FAST PHOTOCHEMICAL OXIDATION OF PROTEINS BY SINGLET OXYGEN

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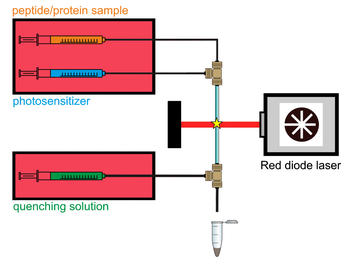
INTRODUCTION

- Protein footprinting is a powerful tool for structural characterization of proteins and their complexes that examine conformational changes and ligand binding by determining the solvent accessibility of the backbone or side chain structures of protein molecules [1]. By comparing the footprint of a protein in two different structural states, changes in the protein topography can be detected and interpreted in the context of other structural information [2].
- There are many footprinting methods used with the mass spectrometric detection. One popular approach is based on monitoring extent of oxidation of protein side chains due to exposure of oxidative reagents from solution.
- We examined the possibility to achieve footprinting by exposing model peptides or proteins to molecular singlet oxygen, a known reactive oxygen species.
- Singlet oxygen, $O_2(^1\Delta g)$ or $^1O_2$, is the lowest excited electronic state of molecular oxygen. It is generated by the transfer of energy to ground (triplet) molecular oxygen and it exhibits significantly greater reactivity towards organic compounds than triplet oxygen [3].
- Singlet oxygen reacts with a wide range of biological targets, including DNA/RNA, proteins, and all classes of lipids. Kinetic data are consistent with proteins being a major target for the singlet oxygen, with modifications occurring preferentially at Trp, His, Tyr, Met, and Cys side-chains. It has been also described that reaction between proteins and singlet oxygen generates peroxides in high yield, mostly on Tyr, Trp, and His residue sites. [4,5]

METHODS



Schema 1: Apparatus and experimental steps
1/ Mixing sample and photosensitizing compound
2/ Irradiation with red laser light, generation of singlet oxygen and reaction with the analyte
3/ Quenching the reaction and sample collection
4/ Detection of collected sample by HRAM-MS

METHODS

- Quench-flow setup consisted of 3 syringe pumps, separately delivering solution of the protein/peptide to be examined, solution of the photosensitizer compound and quenching solution (Schema 1)
- Highly reactive singlet oxygen was generated after laser irradiation by a photodynamic effect of phtalocyanine - a process in which excited photosensitizer interacts with a ground state oxygen dissolved in water, and creates singlet oxygen [6].
- The collected samples were analyzed by direct infusion into solariX XR 15T, Bruker Daltonics
- The LC analysis was performed using Evosep One EV-1000 (Evosep) and timsTOF SCP (Bruker Daltonics)

RESULTS

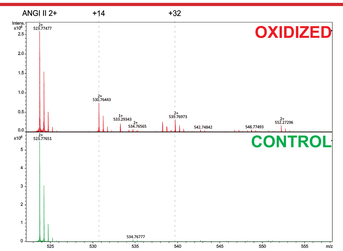


Figure 1: Mass spectrum of Angiotensin II; sequence=DRVYIHPF
Top: After exposure to singlet oxygen
Bottom: Control (intact Angiotensin II, doubly charged ion at m/z=523)

Mass spectrum of Angiotensin II showed mainly the doubly charged ion at m/z=523. Sample exposed to singlet oxygen showed new doubly charged peaks at m/z=530 (addition of 14Da), corresponding to addition of O atom and loss of two H atoms (or addition of 2O and loss of water) and m/z=539 (addition of 32Da), corresponding to addition of 2O atoms.

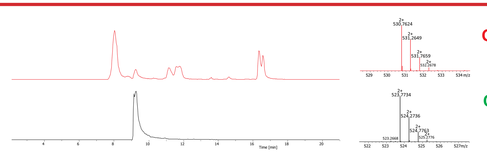


Figure 2: LCMS anlysis of Angiotensin II oxidized by singlet oxygen
Top: XIC (m/z=530.7) of oxidized sample
Bottom: XIC (m/z=523.7) of control sample

RESULTS

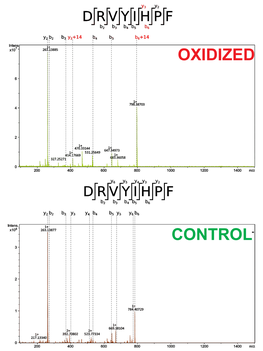


Figure 3: CID MSMS spectrum of doubly charged Angiotensin II precursors
Top: Oxidized precursor; MSMS of 530
Bottom: Control (intact Angiotensin II); MSMS of 523

The MSMS spectrum shows that the oxidative modification in the sequence begins with fragments y3 and b6, which localizes it to histidine

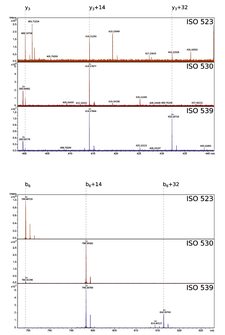


Figure 4: CID MSMS spectrum of doubly charged Angiotensin II precursors
Top: Fragment y3 and its modifications
Bottom: Fragment b6 and its modifications

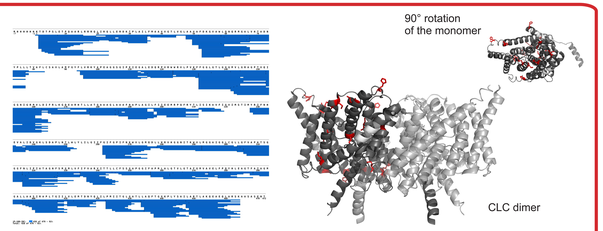


Figure 7: Singlet oxygen labelling of CLC-ec1 using in-house built quench-flow apparatus. Summary of sequence coverage (left) and visualization of modified sites on the CLC-ec1 structure (right)

RESULTS

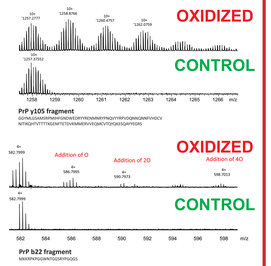


Figure 5: Top-down analysis of recombinant prion protein (PrP) oxidized by singlet oxygen

Top: Fragment y105; oxidized and control
Bottom: Fragment b22; oxidized and control

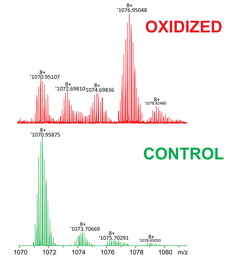


Figure 6: Oxidation of Ubiquitin by singlet oxygen

Top: Peaks of oxidized Ubiquitin 8+ species
Bottom: Peak of control Ubiquitin 8+

CONCLUSIONS

- Singlet oxygen generated by photodynamic effect was tested as a reagent for protein footprinting
- Experimental conditions were optimized for quench-flow conditions
- Model peptides and proteins were oxidized by singlet oxygen and analyzed by MS
- Oxidative modifications were detected by direct infusion and LC-MS and localization was performed by top-down and bottom-up

ACKNOWLEDGMENTS

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