Mass Spectrometry

Fragmentation of Singly Charged Peptide Ions by Photodissociation at $\lambda = 157$ nm**

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Bond-selective chemistry has been a goal of photochemists for decades, particularly since the development and proliferation of tunable laser light sources. Nevertheless, for relatively large molecules, this goal has been elusive. Rapid intramolecular vibrational relaxation appears to redistribute energy throughout large molecules on timescales faster than dissociation so that any selectivity that may be injected by an excitation process is lost. The fragmentation of peptide ions activated by blackbody radiation,[1] IR multiphoton excitation,[2] UV laser excitation,[3–5] and collisions with gas-phase molecules or surfaces[6,7] involves vibrational excitation of precursor ions and consequently, production of similar types of daughter ions. The latter are primarily *b*- and *y*-type fragments as defined by the standard nomenclature shown in

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Figure 1 a.[8,9] In sharp contrast, electron capture dissociation (ECD)[10] and electron transfer dissociation (ETD)[11] apparently involve the capture of an electron by a multiply

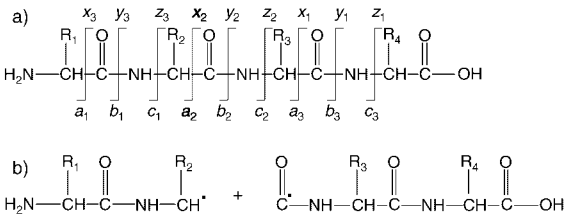

Figure 1. a) Standard nomenclature of peptide fragmentation; b) products of homolytic radical cleavage of a sample peptide. Note that the location of the added proton is not specified.

protonated protein ion, leading to charge reduction and the formation of a hydrogen atom that subsequently induces cleavage between the backbone nitrogen and α-carbon atoms. These techniques induce localized excitation, and dissociation apparently occurs before the internal energy is randomized, producing *c*- and *z*-type fragment ions with high sequence coverage. We now report another unorthodox fragmentation phenomenon in which 157-nm light excitation induces unusual backbone cleavage in singly protonated peptide ions.

Theoretical and spectroscopic studies suggest that small polypeptides absorb rather strongly in the vacuum ultraviolet, and the chromophore involved in the process is associated with the peptide backbone amides.[12] One absorption band occurs near 190 nm.[13] This transition can be excited using 193-nm ArF laser light. Disappointingly, photodissociation experiments at this wavelength have generated a combination of thermal ion fragments and a few fragments that are similar to those produced by high-energy collision-induced excitation.[4,5,14] Small model compounds such as *N*-acetylglycine absorb near 160 nm.[15,16] Theoretical studies of polyamides predict several charge-transfer bands in the 150- to 175-nm region, and an interaction between the excited states of the carboxyl terminus and the backbone amides has been inferred.[17] Robin suggested that one of the absorption bands in this wavelength region might be associated with an $n_o \rightarrow \sigma^*$ transition.[18] In order to investigate whether excitation of these electronic states would lead to novel dissociation products, the present study was undertaken (for details see the Experimental Section).

Tandem mass spectra of the peptide FSWGAEGQR generated with four sources of excitation are displayed in Figure 2. Collision-induced dissociation (CID) and post-source decay (PSD) generate primarily *y* fragments from this peptide, although several *b* and *a* fragments are also observed. Photodissociation with 157-nm light generates a completely different fragmentation pattern dominated by *x*-, *v*-, and *w*-type fragments. The appearance of y_3 and y_6 is probably associated with the enhancement of already intense PSD ions. Ions labeled *x* correspond to cleavage of the backbone bond between the α-carbon and the carbonyl carbon with the charge remaining on the C-terminal fragment. *v* ions are high-energy C-terminal fragments that

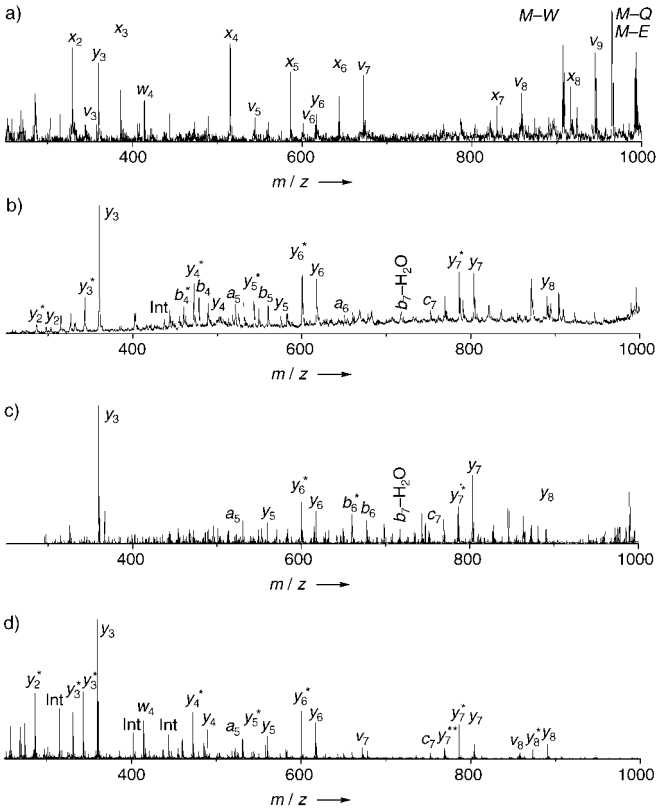

Figure 2. MALDI tandem mass spectra of peptide FSWGAEGQR obtained by: a) 157-nm photodissociation using 2,5-dihydroxybenzoic acid as matrix, b) PSD using α-cyano-4-hydroxycinnamic acid as matrix, c) AP MALDI CID, d) 2-keV TOF-TOF CID. Peaks labeled Int are internal fragments. The * and ** labels represent the loss of one and two NH_3 groups, respectively.

completely lose an adjacent amino acid side chain. Certain amino acids produce *w* ions from partial side-chain loss, with cleavage occurring between the β and γ carbon atoms. Losses of the side chains of some amino acids that would be expected to absorb 157-nm light are also observed and labeled *M*−*X*.

We propose that x_n+1 ions are initially generated by homolytic radical cleavage as shown in Figure 1 b. *x*, *v*, and *w* ions with even numbers of electrons[9] are formed following subsequent loss of H atoms or radicals. Ultraviolet-light-induced homolytic radical cleavage of small amides by means of a Norrish Type I mechanism is well known,[19] but this reaction has not been reported for larger molecules such as peptides. The initially formed radical ions subsequently lose an H atom to form x_n ions, CO and a side-chain radical to form v_n ions, or HN=C=O and part of a side chain to form w_n ions. Biemann and co-workers have suggested that v_n ions can be produced from x_n+1 precursor ions following high-energy peptide excitation.[9]

A number of peptides with basic residues at their C termini were fragmented using 157-nm photodissociation, and four typical mass spectra are shown in Figure 3. Every spectrum is dominated by *x*, *v*, and *w* fragments. *w* fragments are usually observed at leucine residues, rendering them distinguishable from isoleucine. Although *b*- and *y*-type ions occasionally appear in these spectra, they generally corre-

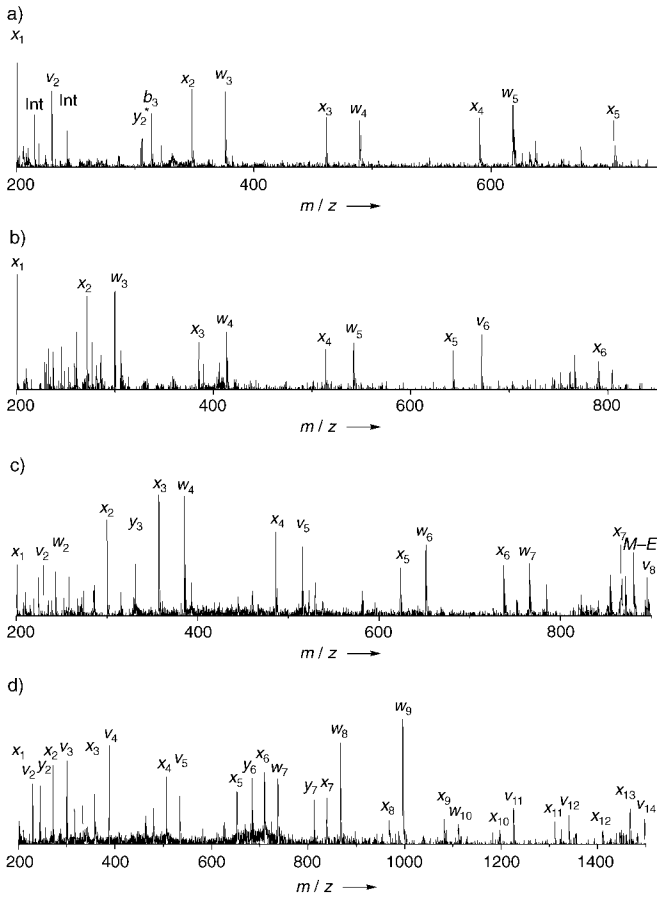

Figure 3. MALDI 157-nm photodissociation tandem time-of-flight mass spectra of a series of peptides containing C-terminal arginine. A) ALELFR; B) LFEELAR; C) IENHEGVR; D) EGVNDNEEGFFSAR. Peaks labeled Int are internal fragments. The * label represents the loss of one NH_3 group.

spond to intense PSD fragments that were not completely eliminated with background subtraction.

Other experiments, for which the data are not shown, were performed on peptides having N-terminal arginines. 157-nm light is found to cleave the backbone bond between the α-carbon and the carbonyl carbon as discussed above. In these cases, the N-terminal arginines sequester the charge, resulting in spectra dominated by *a*-type daughter ions. Whereas *a*-type ions are observed with other peptide-ion fragmentation techniques, we observe high sequence coverage and similar fragment-ion intensities just as with peptides having C-terminal arginines. This is consistent with the Norrish Type I photochemical cleavage model described previously.

Thus far we have photodissociated over two dozen peptide ions using 157-nm light. All peptides with C-terminal arginines yield predominantly *x*, *v*, and *w* ion fragments. This simple and well-defined product distribution apparently results from excitation of a dissociative electronic state by the 157-nm light. Thus, despite the relatively large size of the molecules investigated, at least the initial fragmentation step must be occurring without electronic-to-vibrational relaxation. Prompt dissociation may precede intramolecular vibrational relaxation in the excited electronic state. Alternatively, dissociation may take place from a vibrationally randomized excited electronic state. As our experiment does not provide time-dependence information but simply offers evidence of a novel fragmentation mechanism, it is not possible to rule out either of these possibilities.

The relative similarity of the intensities of many photofragment-ion peaks together with the fact that the charge always remains on the fragment containing arginine suggests that the charge is not involved in the fragmentation mechanism. Observation of *a* ions from peptides with R near the N terminus and *x* ions from peptides with R near the C terminus suggests that the role of the charge is simply to make the fragments detectable. This is in contrast with low-energy peptide fragmentation in which the mobility (or lack of mobility) of a proton usually has a dramatic effect on the observed spectra.[20]

Most peptides generated by digesting proteins with the enzyme trypsin have the basic residues arginine or lysine at their C termini. However, lysine does not sequester protons as effectively as arginine, and we have preliminary evidence that it should be guanidinated[21] to produce *x*-, *v*-, and *w*-type fragments. This is currently under investigation. Since tryptic digests are widely used, 157-nm photodissociation may interface well with protein identification experiments. The rapid timescale and charge conservation obtained with 157-nm photodissociation make it complementary to ECD and ETD, since it is compatible with singly protonated MALDI ions and TOF mass analyzers. The high sequence coverage observed and potential predictability of ion fragmentation patterns may facilitate peptide de novo sequencing. Because light is not affected by electric or magnetic fields, photofragmentation should be compatible with various mass analyzers and ion sources. Further studies of this phenomenon will involve 157-nm photodissociation of singly and multiply protonated peptide and protein ions generated by atmospheric pressure ion sources and will further probe the mechanism through which this fragmentation occurs.

Experimental Section

Experiments were performed on a homebuilt MALDI tandem time-of-flight instrument. Its design is similar to tandem time-of-flight instruments that employ collision cells[22,23] except that ion fragmentation is induced by 157-nm VUV light from an F_2 laser. Precursor ions are separated in a linear TOF apparatus, and those of interest are selected by an ion gate. An unfocused 10-ns, 2-mJ laser pulse with a cross section of 5 mm × 10 mm interacts with the selected ions. Precursor and fragment ions are then reaccelerated, separated, and detected in a reflectron TOF analyzer. Spectra with and without photodissociation are recorded on alternating shots so that the post-source decay contribution can be subtracted away. For comparison, high-energy collision-induced dissociation data were recorded on an Applied Biosystems (Foster City, CA) 4700 Proteomics Analyzer using 2-keV fragmentation energy with air as the collision gas. Low-energy CID was performed on a ThermoFinnigan LCQ Deca XP (Waltham, MA) using an atmospheric pressure MALDI ionization source.

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