Contribution ID: **26** Type: **Poster**

Identification and quantification of methionine oxidation in monoclonal antibody using middle-down proteomics with electron activated dissociation

Methionine oxidation is one of the most frequently observed post-translational modifications of monoclonal antibody (mAb) products during their development and production that can affect the drug's safety and efficacy. Such modifications are typically assessed using bottom-up peptide mapping techniques. However, this methodology is often tedious and prone to the generation of artefacts through the sample preparation process. In this study, we present a middle-down method that is complementary to a bottom-up approach to characterise methionine oxidation in mAbs, utilising electron activated dissociation (EAD) using a ZenoTOF 7600 (Sciex).

Oxidised mAb and non-oxidised mAb samples were mixed to create different oxidation levels. These samples were digested to subunits (Lc, Fd'and Fc/2) with IdeS protease and EndoS. Digested samples were denatured and reduced in guanidine hydrochloride/DTT. The samples were injected on to a Waters Acquity BEH C4 column and separated with 0.1% FA in H2O and 0.1% FA at 7 µL/min. MS analysis was carried out using an optimised MRMHR EAD experiment on ZenoTOF7600. Data obtained were processed for subunit mass analysis, sequencing, oxidation identification and quantification using Sciex OS and Biologics Explorer software packages.

The optimised middle-down EAD method allowed us to measure different oxidised species of the antibody subunits with 15 PPM or better mass accuracy. The MS/SM spectra showed the fragments mass shifts due to various oxidation on the subunits. EAD fragmentation allowed site-specific identification of methionine oxidation sites on Lc at M4; Fd'at M34 and M101; Fc/2 at M16, M122 and M192. Quantification of oxidised mAb, using Fc/2, was achieved down to 1% oxidation level with a strong correlation between expected and measured oxidation percentage at both MS1 and MS2.

A middle-down EAD method has been developed that can be applied for complimentary characterisation of site-specific methionine oxidation of mAb products during formulation process.

User consent

yes

Authors: AKINLOYE, Abdulafeez (University of South Australia, Adelaide, Australia); Dr SHAH, Alok (CSL Ltd, Melbourne, Australia); Dr YOUNG, Clifford (University of South Australia, Adelaide, Australia); Dr DONNELLAN, Leigh (University of South Australia, Adelaide, Australia); Mrs NASSIRI, Mariam (CSL Ltd, Melbourne, Australia); Dr CONDINA, Mark (Mass Dynamics, Melbourne, Australia); Dr EDWARDS, Nathan (CSL Ltd, Melbourne, Australia); Prof. HOFFMANN, Peter (University of South Australia, Adelaide, Australia)

Presenter: AKINLOYE, Abdulafeez (University of South Australia, Adelaide, Australia)

Session Classification: Poster Session 2