International Journal of Mass Spectrometry 391 (2015) 31–40

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Explorations into isomeric peptides of opposite directionalities by high resolution electrospray collision induced dissociation tandem mass spectrometry

Varatharajan Sabareesh[a,b,*], Dinkar Sahal[c,**]

[a] Proteomics and Structural Biology Unit, Council of Scientific and Industrial Research - Institute of Genomics and Integrative Biology (CSIR - IGIB), Mall Road, New Delhi 110007, India
[b] CSIR - IGIB Extension Centre (Naraina), A93/94, Naraina Industrial Area - Phase I, Naraina, New Delhi 110028, India
[c] International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 8 August 2015
Accepted 25 August 2015
Available online 15 September 2015

Keywords:
Electrospray ionization
Collision induced dissociation
Retro peptides
Degree of fragmentation
Peptide conformation
Helical peptides

ABSTRACT

In this study, we have probed the influence of reversal of peptide bond directionality in S peptide vs Retro S (RS) peptide on the characteristics of collision induced dissociation (CID) tandem mass spectrometry (MS/MS) under electrospray ionization (ESI) conditions. S peptide: KETAAAKFERQHMDSS, which corresponds to residues (1–16) of bovine pancreatic ribonuclease A (RNase A) and RS peptide: SSDMHQREFKAAATEK were taken as models. CID was carried out within a linear trap quadrupole (LTQ) on the doubly protonated ($[M+2H]^{2+}$) precursor ions (m/z 918.44) of the two peptides at different collision energies (CEs) and the product ion analysis was by high resolution mass analyzer, orbitrap. The degree of fragmentation – 'η' of each of the fifteen peptide bonds of the peptide molecular ions from each peptide was determined by estimating the relative abundance of product ions (b- and y-ions) with reference to precursor ions, at every CE. The greater fragility of RS peptide than S peptide was evident from determinations of CE^{50} and CE^{*} (the minimum collision energy, at which, the precursor ion population is 50% and 0% of the initial populations, respectively). The values of CE^{50} were 23.6 and 22.6 and the values of CE^{*} were 30 and 28 for S and RS peptides, respectively. In view of the previously determined conformational propensity of S peptide to be more structured than RS peptide (Pal-Bhowmick et al. [31]), our data suggest that the solution structures of these peptides may be preserved also in the gas phase. This augurs well for the application of high resolution CID MS/MS to probe conformational properties of peptides in gas phase.

1. Introduction

Determination of primary structure or the sequential order in which amino acid residues are linked up in a peptide can be accomplished by tandem mass spectrometry (MS/MS). For more than about two decades, this has been achieved by the use of electrospray ionization (ESI) and/or matrix-assisted laser desorption/ionization (MALDI), which can be coupled to various kinds of mass analyzers that can offer different levels of mass resolution. Peptide sequencing by MS/MS is achieved by different types of ion-fragmentation methods [1–4], of which collision induced dissociation (CID) has been successfully used in many cases, particularly in the area of proteomics.

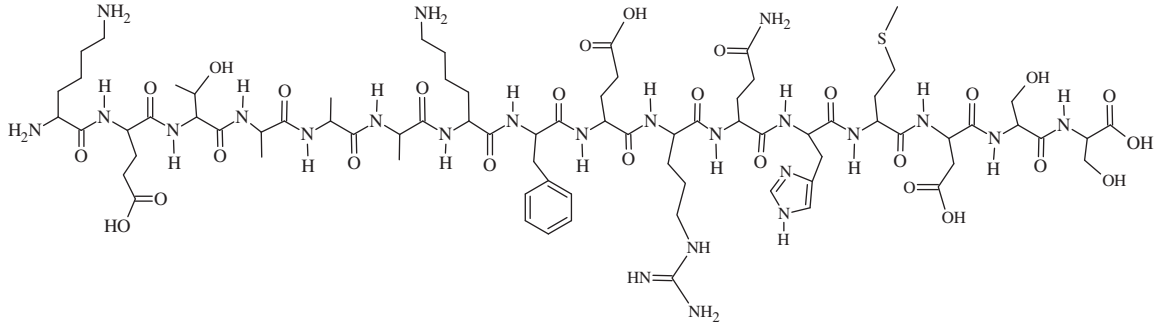
CID of peptides usually results in the fragmentation of peptide units giving rise to b- and y-type product/fragment ions, whose mass-to-charge (m/z) values enable deduction of sequence of the peptide precursor ion. In spite of the great utility of CID in peptide sequencing, the highly disparate yields of the complementary b- and y-series of ions observed in the CID data acquired from instruments of various types of configurations and settings [5–7] continue to be a nagging problem. Furthermore, selective dissociation of certain backbone peptide units of a peptide precursor ion leads to uneven abundance levels of b-, y- and other product ions causing over and under representation of different peptide fragments [6,8,9]. Additionally, charge state of the peptide precursor ions strongly impacts the fragmentation processes and pathways, causing formation of unequal populations of different

* Corresponding author. Current address: Advanced Centre for Bio Separation Technology (CBST), Vellore Institute of Technology (VIT) University, Vellore, Tamil Nadu 632014, India.
** Corresponding author.
E-mail addresses: sabareesh6@gmail.com, v.sabareesh@vit.ac.in (V. Sabareesh), dinkar@icgeb.res.in, dsahal@gmail.com (D. Sahal).

http://dx.doi.org/10.1016/j.ijms.2015.08.024
1387-3806/

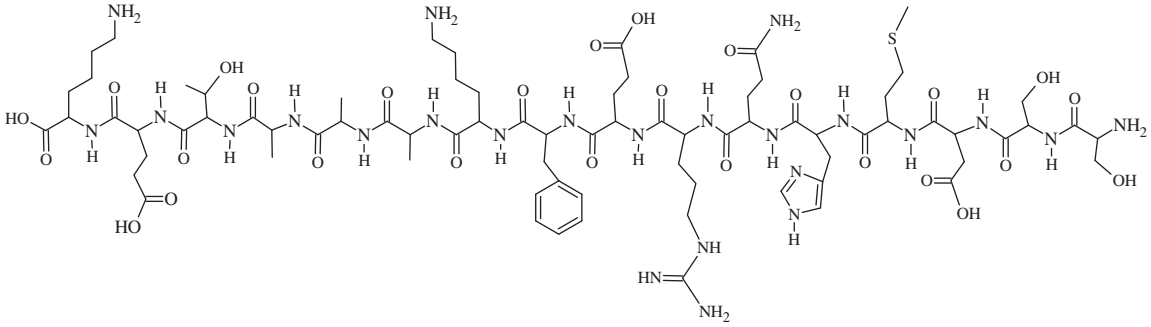
S peptide:

H_2N - KETAAAKFERQHMDSS - COOH



RS peptide:

HOOC - KETAAAKFERQHMDSS - NH_2



Scheme 1. Chemical structures of S and RS peptides.

peptide fragment ions [5,10–12]. Such observations are many a time attributed to the amino acid composition and/or sequence and/or gas-phase conformational state of peptide molecular ions [5,9,12–14]. Thus, majority of the investigations in the past have focused on understanding how specific side chains contribute to the degree of CID induced peptide fragmentation.

Additionally, mass spectrometry has been successfully applied to characterize structural and conformational properties of proteins and their complexes as well [15–18]. The two widely followed MS based methods for probing structure/conformation of polypeptides and proteins are hydrogen/deuterium exchange (HDX) MS and ion mobility (IM)-MS. In the case of HDX, back-exchange is an undesirable process, which can hamper the understanding of protein folding events. To overcome this, a few novel methods have been attempted. Hemling et al. developed two different methods of carrying out HDX in the gas phase, whereby deuterated ammonia (ND_3) was incorporated as nebulizing gas and as curtain gas; accordingly the instrumentation was modified [19]. In another study, D_2O was used as sheath liquid for accomplishing HDX during the course of ESI for LC–MS and LC–MS/MS experiments [20]. Very recently, gas phase HDX-MS has been demonstrated in a Synapt G2-S ESI mass spectrometer, by introducing ND_3 into the transfer ion guide, which is situated between the ion mobility cell and the ToF analyzer [21]. Investigations have also been carried out by combining HDX with CID to determine the sites of deuteration, which has been shown to be successful in certain cases [22–24]. But, hydrogen/deuterium scrambling during the course of CID is a potential problem [25,26]. Alternatively, electron-based and laser-induced fragmentation methods have been tried, which show only miniscule extent of hydrogen/deuterium scrambling [27]. On the other hand, the combination of IM with MS has led to a tremendous growth in applying MS specifically for analyzing structure and conformation of bio-macromolecules and thus MS indeed has currently become a major biophysical technique complementing other established techniques such as circular dichroism spectroscopy, NMR spectroscopy and X-Ray diffraction [28,29]. Furthermore, CID in combination with IM-MS has also been applied to understand the gas-phase structural properties. Recently, Morrison and Wysocki demonstrated that even the b-ions originating from molecular ions of capped polyalanine helical peptides of some definite lengths ($n \geq 10$) retained the helical structure of the parent peptide [30]. Such an observation suggests a clear link between the conformation state of the precursor ions that are subjected to CID and the resulting product ions.

In this study, we have addressed for the first time the question of how reversal of peptide bond directionality may influence the CID pattern. We have chosen to compare the susceptibility towards CID of a peptide and its retro-isomer under ESI conditions. The sequences of the two peptides are, KETAAAKFERQHMDSS {S peptide, residues (1–16) of bovine pancreatic ribonuclease A} and SSDMHQREFKAAATEK (Retro S or RS peptide) (Scheme 1). It is notable that not only these two isomeric peptides have identical amino acid compositions but also the nearest neighbouring residues in each peptide are identical. Thus, the S and the RS peptides differ only in that they have mutually opposing directions of their peptide bonds. Here, we report that these two closely related peptides exhibit CID characteristics unique to each one of them.

2. Materials and methods

S and RS peptides were synthesized as reported earlier [31]. About 2 mg of each peptide was dissolved in 1 ml of methanol:water