Protein profiling using WAX-RPLC 2D separation and FTICR-MS intact protein analysis

Overview

The techniques WAX and RPLC are combined to perform preparative steps. For the purpose of comparative proteomics, 2D displays for cell lysate fractions were generated to model datasets that can be used to check for pathways in protein expression between control samples and those subjected to biological perturbation. In the next stage, detection of proteins/modified proteins of biological interest will be followed by targeted MS/MS characterization using gas phase dissociation techniques (e.g. CID, ECD).

Methods

S. oneidensis cells were disrupted by bead beating with 31.5 mm (inner diameter) beads. The lysate was then subjected to an ultrafiltration using a 10 kDa Amicon filter. Protein concentrations were determined using the Bradford assay. The raw lysate was clarified by ultracentrifugation to protect the chromatographic columns from clogging.

Introduction

Protein profiling using WAX-RPLC 2D separation and FTICR-MS intact protein analysis. 470 positive proteins were detected in the whole cell lysate alone while 710 positive intact proteins were detected in the whole cell lysate plus WAX fractions, indicating that the number of tentative identifications for intact protein also increased from 27 to 44 following the fractionation of the cell lysate. Due to the nature of intact protein analysis, only high mass accuracy (0.1 Da resolution) can also include the ability of FTICR-MS to detect proteins in more than one modification states.

Experimental Scheme

Figure 1: Experimental workflow outlining preparative steps for generating WAX/RPLC/FTICR-MS intact protein analysis.

Data Analysis

Peptides were identified from the RPLC-FTICR-MS analysis using MASCOT and were annotated with a z-score > 2. Those were used to query a panel of proteins for the cell lysate and each WAX fraction. Assignment of sequence data is based on the presence of unique masses (identifying monoisotopic masses) of the intact protein, and spectrum number (relating to RPLC retention time).

Once the UMCs were defined, constant noise spectra identified at each UMC were located in order to improve the signal to noise (S:N) ratio for low-abundance species. To evaluate the performance of the system for accurate mass measurements, all the UMCs we analyzed were tested with the intact protein (Figure 2). A total of six UMCs were obtained. Another 15 UMCs were obtained based on the isotopic fit < 0.25, cluster size 2 or more criteria; with four intact proteins of biological interest found in each UMC.

Conclusions and Future Directions

5. Bottom-up proteomics.
6. For the purpose of comparative proteomics, 2D displays for cell lysate fractions were generated to model datasets that can be used to check for pathways in protein expression between control samples and those subjected to biological perturbation. In the next stage, detection of proteins/modified proteins of biological interest will be followed by targeted MS/MS characterization using gas phase dissociation techniques (e.g. CID, ECD).

Results

For the purpose of comparative proteomics, 2D displays for cell lysate fractions were generated to model datasets that can be used to check for pathways in protein expression between control samples and those subjected to biological perturbation. In the next stage, detection of proteins/modified proteins of biological interest will be followed by targeted MS/MS characterization using gas phase dissociation techniques (e.g. CID, ECD)."