

ICPL Labeling in Functional Proteomics Experiments: Substrate Identification of the Extracellular Protease ADAMTS1 using SDS-PAGE LC-MS/MS

● ABRF 2008, P62 - M

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Overview

- Study of protein – protein interactions in pathological reaction pathways
- Stable Isotopic Labeling of intact proteins was applied for reliable quantitation in an SDS-PAGE LC-MS/MS workflow
- New substrates of a metalloproteinase were identified

Introduction

Proteolytic modification of components of the extracellular milieu by metalloproteinases plays an important role in the regulation of multiple cellular and physiological processes and pathological conditions. ADAMTS1 (A Disintegrin And Metalloprotease domain with Thrombospondin motifs 1, Fig. 1) is a secreted enzyme of the ADAMTS family of proteases, which is related to angiogenesis, inflammation, and cancer. We describe a proteomic screening for ADAMTS1 substrates using an ICPL based workflow (Fig. 2, Fig. 3).

Methods

Conditioned media proteins from 293T cells, parental and overexpressing ADAMTS1, were concentrated by UF and labeled with ICPL: ¹³C₆ (Heavy) parental /¹²C₆ (Light) ADAMTS1 overexpressing cells. The mixture of the two labeled samples was separated by SDS-PAGE. The gel lane was cut in 20 slices that were subjected to in-gel trypsin digestion. Each digest was analyzed by nano RPLC-MS/MS on a Bruker Esquire HCT Ion Trap. Quantitation of relative protein abundances was performed on the basis of the signal areas of labeled peptide pairs, using WARP-LC 1.1 as integrated software platform for LC-MS/MS workflows.

Results

- The ICPL based 1D-SDS LC-MS workflow used allowed an efficient quantitative comparison of the analyzed proteomes. A total of 827 proteins were identified on the basis of 2001 peptide IDs (Fig. 4). Quantitative data was obtained for 511 proteins (61%), based on 1254 labeled peptide IDs.
- Labeling was highly efficient, as almost no peptides with non-derivatized lysine residues were observed.

- In agreement with previous experiments using DIGE (data not shown), basal lamina Nidogen 1 and 2, were identified as putative substrates of the ADAMTS1 protease.
- The ICPL strategy provided the ID of 6 new putative substrates.
- Due to the top-down analysis strategy (SDS-PAGE) proteolytic degradation was monitored using ICPL. Intact protein band showed different regulation than proteolytic fragments (Tab. 1).

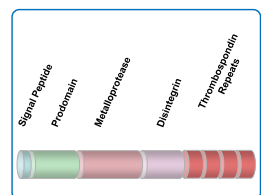


Fig. 1 Domain Structure of ADAMTS1

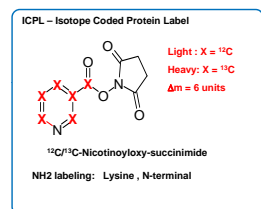


Fig. 2 ICPL Labeling chemistry for quantitative proteomics

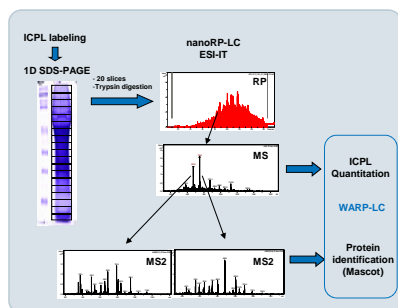


Fig. 3 Experimental Workflow. ICPL permits protein pre-fractionation and quantification via MS spectra. Regulated peptides are preferred precursors in AutoMS(n) measurements, which lead to protein identification with Mascot.

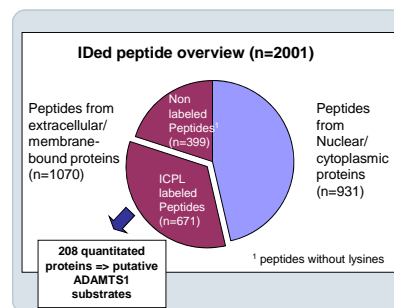


Fig. 4 Summary of the proteomic analysis results. Only extracellular and membrane proteins (~45%) were considered as putative substrates of the protease for further analysis.

Protein	FL full length PF: proteolytic fragment	Mapped region (total aminoacids)	Obs. Mw (kDa)	Total peptides	Labeled Peptides	ADAMTS1/C ₆ label ratio (L/H ICPL) (StdDev)	ADAMTS1/C ₆ label ratio (DIGE)
ADAMTS1	FL		105	78	72	~20	10
Nidogen 1	FL	124-1247 (1247)	180	9	2	1.85 (0.04)	1.61
Nidogen 1	PF1	424-1247 (1247)	1116	21	6	2.69 (0.35)	3.25
Nidogen 1	PF2	124-176 (1247)	40	18	7	1.88 (0.40)	
Nidogen 2	PF1	309-968 (1175)	180	10	2	2.19 (0.40)	
Nidogen 2	PF2	91-171 (1175)	38	4	1	2.05	1.86
Fibulin-1	PF1	396-576 (703)	38	10	6	3.24 (0.37)	
Fibulin-1	PF2	182-619 (703)	55	8	2	3.32 (1.03)	
Calretinin 1	PF	137-484 (981)	80-90	15	11	1.23 (0.33)	
Beta-amyloid protein precursor (APP)	PF1	103-516 (770)	55	5	3	1.33 (0.42)	
Beta-amyloid protein precursor (APP)	PF2	117-535 (770)	80	9	3	1.24 (0.16)	
Nucleohindin-1	FL	54-461 (461)	55-60	20	10	-1.44 (0.10)	
Nucleohindin-1	PF1	97-310 (461)	40	19	10	1.71 (0.40)	
Nucleohindin-1	PF2	179-377 (461)	25	10	5	3.14 (0.78)	
Insulin-like growth factor binding protein 2	FL	45-320 (328)	36	12	5	-3.56 (0.10)	
Insulin-like growth factor binding protein 2	PF1	45-240 (328)	15	7	2	5.42 (0.73)	
Insulin-like growth factor binding protein 2	PF2	284-304 (328)	9	3	2	1.67 (0.02)	
Legumin	FL	45-403 (433)	55	7	4	-2.88 (0.08)	
Legumin	PF1	318-433 (433)	20	8	5	4.69 (0.95)	

Tab. 1 Putative substrates of ADAMTS1 and IDED proteolytic fragments. Fragments exhibit opposite "regulation" as parent proteins.

Conclusions

- ICPL labeling chemistry in combination with SDS-PAGE LC-MS/MS allowed the identification of new putative substrates of metalloproteinase ADAMTS1.
- The proteolytic fragments were assigned based on their MW in SDS-PAGE, their significant up regulation in comparison to the down regulation of the full length sequences, and their reduced coverage of the parent protein sequence.

Ion Trap MS