BioPharmaView™ Software as a Robust Tool for Automated Quantitation of Oxidation Sites in Monoclonal Antibody Characterization

Oxidation studies at peptide level using benchtop X500B QTOF and BioPharmaView™ Software

Kerstin Pohl[1]; Amandine Boudreau[2]; Annu Uppal[3]
[1]SCIEX, Darmstadt, Germany; [2]SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada;[3]SCIEX, 121, Udyog Vihar, Phase IV, Gurgaon, Haryana, India

Introduction

Oxidation of methionine is one of the most common post-translational modifications known to occur in monoclonal antibodies during manufacturing, formulation and storage processes. The monitoring of such critical qualities attributes is of major concern as it can lead to a product with altered binding properties. Peptide based mass spectrometry methods are generally preferred for the site specific quantitation of oxidation levels under forced conditions. However, peptide level studies are often restricted by the lack of adequate software tools for the automated and accurate quantitation of sites susceptible to oxidation.

In this study, differential levels of oxidation were induced by incubating a monoclonal antibody standard with increasing concentrations of oxidizing agent (H_2O_2) prior to digestion. The high quality data obtained from the X500B instrument in combination with BioPharmaView™ software allowed accurate and automated quantitation of sites susceptible to oxidation, making it a robust tool for routine sequence coverage analysis as well as quantitation of critical quality attributes.

Materials and Methods

Sample Preparation:

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

Methionine oxidation was subsequently quenched by adding L-methionine.

Fig 1: SCIEX X500B Q-TOF instrument.

The treated samples were denatured, reduced and alkylated using *DL*-dithiothreitol and 2-iodoacetamide (Sigma Aldrich). Trypsin (Promega) was added in a ratio of 1:30 (w:w; Trypsin:mAb) followed by an incubation at 37°C overnight. Digestion was stopped by adding formic acid and supernatant was subsequently measured using LC-MS.

Chromatography:

System	SCIEX ExionLC™
Column	C18 (100x2.1mm; 1.7 um; 13 nm)
Column Temp.	40 °C
Mobile phase A	0.1 % formic acid in water
Mobile phase B	0.1 % formic acid in acetonitrile
Flow rate	0.3 ml/min
Injection Vol.	6 µl (or as indicated)

Mass Spectrometry:

All measurements were carried out in replicates on X500B Q-TOF (Fig. 1) coupled to a Turbo V™ ion source using a data dependent (DDA) acquisition strategy. High resolution MS/MS data of ten candidate ions per cycle with a total cycle time of 1s was acquired.

Data Processing

The data processing was performed with BioPharmaView™ software. The experimental peptide data was matched to the in-silico generated list of peptide masses with oxidation as variable modification. The maximum error tolerance of 5 ppm was used for peptide matching.

Results

In order to map the oxidation sites under forced conditions, the tryptic digests of both control and H_2O_2 treated antibody

standard were analysed on X500B system and processed with BioPharmaView™ software.

Three tryptic peptides (VGYMHWYQQKPGK, DIQMTQSPSTLSASVGDR, DTLMISR) containing methionine susceptible to oxidation were chosen for further analysis based on ionization and chromatographic behaviours. Oxidized peptides were matched and the percentages of oxidation were calculated automatically and reproducibly by BioPharmaView™ software (Fig. 2).

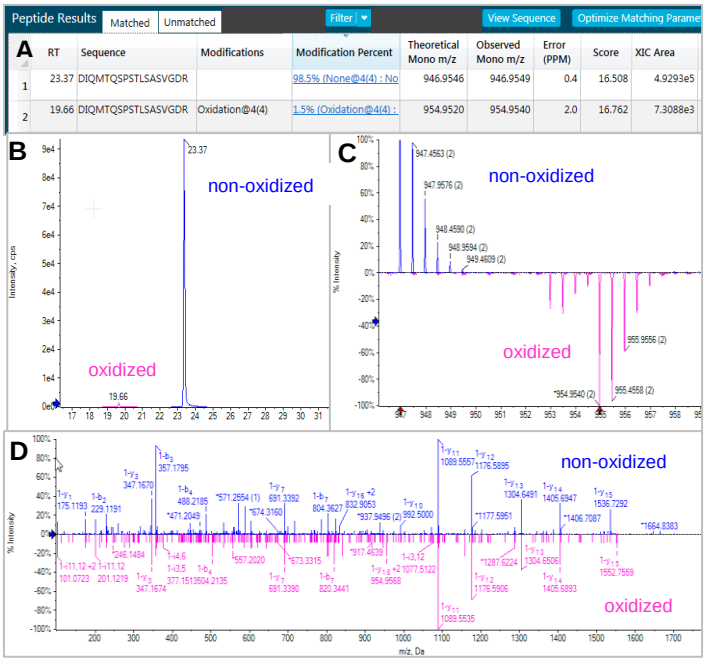

Figure 2. Evaluation of peptide DIQMTQSPSTLSASVGDR. The mAb sample was incubated with 0.003125 % H_2O_2 at 37°C for four hours prior to digestion. A: Table providing results from automated calculation of percentage modification by BioPharmaView™ software. B: Extracted ion chromatograms (XIC). C: Mirror plot of TOF-MS data. D: Mirror plot of MS/MS data

The BioPharmaView™ software calculates the percent modification using the XIC areas of the modified form divided by the sum total of XIC areas of both modified and unmodified forms of the peptide automatically. It is to be noted that the percent modification calculation performed by BioPharmaView™ also considers all the charge states which are present for a particular peptide. The oxidation levels of all the three peptides considered for analysis are plotted as a function of percent H_2O_2 (Fig. 3). As expected higher oxidation levels were observed with increasing concentration of oxidizing agent. However, some degree of oxidation was also observed in the control sample (0% H_2O_2), possibly as a result of sample storage and preparation.

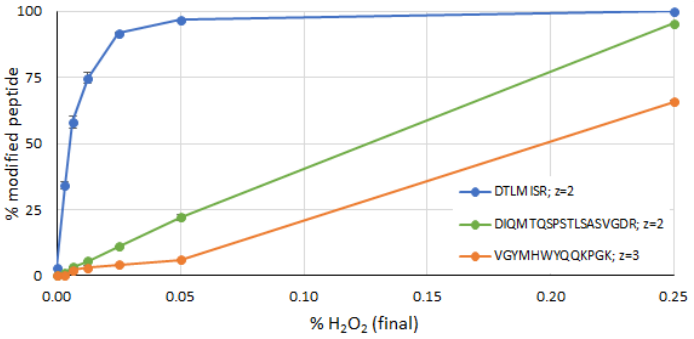

Figure 3. Mean percentages of different modified peptides based on the XIC areas of the MS1 (*n* = 3). mAb samples were incubated with H_2O_2 at 37°C for four hours prior to digestion.

Furthermore, each peptide showed different levels of susceptibility to the forced oxidation. The methionine residue in the DTLMISR peptide was found to be most susceptible to oxidation, which is in accordance with the previous studies as this is the surface exposed residue [1]. The VGYMHWYQQKPGK peptide was found to be most resistant, as 65% oxidation was observed with the highest concentration (0.25% H_2O_2) of oxidizing agent used in this study.

Table 1. Mean percentages and %CV of oxidized peptides at lowest detectable oxidation level (*n* = 3). mAb samples were incubated with H_2O_2 for four hours prior to digestion.

Peptide	% H_2O_2	% modified	% CV
DTLM[Oxi]ISR	0	3.2	3.1
DIQM[Oxi]TQSPSTLSASVGDR	0.003125	1.5	7.1
VGYM[Oxi]HWYQQKPGK	0.00625	2.2	2.8

The automated percentage modification calculation by BioPharmaView™ showed excellent accuracy for triplicate injections, even at the lowest detectable oxidation levels for each peptide, as evident by low percent CV values (Table 1).

The linearity and reproducibility of the method was tested by injecting different volumes of the sample treated with 0.03125% H_2O_2. The peptide DIQMTQSPSTLSASVGDR in both - the modified and unmodified form - showed very good linearity over all injections (Fig. 4).

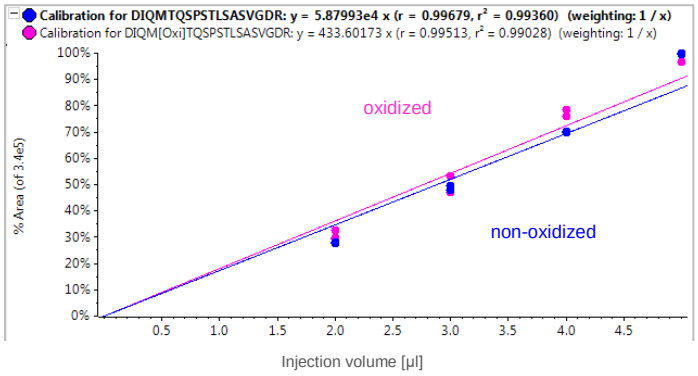

Figure 4. Linearity assessment of peptide DIQMTQSPSTLSASVGDR (*z* = 2). The XIC areas of three injections are plotted with respect to different injection volumes for oxidized (blue) and non-oxidized (pink) peptide. mAb sample was incubated with 0.003125% H_2O_2 for 120 min prior to digestion.

The automatically calculated percentage of modification was observed to be very stable over all injections (Fig. 5).

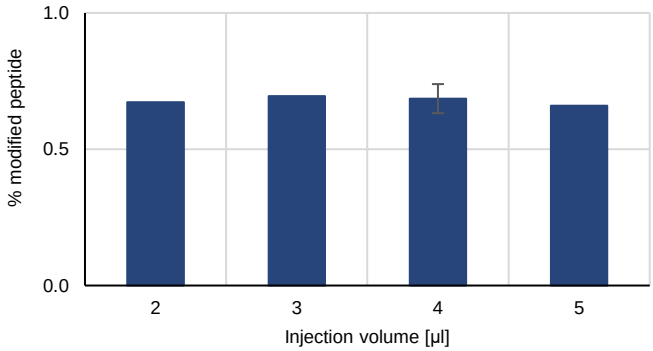

Figure 5. Reproducibility assessment of peptide DIQM[Oxi]TQSPSTLSASVGDR. Mean percentages of oxidized peptide based on XIC areas are shown over different injection volumes (*n* = 3). mAb sample was incubated with 0.003125% H_2O_2 for 15 min.

Conclusions

Peptide based mass spectrometry methods are generally preferred for the site specific quantitation of oxidation levels of biotherapeutics. Unlike subunit level analysis, peptide mapping studies involve complex sample preparation and longer run times, but provide better chromatographic resolution of modified and unmodified features for accurate and reliable quantitation. The high resolution accurate mass data obtained by the X500B Q-TOF system in combination with the fast data processing using BioPharmaView™ software enabled the accurate and reproducible quantitation of sites susceptible to oxidation under forced conditions. Here, we have demonstrated this methodology for the quantitation of oxidation. However, this can easily be extended for the monitoring of multiple attributes such as deamidation, glycosylation, C-terminal lysine clipping and N-terminal pyro-Glu conversion in the analysis of therapeutic antibodies or other protein products.

References

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