Anal. Chem. **2002,** *74,* 17−25

Articles

Capillary Array Electrophoresis-MALDI Mass Spectrometry Using a Vacuum Deposition Interface

Jan Preisler,† Ping Hu, Tomáš Rejtar,‡ Eugene Moskovets, and Barry L. Karger*

Barnett Institute and Department of Chemistry, Northeastern University, Boston, Massachusetts 02115

We previously introduced a vacuum deposition interface for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI/TOF MS) on a moving surface (e.g., quartz wheel, Mylar tape, metal target). In our present work, the approach has been extended to demonstrate parallel analysis for multiple on-line infusion MALDI MS and capillary array electrophoresis (CAE)-MALDI MS. In the infusion mode, individual peptide samples were simultaneously deposited on a Mylar tape cartridge using an array of eight capillaries, yielding eight parallel traces. For CAE-MALDI/TOF MS, the same number of separation capillaries were coupled with an array of eight infusion capillaries using a common liquid junction, containing matrix solution. A fast-scanning mirror was employed to traverse the beam of the desorption laser across the Mylar tape to probe one trace at a time. The positions of the eight sample traces formed on the tape were automatically determined, and all samples were analyzed in rapid sequence using a kilohertz repetition rate laser and a high-throughput data acquisition system. The instrumentation was operated with CAE MS for high-throughput analysis without compromising data quality. The principles of parallel separation−vacuum deposition should be generally applicable to MALDI/TOF MS analysis for proteomics and other areas where separation and high throughput are required.

Proteomics and other global analyses require high throughput and sensitive analytical methods of wide dynamic range for identification, structure characterization, and quantitation of highly complex mixtures.[1,2] While advances in protein separation and identification have been made, integrated automated technologies for proteome analysis are still required. Identification of proteins in biological samples typically involves separation of the sample by 2D gel electrophoresis, in-gel digestion of individual stained spots and MS mapping of the peptide digest, followed by analysis of selected peptides by MS/MS.[3,4] Alternatively, the entire protein fraction of a cell lysate, tissue fragment, etc., can be first digested, followed by separation of the tryptic peptides by liquid chromatography (LC) or capillary electrophoresis (CE) and analysis by MS or MS/MS. One strategy for protein identification relies on assignment of a single peptide, "accurate mass tag" with high mass accuracy.[5] For more detailed analysis, most other approaches to the analysis of complex peptide mixtures are based on multidimensional analytical tools, such as LC-CE,[6] IEF-HPLC,[7] affinity chromatography-HPLC,[8,9] and solid-phase microextraction-multistep elution-CE[10] coupled to MS or MS/MS. Separation preceding mass analysis also minimizes ion suppression effects, as shown for CE-matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI/TOF MS) analysis of peptide mixtures.[11] There is a great need for high-performance separation-MS analysis of complex biological mixtures.

Another major aspect of proteome analysis is sample throughput in terms of the number of samples or number of assays unit time required for individual samples. Throughput of chromatographic methods coupled to ESI MS can be increased using multiple sprayers[12−16] or a single sprayer in which injections into parallel columns are staggered, allowing the mass spectrometer to analyze continuously the chromatographic window of interest.[17]

† On leave from: Department of Analytical Chemistry, Masaryk University, Brno, Czech Republic.

‡ On leave from: Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, Brno, Czech Republic.

(1) Williams, K. L. *Electrophoresis* **1999**, *20*, 678−688.
(2) Kyranos, J. N.; Hogan, J. C. Jr. *Anal. Chem.* **1998**, *70*, 389 A−395 A.
(3) Shevchenko, A.; Jensen, O. N.; Podtelejnikov, A. V.; Sagliocco, F.; Wilm, M.; Vorm, O.; Mortensen, P.; Shevchenko, A.; Boucherie, H.; Mann, M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14440−14445.
(4) Lahm, H. W.; Langen, H. *Electrophoresis* **2000**, *21*, 2105−2114.
(5) Conrads, G. T. P.; Anderson, A.; Veenstra, T. D.; Paša-Tolič, L.; Smith, R. D. *Anal. Chem.* **2000**, *72*, 3349−3354.
(6) Udiavar, S.; Apffel, Chakel, J.; Swedberg, S.; Hancock, W. S.; Pungor, W. S. *Anal. Chem.* **1998**, *70*, 3572−3578.
(7) Wall, D. B.; Kachman, M. T.; Gong, S.; Hinderer, R.; Parus, S.; Misek, D. E.; Hanash, S. M.; Lubman, D. M. *Anal. Chem.* **2000**, *72*, 1099−1111.
(8) Gygi, S. P.; Rist, B.; Gerber, S. A.; Turecek, F.; Gelb, M. H.; Aebersold, R. *Nat. Biotechnol.* **1999**, *17*, 994−999.
(9) Geng, M.; Ji, J.; Regnier, F. E. *J. Chromatogr., A* **2000**, *870*, 295−313.
(10) Tong, W.; Link, A.; Eng, J. K.; Yates, J. R. *Anal. Chem.* **1999**, *71*, 2270−2278.
(11) Preisler, J.; Hu, P.; Rejtar, T.; Karger, B. L. *Anal. Chem.* **2000**, *72*, 4785−4795.
(12) Zeng, L.; Kassel, D. B. *Anal. Chem.* **1998**, *70*, 4380−4388.
(13) Loo, R. R. O.; Udseth, H. R.; Smith, R. D. *J. Phys. Chem.* **1991**, *95*, 6412−6415.
(14) Rulison, A. J.; Flagan, R. C. *Rev. Sci. Instrum.* **1993**, *64*, 683.
(15) Kostiainen, R.; Bruins, A. P. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 549−558.
(16) Shia, J.; Wang, C.-H. *J. Mass Spectrom.* **1997**, *32*, 247−250.

10.1021/ac010692p CCC: $22.00
Published on Web 11/27/2001

A commercial multiplex LC-MS instrument, which uses a spinning rotor to sample liquid streams sequentially from four or eight separate electrospray inlets, has been introduced recently.[18] Although these techniques can increase throughput for simple mixtures, they do not offer true independent parallel analyses and the probability of peak overlap increases with the mixture complexity of samples and number of channels.

Many high-throughput approaches are based on off-line MALDI/TOF MS analysis. With target capacity of thousands of sample spots and commercial robotic stages, MALDI/TOF MS offers automated high-throughput off-line analysis of discrete samples. If samples are deposited with micropipets[19] or piezoelectric dispensers,[20−22] which are capable of delivering sample volumes in the pico- to nanoliter range, both the sensitivity and the density of samples on the target can be significantly increased. In addition, lack of a direct MS/MS method suitable for MALDI/TOF analysis has been overcome with the introduction of qQTOF and TOF/TOF mass spectrometers.[23−25] Off-line collection of an eluent from a separation column on a target of MALDI/TOF mass spectrometer in the form of spots[26−28] or a continuous streak[29] has also been demonstrated. The streaks can be simply deposited with a capillary,[29] sprayed with a nebulizer,[30] or electrosprayed[31] on a suitable substrate. These techniques may lead to reproducible and sensitive analysis; however, they may contain specific limitations. For example, analytes are typically deposited over a relatively large area or diffuse after deposition, which would deteriorate sensitivity and separation efficiency. In addition, several interfaces for allowing on-line coupling of a separation method with MALDI MS have been developed.[32−36] For those cases, one sample or sample stream was analyzed at a time, and the performance was in general not suitable for narrow peaks of only a few seconds.

We recently introduced vacuum deposition of samples leading to high spot-to-spot reproducibility and high mass sensitivity in MALDI/TOF MS.[37,38] In one approach, the liquid mixture of analyte and matrix was deposited as a narrow (∼100 μm) dried trace on a moving surface directly in the evacuated source chamber. The trace was transported on-line to the repeller where laser desorption took place. In an alternative format, the sample was deposited on a conventional target in a stand-alone evacuated chamber and transferred off-line to a commercial instrument for MALDI analysis.[38] The vacuum deposition interface allowed rapid analysis of trace sample amounts as well as coupling of microcolumn high-resolution separation techniques with MALDI MS. Due to the uniformity of the deposited traces, no sweet spot[39] needed to be searched, resulting in an increase in sample throughput. However, the full potential for throughput offered by MALDI/TOF MS is still far from being exploited, since the instrument recorded ion signal using ∼0.1% of the analysis time.

In this work, we used a tape cartridge deposition surface[11] to demonstrate the potential of capillary array electrophoresis[40] (CAE) MALDI/TOF MS analysis. All steps of the analysis, i.e., sample injection, separation, mixing with matrix, and deposition, were carried out in parallel. Analysis of all sample traces was accomplished with a single TOF mass analyzer. In this approach, an array of eight infusion capillaries was used to deposit eight individual sample traces simultaneously on the advancing tape. Using a fast-scanning mirror, the positions of the eight sample traces were automatically determined, samples were desorbed with a kilohertz laser, and spectra were recorded with a high-throughput data acquisition system. Importantly, the increase to eight sample analyses did not compromise the data compared to analysis of a single streak. Mass resolution above 2000 (fwhm) and low-attomole detection limits were obtained for small peptides, similar to that for the single-capillary interface. Furthermore, using CAE-MALDI/TOF MS, multiple samples were separated, deposited on the tape, and analyzed by the mass spectrometer, in parallel. With this coupling of multiple sample streams, high throughput could be achieved without compromising separation performance and sensitivity of analysis and with no sample cross talk. Although the principles of CAE-MALDI/TOF MS are demonstrated in on-line arrangement in this work, the experiment can be carried out with a commercial instrument in the off-line mode as well.

EXPERIMENTAL SECTION

Mass Spectrometer. A modified laboratory-built linear TOF MS, described previously,[37] was used in this work. Briefly, the instrument consisted of a Wiley−McLaren-type ion source,[41] a 2-m flight tube, and a dual microchannel plate (Burle, Lancaster, PA). The distances between the repeller and first grid, and between the first and second grids, were 10.0 and 12.7 mm, respectively. Voltages on the repeller and the first grid were controlled by two power supplies (model CZE1000R/X2263, Spellman, Hauppauge, NY) at 14.0 and 12.0 kV, respectively. High-voltage pulses for

(17) Van Pelt, C. K.; Corso, T. N.; Schultz, G. A.; Lowes, S.; Henion, J. *Anal. Chem.* **2001**, *73*, 582−588.
(18) Micromass LCT with MUX technology, http://www.micromass.co.uk: *Chem. Eng. News* **1999**, *77* (13), 47.
(19) Jespersen, S.; Niessen, W. M. A.; Tjaden, U. R.; van der Greef J.; Litborn, E.; Lindberg, U.; Roeraade. J. *Rapid. Commun. Mass Spectrom.* **1994**, *8*, 581−584.
(20) Little, D. P.; Cornish, T. J.; O'Donnell, M. J.; Braun, A.; Cotter, R. J.; Köster, H. *Anal. Chem.* **1997**, *69*, 4540−4546.
(21) Allmaier, G. *Rapid. Commun. Mass Sectrom.* **1997**, *11*, 1567−1569.
(22) Ekström, S.; Ericsson, D.; Önnerfjord, P.; Bengtsson, M.; Nilsson, J.; Marko-Varga, G.; Laurell, T. *Anal. Chem.* **2001**, *73*, 214−219.
(23) Medzihradsky, K. F.; Campbell, J. M.; Baldwin, M. A.; Falick, A. M.; Juhasz, P.; Vestal, M. L.; Burlingame, A. L. *Anal. Chem.* **2000**, *72*, 552−558.
(24) Krutchinshy, A. N.; Zhang, W.; Chait, B. T. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 493−504.
(25) Shevchenko, A.; Loboda, A.; Shevchenko, A.; Ens, W.; Standing, K. G. *Anal. Chem.* **2000**, *72*, 2132−2141.
(26) Stevenson, T. I.; Loo, J. A. *LC-GC* **1998**, *16*, 54−58.
(27) Bergman, A. C.; Bergman, T. *J. Protein Chem.* **1997**, *16*, 421−423.
(28) Walker, K. L.; Chiu, R. W.; Monnig, C. A.; Wilkins, C. L. *Anal. Chem.* **1995**, *67*, 4197−4204.
(29) Zhang, H. Y.; Caprioli, R. M. *J. Mass Spectrom.* **1996**, *31*, 1039−1046.
(30) Biemann, K.; Gagel, J. J. U.S. Patent 4843243, June 1989. Biemann, K. U.S. Patent 5770272, June 1998. http://www.labconnections.com.
(31) Hensel, R. R.; King R. C.; Owens K. G. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1785−1793.
(32) Murray, K. K. *Mass Spectrom. Rev.* **1997**, *16*, 283−299.
(33) Nagra, D.; Li, L. *J. Chromatogr.* **1995**, *711*, 235−245.
(34) He, L.; Li, L.; Lubman, D. M. *Anal. Chem.* **1995**, *67*, 4127−4132.
(35) Chang, S. Y.; Yeung, E. S. *Anal. Chem.* **1997**, *69*, 2251−2257.
(36) Orsnes, H.; Graf, T.; Degn, H.; Murray, K. K. In *Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics*; June 13−17, 1999; poster MPD 085.
(37) Preisler J.; Foret, F.; Karger, B. L. *Anal. Chem.* **1998**, *70*, 5278−5287.
(38) Rejtar, T.; Hu, P.; Preisler, J.; Karger, B. L., submitted to *Anal. Chem.*
(39) Strupat, K.; Karas, M.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Processes* **1991**, *111*, 89−102.
(40) Mathies, R. A.; Huang, X. C. *Nature* **1992**, *359*, 167−169.
(41) Wiley, W. C.; McLaren, I. H. *Rev. Sci. Instrum.* **1955**, *26*, 1150−1157.

delayed extraction were produced by a voltage pulser (model HTS 151 A, Eurotek, Morganville, NJ). The repeller, initially at the same potential as the extraction grid (12.0 kV), was pulsed to the full voltage (14.0 kV) for ~10 μs. The delay time between the laser and high-voltage pulses was set with a digital delay generator (model 9650A, EG&G, Princeton, NJ) according to the m/z range that was to be focused. It should be noted that the values of the delay time corresponded to those set with the delay generator; i.e., they were not corrected for either ~190-ns delay start time of the high-voltage pulser or ~80-ns rise time of the pulse. Pressure in the flight tube was 1×10^{-7} (no infusion), 1.4×10^{-6} (single-capillary infusion), or 1×10^{-5} Torr (infusion with the eight-capillary array).

Vacuum Deposition Interface for MALDI/TOF MS. The details of the interface for vacuum deposition of samples were described previously.[11] The sample solution was deposited on a Mylar (polyester) tape (Maxell Corp., Fair Lawn, NJ), which was propelled by a geared stepper motor in a manner similar to a casette player. The tape velocity was set to 1 mm/s. For the parallel MALDI/TOF MS experiments, a 10-capillary array was prepared, with 8 capillaries being used for deposition at one time. The two additional capillaries were available for manual replacement of the clogged capillaries in the liquid junction. Ten tapered capillaries with 20-μm i.d. and 150-μm o.d., 14-cm length (Taper-Tips TT150-20-15-N-5, New Objective, Cambridge, MA) were aligned in an array, and an epoxy droplet was applied ~6 mm from the tips, to maintain the capillaries in a fixed position. The tips were silanized by immersing into dichlorodimethylsilane (Sigma Chemical Co., St Louis, MO) for ~5 min. The array was inserted into a tubular probe (9.5-mm o.d., 40-mm length) and fixed with two small bolts ~12 mm from the tips. For vacuum sealing, a silicone rubber septum was tightened with a thumb-screw at the outer end of the probe. Finally, the probe was inserted into the source chamber via quick coupling such that the ends of the capillaries were slightly bent while touching the tape.

Capillary Electrophoresis. Eight separation capillaries (10 cm length) were coupled with eight infusion capillaries using a common liquid junction. The construction of the common liquid junction, similar to that of our previous single-unit liquid junction,[42,43] was made from a polycarbonate block with PEEK liners.[37] The distance between individual separation channels in the array was 2.5 mm. The separation and infusion capillaries were precisely aligned inside the liquid junction with a 50-μm gap. The pressure at both ends of the separation capillary was the same. However, the inlet of the infusion capillary, positioned in the liquid junction, was at atmospheric pressure, while the outlet was under vacuum. Due to the pressure difference, there was a steady flow of ~120 nL/min in the infusion capillary, requiring ~15 s for analytes to travel through the infusion capillary. In addition, the 50-μm gap between the separation and infusion capillaries prevented the formation of hydrodynamic flow in the separation capillary.

The cathode reservoir was common for all separation capillaries; i.e., the same CE buffer was used for all channels.

Capillary electrophoresis was performed using 75-μm-i.d., 375-μm-o.d., 10-cm-length fused-silica capillaries (Polymicro Technologies, Phoenix, AZ), coated with poly(vinyl alcohol) (PVA)[44] to reduce electroosmotic flow substantially. The liquid junction contained a 10 mM solution of α-cyano-4-hydroxycinnamic acid (αCHCA) in 50% (v/v) methanol/water as MALDI matrix. The same matrix solution was also chosen as background electrolyte. The stepper motor was activated to move the tape at 1 mm/s within 5 s after the liquid junction was filled with the matrix solution. The sample was injected hydrodynamically or electrokinetically at 800 V/cm, and electrophoresis was performed at 800−1000 V/cm using a high-voltage power supply (model PS/EH30, Glassman, Whitehouse Station, NJ).

Optical Design. A 355-nm, 5-kHz diode-pumped, frequency-tripled Nd:YAG laser (model EPO-5000, Continuum, Santa Clara, CA) was used for desorption. To achieve tighter focus, the beam was first expanded 3-fold with concave and convex quartz lenses (focal length 5 and 15 cm, respectively), which was necessary due to the relatively high beam divergence. As shown in Figure 1, the reflected laser beam was attenuated with a stepped neutral density filter (Edmund Scientific, Barrington, NJ) and directed with a scanner mirror via a quartz lens (focal length 20 cm) on to the sample target at an angle of incidence of 60°. A galvanometer optical scanner (model 6810P, Cambridge Technology, Cambridge, MA) was used to scan the laser beam. According to the manufacturer, the scanner could move the mirror (8×10 mm) through a small angle to within 99% of the final position on a submillisecond time scale. The angular repeatability of the scanner was less than 20 μrad.

Experimental Control and Data Acquisition. A LabVIEW (version 4.0, National Instruments, Austin,TX) program running on a master PC controlled the entire experiment. The master PC communicated with a digital oscilloscope (model 9350AM, LeCroy, Chestnut Ridge, NY) via a GPIB interface (model PC II, National Instruments), operated the stepper motor controller (model EPC01, Hurst, Princeton, IN), scanned the mirror via a digital/analog output card (model LAB PC 1200, National Instruments), and triggered a custom-made timing box. Alternatively, the inner frequency generator in the stepper motor controller moved the tape at 1 mm/s. The digital oscilloscope was used only in the initial stage for determination of the positions of the traces and for real-time display of the ion signal. In the "determination" mode, the timing box simultaneously sent single trigger pulses to both the delay generator and the laser after receiving a trigger from the PC card. Data were recorded with a high-speed signal averager/compressor, HSSAC (model FastFlight, PerkinElmer Instruments, Crownsville, MD) and transferred to a PC. In the data acquisition mode, the PC card sent trigger pulses with specified frequency to the timing box, which, after receiving each of the trigger pulses, generated n pulses with specified delay. The first pulse was sent to the delayed extraction followed by $n - 1$ pulses to the HSSAC. Within 64 ns after receiving each pulse, HSSAC started the data acquisition and simultaneously returned the pulse back to the timing box. The timing box then forwarded the HSSAC pulses to the laser and the delayed extraction.

This complex scheme assured synchronization of data acquisition with laser and high-voltage pulses, as well as identical wave shapes of each of n high-voltage pulses. A C++ program was then used to convert data into *.SPC file format for analysis and

(42) Lee, E. D.; Muck, W.; Henion, J. D.; Covey, T. R. *Biomed., Environ. MS* **1989**, *18*, 844−850.

(43) Foret, F.; Zhou, H.; Gangl, E.; Karger, B. L. *Electrophoresis* **2000**, *21*, 1363−1371.

(44) Karger, B. L.; Goetzinger, W. U.S. patent 5,840,388, 1998.

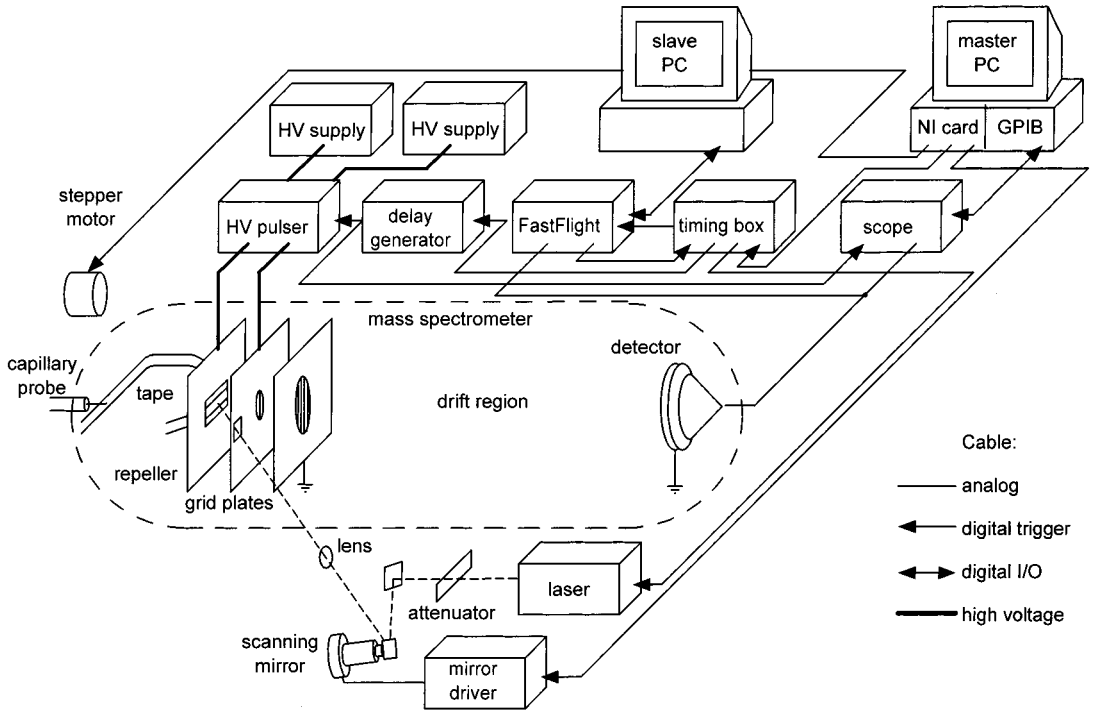

Figure 1. Schematic diagram of the capillary array on-line MALDI/TOF MS. Lines: low-voltage connection (thin solid), high-voltage connection (thick solid), and laser beam (dash). Components listed in the figure are described in detail in the Experimental Section.

visualization with commercial software (GRAMS 3D, Galactic Industries, NH).

Chemicals and Sample Preparation. αCHCA (Sigma) was recrystallized in methanol. Urea was purchased from Pharmacia (Uppsala, Sweden), 1,4-dithio-DL-threitol (DTT) and iodoacetic acid (IAA) were from Pierce Chemical Co. (Rockford, IL), and HPLC grade methanol and acetonitrile (ACN) were from Fisher Scientific (Fair Lawn, NJ). Deionized water was produced with an Alpha-Q system (Millipore Corp., Marlborough, MA).

Angiotensin peptides, purchased from Sigma Chemicals Co., were made up as 1 mg/mL stock solutions in water. Sample test solutions of angiotensin peptides were prepared by addition of a small volume of diluted peptide stock solution to the matrix solution. All proteins were purchased from Sigma and digested according to a standard protocol.[45] Briefly, a 5 mg/mL solution of an individual protein was dissolved and denatured in a solution of 50 mM NH_4HCO_3 and 8 M urea (pH 8.0), reduced by DTT, alkylated by IAA, diluted, and digested with TPCK-treated trypsin (Sigma) in a ratio of 1:25 (w/w) at 37 °C for 16 h. After digestion, the solutions were stored in a freezer (−20 °C) for future use. The digested solutions (containing 2 M urea) were hydrodynamically injected (Δh = 3 cm for 10 s) into CE columns without further purification.

Safety Considerations. Special care should be taken while working with the high-voltage power supply and laser.

RESULTS AND DISCUSSION

Principles of Capillary Array MALDI/TOF MS. In previous work,[11] we showed that continuous on-line vacuum deposition of a sample on a Mylar tape resulted in high spot-to-spot reproducibility and high mass sensitivity. Here, we demonstrate how this deposition approach can be employed to exploit the substantial speed of the TOF mass spectrometer for analysis of multiple separated samples without sacrifice of sensitivity or quality of the mass spectra. The discussion is relevant to both on-line and off-line MS analysis, especially with high-resolution separation.

Consider a single-capillary system. A mixture of analytes and matrix is assumed to be infused into a mass spectrometer and deposited on a target as a narrow trace (<100 μm). The thickness of the layer of deposited sample will be very small (a few micrometers),[37] and the entire sample from a given segment of the deposited trace will typically be consumed in a limited number of laser shots. If the portion of the trace irradiated by the laser is assumed to be 100 μm and if the target velocity is 1 mm/s, then, in 1 s, 10 five-shot average mass spectra can be obtained from the trace using a laser with a repetition rate of 50 Hz, i.e., 10 points/s across a separated peak. Assuming that acquisition of a single-shot mass spectrum requires 100 μs, the duty cycle of the instrument would then be only 0.5% for a single sample trace; i.e., 99.5% of the time the mass spectrometer would not be in use. It is clear that there is a significant opportunity to enhance the throughput of the TOF MS by increasing the repetition rate of the laser, while maintaining spectral quality (sensitivity, resolution, etc.). However, a simple increase of the repetition rate of the laser may not lead to an enhancement in sample throughput. Even worse, the accumulation of too many single-shot mass spectra from a given spot might decrease the signal-to-noise ratio, because of premature depletion of the thin sample trace.

To benefit fully from the high acquisition speed of a TOF mass analyzer equipped with a laser operating at kilohertz repetition rates, the instrument time should be divided among a number of samples (or sample streams) accessible in the mass spectrometer.

(45) Stone, K. L.; Williams, K. R. In *The Protein Protocols Handbook*; Walker, J. M., Eds.; Humana Press: Totowa, NJ, 1996.

Multiple samples can be deposited in parallel on a MALDI target using an array of *n* infusion capillaries, closely spaced to maximize the density of the deposited traces for the on-line or off-line mode.

In the on-line approach, the traces are deposited on a moving target, such as Mylar tape[11] or a quartz wheel,[37] in the source chamber and immediately transferred to the desorption region. The target with the array of deposited traces moves in one dimension and is scanned with the desorption laser beam by a fast-scanning mirror in the perpendicular dimension. Advantages of this approach are its continuity and minimal time between deposition and analysis, which can be useful, e.g., for continuous monitoring.

In the off-line arrangement, the traces are deposited on a conventional target positioned on a fast x, y stage in a standalone subatmospheric chamber and transferred to the mass spectrometer later in a separate step. A fast x, y stage would move the target with multiple deposited sample traces in the source chamber across a stationary laser beam. This approach would offer several advantages; the ion trajectory would be independent of sample position on the target, and a single external calibration could be used for the entire target. Another advantage of this approach would be that the positions on the target could be rapidly addressed and in any order, making reanalysis straightforward and more convenient (i.e., archiving). Although the step positioning speed of an x, y stage with the entire target is not as fast as that of the mirror scanner, the stage could move at a constant speed (∼100 mm/s) along a trace while the laser is pulsing without interruption. The number and length of the deposited traces would be limited by the density of the traces and the size of the target (approximately 8 × 12 cm for commercial instruments). A limitation of standard targets is that the area available for sample deposition (<100 cm^2) is much smaller than a single tape cartridge (∼3000 cm^2).

Vacuum Deposition Using a Capillary Array. To demonstrate experimentally the feasibility of the parallel deposition, we used a closely arranged array of 10 fused-silica capillaries (20-μm i.d., 150-μm o.d., and 14-cm length) coupled to the vacuum deposition interface to produce eight traces on the Mylar tape; two capillaries were used as spares in case of blockage of a tip. Close alignment of the infusion capillaries in the array was used to minimize the width of the entire array and hence the differences in the length of the ion trajectories in the mass spectrometer. Also, large (12- and 25-mm-diameter) extraction grids and a 40-mm-diameter dual microchannel plate were selected to achieve high ion collection for all traces. No difference in ion collection efficiency and mass resolution was observed when a MALDI sample, which was deposited on a conventional metal probe tip with 6.35-mm diameter, was desorbed from spots up to 3.1 mm away from the flight tube axis.

Vacuum deposition of 1 μM angiotensin III with 10 mM αCHCA in 50% methanol on the tape moving at 1 mm/s yielded eight parallel traces. The time required for sample transfer from the inlet of the infusion capillary to the desorption region was 130 s at the above speed. As measured with an optical microscope, the spacing between the centers of the traces was ∼150 μm, i.e., the outer diameter of the capillaries. Since the width of the traces was determined to be <80 μm, the spacing between the edges of the traces was at least 70 μm, and the optimum width of the laser spot on the tape was ∼100 μm. Such a laser beam could hit a specific trace without irradiating an adjacent trace and still fully encompass the selected trace.

Table 1. List of Angiotensins Used for Capillary Array MALDI/TOF MS Analysis

capillary	peptide	m/z[a]	ion intensity[b] (V)
1	angiotensin, bullfrog	1259.6	−0.28
2	angiotensin II, fragment 1−7	899.5	−0.27
3	angiotensin II, fragment 3−8	775.4	−0.30
4	angiotensin I, human	1296.7	−0.23
5	angiotensin III, human	931.5	−0.30
6	angiotensin II, human	1046.5	−0.25
7	angiotensin I, [Val^5]	1282.6	−0.21
8	angiotensin I, salmon	1258.7	−0.23

[a] Mass of monoisotopic $[M - H]^+$ form. [b] As obtained from Figure 2.

Infusion of 50% (v/v) methanol via this eight-capillary array resulted in a pressure increase to ∼1.0×10^{-5} Torr. In the future, the instrument could be readily expanded to an even larger capillary array using differential pumping. It should be noted that differential pumping is not necessary for off-line deposition in a standalone subatmospheric chamber, since the deposition can be carried out under rough vacuum conditions.

The shape of capillary tips was found to be important for proper deposition. It was observed that long sharp tips prepared by etching in hydrofluoric acid were fragile and could be easily damaged and clogged. Tapered tips, on the other hand, were found to be mechanically robust and had a life expectancy of at least several weeks. Nevertheless, arrays with 10 capillaries were manufactured, so that the entire array in this prototype study did not have to be exchanged if one or two capillaries were accidentally clogged. Silanization of capillary tips with dimethyldichlorodisilane was found to reduce occasional accumulation of the deposited material on the tips.

Performance of the Capillary Array Interface with Infused Samples. The instrument design was tested on an array of eight sample traces obtained by deposition of solutions of 1 μM peptides and 10 mM αCHCA matrix in 50% (v/v) methanol at 1 mm/s for 1 min. In each trace, a single peptide was present; see Table 1. For this investigation, the laser frequency was set at 100 Hz, and a digital oscilloscope was used for data acquisition. The position of the scanning mirror was changed by manual adjustment of the input voltage controlling the mirror driver until a trace was found, and a 10-shot average spectrum was then recorded at that position. Figure 2 shows eight 10-shot average mass spectra obtained from a single scan of the array. In each spectrum, the expected angiotensin peptide could be identified from the major peak. Significantly, no cross talk between two adjacent traces on the tape was observed, which confirmed that the laser spot size could be adjusted so that irradiation of a position on a single trace at a time was possible. It should be noted that the absolute magnitude (i.e., not normalized) of the ion signal of the peptides was plotted in Figure 2. The peak heights of all eight peptides were comparable, suggesting very similar conditions (infusion, desorption, detection) for all traces of the array. Mass resolution obtained from each trace was above 2000 for single-shot spectra on this laboratory-built instrument.

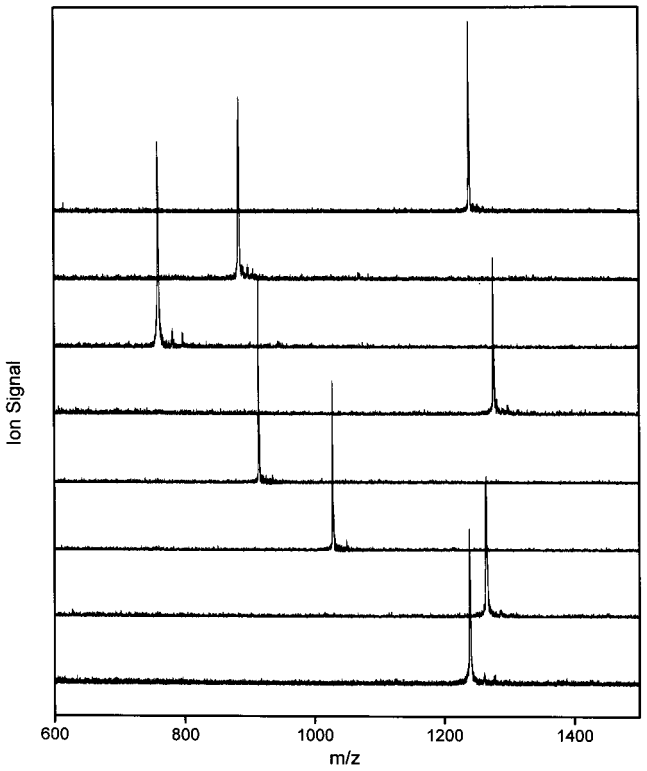

Figure 2. Mass spectra of eight angiotensin samples in Table 1 infused from an eight-capillary array with 100-Hz laser repetition rate. Ion signal unit (V) is the same for all spectra; values of offset of individual spectra were varied in the range 0.0−0.7 V with an increment of 0.1 V for better clarity of the graph. See Table 1 and text for details.

To compare mass calibration among traces, a mixture of three peptides (angiotensin II, fragments 3−8; angiotensin II human; angiotensin I, human) and 10 mM αCHCA matrix in 50% (v/v) methanol/water were infused via all eight capillaries and deposited on the tape, again at 1 mm/s for 1 min (results not shown). The differences among the calibration coefficients for the capillaries were found to be random within the range corresponding to the ±1 Da mass window, probably due to relatively poor voltage stability (voltage stability 0.1%) of the high-voltage power supplies. Upgrade of the high-voltage power supplies with better stability (voltage stability 0.001%) is in progress.

Automatic Determination of Positions of the Traces. Prior to high-throughput MALDI analysis, the position of each trace on the Mylar tape had to be known. After deposition of eight identical sample solutions (1 μM angiotensin III with 10 mM αCHCA in 50% methanol) on the tape, the parallel traces were transferred to the desorption region. A LabVIEW program then generated a voltage ramp consisting of 100 small steps for the galvanometer optical scanner to move the laser beam across the tape at a rate of 500 Hz. Each step of the scanner moved the laser spot ∼15 μm across the tape, as calculated from the dependence of the laser beam deviation at the focal plane on the input galvanometer voltage. After each step of the scanning mirror, the laser was triggered and a single-shot mass spectrum recorded. To reduce data transfer from the oscilloscope to the master PC, only ion intensity of a selected ion (alkali metal, matrix, or internal standard ion) from each of 100 single-shot mass spectra, was sent from the oscilloscope to the computer.

A set of 100 values of potassium ion intensity from the mass spectra taken across the tape with eight deposited traces is shown in Figure 3. The width of the entire array was 1.1 mm, in agreement with the value found with the optical microscope. The position of the eight traces could easily be identified from the graph and the positions of their centers automatically calculated by the computer for use in data acquisition. It can also be observed in Figure 3 that the laser could strike a single trace without desorbing the adjacent trace(s); i.e., there was no cross talk between samples in adjacent traces. It is important to note that, despite the fact that all data result from single-shot spectra (the standard deviation of laser energy is ∼6%), the magnitudes of heights of each peak are very similar. The automatic determination of the positions of the traces required ∼1 s. This time could be further shortened to a fraction of a second with faster software and, if necessary, repeated in real time during the analysis. To investigate the stability of the deposition and the desorption alignment, the positions of the traces were monitored along a 40-mm portion of the tape (corresponding to 40 s of deposition). No significant drifts or variations of the positions of the traces were observed. This indicated excellent stability of the mechanical, optical, and electronic components and proved that a single determination of the positions of the traces prior to acquisition could be sufficient for the entire experiment. After the position of the eight traces on the target was known, the scanning mirror automatically began to move the laser beam in larger steps to probe only the centers of the traces. A scan of the array began by directing the laser beam to the first trace. After the mirror movement, delayed extraction circuitry was initially pulsed one time without a laser shot in order to change the load of the two high-voltage power supplies (∼1 ms). To improve signal-to-noise ratio and signal reproducibility, the laser struck the first trace 5 times at a rate of 1 kHz (∼5 ms), an average MALDI mass spectrum was acquired, and the mirror then moved the beam to the next trace. The laser was then switched off for ∼4 ms, providing sufficient time for the LabVIEW program to recall the next mirror position and for the mirror to make the appropriate step. This procedure continued until the last trace in the array was interrogated; the mirror then jumped the laser beam back to the first trace and a new array scan began.

CAE-MALDI/TOF MS. In this work, we demonstrate the high-throughput capability of a multichannel interface for fast high-resolution CAE-MALDI/TOF MS analysis of the peptide mixtures listed in Table 2. Eight separation capillaries were coupled with the array of eight infusion capillaries in a single common liquid junction. This junction, the anodic reservoir, and the separation capillaries were filled with 10 mM αCHCA in 50% (v/v) methanol/water, which served as MALDI matrix as well as CE buffer. The samples were electrokinetically injected at 800 V/cm for 2 s and separated by CE at 800 V/cm. The cations leaving the separation columns were drawn into the infusion capillary in the flow of matrix solution from the liquid junction and deposited on tape at 1 mm/s for ∼2 min; the liquid junction was then washed with methanol. The LabVIEW program was run to determine automatically the position of the traces and acquire the data, as described above.

The resulting 3D images of eight MS-electropherograms are shown in Figure 4. The peak pattern on each image could be easily

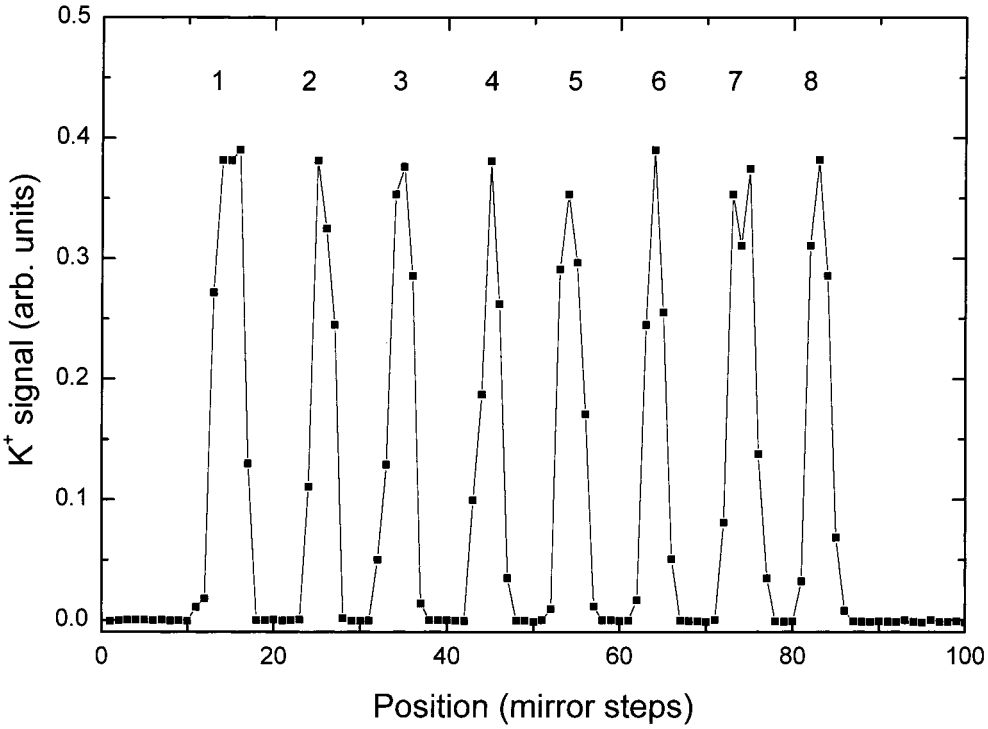

Figure 3. Peak area of the K^+ ion as a function of the transverse position of the laser spot on the tape. Deposited solution: 1 μM angiotensin III with 10 mM αCHCA in 50% methanol at a tape speed of 1 mm/s.

Table 2. CAE-MALDI/TOF MS Analysis of Peptide Mixtures

		migration time (s) for capillary no.								
sample	peptide[b]	1	2	3	4	5	6	7	8	RSD[a] (%)
1	angiotensin fragment 3−8		85.3		85.0		87.3		88.0	1.7
	angiotensin fragment 1−7		79.3		77.9		80.2		81.3	1.8
	angiotensin II [Asn[1], Val[5]]		68.9		72.6		70.8		71.1	2.1
	angiotensin I des-Asp		62.1		61.2		64.1		62.8	2.0
	angiotensin [Val[5]]		70.9		72.9		72.8		73.2	1.4
2	angiotensin III human	78.9		65.5		63.4		67.2		2.9
	angiotensin II [Asn[1], Val[5]]	81.9		69.5		68.5		71.5		2.2
	angiotensin II human	91.0		79.3		76.2		82.6		4.0
	angiotensin I bullfrog	86.0		72.9		72.8		77.6		3.7
	angiotensin I salmon	98.4		84.0		81.6		87.6		3.6

[a] Values of migration time for capillary 1 excluded. [b] Concentration of all peptides was 1.0 μM, except angiotensin, fragment 3−8, and angiotensin I [Val[5]] (C = 0.3 μM).

attributed to one of the two peptide mixtures (Table 2). No cross talk between adjacent traces was observed, even though large amounts (~100 fmol) were injected into the short capillaries. Thus, no mixing of sample solutions in the common liquid junction took place. It can be seen from Table 2 that the migration time of angiotensin II [Asn[1], Val[5]], which was present in both sample mixtures, was significantly longer (~13 s) in the first capillary compared to other capillaries of the array. The peak heights of all peptides were similar with the same sample (capillaries 3, 5, and 7), suggesting proper function of liquid junction for the first capillary. Most likely the first infusion capillary was partially clogged. Yet, the pattern of the peaks and the measured peptide masses point unambiguously to sample 2. If necessary, an array may be calibrated using a standard in all capillaries and the migration times corrected for the differences in the infusion time. The relative standard deviations of the migration times of the peptides in the capillaries of the array ranged from 1.4 to 4.0% (excluding the data for the first capillary). The half-width of analyte peaks was 2.5−4 s.

Averaging and compression of data by the high-speed signal averager/compressor (HSSAC) reduced data size by discarding the data points without useful information. Briefly, the HSSAC detected peaks in the average mass spectra and kept all data points in the interval of the peaks but only every nth point of baseline between the peaks (e.g., n = 100). For example, the overall size of data generated by HSSAC for the 200-s CAE-MALDI/TOF MS of eight protein digests was 32.5 MB. The initial data stream of 12.5 MB/s (12.5 array scans/s, 8 capillaries, 5 shots/spot, record length 25 000 points at resolution 2 ns/point, 1 byte/point) was reduced by averaging to 7.8 MB/s (record length 25 000 points at resolution 2 ns/point, 3 bytes/point) and further by compression to 160 kB/s. It should be stressed that the data were recorded at a rate of 12.5 average MS per second per capillary, which allowed good representation of separated peaks.

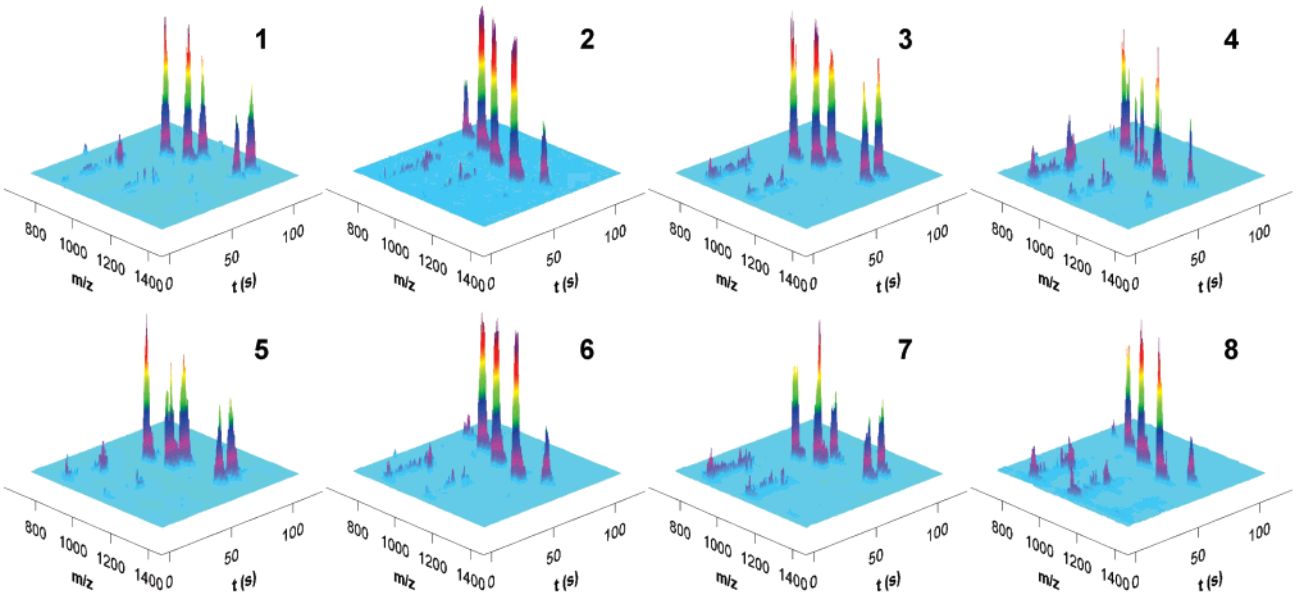

Figure 4. Eight MS-electropherograms of two angiotensin sample mixtures in Table 2 from CAE-MALDI/TOF MS, using 500-Hz laser repetition rate. See Table 2 and text for details.

Table 3. Results and Parameters of the MSFit Search for the First Capillary

rank	MOWSE score	no. (%) of masses matched	protein MW/p*I*	species	protein name
1	3.29e+003	11/20 (55)	16951.6/7.36	horse	myoglobin
2	465	6/20 (30)	17089.7/6.67	rabit	myoglobin
3	129	7/20 (35)	17205.9/7.33	canfa	myoglobin
coverage: 59% (91/153AA's)					
database searched	SwissProt.12.15.2000				
molecular weight search	10 000−20 000				
p*I* range	full				
species	mammals				
peptide mass tolerance	1.0 Da				
enzyme	trypsin				
max no. of missed cleveages	2				
cysteine modified by	carboxymethylation				

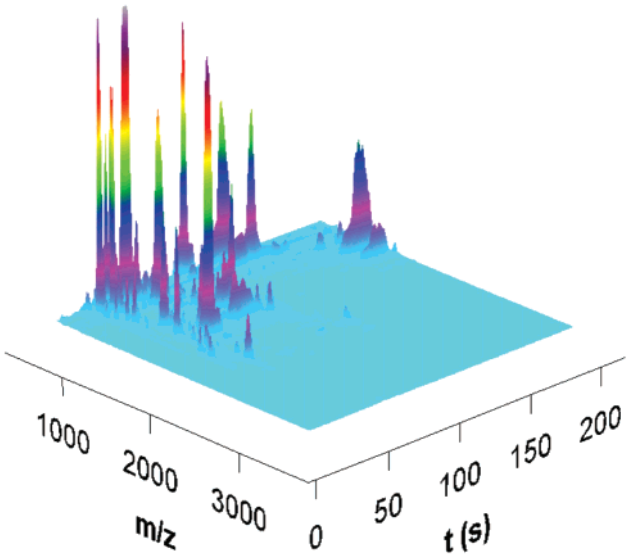

Figure 5. MS-electropherogram of myoglobin digest from CAE-MALDI MS analysis for the first capillary. See Table 3 and text for details.

Finally, individual samples of digests of eight proteins, myoglobin, fetuin, β-lactoglobulin, β-casein, cytochrome *c*, carbonic anhydrase, bovine serum albumin, and ribonuclease b, were hydrodynamically injected into the separation capillaries at $\Delta h =$ 3 cm for 10 s and then separated by CE at 1 kV/cm. All eight deposited traces were again found, and data were recorded for 200 s. The 3D images of eight MS-electropherograms (not shown) revealed unique peak patterns, each corresponding to the injected digests. Again, comixing and cross talk were not observed. As an example, a 3D MS-electropherogram from the first capillary is shown in Figure 5. The masses of 20 observed peaks were entered into a database program, and horse myoglobin was correctly identified (see Table 3 for details).

CONCLUSION

The principles of parallel vacuum deposition for MALDI/TOF MS have been demonstrated in the analysis of eight individual solutions consisting of a mixture of peptides and matrix deposited on a moving tape directly in the source chamber of the TOF mass spectrometer. Using a fast galvanometer optical scanner, the positions of the eight sample traces were found, and all samples

were successfully analyzed. No cross talk between adjacent sample traces was observed. At the repetition rate 100 Hz, the performance of the interface was similar to that obtained in single-capillary experiments.

In addition, an array of eight separation capillaries was coupled to eight infusion capillaries by means of a common liquid junction for CAE-MALDI/TOF MS analysis of peptide samples and protein digests. With a high-repetition laser at 500 Hz and high-speed data acquisition system, parallel processing was shown. Eight MS-electropherograms were recorded at a data rate of 12.5 average mass spectra per second for each sample. Modification of the timing diagram of the experiment and upgrade of our current high-voltage power supplies, which prevented high mass resolution and accuracy at the kilohertz repetition rate, is in progress.

As noted earlier, the approach to a capillary array vacuum deposition interface is effective for both on-line and off-line. Moreover, the deposition can be decoupled from the desorption step; i.e., the movement of the tape at the deposition region does not have to be identical to the movement in the desorption region. This decoupling may enable further analysis, such as ISF, PSD, or MS/MS, on selected bands while the tape is momentarily stopped at the desorption region. Another feature of the tape cartridge, i.e., the large surface area (comparable to ~30 microwell plate-sized flat targets), may be attractive for archiving of separated samples. Advantages of the off-line deposition on flat targets include a wide choice of suitable commercial instruments, calibration independent of trace position, size of the capillary array limited only by the target width, and rapid addressing of samples in an arbitrary order.

The maximum number of samples that can be analyzed in this approach can be further increased. Assuming 100-μs length of a single-shot spectrum and neglecting the time needed to reposition the target, the desorption laser can be run at 10 kHz without interruption. At this rate, ~100 traces (96-capillary array) would be characterized with 10 average spectra (each from up to 10 single-shot spectra) per trace per second. Note that the extension to parallel analysis does not compromise data quality but still provides data rates suitable for fast separations (10 average spectra/s). The properties of the proposed capillary array system, sensitivity, mass resolution, and high throughput, make it attractive for proteomics.

ACKNOWLEDGMENT

The authors gratefully acknowledge NIH for support of this work (Grant HG02033), Dr. Arthur Miller for writing the file converter, Dr. Gary Valaskovic for providing TaperTip capillaries, and Dr. František Foret for stimulating discussions. Contribution no. 794 from the Barnett Institute.

Received for review June 20, 2001. Accepted October 10, 2001.

AC010692P