Comparing an intrinsically disordered protein α-synuclein to fixed structure proteins following FPOP modification using high resolution LCMS intact analysis

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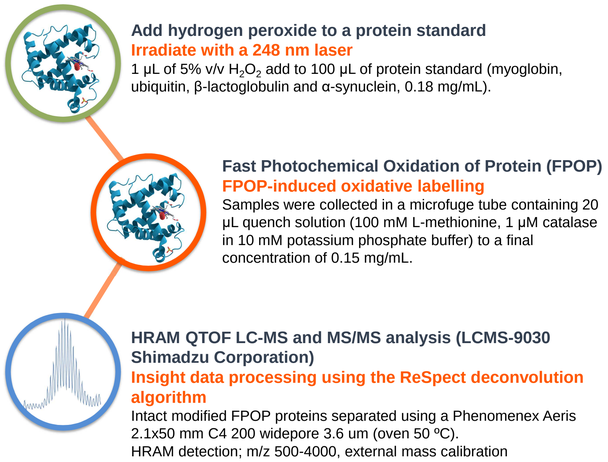
Overview

- Fast photochemical oxidation of proteins (FPOP) was used to oxidatively modify several model proteins in order to investigate oxidation efficiency depending on whether the protein was of fixed structure or an intrinsically disordered protein (IDP).
- High resolution QTOF LCMS analysis used charge state deconvolution software and theoretical spectra to confirm measured masses to theoretical masses.
- Intact native LCMS was show to be an effective tool for monitoring modification efficiency in proteomic studies.

1. Introduction

IDPs are typically defined as lacking a fixed structure however there is growing evidence that IDPs play an important role in cell signalling and various pathological states. Misfolding and aggregation of α-synuclein form a major component in Lewy bodies which form the main pathogenesis of Parkinson's disease (PD) and thus characterisation of differing conformational states of IDPs is vital to the understanding of disorders such as PD. In this work a technique known as FPOP was used to oxidatively modify several model proteins in order to compare modification efficiency of fixed conformation proteins verses the IDP α-synuclein.

2. Methods



Add hydrogen peroxide to a protein standard
Irradiate with a 248 nm laser
1 µL of 5% v/v H_2O_2 add to 100 µL of protein standard (myoglobin, ubiquitin, β-lactoglobulin and α-synuclein, 0.18 mg/mL).

Fast Photochemical Oxidation of Protein (FPOP)
FPOP-induced oxidative labelling
Samples were collected in a microfuge tube containing 20 µL quench solution (100 mM L-methionine, 1 µM catalase in 10 mM potassium phosphate buffer) to a final concentration of 0.15 mg/mL.

HRAM QTOF LC-MS and MS/MS analysis (LCMS-9030 Shimadzu Corporation)
Insight data processing using the ReSpect deconvolution algorithm
Intact modified FPOP proteins separated using a Phenomenex Aeris 2.1x50 mm C4 200 widepore 3.6 um (oven 50 ºC).
HRAM detection; m/z 500-4000, external mass calibration

Figure 1. Methodology used to generate FPOP modified proteins and to detect intact proteins by high resolution accurate mass (HRAM) detection with the LCMS-9030 QTOF system. Insight deconvolution software was used in data processing.

3. Results

The principal of FPOP employs the addition of H_2O_2 followed by irradiation with a pulsed laser generating hydroxyl radicals resulting in typically +16 Da covalent modification. The technique offers some advantages over alternative approaches such as HDX which can be challenging due to back exchange in non-acidic conditions. The hydroxy radicals generated in FPOP have a relatively short half-life making modification more predictable. Furthermore, oxidative modification from FPOP can be more stable during subsequent analysis by either intact MS or proteomic enzymatic digestion workflows.

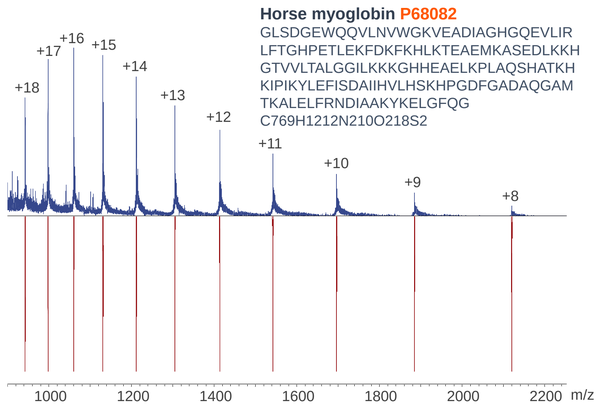


Figure 2. Horse myoglobin following FPOP modification: measured spectrum (blue), theoretical spectrum (red). Sequence taken from Uniprot (https://www.uniprot.org/) converted to formula via Expasy (https://web.expasy.org/protparam/), theoretical spectrum generated using LabSolutions Insight mass calculator.

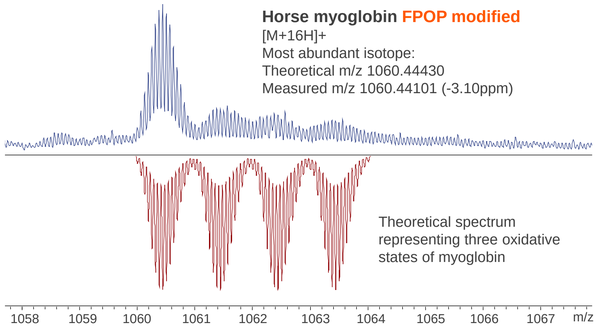


Figure 3. Following FPOP modification, myoglobin remained largely unmodified with only low levels of +O, +O2, +O3 forms; measured spectrum (blue), theoretical spectrum (red). All measurements were performed using external mass calibration carried out before analyses.

3.1 FPOP modification in myoglobin, ubiquitin, β-lactoglobulin and α-synuclein

In these experimental conditions fixed structure proteins were typically oxidatively modified by just one +16 Da after FPOP due to large parts of the protein remaining inaccessible to hydroxy radicals. Figures 2 and 3 show horse myoglobin after FPOP modification. Theoretical mass spectra were generated in order to confirm measured mass to expected masses. Deconvolution using the ReSpect algorithm enabled accurate data interpretation using isotopic separation of the proteins compared to a standard deconvolution approach using average mass.

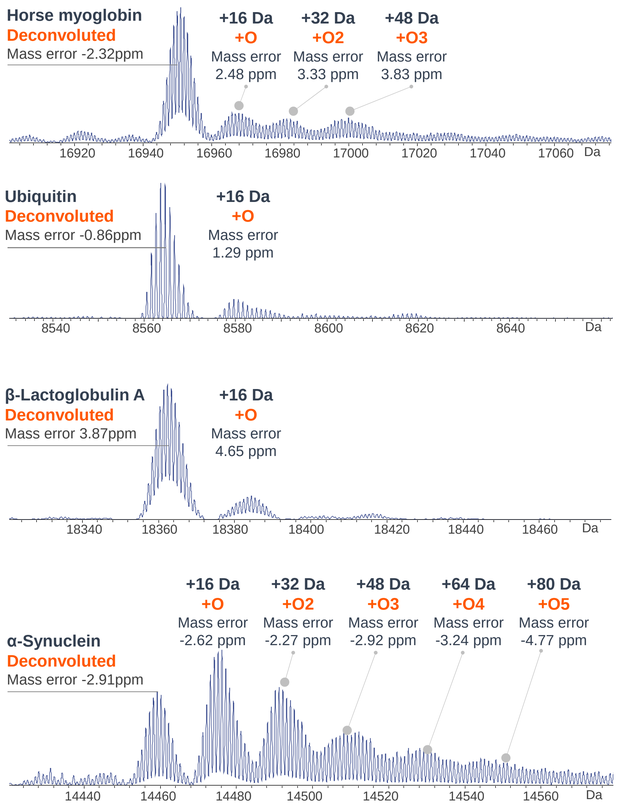


Figure 4. Uncharged deconvoluted mass spectra of three fixed structure proteins and the IDP, α-synuclein following FPOP modification. β-lactoglobulin A (RT 2.817 min) showed comparable levels of oxidative modification to β-lactoglobulin B (RT 2.778min).

3.2 FPOP applied to protein structure and dynamics

FPOP uses hydroxyl radical labeling to probe the surface-accessible regions of proteins and has a number of important advantages.
Key points include;

- Resulting covalent modifications are irreversible enabling downstream analysis by HRAM LC-MS/MS
- Microsecond-millisecond timescale
- Wide application scope (protein folding, membrane proteins, structure elucidation, and epitope mapping)

In this study the advantages of FPOP highlighted differences between three fixed structure proteins whereas the fourth protein, α-synuclein, was an intrinsically disordered protein.

Key points include;

- In fixed structure proteins (myoglobin, ubiquitin, β-lactoglobulin) the level of oxidation is lower compared to α-synuclein (an intrinsically disordered protein).
- During FPOP modification an IDP is thought to undergo higher levels of oxidation compared to fixed structure proteins due to the IDP changing conformation during the chemical process. In these experiments up to five oxidative forms of α-synuclein were generated with the unmodified form showing lower relative intensity to the +1 oxidised form.
- There is growing evidence that IDPs play an important role in biological processes and Lewy bodies, the main pathogenesis marker of PD, are thought to be formed as a result of the protein having no fixed structure.
- Intact LCMS analysis of FPOP modified proteins enables a simple mechanism to provide evidence supporting the hypothesis that a protein may be an IDP and therefore of interest for research such as cell signaling.

4. Conclusions

- FPOP provides the unique capability to obtain residue-level resolution data on protein structures enabling investigations of fast processes such as protein folding and weak protein–protein interactions.
- Native LCMS analysis of FPOP modified proteins demonstrated the ability to differentiate between fixed structure proteins to a known IDP α-synuclein.
- This relatively simple technique may prove to be a useful tool in future studies providing evidence of IDP for previously uncharacterized proteins.

5. References

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