Abstract #: 1005

Online Electrochemical Reduction of mAbs for Rapid LC-MS Analysis

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Introduction

In this poster, we will demonstrate the successful reduction of inter- and intrachain disulfide bonds of various monoclonal antibodies (mAbs) with an inline electrochemical flow cell coupled to a liquid chromatography-mass spectrometry (LC-MS) system. The addition of a trap/release column in the chromatographic set-up allowed the analytical separation and mass spectrometry analysis to be unmodified with run times of only 23 min. The study demonstrates the complete reduction of intact mAbs to the corresponding light and heavy chain (Lc, and Hc) subunits. Middle-up subunit analysis by electrochemical reduction coupled to HRAM LC-MS can be carried out from intact antibodies without the need for enzymatic digestion, specific reducing agents, or specific denaturing agents.

Methods

A µ-PrepCell SS cell with a ROXY Exceed Potentiostat (Antec Scientific), was used for the reduction, controlled by Chromeleon software (Thermo Scientific). The temperature was 20 °C for partially and 60 °C for the fully reduced experiments. 1 µL of sample was injected onto the µ-PrepCell with products trapped on a MAbPac column (50 mm length), after column switching samples were washed onto a MAbPac column (100 mm length). Separation occurred with a linear gradient. LC-MS analysis was acquired on a Vanquish Flex Duo UHPLC system coupled to a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific).

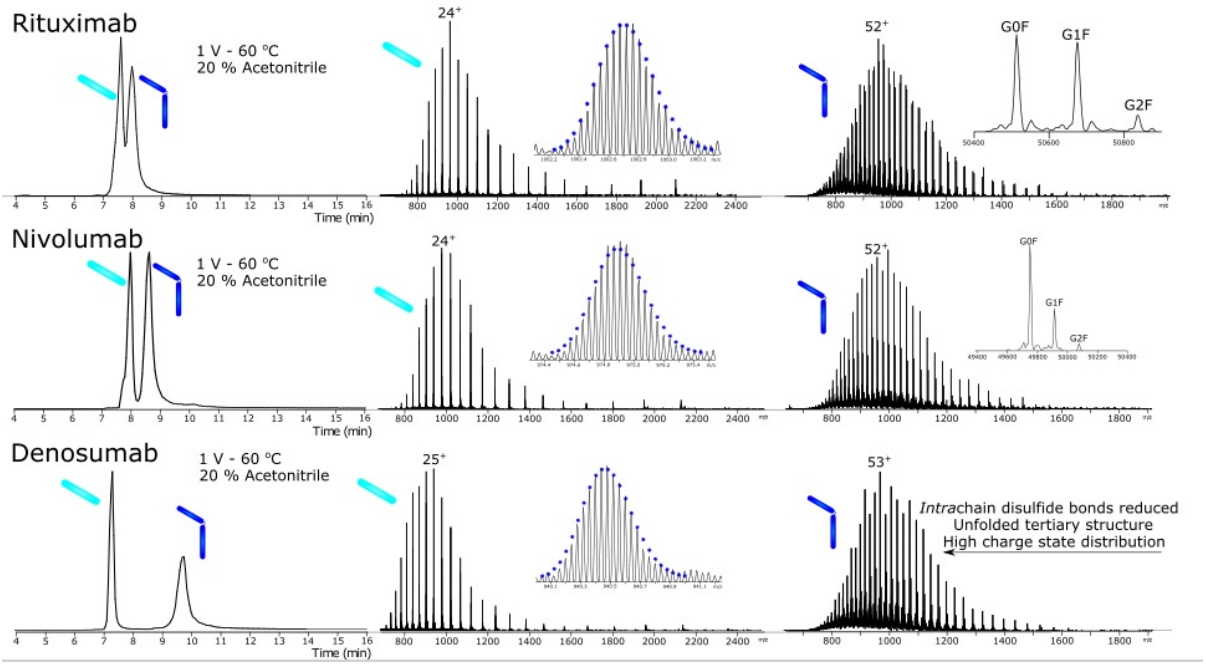
Preliminary data (results)

Electrochemical reduction of both the intra- and interchain disulfide bonds in different mAbs such as Rituximab, Nivolumab, Denosumab, and Cetuximab, could be carried out using electrochemical reduction inline with an LC-MS system. Increasing the electrochemical potential of the electrochemical cell resulted in more complete disulfide bond reduction. Tertiary structure of mAbs was shown to reduce electrochemical efficiency but denaturing the antibodies by termostating the electrochemical flow cell at 60 °C, using the oven of the ROXY Potentiostat, increased the reduction efficiency. The LC-MS system required no modification to the separation and mass spectrometry methods other than the introduction of the electrochemical cell. The reduction of antibodies can be carried out inline with the addition of an electrochemical cell into the chromatographic flow path reducing the intact mAbs. The developed workflow reduces an antibody down to light chain and heavy chain subunits without the need for addition

of enzymes or specific denaturing agents. The electrochemical reduction workflow can be used for the analysis of previously digested fabricator samples as well as intact antibody species. Glycoforms of each antibody were shown to be unaffected, even at the maximum level of reduction. Overall, the data showed completely reduced light and heavy chain formation for the online analysis of intact mAbs, and completely reduced light chain, Fd, and Fc/2 subunits when coupled with offline IdeS digestion. Selectivity of the disulfide bond electrochemical reduction by tandem MS could yield useful information about the formation of non-uniform disulfide bonding structures within antibodies.

Please explain why your abstract is innovative for mass spectrometry?

Instant reduction of S-S bonds in mAbs by online coupling of Electrochemistry with LCMS, without denaturating or reducing agents



Rapid electrochemical reduction of all inter-and intrachain disulfide bonds