Novel method for efficient reduction of disulfide bonds in peptides and proteins prior MS detection

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Introduction
Disulfide bonds are one of the most important post-translational modifications of proteins. They are stabilizing proteins’ 3-dimensional structure and are crucial for their biological function. Their presence can, however, hamper protein characterization by mass spectrometry. Proteins with disulfide bonds show more resistance to fragmentation and therefore need to be reduced prior MS analysis. Off-line reduction is performed using highly concentrated chemical agents (e.g. dithiothreitol (DTT)) that needs to be removed prior LC/MS analysis. Alternatively, on-line reduction agents as TCEP (tris (2-carboxyethyl) phosphine) can be used. However, the sample preparation remains laborious and difficult to combine with on-line LC/MS. Moreover, the possibility of on the fly disulfide bond reduction can be beneficial for the determination of intact proteins without enzymatic digestion.

Methods/Instrumentation
A preparative electrochemical cell (Antec) equipped with a Magic Diamond (MD) or a proprietary semi-preparative S reducing electrode was used for electrochemical reduction of disulfide bonds. Typically, 1 µM solution of the target compound (peptide, protein, etc.) in 1% formic acid /acetonitrile (90/10, v/v) was used for reduction of disulfide bonds. The cell was operating in a pulse mode. The potential between the auxiliary and the working electrode was set to 0 V. The position of disulfide bonds in peptides and proteins was analyzed using Magic Diamond (MD) working electrode and the newly developed S reducing electrode based on biorecognition elements.

Results
In summary, we demonstrated new, electrochemically opening new opportunities for faster and superior characterization of disulfide bonds in biopharmaceuticals.

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References