INTRODUCTION

Oxidation of methionine is one of the common post-translational modifications known to occur in recombinant monoclonal antibodies during late-stage manufacturing processes. Monitoring cysteines is of major concern because its content in the proteins clinical efficacy and/or stability dramatically impacts (90). A recent BioPharmaView™ software was published for the determination of native cysteine by TOF MS/MS analysis and software tools for the automated quantification of native susceptibility to oxidation in antibody characterization is at a prime importance (10). Here, we demonstrated in this study an automated method for both the determination of oxidation levels and the estimation of area susceptibility to oxidation in monoclonal antibody.

MATERIALS AND METHODS

Humanized IgG1 monoclonal antibodies (mAbs) were obtained from the National Institute of Standards (NIST) (20). Samples were incubated for 25 °C to 4 °C for four hours as indicated using different concentrations of hydrogen peroxide (H2O2). Methionine oxidation was subsequently quantified by adding a methylene blue. Samples were split into three parts. One part was subjected to automated TOF MS/MS digestion, the second part was subjected to LC-MS/MS digestion prior to HPLC measurement. The samples of the remaining part were dehydroxylated and adopted using the BioPharmaView™ software (10). The resulting peptide was subjected to the determination of oxidation levels using the BioPharmaView™ software (10).

HPLC Conditions:

The HPLC conditions for intact and subunit peptide were set up as follows:

MS/MS Conditions:

All measurements were carried out on a QTRAP 5500 (for oxidation and multiplex assays) or a QTRAP 6500 (for whole molecule) TOF/MS/MS system. Detection of peptides was done using an information-dependent acquisition (IDA) method for maximum candidate identification of 10 per cycle with a dwell time of 1 s.

RESULTS

The concentration of H2O2, intact peptide, and modified peptide are shown in Table 1. The determination of various modifications, like oxidation on intact level is challenging. During processing, sample preparation and storage processes, cysteine is not completely removed. It is well known that oxidation of cysteines can be monitored at various stages of mass peaks (Fig. 1). Due to the complexity of large molecules, such as mAbs, the determination of various modifications like oxidation on intact level is challenging. During processing, sample preparation and storage processes, cysteine is not completely removed. It is well known that oxidation of cysteines can be monitored at various stages of mass peaks (Fig. 1). The oxidation of cysteines was determined using automated software tools for the determination of native susceptibility to oxidation.

Subunit Analysis

Reducing complexity by extracting subunits of proteins, it is a fast way to have further insight into modifications. Since the detection of oxidation on subunit level is more complex due to the presence of other oxidation sources which are prone to oxidation. The control samples did not show any modification, whereas at least 3% oxidation was observed in the oxidized samples (Fig. 2a). The oxidation of cysteine was monitored in a series of intact mAbs in the total of 90 isolated events (including oxidized in Fig. 3) per fragment. Similarly, the software can calculate the mean rate of modification by averaging the results of multiplicity (Fig. 3b). It is expected the oxidation of cysteines was observed in the range of 200−700 ppm, with the concentration of oxidized cysteines, while glad.

CONCLUSIONS

The results from intact, subunit and peptide level were considered over all samples. Incubation of intact samples with higher concentrations of H2O2 is forming higher oxidation levels of mAbs. Intact levels can already give an indication of oxidation, desired by some clinicians and users. For further insight into oxidation events, subunits can be used. Subunit analysis is offering a fast way of determination of oxidation in terms of sample preparation as well as the time and processing time. By potently oxidizing by the study of many samples (e.g., during batch processing) is the determination of the exact position of an oxidation event and for quantification of very low level oxidation of monoclonal antibodies, performance is important. We have demonstrated that oxidation on intact level is challenging. During processing, sample preparation and storage processes, cysteine is not completely removed. It is well known that oxidation of cysteines can be monitored at various stages of mass peaks (Fig. 1). The oxidation of cysteines was determined using automated software tools for the determination of native susceptibility to oxidation.

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