

Immonium Ion Intensity is a Strong Predictor of Associated Amino Acid Presence Using an ABI QSTAR Hybrid TOF Mass Spectrometer

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Overview

Upon observing a high frequency of immonium ion in datasets run on the Qstar, the prospect of using these internal fragmentation peaks to improve peptide assignment probability seemed interesting. We analyzed the statistical occurrence of the six most common immonium ions observed in our dataset (H, I/L, F, V, Y, W), as well as one negative control (D).

Introduction

Immonium ions are produced as a secondary fragmentation (a combination of a y and a-type cleavage) of the amide bond during low energy CID. Their structure is represented by $RCH=NH_2^+$, with R indicating the amino acid side chain (Fig. 1). Their mass is directly proportional to the mass of an amino acid, making them informative in peptide sequence assignment.

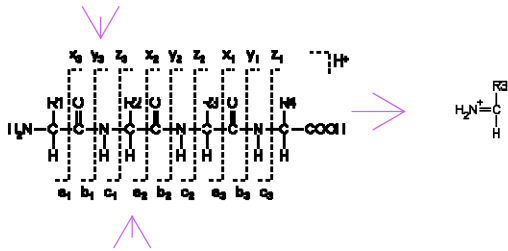


Figure 1: Immonium ion fragmentation schematic. Adapted from www.matrixscience.com

These ions are undetectable in ion trap mass spectrometers due to the inherent low mass cut off. Because this platform dominates the field of proteomics, incorporation into search and validation algorithms has lagged.

However, these peaks are seen with hybrid time of flight type instruments, now available from a number of vendors. We have performed a quantitative analysis of this phenomenon to determine its frequency and impact on sequence assignment (Fig. 2).

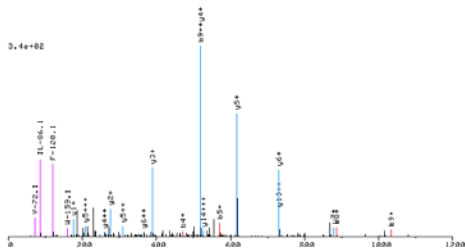


Figure 2: A typical QSTAR MS² spectra. Purple lines indicate immonium ion peaks.

Methods

A total yeast extract from *Saccharomyces cerevisiae* (strain BY4741) was digested and separated into ten fractions using strong cation exchange.

Each fraction was analyzed with a QSTAR Pulsar i using LC/MS/MS and standard collision energies (optimized for most peptide identifications).

Spectra were searched with SEQUEST and assigned a probability of correct assignment using Peptide Prophet. A dataset of high confidence assignments ($P > 0.9$) was assembled for doubly and triply charged spectra, numbering 2240 and 1541, respectively.

The intensity of all immonium ion peaks relative to the base peak was extracted from the high confidence ($P \geq 0.9$) spectra to a database for analysis.

Results

Immonium Ion Occurrence

At least one immonium ion was present in approximately 50% of all spectra in this dataset.

The intensity of the immonium ion peak increased as the number of amino acid residues increased in the peptide sequence at a linear rate that is specific to each amino acid (Fig. 3).

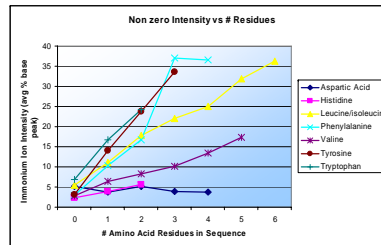


Figure 3: The intensity of the immonium ion increases linearly with the number of residues in the peptide. Assignment for all amino acids studied, except D, the negative control.

Predictive Properties of Immonium Ions

Each immonium ion has a probability of predicting the corresponding amino acid, depending on its intensity relative to the base peak (Fig. 4). Immonium ion data was sorted into quintile bins, based on the immonium ion intensity for this analysis.

The probability tends to increase with the intensity of the immonium ion peak.

This probability can potentially be used to increase the identification scores of peptide assignments using software such as Peptide Prophet.

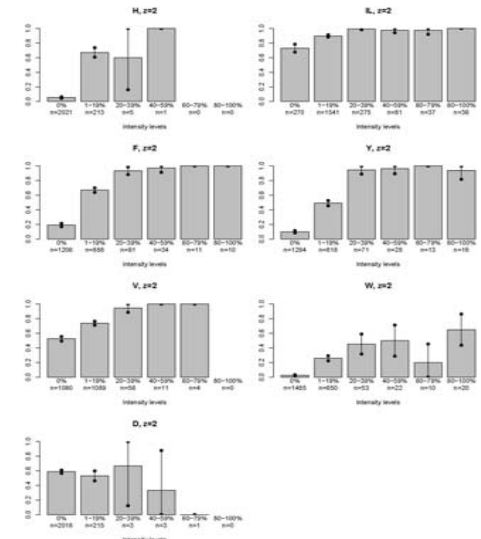


Figure 4: Probability distributions of 7 common immonium ions from 2+ precursor ions. Error bars represent 95% asymptotic confidence intervals.

Conclusions / Future Work

We have demonstrated that immonium ions are a significant indicator of amino acid presence within a peptide assignment. Each immonium ion behaves in a unique manner, and each has a different predictive value.

We are currently attempting to modify the ProbiD program to incorporate these immonium ion probabilities into our peptide assignment algorithms.

Select References

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