Monitoring Antibody Oxidation at Subunit Level Using BioPharmaView™ Software

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Introduction

Monitoring post-translational modifications like oxidation during the development of monoclonal antibody (mAb) therapeutic is critical for the demonstration of product's efficacy and/or stability. The oxidation of methionine especially in the Fc region is of major concern as it can lead to immunogenicity, reduced binding affinity and short half-life of the biotherapeutic. Here, we assessed the susceptibility of methionines in the Fc region to oxidation through a combination of forced oxidation conditions and enzymatic digestion at the subunit level using a liquid chromatography mass spectrometry (LC-MS) approach. In addition, the fast and simple batch processing using BioPharmaView™ software permits rapid evaluation of domain specific oxidation levels. The subunit level analysis offers advantages by reducing the heterogeneity at the intact level. Moreover, this can be a method of choice over a conventional peptide mapping approach as this requires less sample preparation, reduces the chances of method artefacts and can be used for high throughput screening of mAb products during the development cycle.

Materials and Methods

Sample Preparation:

Humanized IgG monoclonal antibodies were obtained from the National Institute of Standards (#RM8671). Samples were incubated at 37°C for four hours using different concentrations of hydrogen peroxide (H_2O_2).

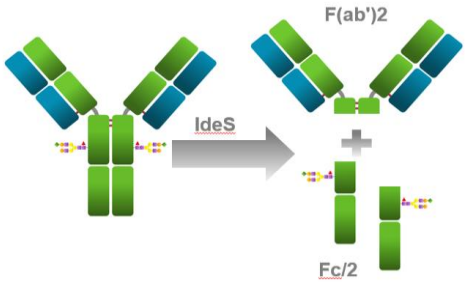

Figure 1: Digestion of mAb with IdeS enzyme reveals two identical Fc/2 and one F(ab')2 subunit).

Methionine oxidation was subsequently quenched by adding L-methionine. Samples were digested into subunit fragments with IdeS enzyme (Genovis AB) producing a F(ab')2 and two Fc fragments per mAb molecule (Fig. 1) prior to LC-MS measurement.

Liquid Chromatography:

System	SCIEX ExionLC™
Column	C4 (50x2.1 mm; 1.7 um; 30 nm)
Column temp.	75 °C
Mobile phase A	0.2 % formic acid in water
Mobile phase B	0.2 % formic acid in acetonitrile
Flow rate	0.3 ml/min
Injection Vol.	2 µl

Mass Spectrometry:

All measurements were carried out in replicates on X500B Q-TOF (Fig. 2) coupled to a Turbo V™ ion source using large protein mode acquisition. Intact and subunit samples were measured on TOF-MS mode over a mass range from 900-4000 with optimized source parameters.

Figure 2: SCIEX X500B Q-TOF instrument.

Data Processing

The complete data processing including spectral deconvolution, mass reconstruction and analysis of

oxidation levels was performed using BioPharmaView™ software.

Results and Discussion

Reducing complexity by analyzing subunits of proteins is a fast way to obtain detailed insight into the domain specific modifications. The chemically oxidized mAb samples after IdeS digestion were analyzed by LC-MS and processed with BioPharmaView™ software. The LC-MS profile showed two peaks corresponding to Fc and F(ab')2 fragment.

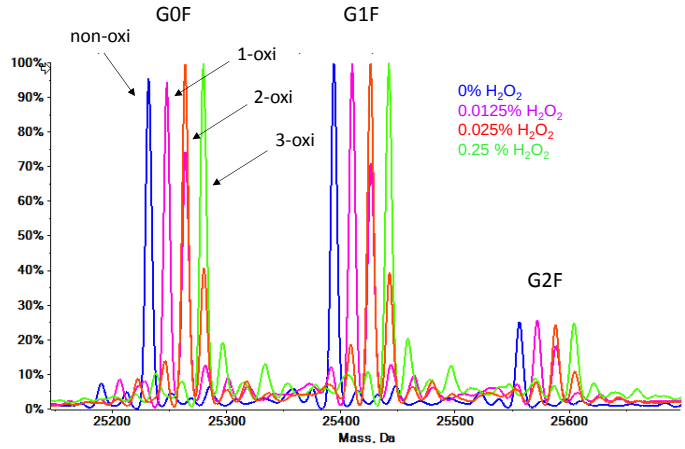

Figure 3. Overlay of reconstructed masses of Fc subunit of NIST mAb standard showing increase in the percentage of oxidized species (1-oxi, 2-oxi and 3-oxi) with increased H_2O_2 concentration.

The Fc subunit contains three methionines residues which can undergo oxidation. The reconstructed spectra of the Fc peak showed a strong correlation between the percent oxidation levels and the concentration of the H_2O_2 (Fig. 3).

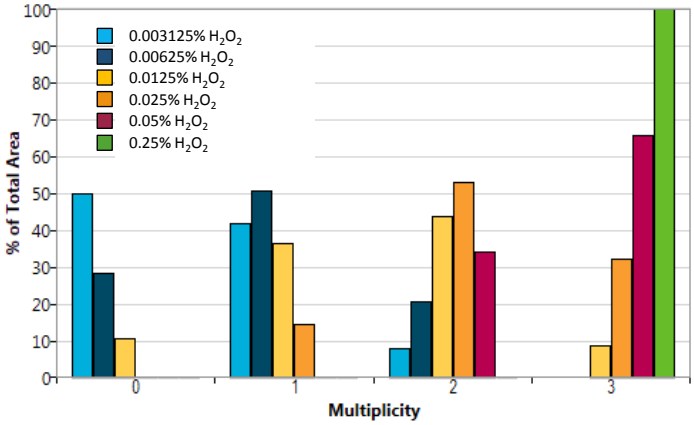

Figure 4. Automated calculation of the percentage of modification of the total reconstructed area for Fc fragment for each sample expressed as multiplicity by BioPharmaView™ software.

The BioPharmaView™ software can automatically calculate and provide the graphical representation of the oxidation levels based on total reconstructed area of Fc fragment for all the treated samples in terms of multiplicity (Fig. 4). The green histogram represents the complete oxidation of Fc subunit i.e. all the three methionines are oxidized with the highest concentration of H_2O_2 used (Fig.4).

One of the advantages of using BioPharmaView™ software is that it can also calculate the mean ratio of modifications automatically by summing the reconstruction areas for each multiplicity and converting to percent contribution for each form. As expected, the oxidation level (red bar in Fig. 5) is decreasing with decreasing concentration of oxidizing

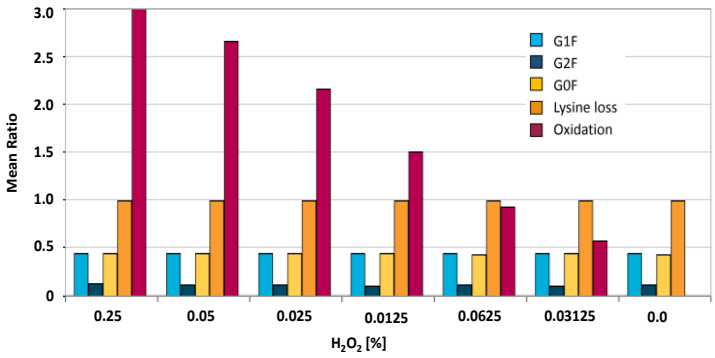

Figure 5: Automated mean ratio calculation of modifications of IdeS digested mAb in BioPharmaView™ software. Ratio calculations are based on reconstructed areas of the Fc fragment.

agent, while other modifications (lysine loss, glycosylations G0F, G1F, G2F) remain constant across all samples. This provides a fast and automated tool which enables the monitoring of batches and quick identification of any differences in a particular lot.

Conclusion

The susceptibility of mAbs to undergo chemical modifications during manufacturing, formulation and storage is one of the biggest challenge faced by the biopharmaceutical industry. This generates the necessity of having robust high-throughput workflows to monitor the stability of these biotherapeutics. The subunit mass analysis workflow demonstrated here provides a fast method of methionine oxidation determination with fast sample preparation as well as a short run time and quick

processing time, especially suitable for the high throughput analysis of a large number of samples.

The high-quality data obtained by the X500B Q-TOF System combined with fast data processing using BioPharmaView™ software were demonstrated to be reliable tools for the automated calculation of percent modifications under stress conditions, thereby facilitating the monitoring of critical quality attributes at the subunit level during the development of biotherapeutics.

References

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