Analysis of the Salmonella typhimurium Proteome using Fractionated LC-MS

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Abstract

The Salmonella typhimurium proteome was analyzed by LC-MS and LC-MS/MS fractionation methods. Both techniques were compared to evaluate the impact of fractionation on the accurate mass and elution time (AMT) tag approach for peptide identifications.

Introduction

The LC-MS coupled with ESI has proven to be a powerful tool for the study of global proteomes. LC is a number of attractive features including reducing the complexity of samples being analyzed into a more accessible data set. LC-MS/MS can also be used as a determinant in the identification of peptides.

Despite the utility of LC-MS, there are some limitations. Due to the limited ionization efficiency when performing LC-MS/MS, that is a peak must be sampled many times for detection. Since many peptides may be present simultaneously for complex samples, it is often impossible to perform MS analyses on all of the existing species. The quality of the fragmentation spectrum depends also on when an initial detection of a precursor mass is made in an analyte's profile. The presence has an impact on the mass accuracy, signal intensity, and ability to establish the mass of the fragment peptide. The LC-MS coupled with ESI typically has a lower sensitivity, but a higher robustness in comparison to LC-MS/MS.

Methods

Salmonella typhimurium Cell Growth and Harvesting

Salmonella typhimurium cultures were grown and harvested using the Rapigest® Digestion. The cells were then digested with trypsin.

Proteomic Sample Preparation

Cell pellets of Salmonella enterica serovar Typhimurium (aka, Salmonella typhimurium) were lysed and digested using the Rapigest® Digestion. The proteins were then fractionated on an Agilent 1100 HPLC system with a C18 Jupiter (Phenomenex, Torrance, CA) column (2.1 x 250 mm, 5 μm). The gradient started at 100% mobile phase A (0.2% aqueous TFA) and was eluted until 100% mobile phase B (acetonitrile:0.2% aqueous TFA) was reached.

Results

The LC-MS coupled with ESI has proven to be a powerful tool for the study of global proteomes. LC is a number of attractive features including reducing the complexity of samples being analyzed into a more accessible data set. LC-MS/MS can also be used as a determinant in the identification of peptides.

Fractionated LC-MS/MS

Each of the 80 fractions was injected into an LTQ Orbitrap operated as described above for 5 minutes using Nanodrop from Advion Bioscience.

Data Analysis

SEQUEST Analysis

The MS/MS spectra were correlated with possible peptide identifications by using the SEQUEST algorithm to search the annotated Salmonella typhimurium database of proteins from TIGR.

Conclusions

Fractionating a reversed phase LC separation could be used to overcome undersampling in LC-MS/Ms at the cost of throughput. The reduction in complexity and the ability to perform at least a rudimentary AMT tag approach would allow these LC fractionation methods to be used with orthogonal separations of peptide to increase overall coverage.

Since this method tends to perform a better sampling of the higher abundance species versus lower abundance species, we have attained a more representative concentration standard for Salmonella proteins.

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Capillary LC-MS/MS

LC-fractionation can be used with orthogonal separations of peptide to increase overall coverage.

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