Controlled Reduction of Disulfide Bonds in Proteins and Peptides Using an Electrochemical Reactor Cell in online LC/EC/MS

Jean-Pierre Chevel1, Agnieszka Kraj1, Arleen Kennedy2, Nico Reinhoud3
1Antec, Zoeterwoude, The Netherlands; 2Antec (USA), Boston, USA

Introduction

The electrochemically-assisted reduction of disulfide bonds in peptides and proteins follows 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 assay and characterization of S-S bonds in bio-pharmaceuticals.

Methods/Instrumentation

In infusion mode experiments (Fig 1B), typically 2 - 20 µM solutions of the target compound in 1% formic acid/5% acetonitrile (90/10, v/v) were pumped into the electrochemical (EC) reactor cell at a flow rate of 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 injected in 0.1% formic acid and 5% acetonitrile using 5µL injection using a 5µL injection valve. The gradient from 5% to 50% of acetonitrile 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. PROX EC system (Antec, The Netherlands) and Ditaquadruple software were used to control reduction conditions and start MS analysis. HCT plus ion trap (Bruker Daltonics, Germany) or LTQ FT-ICR (Thermo, USA) mass spectrometer equipped with electrospray (ESI) source was used to monitor the reduction products during the optimization of the method. Figure 1: A. Reduction of disulfide bonds using µ-PepCath®. B. Instrumental set-up used in direct infusion. C. Post-pulse potential reduction experiments.

Results

Insulin and somatostatin was used for optimization of the electrochemical reduction of the disulfide bonds. The electrochemically-assisted reduction of disulfide bonds in peptides and proteins was performed via a titanium auxiliary electrode, a new population of the [M+1+12]c ion of insulin showing its isotopic pattern. Cysteine residues were selected to cover a broad range of molecular weights, 1637.88, 5731.61, 14176.81 Da, respectively.

Post column reduction experiments. The electrochemically-assisted reduction of disulfide bonds in peptides and proteins, Figure 2A. Insulin & somatostatin sequence with indicated position of disulfide bonds. B. Pulse settings. C. Pulse settings optimization. A. Optimization of E1 potential. The application of potential below –1.5V resulted in near 100% conversion of insulin. E2 = 1V. B. Optimization of E2 potential. Only positive values of E2 potential resulted in near 100% reduction of insulin. E1 = 1.5V.

Insulin and somatostatin was used for optimization of the electrochemical reduction of the disulfide bonds. The electrochemically-assisted reduction of disulfide bonds in peptides and proteins, Figure 3: A. Reduction of disulfide bonds using µ-PepCath®. B. Instrumental set-up used in direct infusion. C. Post-pulse potential reduction experiments.

Conclusions

In summary, we demonstrated new, electrochemically-assisted technique for efficient reduction of disulfide bonds in proteins and peptides. Controlled in Magic Magnum (MD) working electrode platform (commercially available) of the electrodes, e.g. 20 to 30% of 75% (Ti/TiO2)-based working electrodes, opening new opportunities for faster and superior characterization of disulfide bonds in bio-pharmaceuticals.

References
[3] Zhang Y et al., J. Proteome Res. 10 (2011) 1293

Lysozyme

Figure 7: Electrochemical reduction of disulfide bonds in lysozyme. A) Before reduction. A mass spectrum shows multiple charge states up to the [M+12]c ion. B) After reduction (Pattern P1+) – the reduction of the compound of interest (pattern P1+) shifted to higher charge states, with the appearance of a new population of the [M+11]c ion. (MD) working electrode platform.

Figure 8: Zoom of the overlapping isotopic pattern of the +9 ion of lysozyme before and after electrochemical reduction. Mass spectra of lysozyme and Insulin shows after reduction, respectively C & D. E. Zoom of the [M+3H]3+ ion of chain A of insulin showing its isotopic pattern.

Figure 9: Long term repeatability study using flow injection set-up. 5µL of insulin was injected via injection loop. µ-PepCat® was continuously operating in optimized square wave pulses.

Figure 10: Electrochemical reduction of disulfide bonds in lysozyme. A. EC reactor cell OFF (top) and ON with different E1 potentials. B. Insulin sequence with indicated position of disulfide bonds. C. Zoom of the overlapping isotopic pattern of the +9 ion of lysozyme before and after electrochemical reduction. Mass spectra of insulin and somatostatin after reduction, respectively B.

Figure 11: A. a-Lactalbumin. B. Insulin & somatostatin sequence with indicated position of disulfide bonds. C. Zoom of the overlapping isotopic pattern of the +9 ion of lysozyme before and after electrochemical reduction. Mass spectra of insulin and somatostatin after reduction, respectively B.