Supporting Information

Electrochemistry-Assisted Top-Down Characterization of Disulfide-Containing Proteins

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EXPERIMENTAL SECTION

Materials. *β*-Lactoglobulin A from bovine milk (MW: 18363 Da), lysozyme from chicken egg white (MW: 14300 Da), and formic acid (~98%) were all purchased from Sigma-Aldrich (St. Louis, MO). HPLC-grade methanol was obtained from GFS Chemicals (Columbus, OH). The de-ionized water used for sample preparation was obtained using a Nanopure Diamond Barnstead purification system (Barnstead International, Dubuque, IA).

APPARATUS

The configuration of the EC/DESI-MS apparatus is shown in Figure S-1. The electrochemical flow cell consists of a magic diamond working electrode (WE, 12×30 mm^2 surface area, Antec BV, Netherlands), a titanium auxiliary electrode (AE) and a HyREF^{TM} reference electrode (RE). The WE and AE were separated by two spacers (50 μm thickness). A ROXY potentiostat (Antec BV, Netherlands) was used to apply potentials to the electrochemical cell for the reduction of proteins that flowed through the cell. The protein samples were prepared in methanol/water (1:1 by volume) containing 0.5% formic acid. The reduced proteins flowed out of the cell *via* a piece of fused silica

connection capillary (i.d. 0.1 mm, 7.5 cm long) and underwent interactions with the charged microdroplets from the DESI spray for ionization. The capillary outlet was placed about 1 mm downstream from the DESI spray probe tip and kept in line with the DESI sprayer tip and the MS inlet orifice. The flow rate for sample solutions passing through the electrochemical cell for electrolysis was 4 μL/min. The spray solvent for DESI was methanol/water (1:1 by volume) containing 1% acetic acid and injected at a flow rate of 5 μL/min. A high voltage of + 5 kV was applied to the spray solvent for generating charged droplets with nebulization gas N_2 pressure being 170 psi .

In order to acquire ECD spectra, the cathode filament of the FT-ICR instrument was conditioned at 1.6 A. To record the spectra the ECD lens was set to 10 V, and a pulse length of 50 ms and ECD bias of 0.7 V was employed. For CID, the collision voltage was set between 11 and 18 V for ions of different *m/z* values. Each acquired spectrum was the average of 100~120 broadband 1 M time-domain transient. The Bruker SNAP 2.0 algorithm was used to pick the peaks and Bruker Data Analysis 4.0 was used to generate fragment mass lists which were searched using Bruker BioTools and ProsightPTM[1], and manually checked, to assign the cleavage sites in the protein sequence.

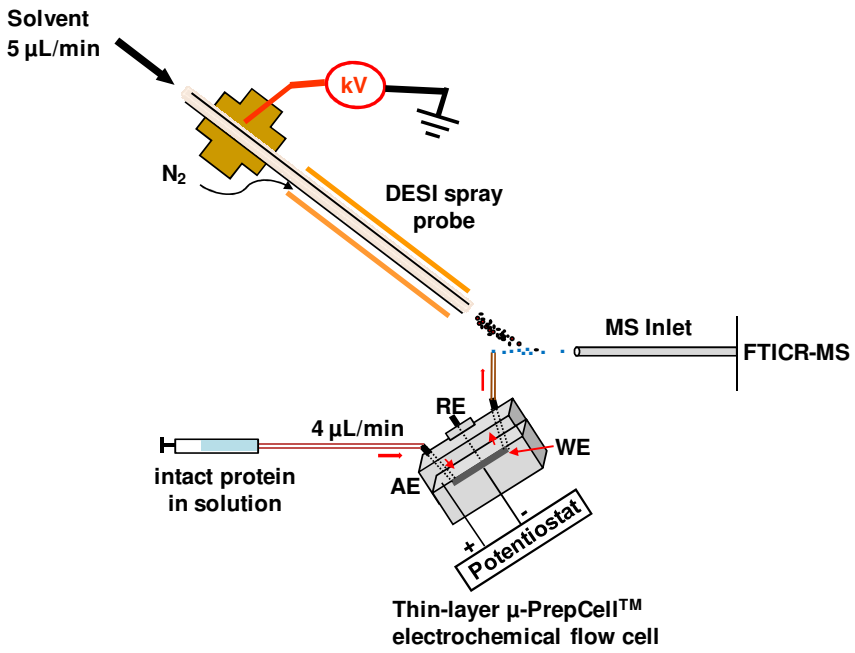

Figure S-1. Scheme showing the apparatus for online coupling of a thin-layer µ-PrepCell™ electrochemical flow cell with a Bruker 12 T FTICR-MS using DESI as the interface. WE, working electrode; AE, auxiliary electrode; and RE, reference electrode.

COMPARING ELECTROLTIC AND CHEMICAL REDUCTION

Using lysozyme as an example, we further tested DTT reduction of protein. Figure S-2a shows the ESI-MS spectrum of the intact protein where +8~+10 ions are seen. When lysozyme at pH 3 was added with DTT in 100 fold excess amount, no CSD shift was noted even after overnight incubation with DTT, indicating that DTT reduction is slow in acidic solution. When the pH of lysozyme was adjusted to 8 by ammonium hydroxide, reduction can occur and the process was monitored by ESI-MS. As shown in Figures S-2c to 2f, the reduction yield increased with increased incubation time and 90 min appears to allow the complete reduction as indicated by the appearance of high abundant peaks with higher charge numbers.

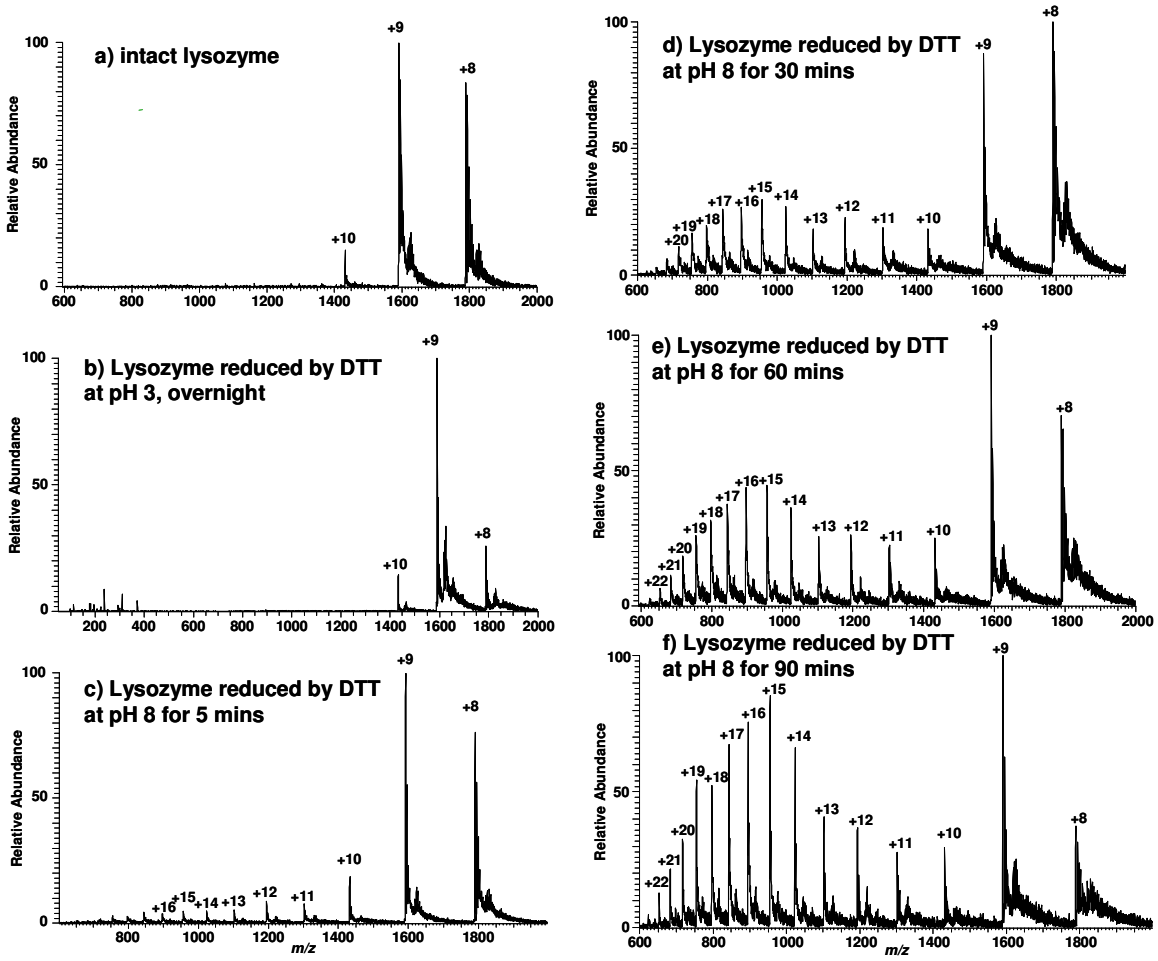

Figure S-2. ESI-MS spectra of (a) intact lysozyme, (b) lysozyme reduced by DTT at pH 3 for overnight, (c) lysozyme reduced by DTT at pH 8 for 5 min, (d) lysozyme reduced by DTT at pH 8 for 30 min, (e) lysozyme reduced by DTT at pH 8 for 60 min, and (f) lysozyme reduced by DTT at pH 8 for 90 min.

The efficiency of electrolytic reduction of protein disulfide bonds can be further improved by using a different electrode. When a 204.5010 μ-Prepcell WE (Antec BV, Netherlands) was used for electrolysis of lysozyme in MeOH/H_2O/HCOOH (50: 50: 1 by volume), many newly produced protein ion peaks with higher charge numbers and high abundance arose as show in Figures S-3.

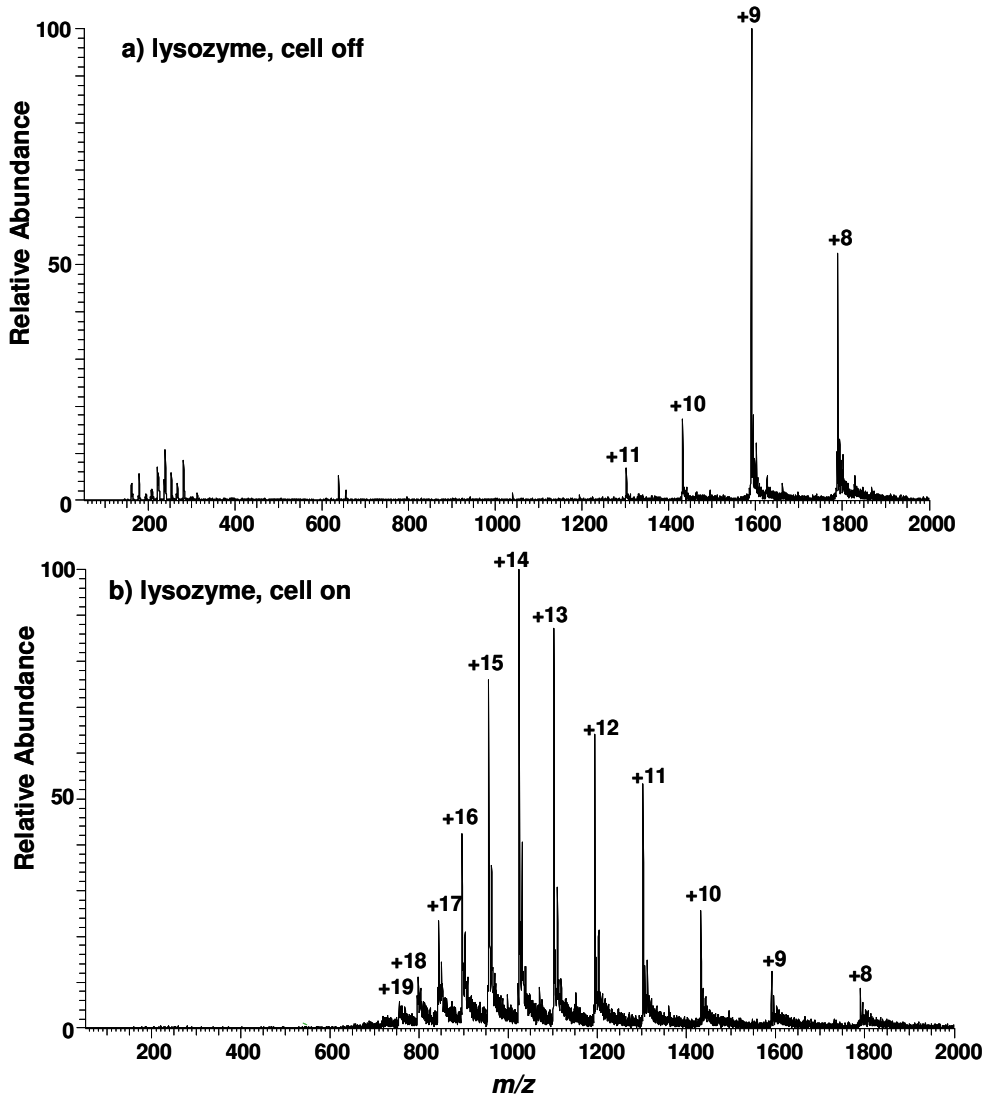

Figure S-3. Online EC/DESI-MS spectra of lysozyme in MeOH/H_2O/HCOOH (50: 50: 1 by volume) flowing out of the electrochemical cell with (a) no electrolysis and (b) with electrolysis. The working electrode used for lysozyme reduction was 204.5010 μ-Prepcell WE (Antec BV, Netherlands).

References

(1) LeDuc, R. D.; Taylor, G. K.; Kim, Y. B.; Januszyk, T. E.; Bynum, L. H.; Sola, J. V.; Garavelli, J. S.; Kelleher, N. L.. *Nucleic Acids Res.* 2004, *32*, W340-345.