

Abstract (In English)

The reaction of highly reactive oxygen radicals with protein solvent-accessible residues can be utilized to map protein landscape. Fast photochemical oxidation of proteins (FPOP) is an MS-based technique, which utilizes highly reactive radical species to oxidize proteins and map protein surface or its interactions with their interaction partners.

In this work, FPOP was employed to study protein-DNA interactions. First, a full-length of FOXO4-DBD was successfully expressed and purified. The ability of the protein to bind its DNA-response element was verified by electrophoretic and MS-based techniques, respectively. Optimal experimental conditions were achieved to oxidize the protein itself and in the presence of DNA, respectively. Oxidized samples were analyzed by bottom-up and top-down approach.

In the bottom-up experiment, modification of individual residues was precisely located and quantified. Different extend of modification was observed for protein alone and in complex with DNA. To avoid experimental artifacts analyzing multiply oxidized protein, standard bottom up approach was replaced by a progressive top-down technology. Only a singly oxidized protein ion was isolated, and further fragmented by collision-induced dissociation (CID) and electron-capture dissociation (ECD), respectively. Quantifying the extent of modification of neighboring sequence ions enabled identification of protein region shielded by DNA. Even the bottom-up approach reached better spatial resolution, both techniques pointed out the same protein regions responsible for DNA binding that are in agreement with previously published crystal structural model and hydrogen-exchange experiments of FOXO4-DBD•DAF16 complex. Our results indicate, that both bottom-up and top-down approaches are useful for probing protein-DNA interface. While bottom-up is able to reach single residue spatial resolution analyzing modified protein on peptide level, top-down allows gas phase purification of singly oxidized protein for further analysis.

Key words: Fast photochemical oxidation of proteins, protein-DNA complexes, FOXO4, transcription factor, quench flow system, protein footprinting, mass spectrometry, bottom-up, top-down.