Development of a 20 kpsi Enzymatic Digester for High Throughput Proteomic Analysis and its Application to Membrane Proteomics

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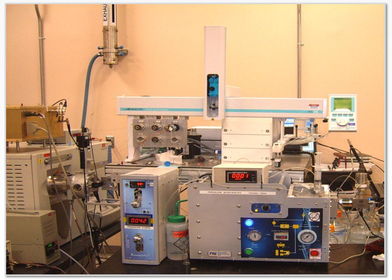
Overview

- Digesting proteins into peptides is an essential step in bottom-up proteomics. Recently, pressure assisted digestion using pressure cycling technology (PCT) was demonstrated to significantly speed time needed for digestions from hours to minutes.
- In this work, we developed a novel ultra-high pressure PCT reactor that couples to an LC-MS platform. The reactor consists of a pressure generator, a temperature controlled flow-through pressure cell, isolated from the flow path by high pressure valves.
- The system was tested initially with a pool of standard proteins and later with a set of digestion resistant proteins, including the hydrophobic membrane-spanning protein bacteriorhodopsin (*Halobacterium halobium*) and a water-insoluble fraction of *Shewanella oneidensis* proteins.



20 kpsi Enzymatic Digester (XStream™) configured with a custom LC system and a ThermoScientific LTQ-FT mass spectrometer.

Methods

Proteomics approach

4-protein standard
- BSA
- Beta-casein
- Cytochrome *c*
- Myoglobin

Shewanella oneidensis
- Insoluble fraction

Bacteriorhodopsin

All samples were dissolved in 25 mM NH_4HCO_3. The 4-protein standard was treated with 5 mM TCEP and 50 mM IAA, followed by sonication for 5 min at 50% power. *S. oneidensis* and bacteriorhodopsin were processed without any chemical treatment. Trypsin was added with the ratio of 1:20, weight-to-weight.

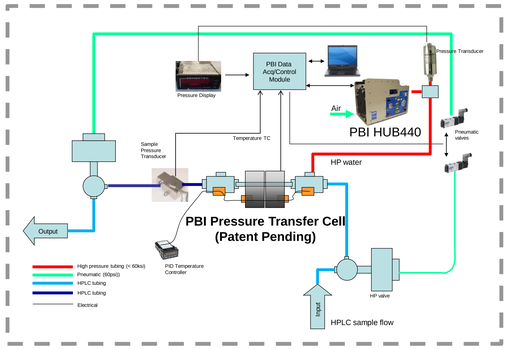
Overnight Digestion
(37°C, 12 h)
- *S. oneidensis* insoluble fraction

PCT Digestion
(20 kpsi)
- 4-protein standard : 5 min
- *S. oneidensis* insoluble fraction and bacteriorhodopsin: 20 min

LC-MS/MS – ThermoScientific LTQ-FT
Sample amounts injected into C18 trap column for LC-MS/MS:
- 4-protein standard: 4.5 µg total protein
- Insoluble *S. oneidensis* fraction: 5 µg total protein
- Bacteriorhodopsin : 1 µg total protein

SEQUEST Peptide/Protein Identification and Data Analysis

Pressure BioSciences XStream™ Ultra-high Pressure Digester



Results

4-protein standard

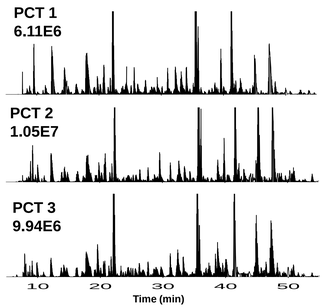


Figure 1. Base peak chromatograms for three technical replicates (PCT 1, 2, 3) of the 4-protein standard following digestion in the PCT reactor.

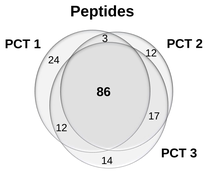


Figure 2. Number of peptide identifications (FDR<1%) for each of the three technical replicates.

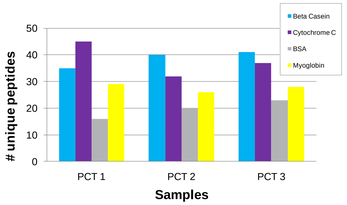


Figure 3. Number of unique peptide identifications for each technical replicate.

S. oneidensis

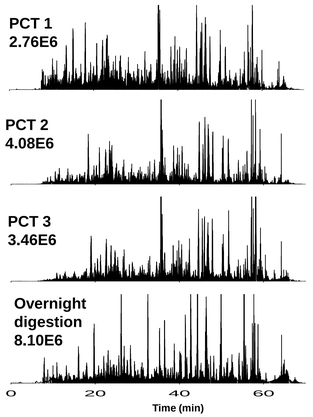


Figure 4. Base peak chromatograms for three technical replicates (PCT 1, 2, 3) of the *S. oneidensis* insoluble fraction following digestion in the PCT reactor, and one following overnight digestion for comparison.

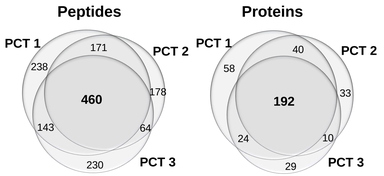


Figure 5. Number of peptide (FDR<1%) and protein identifications for each of the three technical replicates.

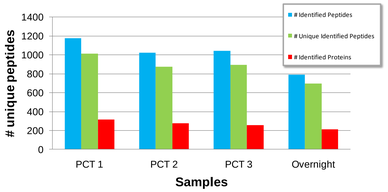


Figure 6. Number of unique peptide and protein identifications for the three PCT technical replicates and overnight digested sample.

Bacteriorhodopsin

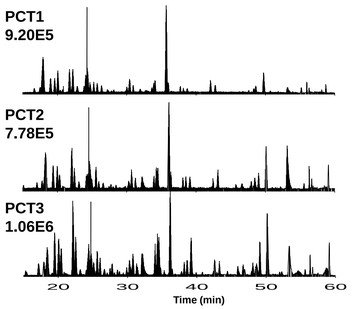


Figure 7. Base peak chromatograms for three technical replicates (PCT 1, 2, 3) of the bacteriorhodopsin standard following digestion in the PCT reactor.

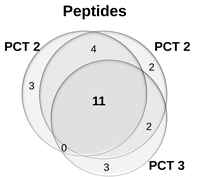


Figure 8. Number of peptide identifications (FDR<1%) for each of the three technical replicates.

>gi|15790468|ref|NP_280292.1| bacteriorhodopsin; Bop [Halobacterium sp. NRC-1]

MLELLPTAVEGVSQAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITTLVPAIA

FTMYLSMLLGYGLTMVPFGGEQNPIYWAR**YADWLFTTPLLLLDLALLVDADQGTILALVGADGI**

MIGTGLVGALTKVYSYRFVWWAISTAAMLYILYVLFFGFTSKAESMRPEVASTFKVLRNVTVVLWS

AYPVVWLIGSEGAGIVPLNIETLLFMVLDVSAKVGFGLILLRSRAIFGEAEAPEPSAGDGAAATSD

Cytoplasmic Transmembrane

Figure 9. Identified peptide sequences of bacteriorhodopsin and their annotation.

Conclusions

- A 20 kpsi enzymatic digester was developed and performance demonstrated using a 4-protein standard and hydrophobic membrane proteins.
- The digester was shown to be robust and compatible with nano-flow LC-MS/MS, enabling online enzymatic lysis of hydrophobic membrane proteins.
- For the *S. oneidensis* insoluble fraction, the sample digested for 20 min in the PCT reactor showed more protein identifications than the sample digested overnight.
- The ultra-high pressure PCT reactor facilitates proteomics research demanding high throughput technology, especially membrane protein analysis.
- Incorporation of protein digestion into a fully automated online LC-MS platform reduces sample handling errors and losses associated with offline processing methods.

Acknowledgements

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