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Electron capture dissociation and collision induced dissociation behavior of peptides containing methionine, selenomethionine and oxidized methionine

Małgorzata A. Kaczorowska *

Faculty of Chemical Technology and Engineering, UTP University of Sciences and Technology, Seminaryjna 3, PL-85326 Bydgoszcz, Poland

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ABSTRACT

The electron capture dissociation and collision induced dissociation of doubly and triply charged ions of peptides RPLGMSPFK, RPLGM(Se)SPFK and RPLGM(O)SPFK were determined by Fourier transform ion cyclotron resonance mass spectrometry. The results suggest that ECD of analyzed species strongly depends on the nature of a single element (i.e., sulfur and selenium atoms, sulfoxide group) and can provide both primary sequence information and structural information regarding the modification. The properties of sulfur and selenium atoms are negligible in the case of CID processes. The CID loss of CH_3SOH, which is unique in peptides to the Met(O) side chain, does not depend on the peptide sequence.

1. Introduction

The combination of high-resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS) methods has an important role in the identification and characterization of peptides, proteins and post-translational modifications (PTMs) of proteins, mainly because of the high accuracy, sensitivity and simplicity of the techniques [1,2]. Between several MS/MS methods developed in recent years, which are useful for structural elucidation, a predominant role in the analysis of modified peptides and proteins is occupied by electron capture dissociation (ECD) [3,4], based on irradiation of multiply charged cations with low-energy (0.2 eV) electrons [5]. In the ECD process, electron capture is followed by a cascade of radically driven, intramolecular reactions and the subsequent cleavage of the peptide backbone. Preferentially, ECD cleaves N—C_α and disulfide bonds and retains post-translational modifications [5,6]. As a result of the electron capture dissociation cleavage of the peptide N—C_α bonds, mainly N-terminus and C-terminus (c' and $z^\bullet$ or $c^\bullet$ and z') fragment ions are formed [5,7,8]. The ECD cleavage of disulfide bonds leads to the formation of $S^\bullet$ and S^- fragments, which are converted to SH by hydrogen abstraction. Although several possible mechanisms have been suggested for these fragmentation processes, their details are not fully understood yet and are under investigation with the use of experimental and theoretical chemistry methods [9,10].

Recently, it has been reported that electron capture dissociation behavior of disulfide bonds is different, in comparison with diselenide bonds, although sulfur and selenium are in the same column of the periodic table and have many similar physicochemical properties (i.e., electron affinities). Li and O'Connor [11] applied the ECD method in the investigation of dissociation mechanisms for a series of disulfide, sulfur-selenium and diselenide bonds containing peptide ions. Their results indicate that selenium and sulfur have very different reactivities towards electrons which, consequently lead to different ECD behaviors and mechanisms. However, a consistent interpretation of the results for different peptide chains was not possible on the basis of the data obtained by the said authors.

Differences in the properties of a disulfide bond with an analogous diselenide bond have been also noted by Beld et al. [12], who performed thermodynamic studies for glutathione (GSSG) and its derivative in which cysteine has been replaced by selenocysteine (GSeSeG). They found that the greater thermodynamic stability of diselenide bonds relative to disulfide bonds is not matched by a corresponding decrease in reactivity. Their results have shown that diselenides can oxidize the cofactor NADPH and efficiently interact with the cellular redox mechanism. Dumont et al. [13] used ab initio methods to investigate the gas-phase electron addition on sulfur and selenium-containing organic compounds, such as

* Tel.: +48 500360666.
E-mail address: Malgorzata.Kaczorowska@utp.edu.pl

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dimethyldisulfide, dimethylselenylsulfide and dimethyldiselenide. They reported that selenium strongly enhances the electron affinity, with an increase of adiabatic electron affinity by about 0.20 eV after replacing a sulfur atom with a selenium atom.

Selenium organic species such as peptides and proteins, containing selenocysteine or selenomethionine, have important nutritional and biological functions, and many of them are enzymes or genes [14–16]. As biological and toxicological effects of seleno-containing species are dependent on Se chemical form, the speciation of selenometabolites can provide information regarding the metabolic pathway of this element [17]. As a result of growing interest in selenometabolomics, there is a need for fast and simple techniques for the characterization of seleno-organic compounds. Tandem mass spectrometry provides tools for structural elucidation and identification of such compounds [18,19], however, in the case of more complex species, i.e., peptides containing diselenide bonds or a greater amount of data obtained for peptides which have different sequences, it is difficult to rationalize the results [11].

In this study, it has been investigated whether electron capture dissociation (ECD) and collision induced dissociation (CID) techniques provide enough structural information to distinguish/identify the peptides: RPLGMSPFK, RPLGM(Se)SPFK, RPLGM(O)SPFK, which differ only by a single element: the first one contains methionine (sulfur atom), the second does selenomethionine (a selenium atom instead of a sulfur atom) and the third one – oxidized methionine (a sulfoxide group instead of a sulfur atom), respectively. Since sulfur, selenium and the sulfoxide group have different properties, application of the ECD and CID methods for the characterization of the three simple peptides with an identical sequence will lead to a better understanding of the effect of S, Se and —S(O)— group on the mechanisms of fragmentation reactions, which occur during the electron capture dissociation and collision induced dissociation. Oxidation is an important post-translational modification which has a regulatory role and is associated with oxidative stress [20,21]. The characterization of oxidative modification to the side chain of methionine has been performed before by the application of different tandem mass spectrometry methods (CID, ECD, ETD) [22,23]. Here, the ECD and CID techniques were used for the structural elucidation of doubly and triply charged ions of RPLGM(O)SPFK also to check whether the peptide sequence has an effect on the electron capture dissociation and collision induced dissociation behavior of oxidized methionine-containing species.

The results indicate that combined data, originating from ECD and CID experiments, can be used for the quick detection and localization of methionine, selenomethionine and oxidized methionine residues in the peptide backbone and for identification and distinguishing betwen peptides, which differ only by a single element.

2. Experimental

2.1. Sample preparation

Peptides with the sequences RPLGMSPFK, RPLGM(Se)SPFK and RPLGM(O)SPFK were synthesized by Bachem (Germany) and used without further purification. Doubly and triply charged ions of the analyzed peptides were generated by electrospray of methanol (Fisher Scientific, Loughborough, UK), water and formic acid (Fisher Scientific) solutions (49:49:2, v/v) of RPLGMSPFK, RPLGM(Se)SPFK and RPLGM(O)SPFK, respectively, for a final concentration of 10 pmol/μl.

2.2. Mass spectrometry experiments

All MS and MS/MS experiments were conducted using a Thermo Finnigan LTQ FT mass spectrometer (ThermoFisher Scientific, Bremen, Germany). The samples were introduced into the mass spectrometer using an Advion Biosciences Triversa Nanomate electrospray source (Advion Biosciences, Ithaca, NY, USA). The Xcalibur 2.0 software (Thermo Fisher Scientific) was used for data acquisition and analysis. Mass spectra in all the experiments were acquired with a resolution of 100 000 at m/z 400. All MS and MS/MS spectra were averaged over 30 scans and analyzed manually. Each ECD and CID scan consisted of five co-added microscans.

ECD: For all the electron capture dissociation experiments, the ions of interest were mass selected, isolated in the linear ion trap and next transmitted to the ICR cell for ECD. Automatic gain control (AGC) was used to accumulate sufficient precursor ions (target value 1×10^6). Electrons were generated on the surface of an indirectly heated barium tungsten cylindrical dispenser cathode (Heat Wave Labs, Inc., Watsonville, CA). The current across the electrode was about 1.1 A. Precursor ions were irradiated with electrons for 70 ms.

CID: All the collision induced dissociation experiments were performed in the front-end linear ion trap and the fragments transferred to the ICR cell for detection. The AGC target value was 1×10^6. Helium gas at a normalized collision energy of 35% was used for all CID experiments.

Isolation width for all ions of interest in every tandem mass spectrometry experiment was 10 m/z.

3. Results and discussion

Electrospray ionization of the peptides RPLGMSPFK, RPLGM(Se)SPFK and RPLGM(O)SPFK leads to the formation of doubly and triply charged ions. The results presented in this paper were obtained for $[M+2H]^{2+}$ precursor ions; however, similar fragmentation behaviors (ECD and CID) were observed for both charge states.

3.1. Electron capture dissociation

The ECD MS/MS spectra of doubly charged precursor ions $[M+2H]^{2+}$ of the peptides: RPLGMSPFK, RPLGM(Se)SPFK and RPLGM(O)SPFK are shown in Fig. 1a–c, respectively. All the fragments detected are described in Supplementary Tables 1–3, which are provided in the electronic version of this article. For all the doubly charged precursor ions, the ECD mass spectra are dominated by the peaks which correspond to doubly-charged parent ions and to a series of singly charged c, z and a$^\bullet$ ions typically observed in peptide ECD [3,24]. Additionally, the peaks corresponding to the fragment ions: $[M-NH_3+2H]^{+\bullet}$, $[M-2NH_3+2H]^{+\bullet}$ and $[M-(C_4H_{11}N_3)+2H]^{+\bullet}$, formed as a result of the loss of one or two molecules of NH_3 or abstraction of $C_4H_{11}N_3$ originated from arginine [24,25] were observed following ECD of all the examined species. The abstraction of the fragment CH_5N_3 noted only for $[M+2H]^{2+}$ ions of the peptide RPLGM(Se)SPFK is also related to the arginine side-chain loss from the reduced precursor ions [25]. It has been reported [26] that the side-chain dissociations of arginine and histidine are preferred over other amino acid side chains because the two species are characterized by higher gas-phase basicities and should be good sites of protonation in the formation of $[M+2H]^{2+}$ ions. In a fast, nonergodic process such as ECD, primary fragmentation cleaves linkages in the proximity of the recombination site [5]; for the peptides examined in this paper, arginine side chains are the case. Unlike other ECD mass spectra, the spectrum obtained for doubly-charged ions of RPLGMSPFK shows also some peaks resulting from cleavages within the methionine side chain: $[M-(C_2H_5S^\bullet)+2H]^+$, $[M-(C_2H_5S^\bullet+NH_3)+2H]^+$ and $[M-(C_3H_6S)+2H]^{+\bullet}$. The loss of the neutral even-electron C_3H_6S fragment (74.019 Da), from the Met residue has been observed