UTILIZATION OF FAST PHOTO-OXIDATION OF PROTEINS AND TOP DOWN MASS SPECTROMETRY FOR STRUCTURAL CHARACTERIZATION OF PROTEIN COMPLEXES

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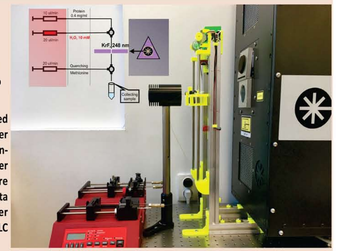
INTRODUCTION

Protein footprinting coupled to mass spectrometry is commonly applied for protein structural studies, providing information on protein conformations and dynamics. Traditional mass spectrometry approaches for structural elucidation include hydrogen deuterium exchange, chemical cross-linking, ion mobility and covalent labeling. Among these, hydroxyl radicals are a perspective probe for the fast protein footprinting as introduced two decades ago. There are different methods to generate them including Fenton reaction, radiolysis of water and fast photo-chemical oxidation of proteins (FPOP). Bottom up mass spectrometry is the dominant method to identify modified amino acids and determine the solvent accessible area of proteins. Here, we present utilization of the Top-down sequencing for localization of modified residues within the protein structure.

Methods

- FPOP uses OH radicals to modify proteins.
  KrF laser at 248 nm was used to dissociate H_2O_2 (10mM) into hydroxyl radicals. The radicals react with amino acids side chains.
- Top Down analysis (Figure 1) was performed using ESI FT-ICR mass spectrometer (Bruker Daltonics, 15T solariX XR), utilizing collision-induced dissociation (CID), Electron transfer dissociation (ETD) and Electron capture dissociation (ECD) (Figure 2). Bottom up data were collected on timsTOF Pro (Bruker Daltonics) connected to Agilent 1290 UPLC system (Agilent Technologies).



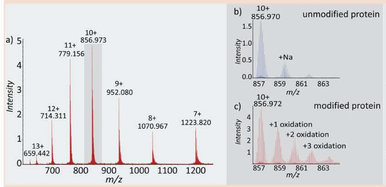
- DataAnalysis 5.2 (Bruker Daltonics) was used for the visualization and calibration of MRMS spectra. Theoretical m/z values of CID, ETD, ECD, Multi CASI/CID and Multi CASI/ECD fragment ions were obtained using the Ms2links tool [1]. Extent of the oxidation was quantified using the oxidized fragment ion intensities in CID, ETD, and ECD. PEAKS Studio Xpro (Bioinformatics Solutions) enabled the interpretation of DDA data. Oxidation yields for all the ions were calculated as the ratio of the oxidized fragment (Iox) to the sum of all fragments (oxidized (Iox) and non-oxidized (I).

eq.1 $$Extent\ of\ oxidation = \frac{\sum I_{ox}}{\sum (I_{ox} + I)}$$

eq.2 $$p = \frac{\sum I_{ox}(n) + \sum I_{ox}(n+1) + \cdots}{\sum (I_{ox} + I\,(n)) + \sum (I_{ox} + I\,(n+1)) + \cdots}$$

RESULTS

MS spectrum: Top Down method



How to identify sites of modification?

There are several fragmentation strategies: CID, ECD and ETD

Top down sequencing: it is a way to characterize an intact protein and determine site of modifications

Figure 1: Ubiquitin was detected in several charge states following FPOP reaction (a). Mass spectrum corresponding to the 10+ charge state of untreated (b) and treated ubiquitin (c).

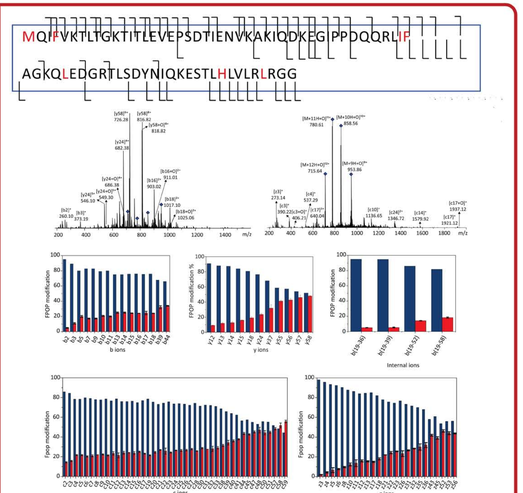


Figure 2: MULTI CASI/CID and CASI/ECD spectra of the singly oxidized ubiquitin and the charts describing the ratio between regular and oxidized fragments. Error bars indicate SD.

References

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[2] Polák M, Yassaghi G, Kavan D, Filandr F, Fiala J, Kukačka Z, Halada P, Loginov DS, Novák P. Anal Chem. 2022; 94 (7): 3203-3210.

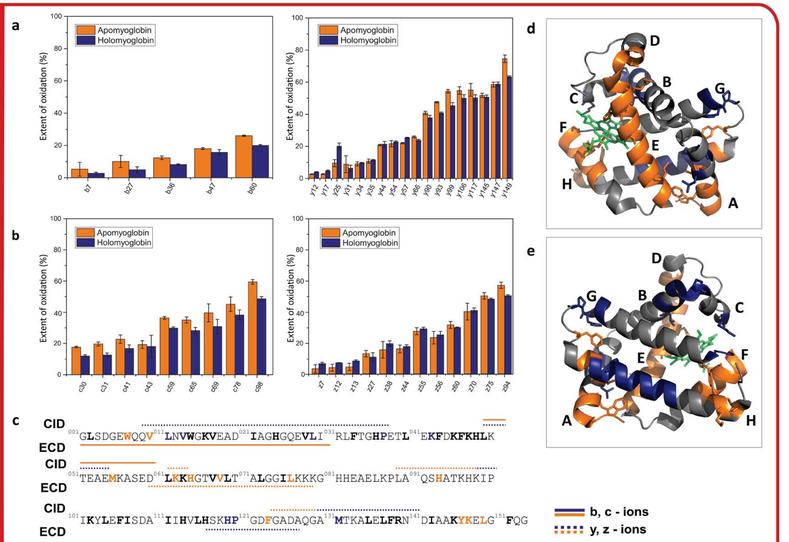

Figure 3: Interpretation of FPOP experimental results for structural analysis of holomyoglobin after heme removal. Extents of peptide oxidation calculated from multi CASI/CID (a) and multi CASI/ECD (b) experiment for apomyoglobin (gold) and holomyoglobin (blue). Myoglobin sequence alongside with indication of increased levels of oxidation in CID (b and y fragments) and ECD (c and z fragments) (c). The amino acids sites that were found impacted in bottom-up control experiment are indicated in the sequence by using the same color coding. Holomyoglobin structural model (1WLA) with regions most affected by heme removal. Front orientation (d) and back orientation (e) are shown.

Conclusion

MRMS is a very effective technique for protein footprinting by top-down detection of oxidation sites on FPOP modified proteins. It provides specific selection of only singly oxidized forms of the modified protein. Utilization of Multi-CASI allows selection of all major charge states and provides accurate oxidation extent data. ECD offers the best sequence coverage and thus better spatial resolution than CID and ETD. Therefore, ECD is a fragmentation technique of choice for FPOP top-down experiments. The method was successfully tested to probe structural differences between apo and holo myoglobin and the results were in good agreement with X-ray structural model as well as with bottom-up FPOP results (Figure 3).

Further, the combination of FPOP and both bottom-up and top-down strategies allows a comprehensive structural charactization of transcription factor / DNA response element complexes hardly accesibled by rutine techniques of structural biology. Analysing FOXO4-DBD·DAF16 complex reveals the ambivalent properties of helix H4 and protection of strand S3 upon DNA binding. Alteration of the intervening loop and S1 strand in the presence of DNA is shown for the first time (Figure 4).

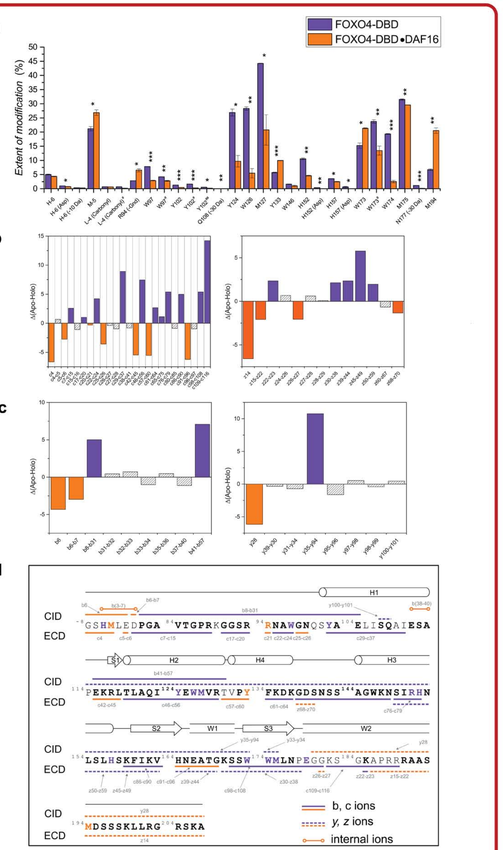

Figure 4: FPOP structural analysis of FOXO4 transcription factor upon DNA responce element binding (DAF16). Quantified extents of residue modifications detected in LC-MSMS analysis (a). Plots indicating changes in oxidation rates between apo and holo forms for ECD (b) and CID (c) ions. Differential oxidation map of FOXO4-DBD with/without DNA (d). The bolded sequence represents spatial resolution achieved by top-down approach (covered by complementary ECD and CID fragments). Coloured residues refer to the ones detected in bottom-up approach. Secondary structure topology are denoted above the sequence.

LABORATORY OF STRUCTURAL BIOLOGY AND CELL SIGNALING

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