INTRODUCTION
Characterization of intact proteins using tandem mass spectrometry approaches can be challenged by the presence of disulfide bonds that maintain and stabilize three-dimensional structures. Moreover, the presence of multiple disulfide bonds significantly reduces fragmentation efficiency. Electrochemistry-assisted intact protein tandem MS was recently reported for the characterization of multiple disulfide bond containing proteins using DESI-ETD. Following electrochemical reduction and on-line ECD-MS, significant improvements in backbone cleavages were reported compared to the ECO of the oxidized forms of the proteins. This technique provides an appropriate alternative for disulfide bond reduction using thiols or other chemical reducers. ESI-ETD-ion mobility MS was used to investigate the fragmentation efficiency of a number of disulfide binding containing peptides and their oxidized and reduced forms.

METHODS

Mass Spectrometry

MS:
- Synapt G2 or G2-MS (Fig. 1: ECD)

Capillary voltage:
- 2.5 kV

Temperature:
- 250°C

Desolvation temperature:
- 200°C

ETD-MS

Reagent:
- 4-methoxy-2nitrophenyl 4-(2-hydroxyethoxy)phenyl ether (HNMP-2)

Glue discharge current:
- 150 µA

Trap wave height:
- 6.2 + 2.5 V

Temperature:
- 450-550°C

Chromatography

UpLC:
- Acquity UPLC; XEVA 350/360, 1.7 µm, 1.5 x 100mm

Mobile phase A:
- H2O, 0.1% formic acid

Mobile phase B:
- ACN, 0.1% formic acid

Gradient:
- 10%-85% in 15 minutes

Electrochemistry

Electrochemical (EC) cell:
- Bovine Insulin EC-MS

Reduction solvent:
- 1 M LiCl

Acidiﬁcation solvent:
- 0.1 M HCOOH

Sample introduction:
- 50 nL of 50 µM solution

RESULTS

Bovine Insulin EC-MS

Figure 4. ETO mass spectra obtained from the source mixture of the charge-selected (pH=6.0) for the (A) unpaired and (B) reduced forms of insulin. The selected ions were directly analyzed for [M+H] + or [M+5H] + in the high mass range (1500 to 1900 m/z). The charge state of the ion was maintained by the pulsed collision cell.

Chicken Lysozyme EC-MS

Figure 7. ETO mass spectra obtained from the source mixture of the charge-selected (pH=6.0) for the (A) unpaired and (B) reduced forms of insulin. The selected ions were directly analyzed for [M+H] + or [M+5H] + in the high mass range (1500 to 1900 m/z). The charge state of the ion was maintained by the pulsed collision cell.

Chicken lysozyme mass spectra obtained with EC-MS.

Figure 8. The mass spectra obtained from the source mixture of the charge-selected (pH=6.0) for the (A) unpaired and (B) reduced forms of lysozyme were directly analyzed for [M+H] + or [M+5H] + in the high mass range (1500 to 1900 m/z). The charge state of the ion was maintained by the pulsed collision cell.

Mouse mAb standard LC-EC-MS

Figure 10. EC-MS spectra obtained for mouse mAb standard preparation (detailed in Methods). Significant sequence coverage was observed for the native and multiply charged ion (m/z 1720, 1614, and 1529). The micro-chromatograms (10-15s) were obtained using synthetic human lysozyme as mass standards.

CONCLUSION

- Initial studies demonstrate the potential of an electrochemical cell for on-line disulfide bond reduction for scalable top-down ETO-MS studies.
- Significant differences were observed between the chemically and electrochemically-reduced forms of the large mAb proteins.
- Further work and method optimization of the pulse profiles for large mAb proteins is currently ongoing.

References

- Umek et al. Electrospray nanoLC-ESI-MS/MS analysis of the human brain protein complement component C1q. Anal Bioanal Chem 389: 499-505
- Evans et al. Electrospray nanoLC-ESI-MS/MS analysis of the human brain protein complement component C1q.