Methionine oxidation analysis in therapeutic protein samples with PEAKS AB® software

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Introduction

One of the major focuses in the biopharmaceutical industry is the ability to quantify modifications within targeted proteins. This is important because modifications strongly influence the protein's biological functions. For example, the oxidation of methionine will alter the physiochemical and functional properties of a mAb. This reaction is commonly induced during storage and sample handling, where any trace of oxidizing agents, will cause methionine to convert to methionine sulphoxide. As a result, problems during subsequent downstream analysis often arise. To overcome this challenge, we demonstrate how PEAKS AB® software can maximize the power and sensitivity of LC-MS technology to quantitatively compare modifications between samples of therapeutic proteins.

Methods

1. Perform forced oxidation of a monoclonal antibody using hydrogen peroxide, followed by LC-MS.
2. Perform peptide mapping and sequence validation to find modifications, and generate an annotated chromatograph.
3. Find hidden and unknown modifications from remaining high quality spectra.
4. Perform quantitative analysis and relative percent oxidation for each Met-containing peptide.

Experimental

Immunoglobulin G (IgG) was obtained from ProMab Biotechnologies, Inc., and equally divided into two groups labelled as 'Control' and 'Treated'. The Control samples were immediately subjected to a trypsin digestion, whereas the Treated samples were first incubated with hydrogen peroxide, dried, and then digested with trypsin. All samples were then analyzed by LC-MS/MS on an AB Sciex 5600 TripleTOF mass spectrometer.

The LC-MS/MS data generated was processed using the PEAKS AB® software, with an error tolerance of 50 ppm for the precursor mass and 0.1 Da for the fragment mass. Peptide mapping was obtained with a 0.1% false discovery rate at the peptide level. The sequence coverage returned was 90% and 93% respectively for the heavy and light chains (see Figure 1).

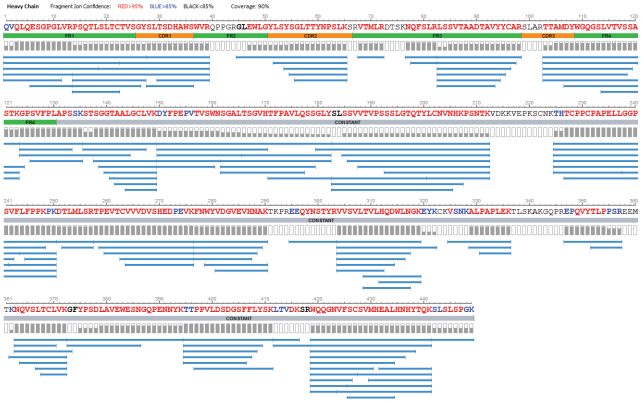

Figure 1. Sequence Coverage of Heavy Chain

Oxidated residues were identified within the peptide map, and further investigated using the PTM profiling tool as shown in Figure 2.

The relative percentage of oxidation for each site was estimated with the formula below:

Relative Percentage Oxidation = Σ Area of Met-oxidized peptide ions / ((Σ Area of Met-oxidized peptide ions)+(ΣArea of Met-(non-oxidized peptide ions))

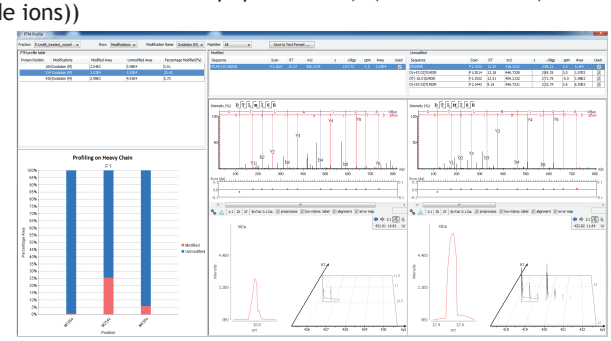

Figure 2. PTM Profiling in PEAKS AB®

Additional modification search capabilities of PEAKS AB® also revealed an unexpected modification - the elimination of CH_3SOH (dethiomethyl) from oxidized methionine residues (Figure 3).

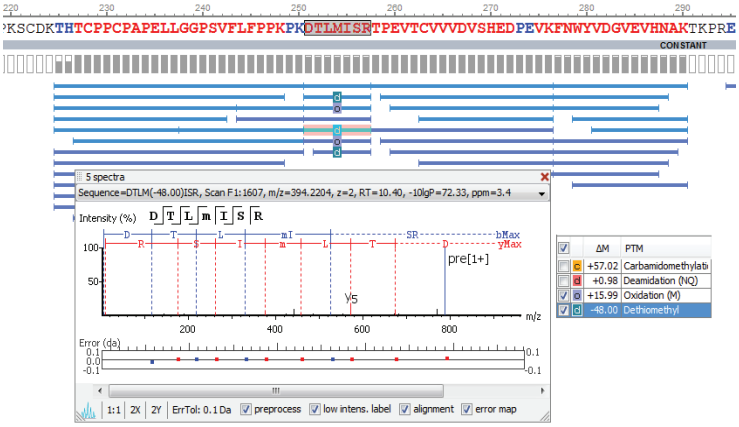

Figure 3. Unexpected Dethiomethyl Modification

Based on the results observed in the PTM Profile, there appears to be differences in susceptibility to oxidation for different peptides containing Met residues. Met 254 was found to be more susceptible to oxidation than Met 106 and Met 430 (shown in Figure 4). Relative percentage of oxidation on Met 254 increased from 2% to 25% after treatment with hydrogen peroxide (listed in Table 1). The difference in resistance to oxidation of the two peptides suggests that the Met 254 is likely to be surface exposed in the antibody.

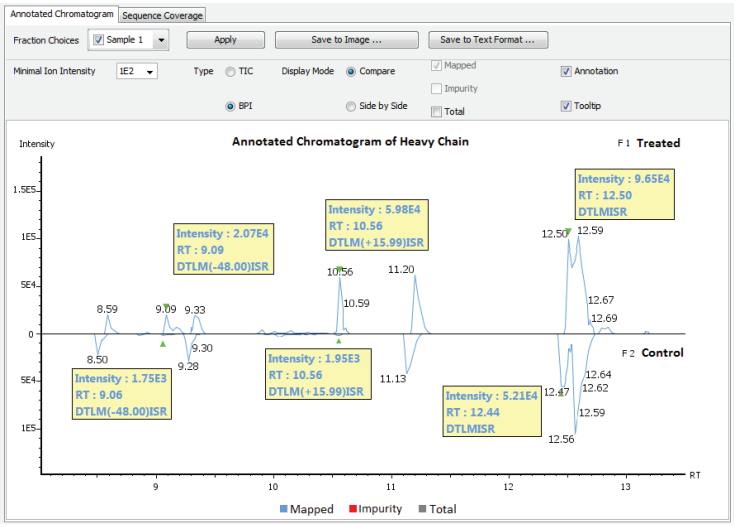

Figure 4. Alignment of Annotated Chromatogram

Table 1. Comparison of Oxidation Susceptibility

Position	Modification	Control	Treated
106	Oxidation	0	0.51
106	Dethiomethyl	0	0.9
254	Oxidation	2.28	25.42
254	Dethiomethyl	2.97	18.51
430	Oxidation	4.4	5.73
430	Dethiomethyl	0	2.12

Conclusion

A software tool for direct quantitative analysis of modifications of therapeutic proteins.