



HAL
open science

Specific detection of cysteine sulphenic acid by coupling Mass Spectrometry with Laser Induced Dissociation

Jean-Valery Guillaubez, Delphine Pitrat, Yann Bretonnière, Jérôme Lemoine,
Marion Girod

► **To cite this version:**

Jean-Valery Guillaubez, Delphine Pitrat, Yann Bretonnière, Jérôme Lemoine, Marion Girod. Specific detection of cysteine sulphenic acid by coupling Mass Spectrometry with Laser Induced Dissociation. SMAP2019: (Spectrométrie de Masse et Analyse Protéomique), Sep 2019, Strasbourg, France. . hal-03898940

HAL Id: hal-03898940

<https://hal.science/hal-03898940>

Submitted on 14 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Specific detection of cysteine sulphenic acid by coupling Mass Spectrometry with Laser Induced Dissociation

Jean-Valery Guillaubez¹, Delphine Pitrat², Yann Bretonnière², Jérôme Lemoine¹, Marion Girod¹

¹Institut des Sciences Analytiques, UMR 5280, CNRS - Université Lyon 1;
²Laboratoire de Chimie ENS Lyon, UMR 5182, ENS Lyon CNRS et Université Lyon 1

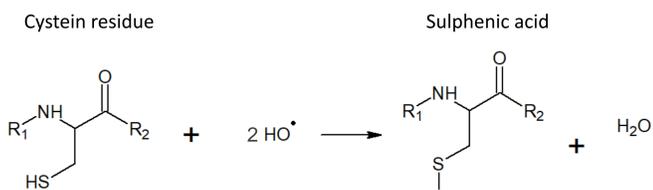
jean-valery.guillaubez@isa-lyon.fr ; marion.girod@univ-lyon1.fr

Overview

Improvement of oxidative stress induced modification of cysteine residues detection. Protein cysteine residues are oxidized into sulphenic acid (SOH), derivatized with a specific chromophore and specifically detected and identified thanks to Laser Induced Dissociation (LID) in the visible range

Introduction

In a context of population aging, discovery and validation of novel oxidative stress biomarkers for screening of neurodegenerative diseases is a key issue. One of the modification induced by reactive oxygen species (ROS) is the oxidation of protein cysteine (Cys) residues¹.



As this oxidative process is minor, the detection of these oxidized proteins at low concentrations is problematic due to the complexity and dynamic concentration range of the samples to be analyzed.

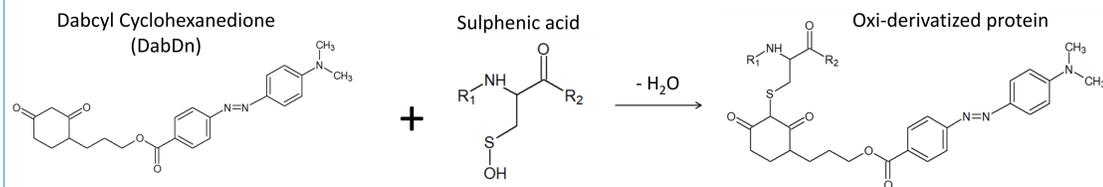
In order to improve the detection specificity for oxidized proteins, we use an experimental setup coupling mass spectrometry and laser induced dissociation (LID) in the visible range (473 nm) to add a stringent optical specificity to the mass selectivity².

Methods

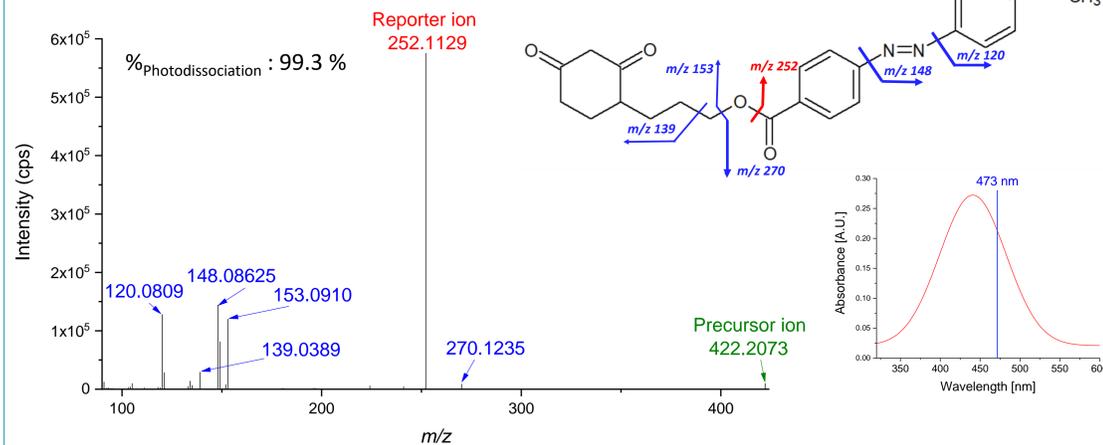
- Protein unfolding and reduction of disulfide bridges with a ratio Prot:DTT of 1:3 during 40 min at 60°C
- Oxidation of cysteines into SOH with an excess of H₂O₂ :
 - . Pure LCTVATLR peptide → 1:100 ratio
 - . Mixture of 3 proteins: albumin/serotransferin/lysozyme → 1:10 ratio
- Simultaneous specific derivatization of formed SOH with Dabcyll Cyclohexanedione (ENS home-made reactant) at 1:1 ratio during 6h. Increasing the ratio only impact the derivatization speed, not quantity of reacted product.
- Enzymatic **digestion** with trypsin (10 μL at 2 mg/mL) overnight at 37°C.
- 2nd removal of disulfide bridges with 1:3 DTT during 40 min at RT, and free-cysteine blocking with 1:3 IAM during 40 min at RT in the dark.
- Samples **desalting** and concentration by Solid Phase Extraction (Oasis™ HLB 3cc)
- High Performance Liquid Chromatographic (HPLC) peptides **separation** on C₁₈ column (2.1 mm, 3.5 μm) : 45 min run with a 5-45% ACN gradient (300 μL/min).
- Parallel Reaction Monitoring (PRM) of peptides in LID@473nm/HCD on a modified hybrid quadrupled orbitrap mass spectrometer (QExactive ThermoScientific) (R= 35,000 ; activation time 25/3 ms, activation energy 3/25 eV)
- Data treatment performed with Skyline and manually

Chromophore derivatization of cysteine sulphenic acid

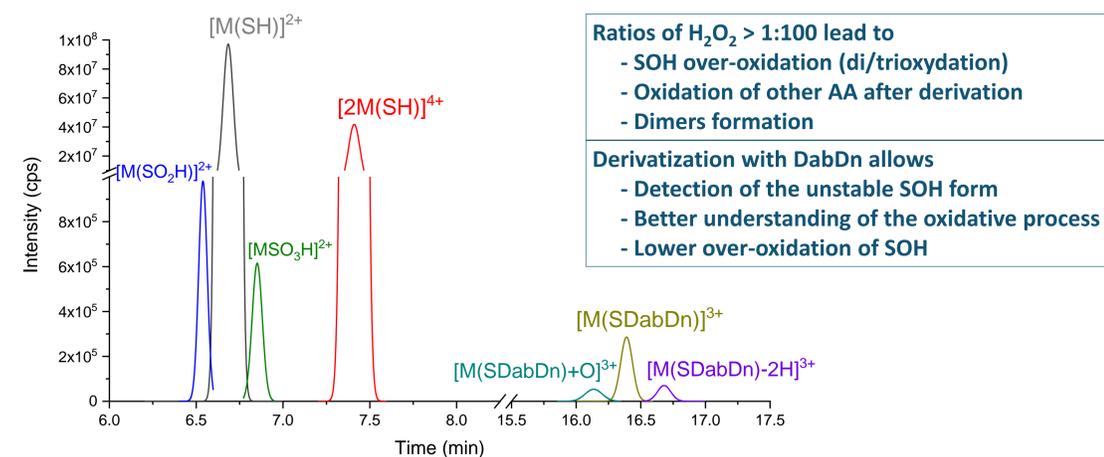
Since peptides do not naturally absorb in the visible range, this new methodological approach relies on the proper chemical derivatization of oxidized Cys with a chromophore : Dabcyll Cyclohexanedione (DabDn), which contains a cyclohexanedione group that specifically binds to SOH³, and a Dabcyll chromophore which absorbs at 473nm.



LID spectrum of the Dabcyll Cyclohexanedione chromophore @473 nm

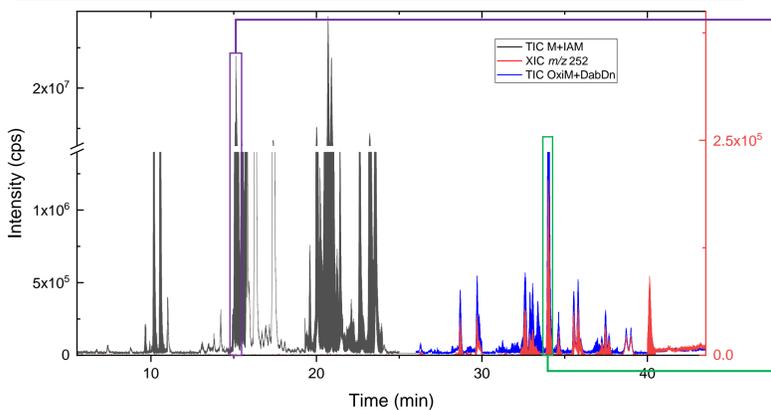


Chromatogram of the different forms of LCTVATLR peptide after oxidation – derivatization process



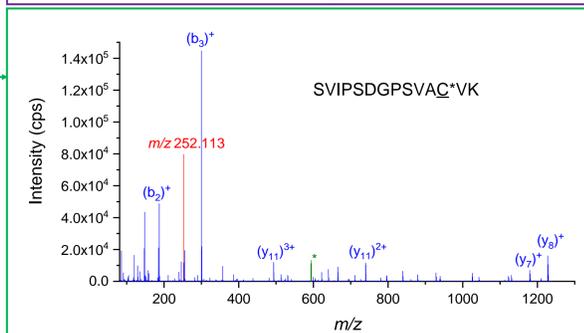
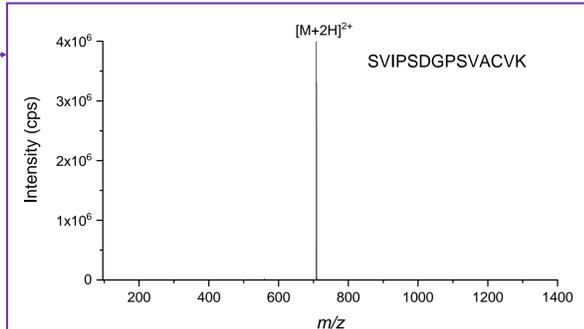
Specific fragmentation of oxi-derivatized peptides by LID

PRM-LID analysis of a 3 protein digest (albumin/serotransferin/lysozyme)

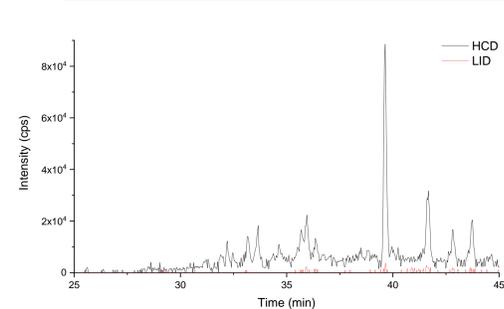


The chromatogram is divided in two parts :
- Low RT : Transitions of carbamidomethylated peptides (grey)
- High RT : Transitions of oxi-derivatized peptides (blue)

Carbamidomethylated peptides are used as an internal control of
- RT and intensity stability
- Sample preparation reproducibility
- Relative intensity comparison with oxi-derivatized forms



XIC m/z 252 in control sample without DabDn



Specific fragmentation of the oxi-derivatized peptides in LID :

- Carbamidomethylated peptides remain intact
- No interfering signal in non-oxi-derivatized sample unlike in HCD

➡ **Easier and more precise identification and quantification of oxi-derivatized peptides in complex samples**

References

1. P. Eaton: Protein thiol oxidation in health and disease: Techniques for measuring disulfides and related modifications in complex protein mixtures, *Free Radical Biology and Medicine*, **2006**, 40, 1889-1899.
2. M. Girod, et al.: Implementing visible 473 nm photodissociation in a Q-Exactive mass spectrometer: towards specific detection of cysteine-containing peptides, *The Analyst*, **2014**, 139, 5523-5530.
3. Y. H. Seo, et al.: Quantification of Protein Sulfenic Acid Modifications Using Isotope Coded Dimedone and Iododimedone, *Angew. Chem. Int. Ed.* **2011**, 50, 1342-1345

Conclusion

- Specific derivation on cysteine sulphenic acid with the dabcyll cyclohexanedione
- Specific detection of derivatized cysteine sulphenic acid-containing peptides in a digested protein mixture in LID @ 473nm, allowing to reduce interfering signal.

Perspectives: Extension of the protocol to complex samples and endogenous Cys oxidation