International Journal of Mass Spectrometry 391 (2015) 11–16

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Study of the gas-phase fragmentation behaviour of sulfonated peptides

Sanja Škulj, Marko Rožman*

Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička 54, HR-10002 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 6 July 2015
Accepted 24 July 2015
Available online 1 August 2015

Keywords:
Posttranslational modifications
Sulfonation
Collision-induced dissociation
Fragmentation mechanism
Molecular modelling

ABSTRACT

A series of singly and doubly protonated peptides bearing sulfonated residue have been studied, using both experiment and molecular modelling, to elucidate fragmentation chemistry of sulfonated peptides. Collision-induced dissociation mass spectra indicate that the sulfo group loss (neutral loss of 80 Da) is the dominant dissociation channel. Modelling results suggest the proton transfer mechanism, where upon vibrational excitation, the acidic side chain proton is transferred from the sulfo group hydroxyl to the ester oxygen resulting in S—O bond cleavage and formation of the unmodified hydroxyl containing residue and SO_3. Conformations associated with potential energy profile of the reaction imply the charge remote nature of the proposed mechanism. The proposed proton transfer mechanism was compared with the intramolecular nucleophilic substitution (S_N2) mechanism, the main pathway suggested for neutral loss of phosphoric acid from phosphopeptides. Both pathways (proton transfer and S_N2) are available for sulfonated and phosphorylated peptides; however, each posttranslational modification favours different mechanism. The change of the bond dissociation enthalpies and the ability of stabilising the transition state structures are demonstrated as main factors responsible for each posttranslational modification activating a different pathway.

1. Introduction

One of the most common uses of mass spectrometry (MS) is obtaining the structure or sequence of an ion being analysed. The gas-phase approach to generate structure-specific information involves use of tandem MS and subsequent interpretation of fragment ion spectra. In the tandem MS of the protonated peptides, the ion of interest is isolated and then (usually) dissociated via low energy vibration excitation (either collision-induced dissociation (CID) or infrared multi-photon dissociation (IRMPD)). Assignment of molecular structures to the tandem MS spectra greatly relies on the fragmentation models used. The most comprehensive set of rules for understanding of dissociation mechanisms of protonated peptides is known as the mobile proton model [1–5]. The model (introduced by Vicki Wysocki and Simon Gaskell) assumes that in the activated protonated peptide, ionising proton(s) can migrate to various sites, thus triggering charge-directed fragmentation mechanisms [1–5]. The mobile proton concept has been successfully applied in numerous studies of fragmentation mechanisms of various tryptic and non-tryptic peptides, cyclic peptides and peptides bearing some posttranslational modification (PTM) [1–7].

Sulfonation, common PTM in multicellular eukaryotes, represents addition of sulfonic acid group to a protein. Modification is detected on Tyr (mainly), Ser and Thr residues and has the same nominal mass increase as phosphorylation (+80 Da) [8–10]. Thus, it was suggested that rates of protein sulfonation could be underestimated due to a coexistence of both phosphorylated and sulfonated (isobaric) forms of the same peptide [8–10]. Use of ultra-high resolution (accurate mass) measurements, ultraviolet and infrared photodissociation spectroscopy techniques demonstrated that PTM modified isobaric peptides could be distinguished [11–13]. Despite the advantages of high-resolution mass measurements, photodissociation techniques and electron capture/transfer dissociation [14], CID remains the generally used approach for sulfonation assignments [8–10,15–17]. Protonated sulfonated peptides analysed by CID undergo the facile neutral loss of the sulfur trioxide (SO_3, loss of 80 Da) from their precursor ions [8–10,15–17]. The neutral loss precedes any peptide backbone fragmentation, thus limiting precise localization of the sulfonation sites. Understanding of the gas-phase dissociation pathways of sulfonated peptides is very limited. Recently, Patrick et al. preformed structural investigation of protonated sulfoserine dissociation pathways [18]. Low-energy CID of protonated sulfoserine produced two major

* Corresponding author at: Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia. Tel.: +385 1 4680096; fax: +385 1 4680245.
E-mail address: marko@irb.hr (M. Rožman).

http://dx.doi.org/10.1016/j.ijms.2015.07.023
1387-3806/

product ions: 3-member aziridine ring structure attributed to the neutral loss of 98 Da (loss of H_2SO_4) and a structure identical to the protonated serine was related to the loss of 80 Da (loss of SO_3). However, insights at the peptide level are still missing.

Better understanding of the fragmentation chemistry of sulfonated peptides would be of value. Previously, we provided a description of the gas-phase dissociation of phosphorylated peptides [7]. One may consider sulfonation and phosphorylation as similar because both modifications represent highly acidic monoesters of their respective acids and give rise to a nominal mass increase of 80 Da However, neutral loss during vibrational excitation from protonated sulfopeptides is associated to SO_3 [8–10] while that from phosphopeptides (phosphorylated Ser and Thr residues) is associated mainly to elimination of H_3PO_4 [6,7], i.e. suggesting activation of different dissociation pathways.

In this work, through the combination of low-energy CID experiments and molecular modelling, we present the description of the fragmentation mechanisms of sulfonated peptides. Furthermore, we complement the present findings on sulfopeptides with our previous results on phosphopeptides and attempt to understand why a certain fragmentation pathway is related to specific PTM.

2. Materials and methods

2.1. Materials

Analytes and reagents were obtained from Sigma-Aldrich (St Louis, USA) and used without further purification. Peptides TSQLL, SAALSLLR, SAALYLLR and their posttranslational modified variants were obtained from PolyPeptide Laboratories (Strasbourg, France). Peptide sulfonation was achieved using the procedure described in ref. [9]. Briefly, peptides were dissolved in trifluoroacetic acid and reacted with 5% chlorosulfonic acid ($ClSO_3H$) at room temperature for 20 min The reaction was terminated adding H_2O and the solution neutralised with NH_4OH.

2.2. Mass spectrometry

MS and tandem MS experiments were carried out on the amaZon ion trap mass spectrometer (Bruker Daltonik, Bremen, Germany). Peptides were dissolved in 50/50 ethanol/water with 0.1% formic acid to obtain 1 μM concentration. Solution was introduced into the electrospray ionisation source by direct infusion at the flow rate of 75 μL/h. The capillary voltage was set at −4500 V while high voltage end plate offset was −500 V. The temperature and the flow rate of the drying gas were set at 205 °C and 5 L/min, respectively. The electrospray ionisation source parameters were optimised to allow an efficient ionisation and to reduce the in-source fragmentation of precursor ions. The isolation width of the precursor ion was set at 2 Da The CID excitation time was 40 ms and the amplitude was in the 0.4–1 V range, depending on a precursor. All spectra were acquired in the positive ion mode using a scan range from *m/z* 100 to 1100. DataAnalysis 4.0 and BioTools 3.2 (Bruker Daltonik GmbH, Bremen, Germany) were used for spectra analysis and extraction of the MS and tandem MS data.

2.3. Computational methods

In order to gain initial understanding of the potential energy surface (PES) associated with loss of the sulfate modification, the proposed pathways were first established on the small model system, $CH_3COsSerNHCH_3$, and then further evaluated on the test peptides TsSQLL and SAALsSLLR. Both the small model system and the peptides were optimised at the B3LYP/6-31G(d) level of theory. Both the functional and the basis set represent a good compromise for obtaining satisfactory geometries and approximate relative energies, as demonstrated in the theoretical studies of similar systems [7,19,20]. Stationary points (i.e. the minima and transition states on the potential energy surface) were identified by the harmonic frequency analysis. Transition state structures were additionally tested by the Intrinsic Reaction Coordinate (IRC) analysis. In order to get a more accurate description of dissociation energies, calculations using the G3(MP2)//B3LYP protocol [21] were performed on a restricted number of the small model system conformations. The G3(MP2)//B3LYP results were correlated with series of the single point energy calculations in order to pinpoint a model suitable for use on the test peptides. The B2PLYP, B3LYP, M062X and MP2 methods were used in combination with the different basis sets: 6-31G(d), 6-31+G(d), 6-31++G(d,p), 6-311++G(d,p) and TZVP. The smallest mean absolute deviation was found for energies calculated at the B3LYP/TZVP level of theory.

Combination of quenched dynamics and simulated annealing with the AMBER 99 force field was used to sample the potential energy surface of the test peptides (TSQLL and SAALSLLR) by the protocol identical to that previously used [7,22]. Final structures were reoptimised using the B3LYP/6-31G(d) level of theory and the lowest energy structure was considered as the representative structure. From the representative structure, the potential energy profiles of dissociation pathways were constructed at the B3LYP/TZVP//B3LYP/6-31G(d) level. The Rice-Ramsperger-Kassel-Marcus (RRKM) kinetic theory was used to describe the reaction rate as a function of internal energy of peptides.

All quantum mechanic calculations were established using the Gaussian 09 [23], molecular dynamic simulations were carried out using the AMBER 12 [24] and RRKM calculations were obtained using the MassKinetics 1.15 [25].

3. Results and discussion

3.1. CID of sulfonated peptides – mobile proton environment

The low-energy CID product ion spectra were examined in order to set initial understanding of the gas-phase fragmentation behaviour of sulfonated peptides. The spectra obtained by dissociation of the doubly protonated SAALsSLLR and SAALsYLLR exhibit a very intense neutral loss of 80 Da, Fig. 1. The sulfo group loss from the precursor ion as well as from b and y product ions dominates all tandem MS spectra analysed in this work (Fig. 1 and Fig. S1 – supplementary data). However, in some cases, small portion of b and y ions retain the sulfo group and enable characterisation of the peptides (Fig. 1). Those ions are associated with 5.7% of the total ion intensity of all identified ions (in this work) and are not readily observed in tandem MS spectra of sulfonated peptides (e.g. Fig. S1 b and ref. [8–10,16]). Therefore, it is sometimes difficult to obtain site-specific information on the location of the sulfo group.

Although the tandem MS spectra of peptides described here represent only an example, together with previous results [9,16] they indicate that the sulfo group loss is the dominant dissociation channel in the collisionally activated peptides where ionising proton can migrate to various sites (the mobile proton environment). It would be of interest to collect more sulfopeptide tandem MS spectra obtained on different instruments in order to statistically characterise fragmentation behaviour but this is out of the scope of the present paper.

The mechanism, which could be associated with the sulfo group loss, includes the proton transfer from the sulfo group hydroxyl to the ester oxygen and consequent formation of the hydroxyl group and SO_3 (Scheme 1 a). The mechanism is similar to the one associated with the loss of metaphosphoric acid in phosphorylated