Parallel Sample Processing of Protein Digests on MALDI TOF MS and MALDI TOF-TOF ™ MS-MS via CD Technology

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ABSTRACT

Identification of proteins based on the MALDI TOF MS and MALDI TOF-TOF ™ MS-MS analysis of peptides from an integrated sample preparation system (CD) has been demonstrated. The sample preparation system allows for parallel processing of 96-samples at a time in a highly integrated and automated manner. The processed samples are then analyzed directly in the mass spectrometer without transfer to a separate MALDI sample plate. Excellent sensitivity in the identification of proteins in MS and MS-MS modes has been demonstrated down to 40 fmole of a tryptic BSA digest. Mass depletion across a CD segment (270 pm RSD) is minimized by using close external calibration wells on the CD. Repeatability is evident by a 22% peak height relative standard deviation across a 40 fmole BSA digested sample.

INTRODUCTION

Gyrolab™ MALDI SP1 is a CD microlaboratory that prepares samples for analysis by MALDI mass spectrometry. Up to 96 samples are processed simultaneously through individual micro electrodes on the CD. Within each electrode, a protein digest is concentrated, desalted, mixed with matrix and crystallized onto a MALDI target area. Every step, from sample application to chromatographic purification and crystallization, is optimized and precisely controlled to increase sample recovery and reproducibility. Highly concentrated, well-crystallized sample ensures the highest sensitivity during MALDI MS analysis.

DIGESTION AND CD PROCESSING CONDITIONS

Bovine serum albumin (BSA) was purchased from Sigma (90% purity). BSA was dissolved in ammonium bicarbonate buffer at pH 8 to a concentration of 10 mg/ml, reduced with DTT and then desalted (superamine treated to protein carbamidomethylated cysteine. The BSA solution was digested for 24 h with TPCK trypsin (Worthington). BSA was treated to yield carboxymethyl cysteines. The BSA solution was digested for 24 h with TPCK trypsin (Worthington). The CD was cut into sections with 10 samples per segment. The sections were mounted on a modified stainless steel sample stage and introduced directly into the mass spectrometer. The samples were then analyzed on a Applied Biosystems Voyager-DE™ STR Biospectrometry™ Workstation or Applied Biosystems 4700 TOF-TOF Proteomics Analyzer respectively, as indicated in the results section. The CD was cut into sections with 10 samples per segment. The sections were mounted on a modified stainless steel sample stage and introduced directly into the mass spectrometer. The samples were then analyzed on a Applied Biosystems Voyager-DE™ STR Biospectrometry™ Workstation or Applied Biosystems 4700 TOF-TOF Proteomics Analyzer respectively, as indicated in the results section. The standard 250 uL whole serum BSA digest was used during the data collection with the Applied Biosystems 4700 TOF-TOF ™ instrument. Approximately 1000 spectra (5 seconds) were used for the MS analysis and 3000 spectra (15 seconds) were used for the MS-MS analysis. 100 spectra (5 seconds) were accumulated for the MS data on the 4700 Applied Biosystems Voyager DE™ STR mass spectrometer, Database searching was performed using UCar's Protein Prospector V3.2.1 software. The results were then imported into Protein Prospector V3.2.1 for the peptide Fingerprint analysis.

RESULTS

The possibility to integrate a number of unit operations in sample preparation for MALDI MS analysis has been demonstrated. The authors gratefully acknowledge Peter Juhasz from Applied Biosystems for assistance with the MS-MS analysis on the ABI 4700 Proteomics Analyzer.

CONCLUSIONS

• The possibility to integrate a number of unit operations in sample preparation for MALDI MS analysis has been demonstrated.
• MALDI TOF MS and MALDI TOF-TOF ™ MS-MS of Protein Digest samples that have been concentrated, desalted and analyzed in a CD has been shown.
• Mass Calibration and Peak Intensities appropriate for reliable protein identification across a CD segment has been demonstrated.
• MALDI TOF-TOF ™ MS-MS analysis of 40 fmol of BSA Digest sample has provided very good protein identification results.
• Low Sensitivity MALDI TOF MS Peptide Fingerprint Identification at sub-femtomole sensitivity has been demonstrated.

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