Determination of ligand and pH-induced conformational changes in the cation-independent mannose-6-phosphate receptor by fast photochemical oxidation of proteins

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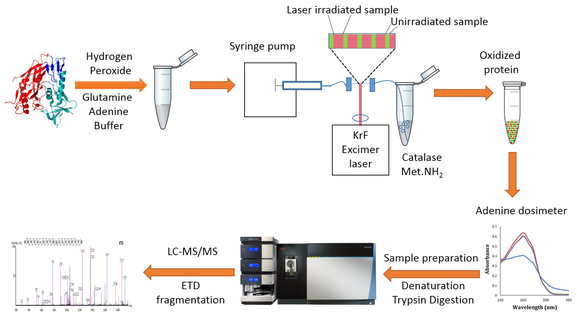
Overview

- The Cation Independent Mannose 6-Phosphate Receptor (CI-MPR) is a member of the P-type lectin family.
- CI-MPR functions by binding to proteins bearing *N*-linked oligosaccharides modified with a phosphomannosyl residue.
- A major function of the CI-MPR is to direct vesicular transport of proteins between the Golgi or cell surface to the early/late endosome (pH <6.0) where they are packaged into newly forming lysosomes.
- Fast photochemical oxidation of proteins mapped two binding sites of CI-MPR with the lysosomal enzyme palmitoyl-protein thioesterase 1 (PPT1): the interface of domain 1 and 3, and domain 5. Allosteric change causes the exposure of domain 4 to the solvent.
- Low pH (such as occurs during endosomal maturation) causes substantial changes in the protein conformation.

Introduction

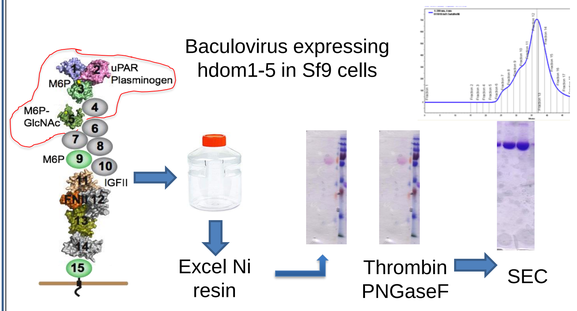
The Cation-Independent Mannose 6-Phosphate Receptor (CI-MPR) is a 300 kDa multi-functional protein which plays a central role in many cellular processes: autophagy, development, tumor suppression and generation of lysosomes. CI-MPR has a large extracellular region comprised of 15 domains. Mannose-6-phosphate is a key targeting signal for acid hydrolases destined for transport to lysosomes. CI-MPR targets about 60 different phosphomannosyl-containing acid hydrolases to the lysosome. The low pH of the endosome causes CI-MPR to release its cargo. Some, but not all domains of the CI-MPR have known functions. In order to understand the role of domains 1-5, a recombinant protein containing these domains was overexpressed in baculovirus and purified. Fast Photochemical Oxidation of proteins (FPOP) was employed to understand the conformational changes induced by low pH and lysosomal enzyme binding (PPT1).

General workflow for FPOP



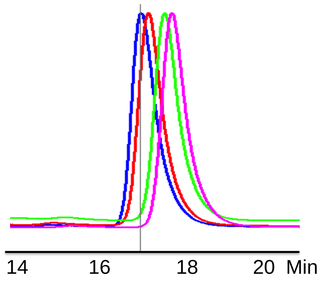
- Glutamine - radical scavenger, Adenine - radical dosimeter, allows for correction for differences in buffers, ligands, etc.
- Photolysis of peroxide at 248 nm by KrF excimer laser.
- Quenching by catalase and methionine amide.
- Solvent protection by ligand binding will result in reduced oxidation and solvent exposure will result in increased oxidation of the peptide.

CI-MPR overexpression and purification



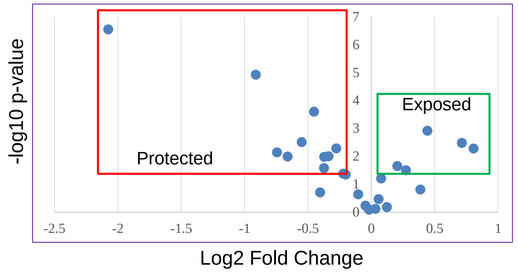
- CI-MPR containing domains 1-5 overexpressed in Sf9 cells using baculovirus expression vector.
- Purified by Ni-NTA Chromatography and then cleaved by thrombin and treated with PNGaseF.
- Final step of purification involved size exclusion chromatography.

Conformational changes induced by low pH



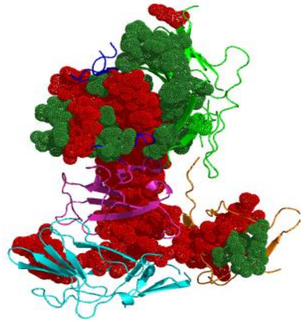
Size exclusion of CI-MPR at pH 6.5, 5.5, 4.8, and 4.5

- Hydrodynamic radius decreases as the pH decreases more acidic suggesting the protein undergoes conformational changes and becomes more compact.



Volcano plot of the –log10 p value and fold change of CI-MPR at pH 6.5 and pH 4.5

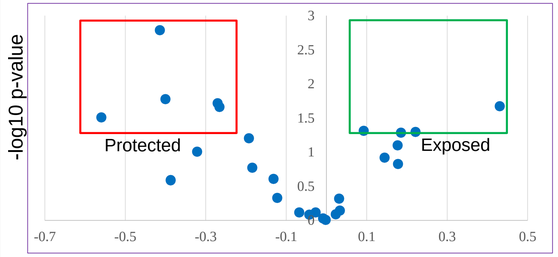
- There is a widespread conformational changes in the protein at pH 4.5 as compared to the pH 6.5.
- The individual domains of dom1-5 (with the exception of dom3) are much less accessible to hydroxyl radical.



Modeling of the FPOP data on CI-MPR

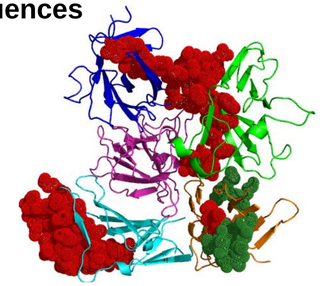
- Peptides less reactive at pH 4.5 as compared to pH 6.5 are colored red and peptides more reactive are in green.

Sites of PPT1 binding and allosteric consequences



Volcano plot of the –log10 p value and fold change of CI-MPR oxidation without and with PPT1.

- Peptides from all the domains were detected.
- Five peptides showed decreased solvent accessibility and two peptides showed increased solvent accessibility.
- Peptides from domain 1 and 5 show decrease in solvent accessibility.



Modeling of the FPOP data on CI-MPR

- Peptides represented by red mesh were less reactive in presence of PPT1, green mesh peptides were more reactive.
- Domains 1, 3 and 5 bind to PPT1 leading to decreased oxidation and allosteric changes in domain 4 results in its solvent exposure.

Conclusions:

- pH causes widespread conformational changes in the protein.
- Domain 5 of CI-MPR binding to PPT1.
- No other protected patch available.
- Can CI-MPR bind with two carbohydrates simultaneously?

Acknowledgements

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