A Method for Continuous Deposition of Reversed-phase HPLC Protein Digest Separations to MALDI Target Plates

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Introduction

This poster investigates the advantages of using an LC-MALDI interface for the continuous deposition of HPLC separations onto a moving MALDI target plate.

Peptide mass fingerprinting (PMF) by MALDI-TOF/MS has been used extensively for protein identification [1-4]. The most common application is for the identification of proteins from 2-D and 1-D gel spots with enzymatic digestion by standard protocols [5, 6]. The digest is spotted onto a sample well of the MALDI target.

In this work the prototype LC-MALDIprep™ device [7, 8] is used to continuously deposit RP HPLC separations of digests onto the matrix pre-coated MALDI target [9]. This sample deposition method enables four significant improvements for MALDI peptide mass fingerprinting. 1) Spatial separation of analyte on the MALDI target reduces ion suppression effects [10-13]. 2) Samples are spatially focused to an area 3-times smaller than traditional sample wells. 3) Larger solution volumes can be desolvated and deposited to the target. 4) Individual peptide peaks are focused to different regions along the length of the sample track and can therefore be sampled in sequence rather than concomitantly. Significant improvements in PMF and MALDI-TOF/MS S/N are shown.

Experimental

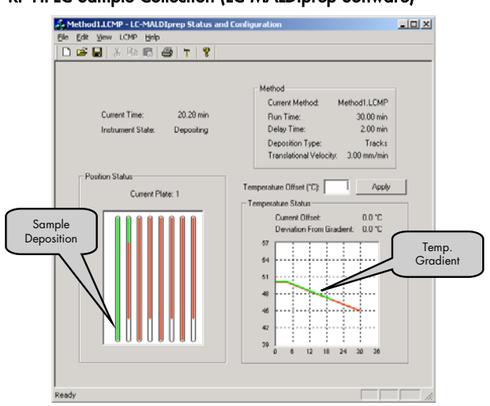
Samples were prepared from standard and ribosomal proteins and digested with trypsin (porcine, sequencing grade). The yeast large ribosomal subunit proteins were separated on 1-D SDS PAGE and the Coomassie stained bands were then excised.

Reversed-phase HPLC was performed using a Waters CapLC™ System with a 0.32 x 50 mm Symmetry® C_{18} column packed with 3.5 µm silica. Column temperature: 30 ° C, flow rate: 10 µL/min and binary gradients formed from A: 98% water w/ 0.1% TFA and B: 98% acetonitrile w/ 0.1 % TFA.

Mass spectrometry was performed using a Micromass MALDI-TOF mass spectrometer with reflectron and delayed extraction. The instrument was operated in positive ion mode with a nitrogen laser (337 nm) and an effective flight path of 2.3 meters. Plate spatial calibration was performed using an automated laser positioning system (ALPS).

Results

Figure 1. Typical Experimental Conditions for Capillary RP HPLC Sample Collection (LC-MALDIprep Software)



Results

Figure 2. Heated Capillary Nebulizer

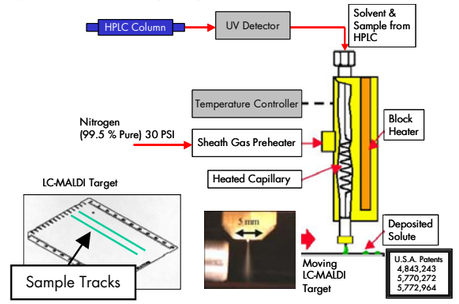


Figure 3. MALDI-TOF/MS Sensitivity for Detection of ACTH (Clip 18-39) and Effects of the Stage Speed

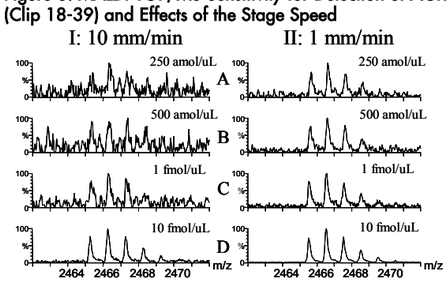


Figure 4. Effect of Stage Speed on Chromatographic Performance

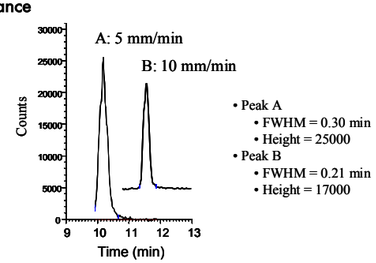


Figure 1 depicts the sample tracks and desolvation temperature for sample collection as well as relevant collection parameter read-backs. Figure 2 shows the heated capillary nebulizer and demonstrates that the spray has a diameter less than 1 mm. The mass spectral sensitivity is shown to be strongly dependent on the stage speed during sample collection (Figure 3). The limit of detection is 2 fmol/µL for the fast stage speed (10 mm/min) and 200 amol/µL for the slow stage speed (1 mm/min). The chromatographic resolution increases with the faster stage speed (Figure 4B) while the sensitivity is greater for the slower stage speed (Figure 4A).

This continuous collection of eluent to the MALDI target allows for the generation of true LC-MS data using the MALDI to sample the spatially distributed analyte peaks (Figure 5). Each scan represents a 0.1 mm increment and the total length depicted is over 40 mm. The x,y coordinates, based on the scan number, can be used to revisit a peak of interest for further mass spectral analysis. A peak is deposited into a rectangular well of 1.5 mm^2 (traditional well area = 5.3 mm^2) thus providing enhanced spatial focusing.

Results

Figure 5. The Base Peak Intensity Chromatogram from MALDI-TOF/MS Analysis of an Apomyoglobin Tryptic Digest Separation

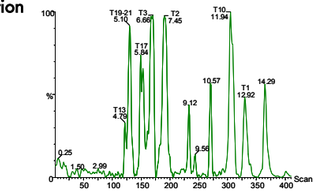


Table 1. Summary of Peptide Mass fingerprinting Results for the LC-MALDI and Spotting Analysis of the FOUR PROTEIN DIGEST.

A. Spot w/ Cleanup	Load (fmol)	Matched Peptides	% Seq. Cov
Actin	10	1	4
ADH	1270	9	33
Myoglobin	10	2	17
CytC	10	0	0

B. LC-MALDI	Load (fmol)	Matched Peptides	% Seq. Cov
Actin	10	9	31
ADH	1270	13	35
Myoglobin	10	6	47
CytC	10	4	44

To simulate a 2-D gel spot with one high abundance and 3 low abundance components a mixture of four standard proteins was prepared (Table 1). Peptide mass fingerprinting results (Table 1) show that the LC-MALDIprep device provides for significant improvements in the number of peptides matched and the percent sequence coverage. The improvement in mass spectral signal to noise (S/N) is also quite significant (Figure 6).

Figure 6. Comparison of Mass Spectral S/N for Tryptic Peptides from Actin (10 fmol) Mixed in the FOUR PROTEIN DIGEST (Table 1)

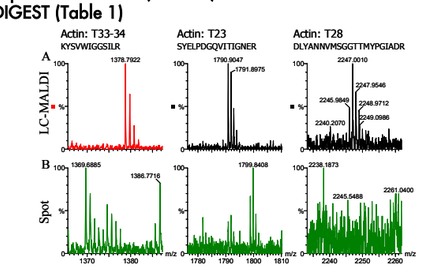
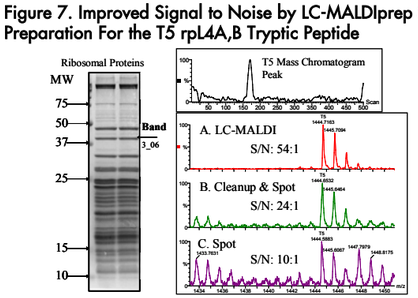


Figure 7. Improved Signal to Noise by LC-MALDIprep Sample Preparation For the T5 rpL4A,B Tryptic Peptide



The in-gel digest of one of the ribosomal proteins was analyzed (Fig. 7) by LC-MALDI (A), spotting with cleanup (ZipTip™) (B) and spotting w/out cleanup (C). The T5 peptide can be located on the plate via the mass chromatogram shown above and the S/N shows significant improvement over spotting.

Results

Figure 8. BPI MALDI Mass Chromatogram and 2-D Plot of a 200 Minute Separation of a Global Ribosome Digest

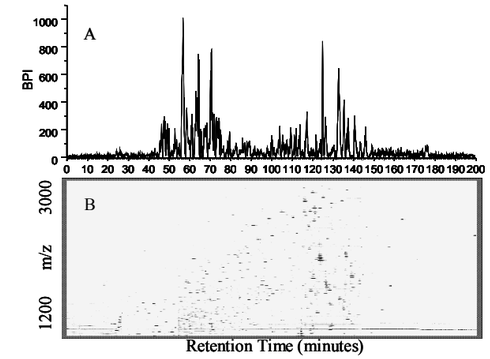


Figure 8 demonstrates how the LC-MALDIprep device can collect a complex tryptic digest onto one MALDI target enabling the MALDI mass spectrometer to detect a large number of peptides (dark bands against a light background) whose m/z values can be related to retention time as well as spatial position on the target. This separation could be analyzed by MALDI-QTOF instrumentation to sequence large numbers of peptides.

Conclusion

- Mass spectral performance is improved with the LC-MALDIprep sample collection device due to reduced ion suppression and enhanced spatial focusing
- Peptide mass fingerprinting from LC-MALDI prepared samples shows enhanced numbers of matched peptides and improved sequence coverage, especially for the low abundance protein digests (low fmol level) mixed with high abundance protein digests (low pmol level).
- Separations are stored onto the MALDI target and can be sampled weeks later due to the offline nature of the method
- Peptide single mass chromatograms can be used to locate a given peptide on the MALDI target for further mass spectral analysis
- Applicability of this LC-MS technology to MALDI-QTOF experiments holds potential for the future

References

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