Developing effective reagents for tyrosine bioconjugation

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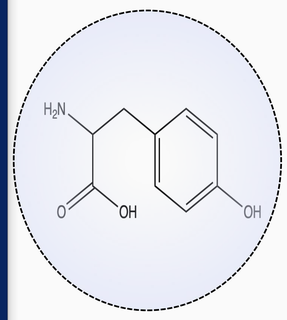
OVERVIEW

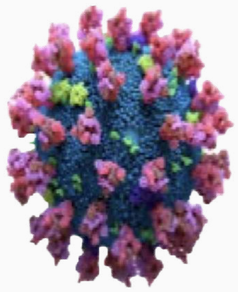
- We evaluated the site-specific labeling of tyrosine residues employing 4-phenyl-3H-1,2,4-triazole-3,5(4H)-dione (PTAD) as the bioconjugation reagent.
- Our study provides an extensive structural proteomics investigation of labeling tyrosine in samples of varying complexity ranging from peptides and proteins to cell lysates.
- We used the tandem mass spectrometry experiments to locate and characterize the labeled tyrosine residues.

INTRODUCTION



Tyrosine amino acid

Mutations in diseases like SARS-COV2



- Tyrosine residues are uniformly distributed in proteins, either on surface areas or embedded within hydrophobic cores.
- They can be enriched and frequently serve as the strongest interaction points at protein interfaces, which are known as hot spots and bind small molecules, nucleic acids, or protein partners.
- Site-specific modifications of peptides and proteins by chemical bioconjugation provide valuable information about their biological function.

METHODOLOGY

- We achieved the bioconjugation of tyrosine residue in intact peptides and proteins using PTAD in PBS buffer (pH 7.4) under physiological conditions, followed by their tryptic digestion, desalting, and MS analysis.
- PTAD labeling of tyrosine residues was validated using proteome discoverer software.

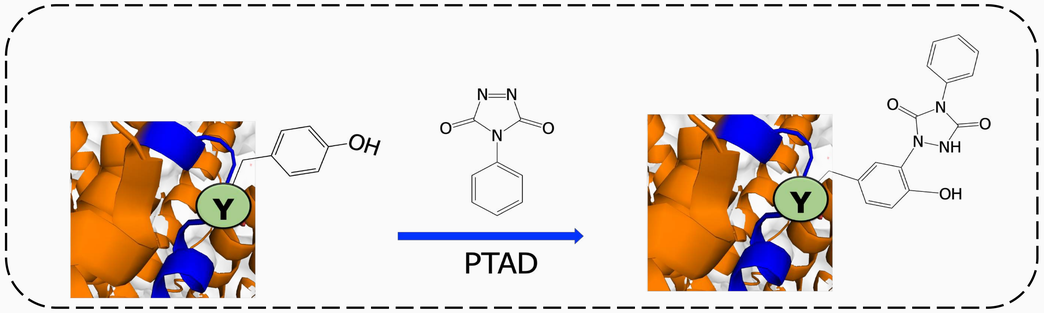
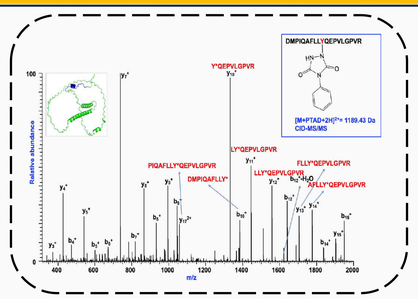
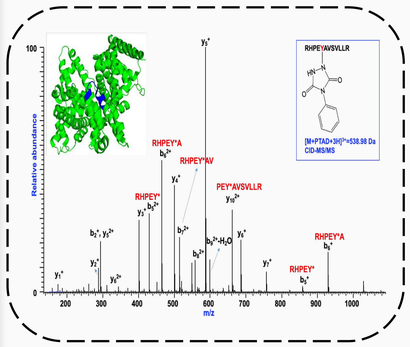
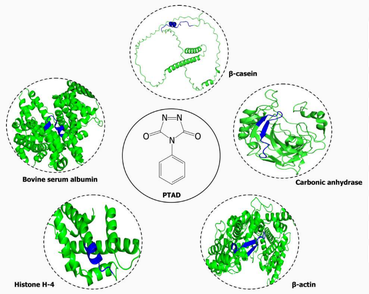


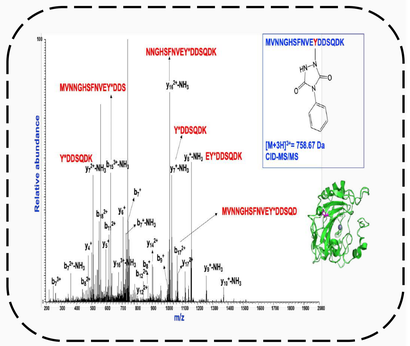
Fig 1: Schematic representation of tyrosine labeling using PTAD

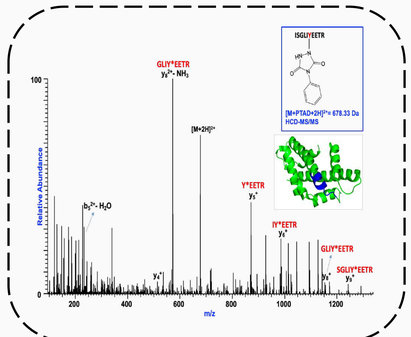
RESULTS

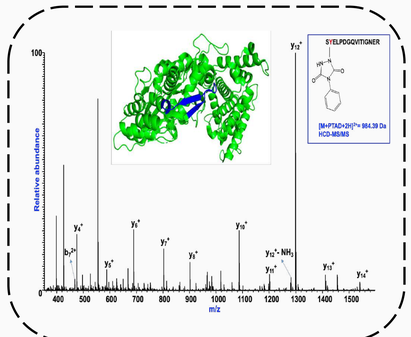












CONCLUSIONS

- This works explores the utility of PTAD to tag tyrosine residues across peptides, proteins and cell lysates.
- Experimental conditions were optimized to ensure optimal tyrosine conjugation keeping under consideration the potential for this type of biocompatible ene-ligation process to also be affected by hydrolysis.
- Upon visualizing the tagged tyrosine sites in proteins, we found that a majority of the tagged tyrosine residues were located on the periphery of the protein.
- We further aim to utilize the PTAD-based reagent to probe protein-protein interactions.

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