Effect of Basic Residue on the Kinetics of Peptide Fragmentation Examined Using Surface-Induced Dissociation Combined with Resonant Ejection

Julia Laskin^{*}

Physical Sciences Division, Pacific Northwest National Laboratory, Richland, WA, 99354

Author for correspondence:

Julia Laskin (Julia.Laskin@pnnl.gov)

Physical Sciences Division

Pacific Northwest National Laboratory

PO Box 999, K8-88

Richland, WA 99352 USA

Abstract

In this work, resonant ejection coupled with surface-induced dissociation (SID) in a Fourier transform ion cyclotron resonance mass spectrometer is used to examine fragmentation kinetics of two singly protonated hexapeptides, RYGGFL and KYGGFL, containing the basic arginine residue and less basic lysine residue at the N-terminus. The kinetics of individual reaction channels at different collision energies are probed by applying a short ejection pulse (1 ms) in resonance with the cyclotron frequency of a selected fragment ion and varying the delay time between ion-surface collision and resonant ejection while keeping total reaction delay time constant. Rice-Ramsperger-Kassel-Marcus (RRKM) modeling of the experimental data provides accurate threshold energies and activation entropies of individual reaction channels. Substitution of arginine with less basic lysine has a pronounced effect on the observed fragmentation kinetics of several pathways, including the b_2 ion formation, but has little or no effect on formation of the $b_5 + H_2O$ fragment ion. The combination of resonant ejection SID, time- and collision energy-resolved SID, and RRKM modeling of both types of experimental data provides a detailed mechanistic understanding of the primary dissociation pathways of complex gaseous ions.

Introduction

Collisions of ions with surfaces result in rapid vibrational excitation followed by a unimolecular or shattering fragmentation of the excited species called surface-induced dissociation (SID).[1-4] SID has been extensively used for structural characterization of peptides, proteins, and protein complexes.[2, 5-9] One of the most notable examples is the contribution of collision energy-resolved SID experiments to the development of the mobile proton model of peptide fragmentation.[2, 10-14] Furthermore, it has been demonstrated that rapid vibrational excitation efficiently suppresses protein unfolding prior to fragmentation, thereby providing unique structural information on the stoichiometry and tertiary structure of protein complexes.[15-17]

SID also has been used for studying the kinetics and energetics of large ion fragmentation.[5, 18] These studies rely on Rice-Ramsperger-Kassel-Marcus (RRKM) modeling of time- and collision energy-resolved SID data that enables accurate determination of energy and entropy effects in the dissociation of complex gaseous ions.[19, 20] The modeling approach uses an analytic form of the internal energy deposition function (EDF) that is able to reproduce both collision-induced dissociation (CID) and SID data. [19, 20] Studies of ion activation and dissociation performed using this approach have established a fundamental understanding of energy transfer in ion-surface collisions [6, 7, 21-23] and determined dissociation energies and activation entropies for the unimolecular decomposition for both covalent and non-covalent bonds in complex ions. [18, 24-41]

Another approach for studying the kinetics of large ion fragmentation combines SID in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) with resonant ejection of selected fragment ions at a variable delay time.[42] In this approach, fragment ions at a particular m/z formed before the ejection pulse is applied are removed from the spectrum, while ions at the same m/z formed after the ejection pulse are detected in the spectrum. In addition, product ions formed through sequential fragmentation of the ejected fragment are affected by the ejection pulse, while all other fragments remain unperturbed. This approach allows the fragment ion population to be probed at different delay times after ion-surface collision. RRKM modeling enables determination of both the dissociation parameters of the individual fragmentation channels and the shape of the EDF from the time-resolved resonant ejection SID data.[42] In this

study, time-resolved resonant ejection SID experiments are used to examine the effect of basic residue on the fragmentation kinetics of protonated peptides.

Gas-phase fragmentation of protonated peptides is initiated by transferring the ionizing proton to the peptide backbone.[10, 13] According to the mobile proton model, the rate of this initial step depends on the types of residues present in the peptide sequence.[12, 43] Specifically, the basic side chains of arginine (R), lysine (K), or histidine (H) sequester the ionizing proton and slow down the proton transfer step. As a result, protonated peptides containing basic residues are relatively more stable toward fragmentation.[12] A previous SID study demonstrated that low activation entropy is the major factor in determining the relative stability of arginine-containing protonated peptides.[37] Specifically, it was found that dissociation of arginine-containing peptides often is characterized by lower threshold energies and substantially less favorable (lower) activation entropies than fragmentation of their histidine- and lysine-containing analogs. Negative activation entropies in the dissociation of arginine-containing peptides are attributed to the kinetically hindered fragmentation pathways that involve salt-bridge intermediates.[44, 45] In contrast, lysine- and histidine-containing peptide fragmentation proceeds through entropically favored pathways involving canonical structures.[37] This study examines the kinetics of fragmentation of two related peptides, RYGGFL and KYGGFL, for which very different kinetics for the formation of the backbone b_2 and b_5 fragments were previously observed. The results presented here demonstrate that time- and collision energy-resolved SID combined with resonant ejection SID experiments provide a more detailed description of the kinetics and energetics of the primary dissociation pathways than either technique individually.

Experimental

Peptides (KYGGFL and RYGGFL) were purchased from Cellmano Biotech Limited (Hefei, China), and 1-dodecanethiol was purchased from Sigma-Aldrich (St. Louis, MO). All chemicals were used as received. The peptides were dissolved in methanol to produce $\sim 140\ \mu\text{M}$ solutions.

The self-assembled monolayer surface of 1-dodecanethiol (HSAM) was prepared on a single gold crystal (Monocrystals Co., Richmond Heights, OH). The target was cleaned in an ultraviolet (UV) cleaner (Model 135500, Boekel Industries Inc., Feasterville, PA) for 10 minutes and allowed to stand in a solution of 1-dodecanethiol for 10 hours. The target was removed from the thiol solution, washed ultrasonically in methanol for 10 minutes to remove extra layers, and introduced into the vacuum system through a vacuum interlock assembly. For SID experiments, the surface was positioned at the rear trapping plate of the ICR cell.

SID experiments were conducted on a specially fabricated 6 Tesla FT-ICR MS described in detail elsewhere. [46] In this instrument, peptide ions produced using electrospray ionization (ESI) transferred into the vacuum system through a heated stainless steel capillary inlet followed by an electrodynamic ion funnel and a collisional quadrupole. Ions are mass selected using a quadrupole mass filter (Extrel CMS, Pittsburgh, PA) and accumulate in an octopole collision cell held at an elevated pressure ($\sim 2\text{--}5 \times 10^{-3}$ Torr). The experimental parameters were as follows: high voltage, 2 kV; flow rate of $40\ \mu\text{L/hr}$; heated capillary temperature, 150°C ; accumulation time, 0.25 s. Ions were extracted from the octopole using a $100\text{-}\mu\text{s}$ -long extraction pulse, transferred into the ICR cell through an electrostatic ion guide, and allowed to collide with the surface. The initial kinetic energy of ions extracted from the accumulation octopole was $\sim 6\ \text{eV}$. [46] The final kinetic energy of the ions striking the surface was adjusted by applying a potential to the ICR cell and the surface. SID of positive ions was performed by applying negative potentials to the ICR cell components. Scattered ions were trapped by applying a 10-15 V trapping potential between the ring electrode of the cell and trapping plates.

Resonant ejection was performed by applying an excitation pulse of $\Delta t = 1\ \text{ms}$ duration at the resonant frequency of a fragment ion of interest to the two excitation plates of the ICR cell

ring electrode.[42] In this study, SID spectra were acquired at 15 delay times between ion trapping and resonant ejection given by equation (1):

$$t_d(ms) = 1.45^{n-1} - 2 \quad (n = 1 - 15) \quad (1)$$

with the shortest delay time placing the ejection pulse right before ion trapping and the longest delay time of 179 ms. This allowed access to both fast and slow fragmentation pathways. In resonant ejection experiments, products of fast fragmentation pathways are difficult to observe when a longer duration ejection pulse is used and the delay time is longer than 5 ms.

SID spectra were acquired at four kinetic energies of the projectile ion by applying a broadband chirp excitation pulse 0.2 s after ion trapping, followed by 262 ms detection with a bandwidth of 2 MHz and a low-mass cutoff of 46.1 amu. The following kinetic energies were examined in this study: 55, 65, 75, and 85 eV for RYGGFL and 50, 58, 66, and 74 eV for KYGGFL. The kinetics plots were constructed by plotting the abundance of a selected fragment ion normalized to the abundance of the precursor ion as a function of t_d .

RRKM Modeling

Modeling kinetics plots was performed as described in a previous study using reaction Schemes 1 and 2 for KYGGFL and RYGGFL, respectively.[37] Specifically, microcanonical rate constants are calculated for each reaction channel shown in Schemes 1 and 2 as a function of internal energy using the RRKM/quasiequilibrium theory (QET) equations and vibrational frequencies adopted from a previous study.

[37] Breakdown curves representing the fragmentation probability of each precursor ion into a particular reaction channel as a function of the internal energy of the ion (E) and the reaction time (t_r), $F(E, t_r)$, were calculated considering two types of fragment ions: ions that do not (type 1) and ions that do (type 2) undergo sequential fragmentation.

For type 1 and 2 fragment ions not subjected to the ejection pulse, breakdown curves were calculated using equations (2) and (3), respectively:

$$F_{1i} = \frac{k_i}{k_{\text{tot}}} (1 - \exp(-k_{\text{tot}} t_r)) \quad (2)$$

$$F_{2i} = \frac{k_i}{k_{\text{tot}} - k_{i1}} (\exp(-k_{i1} t_r) - \exp(-k_{\text{tot}} t_r)) \quad (3)$$

where k_i and k_{i1} are rate constants of the corresponding primary and secondary dissociation pathways, respectively, and t_r is the reaction delay time of 200 ms. The total rate of the precursor ion decomposition, k_{tot} , is given by equation (4) that accounts for slow decline in the total population of vibrationally excited precursor ions undergoing fragmentation through radiative cooling with a characteristic rate constant, k_{rad} :

$$k_{\text{tot}} = k_{\text{rad}} + \sum_i k_i \quad (4)$$

For type 1 and 2 fragment ions subjected to resonant ejection, breakdown curves were calculated using equations (5) and (6), respectively:

$$F_{1i}^{\text{ej}} = \frac{k_i}{k_{\text{tot}}} \exp(-k_{\text{tot}}(t_d + \Delta t)) (1 - \exp(-k_{\text{tot}}(t_r - t_d - \Delta t))) \quad (5)$$

$$F_{2i}^{\text{ej}} = \frac{k_i}{k_{\text{tot}} - k_{i1}} \exp(-k_{\text{tot}}(t_d + \Delta t)) (\exp(-k_{i1}(t_r - t_d - \Delta t)) - \exp(-k_{\text{tot}}(t_r - t_d - \Delta t))) \quad (6)$$

The relative abundance of the remaining precursor ion, $F([M+H]^+)$, was calculated using equation (7):

$$F([M+H]^+) = \exp(-k_{\text{tot}} t_r) + \frac{k_{\text{rad}}}{k_{\text{tot}}} (1 - \exp(-k_{\text{tot}} t_r)) \quad (7)$$

For each collision energy, breakdown curves were integrated over the EDF given by equations (8) and (9):

$$P(E, E_{\text{coll}}) = \frac{1}{C} (E - \Delta)^l \exp\left(-\frac{(E - \Delta)}{f(E_{\text{coll}})}\right) \quad (8)$$

where l and Δ are parameters, $C = \Gamma(l+1)[f(E_{\text{coll}})]^{l+1}$ is a normalization factor, and $f(E_{\text{coll}})$ has the form:

$$f(E_{\text{coll}}) = A_2 E_{\text{coll}}^2 + A_1 E_{\text{coll}} + A_0 \quad (9)$$

where A_0 , A_1 , and A_2 are parameters and E_{coll} is the collision energy. Finally, the fragment ion abundance was normalized to the calculated abundance of the precursor ion.

This approach was used to simulate the dependence of fragment ion relative abundance on delay time, t_d , between the trapping and ejection and impacting ion kinetic energy. Parameters describing the EDF were obtained by fitting fragmentation efficiency curves of individual reaction channels obtained using time- and collision energy-resolved SID experiments. The same parameters were used for all values of t_d . Simulated results were compared to the experimental data, while dissociation parameters for individual pathways, the radiative rate constant, were adjusted until the best fit was obtained.

Results and Discussion

Resonant ejection SID experiments were conducted for two protonated peptides, KYGGFL and RYGGFL, at four collision energies at 15 delay times. Figure 1 shows representative SID spectra acquired following resonant ejection of several primary and secondary fragment ions of KYGGFL at 58 eV collision energy and a delay time of 25 ms. For comparison, SID spectrum obtained without resonant ejection is also shown. At this collision energy, the internal fragments (y_5 , b_4) labeled as b_{YGGF} and (y_5 , a_4) labeled as a_{YGGF} at m/z 425 and 397, respectively, are the most abundant species in the spectrum.[37] Abundant high- m/z fragments include $b_5 + \text{H}_2\text{O}$ at m/z 571, y_5 at m/z 556, and $b_5 - \text{H}_2\text{O}$ at m/z 535, while the b_5 ion at m/z 553 is observed at the baseline level. Additional fragments include: $b_3 - \text{H}_2\text{O}$ at m/z 331, $b_2 - \text{H}_2\text{O}$ at m/z 274, b_2 at m/z 292, y_2 at m/z 279, b_1 at m/z 129, $b_2 - \text{NH}_3$ at m/z 275, $a_2 - \text{NH}_3$ at m/z 247, $a_1 - \text{NH}_3$ at m/z 84, and several immonium ions and small internal fragments. Primary fragments of RYGGFL (data not shown) include: $[\text{M} + \text{H} - \text{NH}_3]^+$ at m/z 695, $b_5 + \text{H}_2\text{O}$ at m/z 599, b_5 at m/z 581, b_2 at m/z 320, and b_1 at m/z 157. Other abundant fragments include: $b_5 - \text{NH}_3$ at m/z 564, $a_5 - \text{NH}_3$ at m/z 536, $b_2 - \text{NH}_3$ at m/z 303, $a_2 - \text{NH}_3$ at m/z 275, $b_1 - \text{NH}_3$ at m/z 140, $a_1 - \text{NH}_3$ at m/z 112, and several immonium ions and small internal fragments.

The results shown in Figure 1 indicate some variances in the suppression of different fragments by the ejection pulse. For example, the $b_5 + \text{H}_2\text{O}$ ion is completely eliminated by the ejection pulse applied 25 ms after ion-surface collision, while the intensity of the y_5 ion is only

slightly reduced following resonant ejection. This observation indicates pronounced differences in the kinetics of these two fragmentation pathways. Specifically, the ejection pulse efficiently eliminates all fragment ions formed through fast dissociation channels, while fragments formed through slow reactions are less affected by resonant ejection. It follows that the formation of the b_5+H_2O ion is kinetically favored, whereas the y_5 ion is formed through a kinetically hindered pathway.

Figure 2 shows the kinetics plots obtained for fragment ions of KYGGFL subjected to resonant ejection. Solid lines in the figure represent the best fit obtained using RRKM modeling and will be discussed later. The kinetics plots were obtained at four collision energies (50, 58, 66, and 74 eV) and represent the abundance of each fragment ion normalized to the signal of the remaining precursor ion as a function of t_d . As expected, fragment ions subjected to resonant ejection show a decrease in relative abundance with increasing t_d . However, the shapes of the kinetics plots vary widely for different fragment ions. In this study, the experimental conditions were optimized for characterizing the kinetics of both fast and slow fragmentation pathways by applying a substantially shorter ejection pulse, 1 ms in duration, compared with the 5 ms ejection pulse used in a previous study[42] and extending the range of short delay times at which the ejection pulse is applied. Under these conditions, good quality kinetics plots could be obtained for fast fragmentation pathways, including the formation of the b_5+H_2O ion (panel b), b_2 ion (panel e), and b_1 ion (panel f), for which the fragmentation kinetics are complete after a 1-5 ms reaction delay. Formation of the b_5-H_2O ion (panel c) and b_{YGGF} ion (panel d) follow slower kinetics, extending to ~30 ms delay time, while the slow formation of the y_5 ion (panel a) is observed up to ~100 ms delay time.

Resonant ejection of the fragment ions of RYGGFL (shown in Figure 3) indicates that formation of the b_5+H_2O ion is the most entropically favorable pathway. The formation of the b_1 ion is somewhat slower, while the slowest kinetics is observed for formation of the b_2 ion and NH_3 loss from the precursor ion. In comparison with KYGGFL precursor ion, slower kinetics are observed for the formation of the b_5+H_2O , b_2 , and b_1 ions from RYGGFL. The slow kinetics obtained for the $[M+H-NH_3]^+$ fragment and b_2 ion and the fast kinetics of the b_5+H_2O ion formation are consistent with the results of time- and collision energy-resolved SID experiments (Figure 4a-c). However, time- and collision energy-resolved SID data indicate similar kinetics of the b_1 and b_5+H_2O ion formation (Figure 4b,d), whereas somewhat slower kinetics of the b_1 ion

formation is inferred from resonant ejection experiments (Figure 3b,d). This difference can be rationalized, assuming that a substantial fraction of the b_1 fragment ions observed in time-resolved resonant ejection SID experiments forms through secondary dissociation pathways that affect the experimental kinetics plots. Meanwhile, time- and collision energy-resolved SID experiments show the entire population of the b_1 fragment ions formed through both primary and secondary dissociation pathways.

Solid lines in Figures 2, 3, and 4 correspond to the best fit obtained using the RRKM modeling of the experimental data described earlier. Both the kinetics plots obtained using resonant ejection (Figures 2 and 3) and fragmentation efficiency curves (Figure 4) were modeled using the same parameters of the EDF. Table 1 summarizes the corresponding dissociation parameters obtained for the individual dissociation pathways, along with results reported in a previous study for several reaction channels explicitly included in the modeling. [37] Good correspondence between dissociation parameters derived from time- and collision energy-resolved SID experiments is observed for most fragmentation pathways considered in the previous work[37] and this study. One exception is formation of the b_1 ion from KYGGFL, for which higher threshold energy and activation entropy were reported in the previous study. The observed difference is attributed to the fast fragmentation kinetics, which makes it difficult to accurately determine the activation entropy and threshold energy for this dissociation pathway based on SID data.

Comparison between dissociation parameters obtained by modeling both resonant ejection and time- and collision energy-resolved SID data shows some similarities and distinct differences between the values derived from the two types of experiments. For example, good correspondence was found for dissociation parameters obtained for the $b_5 + H_2O$ and y_5 ions of KYGGFL and for formation of the $[M+H-NH_3]^+$, $b_5 + H_2O$, and b_2 fragment ions of RYGGFL, while substantially different parameters were obtained for other channels. Many of the fragments observed as primary dissociation products most likely are formed through secondary reactions. For example, resonant ejection experiments (data not shown) indicate that a fraction of b_{YGGF} fragments of KYGGFL forms via sequential dissociation of the y_5 ion. However, the b_2 ion intensity is not affected by ejection of any other primary dissociation products indicating that most likely this fragment is not formed from any other primary product ion subjected to resonant ejection.

For fragment ions produced through both the primary and secondary dissociation pathways, resonant ejection experiments provide composite kinetics plots representing different dissociation pathways, which currently are not accounted for in the modeling. In contrast, modeling time- and collision energy-resolved SID data is less sensitive to the presence of secondary dissociation pathways that contribute to the observed fragment abundance at relatively high collision energies. Another simplifying assumption used in the modeling is that the individual fragment ion formation is adequately described using a single microcanonical rate constant. This assumption excludes the possibility of different conformers of the precursor ion undergoing dissociation with somewhat different rate constants meaning that the modeled microcanonical rate constant is a sum of dissociation rate constants of all possible conformers. It also excludes dissociation products generated through multiple pathways with different energetics and kinetics. For example, a previous study indicated that the b_2 ion may be formed through both canonical and salt-bridge pathways. [37] Canonical pathways are kinetically favorable but associated with relatively high energy barriers, while salt-bridge pathways are kinetically hindered but favored energetically. [37] Modeling both resonant ejection and time- and collision energy-resolved SID results should account for the presence of multiple pathways resulting in the same product ion. It is clear that the discrepancy between dissociation parameters derived from resonant ejection and time- and collision energy-resolved SID data may be attributed to simplifying assumptions used in the modeling. These results indicate that both types of experiments produce consistent dissociation parameters for fragment ions formed through primary dissociation pathways, characterized by a single rate constant.

Dissociation parameters summarized in Table 1 provide further support for the fast kinetics of the b_5+H_2O ion formation from both KYGGFL and RYGGFL. The high energy barrier and more favorable (high) activation entropy for this pathway, known to involve rearrangement of the C-terminal residue [47, 48], can be rationalized, assuming the initial rearrangement is followed by the formation of a strong ion-molecule complex that subsequently fragments to generate the product ion. Strikingly similar dissociation parameters associated with this fragment formation from both precursor ions indicate that the b_5+H_2O ion most likely forms through a charge-remote mechanism as proposed by Gaskell and co-workers. [47, 49] Similarly, b_1 ion formation is characterized by a high barrier. However, the activation entropy for this channel is somewhat less favorable (lower) for RYGGFL, compared with KYGGFL, indicating

this product is probably formed through a relatively fast charge-directed mechanism. In contrast, very slow kinetics is observed for the y_5 ion of KYGGFL and the $[M+H-NH_3]^+$ and b_2 fragments of RYGGFL using both types of SID experiments. It is reasonable to assume that formation of the b_2 ion from RYGGFL follows salt-bridge pathways. [44, 45, 50] In contrast, much faster kinetics, characteristic of the canonical pathway, is observed for the b_2 ion formation from KYGGFL. [37] However, differences in dissociation parameters derived for this product ion from the two types of SID data examined in this study indicate that some of the b_2 fragment ions of KYGGFL also may form through salt-bridge pathways.

Conclusions

In this study, time- and collision energy-resolved SID experiments were combined with resonant ejection experiments to examine the effect of basic residue on the threshold energies and activation entropies of the individual dissociation channels of protonated peptides. Specifically, two extensively studied singly protonated model systems, RYGGFL and KYGGFL, were used to represent peptides undergoing dissociation through salt-bridge and canonical pathways, respectively. Both fast and slow kinetics of the primary fragmentation pathways of RYGGFL and KYGGFL were examined by applying a short (1 ms) ejection pulse at a cyclotron frequency for a selected fragment ion at a variable delay time after ion-surface collision. Results showed that the fast, kinetically favored formation of the b_5+H_2O fragment ion from both precursor ions is characterized by high threshold energy and, most likely, follows a charge-remote fragmentation pathway. More favorable activation entropy associated with this pathway indicates that fragmentation of the ion-molecule complex is the rate-determining step for this reaction. Formation of the b_1 ion also is characterized by a high energy barrier. However, the activation entropy for this channel becomes less favorable when the basic arginine residue is replaced with the less basic lysine residue, indicating this product ion is formed through a charge-directed process. Strikingly different kinetics of the b_2 ion formation from the two precursor ions is consistent with distinct mechanisms of this dissociation channel for RYGGFL and KYGGFL precursors. Specifically, b_2 ion formation from RYGGFL proceeds through a salt-bridge pathway, while the same fragment of KYGGFL likely forms via a combination of canonical and salt-bridge pathways. Comparison between dissociation parameters obtained by modeling both resonant ejection and time- and collision energy-resolved SID data indicates these

techniques may provide complementary information regarding the kinetics and energetics of different reaction pathways.

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Table 1. Dissociation parameters obtained for fragment ions of KYGGFL and RYGGFL by modeling time-resolved resonant ejection and collision-energy resolved SID data.

Fragment Ion		$[M+H-NH_3]^+$	b_5+H_2O	y_5	b_5-H_2O	b_{YGGF}	b_2	b_1
KYGGFL								
Resonant Ejection	$E_0, \text{eV}^{\text{a)}$		1.90	1.05	1.24	1.17	1.61	1.90
	$\Delta S^\ddagger, \text{eu}^{\text{b)}$		10	-36	-20	-21	-1.5	14
Time- and energy-resolved SID			2.01	1.10	1.51	1.52	1.9	2.20
			25	-32	-5	-4	23	40
Previous Work^{c)}			2.48				2.11	2.58
			55				34	64
RYGGFL								
Resonant Ejection		1.22	1.84				1.01	1.00
		-17	24				-26	-28
Time- and energy-resolved SID		1.29	1.90				1.24	1.85
		-19	18				-23	16
Previous Work		1.37	1.95				1.26	1.93
		-16	22				-24	17

^{a)} Uncertainties are $\pm 8\%$ for threshold energies and $\pm 35\%$ for activation entropies.

^{b)} Activation entropy at 450 K. eu=entropy units = cal/(mol K)

^{c)} Ref. [37]

Figure 1. Resonant ejection SID spectra of KYGGFL acquired at 58 eV collision energy and delay time of 25 ms. a) Without ejection and following ejection of the b) b_5+H_2O ion at m/z 571, c) y_5 ion at m/z 556, d) b_5-H_2O ion at m/z 535, e) b_{YGGFL} ion at m/z 425, f) a_{YGGFL} ion at m/z 397, g) b_2 ion at m/z 292, and h) b_1 ion at m/z 129. Red arrows point to the fragment ions subjected to resonant ejection.

Figure 2. Experimental (points) and calculated (lines) kinetics plots obtained in SID of KYGGFL at 50 eV (black open circles), 58 eV (green triangles), 66 eV (red circles), and 74 eV (blue squares) by ejecting a) the y_5 fragment, b) the $b_5 + H_2O$ fragment, c) the $b_5 - H_2O$ fragment, d) the internal b_{YGGF} fragment, e) the b_2 fragment, and f) the b_1 fragment. The plots represent the abundance of the ions being ejected normalized to the abundance of the remaining precursor ion as a function of the delay time between ion trapping and ejection.

Figure 3. Experimental (points) and calculated (lines) kinetics plots obtained in SID of RYGGFL at 55 eV (black open circles), 65 eV (green triangles), 75 eV (red circles), and 85 eV (blue squares) by ejecting a) the $[M+H-NH_3]^+$ fragment, b) the b_5+H_2O fragment, c) the b_2 fragment, and d) the b_1 fragment. The plots represent the abundance of the ions being ejected normalized to the abundance of the remaining precursor ion as a function of the delay time between ion trapping and ejection.

Figure 4. Time-resolved fragmentation efficiency curves obtained for the a) $[M+H-NH_3]^+$ fragment, b) b_5+H_2O fragment, c) b_2 fragment, and d) b_1 fragment of RYGGFL. The results are shown for delay times of 1 ms (black squares), 5 ms (red circles), 50 ms (green triangles), and 1 s (blue squares). Solid lines show RRKM modeling results for the corresponding delay times.

Peptide Fragmentation Kinetics

Fast
Canonical pathway

Slow
Salt-bridge pathway