

Intact Peptide Charge Determination from Ion Trap MS/MS

Introduction

In identifying proteins using tandem mass spectrometry, researchers can match measured masses of peptides, and fragments of peptides, to theoretical masses calculated from a protein sequence database. Because a mass spectrometer measures mass-to-charge ratio (m/z), the peptide's charge (z) must be known to determine the mass used for database searching.

When using an ion trap however, a peptide's charge state is often difficult to determine by the usual method: examination of the initial MS survey scan of a peptide. It has become common practice, then, to allow a database search engine to determine the charge on a peptide by choosing the charge that allows the best match to the database. This is a poor practice since, instead of inferring results from the data, we are determining what data will best fit the results.

Overview

This research presents an algorithm, soon to be part of the PEAKS software, that will allow a researcher to determine a peptide's charge using MS/MS data alone.

Methods

313 MS/MS spectra were obtained from two LCQ, ion-trap mass spectrometers. The charge for each peptide, i.e. each spectrum's precursor ion charge, was known. Charge states were either +1, +2 or +3, and the spectra were grouped accordingly.

Through an iterative process the characteristics of each group evolved and the best determinants of charge state were isolated. The first two determinants are similar to those used by Colinge et al.:

- (1) the distribution of peaks within certain mass ranges of the spectrum as related to the precursor m/z - illustrated in figures 1, 2 and 3;
- (2) the masses of discernable b and y-ion pairs as related to the precursor m/z . With the addition of a third determinant, a greater accuracy can be attained - illustrated in figure 4;
- (3) the m/z difference between a given y-ion and its associated '-water' and '-ammonium' ions - illustrated in figure 5

An algorithm, using these determinants, was tested against the original data set to determine its accuracy in assigning charge states.

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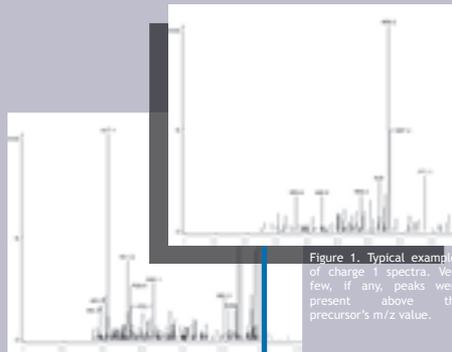


Figure 1. Typical examples of charge 1 spectra. Very few, if any, peaks were present above the precursor's m/z value.

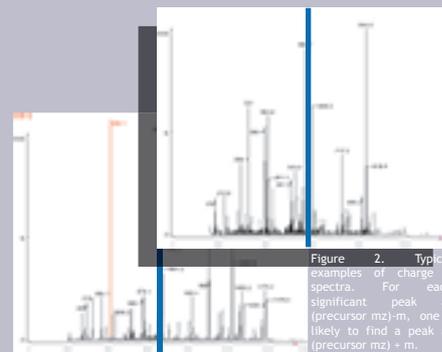


Figure 2. Typical examples of charge 2 spectra. For each significant peak at (precursor m/z)- m , one is likely to find a peak at (precursor m/z) + m .

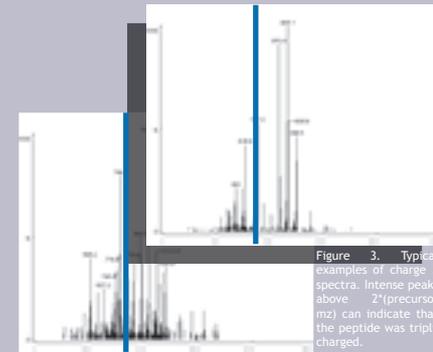


Figure 3. Typical examples of charge 3 spectra. Intense peaks above $2 \times$ (precursor m/z) can indicate that the peptide was triply charged.

For an algorithm to judge the distribution of peaks in a spectrum, it must first assign ranges within the spectrum, then count the number of significant peaks therein. For this purpose it makes sense to divide the spectrum into the ranges: 0 to 0.5 m ; 0.5 m to 1 m ; 1 m to 1.5 m ; 1.5 m to 2 m ; and 2 m to 3 m (where m is the measured precursor's m/z). Peaks within this range should be counted only if they are not noise peaks. As such, peak pairs were counted where two peaks differed by the mass of H_2O , or the mass of NH_3 (or the mass of either when divided by two, or divided by three). In this way a frequency distribution was computed for each spectrum, and the relative frequency of peaks in each range was found to be diagnostic of precursor charge.

Figure 4. An illustration of how the m/z values of two peaks (at 200.9 Da and 722.3 Da), when added together, equal twice the m/z of the precursor ion 461.7 Da. Thus indicating that the peptide was likely carrying charge 2+.

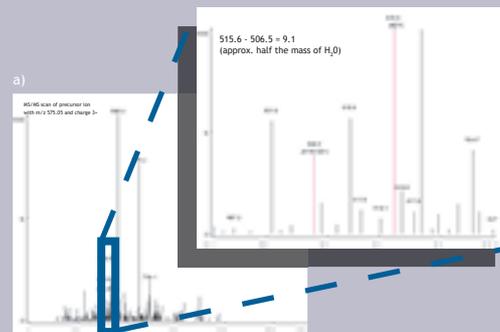
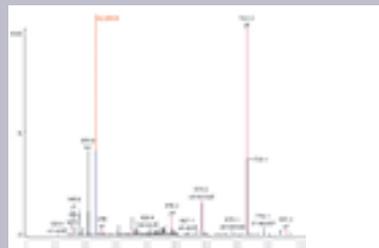
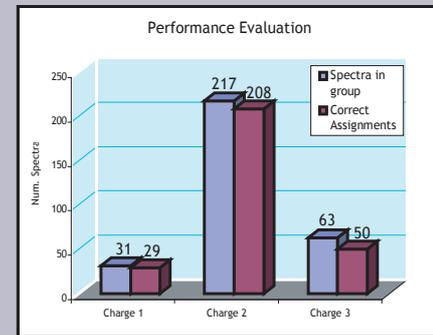


Figure 4. An illustration of how the "loss of water" peak can be used to determine the charge on a peptide. (a) the whole MS/MS spectrum (b) zoomed in on m/z range 494.9 to 527.18 with peaks annotated. Since the loss of water ion appears at -9 Da, one can assume that we've found a doubly charged fragment. This further indicates that the precursor ion was likely carrying at least charge two, and probably charge three.

For charge two spectra, many pairs of peaks are expected such that $m_1 + m_2 = 2m_p -$ the m/z of two peaks equals twice the precursor's m/z . Similarly, in charge three spectra, we can expect to find many peaks such that $m_1 + 2m_2 = 3m_p$.



Results and conclusions

In total, 287/313 or 92% of spectra were assigned the correct charge using MS/MS data alone. 29/31 or 94% of charge 1 spectra were correctly assigned, 208/217 or 96% of charge 2 spectra were correctly assigned and 50/63 or 79% of charge 3 spectra were correctly assigned. The algorithm took approximately 4 seconds to complete charge assignment on 313 spectra.

References

Jacques Colinge, Jérôme Magnin, Thierry Dessingy, Marc Gron, Alexandre Masselot, Improved peptide-charge state assignment, (Proteomics, Vol 3, Issue 8, 2003).