New Strategies for High Pressure-Assisted Digestion in Proteomics

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Overview

- Use of pressurized systems for protein digestion and ^{18}O labeling for quantitative proteomics
- Results obtained using different configurations of a fast on-line digestion system
- Demonstration of rapid on-line glycoprotein characterization in one mass spectrometric analysis, using two different enzymes in sequence (N-glycosidase, followed by a protease) in the fast on-line system
- Comprehensive whole cell processing (capture, lysis, protein reduction and alkylation, and trypsin digestion) with immediate peptide separation and mass spectrometric analysis
- The on-line system and applications represent the latest developments in rapid, reproducible on-line automatable proteomics workflows

Introduction

- Protein digestion for bottom-up proteomics has traditionally been performed using a relatively lengthy sample preparation process that typically includes an incubation period of 6-12 h.
- By using pressure, enzyme/protease kinetics increase dramatically and in some cases increase the number of peptide identifications.
- A natural extension for the use of kinetically enhanced proteomics using pressure is to use the system to also explore multi-enzyme combinations targeting different cellular systems and whole cell rapid proteome.

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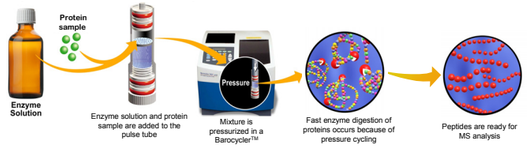
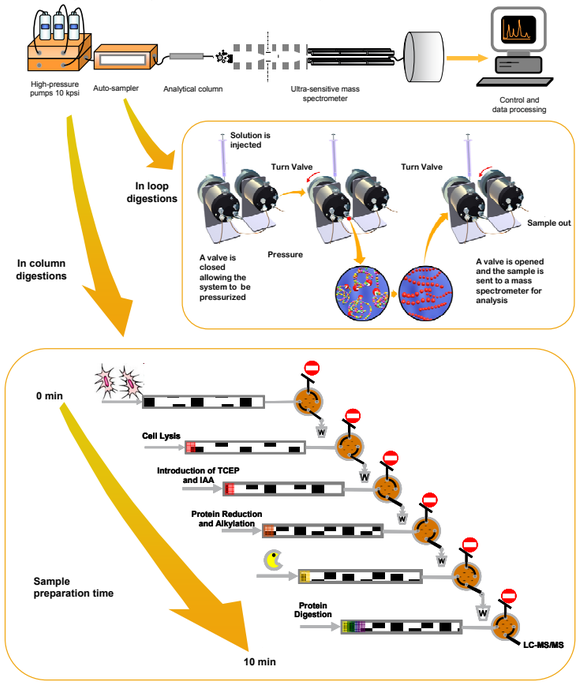
Methods

Pressure cycling protein digestion system (off-line method)



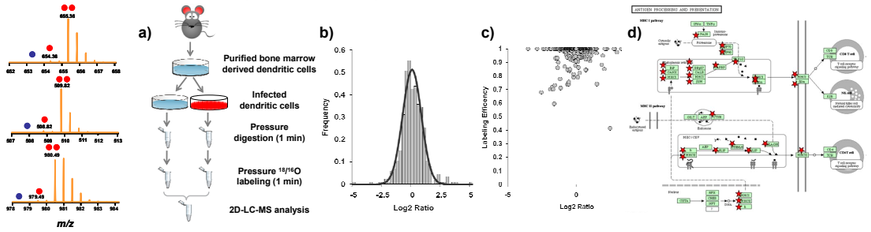
D López-Ferrer, et al. *J Proteome Res* 2008, 7:3276-3281.

High-pressure enzymatic protein digestion system (on-line method)



Results

Pressure assisted ^{18}O quantitative proteomics



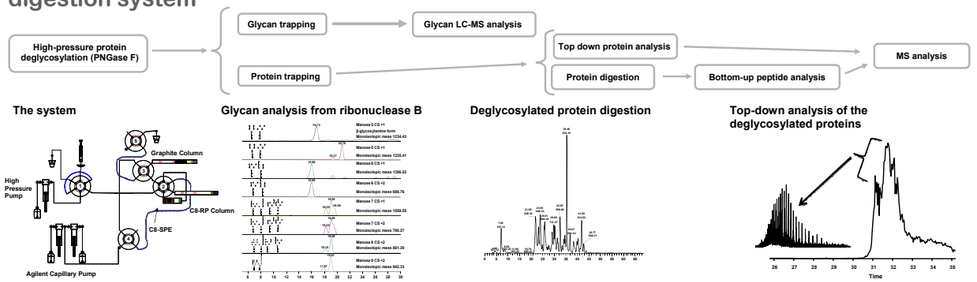
Evaluation of ^{18}O labeling performance using pressure. Spectra corresponding to BSA peptides after ^{18}O labeling under pressure for 1 min.

Application to quantitative proteomics. Proteomics analysis of dendritic cells identified 14,162 peptides resulting in 1558 unique protein identifications and of these 153 were involve in immune pathways. **a)** Workflow scheme for the comparative analysis of uninfected and influenza infected dendritic cells. **b)** Histogram of the $\log_2(^{16}O/^{18}O)$ ratios fitted to a Gaussian function. **c)** Plot of labeling efficiency versus $\log_2(^{16}O/^{18}O)$. **d)** Identified proteins from antigen processing and presentation pathway.

Table.1 Representative expression changes. The majority of the proteins up- or down-regulated following influenza infection play critical roles in the immune response.

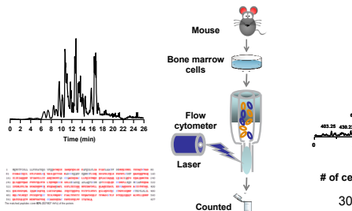
Protein	Literature support (1) with GO term process and function (2)	Peptide	Labeling efficiency	Log2(ER)	p value
prolyl 4-hydroxylase	Prolyl hydroxylases **negatively modulates IKKβ** catalytic activity **(1)**. Cell redox homeostasis;peptidyl-proline hydroxylation to 4-hydroxy-L-proline; procollagen-proline 4-dioxygenase activity;protein disulfide isomerase activity;isomerase activity **(2)**.	**NNFEGEITKEK**	0.87	**-6.19**	1.21E-10
Prosaposin	Prosaposin is modulated in **response to Brucella melitensis, B. neotomae and B. ovis in macrophage (1)**. Sphingolipid metabolic process;lipid metabolic process **(2)**.	**LVLYLEHNLEK**	1.00	**-4.69**	6.47E-06
transaldolase 1	Transaldolase 1 was identified **in activated monocytes from humans (1)**. Carbohydrate metabolic process;pentose-phosphate shunt;metabolic process;transferase activity;transaldolase activity;catalytic activity **(2)**.	**WLHNEDQMAVEK**	1.00	**-4.04**	2.44E-04
Vimentin	Vimentin **controls phagocytosis/vesicle traffic in immature DCs and migration/motility in mature DCs (1)**. Intermediate filament-based process: structural molecule activity;protein binding **(2)**	**EEAESTLQSFR**	1.00	**-3.30**	6.61E-03
cofilin 1	**Cofilin required for DC migratory speed (1).** Positive regulation of actin filament depolymerization;cell motion;cytokinesis;cell projection organization;establishment of cell polarity;negative regulation of cell size **(2)**.	**SSTPEEVKKR**	1.00	**1.07**	3.85E-03
ERO1-like	ERO1-Like protein alpha is stimulated by hypoxia and is likely to help maintain the transfer rate of oxidizing equivalents to PDI in situations of an altered cellular redox state **(1)**. Endoplasmic **reticulum unfolded protein response**;electron transport chain;protein thiol-disulfide exchange;oxidation reduction; electron carrier activity;FAD binding;oxidoreductase activity **(2)**.	**FDGILTEGEGPR**	1.00	**1.65**	2.83E-04
histone cluster 2, H3c1	Macrophages have increased expression of H3c1 in response to oxidative stress **(1)**. Nucleosome assembly; DNA binding **(2)**	**YRPGTVALR**	0.97	**1.79**	1.38E-04
filamin, alpha	**CD4 and the coreceptors interact with the filamin-A**, virus binding induces transient cofilin-phosphorylation and blockade of filamin-A interaction with CD4 and/or coreceptors **(1)**. Early endosome to late endosome transport; actin binding;protein binding;protein kinase C binding **(2)**.	**IQQNTFTR**	1.00	**2.12**	2.27E-05

Multiplex enzymatic digestions using the high pressure enzymatic protein digestion system

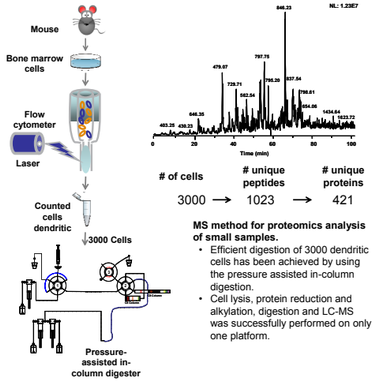


One of the most important goals in the field of proteomics/glycomics is to be able not only to perform glycan profiling, but also to indentify the glycosylated protein and identify the glycosylation site. By using the fast on-line in column digestion system this is possible. The system integrates the following functionalities: 1) glycan release with PNGase F solution, 2) capture of the released glycan, 3) glycan separation based on graphitized carbon chromatography, 4) nanoelectrospray into a mass spectrometer for glycan detection and quantitation, (5) concurrent protein digestion, and 6) peptide separation on a reversed phase column.

Proteomics analysis of intact dendritic cells using pressure-assisted in-column digester



BSA in-column digestion. Chromatogram and proteome coverage (~85%) achieved after BSA processing in the system. The steps consist of in column protein denaturation, derivatization, digestion, and LC-MS/MS analysis.



MS method for proteomics analysis of small samples.

- Efficient digestion of 3000 dendritic cells has been achieved by using the pressure assisted in-column digestion,
- Cell lysis, protein reduction and alkylation, digestion and LC-MS was successfully performed on only one platform.

Conclusions

- The pressure assisted ^{18}O quantitative strategy presented here using PCT represents a promising fast methodology for robust high-throughput digestion platforms.
- The in-column pressure digestion represents a unique capability to allow for the automation of proteomics workflows, as well as proteomics analysis of small samples.
- The combination of pressure on-line digestions, PNGase F and pepsin has been demonstrated to be an effective approach for fast protein and concurrent N-glycan characterization.

Acknowledgements

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