Quantitative Analysis of Proteins Using Stable Isotope-labeled Iodoacetanilide and Mass Spectrometry and the Application to Clinical Samples

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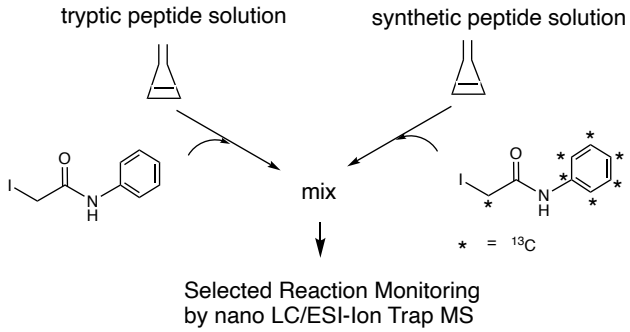
Keywords:; Proteomics; Selected Reaction Monitoring; Mass Spectrometry; Amino Acid Modification; Isotope-labeling

Previously we demonstrated that the Selected Reaction Monitoring (SRM) by modifying cysteine residues with ^{13}C-labeled or -unlabeled iodoacetanilide (IAA)[1] using nano LC-nano-ESI-SRM-MS is effective for absolute quantification of commercial proteins.[2] We also found that this technique is applicable to absolute quantification of candidate proteins for breast cancer biomarkers from nipple discharge (ND) of breast cancer patients.[3]

A custom-synthesized significant peptide, LCENIAGHLK, in catalase was reacted with IAA or $^{13}C_7$-IAA at cysteine, which showed high intensities on MS. The two clusters of peaks representing IAA- and $^{13}C_7$-IAA-modified peptides were observed respectively as singly- and doubly-charged ions. A standard curve was prepared from five IAA-modified peptide solutions with different concentrations and a constant concentration of the $^{13}C_7$-IAA-modified peptide. The standard curve based on the results of above five mixtures showed high correlation. Next, absolute quantification of catalase, a candidate for the breast cancer protein biomarker, of unknown concentration was attempted. Ten catalase solutions obtained from ten breast cancer patients were reacted with IAA and digested with trypsin, followed by being mixed with 100 fmol/μL of $^{13}C_7$-IAA-modified LCENIAGHLK. The result showed a similar trend with the sandwich ELISA results of catalase in ND. Thus this SRM technique using IAA and $^{13}C_7$-IAA was found to be useful for quantitative analysis of proteins without antibodies.



1) Niwayama, S.; Zabet-Moghaddam, M.; Kurono, S.; Cho, H. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 5698.
2) Kurono, S.; Kaneko, Y.; Matsuura, S.; Niwayama, S. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 1110.
3) Kurono, S.; Kaneko, Y.; Matsuura, N.; Oishi, H.; Noguchi, S; .Kim, S. J.; Tamaki, Y.; Aikawa, T.; Kotsuma, Y.; Inaji, H.; Matsuura, S. *Proteomics Clin. Appl.* **2016,** *10,* 605.