International Journal of Mass Spectrometry 299 (2011) 1–4

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Atmospheric pressure photoionization of peptides

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ARTICLE INFO

Article history:
Received 21 July 2010
Accepted 16 August 2010
Available online 15 September 2010

Keywords:
Atmospheric pressure photoionization
Peptide fragmentation
ECD/ETD

ABSTRACT

In this work, fragmentations of peptides under atmospheric pressure photoionization conditions are further investigated for a model peptide. In the positive ion mode, intensive fragmentations are observed, mainly ascribed to *c*-sequence ions along with less abundant *a*- and *b*- type ions. The same features and *c*- ion series are reported for the negative ion mode, thereby suggesting a common mechanistic origin for the fragmentation in both ion modes. Moreover, it is shown that ECD-like fragmentation under APPI conditions may retain non-covalent adducts. This interesting property positions APPI as a promising ECD-like technique for in-source top down sequencing.

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1. Introduction

Mass spectrometry has become an invaluable tool for biopolymers sequencing [1,2]. The exponential growth of the use of mass spectrometry for proteomics originates back to Klaus Biemann, who showed that the mass spectrum of a peptide could be directly indicative of its amino acids sequence [3].

Two antagonist methods are used in proteomics. In the bottom up approach, parts of the protein sequences are obtained by tandem mass spectrometry from proteolytic peptides [4]. This method provides a highly effective mean of generating partial sequences even from globular proteins, and membrane proteins, provided that the latter posses significant polar segments between the helices and their termini [5]. The other approach, referred to as top-down sequencing, consists in fragmenting intact protein ions in the gas phase and to deduce sequences information from the fragment ion spectra [6–8]. Although early aspects of top-down were reported in the middle of the 1980's [9], proteolysis was routinely employed because of poor efficiency of tandem mass spectrometric methods for high mass ions.

One of the dominant applications of top-down mass spectrometry in proteomics has appeared to be the determination of post-translational modifications (PTM) [8,10,11]. Extensive studies of 8.5–29 kDa protein standards have shown that electron capture dissociation (ECD) has the remarkable ability to induce cleavages at the majority of the peptidic backbones [12]. PTMs that survived the process of ionization may then be localized using ECD or ETD [13]. In addition, it appeared that sequencing the entire protein is simply not possible with other fragmentation methods for precursors larger than 5 kDa [14].

For proteins bearing more than 500 residues, the intractability for top-down MS came out to result from the increasing complexity of the gaseous molecular ion's tertiary conformer structure [15–18]. Using a newly developed method, termed pre-folding dissociation (PFD), Mc Lafferty and co-workers were able to push top-down proteomics to proteins with masses larger than 200 kDa [19], with the drawback that dissociation was initiated in source and was fragmenting all the species present, thus requiring pure samples. Moreover, owing to the collisional nature of PFD, it may be expected that this method possess the disadvantages of the other collisional activations, such as disruption of PTMs during excitation, thereby preventing for their localization. Hence, any other method allowing heavy protein in-source fragmentations with retention of PMT would be highly appreciable.

A hypothetical in-source ECD would in principle posses such a combination of properties. Interestingly, atmospheric pressure photoionization of peptides and small proteins has shown to produce extensive in source fragmentations [20,21] with production of *c*- and *z*- sequence ions. In the present context, deeper investigation of the mechanisms at work under APPI conditions becomes especially important, notably verification whether APPI fragmentation of peptides show the same properties as ECD, such as retention of labile adducts.

In the following we report the study of in-source APPI fragmentations of a 15 residues model peptide in both positive and negative

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doi:10.1016/j.ijms.2010.08.010

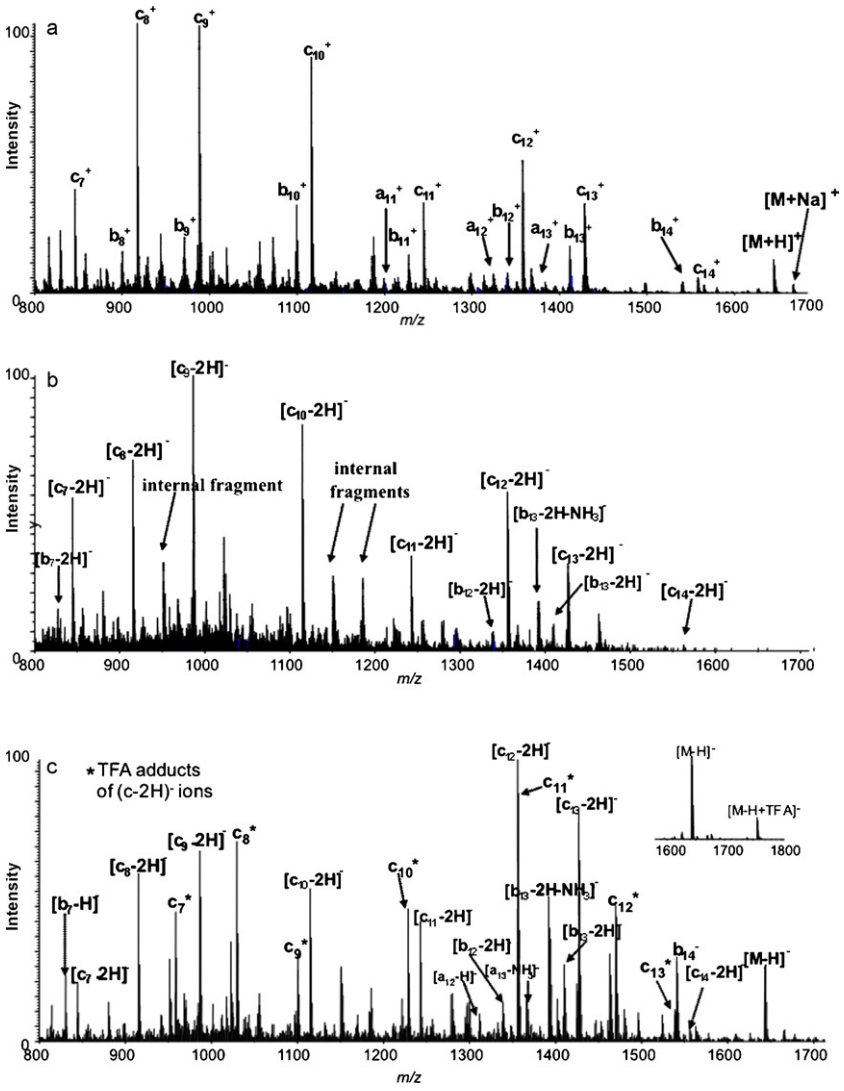

Fig. 1. APPI mass spectra for DF8 in isopropanol under a) positive ion mode, b) negative ion mode and c) negative ion mode for TFA acidified isopropanol solution.

ion modes. Comparison of the fragmentation patterns in both ionization modes and monitoring of the fragmentation with respect to the amount of dopant infused shows that the mechanism beneath in-source APPI dissociation of peptide is closely related to ECD and ETD, sharing with them potential of retaining labile grouping. This work is a first step to evaluate the potentialities of APPI for top down proteomics.

2. Material and Methods

The experimental results were recorded using the Photospray APPI source (Applied Biosystems, Les Ulis, France) fitted with a Cathodeon PKS 106 Krypton lamp. The APPI source is mounted on an hybrid quadrupole time-of-flight Qstar Pulsar i mass spectrometer (ABSciex).

Operating parameters were: Heating temperature = 400 °C and 450 °C, IS = 1600 V (ion spray voltage), DP1 = 20 V (declustering potential), FP = 100 V (focusing potential), DP2 = 15 V (second declustering potential). The TOF calibration was carried out using polypropylene glycol standard solutions. Source gas is pure nitrogen from a generator with less than 5 ppm of oxygen.

The peptide was synthesized by Genepep Prestation (Montpellier, France) with an HPLC purity of 96%. We worked in the flow inject analysis method, in which 5 μl of a 5×10^{-4} M solutions of peptide (denominated DF8 in the following) in isopropanol were injected into a LC flux of isopropanol.

3. Results and Discussion

3.1. Atmospheric pressure photoionization of DF8

The peptide (denominated DF8) is the 56-70 fragment of the Human Platelet Factor (PF4) protein, N-terminal acetylated and C-terminal amidated, namely [22]: Ac-QAPAYKKAAKKLAES-NH_2. Its monoisotopic mass is 1643.85 Da.

Fig. 1a shows the APPI mass spectrum in the 800-1700 *m/z* range recorded in the positive ion mode. Along with *b*- and *a*- sequence ions, abundant *c*- fragment ions are observed. *C* and *z*- ions have already been reported under such conditions [20,21]. Table 1 summarizes the sequence ions observed. Two mechanisms have been proposed to account for the formation of *c*- and *z*- sequence ions under dopant assisted APPI conditions [21]. In such conditions, either the LC solvent, or a post column added organic molecule, is ionized by the VUV photons emitted from the lamp. This ionization phenomenon produces in the medium reactive species able to react by proton transfer with the analyte to produce protonated or deprotonated molecules. Concomitant to the formation of radical cation from ionization of the solvent, photoelectrons are also released in

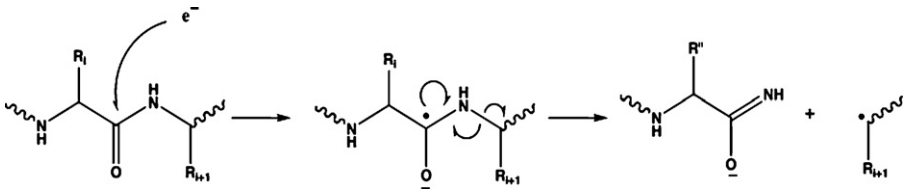

Fig. 2. Suggested mechanism for ion formation for peptide under negative ion APPI conditions.

the medium. These electrons may in turn attach or get transferred to the protonated protein ions if conditions on the electron affinity of the participants are met [23]. Such an atmospheric pressure ECD-like mechanism was suggested to account for the formation of the *c*- and *z*- ions from doubly protonated molecules [21,24]. Surprisingly, it was also reported that singly protonated peptides could also give rise to such fragments ions. Another mechanism involving H atom transfer to singly protonated peptides was then proposed [21,24]. In the present work, isopropanol is ionized by the VUV photons [25] and act as a self-dopant. It is worth noting that photoabsorption by the peptide ion is negligible owing to the strong absorption by the solvent, and its large excess with respect to the analyte. Hence, formation of *c*- and *z*- sequence ions may be entirely ascribed to the APPI processes, that is electron capture (or transfer) or H atom transfer.

Interestingly, analysis of the same sample, under the same experimental conditions, but in the negative ion mode also produces abundant fragments (Fig. 1b). Much less has been done in the study of the dissociation of peptide and proteins in the negative ion mode [26–28]. Fragmentations of deprotonated peptides are complex according to Bowie *et al.* [26], whose group has developed a particular nomenclature. However, other groups [28,29] have adapted the Bieman's nomenclature [3] to the negative ion fragmentations. In the following, we make use of the nomenclature proposed by Ewing and Cassady [27], which is based on the Bieman's nomenclature for the positive, thus allowing a straightforward comparison of both ionization modes. Hence, the negative ion mode mass spectrum at Fig. 1b shows mainly a distribution of *c*-fragment ions ranging from $[c7\text{-}2H]^-$ to $[c14\text{-}2H]^-$, which are identical to that observed in the positive ion mode $c7^+$ to $c14^+$ (Fig. 1a). Fewer *b*- and *a*- ions are observed in the negative mode with respect to the positive ion mode.

Table 1
Fragment ions observed for DF8 in both ion modes.

Fragment ion	m/z_{meas}
Positve ion mode	
$[M+H]^+$	1644.9458
c_{14}	1557.9303
c_{13}	1428.8721
c_{12}	1357.8405
c_{11}	1244.7587
c_{10}	1116.6619
c_9	988.5500
c_8	917.5038
c_7	846.4722
Negative ion mode	
$[M\text{-}H]^-$	1642.9898
$(c_{14}\text{-}2H)^-$	1555.9520
$(c_{13}\text{-}2H)^-$	1426.8880
$(c_{12}\text{-}2H)^-$	1355.8505
$(c_{11}\text{-}2H)^-$	1242.7441
$(c_{10}\text{-}2H)^-$	1114.6266
$(c_9\text{-}2H)^-$	986.5049
$(c_8\text{-}2H)^-$	915.4345
$(c_7\text{-}2H)^-$	844.5013
$(c_7\text{-}2H+TFA)$	958.4155
$(c_8\text{-}2H+TFA)$	1029.4879
$(c_9\text{-}2H+TFA)$	1100.5565
$(c_{10}\text{-}2H+TFA)$	1228.6521
$(c_{11}\text{-}2H+TFA)$	1356.8090
$(c_{12}\text{-}2H+TFA)$	1469.8434
$(c_{13}\text{-}2H+TFA)$	1540.8670

At Fig. 1c, the mass spectrum in the negative ion modes for trifluoroacetic acidified solutions is presented. Although this acidification has no effect in the positive ion APPI mass spectra (data not shown), drastic changes are observed in the negative ion mode (Fig. 1c). The $[M\text{–}H]^-$ ion becomes more abundant. Additional *b*- and *a*- fragments are detected and a new *c*- ion series appears shifted at +114 (see Table 1). This series is assigned to TFA adducts and spans over the same sequence range as the original *c*- series (Fig. 1b). Such adduct is not observed on the *b*- and *a*- series and is preserved only by the *c*- sequence ion formation. *B*- and *a*- sequence ions may then have a thermal or collisional origin, which could disrupt the TFA adduct. In contrast, the mechanism at the origin of the APPI *c*- fragment ions preserves labile adducts. This property is totally parallel to ECD and position APPI as a promising mean for producing in-source ECD-like fragmentations.

4. Conclusion

This work reports the study of a model peptide under APPI in both positive and negative ion modes. Intensive fragmentations are observed and are dominated by the formation of *c*- sequence ions in both ionization modes. Moreover, the negative *c*- ion series retain labile adducts such TFA. A mechanism, involving electron attachment to the carbonyl moiety of the peptide, is proposed to account for those observations. This mechanism is an extension of those classically admitted for ECD. APPI then appear to be a promising mean to produce in-source ECD. This potential could reveal especially useful for top-down proteomic of large proteins.

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