OBJECTIVE:
- Investigate enzyme specificity on modified residues
- Study effect on proteolytic processing relative to size of chemical modifier on cleavage residue

INTRODUCTION:
The identification and localization of post-translational modification (PTM) sites on proteins can be key to understanding the mechanism of various cellular processes. Proteolytic enzymes are typically employed as part of the bottom up proteomic work flow for identifying post-translationally modified peptides. Several criteria are commonly used to help verify and conclude if an identified peptide fragment is modified or not. Part of the characterization “dogma” in that modified residues typically cannot undergo proteolysis due to potential steric hindrance and reduced enzyme accessibility during proteolysis. Here we demonstrate data suggesting the widely accepted rule of thumb that enzymes cannot cleave at modified residues maybe not apply to all proteases.

In this study, we investigated the potential for proteolytic cleavage following protein level chemical labeling at enzyme specific sites. Trypsin and Lys-N were used to study Lyase modified Bovine Serum Albumin (BSA). Different size chemical modifiers were tested to evaluate impact on steric hindrance and enzyme accessibility. BSA was subjected to the following modifications: (1) Reductive Methylation (+28.01 Da), (2) Propionylation (+56.03 Da), and (3) Biotinylation (+226.04 Da). Mass spectrometric data from an analysis of a Histone prep will also be discussed.

MATERIALS AND METHODS:
- Bovine Serum Albumin (BSA) Analysis:
  1. Dithiothreitol was reduced (Dithioreitol) and alkylated (iodoacetamide) in 0.1M TEAB, pH 8.5 or 0.1M AMBAC, pH 7.5.
  2. Samples were unlabeled (control) or subjected to 2 different labeling methods:
     a. Reductive Methylation:
        i.Added 20mL of each sample to 12mL of 1M NH2NH2•HCl (1M NaBH4 complex) (incubated overnight).
     b. Propionylation:
        i. Added Propionic Reagent (0.3, propionic anhydride) to samples incubated at 37°C overnight.
        ii. Added to pH 8.0 buffer and 100 units Trypsin overnight.
     c. Biotinylation:
        i. Added 0.1% SquibyX to samples (incubated on ice for 2 hours).  The excess biotinylation reagent was removed using a 700 Da filter spin column (Thermo Fisher).
  3. Samples were subjected to ESI-MS/MS analysis on an LTQ-FT mass spectrometer (Thermo Fisher) interfaced with a nanoAcquity UPLC (Waters Corp).
  5. Samples were analyzed on an Orbitrap Velos Pro analyzer (Thermo Fisher) interfaced with a nanoAcquity UPLC (Waters Corp).

RESULTS AND DISCUSSION:

CONTROL OF PROTEOLYTIC CLEAVAGE AT MODIFIED RESIDUES

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RESULTS AND DISCUSSION:

REFERENCES:

CONCLUSION:
- Enzymes such as Lys-N and Trypsin can cleave at modified Lysines. In this study, proteolytic cleavage was observed even when Lysines were Dimethylated (Fig.3-6). In Lys-N, the cleavage site is Lysine at position 35 (Fig. 5-6). It is not preferential for proteolytic cleavage to occur at modified residues, as it may not be preferential for proteolytic cleavage to occur at modified residues, as seen in lower PSMs (Fig.2) in labeled versus unlabeled BSA.
- AGA peptides K{\text{K}24\text{O}2\text{H}4}_{\text{N}}, K{\text{K}24\text{O}2\text{H}4}_{\text{N}} and K{\text{K}24\text{O}2\text{H}4}_{\text{N}} were used to confirm the identification of the Lys-N digested peptide fragment. Inset in Figure 8c & d highlight b-fragment ion coverage. More Miscleavages Observed When Protonic Residue is Modified

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