

Global Deglycosylation in High Throughput LC-MS Glycomics: Comparing Microwave, Pressure, and Ultrasound Effects on PNGase F with Human Serum

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Overview

Enhancing the activity of Peptide: N-Glycosidase F (PNGase F) glycan release from human serum glycoproteins with enzyme reactors using a common substrate and a standardized purification pipeline.

- Enzymatic reactors examined
 - Microwave Energy Reactor
 - High Pressure Reactor (Barocycler)
 - Ultrasound Energy Reactor
 - Isothermal Reactor (reference, 37 °C)
- High and low enzyme concentrations were used to characterize the enhancement effect

Glycans were purified with graphitized carbon solid phase extraction, separated on graphitized carbon nanoflow columns, and analyzed with LC-LTQ-Orbitrap MS/MS.

Introduction

Enhancing the reaction rate of N-glycan release from proteins with PNGase F has become more desirable as glycomic profiling on increasingly large sample sets becomes of interest.

Optimizing the enzymatic release step in the pipeline is critical because effective enzymatic release of glycans will dramatically improve both glycan signal and glycan profile quality. Enhanced enzyme activity translates to the utilization of fewer units of enzyme in conjunction with decreased processing time.

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Glycan Sample Processing Pipeline

Serum Collection

- Commercial human serum (Sigma Aldrich)

Chemical Processing

- Protein denaturation
- Assisted PNGase F glycan release**
 - Isothermal Reactor (Eppendorf Corporation)
 - Pressure Reactor (Pressure BioSciences, Inc.)
 - Microwave Reactor (CEM Corporation)
 - Ultrasound Reactor (Hielscher USA, Inc.)
- Ethanol precipitation

Automated Gilson Solid Phase Extraction

- Graphitized carbon cartridge
- Gilson GX-274 liquid handler
 - 40 Samples per batch capacity

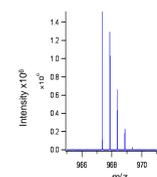


Liquid Chromatography-Mass Spectrometry

- Waters nanoACQUITY UPLC system
- Thermo LTQ-Orbitrap mass spectrometer

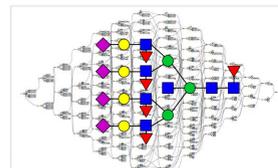
Data Processing

- External mass calibration
- Peak thresholding and centroiding
- “Smart summing” peak detection
- THRASH deisotoping with DeconTools

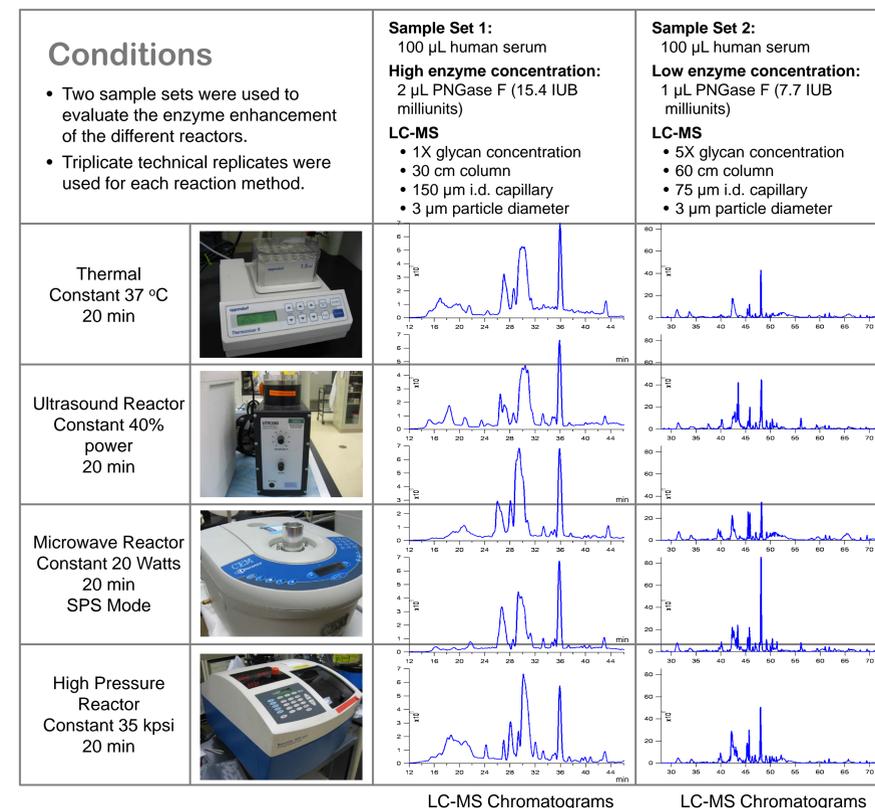


Glycan Profiling

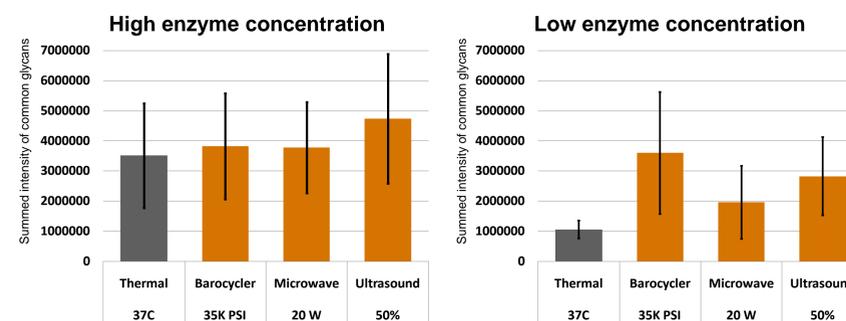
- Theoretical glycan library
- Exact mass analysis
- Monosaccharide differences



Results



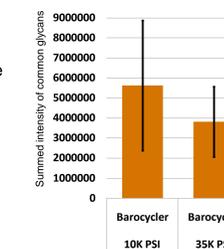
Reactor Performance



- Comparable performance across enzyme reactors in the high enzyme concentration case suggests that the enzyme concentration was in excess, allowing for all methods to perform equally well.
- By decreasing the enzyme concentration and preventing the reaction from going to completion in the reaction time window, the enhancement effects attributable to each of the reactors became more prominent.

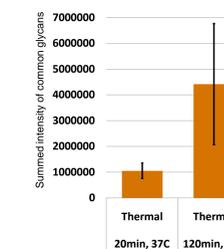
Reaction Pressure

- Operating the pressure reactor at very high pressures may denature the enzyme leading to decreased enzymatic activity.
- Although an optimal pressure still needs to be identified, the addition of moderate pressure enhances the enzymatic reaction.
- Under-pressurizing does not utilize the advantages of enzymatic pressure enhancement, while over-pressurizing denatures the enzyme.



Reaction Time

- Comparison of a 20 min digestion to a 120 min digestion under the low enzyme concentration condition showed that 20 min was not sufficient to release all the glycans.
- Longer enzyme incubation times need to be explored.
- However, shorter reaction times can be beneficial because they minimize side reactions and preserve sample integrity.



Glycan Profiling

Data	Calc	Error	Hex	Hex	Dxy	Neu	Average
(M/Z)	(M/Z)	(PPM)	Hex	NAC	Hex	Ac	Intensity
1234.433	1234.438	3.8	5	2	0	0	Neutral Aldehyde 903425
1275.460	1275.460	0.4	4	3	0	0	Neutral Aldehyde 2675035
1421.518	1421.524	4.1	4	3	1	0	Neutral Aldehyde 4282794
1566.555	1566.561	3.6	4	3	0	1	Neutral Aldehyde 17169854
1712.613	1712.620	3.8	4	3	1	1	Neutral Aldehyde 12795124
1728.608	1728.608	0.1	5	3	0	1	Neutral Aldehyde 1160493
1769.635	1769.644	5.2	4	4	0	1	Neutral Aldehyde 57191037
1874.666	1874.677	5.9	5	3	1	1	Neutral Aldehyde 4413949
1890.661	1890.671	5.1	6	3	0	1	Neutral Aldehyde 3280284
1915.693	1915.692	0.5	4	4	1	1	Neutral Aldehyde 13443288
1931.688	1931.684	1.7	5	4	0	1	Neutral Aldehyde 5345761
1948.703	1948.720	9.0	6	4	1	0	Neutral Aldehyde 3052970
1973.735	1973.710	12.6	4	5	2	0	Neutral Aldehyde 484247
2036.719	2036.729	5.1	6	3	1	1	Neutral Aldehyde 1784842
2077.745	2077.744	0.7	5	4	1	1	Neutral Aldehyde 18616181
2078.766	2078.759	3.2	5	4	3	0	Neutral Aldehyde 1054309
2093.740	2093.750	4.3	6	4	0	1	Neutral Aldehyde 13887200
2094.761	2094.779	8.8	6	4	2	0	Neutral Aldehyde 2399281
2118.772	2118.781	4.0	4	5	1	1	Neutral Aldehyde 5862821
2222.783	2222.780	1.4	5	4	0	2	Neutral Aldehyde 3653114
2239.798	2239.809	4.5	6	4	1	1	Neutral Aldehyde 3248715
2280.825	2280.816	3.8	5	5	1	1	Neutral Aldehyde 2216928
2296.820	2296.817	1.2	6	5	0	1	Neutral Aldehyde 1351142
2313.835	2313.855	8.6	7	5	1	0	Neutral Aldehyde 18269943
2368.803	2368.800	1.2	12	2	0	0	Neutral Aldehyde 595553
2368.841	2368.836	2.0	5	4	1	2	Neutral Aldehyde 58511
2369.861	2369.867	2.5	5	4	3	1	Neutral Aldehyde 1082616
2385.856	2385.866	4.2	6	4	2	1	Neutral Aldehyde 5509854
2426.883	2426.884	0.6	5	5	2	1	Neutral Aldehyde 6589289
2442.878	2442.887	3.9	6	5	1	1	Neutral Aldehyde 11089431
2587.915	2587.917	0.7	6	5	0	2	Neutral Aldehyde 4121681
2661.952	2661.947	1.8	7	6	0	1	Neutral Aldehyde 1853212
2733.973	2733.983	3.8	6	5	1	2	Neutral Aldehyde 7632163
2823.010	2823.031	7.5	6	4	3	2	Neutral Aldehyde 16241780
2879.011	2879.008	0.9	6	5	0	3	Neutral Aldehyde 1464220
2953.047	2953.055	2.6	7	6	0	2	Neutral Aldehyde 8464675
3025.069	3025.068	0.3	6	5	1	3	Neutral Aldehyde 81095
3885.396	3885.374	5.7	7	7	3	3	Neutral Aldehyde 6111608
4031.454	4031.426	6.8	7	7	4	3	Neutral Aldehyde 15870196
4176.491	4176.487	1.0	7	7	3	4	Neutral Aldehyde 5050705
4177.512	4177.514	0.6	7	7	5	3	Neutral Aldehyde 3305577

Conclusions

- Head-to-head comparisons of enzyme reactors were performed under controlled conditions.
- The high enzyme concentration set yielded comparable PNGase F activity in each reactor tested.
- The low enzyme concentration conditions demonstrated each reactor method provided an enhancement of enzymatic activity relative to the control.
- Follow-up experiments will be designed to produce optimal glycan signatures now that driving factors for enhancement of enzymatic activity have been identified.

Acknowledgements

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- The Glycozyler V2.0 software was used to process the glycan LC-MS datasets. DeconTools core libraries were used in part for data preprocessing.
- Theoretical and experimental retrosynthetic glycan network libraries facilitated the serum glycan assignments.
- 42 glycans were detected consistently across all datasets