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Mass spectrometry signal enhancement by reductive amination

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ABSTRACT

Organosulfur compounds (OSCs) subjected to reductive amination in the presence of formaldehyde exhibited increased mass spectrometry signal intensities. In this study, four OSCs including *S*-allyl cysteine, *S*-allylcysteinine sulfoxide, *S*-methylcysteine and *S*-ethylcysteine were generated using isotopic formaldehyde, and mass spectrometry signal intensities of modified and unmodified OSCs were compared. This comparison involved tandem mass spectrometry infusion and detection techniques, such as selected ion monitoring (SIM) and multiple reaction monitoring (MRM). The signal intensities of modified OSCs increased from 2.6 to 39.2 fold by infusion, from 50.0 to 479.6 fold by SIM, and from 146.4 to 2494.8 fold by MRM. Compounds bearing primary amine groups reacted with formaldehyde in high yield and underwent reductive amination in the presence of sodium cyanoborohydride to form a dimethyl group on these amine groups. The modified OSCs showed enhanced intensities because the electron donating dimethyl groups increase their basicity. This signal enhancement is expected to improve the limit of detection in absolute quantification and structural characterization. Therefore, reductive amination involving primary amine groups may find application in the enhancement of mass spectrometry signal intensities.

1. Introduction

Comparative proteomics typically relies on stable isotope labeling and tandem mass spectrometry (MS) coupled with the shotgun approach or two-dimensional gel electrophoresis to achieve global protein identification and profiling [1–3]. Amine containing metabolite profile including 20 amino acids and 15 amines has been generated by stable isotope labeling through the reductive amination of primary amine groups in the presence of formaldehyde [4]. Glycosylation variants have been relatively quantified using isotopic formaldehyde [5].

Reductive amination, described as stable isotope dimethyl labeling, has also been utilized to enhance the signal intensity of saccharides by matrix-assisted laser desorption ionization MS [6]. Reductively aminated oligosaccharides have been detected by high-performance liquid chromatography (HPLC)/electrospray ionization (ESI) MS [7,8] and capillary electrophoresis (CE) [9], and have shown MS signal enhancement. However, organic synthetic conditions are sometimes unsuitable for protein and peptide analysis.

In the early period, however, mobile phase composition adjustment methods were popular for proton transfer efficiency evaluation. Proton scavengers, such as ammonium, methylamine, trimethylamine, diethylamine and triethylamine [10–12], were compared because proteins exhibiting a high charge state during mass spectrometry fragmentation by collision-induced dissociation were more sensitive than those presenting a lower charge state [13]. However, the detection of post-translational modifications, such as protein phosphorylation and glycosylation, and the identification of signal charge states require protein or peptide modification associated with ion charge enhancement. Such a charge enhancement has been observed by electron transfer dissociation (ETD) tandem MS [14,15]. Furthermore, the orifice diameter of the spray tip was altered

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for high resolution nanospray MS [16]. Due to its high surface tension and low relative volatility, *m*-nitrobenzyl alcohol is an excellent solvent, and is often added to the mobile phase to enhance the ion-charging state [17–19]; however, its mechanism is unclear. In this way, it has proven useful in post-translational modification detection by ETD tandem MS [14,15]. However, higher *m*-nitrobenzyl alcohol concentrations result in shorter retention times. Mobile phase addition has produced a less obvious charge shift at the peptide level than direct protein or peptide modification by covalent bonding. This direct modification can generate trialkylammonium ions or blocked C-terminal position in proteins [20,21], but as it requires organic solvents, this can be difficult to handle [6,20,21]. Other methods have also been used to provide protein ion-charge distribution estimates using protein surface areas [22] and in different buffer solutions, such as triethylammonium acetate and triethylammonium bicarbonate to reduce the charge state [23,24].

In this study, a reductive amination approach was developed to enhance the quantitative signal for four representative organosulfur compounds (OSCs). Reductive amination has previously been utilized in comparative proteomics where experimental and control samples were allowed to react with fromaldehyde-D_2 and H_2 [3]. Here, four organosulfur compounds, including *S*-allyl cysteine (SAC), *S*-allylcysteinine sulfoxide (alliin), *S*-methylcysteine (SMC), and *S*-ethylcysteine (SEC), were subjected to dimethyl labeling to generate four fromaldehyde-D_2 and four formaldehyde-H_2 modified OSCs. Signal intensities of unmodified, D_2-fromaldehyde modified, and H_2-fromaldehyde modified OSCs were determined by infusion, selected ion monitoring (SIM), and multiple reaction monitoring (MRM). Modified OSCs exhibited 146.4- to 2494.8-fold signal intensity enhancement compared with unmodified samples. This new method may be utilized for small biomolecules that contain primary amine groups and may be adapted for absolute quantification.

2. Materials and methods

2.1. Chemicals and materials

SAC was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). SMC, SEC, alliin, formaldehyde-H_2 (36.5%–38% in H_2O), and formic acid (FA, 98%–100%) were purchased by Sigma (St. Louis, MO, USA). Sodium acetate (NaOAc), sodium cyanoborohydride ($NaBH_3CN$), and trifluoroacetic acid (TFA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (MeCN, LC/MS grade) was purchased from Merck (Seelze, Germany). Hydrochloric acid, sodium hydroxide, and ethanol were acquired from J.T. Baker (Phillipsburg, NJ, USA). Formaldehyde-D_2 (20% solution in D_2O) was obtained from Isotec Corp. (Miamisburg, OH, USA). Deionized water was produced using a Millipore system at a resistance of 18.2 MΩ (Bedford, MA, USA).

2.2. Instrumentation

Separation and detection were performed by ultra-high-pressure liquid chromatography coupled with a tandem mass spectrometer (UHPLC–MS/MS) using a Thermo Finnigan Acella 1250 UHPLC system (Thermo Fisher Scientific Inc., Waltham, MA, USA). The MS system comprised a triple quadrupole MS (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an ESI ion source in positive ion mode. MS measurements were conducted at an applied voltage of 3000 V, and vaporization and capillary temperatures of 300 °C and 350 °C, respectively. Sheath gas and auxiliary gas pressure were set at 35 and 10, respectively, while the collision pressure was maintained at 1.5, and the collision energy was adjusted between 18 and 25 V. MRM transitions were set to the *m*/*z* values of precursor and product ions (Table 1). UHPLC system control and MS data acquisition were achieved using the Xcalibur software (version 2.2, Thermo Finnigan Inc., San Jose, CA). Samples were sequentially injected into a 10 μL loop using an Acella 1250 autosampler and separated on a Shiseido CAPCELL PAK C18 MG II column (i.d. 1.5 mm × 150 mm, 3 μm, Tokyo, Japan). Mobile phases comprised 0.1% FA in water (A) and 0.1% FA in 100% MeCN (B), and the UHPLC flow rate was set to 200 μL/min. The UHPLC linear gradient was set as follows: 5% (B) for 2 min from injection, 5%–40% (B) for 7 min, 40%–95% (B) for 5 min, and held at 95% (B) for 2 min.

2.3. MS infusion analysis of organosulfur compounds

Organosulfur compounds were dissolved in ethanol and 500 μg/mL solutions were prepared in de-ionized water. Individual OSC solutions (20 μL) were transferred into three tubes and their pH was adjusted to 5.6 using sodium acetate buffer (180 μL). First and second samples were allowed to react for 5 min with 10 μL 4% formaldehyde-H_2 and 10 μL 4% formaldehyde-D_2 solutions, respectively. Modified samples were reduced for 1 h using 0.6 M $NaBH_3CN$ and their pH was adjusted to 2–3 using 10% TFA before mixing with their unmodified counterparts. Individual OSCs mixtures were infused into the mass spectrometer for *m*/*z* characterization.

2.4. UHPLC–MS/MS analysis

In the triple quadrupole MS instrument, OSC fragmentations (precursor ions) were detected via MRM and SIM scanning modes to establish the MRM transitions. In the SIM mode, *m*/*z* values were set to 136, 164 and 168 for unmodified, formaldehyde-H_2, and formaldehyde-D_2 modified SMC, respectively. Meanwhile, *m*/*z* values for other OSCs, such as SEC, SAC, and alliin, were set according to the precursor values listed in Table 1. Establishment of MRM transitions acquired the method in MS, and this method can refer to the fragmented spectra of SIM. Finally, MRM transitions were set according to MS/MS data as follows: 136 > 73 and 136 > 119 for SMC, 164 > 73 and 164 > 119 for H_2-labeled SMC, 168 > 73 and 168 > 119 for D_2-labeled SMC. Other MRM transitions are shown in Table 1. The statistics analysis was conducted using the Xcalibur Thermo LCquan software (version 2.7, Thermo Finnigan Inc., San Jose, CA).

3. Results and discussion

3.1. Reductive amination of organosulfur compounds

Four OSCs were subjected to reductive amination in the presence of isotopic formaldehyde to evaluate the effectiveness of this labeling approach (Fig. 1). Signal enhancement was achieved by reductive amination of OSCs involving formaldehyde. The formation of two methyl groups exhibiting electron-donating properties increased the amine basicity.

Specifically, individual isometric OSCs were in three Eppendrof tubes for reductive amination. After coupling with isotopic formaldehyde in pH 5.6 sodium acetate and subsequent $NaBH_3CN$ reduction, unmodified, H_2-labeled, and D_2-labeled sample solutions were acidified and diluted using TFA before being combined to determine signal differences. The mixtures were analyzed by UHPLC–MS/MS (Fig. 2) using infusion, SIM, and MRM scan modes.

3.2. Detection of signal enhancement by infusion

Infusion spectra clearly showed signal enhancement in all formaldehyde-H_2 and formaldehyde-D_2 modified OSCs (Fig. 3). *S*-Methyl cysteine (SMC) showed *m*/*z* peaks at 136, 164, and 168, which were attributed to unmodified, H_2-labeled and D_2-labeled