Controlled Reduction of Disulfide Bonds in Biopharmaceuticals using an Electrochemical Reactor Cell online with LC/MS

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Introduction

The electrochemically-assisted reduction of disulfide bonds in peptides and proteins followed by on-line mass spectrometric detection is presented. The method is based on square-wave potential pulses applied on a new type of working electrode made from Titanium alloy. The method does not use any chemical agents and is purely instrumental resulting in a fully automated platform for fast assessment and characterization of S-S bonds in biopharmaceuticals.

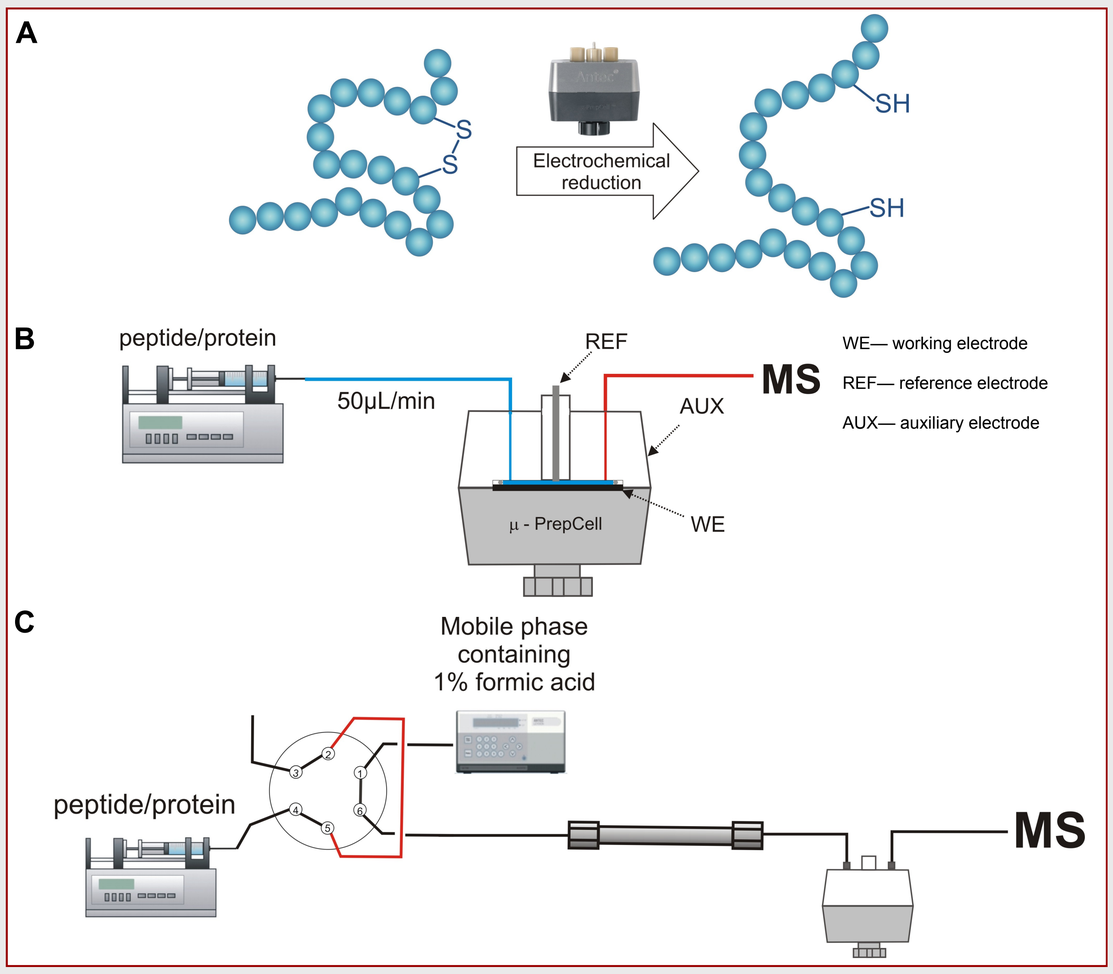

*Figure 1: **A:** Reduction of disulfide bonds using µ-PrepCell™. **B:** Instrumental set-up used in direct infusion. **C:** LC/EC/MS experiment set-up.*

Methods & Results

In infusion mode experiments (Fig 1B), typically 2 - 20 µM solutions of the target protein in 1% formic acid/ACN (90/10, v/v) were pumped into the electrochemical (EC) cell at 50µL/min and the outlet of the cell was directly connected to the ESI-MS. In LC/EC/MS experiments (Fig 1C), the sample was introduced in 0.1% formic acid and 5% ACN using 5µL injection loop. The gradient from 5% to 60% of ACN was used. The mobile phase contained 1% formic acid. The flow rate was 50µL/min. The cell was operating in pulse mode to reduce the compounds of interest. ROXY EC system (Antec, The Netherlands) and Dialogue software were used to control reduction conditions and start MS analysis. Bruker HCT plus (Bruker Daltonics, Germany) or LTQ-FT (Thermo, USA) mass spectrometer equipped with electrospray (ESI) source was used to monitor the reduction products during the optimization steps and to confirm the presence of the reduced proteins/peptides in the control samples.

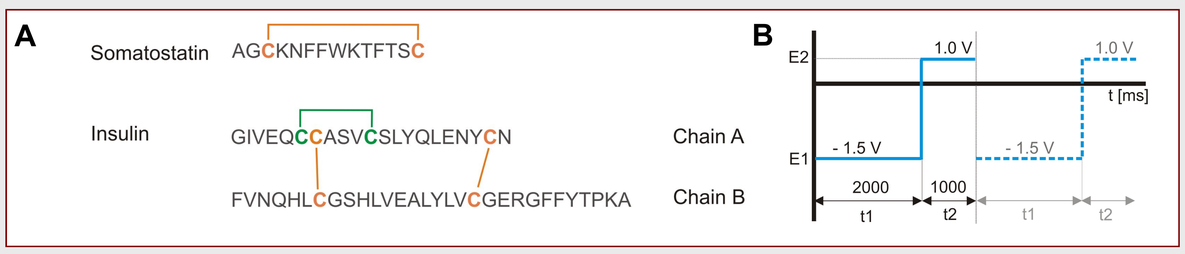

*Figure 2: **A.** Insulin & somatostatin sequence with indicated position of disulfide bonds. **B.** Pulse settings.*

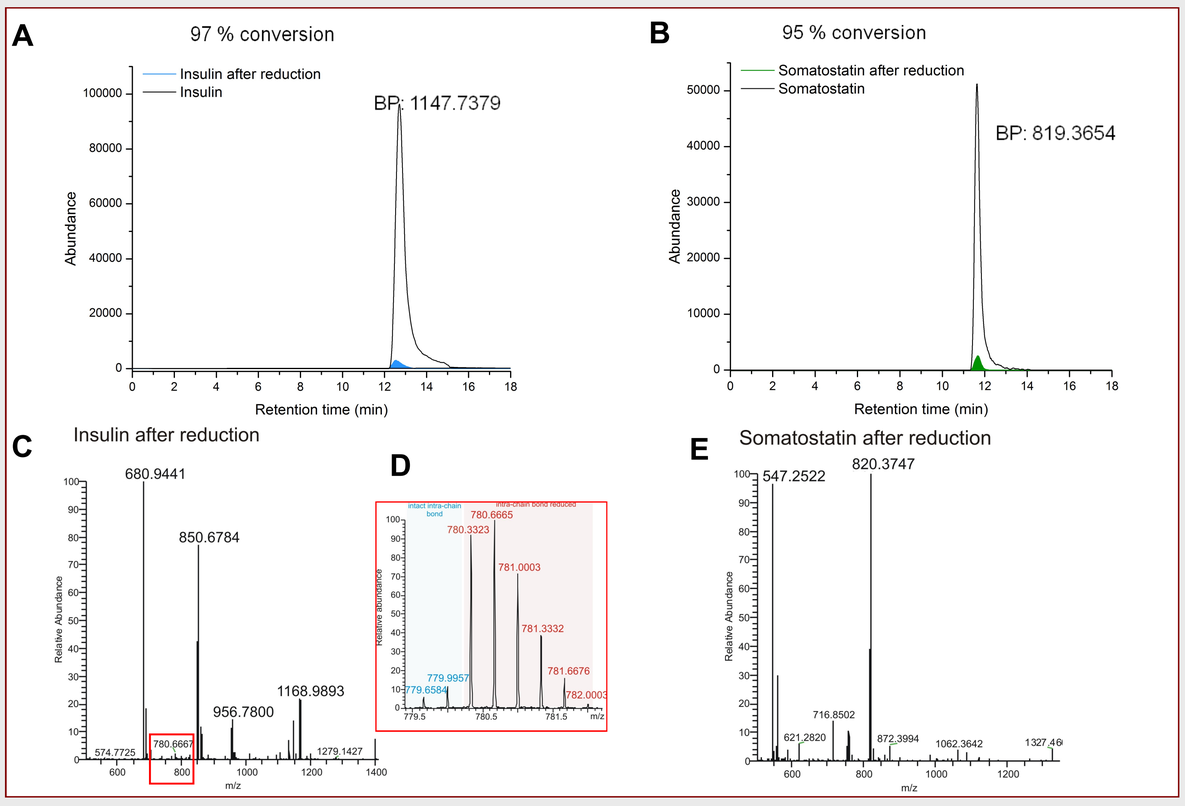

*Figure 3: Somatostatin (m/z 819, **A**) and Insulin (m/z 1147, **B**) before and after electroreduction. Mass spectra of insulin and somatostatin after reduction, respectively **C** & **E**. **D.** Zoom of the [M+3H]3+ ion of chain A of insulin showing its isotopic pattern.*

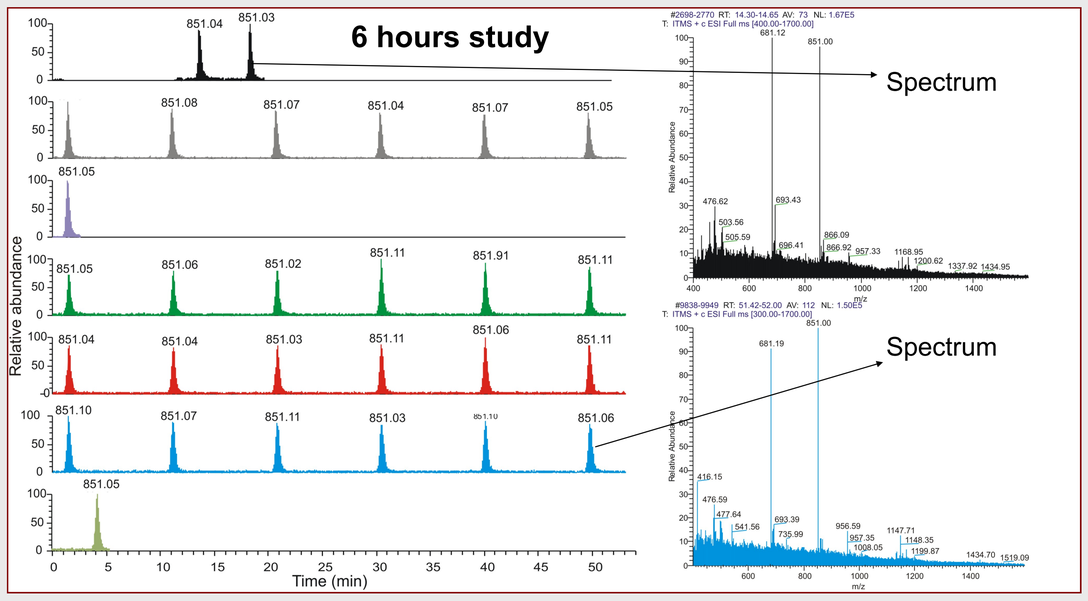

Figure 4: Long term repeatability study using flow injection (without column). 5µL of Insulin was injected via injection loop. µ-PrepCell was continuously operating in optimized square wave pulse (Fig. 2 B).

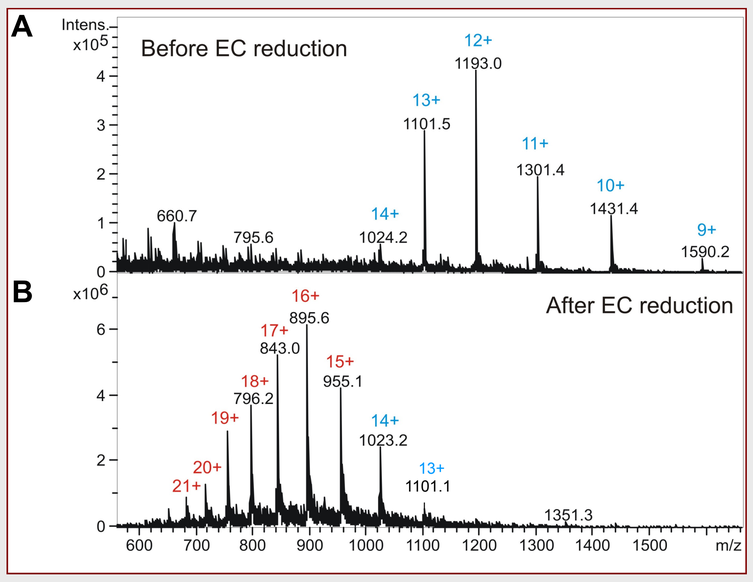

*Figure 5: Electrochemical reduction of S-S bonds in lysozyme. **A:** Before reduction. Mass spectra shows multiple charging up to the $[M+14]^{14+}$ ions. **B:** After reduction (Pulse: E1=-0.9V, t1=2000ms; E2=+1V, t2=1000ms). Shift to higher charges, with the appearance of a new population of the $[M+15]^{15+}$ to $[M+21]^{21+}$ illustrating the reduction of the S-S bonds.*

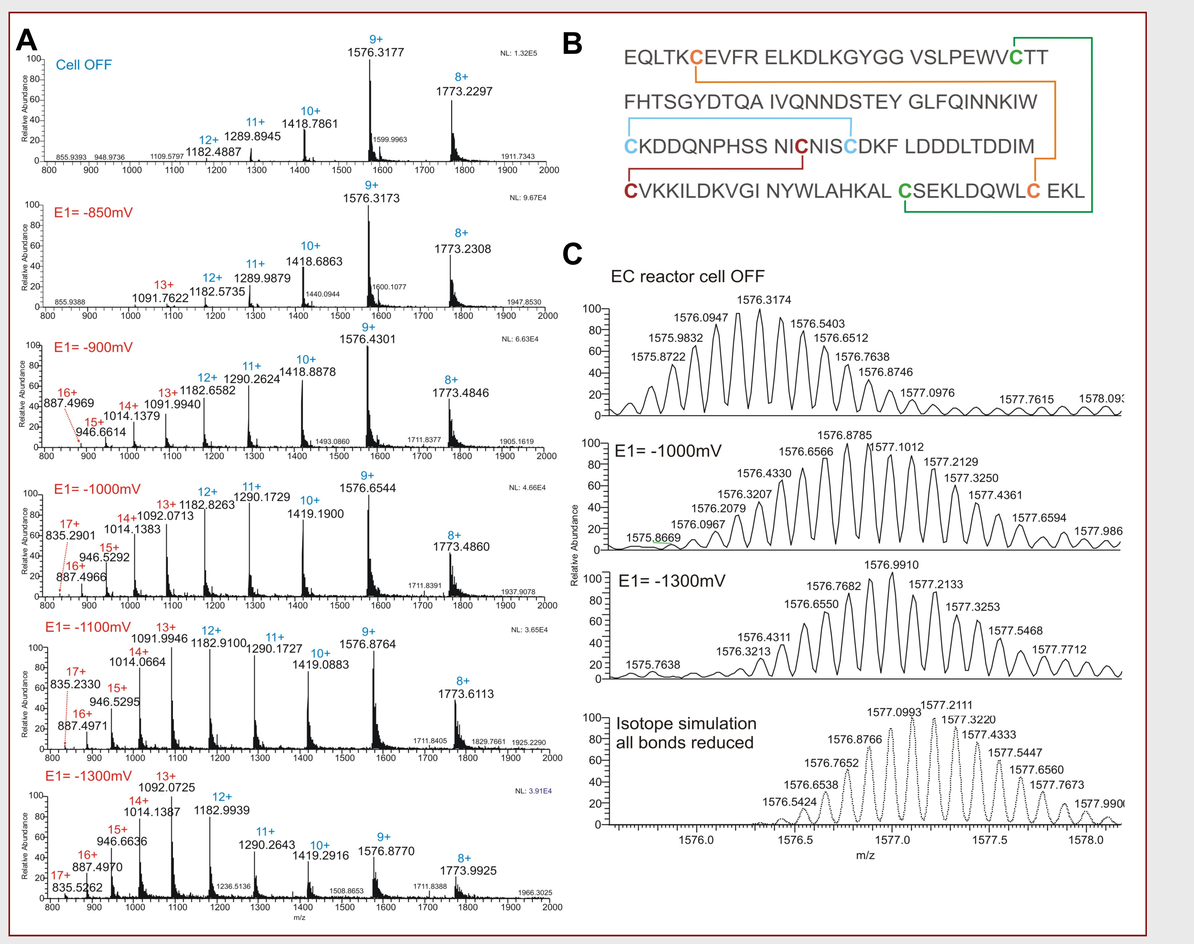

*Figure 6: **A:** Electrochemical reduction of disulfide bonds in α–Lactalbumin. EC reactor cell OFF (top) and ON with different E1 potentials. **B:** α–Lactalbumin sequence with indicated position of disulfide bonds. **C:** Zoom of the overlapping isotopic pattern of the +9 ion of α–Lactalbumin measured with the EC reactor cell turned OFF (top), and ON at E1= -1000mV and E1= -1300mV.*

Conclusions

The new titanium based working electrode provides stable, reproducible and efficient reduction of the S-S bonds in peptides and proteins. The electrochemical reduction was tested in flow injection mode i.e., EC/MS, and in LC/EC/MS settings. In both cases successful reduction was achieved.
The electrochemically-assisted reduction has the major advantage that it works without reducing agents (**chemical free, no DTT or TCEP**) and allows for fast and automated reduction of the S-S bonds in top-down proteomics workflows.
Furthermore, it can be used for the reduction TCEP resistant proteins facilitating HDX MS Proteomics.

References

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