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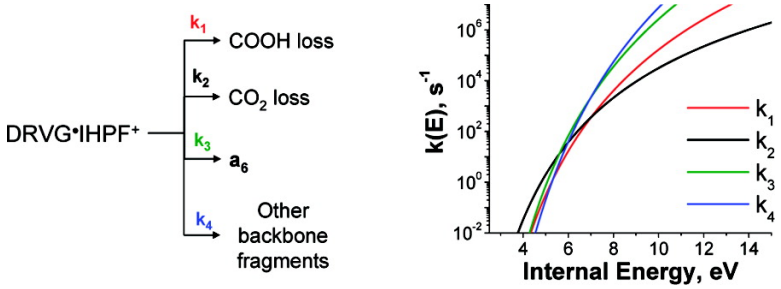
J. Am. Chem. Soc., **2007**, 129 (31), 9598-9599• DOI: 10.1021/ja073748r • Publication Date (Web): 18 July 2007



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Published on Web 07/18/2007

Is Dissociation of Peptide Radical Cations an Ergodic Process?

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Achieving a fundamental understanding of the mechanism of unimolecular dissociation of internally excited complex molecules is one of the most important challenges in modern mass spectrometry. A central question is whether the ergodic assumption is satisfied and dissociation of large molecules is adequately described by statistical theories—RRKM/QET or Phase Space Theories[1]—that have proved to be remarkably successful both for small molecules and a number of small and medium size peptides. The validity of the ergodic hypothesis for dissociation of gas-phase biomolecules has been recently reviewed[2] and will be only briefly discussed here.

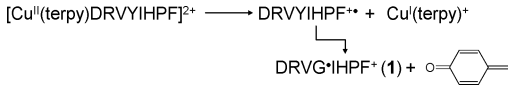
It is generally accepted that in many cases dissociation of protonated peptides can be adequately described using RRKM/QET.[3] However, non-ergodic fragmentation has been frequently invoked to rationalize electron capture dissociation (ECD) of multiply protonated peptide cations. McLafferty and co-workers suggested that the unusual fragmentation behavior of ions following electron capture results from non-ergodic dissociation.[4] This suggestion has been subjected to critical scrutiny by several investigators. An important recent report by Turecek and co-workers utilized high-level ab initio calculations to demonstrate that ECD fragmentation patterns can be rationalized without assuming non-ergodic behavior.[5]

Weinkauf, Schlag, and co-workers proposed that photoionization of small peptides results in non-ergodic dissociation of the corresponding radical cations. In their interpretation, the charge scouts for the site of reactivity without energy dissipation.[6] Theoretical calculations of microcanonical rate constants for gas-phase biomolecules performed by different groups resulted in controversial conclusions.[7,8]

This hypothesis was challenged by Lifshitz and co-workers who carried out first time-resolved photodissociation experiments (TRPD) for radical cations of LY and LLY peptides.[9] Energy-selected dissociation rate constants showed a strong dependence on the internal energy and the size of the ion. These experiments unambiguously demonstrated that dissociation of gas-phase peptides can be described using RRKM/QET. However, small but measurable fragmentation observed at zero delay times raised a question whether a fraction of ions (ca. 10%) underwent non-ergodic dissociation.

In this report, we present for the first time accurate measurements of the energetics and dynamics of dissociation of peptide radical cations. Because the location of the radical site may influence the dissociation rates, we chose an α-radical ($DRVG^{\bullet}IHPF^{+}$, **1**) for which the initial location of the radical site is well-defined as a model system. The α-radical is produced via the loss of *p*-quinomethide from the tyrosine side chain (Scheme 1) as discussed in detail elsewhere.[10] Our results provide solid evidence that a variety of dissociation pathways observed in surface-induced dissociation (SID) experiments of peptide radical cations can be well-described using statistical theories.

Scheme 1



Our study of the energetics and dynamics of dissociation of peptide radical cations utilized SID in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS).[11] The α-radical **1** was produced by in-source fragmentation of the $[Cu^{II}$-(terpy)DRVYIHPF$]^{2+}$ complex (Scheme 1). Mass-selected ions were externally accumulated and collisionally thermalized prior to their collision with a surface (a self-assembled monolayer of dodecanethiol (HSAM) on Au{111} crystal). The ions were then extracted from the source, transferred into the ICR cell using an electrostatic ion guide, and collided with the surface. Scattered ions were collected and mass analyzed in the ICR cell. Time- and energy-resolved fragmentation efficiency curves (TFECs) that represent the relative abundance of the precursor ion and its fragments were recorded by varying the collision energy and the reaction time.

TFECs of the precursor ion (**1**) and its fragments are shown in Figure 1. The major fragments observed in SID spectra include the losses of CO_2, $C_2H_5^{\bullet}$, and $COOH^{\bullet}$, and the formation of a_3-H, a_3, a_5, and a_6 backbone fragments. Most of the fragments are formed by bond cleavages that are remote from the initial location of the radical site. These pathways most likely involve hydrogen abstraction by the α-radical followed by the α-cleavage at the corresponding site.[10e]

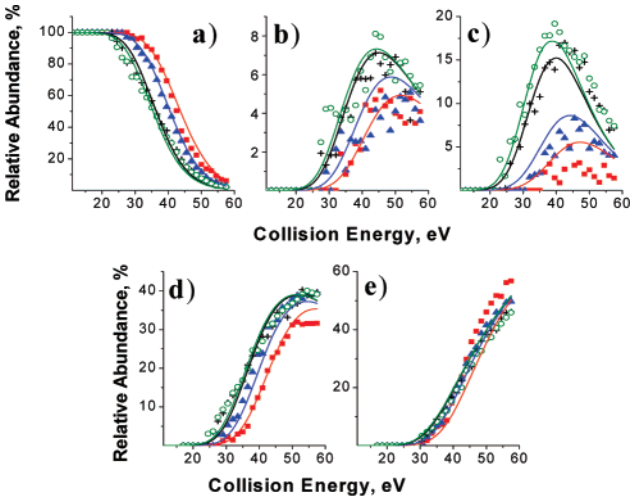


Figure 1. RRKM modeling of the experimental data for dissociation of $DRVG^{\bullet}IHPF$. Experimental (symbols) and calculated (lines) TFECs for (a) **1**; (b) COOH loss; (c) CO_2 loss; (d) a_6 ion; (e) other backbone fragments for reaction delays of 1 ms (red), 5 ms (blue), 50 ms (black), and 1 s (green).

10.1021/ja073748r CCC: $37.00

Table 1. Results of the RRKM Modeling of the Primary Fragmentation Channels of **1**

pathway	COOH loss	CO_2 loss	a_6	other backbone fragments
E_0, eV	1.44	1.25	1.51	1.63
$\Delta S^{\ddagger a}$, eu[b]	9.5	−3.5	20.1	28.6
A, s^{-1}	3×10^{15}	4×10^{12}	6×10^{17}	5×10^{19}
KS ($k = 10\ s^{-1}$), eV[c]	4.4	4.24	4.04	4.06

[a] Calculated at $T = 450$ K. [b] eu = cal/(mol K). [c] KS = kinetic shift.

Dissociation pathways of **1** show remarkably different kinetics. TFECs were modeled using a previously described modeling approach[12] that utilizes RRKM theory and a proposed analytical form for the internal energy deposition function (EDF). The EDF represents the internal energy distribution of ions excited by collisions with a surface. Dissociation parameters obtained from the modeling are summarized in Table 1; microcanonical rate-energy dependences are shown in Figure S1 of the Supporting Information. The radiative rate constant obtained from the best fit is 15 s^{-1}. TFECs of several fragment ions ("other backbone fragments") including a_3-H, a_3, and a_5 were combined together for the modeling. The excellent agreement between the experimental points and the calculated curves shown as solid lines in Figures 1a−e clearly demonstrates that RRKM kinetics adequately rationalizes the time-dependent fragmentation of the radical ion investigated.

It is interesting to note that loss of CO_2 from **1** is a fairly slow, entropically hindered process that most likely requires substantial rearrangement of the precursor ion. The dissociation parameters obtained for this reaction are similar to the typical dissociation parameters of protonated ions that undergo nonselective fragmentation.[3] In contrast, backbone fragmentation is characterized by higher dissociation thresholds and very large positive activation entropy typically associated with decomposition via a loose transition state. The pre-exponential factors for these pathways calculated from the activation entropies vary dramatically from 4×10^{12} to 5×10^{19}.

Despite the large variation in the dissociation parameters obtained for different reaction channels, we conclude that the dissociation rates of both slow and fast dissociation pathways of **1** are well-described using RRKM/QET. Similar results were obtained for other radical cations studied in our laboratory and will be discussed in forthcoming publications.

Schlag and co-workers argued that, if fragmentation were statistical, even relatively small peptide radical cations would not be able to dissociate on a microsecond time scale of their photoexcitation experiments. We tested this assertion by calculating microcanonical rate-energy dependences for L_nW ($n = 1-4$) peptides studied by Schlag et al., assuming that the dissociation parameters for these peptides are similar to the dissociation parameters obtained for backbone fragmentation of **1**. Average fragmentation rate constants corresponding to the experimental internal excitation are 9×10^8, 3×10^6, 7×10^4, and 1×10^4 s^{-1} for $n = 1-4$, respectively, demonstrating that peptides of this size can readily fragment in single-photon excitation experiments discussed earlier.

Finally, it should be noted that, because of the large number of vibrational degrees of freedom, fragmentation of peptide radical cations is characterized by significant kinetic shifts (KS) even on a long time scale of the FT-ICR. The values of the KS corresponding to the rate constant of 10 s^{-1} for different dissociation pathways of **1** are shown in Table 1. The large kinetic shifts observed for all reaction channels strongly support the statistical nature of the observed fragmentation.[2,3]

In this study, we presented first detailed investigation of the energetics and dynamics of dissociation of peptide radical cations. We demonstrated that fragmentation is dominated by bond cleavages that are remote from the initial position of the radical site. RRKM modeling of time- and energy-resolved SID data completely accounts for the kinetics of different dissociation pathways observed in our experiments. Clearly the dissociation of the particular peptide radical cation chosen for our initial experiments is well-described by the RRKM theory. Preliminary results from our laboratory suggest that statistical behavior is quite common for dissociation of peptide radical cations. It is remarkable that the observed fragmentation of the α-radical results from competition between dissociation pathways for which pre-exponential factors differ by more than 7 orders of magnitude.

Acknowledgment. This study was supported by the grant from the Chemical Sciences Division, Office of Basic Energy Sciences of the U.S. DOE, and by the University of Hong Kong and Hong Kong Research Grant Council, Special Administrative Region, China (Project 7019/05P). The work was performed at the Environmental Molecular Science Laboratory located at the Pacific Northwest National Laboratory, operated for DOE by Battelle. IKC acknowledges participation in the PNNL Interfacial and Condensed Phase Summer Research Institute.

Supporting Information Available: Microcanonical rate-energy dependences for the four dissociation pathways of DRVG•IHPF$^+$. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA073748R