

Fragmentation of Peptide Ions via Interaction with Metastable Atoms

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Tandem mass spectrometry is currently one of the most important proteomics tools due to its high sensitivity and specificity. The most prevalent MS/MS methods in use are based on collision-induced dissociation (CID) of protonated peptide ions. The drawbacks of CID include the facile losses of labile groups and incomplete backbone fragmentation. A technique that has been shown to complement CID of multiply charged protonated peptide ions is electron capture dissociation (ECD)¹. However, the maximum cross section of ECD is observed for thermal electron energies. This is the main reason why up-to-date ECD was successfully realized only in FT-ICR instruments. There is a need for an effective ECD-like fragmentation technique which can be used in ion trap mass spectrometers.

A time-of-flight (TOF) mass spectrometer with orthogonal acceleration was used in the described experiments. Peptide ions were produced by electrospray and transferred into the TOF mass analyzer through the heated capillary and the system of quadrupole ion guides. Metastable argon atoms were produced in a glow discharge-type source and injected between the rods of the last quadrupole. The discharge ($I \approx 5$ mA, $V \approx 300$ V) was initiated by applying 4.5-5.0 kV through a limiting 1.0 M Ω resistor. A negative potential was applied to the cathode, while the anode was grounded. The high pressure chamber had a 0.5 mm dia. exit aperture. The pressure in the discharge chamber (where the cathode is located) was ~ 25 Torr, while the pressure in the quadrupole region was around 5 mTorr. Peptide ions were trapped in the last quadrupole for 50-400 ms to increase the time available for interaction with electronically excited metastable atoms. The low kinetic energy beam of metastable argon atoms interacted with the peptide ions collimated near the central axis of the quadrupole ion guide, causing their fragmentation.

To prove that the described above source generates substantially neutral gas flow with electronically excited metastable argon atoms, the entrance capillary was closed and different gases were supplied into the first section of orthogonal chamber. This created a gas flow along the quadrupoles axis. The resulting pressure in the second section of the orthogonal chamber was about 1 mTorr (background pressure in this section was less than 10^{-5} Torr). Recombination energy for the Ar ions equals 15.76 eV and the energy of the two most populated metastable states equal 11.72 and 11.55 eV, respectively. Ionization potentials of nitrogen, methane, ammonia and isobutane equal 15.58 eV, 12.61 eV, 10.07 eV, and 10.68 eV, respectively. Practically no nitrogen ion signal and only a weak methane ion signal were recorded. A strong molecular radical cation signal was recorded with ammonia. An intense spectrum, consisting mainly of fragment ions, was observed for isobutane. These results demonstrate that the glow discharge source produces substantially neutral flow with electronically excited metastable atoms.

Fragmentation of Substance P using metastable atoms produced a dominating series of c-fragment ions: singly charged from c4 to c10 and doubly charged from c8 to c10. The spectrum looked similar to the ECD spectra, for except of the presence of doubly charged c ions. The intensity of the second isotope of molecular ions was larger than for normal isotopic distribution. This indicates the presence of charged reduced molecular ions. The fragmentation spectrum of Bradykinin showed nearly a complete series of c- and z- ions. Fragmentation of larger peptides, Fibrinopeptide A and Insulin oxidized chain B, was also studied (c- and z- ions were mainly observed). The dependence of the fragment ion signal on trapping time was also measured. It was shown, that 100-200 ms was sufficient to induce substantial fragmentation.

Interaction of Bradykinin (3+) ions (selected with RF/DC quadrupole) with metastable argon atoms in continuous mode is shown in Fig.1. The presented spectrum was averaged over 10 s. The total intensity of c- and z- ions constitutes 2.8% of the intensity of the parent ion. This

demonstrates the potential of this method for fast fragmentation of peptide cations. The fragmentation efficiency of Bradykinin (2+) ions was smaller (0.7%).

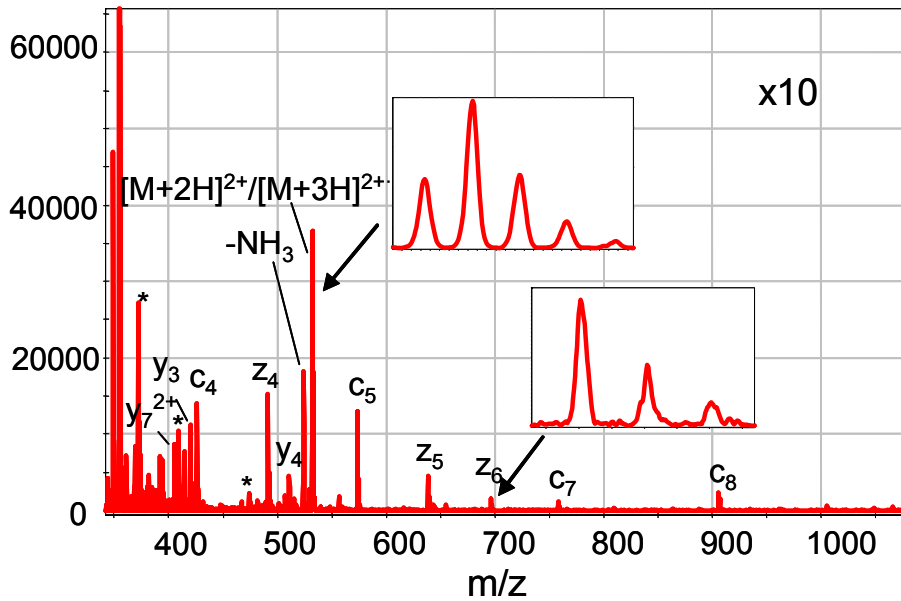


Figure 1. Fragmentation spectrum of Bradykinin (3+) ion obtained in continuous mode.

Fragmentation of phosphorylated peptides from Enolase digest was studied in a trapping mode. The typical trapping time in a linear trap was 200 ms. The intense series of c- and z- ions were observed. The fragmentation spectrum of doubly charged phosphorylated peptide (132-138) from Enolase digest is shown in Fig.2

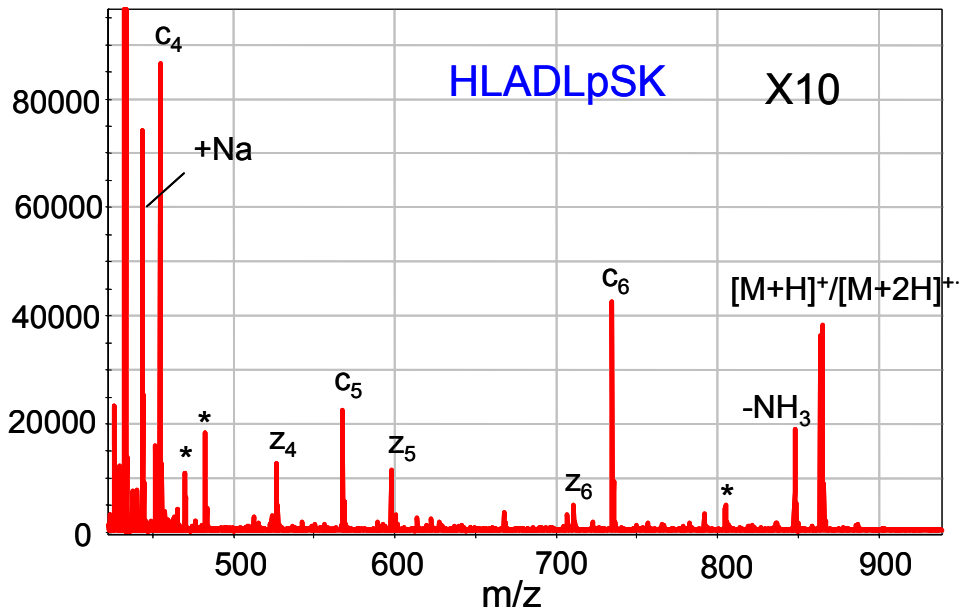


Figure 2. Fragmentation spectrum of phosphorylated peptide from Enolase digest.

1. Zubarev, R.A.; Kelleher, N.L.; McLafferty, F.W. *J. Am. Chem. Soc.* **1998**, 120, 3265-3266.