Abstract #: 959

Instant Reduction of all Inter- and Intrachain S-S Bonds in mAbs by Electrochemistry-MS

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Introduction

An improved electrochemical method is presented that achieves full reduction of both inter- and intrachain disulfide bonds in a set of monoclonal antibodies based on their intact mass and on MS/MS analysis. The system uses an electrochemical flow cell positioned online between a chromatography system and a mass spectrometer to give direct information on pairs of heavy and light chains in an antibody. The complete reduction of the intrachain disulfide bridges is important as the redox state affects the intact mass of the antibody chain. Disulfide bonds also hamper MS/MS fragmentation of protein chains and thus limit the confirmation of the amino acid sequence of the protein of interest if not fully reduced.

Methods

An electrochemical flow cell (µPrepCell-SS) controlled by a ROXY Exceed (Antec Scientific) was used for reduction. Separations were performed on a 100 mm x 150 µm, C4, 300 Å column using a Ultimate 3000 Nano LC system. The sample was trapped and desalted (Pepmap 0.3mm x 5mm, C4, 300 Å). Gradient was 0.1% FA (A) and 0.08% FA, 80% acetonitrile in water (B), 4% to 90% (B) in 25min at 1 µL/min. Post-column, 19 µL/min 1% FA, 50% acetonitrile was added as makeup flow, to total 20 µL/min. Orbitrap Fusion Lumos was used as MS (Thermo Scientific).

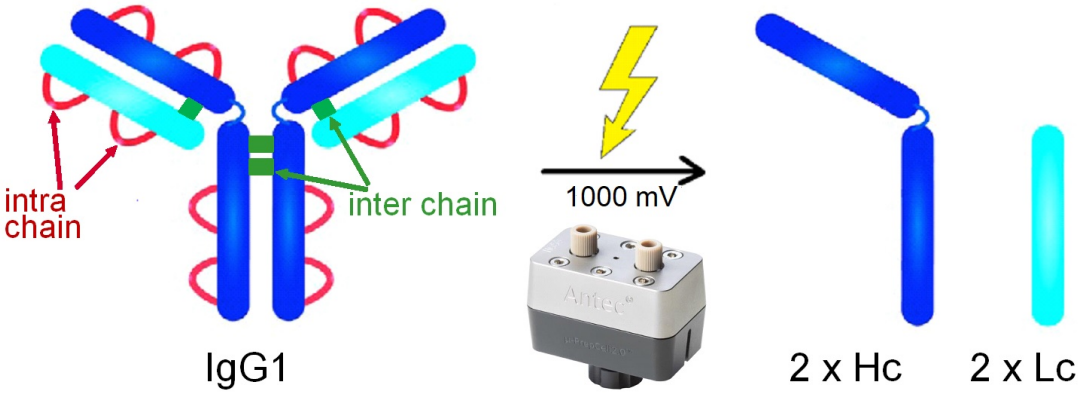
Preliminary data (results)

In this poster, we demonstrate that a selection of monoclonal antibodies (mAbs) such as Bevacizumab (Roche), Panitumumab (Amgen), Pembrolizumab (Merck), Cetuximab (Eli Lily and Co), Adalimumab (Abbott) and Alemtuzumab (Genzyme) could be fully reduced by electrochemical reduction after chromatography of the intact molecule. Both heavy and light chains were released until no signal could be observed from the intact mAb. The intact masses observed in deconvoluted MS spectra were consistent with the reduction of both inter- and intrachain disulfide bridges. Furthermore, the analysis of MS/MS spectra of the light chains confirmed the reduction of the disulfide bonds in the electrochemical cell, and for heavy chains such MS/MS data were obtained from a Fab fragment. The only aspect that was varied was the applied voltage in the electrochemical cell. In general, potentials around 1000 mV were optimal for complete reduction. The post-column reduction setup with makeup flow allows for conventional conditions during separation but full control of the reduction environment. Furthermore,

this post-column configuration result in co-elution of reduced chains that were still connected by a disulfide bridge during chromatography. While this is not beneficial when performing an analysis on a single purified mAb, the design allows for the separation of more complex samples prior to reduction. In this way, the pairing between protein chains in the mixture could be revealed. The current work demonstrates that it is feasible to reduce electrochemically all inter- and intrachain disulfide bridges in various mAbs instantaneously.

Please explain why your abstract is innovative for mass spectrometry?

Instant and complete reduction of mAbs without denaturating or reducing agents using Electrochemistry-MS



Schematics electrochemical reduction of a mAb (IgG1)