Satomi Niwayama et al., J Chromatogr Sep Tech 2017, 8:6 (Suppl)
DOI: 10.4172/2157-7064-C1-035

Methodology for quantitative analysis of proteins using stable isotope-labeled and unlabeled iodoacetanilides by nano LC-nano-ESI-selected reaction monitoring (SRM)-MS and application to clinical proteome in nipple discharge

Satomi Niwayama[1] and **Sadamu Kurono**[2]
[1]Muroran Institute of Technology, Japan
[2]Wako Pure Chemical Industries Ltd., Japan

We previously reported a methodology for quantification of proteins by modifying cysteine residues with stable isotope (13C)-labeled iodoacetanilide (13C7-IAA) or unlabeled iodoacetanilide (IAA) using nano LC-nano-ESI-SRM-MS, which was useful for absolute quantification of commercial proteins such as bovine serum albumin (BSA). We now present that this methodology is applicable to absolute quantification of candidate proteins for breast cancer biomarkers from nipple discharge (ND) of breast cancer patients. A signature peptide, LCENIAGHLK, in catalase as a breast cancer biomarker was reacted with IAA or 13C7-IAA for modification of cysteine, which showed high intensities on MS for the SRM analysis. Two clusters of singly-charged or doubly-charged ion peaks for pairs of IAA and 13C7-IAA-modified peptides were observed to be 7 Da or 3.5 Da apart respectively due to the presence of seven 13C atoms. First, we obtained a standard curve using a pair of IAA-LCENIAGHLK and 13C7-IAA-LCENIAGHLK, which were prepared from the custom-synthesized above peptide. The SRM conditions were as follows: IAA-modified LCENIAGHLK (m/z 615.8 doubly-charged precursor ion >m/z 525.3 and 881.4 singly-charged product ions not containing cysteine), 13C7-IAA-modified LCENIAGHLK (m/z 619.3 doubly-charged precursor ion >m/z 525.3 and 881.4 singly-charged product ions not containing cysteine) and the average chromatogram area ratios between m/z 525.3/525.3 and m/z 881.4/881.4 were used. The standard curve based on the results of above five mixtures had R2 value that was close to 1 (0.9988). Next, we tried absolute quantification of standard catalase with a known concentration, which was reacted with IAA, digested with trypsin and mixed with 100 fmol/μL of 13C7-IAA-modified LCENIAGHLK. The result was close to the known concentration value. By applying this method to measuring catalase in NDs that were extracted from breast cancer patients, we have found that it is useful for analysis of concentration of catalase as a potential candidate for a breast cancer biomarker.

Biography

Satomi Niwayama has completed her PhD from the University of Tokyo, Japan. After working as an Assistant Professor and Associate Professor in USA, she became a Professor at Muroran Institute of Technology, Japan in 2014. She has expertise primarily in organic chemistry and her research projects include those in bioorganic chemistry, applying small organic molecules to solving biological problems. She has been developing a methodology for quantitative analysis of proteins using a combination of small organic molecules that specifically react with certain amino acid residues and their stable isotope-labeled versions combined with mass spectrometry.

sniwayama@hotmail.com

Notes: