New Approach for Studying Slow Fragmentation Kinetics in FT-ICR: Surface-Induced Dissociation Combined with Resonant Ejection

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Abstract

We introduce a new approach for studying the kinetics of large ion fragmentation in the gas phase by coupling surface-induced dissociation (SID) in a Fourier transform ion cyclotron resonance mass spectrometer with resonant ejection of selected fragment ions using a relatively short (5 ms) ejection pulse. The approach is demonstrated for singly protonated angiotensin III ions excited by collisions with a self-assembled monolayer of alkylthiol on gold (HSAM). The overall decomposition rate and rate constants of individual reaction channels are controlled by varying the kinetic energy of the precursor ion in a range of 65 – 95 eV. The kinetics of peptide fragmentation are probed by varying the delay time between [ion activation by collision and short \(5 ms\) resonant ejection of selected fragment ions at a constant total reaction time of 1 s](#). RRKM modeling indicates that the shape of the kinetics plots is strongly affected by the shape and position of the energy deposition function (EDF) describing the internal energy distribution of the ion following ion-surface collision. Modeling of the kinetics data provides detailed information on the shape of the EDF and energy and entropy effects of individual reaction channels.

Introduction

Tandem mass spectrometry (MS/MS) is a powerful tool both for structural characterization and for studying the energetics and mechanisms of gas-phase fragmentation of complex ions. [1, 2] In MS/MS experiments, mass-selected ions are excited by photon absorption, electron capture/transfer, or collisions with a neutral gas or a surface. Vibrational excitation of the precursor ion is followed by unimolecular dissociation with a microcanonical rate constant described by the Rice–Ramsperger–Kassel–Marcus (RRKM) theory.[3, 4] According to RRKM, the rate constant increases almost exponentially with increase in ion internal energy. Several techniques have been developed for measuring dissociation thresholds and internal energy-dependent microcanonical rate constants, $k(E)$. [4-6] These include time-resolved single-photon photoionization[7] and photodissociation,[8] photoelectron-photoion coincidence,[6] and threshold collision-induced dissociation (TCID) under single-collision conditions.[9]

Gas-phase fragmentation of complex ions is associated with the kinetic shift, defined as the amount of internal energy necessary for the ion to fragment on the time scale of the instrument.[10] The kinetic shift increases with increase in the number of vibrational degrees of freedom of the ion, making it impossible to observe dissociation at the thermochemical threshold. It follows that efficient fragmentation of large gaseous ions requires deposition of a larger amount of energy that may be difficult to achieve using traditional approaches. Several techniques have been developed for studying the energetics and dynamics of fragmentation of large ions.

Most of these techniques have been implemented on Fourier transform ion cyclotron resonance mass spectrometers (FT-ICR MS).[11, 12] Blackbody infrared radiative dissociation (BIRD)[13, 14] has been extensively used for studying thermal kinetics of relatively small molecules[13, 15] and large peptide,[14, 16] protein,[17, 18] and cluster ions.[19] In BIRD, ions are maintained at a desired temperature through interaction with blackbody radiation generated by the vacuum chamber walls.[13] Temperature-dependent experiments enable direct determination of Arrhenius dissociation parameters for large ions.[20] Thermal kinetics have been also examined using infrared multiphoton dissociation (IRMPD),[21-23] in which slow absorption of multiple photons results in an energy deposition function (EDF) closely approximated by

a Boltzmann distribution. Sustained off-resonance CID (SORI-CID),[24] in which ions are excited through multiple collisions with neutral molecules, once it has been calibrated against other methods, is a useful technique for studying the energetics of ion fragmentation. [25-27]

It has been demonstrated that surface-induced dissociation (SID)[28-30] in FT-ICR MS has particular advantages for studying energy and entropy effects in dissociation of large ions.[31] Rapid vibrational excitation of hyperthermal (<100 eV) ions in collisions with surfaces[30, 32, 33] results in a quasi-thermal EDF[34] that is adequately described by a relatively simple analytical expression. RRKM modeling of time- and collision energy-resolved FT-ICR SID data[26, 35] enables accurate determination of energy and entropy effects in the gas-phase fragmentation of large ions including even-[31, 35-44] and odd-electron peptide ions,[45-49] non-covalent complexes,[50-54] and organometallic complexes.[55] In the present study, we introduce a new approach for examining the kinetics of large ion fragmentation using FT-ICR SID, which is closely related to earlier studies of metastable ion lifetimes in FT-ICR.[56, 57] In this approach, SID is coupled with resonant ejection of selected fragment ions.[58, 59] By using a fairly short ejection pulse (5 ms) and varying the delay time between [ion activation and resonant ejection of a fragment ion while keeping the total reaction time constant](#), we directly probe the kinetics of fragment ion formation on a timescale of >1 ms. RRKM modeling of the kinetics plots provides information on the energy and entropy effects governing the dissociation of large ions.

Experimental

Angiotensin III and 1-dodecanethiol were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Angiotensin III was dissolved in a 70:30 (v/v) methanol:water solution with 1% acetic acid. 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 and washed ultrasonically in ethanol for 10 minutes to remove extra layers.

SID experiments were conducted on a specially fabricated 6 Tesla FT-ICR mass spectrometer described in detail elsewhere.[60] In this instrument, a sample is propelled through a fused silica capillary positioned in front of a heated stainless steel capillary tube using a syringe pump (Cole Parmer, Vernon Hills, IL) at a flow rate of 30 $\mu\text{L/hr}$. Electrospray ionization (ESI) is induced by applying a high voltage of 2 kV to a stainless steel union connecting between the capillary transfer line and the ESI emitter. Charged droplets produced by ESI are transferred through a heated stainless steel capillary maintained at 180°C to facilitate desolvation. Desolvated ions are subsequently transferred into the vacuum system via an electrodynamic ion funnel. Two quadrupoles following the ion funnel provide collisional focusing and mass selection of the ion of interest. An octopole held at elevated pressure (about $2\text{-}5 \times 10^{-3}$ Torr) is used for accumulation of mass-selected ions prior to their injection into the ICR cell.

In SID experiments, the SID target is introduced through a vacuum interlock assembly positioned at the rear trapping plate of the ICR cell. The experiment is controlled by a modular FT-ICR data acquisition system developed by Heeren and co-workers,[61] which is used to control the voltages and timing of the ion source and transfer optics and ion manipulation in the ICR cell. Figure 1 shows a time diagram of the voltages applied to the trapping plates of the accumulation octopole (AO) and the ICR cell. Mass-selected ions are accumulated in the AO by applying 11 V and 17 V dc potentials to the front and back trapping plates, respectively. Ions are extracted from the AO using a 400- μs long extraction pulse, sufficient for both ion extraction and transfer to the surface. The ion beam is focused using a series of electrostatic lenses. The final acceleration/deceleration of ions is accomplished by voltages applied to the ring electrode (cell offset) and both trapping electrodes of the ICR. SID of positive ions is performed by applying negative potentials to the ICR cell components. Kinetic energy of the ions striking the surface is varied by adjusting the potential difference between the **dc offset of the AO the surface. We have previously shown that the initial kinetic energy of ions extracted from the AO is ~ 0.5 eV higher than the dc offset applied to the AO rods and the width of the kinetic energy distribution is ~ 4 eV.**[60] The potentials applied to the ICR cell's rear trapping plate and the surface is 1 V higher than the potential applied to the cell offset, while the potential applied to the front trapping plate is 3 V higher than the

cell offset. Consequently scattered ions are slightly accelerated toward the center of the cell and reflected back into the cell by the 2 V potential difference between the surface (back trapping plate) and the cell's front trapping plate. Scattered ions are captured by increasing the potentials on the ICR cell's front and rear trapping plates by 15 V relative to the cell offset (trapping potential).

SID spectra are acquired by applying a broadband chirp excitation pulse 150 ms after ion trapping, followed by 262 ms detection with a bandwidth of 2 MHz and a low-mass cutoff of 46.1 amu. In this study, SID was performed at four values of the projectile ion kinetic energy (65 eV, 75 eV, 85 eV, and 95 eV). Ion ejection is performed by applying an excitation pulse of duration $\Delta t = 5$ ms at the resonant frequency of a fragment ion of interest to the two excitation plates of the ICR cell ring electrode. For each collision energy, fragmentation kinetics were examined by varying the delay between the precursor ion trapping and the fragment ion ejection, t_d over a range of 1-130 ms.

Results and Discussion

SID Combined with Resonant Ejection of Selected Fragment Ions

In this study, we examined fragmentation kinetics of singly protonated angiotensin III (RVYIHPF) following collision with an HSAM surface. The kinetics were probed by applying a 5 ms duration excitation pulse at a specified delay time, t_d , for selective ejection of a fragment ion of interest after gated trapping while keeping the delay between ion trapping and detection at 150 ms. Under these conditions, fragment ions formed prior to the ejection pulse are eliminated from the spectrum, while fragment ions formed following the ejection pulse comprise the SID spectrum. This approach enables the investigation of fragmentation kinetics on a timescale longer than 1 ms. SID spectra were acquired as a function of t_d at collision energies of 65 eV, 75 eV, 85 eV, and 95 eV with an HSAM surface. Previous studies demonstrated ~10% kinetic-to-internal energy transfer ($T \rightarrow V$ transfer) following ion collisions with HSAM surfaces.[28, 29] Consequently fragmentation strongly depends on the kinetic energy of the precursor ion and varying ion impact energy enables us to probe the kinetics of these processes.

Figure 2 shows representative SID spectra acquired at 75 eV collision energy and two delay times of 1 ms and 85 ms following resonant ejection of the primary b_5 ion at m/z 669 (panels a and b) and secondary b_5 -NH₃ ion at m/z 652 (panels c and d). In agreement with our previous studies, SID of angiotensin III results in formation of a large number of fragment ions.[40, 62, 63] Although the spectrum shown in Figure 2a was acquired after resonant ejection of the b_5 ion, this primary fragment is fairly abundant at short delay times between ion trapping and ejection, t_d . However, the b_5 ion is completely eliminated from SID spectra at $t_d > 25$ ms (Figure 2b). Since other fragments were not affected by the ejection pulse, we conclude they are not sequential decomposition products of the b_5 ion. Similar results were observed after resonant ejection of the b_5 -NH₃ ion (Figures 2c,d). In contrast, the a_5 ion was completely eliminated from SID spectra acquired after applying an excitation waveform to eject this fragment ion. These results are consistent with the relatively slow formation of the b_5 and b_5 -NH₃ ions and much faster decomposition of the precursor ion to form the a_5 fragment ion.

In a previous study,[40] we examined dissociation of the precursor ion of angiotensin III into six families of fragments: 1) $[\text{MH}-\text{NH}_3]^+$ ion, 2) b_n and a_n ions, (b & a); 3) $b_n-\text{NH}_3$ and $a_n-\text{NH}_3$ ions (b & a $-\text{NH}_3$); 4) y_n ions; 5) internal fragments; and 6) immonium ions. Modeling of SID data indicated that a majority of b_n and a_n ions are formed directly from the precursor ion, but $a_n-\text{NH}_3$ and $b_n-\text{NH}_3$ ions result from sequential fragmentation of the primary $[\text{M}+\text{H}-\text{NH}_3]^+$ fragment. Here we follow a similar approach by combining angiotensin III fragments into six families and modeling the formation kinetics of each family of fragments along with selected individual fragments. This approach is outlined by Scheme 1, in which the ejected ion is explicitly separated from the other fragments in the same family.

Scheme 1

Figure 3 shows the kinetics plots obtained by plotting the abundance of each fragment ion normalized to the total ion signal as a function of t_d . Fragment ions subjected to resonant ejection show a decrease in relative abundance with increase in t_d . In contrast, the relative abundance of the fragment ions produced from the ejected primary or secondary fragments on a timescale longer than 1 ms gradually increase as a function of t_d . Finally, the relative abundance of fragment ions generated by rapid fragmentation or produced directly from the precursor ion is not affected by the ejection pulse. As shown in Figure 3, most fragment ions, including the a_5 , b_4 , $b_4-\text{NH}_3$, $a_4-\text{NH}_3$, b_3 ,

$b_3\text{-NH}_3$, y_2 , $b_2\text{-NH}_3$, and $a_2\text{-NH}_3$ ions, are unaffected by ejection pulses other than those applied at the cyclotron frequency of that fragment. In contrast, the relative abundance of the a_4 fragment increases following ejection of the b_4 ion, indicating that a significant fraction of the a_4 product is produced from the b_4 ion. We also note significant scatter in the relative abundance of the b_5 and $b_5\text{-NH}_3$ fragments, both of which remain essentially constant with increase in t_d . This scatter is attributed to the partial off-resonant ejection of these fragment ions; great care must be taken in these experiments to minimize unintentional off-resonance excitation.

Figure 4 shows kinetics plots obtained for fragment ions subjected to resonant excitation of the precursor ion at four collision energies. In these plots fragment ion abundances are normalized to the intensity of the remaining precursor ion. This presentation is advantageous for visualizing the differences between the plots obtained at different kinetic energies and for the kinetic modeling discussed below. The $[M+H\text{-NH}_3]^+$ fragment exhibits relatively slow kinetics at 65, 75, and 85 eV and is not observed at 95 eV. As previously discussed, the a_5 ion is completely eliminated at all delay times, demonstrating this reaction is complete before the ejection pulse is applied. The b_4 ion is observed only at very short delay times indicating that this fragment is also formed in a relatively fast fragmentation process. In contrast to these results, significantly slower kinetics are observed for the b_5 , $b_5\text{-NH}_3$, and $b_2\text{-NH}_3$ fragments.

RRKM Modeling

Kinetics data obtained following resonant ejection of the primary and secondary fragment ion were simulated using the modified RRKM-based modeling approach described by us previously. [26, 35] Microcanonical rate constants are calculated for each reaction channel shown in Scheme 1 as a function of internal energy using the RRKM/QET formalism and vibrational frequencies for angiotensin III adopted in our previous studies.[40, 63] We then calculate 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), $F(E, t_r)$ is calculated using the equations of formal kinetics Eqs. 1-7 shown below while taking into account radiative cooling of excited ions, [which efficiently competes with very slow](#)

reactions examined in this study.[64] The relative abundance of the precursor ion and all fragment ions is calculated for a fixed reaction delay $t_r = 150$ ms. The total decay rate of the precursor ion (Eq. 1), k_{tot} , is given by the sum of all the rate constants of the primary dissociation channels and the radiative rate constant. The relative abundance of the remaining precursor ion, $F([M+H]^+)$, is given by Eq. 2. We note that radiative cooling contributes to the total decay rate by removing a fraction of the vibrationally excited ions from the total population of precursor ions undergoing fragmentation.[65, 66] This fraction deactivated through radiative cooling is given by the second term of Eq. 2. The relative abundance of the $[M+H-NH_3]^+$ fragment, $F([M+H-NH_3]^+)$, is given by Eq. 3; all other primary fragments, F_P , are described using Eq. 4, in which k_i represents the corresponding rate constant from Scheme 1. The relative abundance of the ejected primary fragment, F_{EP} , is calculated using Eq. 5, in which the second term corresponds to the relative abundance of the precursor ion at the end of the ejection pulse, and the reaction time is calculated starting from the end of the ejection pulse of width $\Delta t = 5$ ms. Eqs. 6 and 7 describe the relative abundance of the secondary fragments; F_{ES} is the ejected secondary fragment and F_S is the sum of all other secondary fragments.

$$k_{\text{tot}} = k_{\text{rad}} + \sum_{i=1,6} k_i \quad (1)$$

$$F([M+H]^+) = \exp(-k_{\text{tot}} t_r) + \frac{k_{\text{rad}}}{k_{\text{tot}}} (1 - \exp(-k_{\text{tot}} t_r)) \quad (2)$$

$$F([M+H-NH_3]^+) = \frac{k_1}{k_{\text{tot}} - (k_{11} + k_{12})} (\exp(-k_{11} t_r) - \exp(-k_{\text{tot}} t_r)) \quad (3)$$

$$F_P = \frac{k_i}{k_{\text{tot}}} (1 - \exp(-k_{\text{tot}} t_r)) \quad (4)$$

$$F_{EP} = \frac{k_6}{k_{\text{tot}}} \exp(-k_{\text{tot}} (t_d + \Delta t)) (1 - \exp(-k_{\text{tot}} (t_r - t_d - \Delta t))) \quad (5)$$

$$F_S = \frac{k_1 k_{11}}{k_{\text{tot}} - (k_{11} + k_{12})} \left(\frac{\exp(-k_{\text{tot}} t_r)}{k_{\text{tot}}} - \frac{\exp(-(k_{11} + k_{12}) t_r)}{k_{11} + k_{12}} \right) + \frac{k_1 k_{11}}{k_{\text{tot}} (k_{11} + k_{12})} \quad (6)$$

$$F_{ES} = \exp(-k_{tot}(t_d + \Delta t)) \left(\frac{k_1 k_{12}}{k_{tot} - (k_{11} + k_{12})} \left(\frac{\exp(-k_{tot}(t_r - t_d - \Delta t))}{k_{tot}} - \frac{\exp(-(k_{11} + k_{12})(t_r - t_d - \Delta t))}{k_{11} + k_{12}} \right) + \frac{k_1 k_{12}}{k_{tot}(k_{11} + k_{12})} \right) \quad (7)$$

Breakdown curves calculated as a function of ion's internal energy are subsequently integrated over the energy deposition function (EDF) corresponding to the internal energy distribution of the precursor ion and described by Eqs. 8 and 9:

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

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

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

where A_0 , A_1 , and A_2 are parameters and E_{coll} is the collision energy. Finally, the overall abundance at a given collision energy, $I_i(E_{coll})$, is obtained for the precursor ion and each fragment ion using Eq. 10:

$$I_i(E_{coll}) = \int_0^{\infty} F(E, t) P(E, E_{coll}) dE \quad (10)$$

The fragment ion abundance is then normalized to the calculated abundance of the precursor ion.

This approach is used to simulate the dependence of fragment ion relative abundance on the delay time, t_d , between the trapping and ejection and impacting ion kinetic energy. The same parameters defining the shape and position of EDF are used for all values of t_d . Simulated results were compared to the experimental data and dissociation parameters for individual pathways, the radiative rate constant, and the parameters describing EDF were adjusted until the best fit was obtained. We also examined the effect of various parameters on the shape of the kinetics plots obtained for

fragment ions subjected to resonant ejection. We note that the relative abundance of other fragments is predicted to be independent of t_d .

Figure 5 shows the influence of shape and position of the EDF on kinetics plots obtained for resonant ejection of primary fragment ions. The kinetics plots shown in Figure 5 were calculated for three EDF's having the same most probable value at a given collision energy and three different widths for three collision energies of 75, 85, and 95 eV. At 75 eV, all three kinetics plots show similar trends as a function of t_d ; the only difference is the absolute value of the relative abundance of the ejected primary fragment ion. However, results obtained at higher collision energies differ in both the shape of kinetics plots and relative abundance of the ejected fragment as a function of t_d . Specifically, a narrower EDF results in sharper decline of the kinetics plot while kinetics plots for broader EDF's decrease relatively slowly with t_d . At 85 eV, a crossover is observed between the kinetics plot obtained for the narrower EDF₁ and EDF₂, and further increase in reaction rates suppresses the kinetics plot associated with EDF₂ in comparison with EDF₁. These results demonstrate that the shapes and relative positions of collision energy-dependent kinetics plots obtained in resonant ejection SID experiments are very sensitive to the shape of EDF.

Figure 6 shows the effect of the microcanonical rate constant on the kinetics plot of the ejected primary fragment calculated for 75 and 95 eV collisions using the intermediate width EDF₂. Two sets of dissociation parameters were used to represent two competing reactions with different threshold energies and activation entropies. Solid lines show the results obtained for the reaction with a higher threshold and a relatively loose transition state (TS), while dashed lines are for the reaction with lower threshold energy and a relatively tight TS. For the same form of the EDF, the relative abundance of the fragment formed via the tight TS reaction is higher than the relative abundance of the fragment formed through a loose TS reaction even though the shapes of the kinetics plots are quite similar.

Figure 7 shows the best fit of the experimental kinetics plots obtained following resonant ejection of the primary b_5 (panels a-d) and secondary b_5 -NH₃ (panels e-h) fragment ions from angiotensin III ions collisions with the HSAM surface at 65, 75, 85, and 95 eV. The same parameters were used for modeling both data sets. Table 1

summarizes dissociation parameters used for individual primary channels adopted from the previous study.[40] Good correspondence is observed between the experimental and the simulated kinetics plots. Note that the crossover between 85 eV and 95 eV kinetics plots of the b_5 ion in Figure 7a reflects the relatively narrow EDF discussed earlier. [Because of the significant scatter in the experimental data obtained for several fragment ions, detailed sensitivity analysis for the fitting parameters was not performed in this study.](#) However, we observed that dissociation parameters have a pronounced effect on the modeling results. Simultaneous fitting of the resonant ejection SID data obtained for different ejected fragment ions is particularly useful to deduce an unique set of energy and entropy parameters.

Conclusions

Kinetics of slow peptide ion fragmentation have been examined using a new approach introduced in this study that combines SID in FT-ICR MS with resonant ejection of a selected fragment ion at a variable delay time. All ions at a particular m/z formed before the ejection pulse are removed from the spectrum while ions at the same m/z formed after the ejection pulse are detected. Fragment ions formed in fast dissociation channels are eliminated from the spectrum even at very short delay times. Fragments formed in slow processes are observed with higher abundance at short delay times and lower abundance at longer delay times. Fragments ions produced in parallel with the ejected fragment ion are not affected by the ejection pulse.

RRKM simulations allowed us to examine the effects not only of dissociation parameters but also the width and position of the EDF on the kinetics plot. Our simulations indicate that the EDF strongly affects both the shape of the kinetics plot and the relative abundance of the fragment ion. Dissociation parameters have a pronounced effect on calculated relative abundances but relatively little impact on the shape of the kinetics plot. For a broad EDF, the shape of the kinetics plot was similar for all collision energies while dramatic changes were observed when a narrow EDF was used in simulations. This results in crossing of simulated kinetics plots obtained at different collision energies.

We also demonstrated that RRKM modeling of the experimental data provides useful information on the dissociation parameters of the individual decomposition pathways and the shape of EDF. Although the agreement between the experiment and simulation obtained in this study is not perfect, we attribute the observed discrepancy between the experimental and calculated plots to experimental uncertainties originating from unintentional ion ejection. These uncertainties will be suppressed in future studies by either reducing the ejection pulse width and/or amplitude of the ejection waveform. Modeling delayed ejection data with different width EDF's and kinetic parameters narrows the range of thermodynamic parameters deduced in SID kinetics, approaching an unique solution. We conclude that combining resonant ejection SID as developed in this study with more traditional time- and collision-energy resolved SID experiments is a powerful new approach for studying slow fragmentation kinetics of large ions.

Acknowledgments

This work was supported by the U.S. Department of Energy (DOE), Office of Basic Energy Sciences, Chemical Sciences, Geosciences & Biosciences Division. The research was performed using EMSL, a national scientific user facility sponsored by the DOE's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory (PNNL). PNNL is operated by Battelle for DOE under Contract DE-AC05-76RL01830.

Table 1. Dissociation parameters from ref. [40] used for modeling the kinetics of angiotensin III fragmentation.

	MH⁺-NH₃	b & a ions	y ions	Immonium ions	Internal Fragments
	k_1	k_2	k_3	k_4	k_5
E₀, eV	1.62	1.55	1.56	1.82	1.60
ΔS[‡], e.u.	-7.0	-14.0	-13.0	-3.3	-10.0

Figure 1. Schematic view of voltages applied to the AO and ICR cell in SID experiments.

Figure 2. SID spectra acquired at 75 eV collision energy following (a, b) ejection of the b_5 ion at m/z 669 with delay times, t_d , of (a) 1 ms and (b) 85 ms; (c,d) ejection of the b_5 - NH_3 ion at m/z 652 with delay times of (c) 1 ms and (d) 85 ms. # denotes a common noise peak. Dashed vertical lines are shown to guide the eye.

Figure 3. Relative fragment ion abundance in 95 eV SID spectra after resonant ejection of $b_2\text{-NH}_3$ (filled squares), b_4 (open circles), a_5 (open triangles), $b_5\text{-NH}_3$ (open diamonds), b_5 (crosses), and $[\text{M}+\text{H-NH}_3]^+$ (filled stars) as a function of the delay time between ion trapping and ejection. The absolute ion abundance is normalized to the total ion signal.

Figure 4. Kinetics plots obtained for ejected ions (a) $[M+H-NH_3]^+$, (b) b_5 , (c) b_5-NH_3 , (d) a_5 , (e) b_4 , and (f) b_2-NH_3 at 65 eV (black squares), 75 eV (green circles), 85 eV (red triangles), and 95 eV (blue triangles). 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 5. Simulated EDF (a, c, e) and the corresponding kinetics plots for the ejected primary fragment (b,d,f) for SID at 75 eV (a,b), 85 eV (c,d), and 95 eV (e,f). The kinetics plots are constructed by plotting the abundance of the fragment ion normalized to the abundance of the remaining precursor ion as a function of t_d .

Figure 6. a) Microcanonical rate constants obtained using $E_0=1.99$ eV and $\Delta S^\ddagger=3.8$ e.u. (solid line), $E_0=1.59$ eV and $\Delta S^\ddagger=-10.2$ e.u. (dashed line) as a function of the internal energy of the ion; the corresponding kinetics plots for the ejected primary fragment calculated for b) 75 eV and c) 95 eV collision using EDF₂. The kinetics plots are constructed by plotting the abundance of the fragment ion normalized to the abundance of the remaining precursor ion as a function of t_d .

Figure 7. Experimental (points) and calculated (lines) kinetics plots representing fragment ion abundances normalized to the precursor ion abundance as a function of the delay time between ion trapping and ejection. Panels (a-d) show the kinetics plots obtained by ejecting the b_5 primary fragment; panels (e-f) show the kinetics plots obtained by ejecting the b_5 -NH₃ secondary fragment. Normalized abundances of (a) b_5 , (b) combined b- and a-ions excluding the b_5 primary fragment, (c,g) combined y ions, (d) combined b_n -NH₃ and a_n -NH₃ ions, (e) b_5 -NH₃, (f) combined b- and a-ions, (h) combined b_n -NH₃ and a_n -NH₃ ions excluding the b_5 -NH₃ fragment.

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