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Liquid chromatography–time-of-flight mass spectrometry with continuous-flow matrix-assisted laser desorption ionization

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Abstract

Matrix-assisted laser desorption ionization (MALDI) mass spectrometry has been combined with liquid chromatography (LC) by using a continuous-flow sample inlet system. The on-line interface consists of two major components: a three-port mixing tee for post-column matrix addition and a flow probe for introducing the solution directly into a time-of-flight mass spectrometer. In the experiment, the LC effluent and the matrix solution flow into the mixing tee through two separate ports. The resulting mixture is directed to the flow probe for MALDI. Both conventional LC and microbore LC have been successfully interfaced to MALDI for on-line detection of proteins. It is demonstrated that the interface does not degrade the chromatographic performance significantly. With microbore LC, LC–MALDI can be performed with total-sample injection in the low-picomole region. An example is also given to illustrate the application of on-line LC–MALDI for peak identification in a protein mixture separation.

1. Introduction

Matrix-assisted laser desorption ionization (MALDI) mass spectrometry along with electrospray ionization has become an increasingly important method for biological macromolecule characterization. The MALDI technique has a wide applicability and is remarkably tolerant to buffers, salts, and other additives in the sample [1,2]. However, compared with electrospray, MALDI is difficult to interface to solution-based separation methods, such as liquid chromatography (LC) and capillary electrophoresis (CE), for mixture analysis [2]. While MALDI can be used for analyzing simple mixtures directly, it is still highly desirable and often required to perform sample clean-up and fractionation by chromatographic methods. There are reports of off-line combination of LC and CE with MALDI mass spectrometry [3–8]. In these techniques, the effluents are collected onto either a rotating disk or an array of collecting cups. The matrix solution is then added to the sample and MALDI can be performed in a way similar to conventional static MALDI experiments. In an attempt to introduce solutions directly into a MALDI mass spectrometer, Murray and Russell recently developed an elegant system in which an aerosol beam is formed from the protein samples and the matrix solution [9,10]. These aerosols are subsequently transferred to a time-of-flight mass spectrometer. MALDI is performed from the aerosol particle surfaces. Although the detection sensitivity is demonstrated to be about 100

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nmol with a mass resolution of 5–10, samples can be continuously introduced into a TOF-MS at 1 ml/min and the technique has been recently used for LC detection [10].

We have developed a continuous-flow matrix-assisted laser desorption ionization (CF-MALDI) technique for introducing solutions directly into a conventional MALDI–TOF-MS system [11]. This technique uses a flow probe to continuously flow the sample and a liquid matrix, 3-nitrobenzyl alcohol (3-NBA), for MALDI. In this earlier work [11], a frit-type flow probe was used to deliver the sample and matrix at a flow-rate of 1–5 μl/min through a capillary tube and onto the probe surface, upon which laser desorption/ionization was carried out. Detection sensitivity of hundreds of picomoles was demonstrated. The use of 3-NBA liquid matrix was found to be very important in achieving reproducible signals under the flow conditions [11].

In this report, we describe the design of a much improved flow probe for CF-MALDI. With this new probe, CF-MALDI can be used for the detection of proteins with an injection of low picomoles of samples in flow injection experiments. Furthermore, we report the development of an interface for combining conventional as well as microbore LC to CF-MALDI for protein analysis. The interface consists of a three-port mixing tee, similar to that employed in continuous-flow fast-atom bombardment for coupling LC [12]. The effluent from the liquid chromatography system is directed to one port of the mixing tee. The second port is connected to a syringe pump which continuously feeds in the MALDI liquid matrix, 3-NBA, in a diluted solution. In this manner the LC effluent is allowed to mix with the liquid matrix. The resulting mixture flows out of the third port and through a flow probe to a TOF mass spectrometer for MALDI. One major advantage of this post-column matrix addition method is that since no matrix is added to the elution solvent, there is no need to change the LC separation process. In addition, flow-rates for LC separation and matrix introduction can be independently changed and optimized for speed and ion detection sensitivity.

2. Experimental

2.1. Probe design consideration

In MALDI, a small amount of sample in the low-picomole to femtomole range, loaded onto a solid probe, often yields a mass spectrum with a good signal-to-background ratio (static MALDI). The detection limit achieved with the MALDI technique depends on several factors, including ionization efficiency, detection efficiency, and the sample loading procedure. From a technical point of view, the major difference between static MALDI and CF-MALDI is the sample loading procedure. In both cases only a small area of the sample is subject to laser desorption, since the laser beam is normally focused onto a spot size of less than 500 μm in diameter. Thus, the question of how one loads the sample into a small, confined area on the probe becomes important in determining the overall detection limit of a MALDI system. In CF-MALDI, the sample is either injected or dissolved into a carrier solvent containing 3-nitrobenzyl alcohol, and flows continuously over the probe surface. Consequently, it is expected that the detection limit of the CF-MALDI method is affected by the size of the area of the probe surface in which the solution diffuses. Diffusion of the sample solution into a large area on the probe surface would result in a high detection limit. We note that other parameters may also play a role in determining the overall sensitivity, such as the degree of sample adsorption onto the capillary tube or to other parts of the probe with which the sample solution makes a direct contact.

In order to obtain better detection sensitivity with CF-MALDI it is thus necessary to maximize the ratio between the area of the sample being desorbed and the total area of the sample diffused on the probe. There are two major ways of achieving this goal. First, an increase in the laser beam size would allow a larger sample area to be desorbed. However, we found that an increase in the beam size above 0.5 mm in diameter does not enhance the signal-to-background ratio sig-

nificantly [11]. An alternative way to maximize the ratio is to decrease the sample probe area. This would allow the sample to flow in a more confined region on the probe surface. The new flow probe described below is geared towards this latter strategy. In addition, the stainless steel frit used in our previous design has been eliminated.

2.2. Design of the new probe

Fig. 1 shows the design of the new flow probe along with the ionization region of the time-of-flight mass spectrometer. A silica capillary tube (100 μm I.D., 360 μm O.D.) (Polymicro Technologies, Phoenix, AZ, USA) is inserted into a 1.27 cm O.D. and 0.635 cm I.D. stainless steel tube and extends from the injector to the probe tip. For electrical insulation the end section of the probe is made of vespel (~2.5 cm long, 2.8 mm O.D.). At the vespel tip a piece of kapton (2.8 mm diameter), with a small hole pierced through the center, is mounted. The use of the kapton sheet improves the flow stability, possibly due to the improvement of surface properties such as the surface flatness over the vespel material. The capillary tube is fitted to the hole in the kapton and is placed no more than 1 mm above the surface. The hole will allow the capillary to slide back and forth but it is not big enough to allow backflow of the liquid. A piece of filter paper is wrapped several times around the probe tip to absorb the excess liquid. The flow probe is inserted into the TOF mass spectrometer between the repeller and extraction plates via a custom-built solid probe lock.

2.3. Mixing tee for LC–CF-MALDI

Fig. 2 shows the schematic diagram of the 3-port mixing tee. It is constructed from a stainless steel 1.59-mm Swagelok tee. The tee is connected to the MALDI flow probe through a transfer tube. This tube is made of stainless steel and has 1.59 mm O.D. and 127 μm I.D. with both ends enlarged. The total length of the tube is 5 cm. In the experiment, the LC effluent from either a micro-LC column or a conventional LC with a sample splitter (see below) flows through a 30 μm I.D., 360 μm O.D. capillary tube to the transfer tube. In order to mix the effluent with the matrix solution, this sample capillary tube is inserted into one end of the transfer tube (500 μm I.D. and about 1 cm depth) and placed about 50–150 μm away from the recessed edge of the transfer tube (see Fig. 2). The liquid matrix solution is pumped through the space between the transfer tube (500 μm I.D.) and the sample tube (360 μm O.D.). At the other end of the transfer tube, a hole with 370 μm I.D. and a

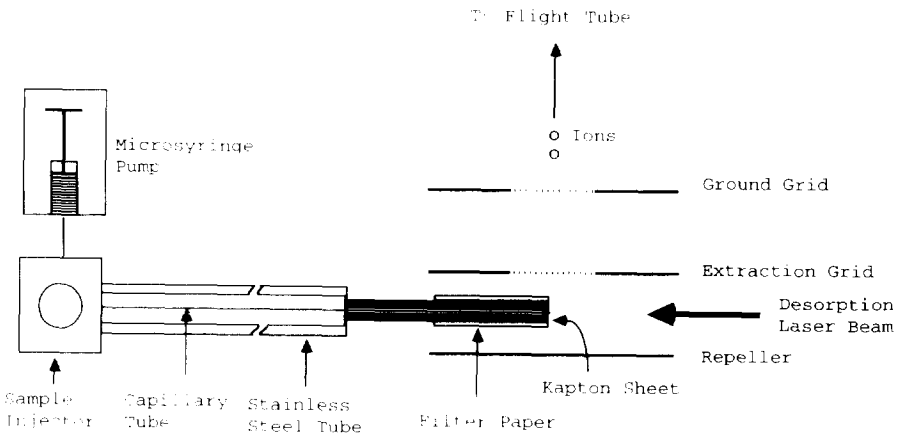

Fig. 1. Schematic of the flow probe used for continuous-flow matrix-assisted laser desorption. Drawing is not to scale. The dimensions of the major components are given in the text.

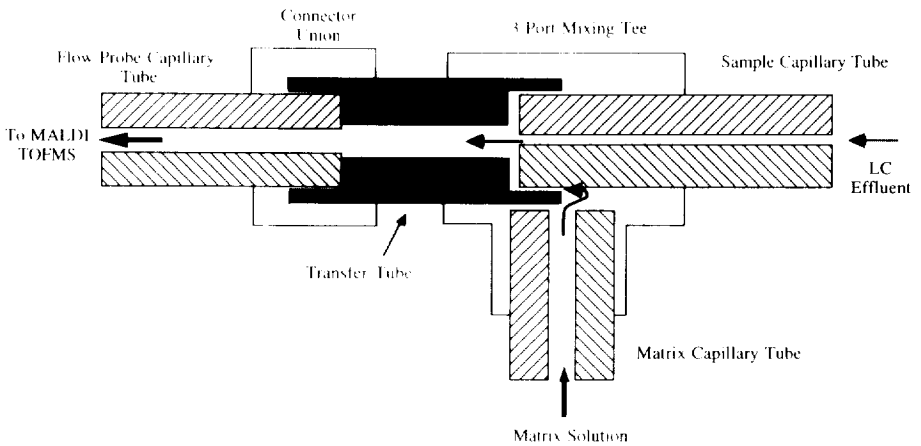

Fig. 2. Schematic of the 3-port mixing tee used for CF-MALDI with post-column matrix addition. Drawing is not to scale.

depth of 1 cm has been drilled to fit to the flow probe capillary tube (100 μm I.D. × 360 μm O.D. × 45 cm) snugly. The mixed solution then flows to the tip surface of the flow probe, at which point MALDI is carried out. Note that different tube I.D.s are used in the interface assembly. This arrangement seems to create a sufficient amount of turbulence in the transfer tube, resulting in better sample/matrix mixing and stable ion signals.

For delivering the matrix solution, a syringe pump (Harvard Apparatus, Model 11, Southnatick, MA, USA) is used. The solution consists of 15% of 0.1% trifluoroacetic acid (TFA), 45% ethylene glycol, 25% 1-propanol, and 15% 3-nitrobenzyl alcohol (all by volume). The flow-rate is generally fixed at 5 μl/min. The LC effluent or sample flow-rate is normally in the range of 1–5 μl/min.

2.4. Conventional LC with CF-MALDI

Fig. 3 shows the overall schematic of the LC system and the interface. A Shimadzu LC-600 dual pump system is used for solvent delivery. For protein separation, a Vydac 25 cm × 2.1 mm I.D. Protein and Peptide C_{18} column is used. A Rheodyne Model 7125 injection valve with a 20-μl internal loop is used for sample injection. The LC separation is carried out at a flow-rate of 0.5 ml/min. In order to achieve a sample flow-rate of 1–5 μl/min prior to matrix addition, the LC effluent is split by a tee using two Swagelok metering valves connected in parallel. This combination of a fine and coarse metering valve gives reproducible and fine flow-rate control of the LC effluent entering the mixing tee.

2.5. Micro-column LC with CF-MALDI

Fig. 4 shows the schematic of the micro-column LC/MALDI system. The LC system consists of a Shimadzu LC-600 dual pump and a home-built solvent splitter. The latter is used to obtain a flow-rate compatible for the micro-column LC separation. The solvent splitter consists of a tee connected to a parallel combination of a coarse and fine metering valve, allowing a controllable flow-rate of 1–10 μl/min for the LC separations. The micro-column (LC Packings, Fusica C_{18}, 5 cm × 320 μm I.D.) is connected directly to a Valco sample injector (60 nl) with finger tight fittings. For MALDI detection, the end of the capillary column is connected to the 3-port mixing tee through a short transfer tube, as shown in Fig. 4.

2.6. Time-of-flight mass spectrometer

A Jordan's reflectron time-of-flight mass spectrometer (R.M. Jordan co., Grass Valley, CA, USA) is used for the CF-MALDI experiments

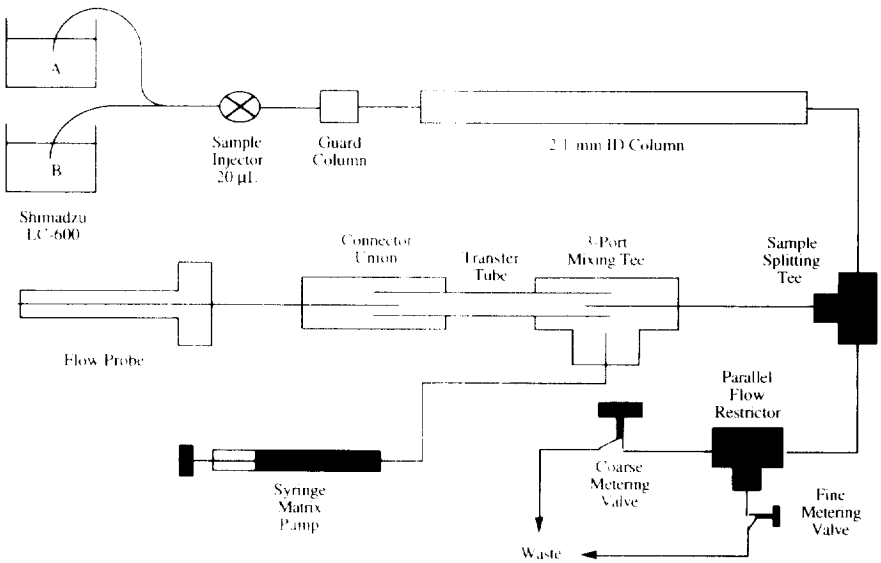

Fig. 3. Schematic of the conventional LC–MALDI system.

reported here. This angular reflectron system, designed primarily for multiphoton ionization of small molecules, has been described previously in detail [13–15]. The reflector can withstand up to 5 kV reflecting voltage. Thus, the present design of the reflector is suitable for reflecting relatively low mass ions. However, for ions with molecular masses above 5000 it is generally found that a voltage much higher than 5 kV is required to achieve good detection sensitivity. Therefore, for the CF-MALDI experiments reported here, the reflectron system is operated in a linear mode. As shown in Fig. 1, the flow probe is placed in between the repeller and the

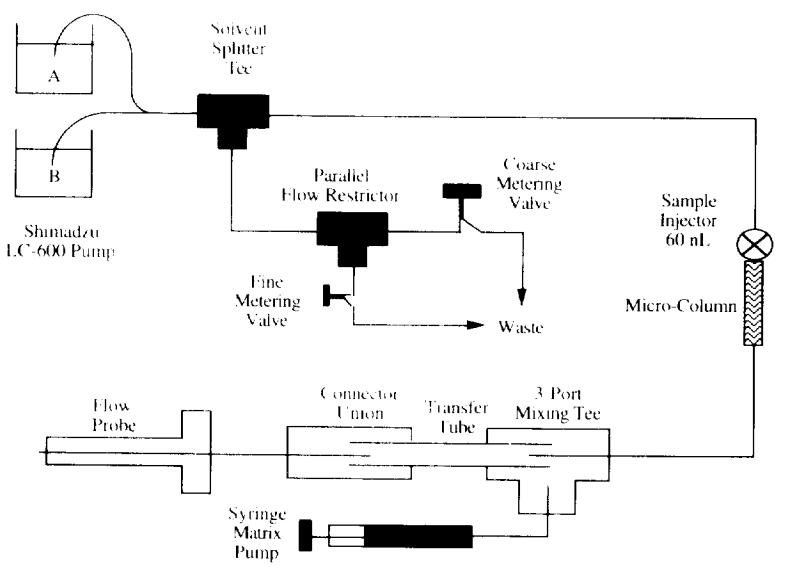

Fig. 4. Schematic of the micro-column LC–MALDI system.

extraction grid. The ions generated from the MALDI process expand in a direction orthogonal to the flight path.

The TOF mass spectrometer is mounted vertically in a six-port cross pumped by a 6-inch diffusion pump (Varian Associates, Lexington, MA, USA). The 1-m flight tube is pumped by a 4-inch diffusion pump (Varian). The pressure in the flight tube is usually below $9 \cdot 10^{-4}$ Pa during operation. A Bayard–Alpert ion gauge with Varian's multi-gauge controller is used for the pressure measurement. It is worth noting that the vacuum pressure reading can sometimes be used as an indication concerning the stability of the continuous flow. A stable flow would result in an almost constant reading or only minor fluctuations in the vacuum pressure.

2.7. Laser desorption and data processing

A frequency quadrupled Nd:YAG laser (GCR-3, Spectra-Physics, CA, USA) which generates 266 nm radiation is used for performing MALDI. The laser is operated at 10 Hz repetition rate. A convex lens (300-mm focal length) is used to focus the laser beam to a ~0.5 mm diameter spot on the flow probe. The spot size is estimated by examining the dark image created on a thermal-sensitive paper, which is placed on the probe using double sided tape, after the paper has been briefly exposed to the laser beam. The laser power density used varies from 10^6 to 10^7 W/cm^2. The laser power is measured with an Ophir Model 10A-MED-AN laser power/energy meter (Diamond Ophir Optics, Wilmington, MA, USA).

The mass spectrum generated by the laser desorption process is recorded with a LeCroy 9400A digital oscilloscope. The analog signal is preamplified 25 × before being fed into the oscilloscope. Data produced on the oscilloscope are then transferred in real time to a PC via General Purpose Interface Bus (GPIB). The PC is a generic 486DX33 machine with a 210-MByte hard drive and 4 MBytes of RAM. The data transfer and data analysis software is developed in-house. Details of the functions of the data system and its performance for chromatography–time-of-flight mass spectrometry have been reported elsewhere [16]. In brief, the data system is capable of transferring and storing transients up to 20k data points at a repetition rate of greater than 10 Hz from the oscilloscope to the PC via GPIB. After storing all the mass spectra in the PC, either a selected-ion or a total-ion chromatogram can be established. Both the mass spectrum and the chromatogram can be saved into a text disk file for use by other commercial software packages for further processing such as mass spectral averaging.

All samples are purchased from Sigma and Aldrich (St. Louis, MO, USA). Protein sample solutions are prepared in 0.1% aqueous TFA.

3. Results and discussion

With the new flow probe for performing CF-MALDI experiments, it is found that flow injection analysis (FIA) can be readily performed. Using the experimental setup shown in Fig. 1 for FIA without the mixing tee, protein samples can be introduced into the matrix flow with the use of a 60-nl sample injector. The microsyringe pump delivers the matrix solution through a capillary tube. This solution consists of 95% ethanol (25%), 0.1% TFA (25%), ethanediol (35%), 3-NBA (15%), and trypsinogen ($6 \cdot 10^{-6}$ M). Trypsinogen is added to the mobile phase and is continuously introduced into the probe for the purpose of optimizing the flow conditions. We note that during the initial development of CF-MALDI, the use of the standard can greatly assist the optimization of the flow conditions. During the course of the experiment, the ion signals in the molecular ion region of trypsinogen is carefully monitored to ensure that experimental conditions such as laser power are not significantly changed. Fig. 5 shows the ion profile of five repeated injections of 3 picomoles of horse heart cytochrome c (M_r 12 361) along with the ion profile from repeated injections of 9 picomoles. The ion profile is obtained by integrating the molecular-ion peak area of each mass spectrum collected and summing 10 peak areas to generate one data point in the ion profile. As

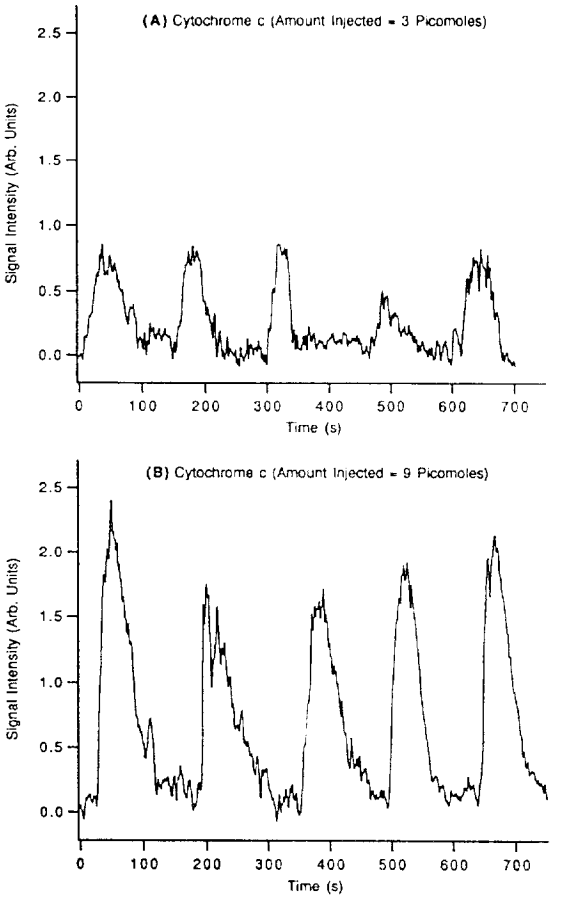

Fig. 5. Flow injection ion profiles of repeated injections of different amounts of horse heart cytochrome c: (A) 3 picomoles and (B) 9 picomoles, without the use of the mixing tee.

Fig. 5 illustrates, the peak areas are dependent on the concentration of the protein injected. We find that under the same experimental conditions (i.e., using the same laser power, same gain for the detector, etc.), a linear calibration can be obtained with the injection of cytochrome c at an amount ranging from 1 to 12 picomoles. Higher concentration requires the reduction of laser power to prevent signal saturation, in order to extend the dynamic range of the technique. This is consistent with the results reported by using static MALDI [17].

In order to examine the effect of the addition of the mixing tee on the performance of CF-

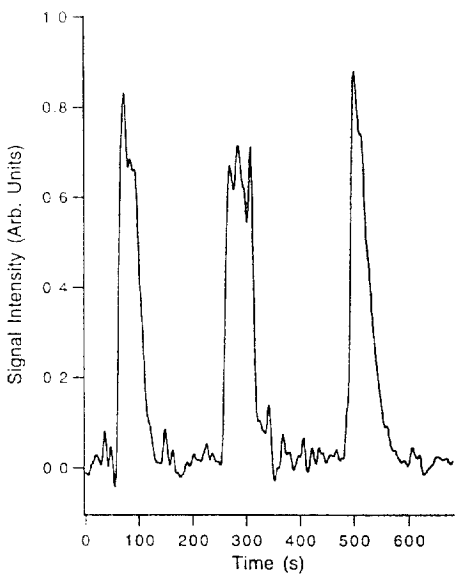

Fig. 6. Flow injection ion profiles obtained from 3 repeat injections of 10 picomoles of cytochrome c with the use of the mixing tee.

MALDI, FIA is first carried out. The experimental setup for FIA is similar to that used for micro-column LC detection as shown in Fig. 4. In FIA, the LC column is not used. Fig. 6 shows three repeat injections of 10 pmol cytochrome c. In this case, the mobile phase solvent contains 60% water and 40% acetonitrile. The peak shapes shown in Fig. 6 are almost the same as those obtained without a 3-port mixing tee as shown in Fig. 5. This indicates that the mixing tee does not introduce a significant amount of dead volume. By examining the ion signal reproducibility, it appears that the injected sample is well mixed with the matrix solution in the tee. By alternately injecting different samples, we do not observe any cross-over ion signals, indicating the absence of memory effect in the interface. It should be pointed out that the current design of the mixing tee has evolved from several earlier attempts. We find that the optimization of the tube diameters and the manner in which the matrix solution is guided to the transfer tube is critical in achieving stable and reproducible results. For example, doubling the I.D. of the

transfer tube would result in larger peak tailing in the FIA ion profile. This can be attributed to the increase of dead volume in the transfer tube.

This mixing tee is then used to combine LC separation with MALDI. In using a conventional 2.1-cm column for protein separation, the flow-rate used is relatively high, i.e., 0.5 ml/min. Since the flow probe can only accept a flow-rate of 1–10 μl/min for stable operation, the LC effluent has to be split. The experimental arrangement shown in Fig. 3 is very easy to set up and we find it to be quite convenient in combining conventional LC with MALDI. Fig. 7A shows the ion chromatogram of a simple mixture containing horse heart cytochrome c and chicken egg white lysozyme obtained by LC–MALDI. In this case, binary gradient elution is used for separation. Pump A contains 0.1% aqueous TFA, while pump B delivers acetonitrile–water (90:10) containing 0.1% TFA. A gradient was performed from an initial 40% solvent B to 65% solvent B in 5 min. From 5 to 8 min the gradient was increased to 70%, and subsequently held thereafter. A 5-nmol injection of each protein was made, with about 0.2% split to the MALDI mass spectrometer. Thus, about 10 pmol of each protein was actually used for producing the ion chromatogram shown in Fig. 7A. We note that, although the rest of the sample was split to the waste in this experiment, it should be possible to recover the sample either directly or after flowing through a UV detector in a real sample application.

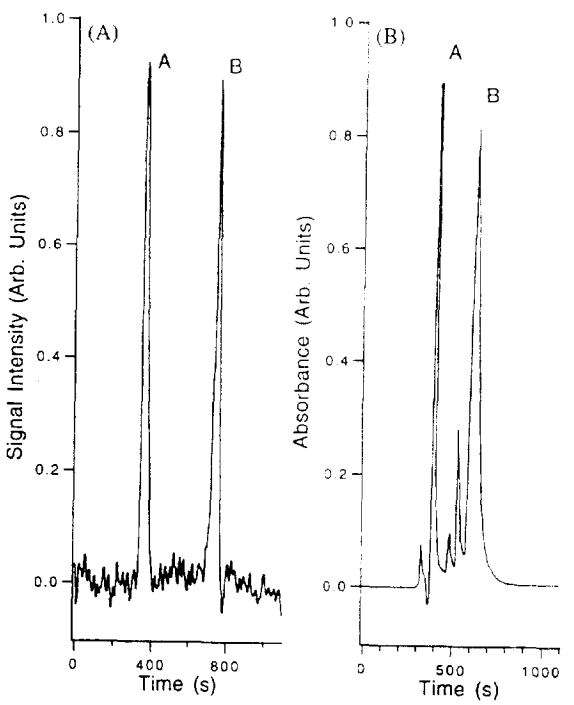

Fig. 7. (A) Ion chromatogram of LC separation of a mixture of horse heart cytochrome c and chicken egg white lysozyme by using a conventional 2.1 mm I.D. column. The total sample injected is 5 nmol each with a 0.2% sample split ratio to MS. (B) UV chromatogram (242 nm) of the same mixture.

Fig. 7A clearly shows that the two proteins are well separated. For comparison, a UV chromatogram of the same mixture obtained by using a conventional LC–UV detection system is shown in Fig. 7B. Since the UV chromatogram was not obtained on-line with the CF-MALDI results, a strict comparison cannot be made. Nevertheless, based on peak shapes and widths, the LC interface for CF-MALDI does not introduce a significant amount of peak broadening or distortion to the LC separation. Note that there are several other small peaks observed in the UV chromatogram. These peaks are not detected in LC–MALDI. They are very likely from low-molecular-mass species present in the sample ($M_r <$ 1000). Since the background signals are quite strong in CF-MALDI in the region with $m/z <$ 1000, these small ions are not resolved from the background signals.

A representative mass spectrum of cytochrome c is shown in Fig. 8. The resolution of the mass spectrum shown in Fig. 8 is 16 FWHM (full width at half maximum) for cytochrome c. With external calibration, the calculated mass for cytochrome c is 12 325. Compared with the known molecular mass of cytochrome c (12 361), the mass measurement error is about 0.29% in this case. Similar results are obtained for other proteins separated by LC. In light of the fact that the current mass spectrometric system is not fully optimized for high-resolution MALDI, these results are quite encouraging.

We also find that the resolution obtained with CF-MALDI is not degraded when compared with static MALDI in this TOF-MS instrument. Therefore, the resolution of CF-MALDI is very

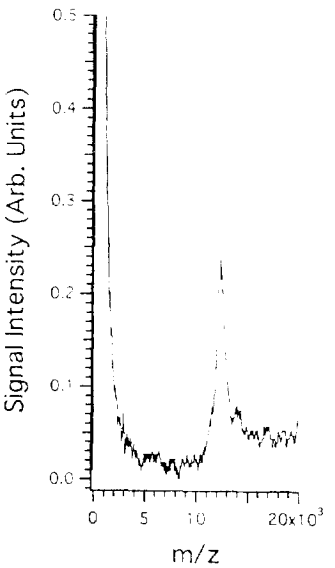

Fig. 8. Mass spectrum cytochrome c obtained at the LC peak shown in Fig. 7.

much related to the design of the mass spectrometer. One of the major reasons of observing low resolution in this TOF-MS system is related to the orientation of the sample probe with respect to the flight tube. In our experimental setup for CF-MALDI, the ions are extracted in an orthogonal configuration with constant voltages applied to the repeller and extraction grid. This configuration allows us to flow the liquid into the ionization region of the TOF without electric breakdown. However, with this configuration, even using a very small laser beam size, one would expect some ion spatial distribution, which would result in poor mass resolution. The development of high-resolution TOF-MS for CF-MALDI is currently underway. Preliminary results indicate that parallel ion extraction provides 5 to 10 times enhancement in mass resolution as well as detection sensitivity [18]. Mass resolution in the range of 100 to 250 and mass measurement error in the range of 0.02% to 0.12% have been obtained for small peptides for CF-MALDI [18]. A full account of this work will be reported in the future.

While LC separation is convenient to perform with a conventional LC system, CF-MALDI TOF-MS requires a high sample split ratio, on the order of 500, in order to avoid overloading the mass spectrometer with excess solvent. We thus explored the possibility of using microbore-LC for separation. With slight modifications the methodology applied to the conventional column LC was also applied to the micro-column LC as shown in Fig. 4. The 3-NBA matrix solution is added to the mixing tee after the column separation, and the entire sample mixture is introduced through the flow probe and onto the probe surface. Fig. 9A shows the MALDI–TOF-MS ion chromatogram of a mixture of cytochrome c (9 pmol) and lysozyme (8 pmol) separated by micro-column LC. The separation is carried out using isocratic conditions [60% solvent A containing 0.1% TFA and 40% solvent B consisting of acetonitrile–water (90:10) containing 0.1% TFA]. Peak A in Fig. 9A is from cytochrome c and peak B from lysozyme. The chromatogram of the same mixture using a UV detector replacing the MALDI–TOF-MS is shown in Fig. 9B for comparison. To obtain the UV chromatogram a piece of capillary tubing (50

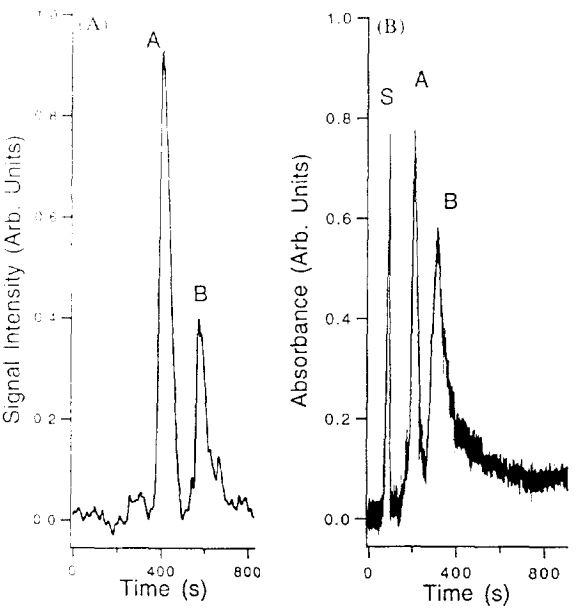

Fig. 9. (A) Ion chromatogram of LC separation of a mixture of horse heart cytochrome c and chicken egg white lysozyme by using a micro-column. The injected sample consists of 9 pmol cytochrome c and 8 pmol lysozyme. (B) UV chromatogram (214 nm) of the same mixture.

μm I.D. × 360 μm O.D. × 60 cm) was connected to the outlet of the LC capillary column, and the other end of the capillary positioned into a 214 nm UV detector (Waters Quanta 4000 capillary electrophoresis). At a point 7 cm from the capillary end a 1-cm portion of the polyimide coating was burnt away to yield a clear quartz surface. Peak S in this UV chromatogram is from the solvent and peaks A and B from cytochrome c and lysozyme, respectively. The comparison of these two chromatograms shown in Fig. 9 shows a small loss in resolution in the ion chromatogram, particularly for peak A. However, the ion chromatogram still retains good overall chromatographic integrity.

A unique application of LC–MALDI is shown in Fig. 10 for the separation of three proteins: horse heart cytochrome c, chicken egg white lysozyme, and horse heart myoglobin on a reversed-phase LC column. The separation is performed with a linear gradient (65%A/35%B to 15%A/85%B in 20 min), where eluent A is 0.1% TFA and eluent B is 0.1% TFA in acetonitrile–H_2O (90:10). The flow-rate is 0.5 ml/min. In the ion chromatogram shown in Fig. 10, peak A is from cytochrome c, and peak B is from lysozyme and peak C is from myoglobin. Under the solution and separation conditions used here, an unexpected peak (peak U) is also observed. The mass spectra obtained during the elution of this peak are the same as those of cytochrome c. This suggests that other forms of cytochrome c have been generated during the course of sample preparation and LC separation.

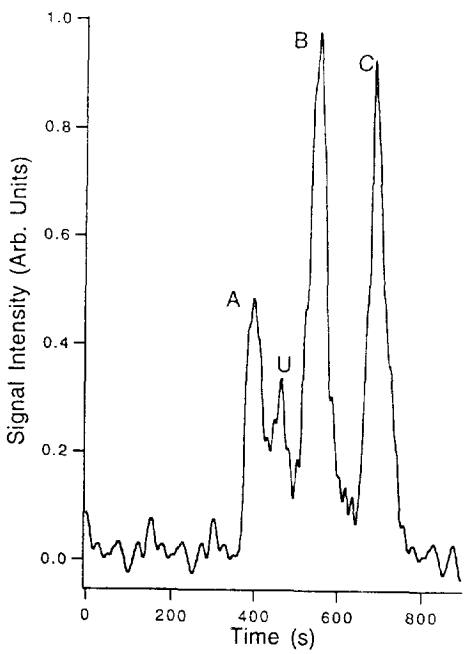

Fig. 10. Ion chromatogram of a mixture of horse heart cytochrome c, chicken egg white lysozyme, and horse heart myoglobin with conventional LC separation and MALDI–TOF-MS detection. The chromatogram represents 5 nmol injection each with 0.2% split to MS.

The peaks A and U may belong to cytochrome c with different degrees of folding. Proteins can alter their conformational structures under non-physiological conditions, such as in acidic solutions or in a column [19]. For example, lysozyme remains folded at 20°C under the acidic condition whereas apo-cytochrome c is unfolded with differing degrees depending on the ionic strength of the protein solution [20,21]. This uncertainty can often create artifacts in the chromatographic trace with a conventional detector. Recently, it was shown that on-line UV spectroscopy using photodiode-array detector in conjunction with temperature studies can be quite useful in confirming whether any additional peaks in protein separation are due to protein itself or to impurities [19]. However, for unknown sample separation, peak identification in UV chromatography would still be difficult. On the other hand, with the use of MALDI for LC detection as illustrated in Fig. 10, the mass spectra provide an additional dimension for identification.

In conclusion, we have developed an on-line LC–MALDI system for protein analysis. We envision that the ability of separating and identifying structurally closely related species by this on-line method should be very useful in protein chemistry. We are exploring the applications of this technique for real-world biological sample analysis. In addition, the on-line technique may provide unique advantages over other LC–MS methods such as electrospray ionization MS in the area of tandem MS for peptide sequencing where MALDI can generate singularly charged species that can be dissociated via collisional-

induced dissociation (CID) to produce full sequencing information, as well as in the area of combining size-exclusion chromatography with MS for industrial polymer analysis. We plan to extend this work to a sector tandem MS instrument for high energy CID experiment with LC–CF-MALDI.

On the instrumental development of LC–MALDI, our future work will mainly concentrate on improving the mass resolution and mass measurement accuracy. To this end, we are developing a second generation time-of-flight mass spectrometric system for high-resolution CF-MALDI. This new instrument will incorporate parallel ion extraction, instead of the orthogonal configuration used in this work. In addition, we will incorporate a time-lag focusing technique for ion extraction. In a linear instrument with static MALDI, we have recently shown that mass resolution in the range of 3000 to 6000 can be obtained with time-lag focusing [22]. With this high resolution, isotopically resolved mass spectra are observed for peptides with masses up to 3000. For proteins such as cytochrome c and myoglobin, mass resolution up to 1200 can be obtained. In view of these new developments, the prospect for developing a high-performance on-line LC–CF-MALDI–TOF-MS is good. With this improved instrument, we will then compare the performance of the LC–CF-MALDI–MS technique with the well-established LC–electrospray ionization mass spectrometry for analytical applications.

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