Rapid Identification and Characterization of tryptic peptides using High Linear Velocity NanoBORE UPLC MALDI MS/MS and Ion Mobility Separation

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NANOBORE UPLC MALDI MS/MS AND ION MOBILITY SEPARATION

RAPID IDENTIFICATION AND CHARACTERIZATION OF TRYPTIC PEPTIDES USING HIGH LINEAR VELOCITY

INTRODUCTION

Here we describe the use of elevated flow rates combined with nanoscale columns packed with 1.7μm particles for rapid peptide separations using a high pressure nanoUPLC system. Increasing the flow rate allows the separation from approximately 0.3μL/min to 1.4μL/min has the potential to reduce the analysis time by a factor of approximately 10. Increasing the flow rate from 0.3μL/min to a flow of 1.4μL/min produced a typical back pressure of 1500 psi to 5900 psi which allows the system to be operated for 8 minutes injection to injection. Conventional HPLC run times for this type of experiment are typically 45 minutes to 2 hours. The eluent is combined with matrix solution and spotted directly onto MALDI target plates using a spotting robot. This combination has an orthogonal acceleration time-of-flight mass spectrometer equipped with a 200nm repetition rate Nd:YAG laser allows for the rapid characterization of simplified protein mixtures. A novel ion mobility separation device was used to separate isobaric peptides.

RESULTS

Initial experiments focused on the reproducibility and quality of the rapid UPLC separations, where used in conjunction with a MALDI mass spectrometer. For this purpose a mixture of four tryptic protein digests each at 50fmol on column was separated. Typical results are shown in Figure 2. From the drift time alignment of both spectra, a reproducibility in retention time was better than ±4 seconds (Figure 3). The feasibility of performing MALDI MS/MS analysis of peptides separated using UPLC. Figure 4 shows that MALDI MS/MS analysis can be performed at sub-nanomole levels from a single LC run. Typical database search results are shown in Figure 4.

CONCLUSION

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