**Overview**

To probe conformational changes of the Ca²⁺-binding protein, Calmodulin by FPOP. To improve FPOP method by introducing new quencher.

**Results**

- FPOP successfully probes conformational changes of calmodulin caused by binding to Ca²⁺ and to two small peptides.

**Introduction**

Fast Photochemical Oxidation of Proteins (FPOP) uses hydroxyl radicals to oxidize solvent-exposed residues of protein. Pulsed laser beam dissociates hydrogen peroxide into hydroxyl radicals that react with solvent-exposed, reactive amino acid residues. Radicals are formed in ~10 ns and self-quench in 100 μs. Different radical scavengers limit radical lifetimes to the ~10 ns and shorter timescales, allowing fast oxidation and surface mapping of reactive residues on a protein.

**Methods**

- Enzyme Digestion
- LC-MS/MS
- TIC F: FTMS + p NSI Full

**Results**

Different radical scavengers were used to limit radical lifetime. Based on theoretical calculation, when Methionine is a scavenger, Lifetime of radical is around 1 microsecond. For Glutamine as a scavenger (Experimental data was shown on Yellow bar), Lifetime of radical is around 10 microseconds.

**Conclusions**

Radical lifetime can be limited by different scavengers. Higher extent of oxidation observed at longer radical lifetime, suggesting that FPOP can be used for kinetic studies. Different extents of oxidation for apo and holo Calmodulin occur, indicating that conformational changes occur with binding to Calcium. Several regions of protection occur for protein binding to small peptides, and FPOP is able to indicated this, in accord with 3D structure. Thus, FPOP can be employed to probe structural changes associated with protein-ligand binding.

**Future**

- Use different enzymes to improve the peptide coverage.
- Develop means to avoid post FPOP oxidation of Methionine residues in protein.
- Apply FPOP to other protein-ligand systems.

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