Dissociative capture of hot (3–13 eV) electrons by polypeptide polycations: an efficient process accompanied by secondary fragmentation

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Abstract

Beside the known maximum of electron capture dissociation (ECD) of gas-phase polypeptide polycations at zero electron energy, a broad local maximum is found around 10 eV. This maximum is due to electronic excitation prior to electron capture, as in dissociative recombination of small cations. In the novel hot electron capture dissociation (HECD) regime, not only N–C_α bonds are cleaved as in ECD, but secondary fragmentation is also induced due to the excess energy. Beneficially, this fragmentation includes abundant losses of $\cdot CH(CH_3)_2$ from leucine and $\cdot CH_2CH_3$ from isoleucine residues terminal to the cleavage site, which allows for distinguishing between these two isomeric residues. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

Gas-phase reactions of polypeptides and their molecular ions with free electrons include such phenomena as ionization [1–3], intramolecular charge and hydrogen atom transfer [4–6], charge recombination [7] and fragmentation. Typically, fragmentation results either from inelastic ion–electron collisions or from dissociative electron capture. Here, we report on a novel reaction involving both processes.

While inelastic ion–electron collisions (EIEIO [8,9]) yield mainly peptide C–N bond cleavages (b and y′ fragments) in a manner similar to ion–neutral collisions, electron capture dissociation (ECD [10]) breaks preferentially N–C_α backbone bonds giving c′ and z· fragments. The N–C_α cleavages occur faster than the losses of labile groups [11,12] and even breakages of non-covalent bonding [13], which allows for determination of the amino acid sequence, the sites of labile post-translational modifications, and can yield information on the gas-phase structure of weakly bound complexes. Secondary fragmentation in ECD is present at a minimal level, which is due to the non-ergodic mechanism [10] and the low value of the excess energy (exothermicity of

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electron capture by protonated polypeptides is 4–7 eV).

It is well known that ECD is efficient only for slow (< 0.2 eV) electrons, with the capture cross-section dropping two to three orders of magnitude for 1 eV electrons [14]. We found that the rate of dissociative capture can also be significant for hot (3–13 eV) electrons, provided their flux is sufficiently high for trapping ions inside the electron beam (this prevents ion cloud diffusion and assists electron capture [15]). The presence of local maximum in the capture cross-section points towards electronic excitation prior to capture and is consistent with the dissociative recombination results for small polyatomic ions (e.g., $HD^{+\cdot}$), where a broad local maximum around 10 eV is often present due to electronic excitation prior to electron capture [16].

2. Experimental

Peptides were either synthesized in-house using EPS221 automatic peptide synthesizer (Intavis, AG, Germany) or obtained from Sigma. Peptide polycations were produced by electrospray ionization and trapped inside a Penning trap of a Fourier transform (FT) mass spectrometer (Ionspec, CA). Upon isolation of the desired charge state by stored waveform, polycations were irradiated for 250 ms by electrons emitted from an indirectly heated cathode (diameter 5 mm) coated with barium oxide (HeatWave, CA).

3. Nomenclature

In this study, we introduce a modified notation that is based on the traditional peptide fragmentation nomenclature [17,18] but offers a bookkeeping of hydrogen atom transfer to and from the fragments. In this notation, the presence of an unpaired electron is always denoted by the radical sign '·', e.g. homolytic $N–C_\alpha$ bond cleavage gives $c^\cdot$ and $z^\cdot$ fragments. Hydrogen atom transfer to the fragment is denoted by '′', e.g., transfer to $c^\cdot$ gives c' species, while hydrogen atom loss from $z^\cdot$ results in z fragments. The number in superscript denotes

the presence of protons in the cationic fragments (absence of protons for anions); the number of residues from the corresponding terminus is given in the subscript, e.g., $c_7'^{2+}$ for a doubly protonated fragment. According to this notation, peptide bond cleavage gives b and y' fragments. Conventional $N–C_\alpha$ bond cleavage in ECD is denoted as c', $z^\cdot$ and less frequent cleavage with hydrogen transfer to the C-terminal fragment as $c^\cdot$, z'.

4. Results and discussion

The properties of the novel reaction are illustrated by the following experiment. Electrospray-produced dications of a tryptic decapeptide from signal recognition particle (SRP) of *Saccharomyces cerevisiae*, (MW 1261) and its modified Ile_7 analogue were subjected to dissociative electron capture at different electron energies.

4.1. $N–C_\alpha$ cleavage rate

Two maxima were observed in the dependence of the abundance of $N–C_\alpha$ bond cleavages (Fig. 1), one at ≈ 0 eV and another at ≈ 7 eV, with full width at half maximum (FWHM) equal to 1 and 6 eV, respectively. The first region of the effective $N–C_\alpha$ bond cleavage corresponded to the conventional ECD regime. The extension to the negative energy values and its width in excess of 0.2 eV were

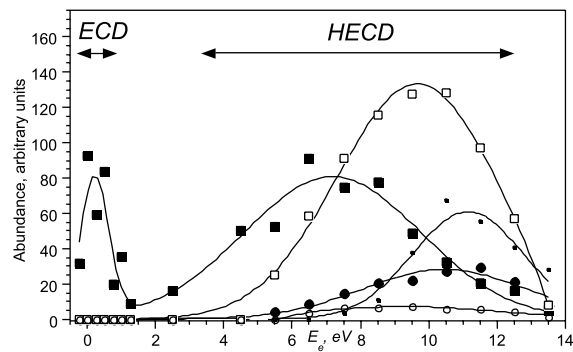Fig. 1. Fragment ion abundances versus electron energy E_e for 250 ms irradiation of SRP molecular ions, 2+: (■) $N–C_\alpha$ bond cleavages, (□) C–N bond cleavages, (○) $z_4^{+\cdot}$ fragments, (●) $w_4^{+\cdot}$ fragments; 1+: (■) C–N bond cleavages.

due to the kinetic energy spread of the electrons emitted from a hot surface [15].

The second maximum was due to the novel reaction of hot electron capture dissociation (HECD). That the observed $N–C_\alpha$ cleavage indeed involved electron capture was confirmed by the observation that even longer (400 ms) irradiation of monocations produced only C–N cleavage (b and y′ fragments) but no $N–C_\alpha$ cleavages (Fig. 1). These b and y′ fragments, as well similar fragments in HECD mass spectra of dications, originated from non-capture EIEIO-type processes [8,9].

4.2. HECD cross-section

The HECD reaction is well separated on the energy scale from the conventional ECD reaction by a region 2–3 eV wide where significantly less fragmentation is observed. The $N–C_\alpha$ fragmentation rate in HECD exceeded that in ECD; however, the electron current through the FT cell was 7.8 μA for HECD and only 70 pA in the ECD case. This gives 100 times larger cross-section for slow electrons, similar to the situation in dissociative recombination of small cations [16].

The mechanism of the $N–C_\alpha$ bond cleavage in HECD was investigated by measuring the correlation factor between the relative abundances of c and z fragments at the electron energy corresponding to the two maxima (Fig. 2). The obtained value of 0.70 is typical for ECD mass spectra obtained at different conditions [19], which indicates that the bond cleavage mechanisms are likely to be similar.

4.3. Secondary fragmentation

Besides $N–C_\alpha$ bond cleavage, HECD gave other fragmentation channels, with many more bonds cleaved than in ECD (Fig. 3). Noticeably, some of the most abundant fragments were due to secondary fragmentation. This was expected due to the large excess energy in HECD, which is equal to the kinetic energy of the electrons prior to capture. The dissipation channels for this energy included losses of H· and larger radical groups near the position of primary cleavage. This had a useful feature of the formation of even-electron d and w species from a· and z· radical fragments by a loss from the side chain adjacent to the radical site (Fig. 4). For isoleucine and leucine residues, the lost groups were $·C_2H_5$ and $·C_3H_7$, respectively, which allowed for distinguishing between these two isomeric residues. For example, the w_4^+ fragment of SRP was the second most abundant ion in the 10 eV HECD mass spectrum (Fig. 3b). In the

Fig. 2. $N–C_\alpha$ cleavage abundances in the mass spectra of 2+ of SRP at different energies of bombarding electrons.

Fig. 3. Mass spectra of 2+ of SRP and its Ile_7 variant at different energies of bombarding electrons.

d-ions

Leu

$$a\cdot$$

R'—NH—ĊH
           |
           CH$_2$
           |
           CH
       H$_3$C   CH$_3$

$\longrightarrow$

R'—NH—CH   +   ·CH(CH$_3$)$_2$
           ‖
     d   CH$_2$ **43 Da**

Ile

$$a\cdot$$

R'—NH—ĊH
           |
           CH
       H$_3$C   CH$_2$
                   |
                   CH$_3$

$\longrightarrow$

R'—NH—CH   +   ·CH$_2$CH$_3$
           ‖
           CH
           |
     d   CH$_3$ **29 Da**

w-ions

Leu

$$z\cdot$$

ĊH—CO—R"
   |
   CH$_2$
   |
   CH
H$_3$C   CH$_3$

$\longrightarrow$

CH—CO—R"   +   ·CH(CH$_3$)$_2$
‖
CH$_2$ *w*   **43 Da**

Ile

$$z\cdot$$

ĊH—CO—R"
   |
   CH
H$_3$C   CH$_2$
             |
             CH$_3$

$\longrightarrow$

CH—CO—R"   +   ·CH$_2$CH$_3$
‖
CH
|
CH$_3$ *w*   **29 Da**

Fig. 4. Schematic representation of fragmentation processes leading to w ions from Leu/Ile residues.

conventional ECD, w ions have never been reported.

To ensure the correct fragment assignment, an Ile$_7$ analogue of the peptide was synthesized. Comparison of the HECD mass spectra (Fig. 3b, inset) confirmed the assignment of the peak at m/z 455 to the w$_4$ fragment. The small peak at m/z 455 in the Ile$_7$ spectrum was likely due to the competing coupling of Fmoc–Leu still remaining in the resin after the previous coupling cycle (Fmoc synthesis progresses from C- to N-terminus) as a result of incomplete washing between the cycles. This parasitic effect could be enhanced by the reduced rate of Ile coupling compared to Leu coupling due to steric hindrance in the former residue [20].

Secondary fragmentation is usually endothermic and therefore demands a certain amount of excess energy. B3LYP/cc-pVT2 calculations gave the following endothermicities of the bond cleav-

ages: 102 kJ/mol for z⁻ (Leu) $\rightarrow$ w + ·CH(CH$_3$)$_2$, 83 kJ/mol for a⁻ (Leu) $\rightarrow$ d + ·CH(CH$_3$)$_2$, 95 kJ/mol for z⁻ (Ile) $\rightarrow$ w + ·CH$_2$CH$_3$ and 80 kJ/mol for a⁻ (Ile) $\rightarrow$ d + ·CH$_2$CH$_3$. These results indicate that d ions should be formed more easily than w fragments; however, three w species (which identified the residues 7, 8 and 9 as Leu, Leu and Ile) and no d ions were observed in HECD of SRP. This is in stark contrast to the presence of four abundant d ions (besides w ions) in the mass spectra of high-energy ion–neutral collisions of SRP monocations [21], which points toward different mechanisms in the latter process compared to HECD. In HECD of other peptides, d ions were observed, but generally at lower abundances than w species. The lower abundance of d compared to w fragments in HECD can be explained by the dominance in ECD of the primary non-ergodic fragmentation channels producing z⁻ ions over those giving a⁻ fragments [14].

4.4. HECH mechanism

The losses from primary fragments in SRP mass spectra were energy-dependent: at 7 eV HECD, four out of five $z^\cdot$ fragments lost a hydrogen atom to become z ions. At higher electron energies, losses of larger radicals were observed, with the abundance of w ions peaking at 11 eV. The appearance energy of the w_4 fragment was 4.5 ± 0.5 eV (Fig. 1), which is significantly higher than ≈ 1 eV required to remove the $\cdot CH(CH_3)_2$ group from the $z_4^{+\cdot}$ fragment. The 4.5 eV of the excess energy has either been deposited far from the cleavage site, or the secondary fragmentation has occurred after the energy has been distributed over many degrees of freedom.

These observations suggested the following HECD mechanism. A hot electron loses its kinetic energy in an inelastic collision with the molecular ion, which facilitates the capture of the decelerated electron by the excited ion. This capture results into fast $N–C_\alpha$ bond cleavage similar to that in the conventional ECD. If no electron capture occurs in the inelastic collision (e.g., the captured electron is ejected back to the continuum), electronic excitation is relaxed via intramolecular conversion into vibrations, with typical for vibrationally excited polypeptide cations C–N bond cleavage. The efficiency of the HECD process decreases after the electron energy exceeds the ionization threshold of ca. 11 eV [2,3].

The experimental and theoretical evidence suggests that $N–C_\alpha$ bond cleavage in ECD may occur faster ($< 10^{-12}$ s) than intramolecular energy redistribution (IVR) [10]. Primary bond fragmentation in HECD can also be non-ergodic. Since intramolecular conversion followed by IVR can take up to 10^{-8} s, secondary fragmentation can occur in already separated fragments. However, not all primary fragments undergo secondary fragmentation: in another experiment, 11 eV HECD of Thr_5-phosphorylated synthetic peptide VYGKTSHLR gave no measurable phosphate group losses from $a^\cdot$, c' and $z^\cdot$ ions, while 70% of $y_6'^+$ ions that were present in the spectrum due to an EIEIO-type process lost H_3PO_4. Secondary fragmentation is therefore most likely to occur near the primary cleavage sites that are additionally 'weakened' by the presence of an unpaired electron.

4.5. Ile/Leu identification

Analytical implications of HECD are not the main subject of this communication, they will be discussed in a separate publication. Here we just notice that, in the SRP peptide, HECD allowed for identification of all three Ile/Leu pairs. In the phosphopeptide example, the presence of an intense w_2^+ ion also identified the Leu_8 residue. In HECD of several other tested peptides 10–17 residues long, w ions and to a lesser extent d ions also afforded Leu/Ile identification. Even for larger molecules, HECD was accompanied by abundant secondary fragmentation, despite the many more degrees of freedom over which the excess energy could distribute. While ECD of 4+ of melittin (2.8 kDa) produced 19 c ions and 22 $z^\cdot$ ions together with a few other products [14], 11 eV HECD gave at least 127 isotopic clusters (≈ 1500 separate mass peaks), with all seven Leu/Ile pairs resolved due to the presence of singly and multiply charged w fragments. This means that secondary fragmentation is not limited to small polypeptides, but may be an inherent feature of the HECD process.

5. Conclusions

HECD is a new fragmentation phenomenon in polypeptides that further extends the already broad range of ion–electron reactions of these species [1–10] and confirms the link between the processes occurring with small cationic species [16] and large biomolecules. HECD can immediately find application in mass spectrometry; it may also have implications for radiobiology. Besides the abundant fragmentation that is advantageous for sequence confirmation, the presence of w and d fragments that reveal the identity of isomeric Leu/Ile residues is invaluable for high-sensitivity de novo sequencing. This is especially beneficial since the alternative technique based on high-energy ion–neutral collisions is not available on many modern instruments.

Acknowledgements

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