Intramolecular hydrogen atom transfer in hydrogen-deficient polypeptide radical cations

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Abstract

Irradiation of protonated polypeptides NH₂–RH‡–COOH by >10 eV electrons leads to further ionization and fast intramolecular charge transfer to the free N-terminus. The resulting species may undergo further hydrogen atom rearrangement to form distonic ions N‡H₃–RH‡–COO⁻. Such transfer is exothermic but can involve an appreciable barrier, e.g., 2.3 ± 0.5 eV for MH²⁺ ions of the peptide ACTH 1–10. Radical polypeptide dications can, therefore, be viewed as hydrogen atom wires. Subsequent capture of low energy electrons results in fragmentation. The pattern of this electronic excitation dissociation (EED) is consistent with hydrogen transfer prior to electron capture. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Besides M⁺ ions that result from ionization of neutrals by electron impact or UV photoexcitation, there are at least two other types of gas-phase peptide radical cations. The MHₙ⁽ⁿ⁻１⁺⁾ ions originate from n-protonated molecules upon non-dissociative electron capture [1,2], e.g.,

\[ \text{MH}ₙⁿ⁺ + e_{\text{slow}} \rightarrow \text{MH}ₙⁿ⁻１⁺ \]  
(1)

Yet another type of radical cation has recently been obtained from protonated polypeptides by sustained irradiation with > 10 eV electrons [3]:

\[ \text{MH}⁺ + e_{\text{fast}} \rightarrow \text{MH}²⁺ + e_{\text{slow}} + e_{\text{fast}} \]  
(2)

The properties of the MH²⁺ dications are largely unknown, but parallels can be drawn with the more conventional M⁺⁺ radical cations. Both species are hydrogen-deficient, i.e., they are lacking one hydrogen atom compared to more stable protonated molecules, as opposed to the hydrogen-excess species formed in (1). Production of M⁺⁺ peptide cations is followed by a fast (â 0.2 ps) intramolecular charge transfer from the site of the original ionization to the N-terminus [4–6]:

\[ (\text{NH}₂–\text{R–COOH})⁺⁺ → \cdot \text{N}⁺\text{H}₂–\text{R–COOH} \]  
(3)

We postulate that a similar charge transfer in dications can be followed by intramolecular hydrogen transfer from, e.g., the C-terminal carboxylic acid to the charged amino group to form a distonic ion:

\[ \cdot \text{N}⁺\text{H}₂–\text{RH}⁺–\text{COOH} \rightarrow \cdot \text{N}⁺\text{H}₃–\text{RH}⁻–\text{COO}⁻ \]  
(4)

First we note that the intramolecular charge transfer must be more effective in dications than the transfer (3) in M⁺⁺ species. While the latter
process is due to the differences in the local ionization energies of amino acid residues [4], there is an additional driving force in dications arising from the coulombic repulsion between the localized charge (proton) and the mobile charge (hole). For example, the barrier for charge migration posed by the residues with higher ionization energies such as Gly is 0.2–0.3 eV [6]. This barrier can hinder the hole transfer in monocations [6]. In a fully stretched dication of a 10-residue peptide, the average inter-charge distance is \(1.5\) nm, corresponding to the coulombic repulsion energy of \(0.8\) eV, i.e., much more than the barrier size. It is likely, therefore, that the hole will rapidly move to a remote site, e.g., to the N-terminus.

As for intramolecular hydrogen transfer, it is a common unimolecular reaction in radical organic cations, with McLafferty rearrangement [7] being one of the best-documented cases. In the presence of amines, hydrogen rearrangement often leads to protonation of the amino group and thus to separation of the charged and radical sites (distonic ion); the driving force is the high proton affinity of the amino group. In glycine \(\text{M}^+\) radical cations, exothermic H transfer has been found from the C-terminus to the charged N-terminus [8]:

\[
\text{N}^+\text{H}_2-\text{CH}_2-\text{COOH} \rightarrow \text{N}^+\text{H}_3-\text{CH}_2-\text{COO}^-. \tag{5}
\]

The activation energy for this rearrangement is 0.4 eV for the ground state, with no barrier for the first excited state. To the best of our knowledge, intramolecular hydrogen atom rearrangement in polypeptides is being reported for the first time, although long-range rearrangement has been reported for non-peptide molecules, e.g., \(\alpha,\omega\)-diaminoalkane radical cations [9]. Our calculations (see thermodynamic cycle in Fig. 1) show that the H transfer in polypeptide dications from the carboxylic acid to the charged primary amine is ca. 1.6 eV more exothermic than in radical cations. Experiments described below support hydrogen transfer in dications of polypeptides 10–24 residues long.

2. Experimental procedure

Protonated ions of commercially available polypeptides (Sigma) were produced externally by 337 nm UV matrix assisted laser desorption ionization (MALDI) using 2,5-dihydroxybenzoic acid (DHB) as a matrix. Doubly protonated molecules were produced by electrospray ionization. The ions trapped inside a Fourier transform (FT) mass spectrometer were isolated and irradiated in situ by >10 eV electrons for 40–300 s. Experimental details of this tandem ionization technique are described elsewhere [3]. The slow electrons ejected in (2) could be reflected back by application of a repelling potential behind the cell, which promoted capture of these slow electrons by the formed dications:

\[
\text{MH}^{2+} + e_{\text{slow}} \rightarrow \text{MH}^{++}\tag{6}
\]

This capture process is 10–12 eV exothermic [3] and may cause fragmentation. Here we term the technique combining tandem ionization (2) with electron capture (6) electronic excitation dissociation (EED).

3. Results

EED of molecular ions MH\(^+\) of ACTH 1–10 (SYSMEHFRWG) gave singularly intense N-terminal \(e_8^+\) fragment (Fig. 2), resulting from the cleavage of the backbone amine bond. When the polarity of the reflecting potential on the quadru-
pole was reversed (no slow electrons inside the cell), no $c^8$ ions were observed, whereas the intensity of the dication $MH_2^+$ remained approximately the same. This proved that the $c^8$ ion originated from capture of the reflected slow electrons by dications. In contrast, the intensities of the $y^5$ ions (cleavage of the peptide (amide) bond) as well as that of $-17$ Da fragments were independent of the polarity of the reflecting potential, consistent with their production through vibronic excitation of $MH^+$ ions by $>10$ eV electrons [10]. Homolytic N–C bond cleavage between Arg-8 and Trp-9 in a neutral ACTH 1–10 molecule corresponds to N-terminal mass of 1053.458; the measured $m/z = 1055.47 \pm 0.02$ revealed that the $c^8$ fragment was both protonated and an even-electron species, i.e., had an extra proton and a hydrogen atom. This even-electron ion was also abundant in the ECD mass spectrum of the corresponding $MH^+$ dications (Fig. 3), but was absent in the collisional (CAD) spectra of the radical $MH^+$ cations (not shown) and protonated molecules $MH^+$ (Fig. 2, right insert). Generally, protonated, even-electron $c$ ions rarely appear in low-energy CAD and only sporadically appear in high-energy CAD of protonated peptides [11]. Formation in EED of intense even-electron $c^8$ ion is, therefore, unexpected. Moreover, even-electron $c$ ions are formed in ECD via incorporation of the extra hydrogen atom (neutralized proton) into the N-terminal $c$-fragment [1,2]:

$$\text{NH}_2\text{–RH}^+\text{–CO(H)}^- = \text{NH}\text{–RH}^+\text{–C(OH)} = \text{NH} + \cdot\text{R}^\cdot\text{–COOH}$$  

(7)

The complementary C-terminal radical fragments $\cdot\text{R}^\cdot\text{–COOH}$ correspond to the $z^\cdot$ ions [1,2]. At first glance, this mechanism cannot work in EED where there is no extra hydrogen atom. However, if intramolecular hydrogen atom rearrangement is involved, a process similar to (7) seems possible.

Let us consider mass spectra in Figs. 2 and 3 in more detail. The ECD fragmentation pattern (N-terminal $c^8$ and $c^9$ ions and a C-terminal series of $z^2$ to $z^0$ ions) is consistent with Arg-8 being one of the protonation sites (arginine has by far the highest gas-phase basicity). The proton is likely to be solvated on the backbone oxygen atoms C-terminal to that residue, otherwise N-terminal fragments below $c^8$ would have been observed. The second proton is likely to reside either on the

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**Fig. 2.** EED of the ACTH 1–10 MH$^+$ ions produced by UV MALDI. The ions were trapped inside a FT mass spectrometer and subsequently irradiated by 19 eV electrons. The slow electrons ejected from cations were reflected back and captured by the formed MH$^{2+}$ dications; this capture resulted in fragmentation. Inserts show (left) determination of the ionization energy of MH$^+$ ions and the appearance energy of the $c^8$ fragment ions (see text), and (right) collisionally activated dissociation of the MH$^+$ ions.
N-terminus or on the His-6 residue (the higher proton affinity of the latter is reduced by the coulombic repulsion with the first proton). Arg-8 is also the most probable protonation site in the MH\(^+\) ions. Therefore, if ionization by electrons occurred in the first eight residues of these ions, the hole is likely to be transferred to the N-terminus. Now, the hydrogen atom transfer from the C- to N-terminus would make the MH\(^{2+}\) radical dications structurally similar to MH\(^{2+}\) ions, with the radical at the C-terminus being the only difference. From the rearranged MH\(^{2+}\) dications, even-electron \(c^\pm\) ions can be formed upon electron capture by a mechanism similar to ECD; however, the complementary C-terminal fragments will be not \(z^\pm\) ions as in ECD but the biradical \(\cdot R'\cdot COO\). Interaction between the two unpaired electrons in the biradical may explain the absence of the Trp–Gly cleavage (no \(c^\pm\) ions) in EED.

The alternative explanation might be that the hydrogen atom rearrangement happened at the moment of electron capture. To test that, the appearance energy of the \(c^\pm\) ion was measured together with the ionization energy of MH\(^+\). The former was found to be equal to 13.8 ± 0.3 eV, while the latter 11.5 ± 0.3 eV (Fig. 2, left insert). Since there is no energy barrier for electron capture by positive ions, the 2.3 eV difference can be interpreted as a barrier for hydrogen atom rearrangement. Dissociation upon electron capture is usually fast (<ps [1]), and thus it is far more likely that hydrogen rearrangement happens prior to electron capture. If not for this time limitation, the released 10–12 eV of the recombination energy would be enough to overcome the 2.3 eV barrier. The larger size of the barrier compared to that found in the glycine radical cation [8] is consistent with the much larger size of the ACTH-1–10 molecule. The rearrangement is unlikely concerted; rather, it is consecutive as in the already cited rearrangement via a 15-member ring of the phenolic hydrogen to the charged primary amine in \(\alpha,\omega\)-diaminoalkane radical cations [9].

In EED of other investigated peptides, \(e^\pm\) ions were also present, although at lower intensities. Substance P (RPKPQQLFFGLM with an amide at the C-terminus) produced three even-electron, protonated \(e^\pm\) ions (\(c^\pm\), \(c^\pm\) and \(c^\pm\)), with a much more abundant \(a\)-series (eight ions). For comparison, the ECD spectrum of all charge states published by the Uppsala group [12] is dominated by a series of \(e\) ions, with a few \(a\) ions. Generally, we noted a difference in behavior of peptides with amidated and acidic C-termini. Gas-phase ionization (3) was accompanied by loss of CO\(_2\) from the formed dications in the acidic form (renin
substrate, ACTH 1–10 and 1–24, Lys-bradykinin, and Lys-des-[Arg-9]-bradykinin), as opposed to the losses of 74 Da by the amidated peptides [Arg-8]-vasopressin, bombesin and substance P. The hydrogen atom transfer (4) can account for the rather unusual peptide cations facile CO\textsubscript{2} loss through radical-site induced cleavage of the R–COO\textsuperscript{−} bond. Such cleavage has been observed in radical polypeptide anions formed through electron ejection from the negatively charged carboxylic group of deprotonated molecules [13]

\[ \text{R}^–\text{COO}^– + e_{\text{fast}}^{-} \rightarrow \text{R}^–\text{COO}^– + e_{\text{slow}}^{-} + e_{\text{fast}}^{-} \]
\[ \rightarrow \text{R}^\cdot + \text{CO}_2 + 2e^{-} \]

(8)

Obviously, this mechanism is disabled when an amide is present at the C-terminus. As the only exception, melittin radical dications lose CO\textsubscript{2} despite the absence in this molecule of carboxylic groups. The much broader range of possible rearrangement reactions (including those involving backbone carbonyl) in this 25-residue molecule can account for this loss.

Summarizing, hydrogen atom transfer appears to be a general phenomenon in peptide radical cations. Such transfer may be important in, e.g., ion formation mechanisms in such desorption techniques as MALDI, and for the attempts to achieve two-step additional protonation of molecular ions in the gas phase [3]. EED can be a useful complementary fragmentation technique for structure characterization of gas-phase biomolecules in the absence of multiply charged ions. It is worth to note that there are important differences between ECD and EED, (electron parity of parent ions and products, exothermicity of electron capture, and so on), which will be discussed in detail in a separate publication [14]. Here, we just note that the observed differences in fragmentation patterns indicate that the fragmentation mechanisms in these two techniques are, in general, similar, with certain common features (like c\textsubscript{8}+ ion in ACTH 1–10) due to intramolecular hydrogen rearrangement.

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**References**