Evaluation of Recombinant, Chemically Treated Trypsin in Proteomics and Protein Characterization Assays

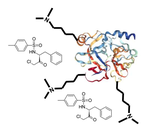
Anders Fridstrom, Jason Wrigley, Judy Boland, Nicolas Caffarelli, Gordon Nicol, Amber Henry, Jeffrey L. Turner, and Kevin Ray
MilliporeSigma, A business of Merck KGaA, Darmstadt, Germany, St. Louis MO 63103

Overview

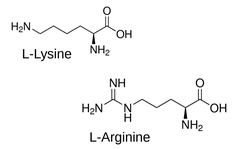
- We compared recombinant trypsin to native-source in various MS workflows.
- Native source trypsin is dimethylated to prevent autolysis and TPCK treated to inhibit chymotrypsin.
- Native and recombinant trypsins gave similar amounts of proteins and peptides identified in a complex mixture.
- TIC of a single-protein digest revealed more complete peptide processing by recombinant trypsins relative to native trypsin.
- Trypsin dimethylation leads to slower processing but with fewer autolytic peptides present.

Introduction

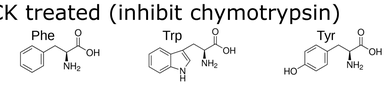
Porcine Pancreatic Trypsin



MODIFIED FROM:
PBD ID: 1AKS
Johnson, A., Krishnaswamy, S., Sundaram, P.V., Pattabhi, V. (1997) Acta Crystallogr D Biol Crystallogr 53 311-315

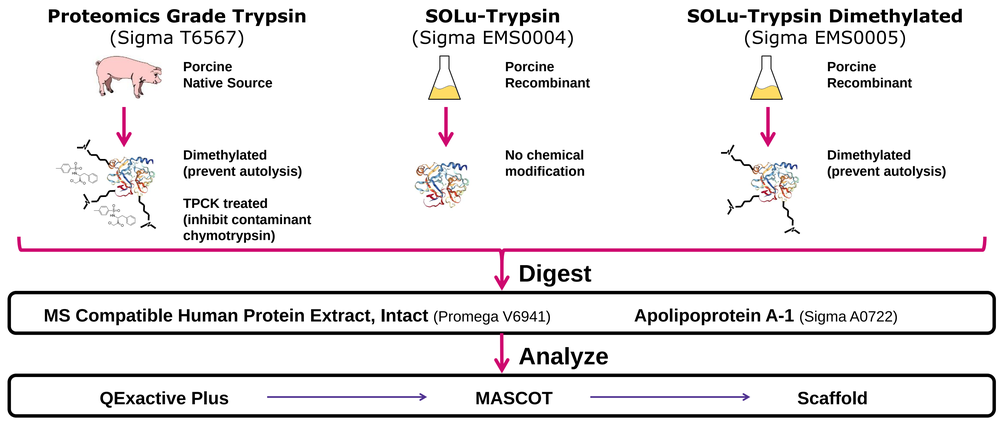


Proteomics grade trypsin is:
1. Dimethylated (prevent autolysis)
2. TPCK treated (inhibit chymotrypsin)



Are there better options?

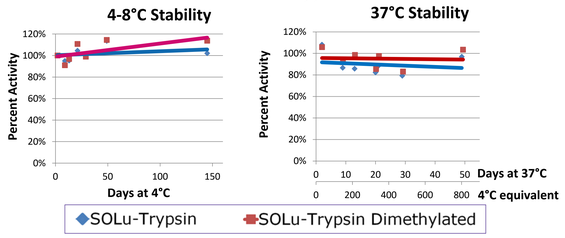
Materials and Methods



Results

SOLu-Trypsin Stability

(BAEE assay)



SOLu-Trypsin and SOLu-Trypsin Dimethylated are stable as supplied, in solution, for at least 2 years at 4-8°C based on accelerated stability studies.

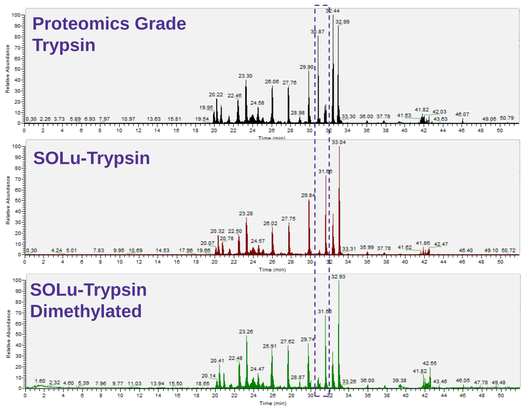
Human Protein Extract Digest

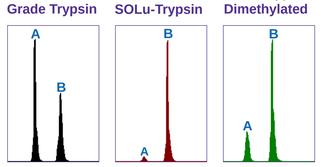
Trypsin	Average number of proteins identified	
	37°C for 18 hr	45°C for 2 hr
Proteomics Grade Trypsin	2,021	2,167
SOLu-Trypsin	2,055	2,128
SOLu-Trypsin Dimethylated	2,025	2,162

Human protein extract was digested using native and recombinant trypsins in a traditional overnight 37°C digest and a rapid 2 hr 45°C protocol. In all cases, similar numbers of proteins were identified.

Apolipoprotein A-1 Digest

(37°C overnight)





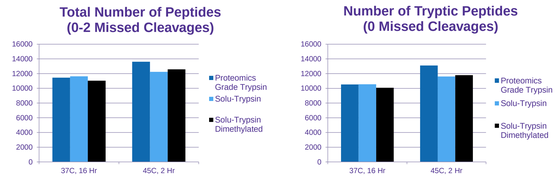
Extracted Ion Chromatogram

A: LREQLGPVTQEFWDNLEK
missed cleavage

B: EQLGPVTQEFWDNLEK
tryptic peptide

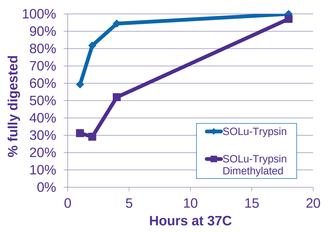
Individual peptides can be identified that are more fully processed by the recombinant trypsin, with or without dimethylation, versus the native trypsin.

To Dimethylate or Not Dimethylate



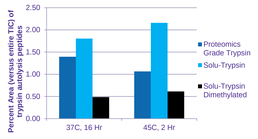
Human protein extract was digested using native and recombinant trypsins in a traditional overnight 37°C digest and a rapid 2 hr 45°C protocol. Slightly more peptides were identified in the 2 hr 45°C, but the results were similar regardless of trypsin source and dimethylation.

Conversion of DSGRDYVSQFEGSALGK to DYVSQFEGSALGK



Non-modified trypsin is capable of cleaving at a problematic site more quickly than dimethylated trypsin.

Trypsin Autolysis Peaks



SOLu-Trypsin Dimethylated produced significantly fewer trypsin autolysis peptides than the non-modified trypsins.

Conclusions

- Recombinant trypsin digests yielded comparable amounts of peptides and proteins identified across all conditions tested versus standard sequencing-grade trypsin.
- Dimethylation was shown to reduce the presence of autolytic fragments, whereas unmodified enzyme yielded faster processing of certain cut sites.
- SOLu-Trypsin is solution-stable for >49 days at 37°C and >800 days at 4°C based on accelerated stability studies.
- Recombinant solution-stable trypsin can be used in lieu of native trypsin with no changes to work flow.