International Journal of Mass Spectrometry 278 (2008) 150–157

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Fragmentation of phosphorylated and singly charged peptide ions via interaction with metastable atoms

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ARTICLE INFO

Article history:
Received 28 December 2007
Received in revised form 24 April 2008
Accepted 25 April 2008
Available online 3 May 2008

Keywords:
Time-of-flight
Fragmentation
Phosphorylation
Metastable atom

ABSTRACT

Fragmentation of phosphorylated peptide ions via interaction with electronically excited metastable argon atoms was studied in a linear trap—time-of-flight mass spectrometer. Doubly charged ions of phosphorylated peptides from an Enolase digest were produced by electrospray ionization and subjected to a metastable atom beam in the linear trap. The metastable argon atoms were generated using a glow-discharge source. An intensive series of c- and z-ions were observed in all cases, with the phosphorylation group intact. The formation of molecular radical cations with reduced charge indicated that an electron transfer from a highly excited metastable state of argon to the peptide cation occurred. Additionally, singly charged Bradykinin, Substance P and Fibrinopeptide A molecular ions were fragmented via interaction with electronically excited metastable helium atoms. The fragmentation mechanism was different in this case and involved Penning ionization.

1. Introduction

Mass spectrometry (MS) is now widely used in protein biochemistry and in proteomics for the identification and characterization of proteins [1]. Collision induced dissociation (CID) is the most commonly used approach to derive structural information from peptide and protein ions through collisions with neutral gas molecules [2]. In this process, which generally leads to cleavage of the peptide backbone amide bond to produce b-type and y-type sequence ions, peptides are kinetically excited and undergo multiple collisions with neutral gas molecules. Energy acquired in each collision is rapidly distributed throughout all covalent bonds. Fragment ions are formed when the internal energy exceeds the activation barrier required for a particular bond cleavage. The drawbacks of CID include the facile losses of labile groups involved in many important posttranslational modifications, such as phosphorylation and glycosylation, and in many cases incomplete backbone fragmentation [3]. Therefore, developing alternative peptide fragmentation methods is of a considerable interest.

McLafferty and co-workers introduced a new technique, called electron capture dissociation (ECD), which has been shown to complement the information obtained with CID of multiply protonated peptide cations [4]. Capture of a thermal electron by a protonated peptide is exothermic by ≈6 eV and causes fragmentation of N–C_α bonds, yielding N-terminal c- and C-terminal z-fragments [5,6]. In contrast to collision-induced dissociation, ECD is believed to be non-ergodic [4], i.e., the cleavage happens prior to any *intra*molecular energy redistribution. As a result, labile modification groups are preserved. ECD generally results in cleavage of a wider range of peptide backbone bonds than CID with less dependence on peptide composition [7]. However, to this day ECD has only been successfully realized in FT-ICR mass spectrometers, where the electric field is very weak and the strong magnetic field confines electrons. The presence of strong (100–1000 V of amplitude) radio frequency (RF) electric fields in 3D and linear quadrupole ion traps hampers the introduction of low energy electrons to the area where ions are located. Recent attempts to implement ECD into radiofrequency ion traps revealed diminished fragmentation efficiency and sensitivity (as compared to FT-ICR) [8,9].

A new fragmentation technique—electron transfer dissociation (ETD), overcoming the technical challenges of introducing low energy electrons into strong oscillating RF fields, was recently proposed [10–12]. In ETD a singly charged anion transfer an electron to the multiply protonated peptide and induces fragmentation of the peptide backbone along pathways that are similar to those observed in electron capture dissociation. Simultaneous trapping of cations and anions is readily accomplished by the RF quadrupole field. However, it has been noted, that the peptide structural information that can be obtained using ETD is charge-state dependent [13]. The doubly-charged peptide cations give much poorer sequence coverage, than triply protonated cations, with fragmentation often

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doi:10.1016/j.ijms.2008.04.019

limited to one or both ends of the peptide. It was also demonstrated that the efficiency of electron transfer dissociation decreases with peptide size [14].

Another recently introduced fragmentation technique, similar to ECD, is based on the electron transfer from metastable, electronically excited atoms [15,16]. The peptide cations are stored in the RF ion trap and irradiated by a beam of particles generated by a fast atom bombardment (FAB) gun [15] or by a beam of metastable atoms produced in a glow discharge [16]. Since the beam is neutral, problems inherent to the charge capacity limitations of ion traps are not encountered. Fragmentation spectra of common peptides were similar to the spectra observed using ECD [16]. In this study we present further development of this fragmentation technique for analysis of phosphorylated peptides and singly charged peptide ions (which cannot be fragmented using ECD/ETD techniques).

2. Experimental

2.1. Reagents

Solutions of peptides were prepared in molecular biology grade water (Cambrex Bio Science, Rockland, ME), reagent grade methanol, and glacial acetic acid (Sigma–Aldrich, St. Louis, MO) (1% in 1/1 water/methanol). Substance P, Bradykinin and Fibrinopeptide A were purchased from Sigma–Aldrich (St. Louis, MO). Phosphopeptide standard was purchased from Waters (Milford, MA) and was used without further purification. Helium (ultra high purity grade) and argon (research grade) were supplied by Airgas (Radnor, PA).

2.2. Mass spectrometry

The time-of-flight mass spectrometer with orthogonal acceleration used in the present study has been described previously [16]. The schematic illustration of the instrument is shown in Fig. 1. Ions were produced in an electrospray source and transferred into the mass spectrometer through an atmospheric pressure (AP) interface. The AP interface consists of a heated capillary (0.4 mm i.d.) and a quadrupole ion guide (6.35-mm rod diameter) operated at ~1 Torr pressure in the RF-only mode.

The octopole ion guide (3.2 mm rod diameter), located after the quadrupole, was differentially pumped by a small turbomolecular pump to a pressure of ~0.1 Torr. Both the quadrupole and octopole were driven by an RF generator, built in-house, according to the design described in Ref. [17]. A capacitive divider allowed the application of different RF amplitudes to the quadrupole and octopole ion guides, respectively. The quadrupole and octopole rods were offset to DC potential, applied through decoupling capacitors.

In contrast to the instrument described earlier [16], a mass resolving quadrupole was placed in a separate differentially pumped chamber. This allowed operating it at pressures of $\sim 10^{-5}$ Torr in typical experimental conditions, thus providing better precursor selection in comparison with the previous design where it operated at a few mTorr. In the initial experiments the mass resolving quadrupole was driven by a SRS (Sunnyvale, CA) Model DS340 sine-wave signal generator coupled through an ENI (Rochester, NY) Model 240L broadband RF power amplifier. An RF coupling transformer, built in-house, gave an output voltage 0–500 V_{0-p} (zero-to-peak and pole to ground voltage) in the frequency range of 100 kHz to 5 MHz. The transformer also provided the required 180° phase difference between the rod pairs. Later, the mass resolving quadrupole was driven by an Extrel (Pittsburgh, PA) Model 150-QC RF/DC power supply, which provided better precursor ion mass selection.

The last quadrupole was operated in a trapping mode. The DC voltages, applied to the entrance and exit apertures of the last quadrupole, were changed by NAND gate integrated circuitry SN74LS03 with open collector outputs (Texas Instruments, Dallas, TX) controlled by SRS (Sunnyvale, CA) Model DG535 digital signal generator. A beam of metastable electronically excited argon atoms was produced in a glow-discharge source and introduced between the quadrupole rods. The glow-discharge source used in the present study has been described previously [16]. One of its

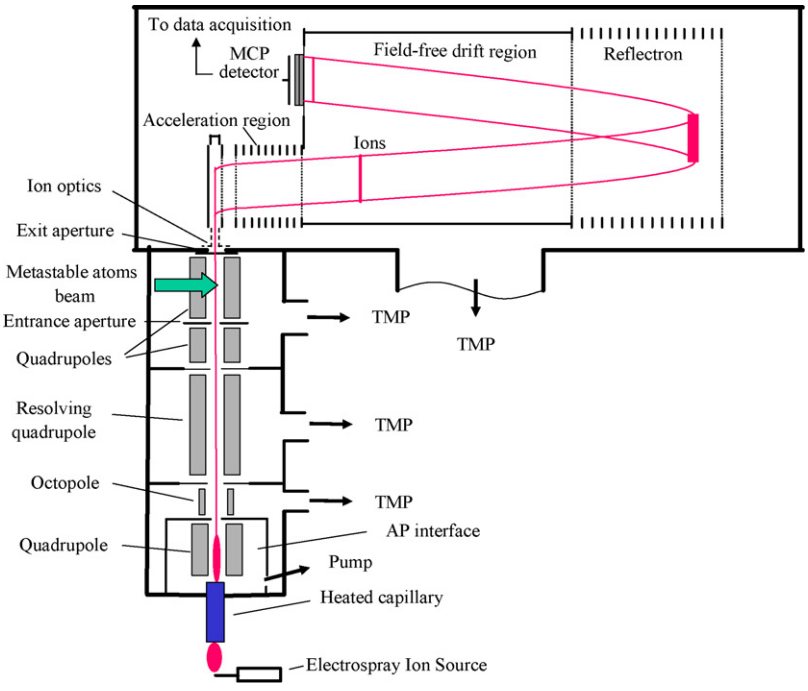

Fig. 1. Schematic view of an ortho-TOF mass spectrometer.