion population can exist as a biradical. We argue that electron capture, to either a protonated site or to a Coulomb stabilized amide π*-orbital, can lead to the formation of a biradical featuring two separate radical sites. This biradical can undergo the expected N–C_α cleavages by ejection of a hydrogen atom and capture by a nearby carbonyl oxygen or by proton transfer to the carbonyl oxygen. Our postulation that a small ion population can exist as biradical can rationalize the reduced intensities of c- and z-type product ions.

An alternative explanation is the presence of competing processes with one process dominating over the other. We reason that radical recombination and N–C_α bond cleavages can be in competition. N–C_α bond cleavages can proceed via the Cornell [9, 27, 28, 33] or UW [25, 26, 29, 32] mechanism. The carbonyl oxygen and the hydrogen-deficient radical site can be in competition for hydrogen atom abstraction (Cornell mechanism). Proton transfer to a carbonyl oxygen (UW mechanism) can be in competition with recombination of the two radical sites in the peptide. Given that c- and z-type product ions were detected with low abundances following ECD of the $[M + H]^{2+\bullet}$ species (Figure 3), we can conclude that radical recombination dominates, whereas N–C_α bond cleavages occur to a lesser degree.

Our hypothesis that a small ion population exists as a biradical or that ECD of hydrogen-deficient species proceeds
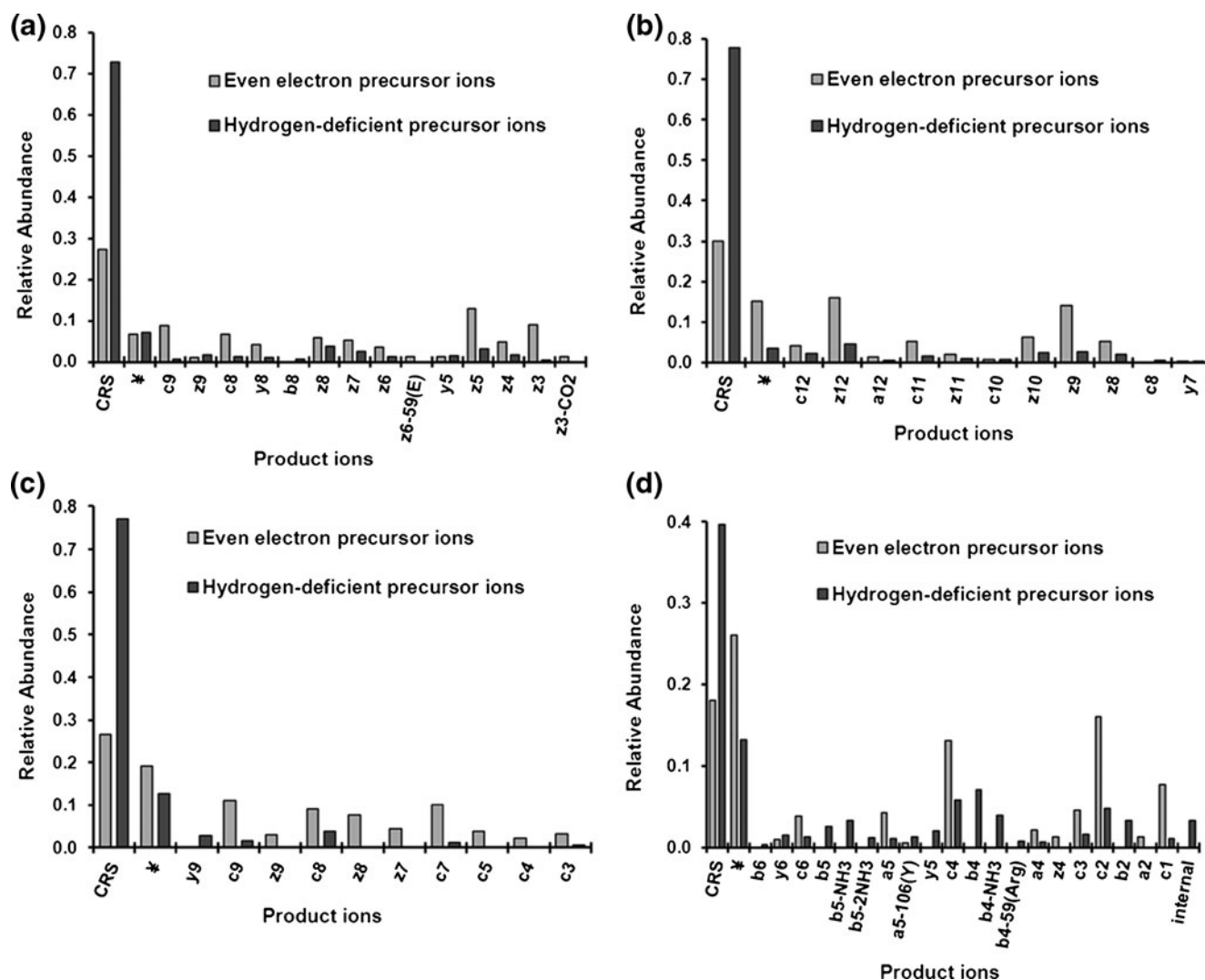
Figure 3. Product ion abundances observed in the ECD spectra of the $[M + 2H]^{2+}$ and $[M + H]^{2+\bullet}$ species of ACTH 1-10 **(a)**, neurotensin **(b)**, angiotensin I **(c)**, and $[Ile^7]$-angiotensin III **(d)**. Product ion abundances are normalized to the total abundances of all product ions (backbone bond cleavages, charge-reduced species, and neutral losses). For simplicity, odd and even electron product ions are not indicated in the Figure. These can be found in Figures 1 and 2, and Supplemental Data (Figures S1, S2, and Tables S1A–S4B). CRS = charge-reduced species, ¥ = side chain and/or H_2O, NH_3 losses (side chain and H_2O/NH_3 losses are shown separately in Figure S3, Supplemental Data)

via two competing pathways can also account for the 1 Da mass decrement observed for several of the product ions in the ECD spectra. We postulate that the unpaired electron of the hydrogen-deficient species does not participate in the N–C_α bond cleavages and results in the formation of product ions, which are 1 Da lower than those observed in ECD of the even electron precursor ions.

With the exception of the $[M + H]^{2+\bullet}$ precursor ions of $[Ile^7]$-angiotensin III, formation of y-type product ions was a minor fragmentation pathway for both the even and odd electron species of the other peptides examined. This is in agreement with previous reports on ECD, in which it was shown that minor dissociation pathways lead to the formation of $a^\bullet$- and y-type product ions [47]. The fragmentation behavior of $[Ile^7]$-angiotensin III will be discussed in more details below.

ECD of Triply Charged Radical Cations $[M + 2H]^{3+\bullet}$

Triply charged $[M + 2H]^{3+\bullet}$ species of neurotensin and angiotensin I were investigated. For ACTH 1-10 and $[Ile^7]$-angiotensin III, the quadruply charged, $[Cu(II)(terpy)(M + 2H)]^{4+\bullet}$, complex was not detected; therefore, generation of the $[M + 2H]^{3+\bullet}$ species for these two peptides was not possible.

Following ECD of the $[M + 2H]^{3+\bullet}$ precursor ions of neurotensin (Figure 4) analogous results to those observed for the doubly charged radical cations were obtained. The hydrogen-deficient species successfully captured an electron to yield an abundant charge-reduced species. In contrast, the abundances of backbone product were reduced compared with those observed for the even electron $[M + 3H]^{3+}$ precursor ions (Figure 5a). The vast majority of product ions detected in the ECD spectrum of

the $[M + 3H]^{3+}$ species were also present for the hydrogen-deficient species. In addition, several product ions exhibited 1 Da mass shift, presumably due to the hydrogen-deficient precursor ions and/or hydrogen atom rearrangements as discussed above. Few y-type product ions were present for both the $[M + 2H]^{3\bullet+}$ and $[M + 3H]^{3+}$ species and their formation can be explained by the previously suggested minor pathways observed in ECD [47].

ECD of the triply charged radical cations of angiotensin I, $[M + 2H]^{3+\bullet}$ (Figure S4, Supplemental Data) resulted in the formation of several b- and y-type product ions, in addition to c- and z-type product ions. As shown in Figure 5b, b- and y-type product ions dominated. With the exception of y_2 and y_4, b- and y-type product ions were absent in the ECD spectrum of the even electrons species. In addition, several c- and z-type product ions, detected for the even electron species, were absent for the $[M + 2H]^{3+\bullet}$ counterpart. These results are in contrast with those obtained for the triply

charged radical cations of neurotensin, for which the vast majority of product ions detected for the even electron species were also present for the odd electron counterpart.

Despite these differences, as illustrated in Figure 5, triply charged radical cations of neurotensin and angiotensin I both exhibited suppressed N–C$_\alpha$ bond cleavages following ECD in agreement with the results obtained for the doubly charged radical cations.

Formation of b- and y-Ions in ECD of $[M + 2H]^{3+\bullet}$ Ions of Angiotensin I and $[M + H]^{2+\bullet}$ Ions of $[Ile^7]$-Angiotensin III

For the $[M + H]^{2+\bullet}$ species of $[Ile^7]$-angiotensin III, (Figures 2 and 3d), and for the $[M + 2H]^{3+\bullet}$ species of angiotensin I, (Figure 5b and Figure S3), abundant formation of b- and y- type product ions was observed in stark contrast to the results obtained for the other

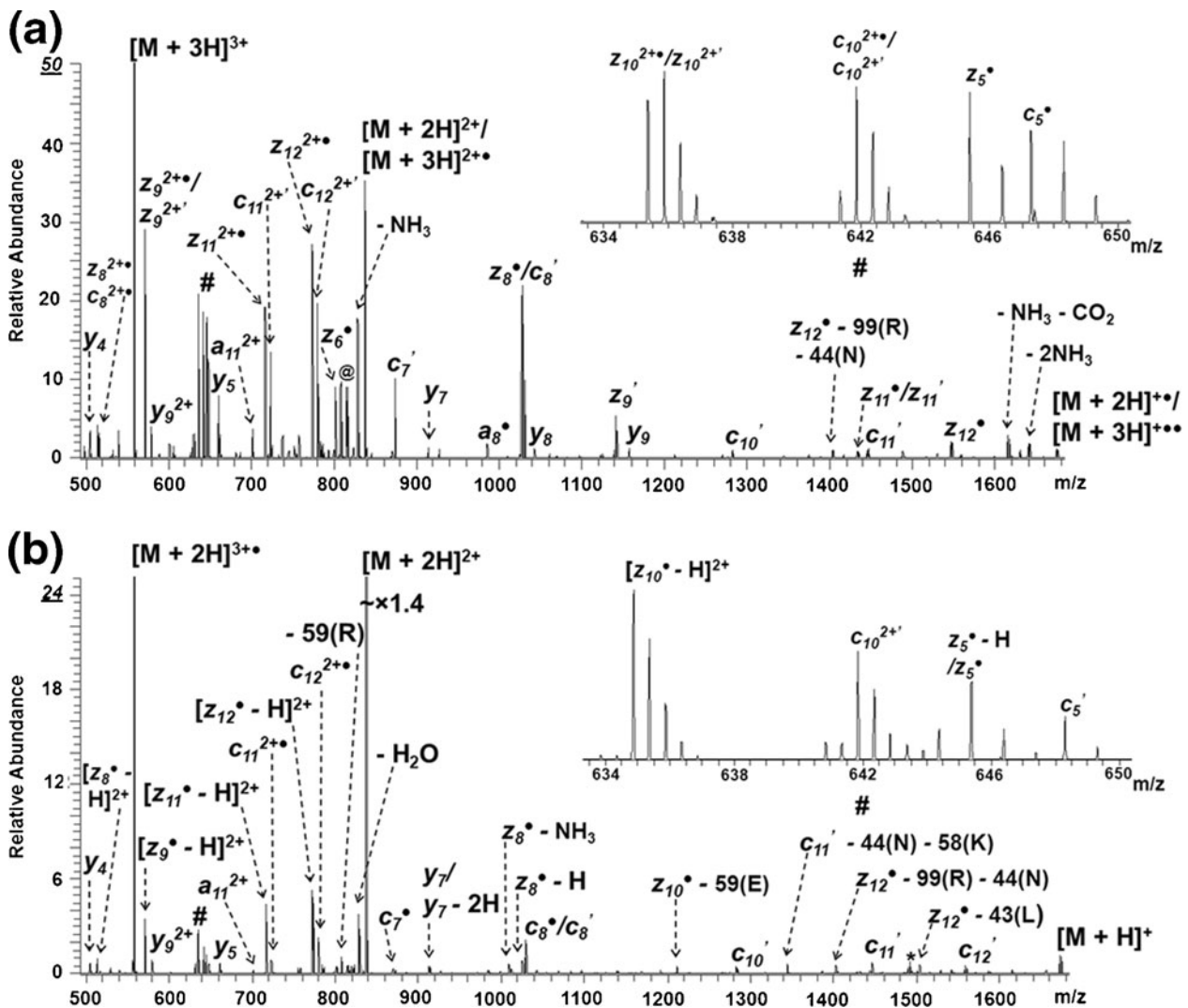**Figure 4.** ECD spectra of the triply charged even electron **(a)** and hydrogen-deficient **(b)** precursors ions of neurotensin, pELYENKPRRPYIL-OH. The majority of product ions detected for the even electron species were also present in the ECD spectrum of the hydrogen-deficient species, but their intensities were noticeably decreased. * = noise peak, @ = amino acid side chain losses
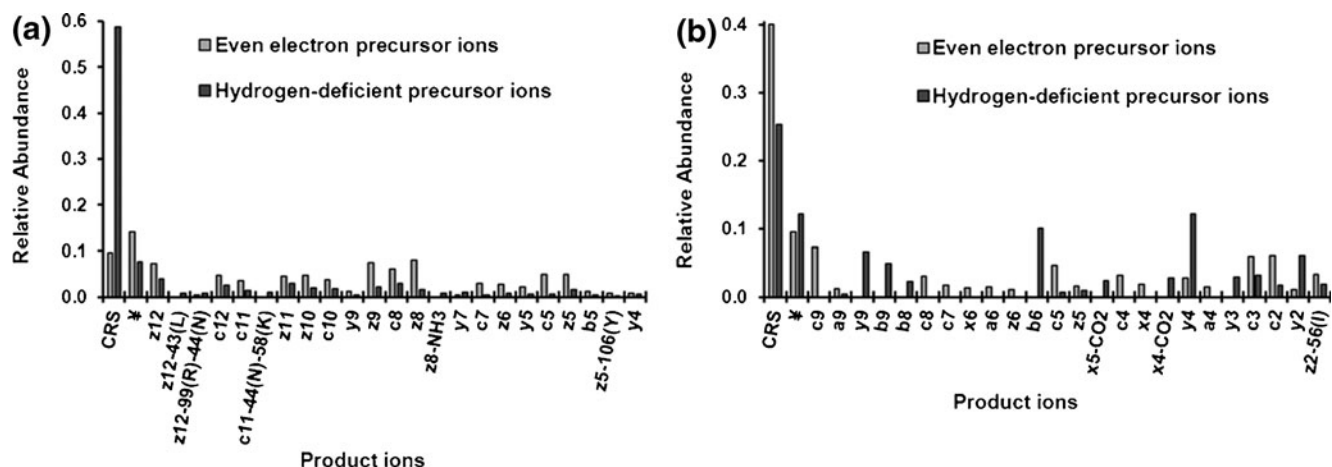
Figure 5. Product ion abundances observed in the ECD spectra of the $[M + 3H]^{3+}$ and $[M + 2H]^{3+\bullet}$ species of neurotensin **(a)**, and angiotensin I **(b)**. Product ion abundances are normalized to the total abundances of all product ions (backbone bond cleavages, charge-reduced species and neutral losses). For simplicity, odd and even electron product ions are not indicated in the Figure. These can be found in Figure 4, and Supplemental Data (Figure S3 and Tables S5A–S6B). Only product ions detected with normalized relative intensities ≥0.008 are displayed. Complete lists of all product ions are given in Supplemental Data (Tables S5A–S6B). CRS = charge-reduced species; ¥ = side chain and/or H_2O, NH_3 losses (side chain and H_2O/NH_3 losses are shown separately in Figure S5, Supplemental Data)

hydrogen-deficient peptides examined. Therefore, additional fragmentation pathways should exist for the $[M + 2H]^{3+\bullet}$ ions of angiotensin I and $[M + H]^{2+\bullet}$ ions of $[Ile^7]$-angiotensin III to account for the formation of abundant b- and y-ions.

In previous reports, the unexpected formation of b-ions in ECD of even electron precursor ions was rationalized by the presence of a vibrationally excited charge-reduced species [48–51]. These reports proposed that electron capture by the precursor ions followed by hydrogen atom loss leads to the formation of a vibrationally excited even electron species, $[M + (n-1)H]^{(n-1)+}$, which dissociates via the mobile proton pathway [48–51]. This hypothesis and additional plausible explanations for the formation of b-and y-type ions in the ECD spectra of angiotensin I and $[Ile^7]$-angiotensin III are discussed below.

It has recently been shown that peptide size affect fragmentation channels in ECD [52]. For peptides containing five residues, ECD resulted in the formation of a-, b-, y-, and w-type product ions and formation of b- and w-type product ions was favored for small size peptides [52]. $[Ile^7]$-angiotensin III was the smallest peptide examined here, containing seven residues. Therefore, we reason that one plausible explanation for the differences observed between $[Ile^7]$-angiotensin III and the other hydrogen-deficient peptides examined can be due to peptide size. In the case of angiotensin I, it is unlikely that peptide size contributed to the observed differences because for the doubly charged radical cations of angiotensin I very similar dissociation channels to those observed for ACTH 1-10 and neurotensin were observed (Figure 3).

Another plausible pathway for the formation of b-type ions in ECD is by secondary fragmentation of c-type ions (the mass of a b_n product ion is identical to the mass of a $c_n - NH_3$ product ion). Although double resonance experiments with c-ion ejection eliminated the latter possibility (i.e., formation of b-ions was still observed after c-ion ejection in ECD of even electron precursor

ions [48]) we cannot exclude this possibility in ECD of hydrogen-deficient precursor ions. Considering that the c-type product ions detected in ECD of hydrogen-deficient precursor ions were mainly radical species, we can postulate that they are more readily amenable to small neutral losses compared with even electron c-type product ions. In fact, a closer inspection of the ECD spectrum of the $[M + H]^{2+\bullet}$ revealed that the c_2 product ion was a mixture of the $c_2'/c_2^\bullet - H$ and the b_2 product ions was a mixture of the $b_2/b_2 - 2H$ (Figure S6, Supplemental Data). This observation suggests that the $b_2/b_2 - 2H$ ions might originate from the $c_2'/c_2^\bullet - H$ product ions after NH_3 elimination from the latter species. In addition, for $[Ile^7]$-angiotensin III (Figures 2 and 3d), loss of NH_3 was observed from the b_5 and b_4 product ions indicating that for this peptide secondary fragmentation contributes to the dissociation outcome. Thus, for hydrogen-deficient species production of some of the b-type ions due to secondary fragmentation of $c^\bullet$-type ions seems like a plausible scenario.

A closer inspection of the ECD spectra of the $[M + 2H]^{3+\bullet}$ ions of angiotensin I and the $[M + H]^{2+\bullet}$ species of $[Ile^7]$-angiotensin III revealed that for these two peptides the charge reduced species was detected as a mixture of the odd and even electron species, with the latter being the most dominant (Figure S7, Supplemental Data). The formation of this species is due to hydrogen atom loss from the charge-reduced ions [41] leading to a mixture of the odd, $[M + (n-2)H]^{(n-1)+\bullet}$, and even electron, $[M + (n-1)H]^{(n-1)+}$, species. Interestingly, for the other hydrogen-deficient peptides examined, only the even electron species were observed.

It has been previously suggested that in ECD, hydrogen atom loss leaves the reduced species with a significant thermal energy. Dissociation of the thermally excited species proceeds via vibrational excitation to produce b- and y-ions [50]. Furthermore, suppression of hydrogen atom loss from

$[M + 2H]^{+\bullet}$ has been attributed to the low reactivity of the charge-reduced species. Following collisional activation, the charge-reduced species could be activated and dissociate through hydrogen atom loss, amide (C(O)–N) and amine (N–C_α) bond cleavages [38]. Therefore, hydrogen atom loss can indicate an activated charge-reduced species.

Based on these reports [38, 50] we hypothesize that for $[M + 2H]^{3+\bullet}$ ions of angiotensin I and $[M + H]^{2+\bullet}$ ions of [Ile^7]-angiotensin III exhibiting hydrogen atom loss and abundant formation of b- and y-type product ions, the charge-reduced species were vibrationally excited, leading to amide bond cleavages. We believe that the vibrationally excited species is a mixture of the odd, $[M + (n-2)H]^{(n-1)+\bullet}$, and even, $[M + (n-1)H]^{(n-1)+}$, electron species since the isotopic distribution indicates presence of both, and that both species can contribute to the dissociation outcome.

The vast majority of the b- and y-ions detected for [Ile^7]-angiotensin III should originate from the excited $[M + 2H]^{2+*}$ species since such ions were either of very low abundance or absent in the CID, IRMPD, and EID spectra of the M$^{+\bullet}$ precursor ions [21]. EID is believed to occur via both electronic and vibrational excitation [53].

For the $[M + 3H]^{2+\bullet}$ precursor ions of angiotensin I the b- and y-type product ions detected can derive from the $[M + H]^{2+\bullet*}/[M + 2H]^{2+*}$ species. Indeed most of the b- and y-ions detected in the ECD spectrum were also present following CID or IRMPD of the $[M + 2H]^{2+}$ precursor ions of angiotensin I (data not shown), and they were also observed in CID, IRMPD, or EID of the $[M + H]^{2+\bullet}$ species [21]. The b_8 was only observed for the even electron species (data not shown), whereas the y_9 and y_3 were only detected for the hydrogen-deficient species [21]. Therefore, the presence of a vibrationally excited charge-reduced species containing both the $[M + H]^{2+\bullet}$ and $[M + 2H]^{2+}$ species can better explain the formation of all observed b- and y-type product ions in the ECD spectrum of the $[M + 3H]^{2+\bullet}$ ions of angiotensin I.

Furthermore, if we assume that hydrogen atom loss provides evidence for the presence of an excited charge-reduced species, we can reason that for the other hydrogen-deficient peptides examined, the charge-reduced species were not vibrationally excited, since no hydrogen atom loss was observed. Consequently, formation of b- and y-type product ions is not expected to represent a dominant dissociation pathway, in agreement with our experimental data. Furthermore, it has been shown that the probability of hydrogen atom loss from dipeptides and tripeptides is not directly correlated with the probability of c- and z-type product ion formation [54]. Our experimental results are in accordance with this observation given that all hydrogen-deficient peptides examined here resulted in the formation of c- and z-type product ions regardless of the charge-reduced species exhibiting, or not, a hydrogen atom loss.

Lastly, factors such as peptide gas phase conformations and structures, amino acid composition, and charge location have been shown to influence the ECD outcome [38, 41, 55–63]. Thus, these factors could also contribute to the different fragmentation behavior of the $[M + 2H]^{3+\bullet}$ ions of angiotensin I and the $[M + H]^{2+\bullet}$ ions of [Ile^7]-angiotensin III.

Conclusions

ECD of doubly and triply charged hydrogen-deficient peptide radical cations have been investigated. Fragmentation pathways of hydrogen-deficient species in ECD were distinct from those observed in CID, IRMPD, and EID of the same species [21]. In general, in CID, IRMPD, and EID of highly charged hydrogen-deficient species fragmentation is governed by a competition between radical and charge-driven processes [10–15, 21]. Abundant amino acid side-chain losses, enhanced cleavages at aromatic residues, and formation of a-, b-, c-, y-, c-, and z-type ions are observed [5, 16, 20–22]. Consequently, dissociation of hydrogen-deficient species in CID, IRMPD, and EID show differences compared with the dissociation of the even electron counterpart. In stark contrast to this behavior, ECD of hydrogen-deficient and even electron species resulted mainly in the formation of the same product ions. Given that the method of ion activation is different in ECD than in CID, IRMPD, and EID, it is not very surprising that different dissociation pathways are observed in the ECD spectra of hydrogen-deficient species compared with CID, IRMPD, and EID.

A major difference between the ECD spectra of the hydrogen-deficient and even electron precursor ions was the reduced intensity of the N–C_α bond cleavages observed for the former species. The suppressed backbone bond cleavages observed following ECD of the hydrogen-deficient species is analogous to the results obtained in ECD of peptides modified with tags having high electron affinity [36], peptides derivatized with fixed charge tags [34, 35], radical and hydrogen atom traps [35], nitrated peptides [37], and peptides featuring a high number of glutamic acid and asparagine residues [38]. These reports [35–38] and our results highlight the importance of the reactive aminoketyl intermediate in ECD and indicate that N–C_α bond cleavages are largely quenched, or eliminated, when the formation of the reactive aminoketyl radical intermediate is inhibited.

Acknowledgment

References

1. Bagheri-Majdi, E., Ke, Y.Y., Orlova, G., Chu, I.K., Hopkinson, A.C., Siu, K.W.M.: Copper-Mediated Peptide Radical Ions in the Gas Phase. *J. Phys. Chem. B* **108**, 11170–11181 (2004)
2. Barlow, C. K., Wee, S., McFadyen, W. D., O'Hair, R. A. J.: Designing Copper(II)Ternary Complexes to Generate Radical Cations of Peptides in the Gas Phase: Role of the Auxiliary Ligand. *Dalton Trans.* 3199–3204 (2004)
3. Barlow, C.K., McFadyen, W.D., O'Hair, R.A.J.: Formation of Cationic Peptide Radicals by Gas-Phase Redox Reactions with Trivalent

Chromium, Manganese, Iron, and Cobalt Complexes. *J. Am. Chem. Soc.* **127**, 6109–6115 (2005)

4. Chu, I.K., Rodriquez, C.F., Lau, T.C., Hopkinson, A.C., Siu, K.W.M.: Molecular Radical Cations of Oligopeptides. *J. Phys. Chem. B* **104**, 3393–3397 (2000)

5. Hao, G., Gross, S.S.: Electrospray Tandem Mass Spectrometry Analysis of S- and N-Nitrosopeptides: Facile Loss of NO and Radical-Induced Fagmentation. *J. Am. Soc. Mass. Spectrom.* **17**, 1725–1730 (2006)

6. Masterson, D.S., Yin, H.Y., Chacon, A., Hachey, D.L., Norris, J.L., Porter, N.A.: Lysine Peroxycarbamates: Free Radical-Promoted Peptide Cleavage. *J. Am. Chem. Soc.* **126**, 720–721 (2004)

7. Budnik, B.A., Zubarev, R.A.: MH$^{2+\bullet}$ Ion Production from Protonated Polypeptides by Electron Impact: Observation and Determination of Ionization Energies and a Cross-Section. *Chem. Phys. Lett.* **316**, 19–23 (2000)

8. Ly, T., Julian, R.R.: Residue-Specific Radical-Directed Dissociation of Whole Proteins in the Gas Phase. *J. Am. Chem. Soc.* **130**, 351–358 (2008)

9. Zubarev, R.A., Kelleher, N.L., McLafferty, F.W.: Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process. *J. Am. Chem. Soc.* **120**, 3265–3266 (1998)

10. Song, T., Ng, D.C.M., Quan, Q.A., Siu, C.K., Chu, I.K.: Arginine-Facilitated alpha- and pi-Radical Migrations in Glycylarginyltryptophan Radical Cations. *Chem. Asian J.* **6**, 888–898 (2011)

11. Wee, S., O'Hair, R.A.J., McFadyen, W.D.: Comparing the Gas-Phase Fragmentation Reactions of Protonated and Radical Cations of the Tripeptides GXR. *Int. J. Mass Spectrom.* **234**, 101–122 (2004)

12. Xu, M., Song, T., Quan, Q., Hao, Q., Fang, D.C., Siu, C.K., Chu, I.K.: Effect of the N-terminal Basic Residue on Facile C(alpha)-C Bond Cleavages of Aromatic-Containing Peptide Radical Cations. *Phys. Chem., Chem. Phys.* **13**, 5888–5896 (2011)

13. Laskin, J., Yang, Z.B., Ng, C.M.D., Chu, I.K.: Fragmentation of alpha-Radical Cations of Arginine-Containing Peptides. *J. Am. Soc. Mass Spectrom.* **21**, 511–521 (2010)

14. Lam, A.K.Y., Ryzhov, V., O'Hair, R.A.J.: Mobile Protons Versus Mobile Radicals: Gas-Phase Unimolecular Chemistry of Radical Cations of Cysteine-Containing Peptides. *J. Am. Soc. Mass Spectrom.* **21**, 1296–1312 (2010)

15. Wee, S., O'Hair, R.A.J., McFadyen, W.D.: The Role of the Position of the Basic Residue in the Generation and Fragmentation of Peptide Radical Cations. *Int. J. Mass Spectrom.* **249**, 171–183 (2006)

16. Laskin, J., Yang, Z.B., Lam, C., Chu, I.K.: Charge-Remote Fragmentation of Odd-Electron Peptide Ions. *Anal. Chem.* **79**, 6607–6614 (2007)

17. Siu, C.K., Ke, Y.Y., Orlova, G., Hopkinson, A.C., Siu, K.W.M.: Dissociation of the N-C-alpha Bond and Competitive Formation of the [z(n) – H]•+) and [c(n) + 2H](+) Product Ions in Radical Peptide Ions Containing Tyrosine and Tryptophan: The Influence of Proton Affinities on Product Formation. *J. Am. Soc. Mass Spectrom.* **19**, 1799–1807 (2008)

18. Ng, D.C.M., Song, T., Siu, S.O., Siu, C.K., Laskin, J., Chut, I.K.: Formation, Isomerization, and Dissociation of alpha-Carbon-Centered and π-Centered Glycylglycyltryptophan Radical Cations. *J. Phys. Chem. B* **114**, 2270–2280 (2010)

19. Knudsen, E.R., Julian, R.R.: Fragmentation Chemistry Observed in Hydrogen Deficient Radical Peptides Generated from *N*-Nitrosotryptophan Residues. *Int. J. Mass Spectrom.* **294**, 83–87 (2010)

20. Sun, Q.Y., Nelson, H., Ly, T., Stoltz, B.M., Julian, R.R.: Side Chain Chemistry Mediates Backbone Fragmentation in Hydrogen Deficient Peptide Radicals. *J. Proteome Res.* **8**, 958–966 (2009)

21. Kalli, A., Hess, S.: Fragmentation of Singly, Doubly, and Triply Charged Hydrogen Deficient Peptide Radical Cations in Infrared Multiphoton Dissociation and Electron Induced Dissociation. *J. Am. Soc. Mass Spectrom.* **23**, 244–263 (2012)

22. Chu, I.K., Lam, C.N.W.: Generation of Peptide Radical Dications via Low-Energy Collision-Induced Dissociation of [Cu-II(terpy)(M +H)](center dot 3+). *J. Am. Soc. Mass Spectrom.* **16**, 1795–1804 (2005)

23. Moore, B. N., Blanksby, S. J., Julian, R. R.: Ion–Molecule Reactions Reveal Facile Radical Migration in Peptides. *Chem. Commun.* 5015–5017 (2009)

24. Ly, T., Julian, R.R.: Tracking Radical Migration in Large Hydrogen Deficient Peptides with Covalent Labels: Facile Movement does not

25. Chen, X.H., Turecek, F.: The Arginine Anomaly: Arginine Radicals are Poor Hydrogen Atom Donors in Electron Transfer-Induced Dissociations. *J. Am. Chem. Soc.* **128**, 12520–12530 (2006)

26. Sobczyk, M., Anusiewicz, W., Berdys-Kochanska, J., Sawicka, A., Skurski, P., Simons, J.: Coulomb-Assisted Dissociative Electron Attachment: Application to a Model Peptide. *J. Phys. Chem. A* **109**, 250–258 (2005)

27. McLafferty, F.W., Horn, D.M., Breuker, K., Ge, Y., Lewis, M.A., Cerda, B., Zubarev, R.A., Carpenter, B.K.: Electron Capture Dissociation of Gaseous Multiply Charged Ions by Fourier-Transform Ion Cyclotron Resonance. *J. Am. Soc. Mass Spectrom.* **12**, 245–249 (2001)

28. Kruger, N.A., Zubarev, R.A., Horn, D.M., McLafferty, F.W.: Electron Capture Dissociation of Multiply Charged Peptide Cations. *Int. J. Mass Spectrom.* **185**, 787–793 (1999)

29. Simons, J.: Mechanisms for S–S and N–C$_\alpha$ Bond Cleavage in Peptide ECD and ETD Mass Spectrometry. *Chem. Phys. Lett.* **484**, 81–95 (2010)

30. Turecek, F.: N–C$_\alpha$ Bond Dissociation Energies and Kinetics in Amide and Peptide Radicals. Is the Dissociation a Nonergodic Process? *J. Am. Chem. Soc.* **125**, 5954–5963 (2003)

31. Chung, T.W., Turecek, F.: Proper and Improper Aminoketyl Radicals in Electron-Based Peptide Dissociations. *Int. J. Mass Spectrom.* **301**, 55–61 (2011)

32. Syrstad, E.A., Turecek, F.: Toward a General Mechanism of Electron Capture Dissociation. *J. Am. Soc. Mass Spectrom.* **16**, 208–224 (2005)

33. Zubarev, R.A., Haselmann, K.F., Budnik, B., Kjeldsen, F., Jensen, F.: Towards an Understanding of the Mechanism of Electron-Capture Dissociation: a Historical Perspective and Modern Ideas. *Eur. J. Mass Spectrom.* **8**, 337–349 (2002)

34. Li, X.J., Cournoyer, J.J., Lin, C., O'Connor, P.B.: The Effect of Fixed Charge Modifications on Electron Capture Dissociation. *J. Am. Soc. Mass Spectrom.* **19**, 1514–1526 (2008)

35. Jones, J.W., Sasaki, T., Goodlett, D.R., Turecek, F.: Electron Capture in Spin-Trap Capped Peptides. An Experimental Example of Ergodic Dissociation in Peptide Cation Radicals. *J. Am. Soc. Mass Spectrom.* **18**, 432–444 (2007)

36. Sohn, C.H., Chung, C.K., Yin, S., Ramachandran, P., Loo, J.A., Beauchamp, J.L.: Probing the Mechanism of Electron Capture and Electron Transfer Dissociation Using Tags with Variable Electron Affinity. *J. Am. Chem. Soc.* **131**, 5444–5459 (2009)

37. Jones, A.W., Mikhailov, V.A., Iniesta, J., Cooper, H.J.: Electron Capture Dissociation Mass Spectrometry of Tyrosine Nitrated Peptides. *J. Am. Soc. Mass Spectrom.* **21**, 268–277 (2010)

38. Chan, W.Y.K., Chan, T.W.D.: Natural Structural Motifs that Suppress Peptide Ion Fragmentation after Electron Capture. *J. Am. Soc. Mass Spectrom.* **21**, 1235–1244 (2010)

39. Castro, I., Faus, J., Julve, M., Gleizes, A.: Complex-Formation Between Oxalate and (2,2'-6',2"-Terpyridyl)-Copper(II) in Dimethyl-Sulfoxide Solution—Synthesis and Crystal Structures of Mononuclear and Dinuclear Complexes. *J. Chem. Soc. Dalton Trans.* 1937–1944 (1991)

40. Kjeldsen, F., Haselmann, K.F., Budnik, B.A., Jensen, F., Zubarev, R.A.: Dissociative Capture of Hot (3-13 eV) Electrons by Polypeptide Polycations: An Efficient Process Accompanied by Secondary Fragmentation. *Chem. Phys. Lett.* **356**, 201–206 (2002)

41. Breuker, K., Oh, H.B., Cerda, B.A., Horn, D.M., McLafferty, F.W.: Hydrogen Atom Loss in Electron-Capture Dissociation: a Fourier Transform-Ion Cyclotron Resonance Study with Single Isotopomeric Ubiquitin Ions. *Eur. J. Mass Spectrom.* **8**, 177–180 (2002)

42. Savitski, M.M., Kjeldsen, F., Nielsen, M.L., Zubarev, R.A.: Hydrogen Rearrangement to and from Radical z Fragments in Electron Capture Dissociation of Peptides. **18**, 113–120 (2007)

43. Kalli, A., Hakansson, K.: Comparison of the Electron Capture Dissociation Fragmentation Behavior of Doubly and Triply Protonated Peptides from Trypsin, Glu-C, and Chymotrypsin Digestion. *J. Proteome Res.* **7**, 2834–2844 (2008)

44. O'Connor, P.B., Lin, C., Cournoyer, J.J., Pittman, J.L., Belyayev, M., Budnik, B.A.: Long-Lived Electron Capture Dissociation Product Ions Experience Radical Migration via Hydrogen Abstraction. *J. Am. Soc. Mass Spectrom.* **17**, 576–585 (2006)

45. Kleinnijenhuis, A.J., Heck, A.J.R., Duursma, M.C., Heeren, R.M.A.: Does Double Electron Capture Lead to the Formation of Biradicals? An

25. ... Equal Indiscriminate Fragmentation. *J. Am. Soc. Mass Spectrom.* **20**, 1148–1158 (2009)

ECD-SORI-CID Study on Lacticin 481. *J. Am. Soc. Mass Spectrom.* **16**, 1595–1601 (2005)

46. Liu, Z.J., Julian, R.R.: Deciphering the Peptide Iodination Code: Influence on Subsequent Gas-Phase Radical Generation with Photodissociation ESI-MS. *J. Am. Soc. Mass Spectrom.* **20**, 965–971 (2009)

47. Zubarev, R.A., Kruger, N.A., Fridriksson, E.K., Lewis, M.A., Horn, D.M., Carpenter, B.K., McLafferty, F.W.: Electron Capture Dissociation of Gaseous Multiply-charged Proteins is Favored at Disulfide Bonds and Other Sites of High Hydrogen Atom Affinity. *J. Am. Chem. Soc.* **121**, 2857–2862 (1999)

48. Cooper, H.J.: Investigation of the Presence of b Ions in Electron Capture Dissociation Mass Spectra. *J. Am. Soc. Mass Spectrom.* **16**, 1932–1940 (2005)

49. Haselmann, K.F., Schmidt, M.: Do b-ions Occur from Vibrational Excitation upon H-Desorption in Electron Capture Dissociation? *Rapid Commun. Mass Spectrom.* **21**, 1003–1008 (2007)

50. Lee, S., Chung, G., Kim, J., Oh, H.B.: Electron Capture Dissociation Mass Spectrometry of Peptide Cations Containing a Lysine Homologue: a Mobile Proton Model for Explaining the Observation of b-Type Product Ions. *Rapid Commun. Mass Spectrom.* **20**, 3167–3175 (2006)

51. Liu, H.C., Hakansson, K.: Abundant b-Type Ions Produced in Electron Capture Dissociation of Peptides without Basic Amino Acid Residues. *J. Am. Soc. Mass Spectrom.* **18**, 2007–2013 (2007)

52. van der Rest, G., Hui, R.J., Frison, G., Chamot-Rooke, J.: Dissociation Channel Dependence on Peptide Size Observed in Electron Capture Dissociation of Tryptic Peptides. *J. Am. Soc. Mass Spectrom.* **22**, 1631–1644 (2011)

53. Lioe, H., O'Hair, R.A.J.: Comparison of Collision-Induced Dissociation and Electron-Induced Dissociation of Singly Protonated Aromatic Amino Acids, Cystine, and Related Simple Peptides Using a Hybrid Linear Ion Trap-FT-ICR Mass Spectrometer. *Anal. Bioanal. Chem.* **389**, 1429–1437 (2007)

54. Chakraborty, T., Holm, A.I.S., Hvelplund, P., Nielsen, S.B., Poully, J.C., Worm, E.S., Williams, E.R.: On the Survival of Peptide Cations after Electron Capture: Role of Internal Hydrogen Bonding and Microsolvation. *J. Am. Soc. Mass Spectrom.* **17**, 1675–1680 (2006)

55. Tsybin, Y.O., Haselmann, K.F., Emmett, M.R., Hendrickson, C.L., Marshall, A.G.: Charge Location Directs Electron Capture Dissociation of Peptide Dications. *J. Am. Soc. Mass Spectrom.* **17**, 1704–1711 (2006)

56. Breuker, K., Oh, H.B., Horn, D.M., Cerda, B.A., McLafferty, F.W.: Detailed Unfolding and Folding of Gaseous Ubiquitin Ions Characterized by Electron Capture Dissociation. *J. Am. Chem. Soc.* **124**, 6407–6420 (2002)

57. Mihalca, R., Kleinnijenhuis, A.J., McDonnell, L.A., Heck, A.J.R., Heeren, R.M.A.: Electron Capture Dissociation at Low Temperatures Reveals Selective Dissociations. *J. Am. Soc. Mass Spectrom.* **15**, 1869–1873 (2004)

58. Adams, C.M., Kjeldsen, F., Zubarev, R.A., Budnik, B.A., Haselmann, K.F.: Electron Capture Dissociation Distinguishes a Single D-amino Acid in a Protein and Probes the Tertiary Structure. *J. Am. Soc. Mass Spectrom.* **15**, 1087–1098 (2004)

59. Kalli, A., Hakansson, K.: Electron Capture Dissociation of Highly Charged Proteolytic Peptides from Lys N, Lys C and Glu C Digestion. *Mol. BioSyst.* **6**, 1668–1681 (2010)

60. Nishikaze, T., Takayama, M.: Influence of Charge State and Amino Acid Composition on Hydrogen Transfer in Electron Capture Dissociation of Peptides. *J. Am. Soc. Mass Spectrom.* **21**, 1979–1988 (2010)

61. Ben Hamidane, H., He, H., Tsybin, O.Y., Emmett, M.R., Hendrickson, C.L., Marshall, A.G., Tsybin, Y.O.: Periodic Sequence Distribution of Product Ion Abundances in Electron Capture Dissociation of Amphipathic Peptides and Proteins. *J. Am. Soc. Mass Spectrom.* **20**, 1182–1192 (2009)

62. Robinson, E.W., Leib, R.D., Williams, E.R.: The Role of Conformation on Electron Capture Dissociation of Ubiquitin. *J. Am. Soc. Mass Spectrom.* **17**, 1469–1479 (2006)

63. Polfer, N.C., Haselmann, K.F., Langridge-Smith, P.R.R., Barran, P.E.: Structural Investigation of Naturally Occurring Peptides by Electron Capture Dissociation and AMBER Force Field Modelling. *Mol. Phys.* **103**, 1481–1489 (2005)