Development of liquid chromatography–mass spectrometry using continuous-flow matrix-assisted laser desorption ionization time-of-flight mass spectrometry

Randy M. Whittal, Larry M. Russon, Liang Li[*]

Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada

Abstract

The general approach of combining liquid chromatography (LC) with matrix-assisted laser desorption ionization (MALDI) mass spectrometry for on-line detection of peptides and proteins based on the use of a continuous-flow (cf) probe is presented. Recent advances in time-of-flight mass spectrometric instrumentation for cf-MALDI are reported. Using 3-nitrobenzyl alcohol (3-NBA) as the liquid matrix, stable flow can be readily achieved and the three-port interface does not introduce chromatographic peak shape degradation for LC–MS. Separation and detection of low picomoles of peptides and proteins can be done with cf-MALDI-LC–MS. Parallel ion extraction and time-lag focusing are shown to provide enhanced performance with regard to mass resolution. However, mass resolution is generally poor for proteins with masses above ~6000 u. Strong adduct ion formation with the use of 3-NBA as the liquid matrix is believed to be the main cause of this resolution reduction. It is argued that cf-MALDI is a technically viable approach for LC–MS, but the overall performance and wide use of this method depend on the discovery of new liquid matrices that are suitable for continuous flow and provide much enhanced utility for MALDI over 3-NBA, particularly for proteins. © 1998 Elsevier Science B.V.

Keywords: Continuous-flow; Matrix-assisted laser desorption ionization mass spectrometry; Mass spectrometry; Proteins; Peptides

1. Introduction

Matrix-assisted laser desorption ionization (MALDI) mass spectrometry is an important technique for chemical analysis [1]. MALDI has been widely used for the detection of biochemicals as well as industrial or synthetic polymers [2]. However, in contrast to electrospray ionization (ESI), the MALDI technique cannot be readily combined with solution-based separation methods, such as HPLC, for on-line detection. An effective and reliable on-line LC–MALDI system is expected to complement LC–ESI in many analytical applications. For example, LC–

MALDI can potentially become a tandem separation technique where the LC separation can be based on mechanisms other than molecular weight. Individual LC peaks consisting of a mixture of several similar compounds can be ionized and separated by MALDI in a time-of-flight (TOF) mass analyzer.

Within the scope of MALDI, an on-line LC-MALDI system can provide several potential benefits for the complete analysis of complex samples. Direct analysis of mixtures by MALDI can suffer from signal suppression of one or more components. This ion suppression effect can be reduced or eliminated with the use of LC-MALDI where the individual components are either completely separated or partially separated into groups, each having similar

[*]Corresponding author.

MALDI properties in terms of hydrophobicity or protein p*I* or protein conformation. With an on-line detection system, sample throughput and MALDI analysis speed can be significantly increased. On-line LC-MALDI also improves sample handling efficiency and minimizes sample loss.

Despite the potential benefits that may be brought about by on-line LC-MALDI, the progress in developing such a system has been slow, mainly due to the technical and perhaps fundamental difficulties associated with interfacing LC to MALDI. One approach developed by Murray and Russell was to introduce the samples along with matrix solutions to a time-of-flight mass spectrometer as an aerosol beam [3,4]. MALDI is performed from the aerosol particle surfaces. Samples can be continuously introduced into the mass spectrometer at 1 ml/min and the technique has been used for LC detection [5]. The detection sensitivity was demonstrated to be about 100 pmol with a mass resolution of 5–30 fwhm (full width at half maximum) for peptides and proteins up to 17 000 u on a linear TOF-MS. Murray and co-workers [6] have recently improved the performance of this aerosol MALDI technique by incorporating a reflectron TOF-MS. A mass resolution of 300–400 fwhm was shown for peptides.

We have been involved in developing continuous-flow matrix-assisted laser desorption ionization (cf-MALDI) technology, for introducing solutions directly into a conventional MALDI-MS system [7]. Conceptually, cf-MALDI is similar to continuous-flow fast atom bombardment (cf-FAB) technology [8–11]. A flow probe is used to continuously flow the sample and a liquid matrix, 3-nitrobenzyl alcohol (3-NBA), for MALDI analysis. In earlier work [7], a frit-type flow probe was used to deliver the sample and matrix at a flow-rate in the range of 1–5 μl/min through a capillary tube and onto the probe surface, upon which laser desorption/ionization was carried out. 3-NBA liquid matrix was found to be important in achieving reproducible signals under the flow conditions used [7]. This method was further developed to combine it with microbore LC [12]. It was demonstrated that on-line LC-MALDI can be performed with a total sample injection in the low picomole region for protein analysis.

In this paper, we first briefly review the general approach of LC–MS based on cf-MALDI. Some

recent instrumental developments for cf-MALDI are then described along with the illustration of the improved performance brought about by these modifications. Experimental results relevant to the search for optimal liquid matrices are presented. The current limitations of this cf-MALDI approach are discussed.

2. cf-MALDI-LC–MS

All initial studies on cf-MALDI were done using a linear TOF mass spectrometer where the sample probe was placed in a position orthogonal to the ion flight path [7,12,13]. Details of the design of the continuous flow probe and the TOF-MS instrument have been reported [7,12,13]. An on-line post-column matrix addition method was also developed to interface LC to cf-MALDI for protein detection [12]. Fig. 1 shows the schematic diagram of the LC–MALDI interface, including the three-port mixing tee. 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 to 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 separation. The micro-column (LC Packings, Fusica C18, 5 cm×320 μm I.D.) is connected directly to a Valco sample injector (60 nl injection volume) with finger tight fittings. For MALDI detection, the end of the capillary column is connected to the three-port mixing tee through a short transfer tube. The second port of the tee is connected to a syringe pump (Harvard Apparatus, Model 11, Southnatick, MA, USA) that continuously feeds in the 3-NBA matrix solution (3–5 μl/min). This solution consists of 15% of 0.1% trifluoroacetic acid (TFA), 45% ethylene glycol, 25% 1-propanol, and 15% 3-NBA (all in volume). The resulting mixture flows out of the third port to the flow probe. The matrix is present in at least a 10^5-fold excess over the analyte. A silica capillary extends from the mixing tee to the flow probe tip. The flow probe is inserted between the repeller and extraction plates of a linear TOF-MS, where a 266-nm UV laser beam from a Nd:YAG laser operating at 10 Hz generates the MALDI ions. The resulting mass spectra are recorded and trans-
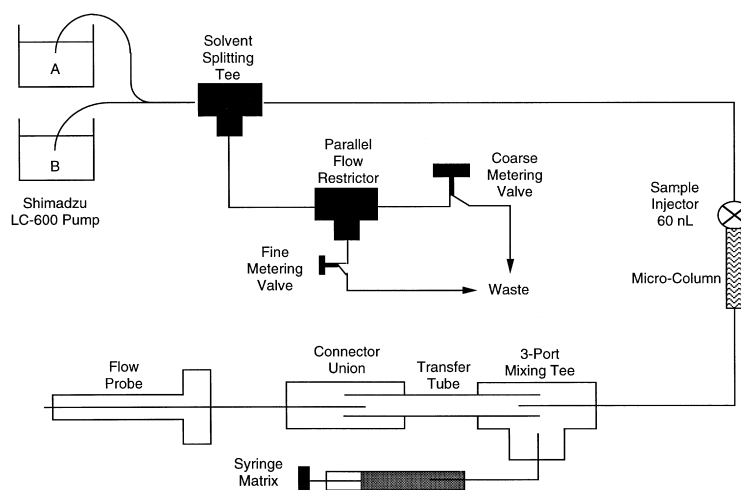
Fig. 1. Schematic of the micro-column LC-MALDI system using a continuous flow probe to interface with a time-of-flight mass spectrometer (reprinted from Ref. [12]).

ferred in real time to a PC via GPIB from a digital oscilloscope using a data system developed in house [13].

Fig. 2 shows the LC–MS ion chromatogram of a mixture of cytochrome *c* (9 pmol) and lysozyme (8 pmol) on a micro-bore LC column (320 μm I.D.). The separation was carried out using isocratic conditions (60% solvent A containing 0.1% TFA and 40% solvent B consisting of acetonitrile–water (9:1) containing 0.1% TFA). Peak A in Fig. 2 is from cytochrome *c* and peak B from lysozyme. The ion
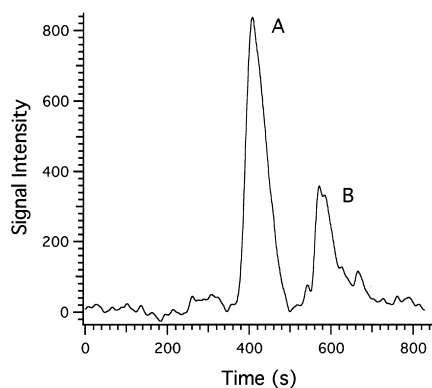

Fig. 2. Ion chromatogram of LC separation of a mixture of horse heart cytochrome *c* and chicken egg white lysozyme by using a micro-column. The injected sample was a mixture of 9 pmol cytochrome *c* and 8 pmol lysozyme (reprinted from Ref. [12]).

chromatogram was found to be similar to the UV chromatogram obtained with the same sample. Based on peak shapes and widths, it was determined that the interface shown in Fig. 1 does not introduce a significant amount of peak broadening or distortion to the LC separation [12].

The above example demonstrates the possibility of doing on-line LC–MS detection based on cf-MALDI. However, the mass resolution obtained in this experimental configuration was poor, about 10–20 fwhm for both peptides and proteins. Thus the analytical utility of this system was limited. Our subsequent research effort in instrumental development was focused on improving the resolution of the system.

3. Parallel ion extraction cf-MALDI

In TOF-MS, ions can be extracted into the flight tube from a probe that is inserted in a position orthogonal or parallel to the ion flight axis. In orthogonal extraction, the sample probe is conveniently placed between the repeller and extraction grids of the TOF instrument, thus allowing flow of the cf-MALDI liquid into the ionization region without electric breakdown. The MALDI ions expand perpendicular to the electric field in the acceleration

region. However, ions are created in different regions of the electric field due to the finite width of the laser beam. Since this results in a spatial distribution of the ions, the resolution will correspondingly decrease. With the orthogonal configuration, the mass resolution observed in cf-MALDI as well as in static MALDI using 3-NBA liquid matrix is generally less than 20 fwhm [7,12]. To reduce the spatial distribution the sample molecules can be desorbed and ionized from an isoelectric surface, i.e., from a sample plane that experiences the same electric field at any point on the plane. This is achieved with parallel ion extraction. The ions formed in the source experience the same electric field as they expand in a direction parallel to the flight tube. The parallel ion extraction method requires the sample probe to be floated to the same high voltage as that applied to the repeller. Thus, the key in using parallel ion extraction for continuous flow experiments is to develop a flow probe that is able to handle the high voltage used.

Fig. 3 shows a schematic diagram of the probe tip and the acceleration region of the linear TOF mass spectrometer that we developed for parallel ion extraction cf-MALDI. The acceleration plates are gridless and spaced by 4.7 mm. The continuous flow probe is inserted into the center of the first acceleration plate. This probe can be floated up to ~15 kV,

above which arcing can sometimes occur in the source region. The voltages applied to the acceleration plates were usually 12.0, 10.5, 8.0 kV, and ground, respectively. The tip of the flow probe is constructed of stainless steel and thus floated to the voltage of the first acceleration plate. An electrically insulated probe heater (usually heated to 65°C) prevents freezing of the matrix/analyte mixture on the probe tip. A video camera is used to monitor the liquid flow.

A matrix solution containing a known molecular weight standard is introduced through a syringe pump at 1.7 µl/min. The injected sample is introduced into the flow stream of a liquid chromatography pump (0.1–1.0 µl/min) and mixed with the matrix in a mixing tee as shown in Fig. 1. The matrix solution contains: (i) 70% 3-NBA, (ii) 25% 1-propanol and (iii) 5% water acidified to pH 2 with trifluoroacetic acid (TFA), with gramicidin S added as an internal standard. The LC effluent contains: (i) 50% water (acidified with TFA) and (ii) 50% acetonitrile. Under cf-MALDI conditions, the vacuum pressure is normally 1×10^{-3} Pa in the ionization region and 6×10^{-4} Pa in the flight tube.

With parallel extraction cf-MALDI, stable flow and reproducible analyte signals (20% S.D. in peak height) can be readily obtained. This is evident in Fig. 4 where a flow profile from five repeat in-
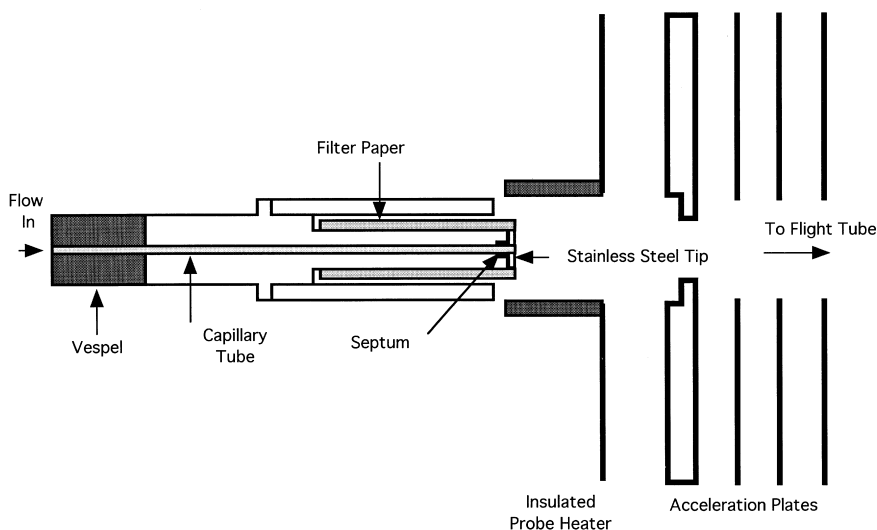

Fig. 3. Schematic of the flow probe tip and the acceleration region of the linear time-of-flight mass spectrometer used for parallel ion extraction cf-MALDI.
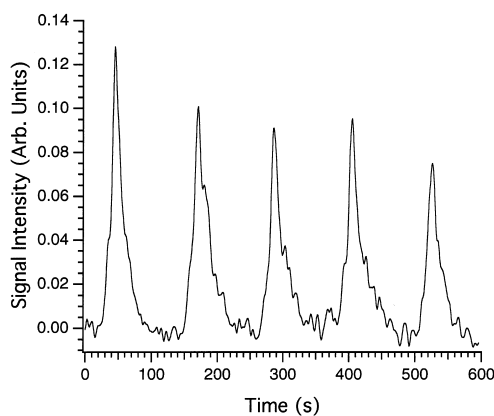
Fig. 4. Flow injection ion profile of five repeated injections of horse heart myoglobin.

jections of 0.4 pmol myoglobin is shown. One benefit of parallel extraction is that it provides about 10-fold increase in detection sensitivity over orthogonal extraction. Mass resolution is significantly improved for small peptides, compared with the
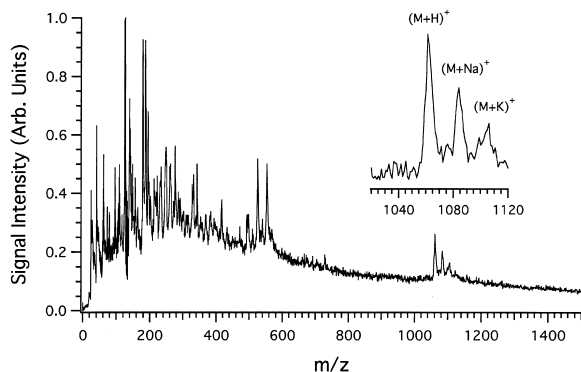

Fig. 5. Mass spectrum of bradykinin obtained by cf-MALDI with an injection of 9 pmol.

results we obtained earlier with the orthogonal ion extraction configuration. Fig. 5 shows the cf-MALDI mass spectrum of bradykinin (9-pmol injection). The mass resolution is 213 fwhm (at 1061 u) in this linear time-of-flight instrument. As expected, improved mass resolution should result in an increase in mass accuracy. Table 1 lists the mass accuracy that was observed for a number of peptides using gramicidin S as an internal standard. They range from 0.02 to 0.13%.

Clearly, parallel ion extraction cf-MALDI improves the analytical performance in terms of mass resolution, mass accuracy, and sensitivity with respect to our earlier work using orthogonal extraction, and it also sets the stage for possible application of time-lag focusing to further improve mass resolution and accuracy. However, the performance of cf-MALDI is still inferior to static MALDI. MALDI performed on a solid insertion probe with a crystalline matrix in the same instrument without time-lag focusing yields spectra with a resolution of ~500 fwhm, for peptides and proteins up to ~10 000 u. When using 3-NBA as a matrix in either continuous flow or static mode of operation, a spectral resolution of 150–280 fwhm is typical for peptides up to 1500 u and resolution is generally less than 100 fwhm for higher mass peptides and proteins. Furthermore, the mass accuracy is typically 0.01% for internally calibrated peptides on the solid insertion probe with solid matrices.

It should be noted that the analysis of peptides using crystalline matrices at the same source and system pressure as the liquid matrix introduces minimal peak broadening; thus, the change of system pressure from the use of the liquid matrix does not seem to play a major role in the observed peak broadening. One of the reasons lower resolution is

Table 1
Mass accuracy results for peptides determined by cf-MALDI with parallel ion extraction

Peptide	Average protonated mass (u)		
	Calculated	Measured	Error (%)
Ac-KLEALEA-amide	814.96	815.9	0.12
Ac-TQDEQFIP-amide	1019.10	1019.3	0.02
Bradykinin	1061.23	1061.5	0.03
LYPVKLPVK	1220.54	1220.0	0.04
Ac-KLEALEAKLEALEA-amide	1569.84	1571.9	0.13

observed when using 3-NBA as the matrix is related to adduct ion formation. Fig. 5 shows that alkali metal attachment to the peptide is a significant feature of the spectrum. As the peptide mass increases, alkali metal attachment becomes more extensive and it becomes more difficult to resolve the adduct peaks, limiting the utility of this liquid matrix for the analysis of high mass peptides and proteins. Consequently, our recent work in the area of cf-MALDI has been focused on searching for better matrices and matrix solution preparations. This is aided with the use of time-lag focusing MALDI-TOF-MS, capable of resolving adducts peaks at an extended mass range.

4. Liquid matrices and time-lag focusing MALDI

In cf-MALDI, the choice of matrix is very limited at present. There are only two known liquid matrices, 3-NBA and 2-nitrophenyl octyl ether (introduced for FAB by Meili and Seibl [14]), that are suitable for MALDI analysis of peptides and proteins [15]. We found that the liquid 2-nitrophenyl octyl ether does not provide stable flow, partially due to its fast evaporation in a vacuum. In addition, it does not provide strong analyte signals for peptides and proteins. In contrast, stable flow can be readily obtained with 3-NBA, which is not surprising because this matrix has been successfully used for cf-FAB-MS [16]. Note that a thin film of 3-NBA placed on the probe tip can remain as a liquid under the vacuum of 1×10^{-4} Pa for ~10 min. For a liquid to be effective as a cf-MALDI matrix it must remain a liquid under high vacuum conditions. This allows the analyte and matrix to continuously flow to the filter paper or other absorber wrapped along the probe adjacent to the tip surface. Otherwise, a sample memory effect would make chromatographic detection difficult.

To search for optimal matrix solutions that may be used for cf-MALDI, a great number of experiments were carried out. All these studies were done in a time-lag focusing MALDI-TOF instrument that has been described previously [17].

Glycerol has been used extensively for FAB-MS and provides stable flow in cf-FAB [11]. However, glycerol lacks a chromophore at the UV wavelengths of 266 nm from the Nd:YAG laser or 337 nm from the nitrogen laser. An additive is required to provide UV excitation [18]. Unfortunately, an investigation of a great number of glycerol mixtures including the use of laser dyes and solid matrices failed to yield any useful liquid matrix combination. For example, a mixture of coumarin 460 and glycerol can produce only very weak signals with poor resolution. In the case of mixtures of glycerol with solid matrices such as sinapinic acid (SA) or α-cyano-4-hydroxy-cinnamic acid (HCCA), the general observation was that analytically useful spectra can be obtained only after the glycerol had evaporated, leaving a dry preparation. This suggests that a mixture of glycerol and a common solid matrix such as SA or HCCA cannot be used for cf-MALDI.

To utilize the 337-nm nitrogen laser, which is by far the most popular ionization laser used for MALDI, initial attempts were made at collecting spectra using 3-NBA spiked with common solid MALDI matrix compounds such as SA and HCCA. Again, good spectra were obtained only after evaporation of 3-NBA. Mixtures with solid matrix compounds were thus abandoned and we focused on using the 266 nm line (the absorption maximum of 3-NBA is near 266 nm) from a frequency quadrupled Nd:YAG laser as the ionization source.

In the remaining experiments, for the study of 3-NBA under time-lag focusing conditions, a peptide or protein solution was prepared in water from a Milli-Q Plus Ultrapure water system (18.2 MΩ·cm) or 0.06% TFA. A 0.5-μl aliquot of 3-NBA was first applied to the probe tip followed by 0.5 μl of the peptide or protein solution. The tip was placed in a vacuum where the water evaporated. Partial removal of alkali salts from the 3-NBA was done with water extraction. Equal volumes of 3-NBA and pure water were mixed and the two solvents allowed to separate. The water was drawn off and another aliquot was added. This was repeated three or four times.

Fig. 6 shows a spectrum of bradykinin using 3-NBA as the matrix obtained by time-lag focusing MALDI. The resolution for the molecular ion peaks is about 2060 fwhm, which is sufficient to resolve the isotope peaks for this peptide. This is a significant improvement over the resolution of about 150–280 fwhm, typically obtained with static MALDI
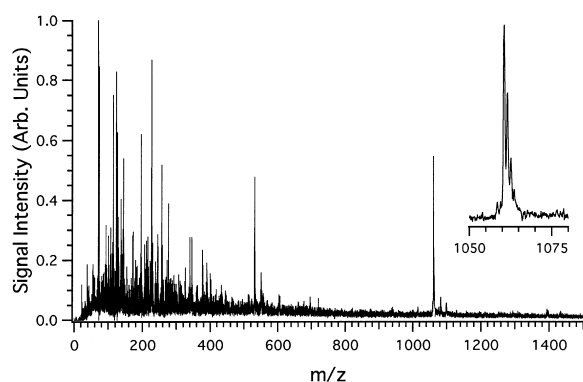
Fig. 6. Mass spectrum of bradykinin by time-lag focusing MALDI with the use of 3-NBA as the matrix.

using 3-NBA in the continuous extraction mode. Another example is shown in Fig. 7 for insulin. Fig. 7A was obtained using continuous extraction and Fig. 7B was obtained using the pulsed ion extraction with time-lag focusing. Fig. 7B shows a resolution of 835 fwhm which compares favorably with a res-
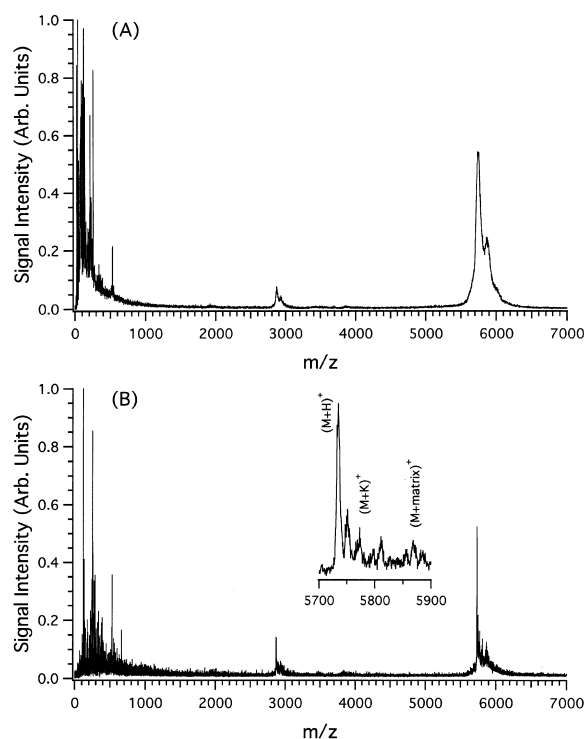
olution of ~1000 fwhm typically obtained with solid matrix preparations on this instrument for insulin. Note that the resolution obtained in the continuous extraction mode is about 70 fwhm for insulin using 3-NBA. In the insert of Fig. 7B, it is evident that strong alkali and matrix adduct peaks are formed. The alkali adduct peaks can be as intense as the protonated molecular peak for some samples. As an example, Fig. 8 shows a spectrum of bovine insulin chain B (oxidized form). In Fig. 8A potassium adduct peaks are prominent features. In a fashion consistent with that described for FAB-MS [19], Fig. 8B shows how the addition of 18-crown-6 ether to the 3-NBA significantly reduced the adduct attachment to the peptide yielding a cleaner spectrum with stronger analyte peaks.

However, for proteins such as cytochrome *c*, we have not been able to observe any resolution enhancement by using the time-lag focusing technique

Fig. 7. MALDI spectra of insulin obtained by using 3-NBA as the matrix with (A) continuous extraction and (B) time-lag focusing.
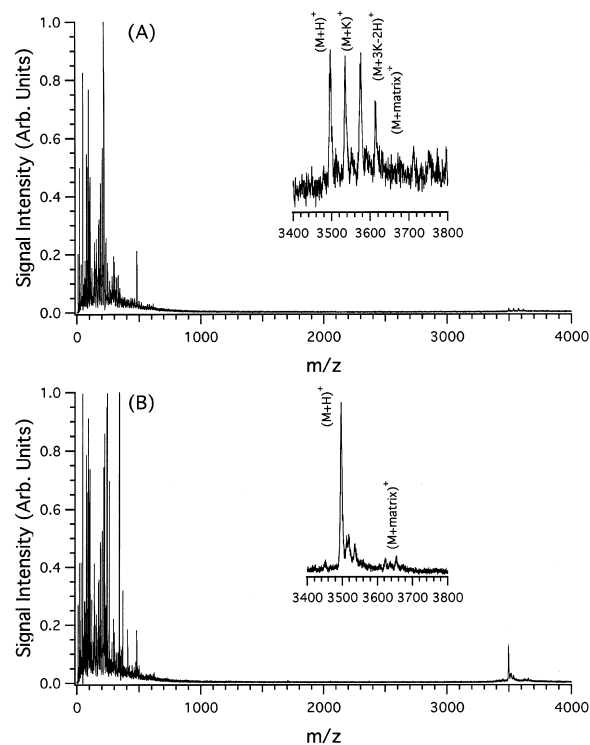

Fig. 8. MALDI spectra of oxidized B chain of bovine insulin obtained by using 3-NBA as the matrix (A) before the addition of 18-crown-6 ether and (B) after the addition. The total sample loaded is 10 pmol.

with 3-NBA as the matrix. In this case, to aid the dissolution of the proteins, a 50-mM solution of the nonionic surfactant n-octyl β-D-glucopyranoside in 3-NBA was used in place of neat 3-NBA [20]. This gave high sensitivity for proteins although the resolution is still poor (~10 fwhm). We speculate that the extensive overlap of multiple alkali adduct and matrix peaks prevent their resolution even by the time-lag focusing instrument. Despite a number of attempts to clean up the samples and matrices, including the dialysis of the samples and desalination of 3-NBA using an ion-exchange column, in the hopes of reducing adduct ion formation, we have not been able to observe signals with resolution better than 20 fwhm for proteins with masses above 8000 (i.e., ubiquitin). Clearly, 3-NBA is a good viscous liquid useful for producing stable flow for cf-MALDI but it is a poor matrix for protein analysis.

5. Conclusions

On-line combination of LC and MALDI-MS is feasible with the use of a continuous flow probe. Using 3-NBA as the liquid matrix, stable flow can be readily achieved. The flow probe interface does not degrade the performance of the chromatographic separation. A limit of detection in the low picomole range for peptides and proteins can be routinely achieved.

The major limitation of this LC-MALDI system for analytical applications is the poor mass resolution observed with peptides and proteins. Parallel ion extraction where the flow probe is floated to a high voltage, up to 15 kV, can be done. It was demonstrated in this work that this mode of operation provides improved mass resolution over the orthogonal ion extraction mode of operation. However, for peptides with masses higher than about 1500 u, it did not offer any improvement in resolution. The use of time-lag focusing TOF was anticipated to significantly improve the resolution. Good resolution was obtained for peptides up to and including the protein insulin (M_r 5734). But, with the use of 3-NBA as the matrix, poor mass resolution was observed for proteins (above M_r 8000) even with time-lag focusing TOF-MS.

The resolution improvement brought about by the parallel ion extraction and the use of time-lag focusing provides some insights on the general performance of 3-NBA as the MALDI matrix. This matrix requires the use of a 266-nm laser beam for desorption, which limits its use in most commercial systems where a nitrogen laser emitting at 337 nm is commonly used. In addition, to accommodate the special need of the flow experiments, cf-MALDI-LC–MS requires the modification of the ion source region and cannot be readily fitted to any commercial systems. A dedicated instrument would be needed. This work with parallel extraction as well as time-lag focusing clearly indicates that 3-NBA is a poor matrix for MALDI of proteins. With 3-NBA, a broad peak is observed in the molecular ion region due to the formation of extensive adduct ions. Unfortunately, unlike the situation in static MALDI with solid matrices, where a better matrix displaying less adduct ion formation can be selected among a pool of matrices, there is a very limited number of liquid matrices available. Choosing other matrices for cf-MALDI is not an option at present.

With all the instrumental development, cf-MALDI will likely be best suited for the analysis of peptides (i.e., $m/z < 6000$) in a linear TOF-MS with time-lag focusing. Note that an ion trap reflectron TOF-MS has been used for cf-MALDI [21]. However, the potential advantages of this system as a viable alternative to time-of-flight analyzers for LC–MS based on cf-MALDI remain to be seen. It is our view that the future advance of cf-MALDI with TOF-MS is dependent on the discovery of new matrices that can be used for a flow system. Recent work using an infrared laser beam [22], instead of a UV beam, as the ionization source for MALDI may open new opportunities of discovery for liquid matrices useful for cf-MALDI.

Acknowledgements

References

[1] F. Hillenkamp, M. Karas, R.C. Beavis, B.T. Chait, Anal. Chem. 63 (1991) 1193.

[2] A.L. Burlingame, R.K. Boyd, S.J. Gaskell, Anal. Chem. 68 (1996) 599.

[3] K.K. Murray, D.H. Russell, Anal. Chem. 65 (1993) 2534.

[4] K.K. Murray, D.H. Russell, J. Am. Soc. Mass Spectrom. 5 (1994) 1.

[5] K.K. Murray, T.M. Lewis, M.D. Beeson, D.H. Russell, Anal. Chem. 66 (1994) 1601.

[6] X. Fei, G. Wei, K.K. Murray, Anal. Chem. 68 (1996) 1143.

[7] L. Li, A.P.L. Wang, L.D. Coulson, Anal. Chem. 65 (1993) 493.

[8] Y. Ito, T. Takeuchi, D. Ishi, M. Goto, J. Chromatogr. 346 (1985) 161.

[9] Y. Ito, T. Takeuchi, D. Ishi, M. Goto, T. Mizuno, J. Chromtagr. 358 (1986) 201.

[10] R.M. Caprioli, T. Fan, J.S. Cottrell, Anal. Chem. 58 (1986) 2949.

[11] R.M. Caprioli, Anal. Chem. 62 (1990) 477.

[12] D.S. Nagra, L. Li, J. Chromatogr. A 711 (1995) 235.

[13] L.D. Coulson, D.S. Nagra, X. Guo, R.M. Whittal, L. Li, Appl. Spectrosc. 48 (1994) 1125.

[14] J. Meili, J. Seibl, Org. Mass Spectrom. 19 (1984) 581.

[15] M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, Int. J. Mass Spectrom. Ion Processes 78 (1987) 53.

[16] D.L. Smith, in: R.M. Caprioli (Ed.), Continuous-Flow Fast Atom Bombardment Mass Spectrometry, John Wiley & Sons, New York, 1990, p. 137.

[17] R.M. Whittal, L. Li, Anal. Chem. 67 (1995) 1950.

[18] D.S. Cornett, M.A. Duncan, I.J. Amster, Anal. Chem. 65 (1993) 2608.

[19] R. Orlando, Anal. Chem. 64 (1992) 332.

[20] J.R. Chapman, Practical Organic Mass Spectrometry, 2nd ed., John Wiley & Sons, New York, 1993, p. 330.

[21] L. He, L. Liang, D.M. Lubman, Anal. Chem. 67 (1995) 4127.

[22] R. Cramer, F. Hillenkamp, R.F. Haglund Jr., J. Am. Soc. Mass Spectrom. 7 (1996) 1187.