# Surface-Induced Dissociation of Peptide Ions: Kinetics and Dynamics

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Kinetics and dynamics studies have been carried out for the surface-induced dissociation (SID) of a set of model peptides utilizing a specially designed electrospray ionization Fourier Transform ion cyclotron resonance mass spectrometer in which mass-selected and vibrationally relaxed ions are collided on a orthogonally-mounted fluorinated self-assembled monolayer on Au {111} crystal. The sampling time in this apparatus can be varied from hundreds of microseconds to tens of seconds, enabling the investigation of kinetics of ion decomposition over an extended range of decomposition rates. RRKM-based modeling of these reactions for a set of polyalanines demonstrates that SID kinetics of these simple peptides is very similar to slow, multiple-collision activation and that the distribution of internal energies following collisional activation is indistinguishable from a thermal distribution. For more complex peptides comprised of several amino acids and with internal degrees of freedom (DOF) of the order of 350 there is a dramatic change in kinetics in which RRKM kinetics is no longer capable of describing the decomposition of these complex ions. A combination of RRKM kinetics and the "sudden death" approximation, according to which decomposition occurs instantaneously, is a satisfactory description. This implies that a population of ions—which is dependant on the nature of the peptide, kinetic energy and sampling time—decomposes on or very near the surface. The shattering transition is described quantitatively for the limited set of molecules examined to date. (J Am Soc Mass Spectrom 2003, 14, 1340–1347) © 2003 American Society for Mass Spectrometry

Ion-surface impact provides an efficient means of transferring large amounts of internal energy into large ions in a matter of a few picoseconds [1–3]. The efficiency of translational to vibrational ($T \rightarrow V$) energy transfer for collisions of large peptide ions with self-assembled monolayer (SAM) surfaces shows only minor dependence on the collision energy and the identity of the ion [2–5]. Surface-induced dissociation (SID) [1, 3, 6, 7] allows for easy control over the internal excitation of the ion by varying its initial kinetic energy, which makes it a convenient method for ion activation. This method is especially valuable in Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry because it avoids the requirements of introducing collision gas using pulse valves and delaying mass analysis until the gas pressure can be reduced to a low enough value for the high mass accuracy advantage of FTICR to be utilized. A secondary advantage is that double resonance ICR to probe mechanisms of ion decomposition can be used in SID-FTICR immediately following the activation step, quantitatively defining decomposition pathways. [Double resonance refers to deliberate ejection of the precursor ion, which necessarily results in a strong reduction in the ICR signal of its frements which enables establishing parent–daughter relationships between ions observed in MS/MS spectra.]

Initially SID was viewed as a three-step process in which impulsive excitation by collision with the surface is followed by inelastic scattering of ions off the surface and unimolecular dissociation of ions in the gas phase [8]. Unimolecular dissociation is by far the slowest of these processes. This is particularly true for large ions that serve as a sink for large amounts of internal excitation and require substantial energy in excess of the thermochemical threshold in order to produce detectable dissociation—the kinetic shift [9, 10]. However, it has been demonstrated for small molecules [11] and clusters [12–17], predicted for protonated glycine [18] and demonstrated by us for peptide ions [19, 20] that under some conditions SID occurs in a single step. In this case ions dissociate during or immediately following their collision with the surface. This transition from slow to instantaneous decomposition or shattering of ions on surfaces opens up a variety of fragmentation pathways for large peptide ions that were not accessible via slow dissociation pathways.

In this contribution we present some of our most

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recent data and address some questions raised during the Sanibel Conference. What are the factors that govern the transition from recoil to shattering? What kind of fragmentation does shattering of peptide ions on surfaces induce? Can we predict and quantify fragmentation behavior of peptide ions in this regime? Can we take advantage of this transition to improve the analytical utility of SID for sequencing of peptides and proteins?

## SID in Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Unimolecular dissociation of large ions is significantly hindered by the presence of the kinetic shift. This means that commonly ions having enough internal energy do not have enough time to dissociate. This effect becomes more pronounced with increase in the size of the ion because of the dramatic decrease in dissociation rates as a function of the number of vibrational degrees of freedom in the ion. The effect of the kinetic shift is minimized by extending the observation time of the mass analyzer. (For infinite time in the absence of other cooling mechanisms rather than dissociation the kinetic shift is reduced to zero independent of complexity of the molecule.) For this reason, FTICR is the most appropriate technique for investigating unimolecular decay of surface-collision activated ions. It is especially advantageous to vary the time delay systematically and define the kinetics of the unimolecular dissociation.

The average internal energy deposited into the ion is determined by its initial kinetic energy and the properties of the surface. For a given surface the total degree of fragmentation can be enhanced by increasing both the collision energy and the reaction time. As noted above, this is most conveniently done by combining SID with FT-ICR MS. FT-ICR MS is characterized by a high collection efficiency of SID fragments that are efficiently confined by the strong magnetic field, very high mass accuracy and resolution required for unambiguous identification of product ions, long and variable delay between ion-surface collision and detection of the resulting fragments.

Our experimental approach described elsewhere [21] involves collision of externally produced ions with a SAM surface positioned at the rear trapping plate of the ICR cell. As discussed in detail in reference [21] ions produced using high-transmission electrospray source are mass-selected and efficiently thermalized in the electrospray interface prior to collision with the surface. Ions recoiling from the surface are trapped in the ICR cell and analyzed after a pre-defined reaction delay. Ion's kinetic energy is controlled by varying the offset of the ICR cell and the surface, which ensures that the quality of the ion beam does not depend on collision energy [21]. Kinetic SID studies are performed by varying the delay between the trapping of scattered ions and their analysis.

## Efficient Thermalization of Ions by Collisions with Surfaces

Our initial studies were focused on SID of small alanine-containing peptides at a fixed reaction delay of 1 s [22–24]. We compared SID with multiple-collision activation in the gas phase (MCA-CID) and found that both activation methods result in the same fragmentation patterns for small peptides. In fact, for any MCA-CID spectrum we could find a matching SID spectrum. This is illustrated in Figure 1 for tri-, tetra-, and pentaalanine. Similar SID mass spectra are obtained for small polyalanines using the tandem quadrupole setup (QQ-SID) with characteristic observation time of 1–10 $\mu$s [25]. Small differences between MCA-CID and SID mass spectra are readily attributed to the differences between the slow activation by gas-phase collisions and fast energy deposition by collisions of ions with surfaces [23]. Specifically, we demonstrated that slow activation effectively discriminates against higher-energy competing reaction channels. We have also shown that internal energy distributions of small peptides activated by both techniques are remarkably similar [22] and can be approximated by Boltzmann distributions of varying temperature [20]. Similar results are obtained for larger peptides. Figure 2 shows internal energy distributions of protonated des-Arg$^1$-bradykinin (PPGFSPFR) following collisions with fluorinated SAM surface at several collision energies. Dashed lines show matching thermal distributions calculated for temperatures indicated on the plot. Clearly, ion-surface impact results in fast and efficient thermalization of vibrational degrees of freedom in SID.

This result is in excellent agreement with the fast equilibration following ion-surface impact found in molecular dynamics simulations for collisions of argon clusters with surfaces [26]. Thermalization manifested by the Maxwell-Boltzmann velocity distribution of atoms in the cluster is achieved in less than 100 fs. [It should be noted that the translational thermalization refers to thermalization of relative velocities of atomic or molecular cluster units and not to the translational motion of the cluster as a whole.] Extensive deformation of the cluster ion during its collision with the surface is the major reason for this fast equilibration. During the collision the atoms at the front of the cluster recoil from the surface and reverse the direction of their motion, while the rest of the cluster is still moving toward the surface. This results in many collisions between the atoms or monomeric units composing the cluster and fast thermalization of their translational motion. Because peptide ions cannot be represented as a simple collection of constituent atoms it is uncertain whether this is also a good model for thermalization of vibrational modes of peptide ions colliding with surfaces. It is obviously an effective mechanism for coupling and redistributing energy among vibrational modes and is at least a plausible rationalization for rapid thermalization. Molecular dynamics simulations

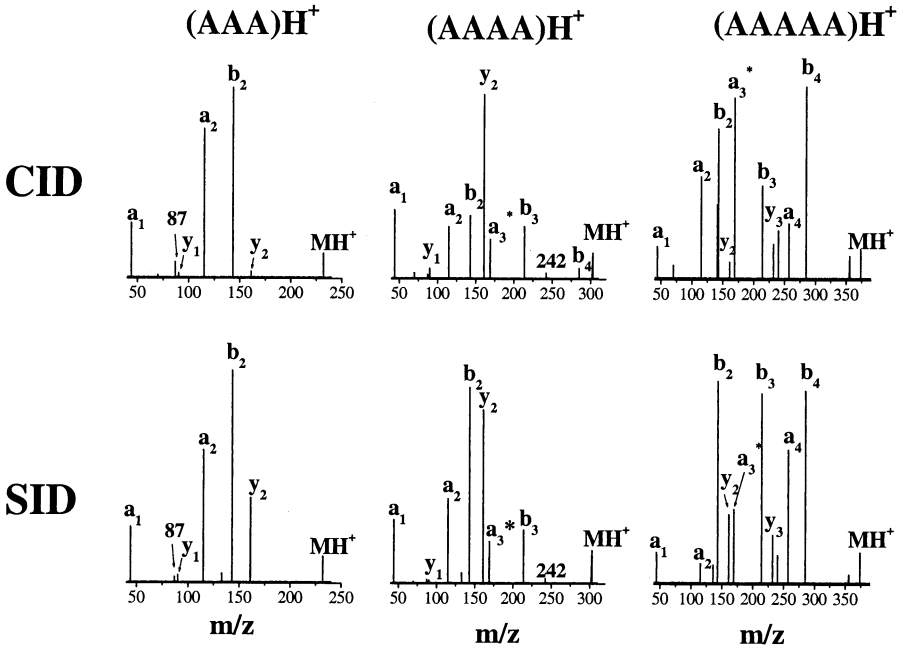


**Figure 1**. MS/MS spectra of small protonated polyalanines obtained using SORI-CID (top panel) and the matching FT-ICR SID spectra (bottom panel).

showed that the internal excitation of peptide ions occurs in less than 1 ps [2]. However, thermalization of vibrational degrees of freedom was not addressed in that study.

## Kinetic Control of SID

With increasing peptide size SID mass spectra obtained using the tandem quadrupole instrument [1] become significantly different from MCA-CID spectra. Figure 3 shows an example of the fragmentation behavior obtained for singly protonated Fibrinopeptide A (ADSGEGDFLAEGGGVR) using SORI-CID in FT-ICR MS (top) and QQ-SID (bottom) for 75 eV collisions with a fluorinated SAM surface. SORI-CID results in the well-studied selective fragmentation C-terminal to aspartic and glutamic amino acid residues [27–30]. This strongly contrasts with QQ-SID, which produces mainly low-mass fragments at all collision energies. Lowering collision energy to 50 eV in the QQ-SID decreases the overall amount of fragments but has only a minor effect on the type of fragments present in the spectrum [31]. The, SID fragmentation pattern obtained at 1 s dissociation delay (Figure 4) in FT-ICR MS is quite different. At low collision energy (43 eV) SID resembles the SORI-CID data showing formation of a very few fragment ions. This presents a further evidence for efficient heating and thermalization of vibrational DOF by collisions with surfaces.

Increasing the collision energy from 43 to 98 eV results in production of a large number of fragments (more than 50 peaks) with *m/z* below 500. Comparison between SID spectra shown in Figures 3 and 4 demonstrates the influence of the observation time on the type and amount of fragments observed experimentally. Long observation time characteristic of FT-ICR MS allows us to observe fragmentation at lower collision energies and sample low-energy dissociation pathways. Similar results were obtained for most peptides that undergo selective dissociation at low collision energy. Namely, only a small number of fragments (2–5) are observed at low collision energies and a large number of fragments (>50) are observed at high collision ener-

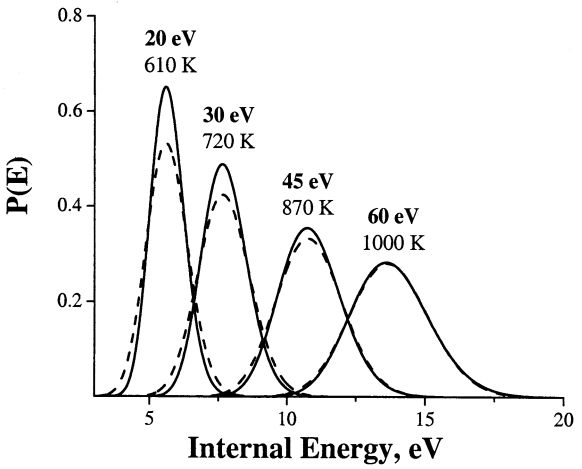


**Figure 2**. Internal energy distributions of singly protonated des-Arg1-bradykinin excited by collision with fluorinated SAM surface (solid line) at different collision energies and the corresponding thermal distributions (dashed line).

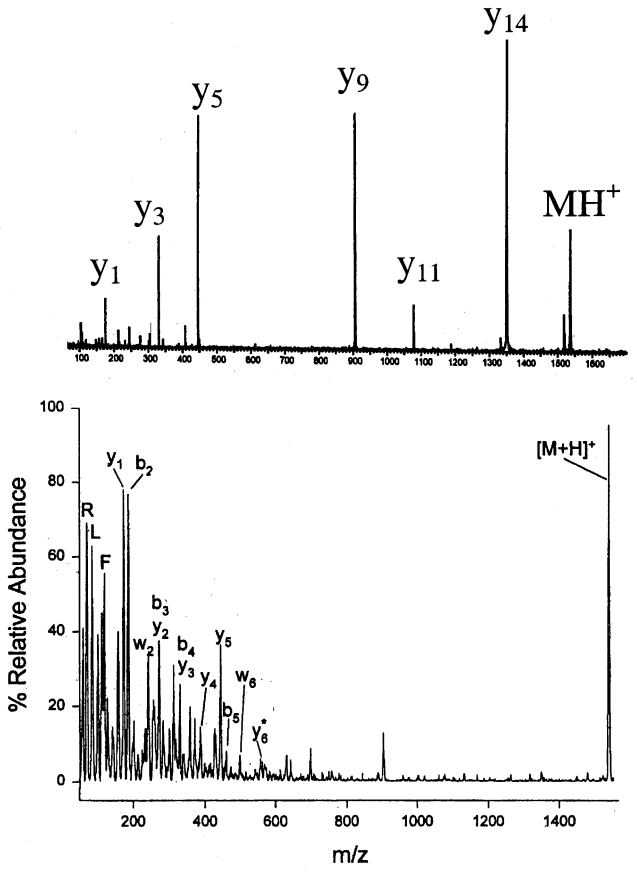


**Figure 3**. SORI-CID (top) and tandem-quadrupole SID (bottom) spectra of singly protonated Fibrinopeptide A (ADSGEGDFLAEGGGVR). Reproduced from reference [25].

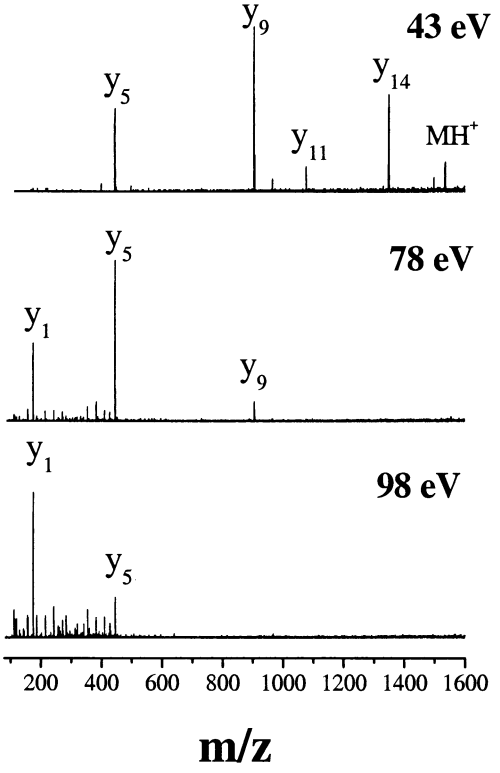


**Figure 4**. FT-ICR SID spectra of singly protonated Fibrinopeptide A at several collision energies and reaction delay of 1 s.

gies. There is also a very sharp transition between the two regimes.

These results are summarized graphically in Figure 5 [19], which plots the number of ions present in the FT-ICR SID spectrum as a function of ion kinetic energy at 1 s observation time. The insert mass spectra are typical of three distinct energy (velocity) regimes in the SID spectra. At the lowest energy, below 10 eV, only inelastically scattered primary ions are observed. At higher energy and up to about 25 eV a plateau region is observed for which the lowest energy fragmentation processes well described by our RRKM model are the characteristic spectra. At 30 eV a sharp transition to shattering is observed and spectra above this energy contain a large number of small fragment ions that form instantaneously following ion impact. By changing the ion kinetic energy and observation time the different characteristic regimes are easily explored and modeled, as described below.

## Shattering Transition

Formation of large number of ions at higher collision energy can result either from the natural branching of the fragmentation by secondary reactions of primary (low-energy) product ions or from opening up of new competing dissociation pathways. In our previous study [19] we presented solid evidence that consecutive dissociation can account for only a small increase in the

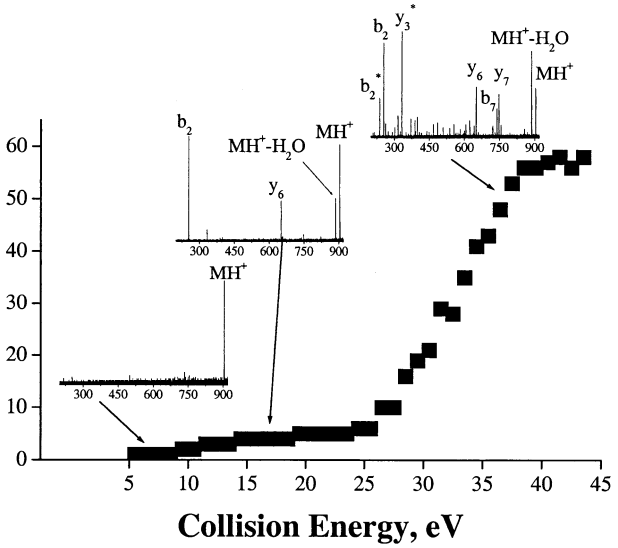


**Figure 5**. Number of peaks in SID spectrum of the singly protonated des-Arg$^9$-bradykinin as a function of collision energy for 1 s reaction delay. Insets show typical mass spectra for three distinct energy (velocity) regimes. Reproduced from reference [19].

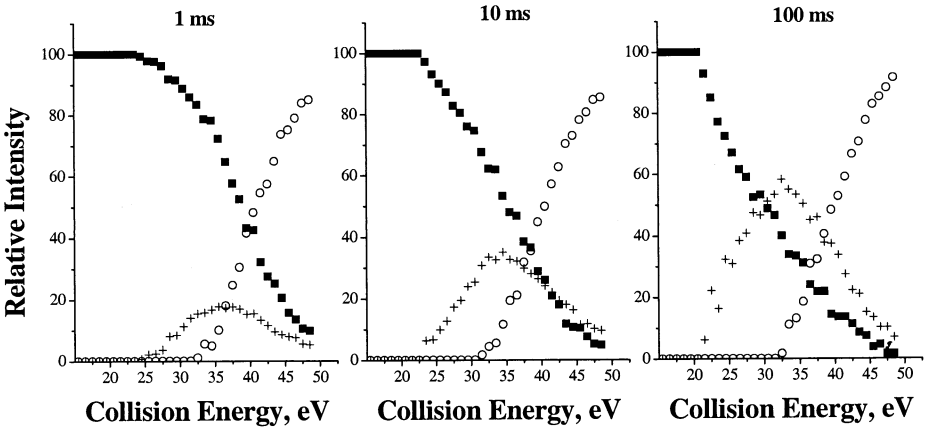


**Figure 6**. Collision energy resolved curves for the parent ion of singly protonated des-arg[1]-bradykinin (filled squares), time-dependent fragments (crosses) and time-independent fragments (open circles) at three different reaction delays.

number of fragments as a function of collision energy. Detailed examination of relative abundances, appearance energies and kinetic behavior of different fragment ions suggests that most of the fragments observed at high collision energies originate directly from the excited parent ion. In addition, kinetic studies demonstrated that relative intensities of many of the high-energy fragments do not depend on the reaction time. This fast fragmentation is rather unexpected for large peptides with 350–600 DOF. RRKM modeling of experimental data using the approach developed by our group [32, 33] using two decay rates (slow and fast) demonstrated that fast fragmentation could be modeled in the so-called "sudden death" approximation, according to which molecule fragments instantaneously after reaching a certain threshold [19].

This instantaneous fragmentation of large molecules at high collision energies occurs on or very near the surface rather than in the gas phase and indicates a transition in the dynamics of ion-surface interaction—namely, the shattering transition. The experimental signature for this transition in our FT-ICR SID experiments is the transition from very selective dissociation resulting in formation of a few fragments with pronounced time dependence to formation of a large number of time-independent fragments. Because of the nature of FT-ICR SID experiments the shattering transition can be experimentally observed only for peptides that undergo selective fragmentation at low collision energies resulting in a small number of primary fragments. For this reason, SID studies discussed in this paper have been limited to selectively fragmenting peptide ions. The contribution of shattering to the overall decomposition of the precursor ion depends on the experimental time frame. Relative contributions of the slow and fast decay rates to the decomposition of des-Arg[1]-bradykinin as a function of the observation time are shown in Figure 6, which shows the collision energy-dependent relative abundances of the precursor ion (solid squares), integrated time-dependent (TD) fragments (crosses) and time-independent (TI) fragments (open circles) at three reaction times. Shattering accounts for almost 100% fragmentation of the precursor ion at high collision energies. The relative abundance of the TD fragments decreases with decrease in the reaction time. This is compensated by the corresponding shift of the parent ion curve towards higher collision energies. RRKM modeling showed that at 10 $\mu$s observation time all TD fragments of des-Arg[1]-bradykinin disappear and dissociation results entirely from shattering [19].

For all peptide ions studied thus far we found that the shattering transition occurs when ion internal energy exceeds 10 eV. (It should be noted parenthetically that this value is based on a limited number of studies for peptide ions with similar numbers of vibrational DOF.) Figure 7 shows microcanonical rate-energy dependences for pentaalanine, des-Arg[1]-bradykinin and Fibrinopeptide A. Rate constants were calculated using a procedure discussed in our previous study [19]. RRKM parameters for des-Arg[1]-bradykinin were adopted from reference [19] ($E_0$ = 1.17 eV, $\Delta S^{\ddagger}$ = −22 cal/molK). Threshold energy and activation entropy for Fibrinopeptide A were assumed to be the same as the ones found for selective fragmentation of singly-protonated LDIFSDFR C-terminal to the aspartic acid ($E_0$ = 1.24 eV, $\Delta S^{\ddagger}$ = −7.9 cal/molK) [5]. Parameters for pentaalanine were $E_0$ = 1.2 eV, $\Delta S^{\ddagger}$ = 9.0 cal/molK [34]. The hatched region in Figure 7 represents the range of rate constants sampled in the tandem quadrupole SID experiments, while the range of rate constants characteristic of FT-ICR SID is shown as a shaded area. Shattering onset is set at 10 eV. Typical rate constants for dissociation on the surface should be in the range $10^{11}$–$10^{12}$ $s^{-1}$. Figure 7 demonstrates that for pentaalanine shattering does not compete with unimolecular dissociation even on a microsecond timescale. Consequently QQ-SID and FT-ICR SID for this ion result in

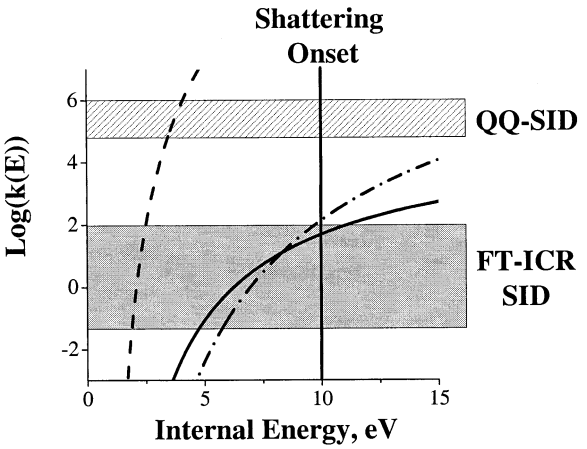


**Figure 7**. Semilogarithmic plot of the microcanonical rate-energy dependencies for dissociation of singly protonated pentaalanine (dashed line), des-arg[1]-bradykinin (solid line) and Fibrinopeptide A (dash-dot line). RRKM parameters are listed in the text. Vertical solid line shows the assumed shattering onset. The hatched area corresponds to the experimental time window of the QQ-SID setup, while the shadowed area shows the observation window in the FT-ICR SID experiment.

similar spectra, as is observed experimentally. In contrast, for both des-Arg[1]-bradykinin and Fibrinopeptide A, shattering is the only fragmentation mechanism which can be observed on the QQ timescale. This is quite different for longer reaction times characteristic of FT-ICR SID, where slow and fast dissociation are competing mechanisms.

## Dissociation Products

The type of fragment ions observed in high-energy SID spectra is a strong function of the properties of the peptide ion. For example, while high-energy SID spectrum of Fibrinopeptide A is dominated by low-mass ions (Figure 4), high-energy SID spectra of bradykinin and its analogs contain fragment ions across the entire mass range. From our experience high-mass fragments usually exhibit strong time dependence, while immonium ions and many other low-mass backbone fragments are commonly time-independent. It should be emphasized that high-energy fragmentation is dominated by backbone cleavages although in some cases side chain cleavages are also observed. Time dependence of relative intensities of internal fragments is strongly influenced by peptide composition and therefore is difficult to predict. Some peptides have a strong propensity to form internal fragments by consecutive dissociation of unstable primary product ions, leading to formation of TD internal fragments. Other peptides (in particular ones with basic residues on the C-terminus) yield stable primary fragments. In this case internal fragments do not exhibit any time dependence. The best sequence coverage is obtained using longer observation times, for which both TD and TI fragmentation are well represented in the spectrum. We have also found that some fragment ions can be formed both by the unimolecular dissociation in the gas phase and shattering [19]. For example, the $b_2$ ion from des-Arg[9]-bradykinin contains contributions from both slow and fast decomposition pathways and is expected to appear in both QQ and FT-ICR SID spectra.

## Quantifying Shattering

Predicting high-energy product distributions from the known sequence of the peptide ion is an ultimate goal for quantifying shattering. As discussed earlier, ion excitation by collision with a surface results in fast thermalization of ion's vibrational DOF [26]. Levine and co-workers used this fact to quantify the shattering transition from the point of view of the maximum entropy method [14–16]. This method searches for the fragment distribution with maximum entropy assuming that ions' internal energies can be characterized by a temperature. It has been demonstrated that for large systems the configurational entropy term is dominant at low internal excitations. This term favors formation of large fragments because of a rich conformational space that they explore. At higher excitations the translational entropy term becomes important, which results in a preferential formation of a large amount of small fragments. The shattering transition occurs when these terms compensate each other [14–16].

The maximum entropy method has been utilized to calculate fragment distributions for fullerenes at different levels of excitation [17]. The transition from formation of large even-numbered clusters by consecutive loss of $C_2$ units from the parent ion to formation of a large number of small odd-numbered product ions was successfully reproduced using this method. The information required for statistical calculations includes a complete list of the heats of formation of all expected fragments (both ionic and neutral). This is a difficult task for quantifying shattering of peptide ions because to date there is very little accurate thermochemical information on various peptide fragments and the types of neutral molecules accompanying ionic fragments are usually hard to predict. Our current research explores some relatively simple systems, for which this information can be reasonably estimated and the shattering transition can be observed experimentally.

A different approach for quantifying shattering relies on combined quantum mechanics/molecular mechanics (QM/MM) simulations. These have been reported by Hase and co-workers for collisions of protonated glycine with the hydrogenated SAM surface [18]. Trajectory simulations demonstrated that shattering occurs when the ion is properly oriented at the time of collision, which promotes the system to a very specific transition state not accessible otherwise, followed by a very fast dissociation on the surface. This kind of calculation also requires prior knowledge of dissociation barriers for each reaction channel. For protonated

glycine these were estimated using both semiempirical and ab initio calculations. However, for larger systems that display a pronounced shattering transition in our FT-ICR SID experiments these calculations are very time-consuming. In collaboration with Professor Hase we shall attempt QM/MM simulations using advanced computational facilities at the Environmental Molecular Science Laboratory (EMSL).

## Summary

The transition from recoil to shattering observed for peptide ions is of both fundamental and practical importance for understanding and improving ion activation by collisions with surfaces. It is particularly important for ions (both singly and multiply protonated) that undergo very selective dissociation at low levels of internal excitation resulting in poor sequence coverage. Shattering of ions results in formation of mainly backbone fragments. Improved sequence coverage can be obtained using SID at collision energies in excess of 40 eV and long observation time characteristic of FT-ICR MS. Understanding factors that affect the transition from slow to fast fragmentation and quantifying product distribution as a function of peptide composition are key questions for future research.

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## References

1. Dongre, A. R. Somogyi, Á.; Wysocki, V. H. Surface-Induced Dissociation: An Effective Tool to Probe Structure, Energetics, and Fragmentation Mechanisms of Protonated Peptides. *J. Mass Spectrom.* **1996,** *31,* 339–350.
2. Meroueh, O.; Hase, W. L. Dynamics of Energy Transfer in Peptide-Surface Collisions. *J. Am. Chem. Soc.* **2002,** *124,* 1524–1531.
3. Grill, V.; Shen, J.; Evans, C.; Cooks, R. G. Collisions of Ions with Surfaces at Chemically Relevant Energies: Instrumentation and Phenomena. *Rev. Sci. Instrum.* **2001,** *72,* 3149–3179.
4. Laskin, J.; Futrell, J. H. On the Efficiency of Energy Transfer in Collisional Activation of Small Peptides. *J. Chem. Phys.* **2002,** *116,* 4302–4310.
5. Bailey, T. H.; Laskin, J.; Futrell, J. H. Energetics of Selective Cleavage at Acidic Residues Studied by Ttime- and Energy-Resolved Surface-Induced Dissociation in FT-ICR-MS. *Int. J. Mass Spectrom.* **2003,** *222,* 313–327.
6. Cooks, R. G.; Ast, T.; Mabud, A. Collisions of Polyatomic Ions with Surfaces. *Int. J. Mass Spectrom. Ion Processes* **1990,** *100,* 209–265.
7. Cooks, R. G.; Ast, T.; Pradeep, T.; Wysocki, V. Reactions of Ions with Organic Surfaces. *Acc. Chem. Res.* **1994,** *27,* 316–323.
8. Burroughs, J. A.; Wainhaus, S. B.; Hanley, L. Impulsive Excitation of $Cr(CO)_6^+$ During Surface-Induced Dissociation at Organic Monolayers. *J. Phys. Chem.* **1994,** *98,* 10913–10919.
9. Lifshitz, C. Recent Developments in Applications of RRKM-QET. *Int. J. Mass Spectrom. Ion Processes* **1992,** *118,* 315–337.
10. Lifshitz, C. Kinetic Shifts. *Eur. J. Mass Spectrom.* **2002,** *8,* 85–92.
11. Schultz, D. G.; Hanley, L. Shattering of SiMe3+ During Surface-Induced Dissociation. *J. Chem. Phys.* **1998,** *109,* 10976–10983.
12. Hendell, E.; Even, U.; Raz, T.; Levine, R. D. Shattering of Clusters Upon Surface Impact—An Experimental and Theoretical Study. *Phys. Rev. Lett.* **1995,** *75,* 2670–2673.
13. Beck, R. D.; Warth, C.; May, K.; Kappes, M. M. Surface Impact Induced Shattering of $C_{60}$. Detection of Small Cm Fragments by Negative Surface Ionization. *Chem. Phys. Lett.* **1996,** *257,* 557–562.
14. Raz, T.; Even, U.; Levine, R. D. Fragment Size Distribution in Cluster-Impact-Shattering Versus Evaporation by a Statistical Approach. *J. Chem. Phys.* **1995,** *103,* 5394–5409.
15. Raz, T.; Levine, R. D. On the Shattering of Clusters by Surface Impact Heating. *J. Chem. Phys.* **1996,** *105,* 8097–8102.
16. Christen, W.; Even, U.; Raz, T.; Levine, R. D. The Transition from Recoil to Shattering in Cluster-Surface Impact: An Experimental and Computational Study. *Int. J. Mass Spectrom.* **1998,** *174,* 35–52.
17. Campbell, E. E. B.; Raz, T.; Levine, R. D. Internal Energy Dependence of the Fragmentation Patterns of $C_{60}$ and $C_{60}^+$. *Chem. Phys. Lett.* **1996,** *253,* 261–267.
18. Meroueh, S. O.; Wang, Y. F.; Hase, W. L. Direct Dynamics Simulations of Collision- and Surface-Induced Dissociation of N-Protonated Glycine. Shattering Fragmentation. *J. Phys. Chem. A* **2002,** *106,* 9983–9992.
19. Laskin, J.; Bailey, T. H.; Futrell, J. H. Shattering of Peptide Ions on Self-Assembled Monolayer Surfaces. *J. Am. Chem. Soc.* **2003,** *125,* 1625–1632.
20. Laskin, J.; Futrell, J. H. Collisional Activation of Peptide Ions in FT-ICR Mass Spectrometry. *Mass Spectrom. Rev.* **2003,** *22,* 158–181.
21. Laskin, J.; Denisov, E. V.; Shukla, A. K.; Barlow, S. E.; Futrell, J. H. Surface-Induced Dissociation in a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer: New Instrument Design and Evaluation. *Anal. Chem.* **2002,** *74,* 3255–3261.
22. Laskin, J.; Denisov, E.; Futrell, J. H. A Comparative Study of Collision-Induced and Surface-Induced Dissociation. I. Fragmentation of Protonated Dialanine. *J. Am. Chem. Soc.* **2000,** *122,* 9703–9714.
23. Laskin, J.; Denisov, E.; Futrell, J. H. Comparative Study of Collision-Induced and Surface-Induced dissociation. II. Fragmentation of Small Alanine-Containing Peptides in FT-ICR MS. *J. Phys. Chem. B* **2001,** *105,* 1895–1900.
24. Laskin, J.; Denisov, E.; Futrell, J. H. Fragmentation Energetics of Small Peptides from Multiple-Collision Activation and Surface-Induced Dissociation in FT-ICR MS. *Int. J. Mass Spectrom.* **2002,** *219,* 189–201.
25. Zhong, W. Ph.D Thesis, Virginia Commonwealth University, 1999.
26. Raz, T.; Levine, R. D. Fast Translational Thermalization of Extreme Disequilibrium Induced by Cluster Impact. *Chem. Phys.* **1996,** *213,* 263–275.
27. Yu, W.; Vath, J. E.; Huberty, M. C.; Martin, S. A. Identification of the Facile Gas-Phase Cleavage of the asp pro and asp xxx Peptid-Bonds in Matrix-Assisted Laser-Desorption Time-of-Flight Mass-Spectrometry. *Anal. Chem.* **1993,** *65,* 3015–3023.

28. Qin, J.; Chait, B. T. Preferential Fragmentation of Protonated Gas-Phase Peptide Ions Adjacent to Acidic Amino Acid Residues. *J. Am. Chem. Soc.* **1995,** *117,* 5411–5412.
29. Summerfield, S. G.; Whiting, A.; Gaskell, S. J. Intra-Ionic Interactions in Electrosprayed Peptide Ions. *Int. J. Mass Spectrom. Ion Processes* **1997,** *162,* 149–161.
30. Tsaprailis, G.; Nair, H.; Somogyi, A.; Wysocki, V. H.; Zhong, W.; Futrell, J. H.; Summerfield, S. G.; Gaskell, S. J. Influence of Secondary Structure on the Fragmentation of Protonated Peptides. *J. Am. Chem. Soc.* **1999,** *121,* 5142–5154.
31. McCormack, A. L.; Somogyi, A.; Dongre, A. R.; Wysocki, V. H. Fragmentation of Protonated Peptides—Surface-Induced Dissociation in Conjunction with a Quantum-Mechanical Approach. *Anal. Chem.* **1993,** *65,* 2859–2872.
32. Laskin, J.; Byrd, M.; Futrell, J. Internal Energy Distributions Resulting from Sustained Off-Resonance Excitation in FTMS: I. Fragmentation of the Bromobenzene Radical Cation. *Int. J. Mass Spectrom.* **2000,** *196,* 285–302.
33. Laskin, J.; Futrell, J. Internal Energy Distributions Resulting from Sustained Off-Resonance Excitation in FT-ICR MS: II. Fragmentation of the 1-Bromonaphthalene Radical Cation. *J. Phys. Chem. A* **2000,** *104,* 5484–5494.
34. Laskin, J.; Denisov, E.; Futrell, J. H. unpublished data.