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Electron detachment dissociation of peptide di-anions: an electron–hole recombination phenomenon

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Abstract

A novel electron–ion reaction mimicking positron capture is reported for gas-phase polypeptide di-anions. Bombardment of the latter by >10 eV electrons produced electron detachment followed by backbone dissociation, noticeably cleavage of the N–C_α bond. On the other hand, reaction with hydrogen cations resulted in losses of neutral groups but not in backbone cleavage. It is proposed that the N–C_α dissociation is due to recombination along the peptide chain of a positive radical charge (hole) with a negative charge. The new reaction is likely to find application in mass spectrometry for primary structure determination of acidic polypeptides.

1. Introduction

Reactions of gas-phase molecules with electrons are important in studies of ionisation [1], intramolecular charge transfer [1,2] and fragmentation [1–3] processes as well as for determination of the molecular structure. For example, primary polypeptide sequence can be established using electron capture dissociation (ECD [4]) that utilises the recombination reaction of multiply protonated polypeptides with subthermal (<0.2 eV) electrons [5]

$$[M + nH]^{n+} + e^-_{slow} \rightarrow ([M + nH]^{(n-1)+\cdot})_{transient} \rightarrow \text{S–S, N–}C_\alpha \text{ bond fragmentation} \quad (1)$$

This dissociation is moderately exothermic (⩽7 eV) but yet non-ergodic [4], which is manifested by e.g. abundant backbone cleavage in large polypeptides [6] as well as by preferential strong bond dissociation in the presence of much weaker bonds [7]. The latter feature found application in determination of the sites of biologically important labile modifications, such as phosphorylation [8,9], O-glycosylation [10], γ-carboxylation and sulphation [11]. While many biologically important peptides are acidic, ECD is restricted to basic peptides that form stable polycations in electrospray ionisation (ESI).

It has been suggested that poly-anions of acidic peptides may undergo similar non-ergodic dissociation upon positron capture [12]

$$[M - nH]^{n-} + e^+_{slow} \rightarrow ([M + nH]^{(n-1)-\cdot})_{transient} \rightarrow \text{fragmentation} \quad (2)$$

It has also been proposed to employ, in the absence of a convenient source of low energy positrons, energetic electrons for production of similar species [12]

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$$[M - nH]^{n-} + e^-_{fast} \rightarrow [M - nH]^{(n-1)-\cdot} + 2e^- \quad (3)$$

Although the electron detachment (3) has been achieved for peptide di-anions, the radical anions appeared stable against backbone cleavage showing only facile CO_2 loss [12]

$$R^-COO^- + e^-_{fast} \rightarrow R^-COO\bullet + 2e^- \rightarrow R^{-\cdot} + CO_2 + 2e^- \quad (4)$$

Now, with an improved apparatus and greater sensitivity [13] we are reporting the first observation of the predicted electron detachment dissociation (EDD) in polypeptide di-anions.

2. Experimental

The model system was the synthetic peptide caerulein pEQDYTGWMDF sulphated at the Tyr_4 residue (Sigma, St. Louis, MO). The peptide was in the amide form, but due to the presence of three acidic sites (sulphate and two aspartic acid residues) produced in ESI abundant di-anions $[M - 2H]^{2-}$. The much less abundant di-cations of this molecule were unstable in the gas-phase against facile loss of the SO_3 group, which precluded the use of ECD for determination of the sulphation site [14]. The di-anions were isolated in the cell of a Fourier-transform (FT) mass spectrometer (IonSpec, USA) and irradiated by 10–27 eV electrons for 0.2–1.0 s.

3. Results

The mass spectrum obtained at 21 eV is shown in Fig. 1. The most prominent losses from the oxidised species $[M - 2H]^{-\cdot}$ are $-CO_2$ and $-SO_3$. Importantly, $\boldsymbol{a}$, $\boldsymbol{c}$ and $\boldsymbol{z}$ series were observed among the backbone cleavages (Fig. 1). Most fragment ions retained the sulphate group, with two $\boldsymbol{a}$ ions showing minor losses. All $\boldsymbol{a}$ ions but $\boldsymbol{a_7}$ were radicals. The $\boldsymbol{a_7}$ ions were even-electron species 1 Da lighter than the corresponding radicals. Most $\boldsymbol{a}$ ions were accompanied by the loss of 44 Da, presumably the CO_2 group. The $\boldsymbol{c}$ ions were radicals besides $\boldsymbol{c_2}$ and $\boldsymbol{c_5}$. Of four $\boldsymbol{z}$ ions, three were radicals, with $\boldsymbol{z_6}$ being even-electron species 1 Da lighter than the corresponding radical.

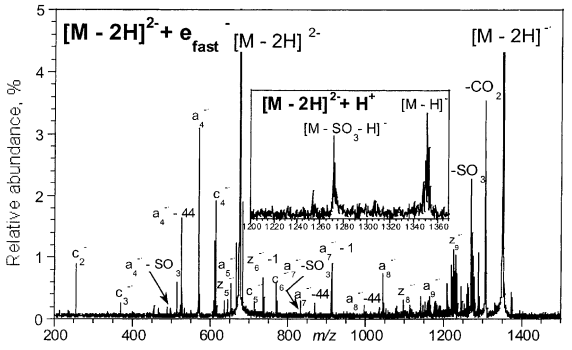

Fig. 1. EDD spectrum of di-anions of the sulphated peptide caerulein. The inset shows the result of the capture of hydrogen ions by the di-anions.

The appearance energies of the major products were determined with ±0.5 eV accuracy. The obtained values: $AE(\boldsymbol{a_4}^{-\cdot}) = 11.8$ eV, $AE(\boldsymbol{c_4}^{-\cdot}) = 12.4$ eV, $AE(\boldsymbol{c_6}^{-\cdot}) = 10.3$ eV, $AE(\boldsymbol{a_7}^{-}) = 11.5$ eV and $AE(\boldsymbol{a_8}^{-\cdot}) = 11.0$ eV are all larger than the appearance energy of the $[M - 2H]^{-\cdot}$ ion, 10.0 eV. This, together with the presence of many radical fragments, indicate that the fragmenting species were oxidised radical anions. Electron-induced vibrational excitation (so-called EIEIO [3]) of di-anions, on the contrary, was unlikely the cause of fragmentation. Consistent with that, collisional activation dissociation (CAD) of di-anions produced losses of neutral groups but no $N–C_\alpha$ cleavage, while CAD of the oxidised $[M - 2H]^{-\cdot}$ species gave also $\boldsymbol{a_4}^{-\cdot}$ and $\boldsymbol{c_4}^{-\cdot}$ ions.

4. Discussion

The appearance energy of the $[M - H]^{-\cdot}$ ions is similar to the ionisation energies of protonated peptides in electron bombardment (10–12 eV) [15]. This means that electron detachment may occur not only from the anionic surface or carboxy-groups (electron affinity ≈4.5 and 3.3 eV, respectively, [16]) but also from the rest of the polypeptide (adiabatic ionisation energy ≈8.5 eV [17]).

We propose the following mechanism of the EDD cleavage. The onset of the polypeptide chain ionisation leads to creation of a positive radical

charge (hole). This hole is mobile, with the driving forces for its transfer being the Coulombic attraction to the negative charges as well as the differences in local ionisation energies of amino acid residues [1,2,18]. Mutual neutralisation of the hole and an electron results in electronic excitation that, in turn, causes backbone cleavage, e.g.:

$$\begin{aligned}&R(OSO_3^-) - \cdots - R(COO^-) + e^- \rightarrow \\ &R(OSO_3^-) - \cdots (+^{\cdot}) \cdots - R(COO^-) + 2e^- \rightarrow \\ &R(OSO_3^{\cdot}) - \cdots [(-^{\cdot})(+^{\cdot})] \cdots - \\ &R(COO^-) + 2e^- \rightarrow \text{fragmentation} \end{aligned} \quad (5)$$

The exothermicity of the electron–hole recombination is approximately equal to the difference between the ionisation energy of the polypeptide chain and the electron affinity of the carboxyl-group, i.e. ≈5 eV. This is comparable to the exothermicity in ECD and can thus be sufficient to cause N–C_α cleavage. At the same time, the much more energetic capture reaction between di-anions and hydrogen cations (exothermicity of proton capture >15 eV) yielded just SO_3 loss, but neither CO_2 loss nor backbone cleavage (Fig. 1, inset):

$$\begin{aligned}&R(SO_3)^-COO^- + H^+ \rightarrow (R(SO_3)^- - COOH)_{hot} \\ &\quad \rightarrow R^-COOH + SO_3 \end{aligned} \quad (6)$$

or

$$\begin{aligned}&R(SO_3)^-COO^- + H^+ \rightarrow (R(SO_3H) - COO^-)_{hot} \\ &\quad \rightarrow RH - COO^- + SO_3 \end{aligned} \quad (6a)$$

This reaction, reported for the first time, was accomplished by in-cell ionisation of the hydrogen gas by 70 eV in the presence of trapped $[M - 2H]^{2-}$ di-anions. The recombination energy here was likely released as vibrational excitation mainly, in contrast to the electronic excitation in electron–hole recombination. The absence of decarboxylation is consistent with the suggestion that the latter process in polypeptide anions is only facile if a radical site is present [12].

Consistent with the drift of the hole towards the electron, the most abundant backbone EDD cleavage (Fig. 2) is located near the sulphated tyrosine residue, the most likely deprotonation site. The timescale of the fragmentation can be of the order of charge transfer in peptides, $<10^{-12}$ s [2],

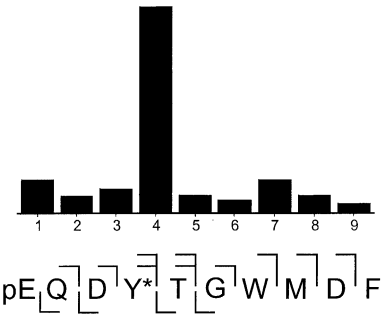

Fig. 2. Cleavages and relative cleavage frequencies of the di-anions of caerulein in EDD.

but no direct indication of this has been observed in the reported experiments.

The proposed EDD mechanism is different from the mechanisms in ECD and another related technique, electronic excitation dissociation [18]. In the latter technique, protonated polypeptides $[M + H]^+$ are first ionised by >10 eV electrons and then the formed radical dications $[M + H]^{2+\cdot}$ capture low-energy (<1 eV) electrons. Although this capture also leads to electron–hole recombination, the fragmented species are the electronically excited protonated polypeptide molecules $[M + H]^{+*}$, in contrast to the radical anions $[M - H]^{-\cdot *}$ in EDD.

Besides caerulein, EDD yielded N–C_α cleavages in an eight-residue peptide in both sulphated and non-sulphated forms; more experiments are under way. Being a substitute of ECD for negative ions, the phenomenon of EDD is likely to find an application in sequence determination and location of the sites of post-translational modifications in acidic peptides.

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