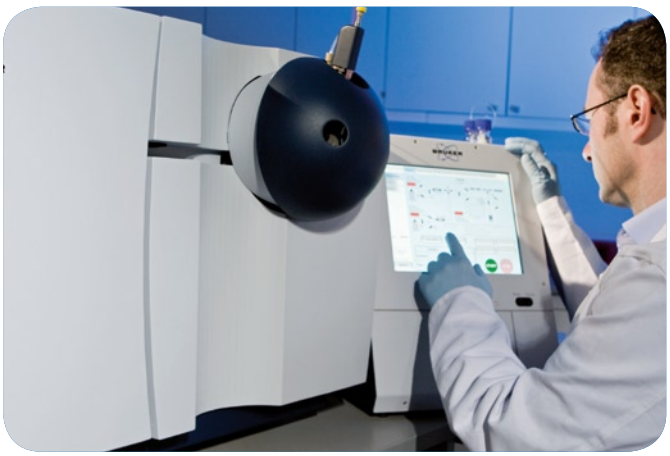
Bruker Daltonics



Application Note # LCMS-56

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

Abstract

Isotope-Coded Protein Label (ICPL [1,2]) is known as an accurate protein labeling strategy for quantitative proteomics which enables protein pre-fractionation and therefore provides reduction of complexity, detection of protein isoforms, posttranslational modifications, and investigations of enzymatic reactions. Such an investigation is demonstrated in a workflow using SDS-PAGE for protein pre-fractionation, a Bruker Ion Trap mass spectrometer, and ProteinScape™ for quantitative analysis.

Introduction

Proteolytic modification of components of the extracellular milieu by metalloproteinases plays important roles in the regulation of multiple cellular and physiological processes and pathological conditions. Exploring the substrate repertoire of metalloprotenaises is required to unveil the mechanisms involved in their biological function. ADAMTS1 (A Disintegrin and Metalloprotease domain with Thrombospondin motifs 1), is a secreted enzyme of the ADAMTS family of proteases, which is related to angiogenesis, inflammation, and cancer [3]. We describe a proteomic screening for ADAMTS1 substrates by analyzing the protein profiles obtained from cultures of transfected cells overexpressing the protease as compared to parental cells. The secreted proteins from the two cell lines were modified with two different isotopomers of the non isobaric ICPL duplex label, mixed and quantitatively compared in a SDS-PAGE LC-MS/MS workflow. Seven proteins were identified as putative substrates of ADAMTS1.

Experimental

Conditioned media from 293T cells (human embryonic kidney), parental and overexpressing the secreted protease ADAMTS1, were collected. Proteins were concentrated by UF, then subjected to TCA-acetone precipitation, resuspended in guanidinium chloride buffer and quantified. 100 micrograms of each sample were then labeled with ICPL. The ICPL reagent is shown in Figure 1. Parental cell proteins were labeled with the heavy isotopomer of ICPL doublex, and ADAMTS1 overexpressing cells with the light isotopomer of ICPL. The mixture of the two labeled samples was separated by SDS-PAGE. The gel lane was cut into twenty fractions and subjected to in-gel trypsin digestion. Each digest was analyzed by nano RPLC-MS/MS on a Bruker Ion Trap system. Quantitation of relative protein abundances was performed based on the chromatographic signal intensities of labeled peptide pairs, using ProteinScape as integrated software platform for quantitative LC-MS/MS workflows. The applied workflow is visualised in Figure 2.

Results

The ICPL based 1D-SDS LC-MS workflow allowed an efficient quantitative comparison of the analyzed proteomes. A total of 827 proteins were identified on the basis of 2001 peptide IDs. Quantitative data were 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 (>99%). In agreement with previous experiments using DIGE [2], basal lamina Nidogen 1 and 2, were identified as putative substrates of the ADAMTS1 protease. With the ICPL strategy provided here, the ID of new putative substrates was achieved. These results are summarized in Table 1. Using a quantitative top-down analysis strategy (SDS-PAGE) proteolytic degradation can be detected: A substrate protein occurs in several bands and shows in the intact protein band a different regulation than in the bands of the proteolytic fragments. Figure 3 shows Nidogen-1 as example, which was identified in several fractions: In fraction 2 we observe the intact protein, in fraction 3 the high mass proteolytic fragment PF1 and in fraction 10 the low mass proteolytic fragment PF 2. The abundance of the intact Nidogen-1 is about 2 times lower in the ADAMTS1 overexpressing cells, while in the fractions where its proteolytic fragments are observed, their abundance is increased 2 times compared to the control cells. The analysis of the band containing the Nidogen-1 proteolytic fragment PF1 is presented in Figure 4. By using ProteinScape for data processing, the complete information of the proteomic experiment, e.g. meta information of the experiment, the gel, the MS and MS/MS data as well as the derived identification and quantitation is stored and linked at a central place and can be easily retrieved with the aid of different views and queries.

ICPL – Isotope Coded Protein Label, Duplex

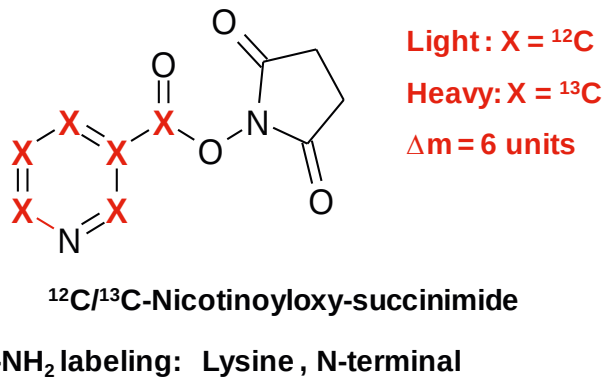


Figure 1: ICPL Duplex labeling chemistry for quantitative proteomics

ICPL workflow

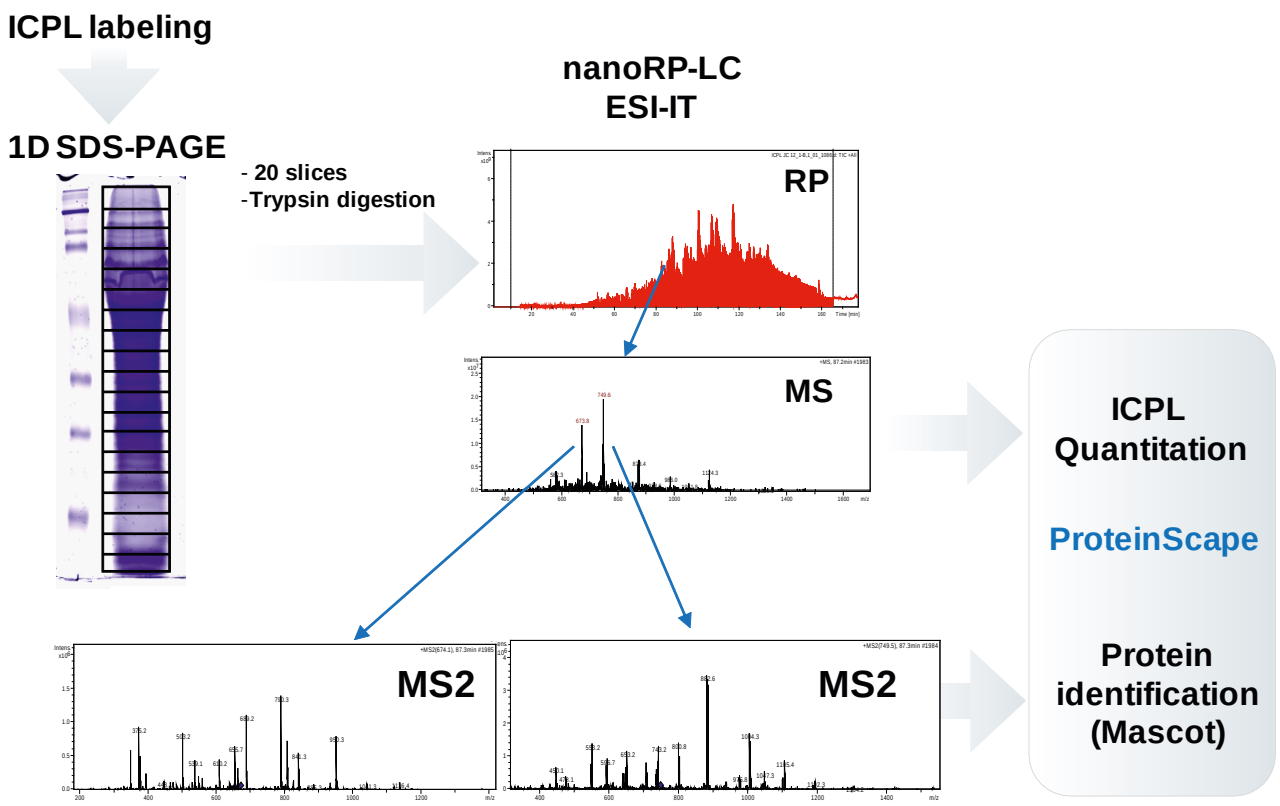


Figure 2: Experimental Workflow. ICPL permits protein pre-fractionation and quantification via MS spectra. Regulated peptides are preferred precursors in AutoMS(n) measurements, which lead to protein identification with Mascot.

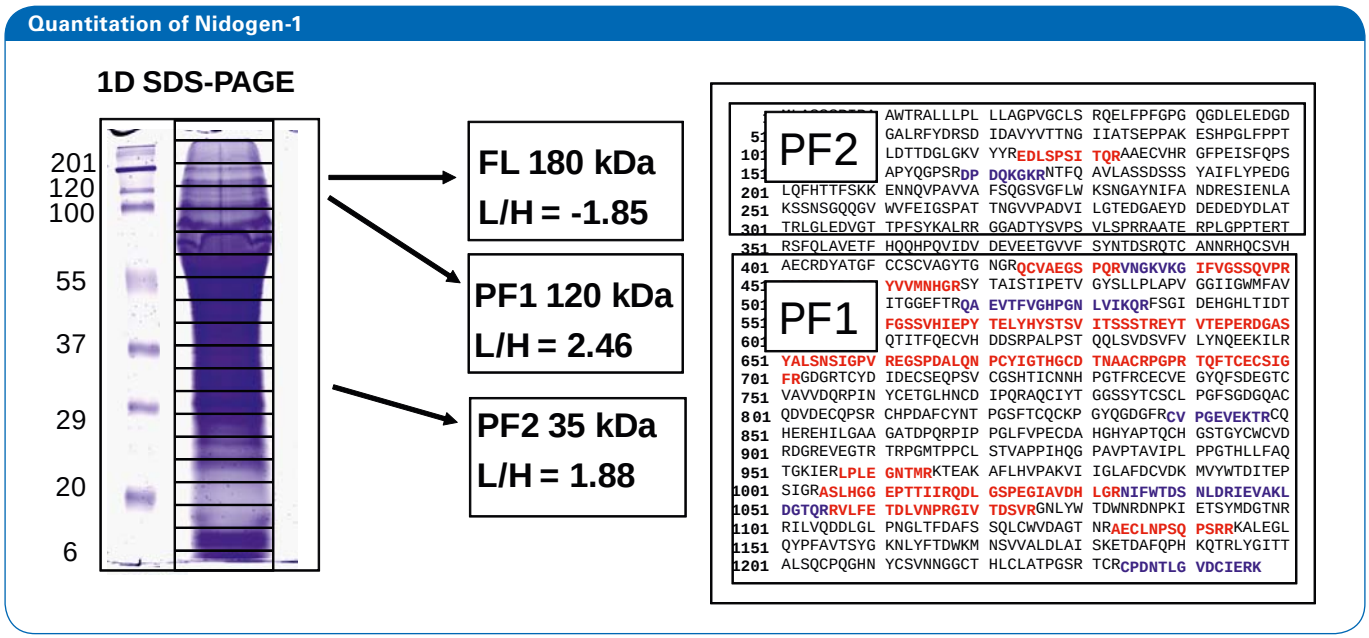


Figure 3: Identification and quantitation of Nidogen-1 in different bands of the 1D gel. Compared to the control, Nidogen-1 is down regulated in the fraction containing the intact protein (FL), while it is up regulated in the fractions containing the proteolytic fragments (PF1 and PF2).

Table 1: Putative substrates of ADAMTS1 and IDed proteolytic fragments. Fragments exhibit opposite regulation as parent proteins. The results are in agreement with previous experiments using DIGE [3].

Protein	FL: full length PF: proteo-lytic fragment	Mapped region (total aminoacids)	Obs. Mw (kDa)	Total Peptides	Labeled Peptides	ADAMTS1 / Control ratio (L/H ICPL) (StdDev)	ADAMTS1/ Control 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)	116	21	6	2.46 (0.74)	3.25
Nidogen 1	PF2	124-176 (1247)	40	18	7	1.88 (0.40)	
Nidogen 2	PF1	309-968 (1375)	180	10	2	2.19 (0.40)	
Nidogen 2	PF2	91-171 (1375)	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)	
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)	
Nucleobindin-1	FL	54-461 (461)	55-60	20	10	-1.44 (0.10)	
Nucleobindin-1	PF1	97-310 (461)	40	19	10	1.71 (0.40)	
Nucleobindin-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)	
Legumain	FL	45-403 (433)	55	7	4	-2.88 (0.08)	
Legumain	PF1	318-433 (433)	20	8	5	4.69 (0.95)	

ProteinScape stores the complete information of the experiment

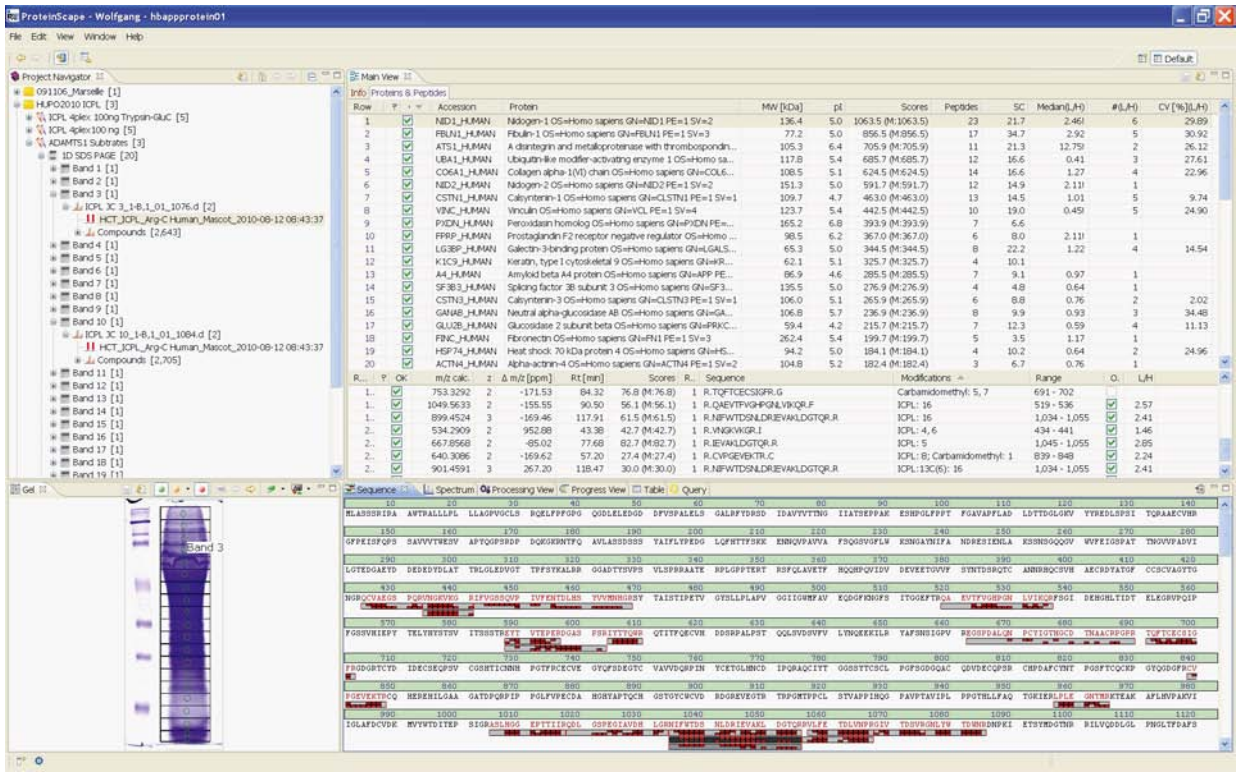


Figure 4: Analysis of the experiment using ProteinScape. The screenshot shows the Mascot Search result for the fraction containing the Nidogen-1 proteolytic fragment PF1. The software allows storage and linkage of the complete information gathered during a proteomic experiment at a central place. The gel separation, identification and quantitation of proteins and peptides of the selected band are displayed.

Conclusion

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.

Keywords
Cancer
ICPL labeling
Metalloproteinase
Quantitative Analysis
Functional Proteomics

Instrumentation & Software
Bruker Ion Trap mass spectrometer
ProteinScape

For research use only. Not for use in diagnostic procedures.

References

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