Idenification and quantitation of cysteine sulfoxidation sites

Chia-Fang Lee¹, Tanya T. Paull², and Maria D. Person¹

¹Protein and Metabolite Analysis Facility, College of Pharmacy, ²Institute for Cellular and Molecular Biology, Howard Hughes Medical Institute and Department of Molecular Genetics & Microbiology, The University of Texas at Austin

Introduction

Reactive oxygen species (ROS) serve as an endogenous source of DNA-damage agents that promote genetic instability. Cysteine is a major target of ROS protein modification. It is challenging to study due to low abundance, variety of oxidation products, and sample handling alterations of oxidation state. Here we describe a method to detect and quantify irreversible cysteine oxidation to sulfonic and sulfonic acid without additional enrichment steps using a long column UPLC-mass spectrometry-based approach.

1. Detection of $SO_2H$ in BSA

- 22 oxidized cysteines detected with long column UPLC-MS/MS

2. Skyline MS1 quantitation on amol BSA

- Microm 6 bovine protein mix has β-lactoglobulin:lactoperoxidase:carbonic anhydrase:glutamate dehydrogenase:o-casein:serum albumin at 100,000:1000:100:100:10:1 molar ratios

3. Detection and quantitation of $SO_4H$ in ATM protein complex

- Overexpressed ATM affinity purification pulls down endogenous PRMT5
- 46% of cysteines were identified in ATM including, with 9 oxidation sites
- 75% of PRMT5 cysteines were observed, with 5 oxidized
- Long column UPLC separates oxidized and unmodified peptides
- High resolution MS1 filtering measures $H_2O_2$ dose dependence
- Baseline oxidation observed presumably from sample preparation

4. Jurkat lysate $SO_2H$ identification

- 153 peptides containing irreversibly oxidized cysteines were discovered in Jurkat cell lysate treated with $H_2O_2$
- Previously observed cysteine oxidation targets are identified

5. pSRM quantitation in cell lysate

- Oxidation sites quantified via Skyline pseudo Selected Reaction Monitoring (pSRM) extracted fragment ion chromatograms
- Hydrogen peroxide dose dependence shows dynamic conversion from sulfonic to sulfonic acid

Discussion

- The mass spectrometry-based approach offers robust quantitation and replicate validation for large unbiased proteomic screens designed to uncover redox sensitive cysteine residues in susceptible proteins
- The method was first applied to identify and quantify oxidized cysteine residues in purified proteins and complexes then demonstrated on cell lysates oxidized in vitro
- Long column UPLC-M5/MS using high resolution MS1 provides for separation of modified peptides and accurate identification with MS1 or MS2 based quantitation using Skyline