Trypsin – a Tired Workhorse? The Selectivity of Atypical Cleavages by Trypsin

Meghan C. Burke, Yuxue Liang, Stephen E. Stein
National Institute of Standards and Technology, Gaithersburg, MD

National Institute of Standards and Technology
U.S. Department of Commerce
Mass Spectrometry Data Center

Introduction

- Results show that semi-tryptic peptide formation, distinct from in-source fragmentation, contributes to sample-specific variation in multiplexed analyses.
- Here, we perform in-depth characterization of both the variability and selectivity of atypical tryptic cleavage sites across more than 120 LC-MS/MS analyses.
- Although semi-tryptic peptides are generally lower in abundance than the fully tryptic form, they are found to comprise a significant fraction of the total peptide spectral matches (PSMs) thereby reducing the dynamic range of a given analysis.
- Our results show how NIST MS Metrics may be used to compare variation across samples to identify contributing factors in multiplexed experiments.

Semi-Tryptic Peptide Formation Contributes to Variation

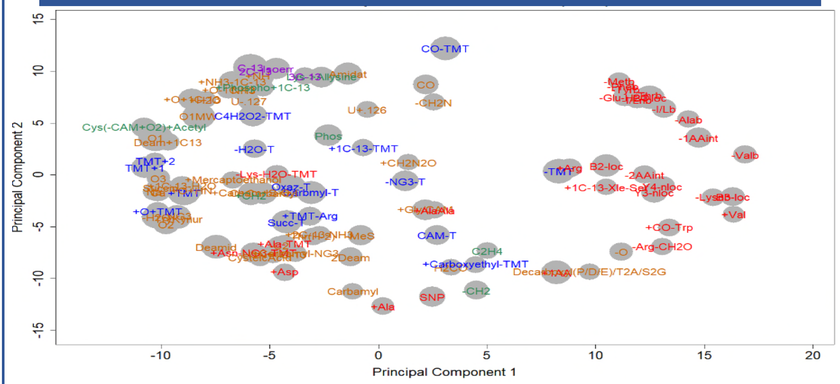

Figure 1: Principal component analysis of the 100 most frequently identified modifications from study CPTAC3 B TMT. The size of the shape is proportional to the total number of identifications per modification (log scale) and the color of the text label is used to distinguish classes of modifications, which include under-/over-labeling (blue), processing errors (purple), artifactual modifications (orange), amino acid loss/addition/substitution (red) and post translational modifications (green).

Study	Total Distinct Semi-tryptic Peptide ID	% Precursor Area	% Total Peptide Ions
CPTAC3 A TMT (25, 2D)	78,797	9.23%	16.80%
CPTAC3 B TMT (16, 2D)	83,803	7.87%	19.50%
CPTAC3 C TMT (23, 2D)	44,783	7.53%	12.22%
CPTAC2 A TMT (17, 2D)	65,816	4.26%	11.99%
CPTAC2 B iTRAQ (37, 2D)	68,506	4.75%	10.25%
NCI-7 TMT (1, 2D)	14,584	18.04%	27.14%
Jurkat (1, 1D)	113	0.57%	0.32%
CompRef (1, 1D)	368	1.59%	1.37%

Table 1: Summarizes the total distinct in-solution semi-tryptic peptide identifications across multiple studies as well as percent of precursor area and total peptide ions, relative to fully tryptic identifications. Shown in parenthesis is the total number of analyses examined.

Trypsin Cleaves C-terminal to Amino Acids Other Than Lys and Arg

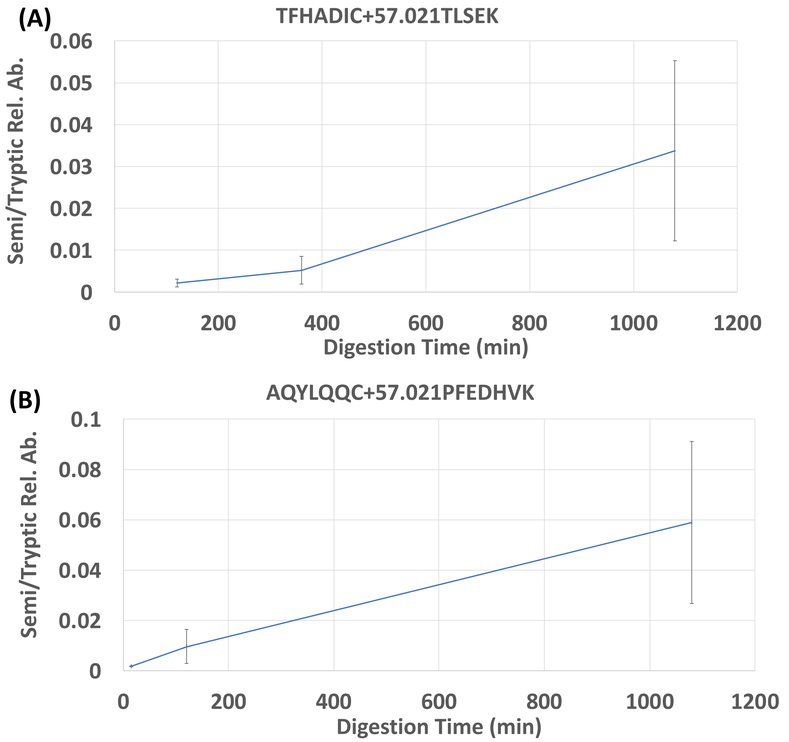

Figure 2: The mean in-solution semi-tryptic peptide ion abundance relative to that of the major fully tryptic sequence for up to four digestion times (15, 120, 360, 1080 min), measured from three replicates, is shown for two peptide sequences. The semi-tryptic sequence shown in **(A)** is TFHADICTLSEK (N-term flanking = Phe), which is compared here to the fully tryptic sequence EFNAETFTFHADICTLSEK. The semi-tryptic sequence shown in **(B)** is AQYLQQCPFEDHVK (N-term flanking – Phe), which is compared to the fully tryptic sequence ALVLIAFAQYLQQCPFEDHVK.

References & Acknowledgements

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Abbreviations

B1-loss – N-terminal amino acid loss; CompRef – Comparative Reference; HSA – human serum albumin; iTRAQ – isobaric tags for relative and absolute quantitation; TMT – tandem mass tag

Semi-tryptic Cleavage Site Distribution from Complex Samples Is Similar to Tryptic Digest of Pure Protein

Study	Total Peptide Ions	Total Distinct Peptide IDs	% Prec. Area (Rel. to Tryptic)	% Total Peptide Ions (Rel. to Tryptic)
HSA 45min	71	44	0.19%	4.92%
HSA 2hr	85	58	0.19%	5.61%
HSA 6hr	95	63	0.53%	6.77%
HSA 18hr	108	81	1.01%	7.35%
HSA 48hr	243	127	1.79%	11.74%

Table 2: Summarizes the total distinct HSA-derived in-solution semi-tryptic peptide identifications across multiple digestion times (45 min, 2 hr, 6 hr, 18 hr and 48 hr) as well as percent of precursor area and total peptide ions, relative to fully tryptic identifications.

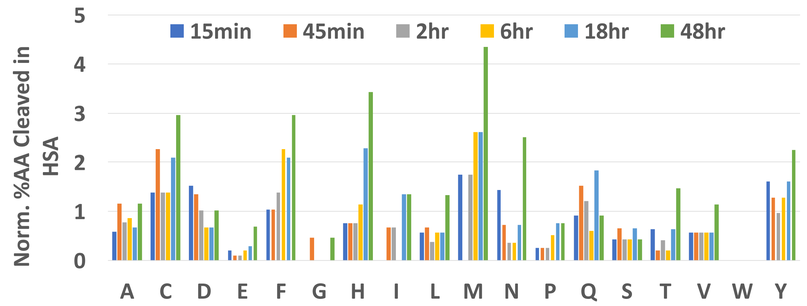

Figure 3: Comparison of the C-terminally cleaved amino acid for in-solution semi-tryptic peptides derived from HSA. The percent of distinct in-solution semi-tryptic identifications for each amino acid were normalized to the frequency of the given amino acid in the HSA sequence.

Conclusions

- The time-course study illustrates that trypsin does cleave C-terminal to amino acids other than Lys and Arg, albeit at a slower rate.
- The distribution amino acids cleaved by trypsin, for in-solution semi-tryptic peptides, from a time-course study using HSA is similar to that obtained from complex human samples.
- This suggests that trypsin may contribute to the semi-tryptic peptides, and the associated variation, identified in in-depth proteomic studies.