Post-translational Modification Analysis with PEAKS Studio

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Abstract

The identification and quantification of modified peptides and proteins are essential to understand the biological functions and impacts of post-translational modifications (PTMs). Particularly, detection of low-abundant modified peptides is critical for therapeutic biologics characterization. PEAKS PTM module allows screening more than 313 known PTM types in one run and supports quantitative PTM analysis.

Introduction

PTMs induced by biotransformation on a therapeutic protein can cause significant impact on the activity of clearance property of the biologics. In this study, the global PTM changes introduced by t-butyl hydroperoxide (tBHP) were identified with PEAKS Studio X. More importantly, quantitative analysis highlighted modified peptides that had undergone significant abundance changes and they were found to be related to the oxidation treatment. This method provides a model for characterizing protein PTMs in a high-throughput manner.

Methods

The dataset used in [2] was re-analyzed with PEAKS Studio X. Briefly, a human monoclonal antibody was treated with/without tBHP, spiked into rat serum, immunocaptured, reduced with DTT, alkylated with iodoacetamide, digested with trypsin and analyzed by LC-MS/MS on a TripleTOF 5600 mass spectrometer in a data-dependent manner. Three replicates were performed for each treatment and control group. PEAKS X was used to analyze the dataset and associating feature with chimera scan was enabled. 1% FDR was applied at peptide-spectrum match (PSM) level.

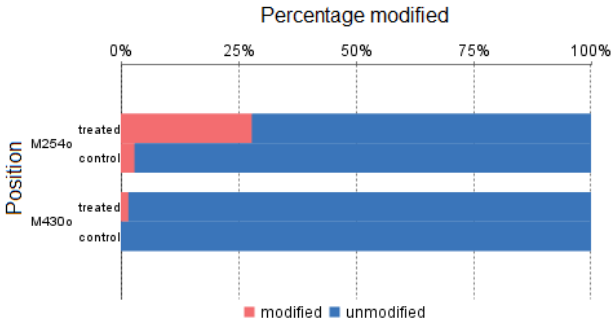
Analysis parameters	Settings
Precursor mass tolerance	50 ppm
Fragment mass tolerance	0.1 Da
Fixed PTM	Carbamidomethylation (C)
Variable PTMs	All 313 built-in PTMs
Database	Uniprot Human Complete Proteome database (v201808)

Results

➢ Quantitative analysis of confident PTMs with PEAKS PTM Profile

Peptides modified via tBHP treatment, which were either absent in the non-treated control sample or had significantly higher expression levels in the treated sample, were determined with PEAKS PTM analysis. As an example, the PTM profile table shown on the next page lists methionine oxidation sites, highest peptide scores, modification site-determining ion intensities and summed abundance of modified and unmodified peptides in each sample.

Protein Position	PTM	-10lgP	Ion Intensity (%)	Treated Modified	Treated Unmodified	Control Modified	Control Unmodified
M254	Oxidation (M)	83.28	59	1.98E4	5.13E4	1.17E3	3.9E4
M430	Oxidation (M)	145.90	6	9.03E2	5.64E4	5.62E0	5.86E4



In the bar chart, the abundance percentage of modified and unmodified peptides was calculated and visualized directly. The result suggests that methionine at position 254 is more sensitive to tBHP-induced oxidation compared to methionine at position 430.

The peptide that covers M254 is DTLMISR in the heavy chain constant region. Figure 1 shows the MS/MS spectra with the best PSM score for DTLMISR peptide in either modified (top) or unmodified (bottom) form. The mass difference between y3 and y4 ions shows an additional 15.99 Da in the bottom spectrum, suggesting an oxidation occurred at this methionine residue.

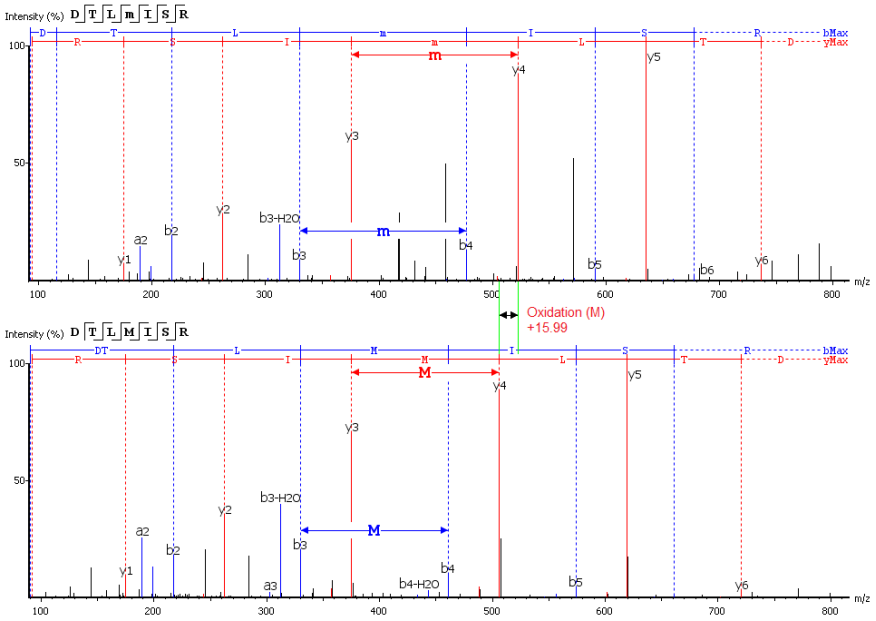


Figure 1. Representative MS/MS spectra of oxidized (upper) and unmodified (bottom) DTLMISR peptide.

Conclusions

PEAKS PTM Profile provides a direct visualization and summary of the quantitative information of modified and unmodified peptides containing the confident PTM sites identified.

References

[1] Han X, He L, et al., PeaksPTM: Mass spectrometry-based identification of peptides with unspecified modifications. J Proteome Res. 2011 Jul 1;10(7):2930-6.

[2] Yao M, et al., LC-MS differential analysis for fast and sensitive determination of biotransformation of therapeutic proteins. Drug Metab Dispos. 2018 Apr;46(4):451-457.