Superior Characterization of Protein Therapeutics by Electrochemical Reduction of Disulfide Bonds

On-line electrochemical reduction of S-S bonds in top-down and bottom-up proteomics for enhanced peptide sequencing and S-S bond assignment

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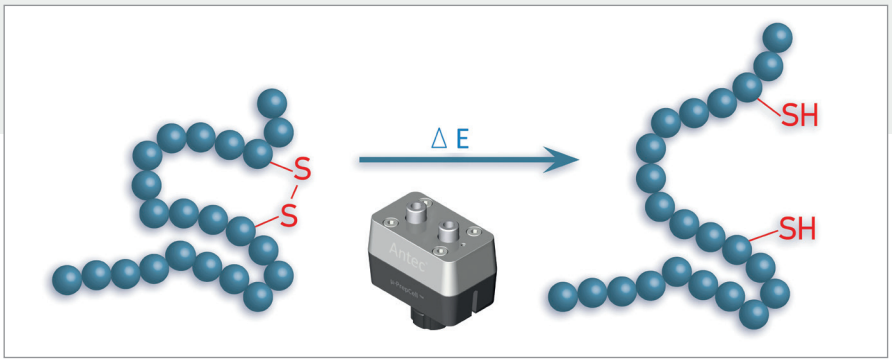

Figure 1: Schematics of a disulfide bond reduction in a peptide chain. During the flow through the electrochemical cell the S-S bond of the intact peptide gets cleaved instantaneously thereby forming the reduced peptide with 2 thiol (S-H) groups. Typical reduction potential applied 1 V (pulse mode).

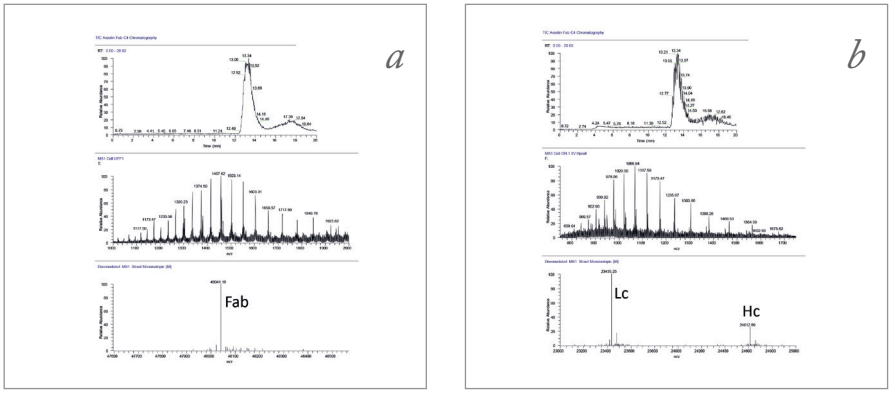

Figure 2: On-line LC-EC-MS of intact Fab fragment (A) and Fab fragment with electrochemically reduced S-S bonds (B). From top to bottom for (A) and (B): TIC, MS spectrum and deconvoluted MS spectrum with monoisotopic mass, measured on Orbitrap Fusion Lumos (Thermo). Courtesy: Dr. Theo M. Luider, Yesim Ikiz and Dr. Martijn van Duijn, Erasmus Medical Centre, Rotterdam, The Netherlands

Disulfide bonds are one of the most important post-translational modifications of proteins. They stabilize the protein's 3D structure and are crucial for their biological function. The reduction of inter- and intramolecular disulfide bonds is necessary for successful characterization and assignment of the bonding sites by MS. Off-line reduction is performed using highly concentrated chemical agents, e.g. dithiothreitol (DTT) that needs to be removed prior LC/MS analysis. Alternatively, thiol - free reducing agents such as TCEP (tris (2-carboxyethyl) phosphine) can be used. However, sample preparation remains laborious and difficult to combine with on-line LC/MS. Moreover, the possibility of on-line electrochemical (EC) disulfide bond reduction can be beneficial for the determination of disulfide bond arrangements in top down proteomics strategy, which relays on fragmentation of intact proteins without enzymatic digestion.

The use of (LC)-EC-MS has shown great potential for the fast assignment of S-S bonds in biopharmaceuticals. With the introduction of a new electrochemical cell, highly efficient and robust online reduction of disulfide bonds in proteins/peptides is now possible. The new µ-PrepCell-SS, see Figure 1, consists of a two-electrode configuration with a new counter electrode design and allows for continuous operation of several days without contamination or loss of reduction efficiency. The flow cell can be used in pre- and post-column HPLC configurations prior to MS detection and is ideally suited for reduction of high disulfide-stabilized proteins. Online reduction occurs within several seconds as compared to conventional offline chemical methods which can take hours or longer to achieve a similar result. A high-pressure version for HDX-MS and a low-dispersion variant to preserve chromatographic separation are also available. Overall much higher sequence coverage has been found in HDX-MS when compared to chemical reduction (TCEP, DTT) and new cysteine peptides could be identified in-post column settings.

In Figure 2 the spectra of the intact and reduced Avastin (bevacizumab, Roche) Fab fragment after HPLC separation on a C4 column are shown. In Figure 2A the intact Fab fragment is shown with the post-column electrochemical cell off and in Figure 2B the reduced Fab fragment is depicted with the cell on (1 V, pulse mode)

From the deconvoluted MS spectrum in Figure 2B and the two fragments with mass 23435.25 Da for the light chain and mass 24612.86 Da for the heavy chain, the reduction of both the inter- and intramolecular disulfide bonds is unambiguously confirmed.

Conclusion: With Antec Scientific's new dual electrode µ-PrepCell-SS efficient and robust reduction of S-S bonds in top-down and bottom-up proteomics becomes possible in routine.