Analysis of post translational modifications with AB SCIEX TripleTOF™ 5600 System

Bioinformatics Solutions Inc.

MOUNT SINAI HOSPITAL
Joseph and Wolf Lebovic Health Complex
Samuel Lunenfeld Research Institute

Cunjie Zhang[1], Baozhen Shan[2]

Samuel Lunenfeld Research Institute, Mount Sinai Hospital, ON [1]
Bioinformatics Solutions Inc, Waterloo, ON [2]

Overview

Purpose: To develop a method to analyze PTMs using an AB SCIEX TripleTOF 5600 system with PEAKS PTM software.

Methods: Use high-resolution tandem mass spectrometry to resolve ambiguity of PTM identification and De novo sequencing to localize the modification sites.

Results: Excellence in both sensitivity and accuracy of PTM identification was obtained.

Introduction

Finding post translational modifications (PTMs) is still a challenging task in mass spectrometry-based proteomics. The Association of Biomolecular Resource Facilities (ABRF) sPRG 2010 study evaluated the performance of available PTM analysis methods against a comprehensive PTM standard. The study showed the need for improving the sensitivity and accuracy of PTM analysis, for example, the identification of acetylated Lys or trimethylated Lys without ambiguity and higher detection success rate for sulfated peptides. In this abstract, a successful procedure using an AB SCIEX TripleTOF 5600 system with PEAKS PTM software was demonstrated.

Methods

1. 80 mM EDTA was added in the sample solution to improve the phosphopeptides detection.
2. Perform high resolution (>30K) on MS mode and high sensitivity on MS/MS mode with resolution >15K tandem mass spectrometry on both precursor and fragment steps.
3. A list of highly confident proteins is identified by database search. Each peptide from the highly confident proteins is "modified" in-silico by trying all possible modifications in Unimod database. These theoretically modified peptides are compared with the spectra to identify modified peptides.
4. High mass accuracy of precursor and fragmentation ions was used to resolve the ambiguity of modifications with very close mass differences. De novo sequencing was used to define sequence variations and to localize modifications.

Results

Two samples from ABRF sPRG2011 were used for LC-MS and MS/MS by AB SCIEX TripleTOF 5600 with IDA experiment. and CID fragmentation. One contains only a lyophilized mixture of 70 synthetic modified peptides, and the second contains the same mixture combined with a tryptic digest of the six proteins from which the synthetic peptides were derived. The data was analyzed with PEAKS 6 with 20 ppm of precursor mass error and 0.1 Da fragment mass error. The suspected modifications were static Carbamidomethylation on C and variable Oxidation on M and Deamidation on NQ. The result was reported with 1% of FDR at peptide-spectrum match level.

61 and 63 synthetic peptides were identified respectively in sample one and two. 40 and 42 modification sites were localized respectively in sample one and two. The recovery of spiked modifications was shown in Figure 1. All five sulfated peptides were successfully identified in two samples. 23 of 29 phospho-peptides were reported. The peptides with acetylated Lys or tri-methylated Lys were also identified without ambiguity as shown in Figure 2. 67% of modifications was localized as shown in Figure 3. In a summary, the characterization of amino acids of a protein sequence was shown in Figure 4.

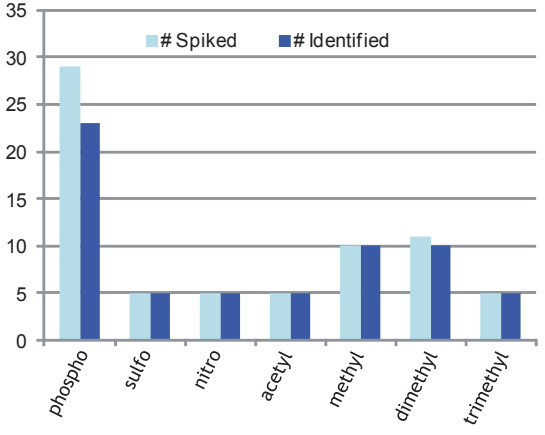

Figure 1. Recovery of the Identificationn for the Spiked Peptides

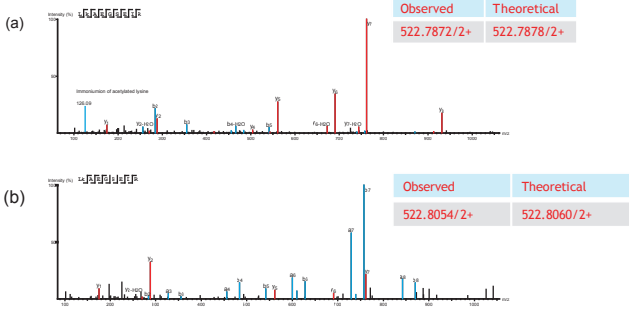

Figure 2. Identification of 2-Acetyl (a) vs 2-Trimethyl (b) Peptides

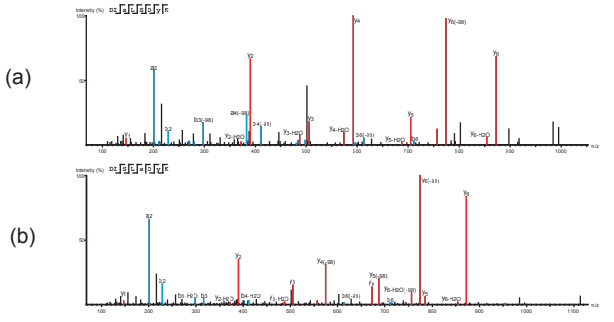

Figure 3. Localization of Modifications for (a) 3, 7-Phospho vs 5, 7-Phospho (b) Peptides

Figure 4. Characterization of amino acids of the sequence of ALBU-BOVIN

Conclusions

Excellent performance of PTM analysis was achieved by using the combination of an AB SCIEX TripleTOF 5600 instrument with PEAKS 6 software.

Reference

X. Han et al. PeaksPTM: Mass Spectrometry Based Identification of Peptides with Unspecified Modifications. Journal of Proteomics Research, 2011, 10(7): 2930-2936.