Previous work has described a parallel approach to peptide sequencing using a data independent LC-MS strategy. This differs from traditional LC-MS/MS experiments, in that the MS mass analyzer does not select precursor ions in a serial fashion, based upon the MS isolation and collision induced dissociation, but rather all of the precursor ions present in the ion source at one moment in time are transmitted to the collision cell and simultaneously fragmented by CID, with the fragments detected in parallel by the MS/MS. The instrument used here was a Xevo Q-ToF MS incorporating a new high field pusher device and dual stage reflectron. This arrangement enables a resolution of greater than 50,000 FWHM to be achieved with a flight tube 35 cm in length.

Alternating between low and elevated energy on the collision cell provides accurate mass precursor and associated product ion spectra on all isotopes of every charge-state across the entire chromatographic peak width. The correlation of precursor to product ions is achieved by chromatographic time alignment, resulting in a highly specific set of accurate mass fragments for identification purposes. The precursor ion intensity can then be used for label-free absolute protein quantification.

In this paper we will compare and contrast qualitative and absolute quantitative protein level separations utilised a 1-85% acetonitrile 90-minute gradient. Protein concentrations over 3 orders of magnitude of protein concentration were investigated and the differences that can be observed between these proteomes.

METHODS

Sample preparation

The bacterial samples were used, a standard tryptic digest of Escherichia coli and a Methylophaga thiooxidans strain. The whole cell lysate of Methylophaga thiooxidans was prepared as described previously and reduced and alkylated prior to digestion by 10 ul of trypsin (Promega, Madison, WI) 1:50 (w/w) for 16 hrs. The sample was then desalted and concentrated as described previously. The digest was then analysed using 1:50 trypsin digestion for 200 ng and 1:100 trypsin digestion for 100 ng loading in direct injection mode.

LC-MS Conditions

Experiments were performed on a nanoAcQUITY Ultra-Performance liquid chromatography (UPLC) system coupled to a Xevo Q-ToF mass spectrometer (Waters Corp., Manchester, UK). The system consists of a nanoflow autosampler automatically loading a series of six microvials on the UPLC system which elutes directly into the mass spectrometer. The mobile phases used were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with a gradient from 5% (B) to 80% (B) in 60 min at a flow rate of 0.3 ul/min. The sample was loaded onto a BEH C18 column (100 mm × 2.1 mm, 1.7 μm) to which is connected a PicoFrit column (150 mm × 0.3 mm, 3 μm) with a nanoflow autosampler (Waters Corp., Manchester, UK). The mobile phases used were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with a gradient from 5% (B) to 80% (B) in 60 min at a flow rate of 0.3 ul/min. The sample was loaded onto a BEH C18 column (100 mm × 2.1 mm, 1.7 μm) to which is connected a PicoFrit column (150 mm × 0.3 mm, 3 μm) with a nanoflow autosampler (Waters Corp., Manchester, UK).

RESULTS

MSE data independent analysis provides accurate mass measurements of all detectable precursor and product ions which is achieved using push acquisition mass chromatograms (Bruker Daltonics, Bremen, Germany). Isolated precursor ions are selected for MS/MS analysis and the resulting product ion data are searched using a revised version of the Proteome Discoverer (v1.4) software. The resulting peptide spectral matches (PSMs) are used to calculate the absolute amounts of identified proteins.

CONCLUSION

The NanoAcQUITY Xevo-QToF mass spectrometer combination used in this study provides a highly reproducible protein identification strategy.

REFERENCE