Dissociation mechanisms and implication for the presence of multiple conformations for peptide ions with arginine at the C-terminus: time-resolved photodissociation study

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Time-resolved photodissociation (PD) patterns of singly protonated peptides with arginine at the C-terminus (C-arg peptide ions) have been used to classify the dissociation channels into two categories, i.e. high-energy channels generating v, w and x and low-energy ones generating b, y and z. x + 1 formed by Cα-CO cleavage seems to be the intermediate ion in high-energy channels just as a + 1 is for N-arg peptide ions. Difference in time-resolved pattern indicates that the two sets of channels, high- and low-energy ones, are not in direct competition. Noncompetitive dissociation is also indicated by the observation of anomalous effect of matrix used in matrix-assisted laser desorption ionization, a cooler matrix generating more high-energy product ions both in spontaneous dissociation and in PD. Results from detailed investigation suggest that the two sets of channels start from two (or more) different conformations.

Introduction

Tandem mass spectrometry for protonated tryptic peptides is widely practiced for protein sequencing. 1,2 Ion activation methods 3–6 used in tandem mass spectrometry are classified into two categories, viz. the low- and high-internal energy regimes. Post-source decay (PSD), low-energy collisionally activated dissociation (CAD) and infrared multi-photon dissociation belong to the former regime, whereas high-energy CAD and ultraviolet photodissociation (PD) belong to the latter.

Tandem mass spectral trend for singly protonated peptides can be summarized as follows. 5–7 Without arginine, both b- and y-type product ions appear distinctly regardless of the energy regime (symbols a–d and v–z are used for product ions with charge retention at the N- and C-terminal moieties, respectively). With arginine, product ions containing this residue appear dominantly. In the low-energy regime, b-type ions dominate for peptide ions with arginine at the N-terminus (N-arg peptide ions), whereas both b- and y-type ions appear for C-arg peptide ions even though the latter ions tend to be more intense. In the high-energy regime, a- and d-type ions dominate for N-arg peptide ions, whereas v-, w-, x- and y-type ions dominate for C-arg peptide ions.

Mechanisms for the formation of b- and y-type ions (low energy-characteristic or low-energy product ions) have been extensively investigated. 6,8–12 A complementary pair of b and y is thought to form via a common reaction path, i.e. charge-directed rearrangement to a proton-bound dimer (PBD) of an oxazolone derivative and a smaller peptide. b or y is formed depending on which moiety retains the charge when PBD breaks up. In contrast, there are conflicting views 13–15 on the mechanisms for the formation of high-energy product ions, v, w and x in particular.

Recently, we developed time-resolved PD tandem time-of-flight (TOF) mass spectrometry 16–18 in which photo-excitation of a precursor ion (m1 +) was done inside a cell floated at high voltage. The same product ions (m2 +) formed inside (in-cell component, I) and outside (post-cell component, P) the cell during the time span of 0–0.1 and 0.1–5 μs after photo-excitation, respectively, had different kinetic energies and could be separated by the second stage analyzer. In addition, we observed peaks arising from consecutive dissociation (consecutive components, C) of m1 + to m2 +, i.e. m1 + → m1 i → m1 f or m2 +, with the first step occurring inside the cell and the second step outside.

When a precursor ion dissociates competitively via more than one channel, time-evolution patterns for product ions are governed by the same rate constant, i.e. sum (total) of individual rate constants. 19 Accordingly, peak splitting patterns for b- and y-type product ions such as in-cell (I):post-cell (P) ratios were similar for peptide ions without arginine. 20–23 This ratio was used to determine PD rate constants, critical energy and entropy, and effective temperature of peptide ions formed by MALDI. In PD of N-arg peptide ions, mostly of α-, β-, and d-type product ions were observed. It is known that α ions have two different forms 6,13 as can be differentiated by H/D exchange, i.e. those formed via high-energy (to be explained later) and...
Peptide ion dissociation and conformation

low-energy (CO loss from b) channels. Time-resolved PD patterns for the high- and low-energy product ions were found to be drastically different, suggesting that the two sets of channels may not be in statistical competition. Invoking broad internal energy distribution for peptide ions formed by matrix-assisted laser desorption ionization (MALDI), we suggested\textsuperscript{[24]} that the high- and low-energy channels might dominate at the high- and low-energy side of the distribution, respectively. Then, apparently noncompetitive behavior may occur even when the two sets of channels are in statistical competition at each internal energy.

As a preliminary to the kinetic study for the dissociation of peptide ions with arginine, we recorded time-resolved PD patterns for some C-arg peptide ions. The results were useful in identifying major high-energy channels. In view of the data\textsuperscript{[20–23]} obtained by kinetic analysis for peptide ions without arginine, however, it was realized that our previous explanation for the drastic difference in time-resolved pattern between high- and low-energy channels could not be upheld. Investigations have been made to trace the causes for the difference. The results are reported in this article.

Experimental

Details of the homebuilt tandem TOF instrument and its operation for time-resolved study were reported previously.\textsuperscript{[16]} The instrument consists of a MALDI source with delayed extraction, a linear TOF analyzer to time-separate the prompt ions generated by MALDI, an ion gate, a PD cell and a second stage TOF analyzer equipped with a reflectron. The ion gate and the PD cell are located immediately in front of and at the first time focus, respectively. An output of 337 nm from a nitrogen laser (MNL100, Lasertechnik Berlin, Berlin, Germany) is used for MALDI. 20 kV DC, and 1.5 kV AC pulse are used for delayed extraction/acceleration and the final electrode of the reflector is kept at 25 kV. A pulsed PD laser, either 266 nm output from a Nd: YAG laser (Surelite III-10, Continuum, Santa Clara, CA, USA) or 193 nm output from an ArF excimer laser (PSX-100, MPB Communication Inc., Canada), is irradiated at the center of the PD cell perpendicularly to the ion beam direction. The PD laser pulse is synchronized with the lowest-mass isotopomer of the precursor ion selected for PD. PD laser intensity is set such that multi-photon effect is insignificant (details will be given later). The PD cell is floated at 3 kV in time-resolved study. The PSD spectrum under the same experimental condition is recorded without the PD laser pulse. The output from the MCP detector is digitized by an A/D card and treated to minimize electrical noise.\textsuperscript{[25]} Finally, the laser-off spectrum (PSD) is subtracted from the laser-on spectrum to obtain the PSD spectrum.

Peptide samples FVVVVVVR (or FV6R), FT6R, Y6R and V7R with better than 90% purity were purchased from Peptron (Daejon, Korea). Y6, ASHLGLAR, FSWGAEQR and PPGFSPFR with better than 98% purity, the matrices \(\alpha\)-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) and other chemicals were purchased from Sigma (St Louis, MO, USA). A matrix solution prepared daily using acetonitrile and 0.1% trifluoroacetic acid was prepared daily using acetonitrile and 0.1% trifluoroacetic acid. The final peptide concentration prepared for PD experiment was 50 pmol/µL; 1 µL of the solution was loaded on the sample plate. The method to prepare a deuterated sample is the same as above except that D\(_2\)O (better than 99.96% isotopic purity) was used as the solvent. Deuteration was done inside the sample introduction system as reported previously.\textsuperscript{[24]}

Results and Discussion

We carried out time-resolved PD study for singly protonated ions generated from a number of C-arg peptides including ASHLGLAR, FSWGAEQR, PPGFSPFR, FV6R, FT6R, Y6R and V7R. Here, the results from [FV6R + H]\(^+\) will be mainly used to explain the mechanism findings for C-arg peptide ions. This peptide ion has been chosen because most of its product ions could be assigned definitely and because important product ions such as v and w were well separated.

High-energy dissociation mechanisms for C-arg peptide ions: a brief review

Biemann et al.\textsuperscript{[5,13]} found that \(a_n\) and \(d_n\) prevalent in high-energy CAD of N-arg peptide ions were formed from \(a_n + 1\) generated by homolytic C\(_\alpha\)–CO cleavage of peptide ions. If the same cleavage occurs for C-arg peptide ions, \(x_n + 1\) will be formed, another reactive radical cation. Accordingly, Reilly et al.\textsuperscript{[15]} proposed that \(v_n\), \(w_n\) and \(x_n\) prevalent in their 157 nm PD spectra for C-arg peptide ions are formed from \(x_n + 1\). Assuming that the ionizing proton remains sequestered at the arginine side chain (argH\(^+\)), these reactions can be drawn as in Scheme 1.

Biemann et al.\textsuperscript{[5,13]} reported that \(v_n\) ions were mainly formed from \(y_n\) with a minor contribution from \(x_n + 1\). The mechanism they proposed is drawn in Scheme 2.

Biemann et al. also reported\textsuperscript{[16]} that \(w_n\) ions were formed from \(z_n + 1\) ions, another radical species which may be formed via homolytic NH–C\(_\alpha\) cleavage. The mechanism they proposed is drawn in Scheme 3. In view of the dissociation of \(a_n + 1\) to \(a_n\), \(z_n + 1 \rightarrow w_n\) may also occur, as shown in Scheme 3.

The results from Reilly’s\textsuperscript{[15]} 157 nm PD and our own\textsuperscript{[24]} 193 and 266 nm PD studies for N-arg peptide ions were consistent with Biemann’s suggestion.\textsuperscript{[5,13]} If Scheme 1 is valid for C-arg peptide ions, homolytic C\(_\alpha\)–CO cleavage will be the first step in the high-energy dissociation of singly protonated peptides with arginine regardless of its position, as suggested by Reilly. In contrast, \(y_n \rightarrow v_n\) channels in Scheme 2 and \(z_n + 1 \rightarrow w_n\) channels in Scheme 3 would complicate the situation. In particular, participation of the latter channels means that another set of the first generation channels – homolytic NH–C\(_\alpha\) cleavage – must be considered in the high-energy regime.

PSD spectra

Figure 1(a) shows the PSD spectrum of [FV6R + H]\(^+\) formed by CHCA-MALDI. The spectrum consists of sequence ions, viz. \(a_n\) \((n = 2, 4 \text{ and } 5)\), \(b_n\) \((n = 2–6)\), \(y_n\) \((n = 1–6)\) and \(z_n\) \((n = 2–6)\), and immonium ions \(V_n\) \((n = 1–5)\). It is a bit of surprise to find that \(b_n\) peaks are more intense than \(y_n\). The overall PSD spectral pattern obtained with DHB-MALDI (Fig. 1(b)) looks similar except that relative intensities are weaker than those in CHCA-MALDI/PSD roughly by a factor of 5 when MALDI laser fluence was kept at two times the threshold value in each experiment. It is to be noted that high-energy product ions, viz. \(v_n\) \((n = 2–7)\) and \(w_n\) \((n = 2–7)\), can be clearly seen in DHB-MALDI/PSD of [FV6R + H]\(^+\). Upon magnification of the CHCA-MALDI/PSD spectrum, the same ions were also found. Their relative abundances (normalized to that of the precursor ion) were comparable to those in DHB-MALDI/PSD. Morgan and Russell\textsuperscript{[20]} also observed v and w in CHCA-MALDI/PSD of [PPGFSPFR + H]\(^+\).

In our previous studies of peptide ions without arginine,\textsuperscript{[20–23]} weaker PSD intensities for b and y and slower PD were observed.
Scheme 1. Formation of $x_n, v_n$, and $w_n$ from $x_n + 1$ in the high-energy dissociation of C-arg peptide ions proposed by Reilly et al.\(^\text{[15]}\)

Scheme 2. Formation of $v_n$ from $y_n$ in the high-energy dissociation of C-arg peptide ions proposed by Biemann et al.\(^\text{[5,13]}\)

Scheme 3. Formation of $w_n$\(^\text{[14]}\) and $z_n$ from $z_n + 1$ in the high-energy dissociation of C-arg peptide ions.
with DHB than with CHCA. Kinetic analysis of the data showed that the effective temperature of the peptide ions formed with DHB was lower than that with CHCA, in agreement with the classification of DHB and CHCA as ‘cold’ and ‘hot’ matrices, respectively.\cite{27} Weaker PSD intensities for \( b \) and \( y \) from [FV6R + H]\(^+\) formed with DHB observed in this work are also in agreement with the above classification. In this regard, the fact that high-energy product ions are as prominent, whereas low-energy product ions are weaker, with DHB (‘cold’) is unexpected. The same trend was observed for all the other C-arg peptide ions investigated. The results were also confirmed with a commercial MALDI-tandem TOF.

In the present instrument, PSD product ions formed between the ion source and the first time focusing position are eliminated by a deflection system.\cite{28} Hence, the fact that \( v \) and \( w \) appeared in PSD spectra means that they were formed slowly, i.e. on the time scale of 10–16 \( \mu s \) after precursor ion formation by MALDI, which is another interesting aspect of the observation.

**PD spectra**

PD spectra (193 and 266 nm) of [FV6R + H]\(^+\) formed by CHCA- and DHB-MALDI are shown in Fig. 2. In the CHCA-MALDI/193 nm PD spectrum (Fig. 2(a)), both high-energy product ions, viz. \( v_1 \) (\( n = 2–7 \)), \( w_1 \) (\( n = 3–7 \)), and \( x_n \) (\( n = 2–7 \)), and low-energy ones, viz. \( a_n \) (\( n = 2 \) and 4), \( b_n \) (\( n = 2–5 \)), \( y_n \) (\( n = 1–5 \)) and \( z_n \) (\( n = 2 \) and 3), appear. Other prominent peaks involve immonium ions \( v_n \) (\( n = 1–3 \)) and a peak due to the loss of toluene ([M + H – 92]\(^+\)). \( x_n \) and [M + H – 92]\(^+\) are weaker in CHCA-MALDI/266 nm PD (Fig. 2(b)). DHB-MALDI/PSD generates similar product ions (Fig. 2(c) and (d)). However, their spectral patterns are noticeably different, i.e. high-energy product ions such as \( v_7 \), \( x_6 \) and \( y_7 \), and [M + H – 92]\(^+\) also, are more prominent in DHB-MALDI/PSD especially at 193 nm. Burlingame et al.\cite{29} also reported more prominent high-energy product ions with DHB than with CHCA in high-energy CAD of peptide ions with arginine. In the experiment, a CAD signal contained contribution from PSD. Hence, the result was attributed to weaker low-energy product signals from PSD with DHB. In the present work, high-energy product ions are relatively more prominent with DHB even though the contribution from PSD has been subtracted.

The matrix effect on PD observed in this work is different from our previous observation\cite{20–22} for peptide ions without arginine, viz. [Y\(_6\) + H]\(^+\), [HF\(_6\) + H]\(^+\), [F\(_3\)HF\(_3\) + H]\(^+\), [F\(_6\)H + H]\(^+\), [KF\(_6\) + H]\(^+\), [F\(_3\)KF\(_3\) + H]\(^+\) and [F\(_6\)K + H]\(^+\). There, the spectral patterns changed only slightly when the precursor internal energy was increased either by changing the matrix from DHB to CHCA or by changing the PD wavelength from 266 to 193 nm. Small changes observed, i.e. slight increase in intensities of low \( m/z \) product ions at the cost of those at high \( m/z \), were probably due to more efficient consecutive dissociations. In particular, the spectral patterns for CHCA/266 nm PD and DHB/193 nm PD were very similar because the internal energies involved were similar (to be explained). As an example, PD spectra of [Y\(_6\) + H]\(^+\) obtained under these two conditions are included in Supporting Information. Comparing these with the pair in Fig. 2(b) and (c), it is evident that high-energy channels of [FV6R + H]\(^+\) are more favored by DHB than by CHCA, as also observed in PSD. The apparently anomalous matrix effect was observed in PD of all the other C-arg peptide ions investigated in this work. This will be further discussed later.

**Time-resolved PD spectra and their mechanistic implications**

In time-resolved PD,\cite{16} kinetic energy of a product ion (\( m_2 \)) formed outside the cell (P) is the same as that of \( m_2 \) formed without the cell voltage. However, their flight times to the detector are different because of different precursor ion transit times through the cell. When the time coordinate of a voltage-on PD spectrum is linearly shifted such that precursor ion TOF matches that in the voltage-off spectrum, TOF of the P component of each \( m_2 \) also matches that of \( m_2 \) in the voltage-off spectrum, allowing its identification. \( m_2 \) formed inside the cell (I) and the intermediate ion (\( m_1 \)) involved in consecutive (C) formation of \( m_2 \) can be identified through SIMION\cite{30} calculations.\cite{17,18} However, we are not interested in analyzing consecutive reactions here.

In Fig. 3, voltage (3 kV)-on splitting patterns of some product ion peaks in 193 nm PD of [FV6R + H]\(^+\) formed by CHCA-MALDI are
shown. The corresponding regions in the voltage-off PD spectrum are also shown. It is to be noted that \( y_6, w_6 \) and \( x_6 \) ions are completely dominated by I components whereas \( b_2 \) and \( y_2 \) ions are dominated by P components. The same trend was observed for other sequence- and side chain-specific ions, i.e. slow and rapid dissociations along low- and high-energy channels, respectively. The splitting patterns for \( z \)-type ions and immonium ions were similar to those for \( b \) and \( y \) as shown in Fig. 3. The same trend was observed for all the other C-arg peptide ions investigated, regardless of the matrix and the PD wavelength. Some examples are shown in Fig. 4. Rapid formation of \( v_n \), \( w_n \) and \( x_n \) is compatible with the competitive radical site dissociations of \( x_n + 1 \) postulated in Scheme 1. Scheme 2 – formation of \( v_n \) from \( y_n \) – is incompatible with the observation that \( v_n \) is formed more rapidly than \( y_n \).

Two different mechanisms were proposed for the formation of \( w_n \), viz. via \( z_n + 1 \) (Scheme 3) and via \( x_n + 1 \) (Scheme 1). If \( z_n + 1 \)
is formed by homolytic \( \text{NH} - \text{C}_n \) cleavage, not only \( w_n \) but also \( z_n \) may be formed competitively from \( z_n + 1 \) as shown in Scheme 3. Drastic difference in time-resolved pattern between \( z_3 \) (mostly P) and \( w_3 \) (mostly I) in Fig. 3 suggests that they are formed from different precursors, in disagreement with Scheme 3. Post-cell formation of \( z_3 \) also suggests that it is related to a low-energy process. Loss of \( \text{NH}_3 \) from \( y_3 \) can form \( z_3 \). Also, the mass of \( z_n \) formed from \( y_n \) is 1 Da less than that from \( z_n + 1 \) when peptide ions are deuterated by H/D exchange. Satisfactory deuteration of \( v_n \) to \( w_n \) from \([\text{PPGSPFR} + \text{H}]^+\), DHB-MALDI/266 nm PD, (c) \( v_2 \) and \( w_2 \) from \([\text{VTR} + \text{H}]^+\), DHB-MALDI/193 nm PD. (d) \( y_2 \) and \( w_2 \) from \([\text{ASHLGLAR} + \text{H}]^+\), DHB-MALDI/193 nm PD. Vertical dotted lines mark the expected positions of post-cell (P) components. In-cell components are marked I.

**Figure 4.** Some product ion peaks in time-resolved PD of C-arg peptide ions. (a) \( y_3 \) and \( x_3 \) from \([\text{FSWGAEGQR} + \text{H}]^+\), DHB-MALDI/193 nm PD. (b) \( v_2 \) and \( b_2 \) from \([\text{PPGSPFR} + \text{H}]^+\), DHB-MALDI/266 nm PD. (c) \( v_2 \) and \( w_2 \) from \([\text{VTR} + \text{H}]^+\), DHB-MALDI/193 nm PD. (d) \( y_2 \) and \( x_2 \) from \([\text{ASHLGLAR} + \text{H}]^+\), DHB-MALDI/193 nm PD. Vertical dotted lines mark the expected positions of post-cell (P) components. In-cell components are marked I.

In our previous kinetic studies for peptide ions without arginine,[19 – 22] sums of the intensities of all the product ions formed inside \( (\sum \{I[I] + [C]\}) \) and outside \( (\sum \{P\}) \) the cell were measured and their ratio was taken as CPD, i.e. 
\[
\text{CPD} = \frac{\sum \{P\}}{\sum \{I[I] + [C]\}}.
\]
Let us define a similar ratio for each product ion as CPD\(_i\), i.e. 
\[
\text{CPD}_i = \frac{\{P_i\}}{\{I[I_i] + [C_i]\}}.
\]
In PD of peptide ions without arginine, CPD\(_i\) for different product ions were similar, indicating statistical competition in their formation. This is in sharp contrast with the different time-resolved patterns between high- and low-energy channels observed here and in Ref. [24] for N-arg peptide ions, which indicates that they are not in direct competition in the dissociation of peptide ions with arginine. This suggests that the precursor ions undergoing dissociation via these two sets of channels differ either in energy, or in quantum state, or in structure (conformation).

**Energy difference: broad thermal energy distribution**

In our previous study on N-arg peptide ions,[24] we speculated that such an apparently nonstatistical behavior might arise due to broad internal energy distribution for peptide ions formed by MALDI. By roughly estimating the internal energy distribution based on our results from previous kinetic studies,[20 – 23] we are now in a position to critically evaluate this speculation. In our previous studies,[20 – 23] the effective temperature of peptide ions generated by MALDI lied in 350–500 K range, CHCA generating hotter peptide ions than DHB and ions with a basic residue being cooler. Let us take 400 K as the effective temperature of peptide ions with arginine. For peptide ions with \( m/z \) around 1000 investigated in this work, the effective widths of the internal energy distributions calculated at 400 K are around 2 eV or less. This is close to 1.76 eV difference in photon energy between 193 and 266 nm. Let us denote the effective minimum and maximum internal energies after 266 nm excitation as \( E_1 \) and \( E_2 \), respectively, and those after 193 nm excitation as \( E_3 \) and \( E_4 \), respectively. For simplicity, we will take \( E_2 = E_1 \). Now let us consider the competition between the high- and low-energy channels assuming that both of these occur statistically. In our previous studies,[20 – 23] very small critical energy \( (E_0) \) and entropy \( (\Delta S^\circ) \) were found for dissociation.
along b–y channels. $E_0$ and $\Delta S^\ddagger$ decreased further, even though slightly, in the presence of a basic residue such as histidine\(^{[21]}\) and lysine.\(^{[22]}\) It would be reasonable to assume that the low-energy channels of C-arg peptide ions are also very small. On the other hand, both $E_0$ and $\Delta S^\ddagger$ for the high-energy channels will be larger if the formation of a high-energy product involves C$_\alpha$–CO bond cleavage as suggested in this work. Qualitative drawings of the rate-energy relations for the high-energy ($k_{H}$) and low-energy ($k_{L}$) channels and their sum ($k_{\text{tot}}$) are shown in Fig. 5. The internal energy at which $k_{H}$ and $k_{L}$ cross is denoted as $E_c$. Three energetic situations are drawn in the figure, viz. $E_a \leq E_c \leq E_1$ and $E_1 \leq E_c \leq E_4$. In the first case, $k_{\text{tot}}$ is mainly due to $k_{H}$ and hence the time-evolution patterns for high- and low-energy channels should be the same in disagreement with our observation. The second case can also be discarded based on a similar argument. Hence, our previous model is equivalent to the third case, $E_1 \leq E_c \leq E_4$. In this case, however, if high-energy product ions are formed more rapidly than low-energy ones in 193 nm PD, the opposite must be true in 266 nm PD, in complete disagreement with the observations. This invalidates our previous suggestion.

Energy difference: multi-photon absorption

In photo-excitation at high laser intensity, precursor ions may absorb one, two or more photons. Let us suppose that a precursor ion dissociates slowly along low-energy channels after single-photon absorption, but rapidly along high-energy channels after multi-photon absorption. Then, apparently nonstatistical behavior can occur even when both high- and low-energy channels are intrinsically statistical and competitive.

Multi-photon effect is observed in our experiments when the laser power is increased, i.e. intensities of low m/z product ions increase steadily at the cost of those of high m/z ions. Laser power dependence is especially noticeable for low m/z immonium ions, their voltage-on splitting patterns in particular. Hence, in kinetic and mechanistic studies of peptide ions, we use CPD, of a low m/z immonium ion as the guideline to establish the single-photon condition. As examples, CPD$^{-1}$ versus laser power plots are shown in Fig. 6 for immonium Y and $Y_1$ from [Y$_6$R + H]$^+$ at 266 nm. Increase in CPD$^{-1}$ of Y with laser power, i.e. faster dissociation to Y with multi-photon absorption, is to be noted. In this case, multi-photon absorption is insignificant when the laser power is kept at or below 300 µJ/pulse. Corresponding value at 193 nm was around 10 µJ/pulse. Use of 300 µJ/pulse at 266 nm and 10 µJ/pulse at 193 nm resulted in around 5% depletion of the precursor ion signal. That is, we have been using only tiny fractions of commercial laser outputs to avoid multi-photon effect, at the cost of spectral quality. Hence, participation of the multi-photon effect can be ignored in all the PD spectra reported in this work and in our previous kinetic studies.\(^{[20–23]}\)

Dissociation in excited electronic states

Time-resolved patterns for high- and low-energy product ions can be different if they are formed in different electronic states, i.e. in excited and ground states, respectively. Previously\(^{[7,24]}\) we noted that absorptions at 193 and 266 nm involved amide backbone ($\tau_{b}^\ddagger \leftarrow \tau_{b}$) and aromatic side chain ($\tau_{b}^\ddagger \leftarrow \tau_{p}$) excitations, respectively. Accepting Scheme 1, high-energy channels might proceed rapidly if the excited state is repulsive along the C$_\alpha$–CO coordinates or if C$_\alpha$–CO bonds become labile upon electronic excitation. Unlike the excitation at 193 nm, the excitation of [FV$_6$R

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**Figure 5.** Qualitative drawings of the rate–energy relations for the high-energy ($k_{H}$, with large $E_0$ and large $\Delta S^\ddagger$) and low-energy ($k_{L}$, with small $E_0$ and small $\Delta S^\ddagger$) channels and their sum ($k_{\text{tot}}$). $E_c$ denotes the internal energy at which $k_{H}$ and $k_{L}$ cross. The internal energies of a peptide ion excited at 266 and 193 nm are taken to lie in the ranges $E_1 – E_2$ and $E_3 – E_4$, respectively, with $E_2 – E_3$. Three energetic situations are drawn, viz. (a) $E_4 \leq E_c$, (b) $E_c \leq E_1$ and (c) $E_1 \leq E_c \leq E_4$.

**Figure 6.** Log–log plots of CPD$^{-1}$ versus PD laser power measured for Y (filled circle) and $Y_1$ (open circle) from [Y$_6$R + H]$^+$. Larger CPD$^{-1}$ means faster dissociation.
The absence of an aromatic chromophore, as demonstrated in Russell et al. [26], acknowledged the influence of intramolecular interactions involving arginine on conformation and postulated that a peptide ion samples numerous conformations prior to PSD. However, investigations in both works were limited to the formation of low-energy product ions.

Dissociation from different conformations

Influence of arginine on the conformation and dissociation of peptide ions was investigated extensively. Ion mobility spectrometry [31–34] was used to study the gas-phase conformations of peptide and protein ions. Presence of more than one stable conformation was reported for peptide ions such as [RPPGFSPFR + 2H]2+ [32] [RPPGF + H]1+ [33] and proline-containing tryptic peptide ions. [34] However, there has been no report on the observation of multiple conformations for singly protonated C-arg peptides without proline. Based on the translational energy losses for b and y, Glish et al. [35] suggested that the ionizing proton is shared between the side chain of arginine and a carbonyl oxygen(s). In MALDI-PSD study of singly protonated bradykinin and its analogs, Russell et al. [26] acknowledged the influence of intramolecular interactions involving arginine on conformation and postulated that a peptide ion samples numerous conformations prior to PSD. However, investigations in both works were limited to the formation of low-energy product ions.

Let us suppose that two distinct conformations, A and B, of a C-arg peptide ion generate low- and high-energy product ions, respectively, and that the rate-energy relations kL and kH in Fig. 5 roughly represent dissociations of A and B, respectively. Then, low-energy channels will dominate in low-energy regime such as in PSD whether both A and B are present as separate (non-interconverting) entities or are in quasi-equilibrium (inter-converting). Since P components are missing for high-energy channels in time-resolved PD whereas they are dominant for low-energy ones, the minimum requirements for this model are (1) A and B are separated by a barrier and (2) A-to-B interconversion after photo-excitation is inefficient. Then, even if all the processes involved proceed statistically, apparently nonstatistical behavior can appear.

One of the surprising findings in this work is an anomalous matrix effect on the high-energy channels of C-arg peptide ions. This is all the more puzzling because the matrix effect on low-energy channels looks normal. Appearance of two widely different matrix effects in the dissociation of the same precursor ion is difficult to explain based on simple energetic and kinetic arguments. Accepting the multiple conformation model, however, an explanation can be found easily, i.e. B is somehow formed more favorably with DHB than with CHCA. At the moment, we do not have enough information to judge whether this is the proper explanation for the anomalous matrix effect, and if it is, why B is more favored by DHB than by CHCA. An anomalous matrix effect observed in matrix-assisted laser desorption of preformed ions by Vertes et al. [36] — even though DHB was cooler than CHCA at well above the threshold MALDI laser fluence, it was hotter near the threshold — is interesting in this regard. However, we do not have enough information to relate the two anomalous matrix effects either.

Conclusion

Time-resolved PD patterns for all high-energy product ions from C-arg peptide ions were similar but different from those for low-energy ions. Hence, it was suggested that v, w and x were formed from a common intermediate, i.e. x + 1 generated by Cα – CO cleavage. Identification of NH3 loss from y as the main mechanism for the formation of z suggested that NH–Cα cleavage might not be important. It seems that homolytic Cα – CO cleavage followed by radical site reactions are the main high-energy dissociation mechanisms for peptide ions with arginine, wherever the residue is located.

To find an explanation for the difference in time-resolved pattern between high- and low-energy channels, an apparently nonstatistical behavior, several possibilities were tested. It is concluded that the participation of two (or more) conformations of C-arg peptide ions, one generating low-energy and the other high-energy product ions, is the best explanation. The anomalous matrix effect observed in PSD and PD of C-arg peptide ions may be an indication of the validity of the multiple conformation model. A weakness of this model is that there has been no report on the observation of multiple conformations for such ions by ion mobility spectrometry. Acquisition of optical spectra may help to study the influence of arginine on the gas-phase conformation of peptide ions.

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Supporting information

Supporting information may be found in the online version of this article.

References


