International Journal of Mass Spectrometry 379 (2015) 26–32

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Insights into the fragmentation pathways of gas-phase protonated sulfoserine

Amanda L. Patrick[a], Corey N. Stedwell[a], Baptiste Schindler[b,c,d], Isabelle Compagnon[b,c,d,e], Giel Berden[f], Jos Oomens[f,g], Nicolas C. Polfer[a,*]

[a] Department of Chemistry, University of Florida, P.O. Box 117200, Gainesville, FL 32611, USA
[b] Université de Lyon, F-69622 Lyon, France
[c] Université Lyon 1, Villeurbanne, France
[d] Institut Lumière Matière, UMR5306 Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne Cedex, France
[e] Institut Universitaire de France IUF, 103 Blvd St. Michel, 75005 Paris, France
[f] Radboud University Nijmegen, Institute for Molecules and Materials, FELIX Laboratory, Toernooiveld 7, 6525ED Nijmegen, The Netherlands
[g] van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form 4 December 2014
Accepted 5 December 2014
Available online 23 December 2014

Keywords:
Post-translational modifications
Sulfation
IRMPD spectroscopy

ABSTRACT

The fragmentation chemistry of protonated sulfoserine was probed using a combination of collision-induced dissociation (CID) mass spectrometry, infrared multiple photon dissociation (IRMPD) spectroscopy, and density functional theory (DFT) calculations. The IRMPD spectra of the dominant fragment ions at *m*/*z* 106 and 88 (*i.e.*, loss of SO_3 and H_2SO_4) were obtained and used to determine the corresponding structures. By comparison to a synthetic standard and calculations, it was determined that the *m*/*z* 106 ion is structurally identical to protonated serine. The *m*/*z* 88 fragment ion was assigned an aziridine structure based on a comparison to theory, analogous to the structure previously proposed by others for phosphoric acid loss from phosphoserine. This work provides the first spectroscopic insights into the dissociation pathways of a sulfated amino acid, laying the groundwork for future studies on related amino acids and peptides with this important, labile post-translational modification.

1. Introduction

Peptide sulfation at tyrosine is a relatively common post-translational modification (PTM), with known roles in various biological processes, including the binding of blood coagulation proteins [1–3] and hormone recognition by receptors [4]. Sulfation of serine and threonine was only first reported in 2004 [5]. This novel modification was detected in proteins from systems ranging from the unicellular malaria parasite, *Plasmodium falciparum*, to humans, which suggests that this too is a key biological modification warranting further study. Furthermore, whilst tyrosine sulfation is slowly beginning to be recognized as a worthwhile analytical and mass spectrometric challenge, the behavior of sulfothreonine- and sulfoserine-containing peptides under various mass spectrometric conditions has gained significantly less attention. Thus, it is useful to begin forming a foundational understanding of the gas-phase fragmentation chemistry of these species, beginning here with a model system, protonated sulfoserine.

Despite the biological significance of peptide sulfation, sulfoproteomics [6] has thus far been less successful than phosphoproteomics [7–9] at gaining recognition as a routine sub-field of proteomics. Due, for example, to the acid and gas-phase lability of the sulfate group, sulfopeptides present distinctive challenges for the mass spectrometry community. Nonetheless, recently some significant strides have been made toward the analysis of sulfotyrosine-containing peptides by mass spectrometric methods. Differentiating sulfotyrosine-containing peptides from phosphotyrosine-containing peptides has been demonstrated by use of high-resolution mass spectrometry [10] and resonant infrared photodissociation spectroscopy [11]. Furthermore, attempts to sequence sulfopeptides have been pursued, with some success, by employing radical dissociation chemistry, such as negative ion electron capture dissociation (niECD) [12], electron transfer/capture dissociation (ETD/ECD) [13], and ultraviolet photodissociation (UVPD) [14]. Alternative approaches have included chemical modification of free tyrosine residues, followed by quantitative removal of sulfate groups from sulfated tyrosine residues, to allow for the indirect determination of sulfation

* Corresponding author. Tel.: +1 352 392 0492; fax: +1 352 392 0872.
E-mail address: polfer@chem.ufl.edu (N.C. Polfer).

http://dx.doi.org/10.1016/j.ijms.2014.12.001
1387-3806/

site(s) through collision-induced dissociation (CID) sequencing [15].

A key aspect of understanding gas-phase fragmentation pathways involves elucidating the structure of the resulting product ions. One of the most powerful techniques for this purpose is infrared multiple photon dissociation (IRMPD) spectroscopy [16,17]. Since infrared photodissociation is only induced when the impinging photon is of a resonant energy with a vibrational mode of the analyte, by recording photofragmentation as a function of photon energy, an IRMPD spectrum, analogous to a more traditional absorption IR spectrum, can be constructed. IRMPD spectroscopy thus serves as a direct structural probe of gas-phase ions and can be used for structural elucidation of said ions, especially when combined with theoretical infrared (IR) frequency calculations and/or comparison to experimental spectra of synthetic standards. This technique has proven successful in many areas, including the study of PTMs [18–21] and of the fragmentation chemistry of peptides [22–25] and remains promising for many additional future applications.

As phosphoproteomics has outpaced sulfoproteomics, studies on the fragmentation pathways of phosphopeptides upon collisional activation [20,26–28] have likewise been more prevalent than comparable studies for sulfopeptides, which are essentially nonexistent thus far. The present study aims to begin closing this gap and shed light on the CID fragmentation patterns of protonated sulfoserine. This is achieved through the combination of various approaches, including IRMPD spectroscopy, CID tandem mass spectrometry, and DFT calculations.

2. Methods

2.1. Experimental methods

Sulfoserine and serine were obtained from Bachem (Bubendorf, Switzerland). Solutions were prepared at concentrations of 100–1000 μM in either 70/30 or 50/50 methanol/water with either 0.1 or 1% acetic or formic acid added to obtain optimal mass spectrometric signal for high quality IRMPD spectra.

To provide an in-depth understanding of the structure and fragmentation pathways of the protonated sulfoserine ion, several complementary mass spectrometry and ion spectroscopy experiments were conducted. Thus, three instrumental setups were used:

(1) IRMPD spectra in the fingerprint region were recorded using a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer coupled to the beamline of the Free Electron Laser for Infrared eXperiments (FELIX) [29,30]. Briefly, precursor ions were generated by electrospray ionization (ESI) using a Micromass Z-Spray source, trapped in the ICR cell, and mass isolated by a stored waveform inverse Fourier transform (SWIFT) excitation pulse [31]. Fragment ions were generated *via* fixed-wavelength irradiation with a CO_2 laser at 10.6 μm, and subsequently mass isolated before analysis. Finally, the ions of interest were irradiated with the tunable output from FELIX. The corresponding (power-corrected) photodissociation yield was calculated by monitoring the abundances of the parent and precursor ions. Irradiation times were chosen to induce sufficient dissociation for each ion (*e.g.*, 2–4 s), with 10 macropulses per second. Typical macropulse energies were ~40 mJ.

(2) IRMPD spectra in the NH/OH stretching region and CID experiments were measured using a Thermo Finnigan LCQ 3D ion trap with an ESI source. This mass spectrometer was modified to allow for the entrance of the laser beam from a YAG-pumped tunable IR optical parametric oscillator/amplifier (OPO/A) (LaserVision), which was focused on the ion cloud. This OPO operates at a repetition rate of 10 Hz with an average pulse energy of approximately 15 mJ (which corresponds to a radiation energy of 0.15 J per second). This set up has been described previously [32]. The mass-isolated protonated sulfoserine precursor was subjected to CID to form the fragment ion of interest, which was then mass isolated before irradiation with the output of the OPO. Typical irradiation times were 800–1900 ms, depending on the analyte. By recording fragmentation as a function of irradiation wavelength, IRMPD spectra were obtained.

(3) To demonstrate inter-setup reproducibility of results, the IRMPD spectrum of the *m*/*z* 88 fragment ion was also recorded using a custom-built hybrid mass spectrometer for IRMPD spectroscopy, described elsewhere [33]. In short, protonated ions were generated by ESI using a custom source, fragmented in-source *via* nozzle-skimmer CID, mass selected by a quadrupole mass filter (QMF), and trapped in a reduced-pressure quadrupole ion trap (QIT). Here, the ions were irradiated (1 s irradiation time, in this experiment) with a focused IR beam from a tunable optical parametric oscillator/amplifier (OPO/A) (LINOS Photonics OS4000) to induce photodissociation. This OPO generates continuous wave (cw) output with powers generally between 20 and 50 mW (which corresponds to a radiation energy of 0.02–0.05 J per second), depending on the wavelength. Due to variable power from one wavelength to the next, each data point was power corrected to account for such fluctuations.

For all IRMPD spectra, the linear IRMPD yield was calculated (see Eq. (1)) and plotted as a function of photon energy, given in wavenumber (cm^{-1}).

$$\text{IRMPD yield} = \frac{\sum(\text{photofragments})}{\sum(\text{photofragments} + \text{precursor})} \quad (1)$$

2.2. Computational methods

Chemical structures and conformations of protonated sulfoserine, protonated serine, and three potential isomers of *m*/*z* 88 were generated using Gabedit [34]. Energy optimization and IR frequencies were then calculated using Gaussian 09 [35] for each unique conformer using density functional theory at the B3LYP level of theory with the aug-cc-pVTZ basis set. This basis set was chosen because it has been previously employed for sulfur-containing systems [36]. Infrared spectra and molecular motions were visualized in Gabedit [34] and/or Avogadro [37]. Scaling factors of 0.973 for the region 550–1900 cm^{-1} and 0.960 for 3200–3700 cm^{-1} were applied, consistent with previous reports [20]. Computed line spectra were convoluted with a 10 cm^{-1} or 30 cm^{-1} (for the NH/OH-stretching region and mid-IR region, respectively) full-width-at-half-maximum Gaussian line shape for ease of comparison with experimental spectra.

3. Results and discussion

3.1. CID of protonated sulfoserine

The mass spectrum of protonated sulfoserine is shown in the upper panel of Fig. 1; the lower panel shows the resulting MS^2 spectrum after CID. Protonated sulfoserine was observed to produce four main fragment ions at *m*/*z* 140, 129, 106, and 88, with the most intense fragments arising from loss of the sulfate modification as either SO_3 or H_2SO_4 (*i.e.*, −80 and −98 amu, respectively). As the corresponding HPO_3 and H_3PO_4 loss ions are of interest to the phosphoproteomics community, these sulfate-loss fragment ions are of potential sulfoproteomic interest and, thus, will each be analyzed