Atmospheric Pressure Radical Induced Oxidative Cleavage of Disulfide Bond in Peptides using a Low-Pressure Mercury Lamp

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Overview

- Disulfide bond oxidative cleavage in small peptide ions from an electrospray ionization (ESI) plume was achieved with the use of a low-pressure mercury lamp under ambient conditions.
- Mechanistic experiments indicated that the 185 nm wavelength emission from the UV lamp photodissociates molecular oxygen causing the formation of radicals that leads to the oxidation and cleavage of the disulfide bond.
- Collision induced dissociation (CID) on the peptide ion after radical reaction results in rich peptide sequence information.
- In the case of radical transfer to the peptide a sulfinyl radical is formed and radical induced neutral losses are observed in competition with peptide backbone fragmentation during CID.

Introduction

- Disulfide bond formation is an important post-translational modification that gives peptides and proteins a tertiary structure necessary for biological function.
- Correct identification of disulfide linkages in proteins and peptides is necessary for structural characterization.
- Enzymatic digestion of proteins followed by high performance liquid chromatography (HPLC) and mass spectrometry (MS) analysis is typically employed to identify disulfide bond linkages in a proteomics experiment.
- New methods for identifying disulfide bond linkages in the gas phase are desired to reduce experimental time and sample waste.
- In this research a new method is introduced to cleave disulfide bond linked peptides in the gas phase under atmospheric pressure from an ESI source using a low pressure mercury lamp.

Methods

- Mass spectrometry (MS) experiments were performed on a 4000 Qtrap (Applied Biosystems/Sciex, Toronto, Canada)
- 20 mA / 6.4 watt double bore tubing low pressure mercury lamp (model 81-1057-51, BHK, inc. Ontario, CA) was used to induce the radical reaction with the 185 nm emission. A similar lamp was used for mechanistic studies but with the 185 nm wavelength filtered (model 80-1057-01, BHK, inc. Ontario, CA).
- An electrospray ionization (ESI) source in positive mode (3-4 kV) with a nitrogen nebulizing gas was used to produce peptide ions. Solution flow rate was 1 µL/min.
- A nano electrospray ionization (nanoESI) source in positive mode (1-2 kV) was used to produce peptide ions. The nanoESI source was wrapped in aluminum foil to minimize solution reactions from the UV lamp.
- Disulfide peptide concentrations were 10-50 µM in 50/49/1 MeOH/H_2O/HOAc (v/v/v).

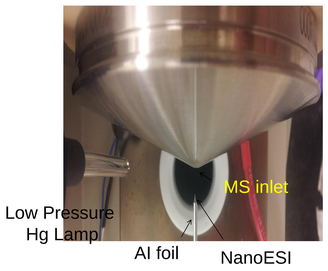

Figure 1: Experimental set-up with the lamp and nanoESI source. The lamp is approximately 6 cm from the source plume.

Results

Oxidative cleavage of disulfide bond

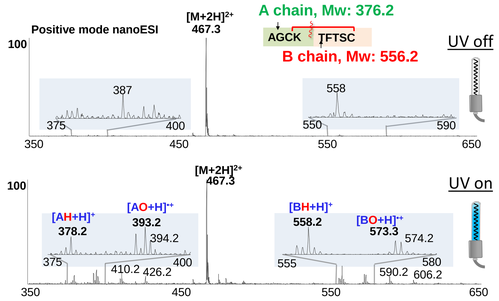

Figure 2: Mass spectrum before and after application of UV light. Top spectrum shows no chain separation before UV light. Bottom spectrum shows disulfide bond cleavage and peptide chain separation with UV light. Sulfinyl radical and thiol are resulting products of the ion/radical reaction.

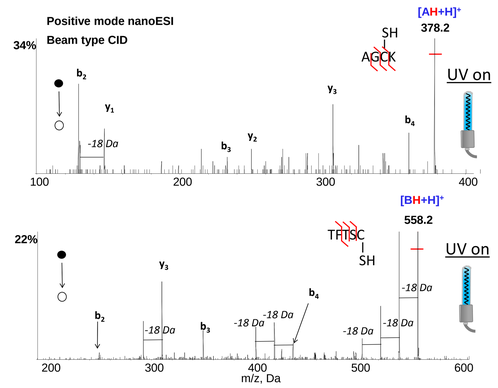

Figure 3: CID on the thiol for each peptide chain after radical reaction. Sequence information is obtained verifying chain separation.

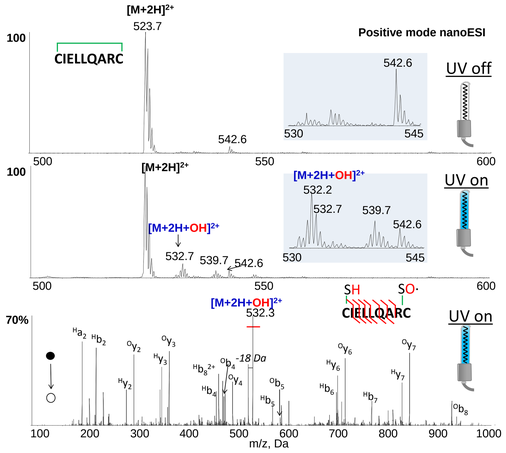

Figure 4: UV experiments with selectin intrachain disulfide linked peptide. The top spectrum is MS^1 before application of UV light. The middle spectrum shows the addition of OH to the peptide with UV light. The bottom spectrum is the CID spectrum on $[M+2H+OH]^{2+}$ and shows rich sequence information indicating oxidative cleavage of the disulfide bond.

Radical formation and reaction

$$O_2 + h\nu_{184.9\ nm} \rightarrow 2O\cdot$$
$$\cdot O + H_2O \rightarrow 2\ \cdot OH$$
$$H_2O + h\nu_{184.9\ nm} \rightarrow \cdot OH + \cdot H$$
$$H\cdot + O_2 + M \rightarrow HO_2 + M$$
$$O\cdot + O_2 \rightarrow O_3\ *$$

Scheme 1: Radical reactions induced by the 185 nm wavelength under ambient conditions. Mechanistic experiments (see below) indicate that photodissociation of molecular oxygen initiates the radical reaction.
(*Dusanter, et al.; Creasey, et al)

Scheme 2: Possible mechanisms for radical induced oxidative disulfide bond cleavage.

Mechanistic studies

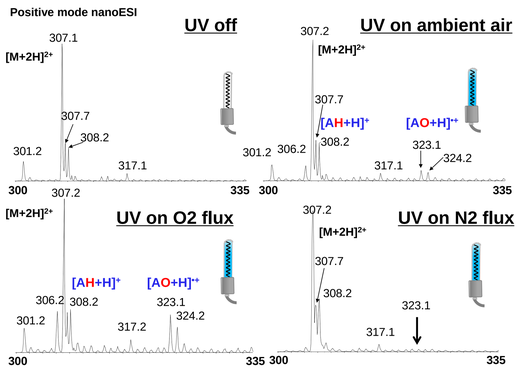

Figure 5: Mechanistic studies for radical formation with the interchain disulfide linked peptide glutathione. With oxygen in flux the peptide chain signal with the sulfinyl radical increases. With nitrogen in flux the sulfinyl radical signal decreases.

185 nm filtered UV lamp: No Radical Reaction

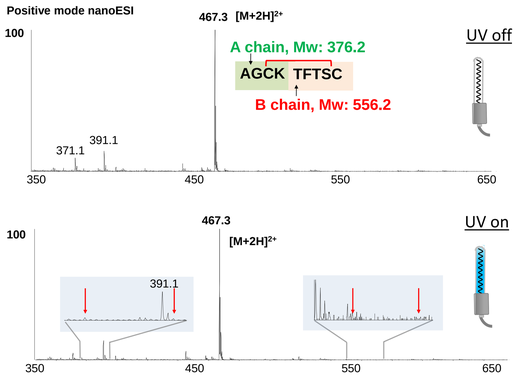

Figure 6: Mechanistic studies with the 185 nm wavelength filtered out. The bottom spectrum shows that with the lamp on there is no evidence of disulfide bond cleavage.

NanoESI: solution reactions from UV light

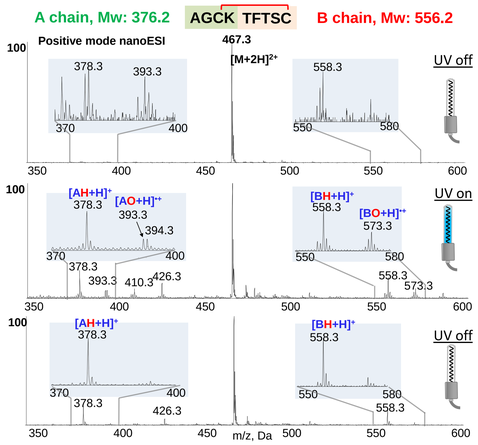

Figure 7: Disulfide bond cleavage can occur in solution when using the UV lamp with a nanoESI source. Top slide is before exposure to UV lamp. Middle slide shows disulfide bond cleavage with the production of thiol and sulfinyl radical peptide chains with UV light. The bottom slide is the same spray but with the lamp off and shows the thiol reaction products but not the sulfinyl radical.

ESI: no solution reactions from UV light

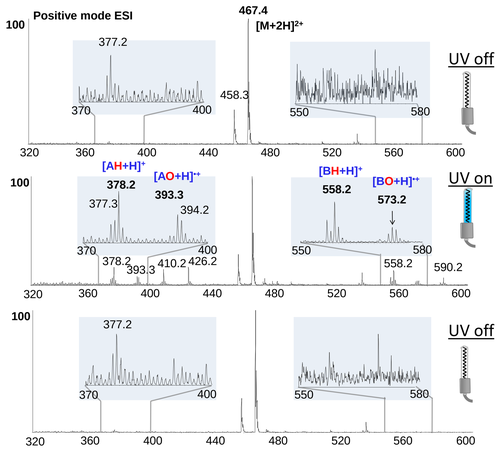

Figure 8: UV lamp experiments with an ESI source. Top slide is before UV light. Middle slide indicates oxidative cleavage of the disulfide bond with UV light. The bottom slide is the same experiment right after the UV lamp is turned off and shows that there was no solution reaction.

Gas phase production of sulfinyl radical

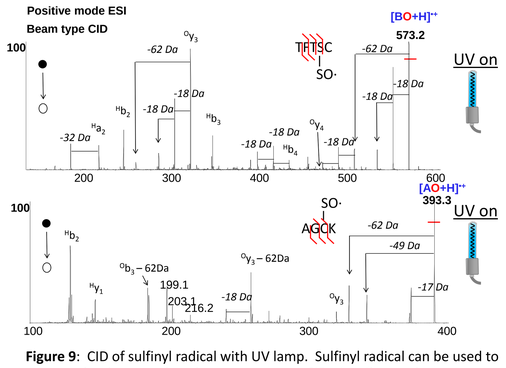

Figure 9: CID of sulfinyl radical with UV lamp. Sulfinyl radical can be used to study radical ion chemistry in the gas phase. CID of sulfinyl radical can produce unique neutral losses such as - 62 Daltons which corresponds to the loss of $CH_2SO\cdot$ (Ma et al)

Identification of disulfide peptides from protein tryptic digests

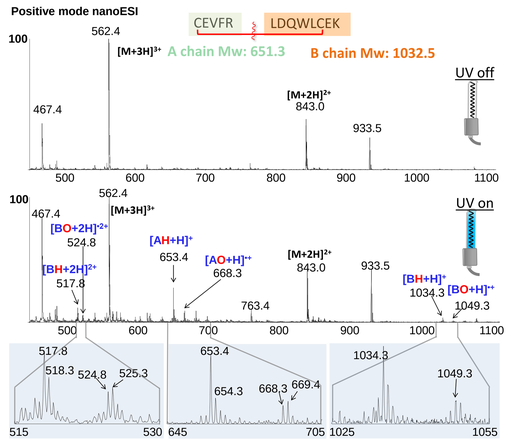

Figure 10: UV lamp experiments of tryptic digested protein α lactalbumin (14 kDa). Oxidative cleavage of the disulfide bond is observed in the middle slide.

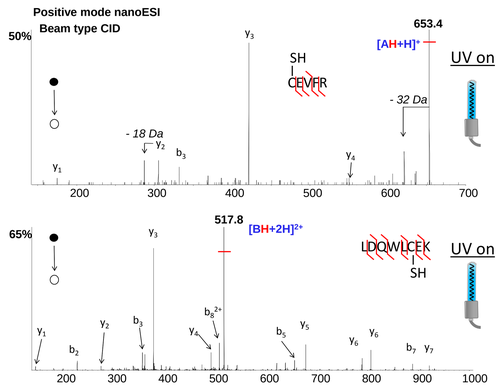

Figure 11: CID on the peptide chains containing the thiol group after chain separation show rich sequence information.

Conclusion

- Disulfide bond linked peptide ions from an ESI source show oxidative cleavage of the disulfide bond after exposure to a low pressure mercury lamp.
- Mechanism experiments indicate that the 185 nm emission from the lamp photodissociates molecular oxygen inducing radical reactions.
- It is hypothesized that ·OH or O· cleaves the disulfide bond creating a thiol and sulfinyl radical.
- Use of the lamp with a nanoESI source can show solution reactions resulting in disulfide bond cleavage forming a thiol group in solution.
- An ESI source does not show any solution reaction after exposure to the UV lamp.
- Rich sequence information is achieved after CID on the peptide ion with the thiol group.
- Gas phase production of sulfinyl radical is also achieved which can be used for radical/ion studies.

References

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- Creasey, D. J., D. E. Heard, et al. (2000). "Absorption cross section measurements of water vapour and oxygen at 185 nm. Implications for the calibration of field instruments to measure OH, HO2 and RO2 radicals." Geophys. Res. Lett. **27**(11): 1651-1654.
- Ma, X., C. Love, et al. (2011). "Gas-Phase Fragmentation of [M + nH + OH]$^{n+}$ ions Formed from Peptides Containing Intra-Molecular Disulfide Bonds." Journal of The American Society for Mass Spectrometry **22**(5): 922-930.

Acknowledgements

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