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MALDI-TOF MS with 2-KHz Laser for Fast Analysis of Separated Peptide Digests

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Introduction

In ICAT or in other methods of isotopic peptide labeling, differentially expressed (DE) proteins are determined in MS and MS/MS scans. In the MS scan, the pairs of peaks separated by known mass are determined. Selected peptides are then identified in a subsequent MS/MS scan. The number of DE proteins is usually much smaller than the total number of proteins in the sample. The search for the DE proteins can be significantly accelerated with a fast MS scan of LC-separated peptides from isotopically labeled samples. Axial TOF mass spectrometers are an efficient tool for fast MS analysis of peptides over 1-5 kDa mass range since the acquisition rate of mass spectra in the axial TOF-MS is only limited by flight time of heaviest peptides, typically 100 µs. A prototype mass spectrometer for rapid MS analysis was tested, with LC separated sample deposited on a tape moving inside the mass spectrometer. Different peptides were mixed with CHCA matrix and deposited on the tape at subatmospheric pressure. The streak was scanned with a 2-KHz laser in a home-built axial MALDI-TOF mass spectrometer and mass spectra were stored at a rate of 10 data files per second, each file contained 200 averaged mass spectra.

Instrumentation and Results

The home-built mass spectrometer with a system providing continuous deposition of the sample [1] was upgraded with a high-repetition rate laser and a data acquisition system with on-board averaging. A delayed extraction system was built for pulse acceleration of ions with repetition rates up to 2 kHz. The pulse ion extraction system was capable of producing 8 kV pulses with 10 µs duration, biased at 16 kV, with a 75 ns rise time. The "pulse down" scheme was used for ion acceleration. In this scheme, the sample electrode was kept at high DC voltage (bias) and the voltage on the extraction electrode dropped from bias voltage (16 kV) to lower voltage (8 kV) several hundred nanoseconds after the laser pulse. High electrical field during initial ion acceleration in delayed extraction scheme allowed reduction of the turn-around time, and thus increases mass resolution. In addition, the short rise time of the HV pulse diminished the mass-dependent distortion of the standard 3-term calibration curve due acceleration of ions under time-dependent conditions [2]. Reducing the rise time and adjusting the residual electrical field near the surface during the delay time, the 3-term calibration could be used over a broad mass range.

The model sample trace deposited on a Mylar tape at subatmospheric conditions using a fused silica capillary (130 µm O.D. and 30 µm I.D.) was a narrow (approximately 200 µm) streak containing microcrystals similar to those deposited by the dried droplet method. After evacuation of the chamber with the tape cartridge, the tape was positioned flush with a flat surface of the sample electrode to avoid field distortion. Sample depletion was observed after approximately 50 shots at one position on the streak with a 200-µm laser spot. To allow an efficient use of the sample material, yet to avoid signal acquisition from depleted portions of the sample trace, the tape was moved at a speed of 2 mm per second during laser irradiation.

High-speed data acquisition was implemented using a 2-Gsamples/sec digitizer with on-board memory. In the averaging mode, the digitizer was capable of summing and transferring to the hard disk 10 data files per second; each 32-bit data file contained 200 averaged mass spectra sampled at 256,000 points.

Operating at 2 kHz, no surface charging effect was observed. In our fast axial MALDI TOF mass spectrometer several thousands mass spectra were recorded continuously on the hard disk. This made it possible to conduct the MS scan of complex samples deposited from one-hour LC separation in 8-10 min. Additionally, with mass accuracy of 10 ppm in MALDI-TOF MS routinely obtained using closely deposited external standards across the entire MALDI plate [3], more reliable peptide identification is possible. This approach provides a means of analyzing complex peptide digests in a high-throughput manner, without the time constraints of the on-line approach.

1. Preisler J., Hu P., Rejtar T., Moskovets E., Karger B. L. *Anal Chem* 2002, **74(1)** ,17-25
2. Moskovets E., Karger B. L. RCM 2003, **17(3)**, 229-237
3. Rejtar T., Chen H-S., Moskovets E., Li L., Andreev V., Karger B. L. *Proceedings of 51-th ASMS Conference on Mass Spectrometry 2003 June 8-13, Montreal, Canada*