Preferential Fragmentation of Protonated Gas-Phase Peptide Ions Adjacent to Acidic Amino Acid Residues

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Matrix-assisted laser desorption/ionization (MALDI) is an effective method for producing protonated peptide and protein ions in the gas phase.[1] Together with electrospray ionization, MALDI has become an ionization method of choice for analyzing peptides and proteins by mass spectrometry (MS).[2] The analyses are usually carried out by measuring the molecular masses of the intact polypeptides or peptide fragments produced by proteolysis or chemical degradation of proteins in solution. An alternative method for obtaining detailed primary structural information uses fragmentation of the protonated peptides in the gas phase followed by MS analysis of the fragment ions. A powerful variation of this approach, called tandem MS, employs mass spectrometric selection of the peptide ion of interest prior to fragmentation, followed by analysis of the fragment ions.[3,4]

Fragmentation of peptide ions produced by MALDI has been studied in time-of-flight (TOF) mass analyzers (fitted with ion mirrors), instrumentation well-suited to the pulsed ionization source.[5,6] Mass analyzers that utilize the principle of ion trapping are also well-suited to the pulsed MALDI ion source.[7] Precursor ion selection can be achieved with high resolution.[8] The prolonged time window of the measurement in the quadrupole ion trap (10^{-4} to >1 s) compared with that in the ion mirror TOF analyzer ($<10^{-4}$ s) enables the observation of fragmentation processes with low rate constants. In the reflecting TOF analyzer, highly preferential peptide bond cleavage has been observed at the bond carboxy (C)-terminal to aspartic acid residues, with Asp−Pro bonds exhibiting particularly high lability.[6] In the present study, we investigate the fragmentation of MALDI-produced protonated peptide ions (containing an Arg residue and at least one acidic amino acid residue) in a quadrupole ion trap mass spectrometer and also observe preferential cleavage of the peptide bond adjacent to aspartic acid residues. In addition, we report the first observation of preferential fragmentation adjacent to glutamic acid residues.

The investigation was carried out on a MALDI quadrupole ion trap mass spectrometer constructed at Rockefeller University.[9] Ions are generated external to the ion trap using 355 nm laser irradiation,[10] accelerated to 60 eV, focused with an einzel lens, and injected axially into the trap. The trapped ions are analyzed via an instability rf ramp (10−100 ms) incorporating resonance ejection for mass range extension.[11]

Two kinds of experiments were carried out. The first involved direct injection and measurement of the laser-desorbed ions, without precursor selection. In this experiment, fragment ions produced both during and after trapping were measured. The observed fragmentation is brought about by a combination of the initial excitation imparted during the MALDI process and collisional excitation prior to, during, and after trapping. The second experiment involved a tandem MS measurement, wherein a precursor ion was selected by ejection of all lower mass fragment ions followed by resonant excitation (tickling)[12] and product ion analysis. This experiment measured fragment ions produced exclusively in the ion trap from a selected precursor.

Figure 1 shows mass spectra resulting from the direct injection experiments. The mass spectrum of osteocalcin fragment 7-19 (Figure 1a) exhibits three dominant peaks, which arise from the protonated molecule (measured monoisotopic molecular mass (MM) = 1407.0 Da, calculated MM = 1406.7 Da) and two fragment ions with *m/z* 611.6 and 272.8, respectively. The masses of the fragment ions correspond to peptide bond cleavage at the C-termini of Asp-8 and Glu-11 with charge retention on the C-terminal fragments to give fragments Y_5 and Y_2[13] with calculated *m/z* of 611.4 and 272.2, respectively. These results show that the fragmentation pathways that lead to Y_5 and Y_2 dominate strongly over other fragmentation pathways, including fragmentation adjacent to Pro residues (residues 3, 5, and 7), a decomposition pathway that has previously been found to have an enhanced rate in low-energy multiple collision-induced dissociation tandem MS measurements of multiply charged ions.[14] Figure 1b shows the mass spectrum of [Glu-1]fibrinopeptide B (measd MM = 1569.7 Da, calcd MM = 1568.8 Da), a 13-residue peptide that contains three Glu residues and one Asp residue. Four dominant fragments are observed, all arising from cleavage of Asp−Xxx or Glu−Xxx bonds. The peaks labeled with asterisks to the left of the protonated molecule and fragment Y_n are from ions with mass 17 Da lower than the peaks immediately to their right and likely arise through the loss of the elements of NH_3. An even more striking example is shown for pancreastatin fragment 34-49 (measured MM = 1845.3 Da, calcd MM = 1845.8 Da), which has a series of five adjacent Glu residues (Figure 1c). Dominant fragment ions are observed to arise from cleavage reactions adjacent to each of the Glu residues. In this case, other fragment peaks that do not arise from cleavage at the Glu residues are also present with comparable intensities. Figure 1d shows an example of the fragmentation of a larger peptide, the reduced N-terminal epidermal growth factor-like domain in human coagulation factor X (measd MM = 4800.5 Da, calcd MM = 4800.3 Da). The peptide contains 43 amino acid residues, of which eight are acidic. Again, the fragmentation is dominated by cleavages adjacent to acidic residues (fragmentation is observed at seven of the eight acidic sites).

Data comparable to those shown in Figure 1 were obtained using true tandem mass spectrometry[9] (Figure 2), wherein a precursor ion is selected and resonantly excited (tickled) and the resulting collision-induced fragmentation products are measured. Selective fragmentation is again observed at Asp and Glu. A total of 25 peptides (MMs ranging from 800 to

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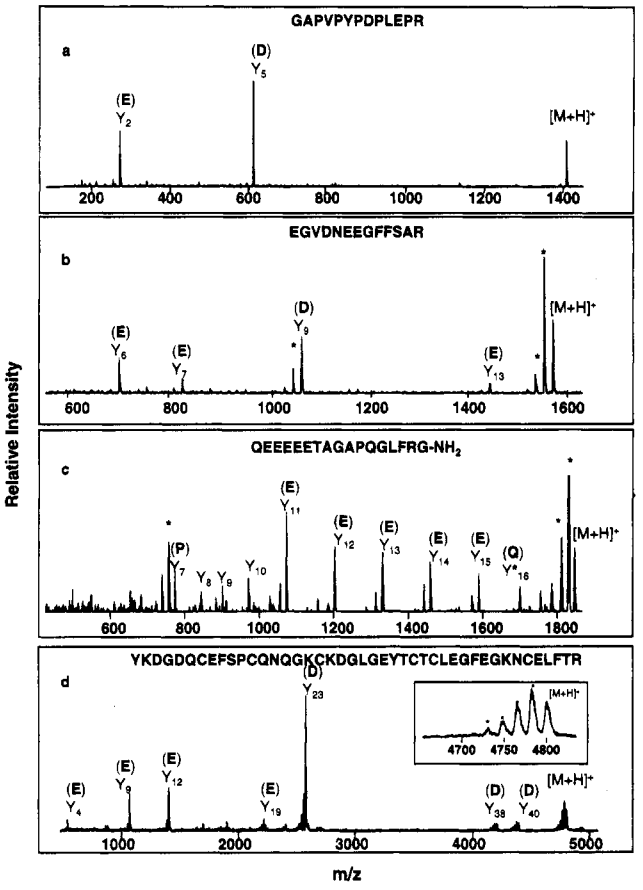

Figure 1. Positive ion MALDI mass spectra of four peptides obtained in a quadrupole ion trap mass spectrometer without precursor selection. The matrix used was 2,5-dihydroxybenzoic acid. (a) Osteocalcin fragment 7-19. (b) [Glu-1]Fibrinopeptide B. (c) Pancreastatin fragment 34-49. (d) Reduced N-terminal epidermal growth factor-like domain in human coagulation factor X. The inset shows a detail of the region around the protonated molecule. The peaks are labeled Y_n instead of Y''_n[13] to save space. The peaks labeled with asterisks are from ions with mass 17 Da lower than the peaks immediately to their right and likely arise through the loss of the elements of NH_3. The letter given above the sequence assignment indicates that the ion is produced from cleavage at the C-terminus of that residue.

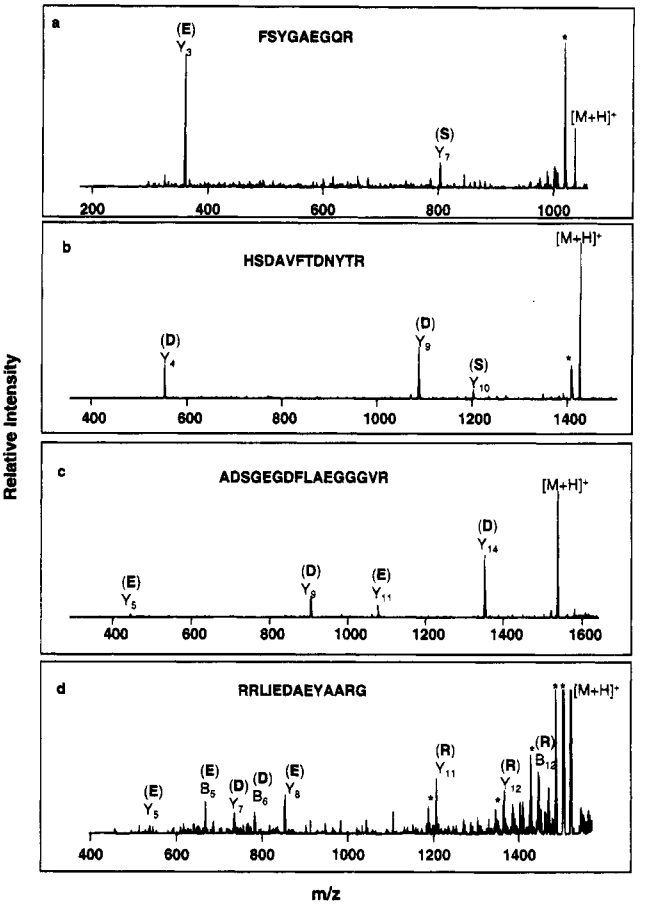

Figure 2. Collision-induced dissociation MALDI positive ion mass spectra of four peptides obtained in a quadrupole ion trap mass spectrometer with precursor selection. Precursor ions are isolated by ejecting all lower mass ions. The isolated intact protonated peptides were resonantly excited and the product ions detected. (a) Peptide with sequence FSYGAEGQR. (b) Vasoactive intestinal peptide fragment 1-12. (c) Fibrinopeptide A. (d) Peptide with sequence RRLIEDAEYAARG. The peaks labeled with asterisks are from ions with mass 17 Da lower than the peaks immediately to their right and likely arise through the loss of the elements of NH_3.

4800 Da) that contained both Arg and one or more acidic amino acid residues were measured. All the peptides studied yielded results comparable to those shown in Figures 1 and 2. The fragmentation patterns obtained using true MS/MS were virtually identical to those obtained using direct injection. Peptides that do not contain Arg were not investigated. The behavior of Arg-free peptides will be the subject of future studies.

Although the process leading to the preferential fragmentation at acidic residues has not yet been unambiguously established,[6] the long time window of the ion trap measurement favors the observation of these reactions which appear to be entropically disfavored but have low enthalpies of dissociation.

The preferential fragmentation at Asp and Glu residues observed in the ion trap MS has important practical implications. For peptides containing acidic amino acid residues, competing fragmentation channels that are not adjacent to the acidic residues will be strongly discriminated against, leading to a reduction in the amount of primary structure information that can be extracted. Such preferential fragmentation presents a serious impediment to obtaining extensive sequence information from trapped peptides ions that contain acidic amino acid residues, perhaps necessitating the use of MS/MS/MS or proteases that cleave at Asp and/or Glu. On the other hand, the specific cleavage at Asp and Glu generates gas-phase decomposition products analogous to those produced in solution by an Asp/Glu-specific protease. By analogy with peptide mapping studies using such proteases, the specificity of the present gas-phase cleavage reactions is potentially valuable for determining features of the primary structures of proteins (especially posttranslational modifications). The specific fragmentation is also potentially valuable for the rapid identification of proteins via elucidation of their component peptides and for reducing the size of peptides to modules that can be conveniently studied via MS/MS/MS.

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