International Journal of Mass Spectrometry 391 (2015) 24–30

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

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, USA

ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 11 July 2015
Accepted 17 July 2015
Available online 26 July 2015

Keywords:
Surface-induced dissociation
Protonated peptide
Resonant ejection
Kinetics
Threshold energy
Activation entropy

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.

1. 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

* Correspondence to: Physical Sciences Division, Pacific Northwest National Laboratory, PO Box 999, K8-88, Richland, WA 99352, USA. Tel.: +1 509 371 6136; fax: +1 509 371 6139.
E-mail address: Julia.Laskin@pnnl.gov

http://dx.doi.org/10.1016/j.ijms.2015.07.017
1387-3806/

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.

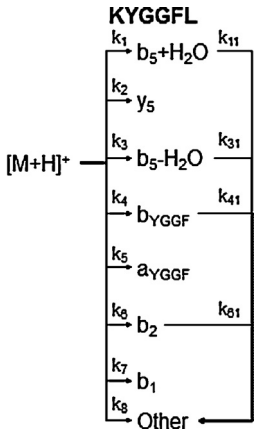
Gas-phase fragmentation of protonated peptides is initiated by transferring the ionizing proton to the peptide backbone [10,13]. According to the mobile proton model, the rate of this initial step depends on the types of residues present in the peptide sequence [12,43]. Specifically, the basic side chains 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.

2. 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 ~140 μM 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 min and allowed to stand in a solution of 1-dodecanethiol for 10 hr. The target was removed from the thiol solution, washed ultrasonically in methanol for 10 min 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 accumulated in an octopole collision cell held at an elevated pressure (~$2–5 \times 10^{-3}$ Torr). The experimental parameters were as follows: high voltage, 2 kV; flow rate, 40 μL/hr; heated capillary temperature, 150 °C; accumulation time, 0.25 s. Ions were extracted from the octopole using a 100-μs-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 ~6 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.



Scheme 1.

Resonant ejection was performed by applying an excitation pulse of Δt = 1 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 Eq. (1):

$$t_d \ (\text{ms}) = 1.45^{n-1} - 2 \qquad (n = 1\text{–}15) \tag{1}$$

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.

2.1. RRKM modeling

Modeling kinetics plots were 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/quasi equilibrium theory (QET) equation 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