

Integration of Nanoscale HPLC-FTICRMS and HPLC-QIT for Accurate Mass Measurements and High Throughput MS/MS to Achieve Enhanced Proteome Characterization*

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Novel Aspect: Integration of nanoscale LC-FTICRMS and LC-QIT provides high mass accuracy and high throughput MS/MS for enhanced microbial proteome characterization

Introduction

LC-MS/MS on a quadrupole ion trap has become a standard method for the high-throughput tandem mass spectrometry of peptides from complex proteome samples. LC-MS on a higher performance FTICRMS affords enhanced capabilities not only in mass accuracies but also in detection sensitivity and improved dynamic range. In order to exploit the capabilities of both techniques, we have developed a methodology to integrate these platforms to obtain more extensive proteome characterization in a high throughput fashion. The approach involves replicating the same nanoscale HPLC conditions for each MS in order to be able to correlate peptide identification. The benefit of this approach was evaluated for the microbe *Rhodopseudomonas palustris*.

Methods

Tryptic-digested proteome samples were prepared from the microbe *R. palustris*. One portion of each sample was examined by LC-MS/MS on an Finnigan LCQ platform. The other portion was examined by LC-MS on an IonSpec FT-ICR platform. The LC operations are identical on both systems, consisting of automatic sample injection, sample trapping on a C18 300 μ m-i.d. cartridge, and high resolution separation on a C18 nanoscale analytical column. MS/MS on the LCQ utilized a full-range MS scan followed by 4 data-dependent MS/MS scans. SEQUEST was used to identify peptides from the *R. palustris* genome. MS on the FT-ICRMS yielded mass accuracies of ± 3 ppm and resolutions of 150,000 (FWHM). Algorithms were developed to compare and correlate the data.

Preliminary results

The nanospray experiment for the FTICRMS was optimized for the Analytica ESI source with an 1800 volt potential difference between a 10 μ m-i.d. uncoated Picotip and the metal capillary tip (3 mm distant). All other conditions were identical to conventional ESI-FTICRMS. Nanoscale HPLC was interfaced as described in methods section and was designed to be virtually identical with the LCQ experiment. The LC-FTICRMS experimental conditions were optimized with a tryptic digest of a 6-component protein mixture. With 1 μ g sample loaded onto the nano-column, sufficient peptides were detected to provide sequence coverages of greater than 90% for each protein. Mass accuracies were found to be within 3 ppm, even with external calibration conditions. With similar gradient elution conditions, the peptide retention times were found to be very similar to the LCQ measurements. Peptide samples generated by tryptic digestion of both soluble and membrane protein fractions from the microbe *Rhodopseudomonas palustris* were examined on both MS systems. The wide dynamic range afforded by FT-ICR allows a substantial increase in the number of peptide measured in the MS scans. Although mass accuracies of ± 3 ppm were routinely achieved, many of the measured masses could be matched to multiple peptides in the *Rhodopseudomonas palustris* genome. To eliminate this ambiguity, the peaks from LCQ experiments and the FTMS experiments were correlated by their retention times and their m/z values. An integrated filtering algorithm was created to validate peptide identities based on three primary factors: mass errors less than 3 ppm, Xcorr scores greater than 1.2, and retention time difference less than 2 min. The analytical information afforded by two independent mass spectrometry measurement enabled not only a larger number of protein identification, but also a higher confidence in the assignment.

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