New Strategies for High Pressure-Assisted Digestion in Proteomics
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Overview

• Use of pressurized systems for protein digestion and 18O 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 tryptic digestion) with immediate peptide separation and mass spectrometric analysis
• The on-line system and applications represent the latest developments in rapid, reproducible on-line autotomable 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 proteomes.

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Methods

Pressure cycling protein digestion system (off-line method)

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

Results

Pressure assisted 18O quantitative proteomics

Table S1: Summary of protein identification

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide Count</th>
<th>Unique Peptide Count</th>
<th>Protein Count</th>
<th>Unique Protein Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>100</td>
<td>100</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vimentin</td>
<td>500</td>
<td>500</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>1000</td>
<td>1000</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Conclusions

• The pressure assisted 18O 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 peptic has been demonstrated to be an effective approach for fast protein and concurrent N-glycan characterization.

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N-glycan analysis
Conventional glycan analysis
High pressure digestion
LC-MS/MS