AP and Vacuum MALDI on a QqLIT Instrument

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This article presents a comparative study of the performance, operational, and instrumental characteristics of AP and vacuum MALDI for the analysis of peptides and protein digests. Spectra generated with the two ion sources were surprisingly similar, both qualitatively and quantitatively, with vacuum MALDI generating ion count rates that were approximately a factor of 2 greater than those generated with AP MALDI on this system. Even though the peptide signals were 2X greater with vacuum MALDI, the background intensities also increased by a similar amount, leading to approximately equivalent signal/background ratios for digests and peptide mixtures. The results suggest that when AP MALDI conditions are properly optimized, the sensitivity can approach that of vacuum MALDI. However, AP MALDI performance is critically affected by source gas flows, potentials, and temperature, making it operationally more complex. In addition, evidence is provided for thermal degradation of samples stored on a target plate within a heated AP MALDI ion source. An improved interface for atmosphere to vacuum ion transfer substantially improved these characteristics. (J Am Soc Mass Spectrom 2005, 16, 176–182) © 2005 American Society for Mass Spectrometry

Matrix-assisted laser desorption ionization (MALDI) [1, 2] is an important and widely used ionization method for the mass spectral analysis of biomolecules. Although MALDI was first described in the late 1980s, a number of significant improvements have been made to increase its applicability for peptide and protein analysis. These improvements include delayed-extraction [3–5], the use of Reflectron geometry in time-of-flight [6], and the coupling of MALDI to tandem mass spectrometry [7–9]. In the early MALDI experiments, ions were generated directly in the vacuum region of the mass spectrometer. However, recently Krutchinsky et al. [10] described a new MALDI source that provided significant performance improvements over previous versions attributable to the incorporation of an RF ion guide for collisional cooling [11]. This ion source was further improved with the addition of a cone in front of the RF ion guide for reduced fragmentation of molecular ions [12]. Techniques for reduction of the internal energy of MALDI ions have also been discussed by Verentchikov et al. [13]. The majority of MALDI applications to date have used time-of-flight mass analyzers because of the pulsed nature of MALDI and time-of-flight.

Laiko et al. [14] described an extension of the MALDI technology involving the generation of ions at atmospheric pressure. Later, he showed the potential benefits of incorporating an AP MALDI ion source with ion trap mass spectrometry for peptide mixture analysis [15]. However, at least 10–50 fmol of sample were required, substantially more than with vacuum MALDI. Subsequent hardware improvements to the AP MALDI ion source have resulted in a number of publications and reviews [16–19]. In general, the increased cooling of MALDI ions generated at atmospheric pressure results in the need to apply heat for declustering. In addition, the system performance depends on careful design of the interface between the atmospheric pressure region and the high vacuum region.

There have been a few comparisons of AP MALDI and electrospray [18, 20] in the literature. However, there have been very few details regarding comparisons of AP MALDI and MALDI at reduced pressures (vacuum MALDI) [21]. None of these reports has made a direct comparison of the optimized performance of the two MALDI sources on a single instrument using the same optics configuration and laser.

This paper describes the optimization of the operational and instrumental parameters for an AP MALDI source on a hybrid quadrupole-linear ion trap (QqLIT) instrument using a modification of a recently described interface for nanoflow ESI [22]. Details of the interface can have a substantial effect on the performance of an AP MALDI system. In particular, factors such as target plate potential, laminar flow chamber diameter, laminar flow chamber length, temperature, and gas flows can be important. Results generated with the optimized AP MALDI source are compared with those generated using a vacuum MALDI source on the same instrument.

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This AP MALDI system generates ion count rates within a factor of approximately two of those generated using vacuum MALDI on the same instrument, however, the AP MALDI source requires more tuning to generate comparable performance. In addition, evidence for thermal degradation of samples on target within the AP MALDI ion source and means to minimize the degradation are reported.

Experimental

Protein digests were purchased from Michrome Bioresources Inc. (Auburn, CA). The digests were diluted to various concentrations in water containing 0.1% formic acid (Aldrich, Milwaukee, WI). A four peptide mixture containing bradykinin, angiotensin II, angiotensin I, and fibrinopeptide A (Sigma, St. Louis, MO) was prepared at 1 pmol/μL of each peptide in water containing 0.1% formic acid. The four peptide mixture was diluted to 20 fmol of each component with 0.1% formic acid. Samples were mixed with a 1:1 ratio with undiluted α-cyano-4-hydrocinnamic acid MALDI matrix solution (Agilent, Palo Alto, CA). Each of the sample spots was prepared by the dried droplet method using 1 μL of the mixed samples.

AP MALDI Source

A commercial AP MALDI source was purchased from MassTech Inc. (Columbia, MD). A new optics flange was designed which eliminated the need for mirrors, focusing the laser light directly onto the sample plate at a 28 degree angle. This is the same optical arrangement as the vacuum MALDI source. The standard source flange was modified to incorporate a stainless steel target plate (ABI, Foster City, CA) and a bath gas of room temperature nitrogen (Praxair, Mississauga, ON, Canada). The standard optical fiber (400 μm) and laser were replaced with a 200 μm optical fiber (FiberGuide Industries, Stirling, NJ) and a nitrogen laser from LSI (Frankin, MA) operating at 10 Hz.

Atmosphere to Vacuum Interface

The standard particle discriminator interface [22] for the 4000 QTRAP had to be modified for incorporation of the AP MALDI source. The length of the laminar flow chamber was extended from 1 cm to 3.3 cm to accommodate the extra distance between the target plate and the interface. Some signal loss was expected as a result of lengthening the laminar flow chamber [22]. Similar decreases have also been shown for capillary extenders [16] and gas conductance limiting tubes [23].

Vacuum MALDI

An O-MALDI II source (MDS SCIEX, Concord, ON, Canada) was installed onto the 4000 QTRAP. The MALDI source was installed as described by Loboda et al. [12], with a cone (4 mm aperture) installed in front of the collisional cooling quadrupole (Q0). The target plate region was pressurized with nitrogen gas to give final pressures of approximately 1 torr and 7 mTorr in the target plate and Q0 regions, respectively. The standard orifice power supply (±400 V) was utilized to apply an adjustable potential to the target plate. This system used the same optical fiber, illuminator, and laser as the AP MALDI system. In addition, the angles and spacings of the optics were the same as described above for the AP MALDI optics flange.

Results

AP MALDI Optimization

A number of parameters were optimized with the AP MALDI ion source. The nitrogen laser settings were first optimized by finding the attenuator setting that provided the best signal/background ratio for a bovine catalase digest prepared at 5 fmol on target. The optimum target plate–inlet spacing was determined by varying the position of the target plate using variable thicknesses of Teflon shims on the mounting surface of the heated laminar flow chamber. The plate–inlet spacing was varied from approximately 1.6 to 3.5 mm, ultimately choosing 3 mm as a convenient gap through which to direct the laser. The performance did not vary substantially provided that the electric field between the sample plate and inlet was constant (typically 800–1000 V/mm in these experiments).

The alignment of the MALDI plume relative to the mass spectrometer inlet is critical for AP MALDI performance in order for ions to be efficiently drawn in by the vacuum and not scattered by the electric fields in this region. It has been shown recently that eliminating the electric field within the target plate region shortly after the desorption event can help to solve this problem [24]. In the present research, the inner diameter of the laminar flow chamber was varied in an attempt to improve the sampling efficiency for MALDI ions regardless of plume alignment. Chamber i.d.s of 1 and 2 mm were tested with respect to plume positioning. The system performance was equivalent when the MALDI plume was aligned with the central axis of either of the two heated chambers (data not shown). Figure 1 shows data generated with the plume deliberately misaligned by approximately 400 μm. Misalignment of the plume reduces the ion count rates, particularly for the heavier ions with the 1 mm diameter chamber. However, no decrease in ion current is observed with the larger I.D. chamber. It seems likely that the larger chamber bore is capable of consuming the entire MALDI plume. With this type of configuration, the bulk of ion losses are likely on the inner surface of the long laminar flow chamber provided that the plume is not misaligned by greater than 1 mm.

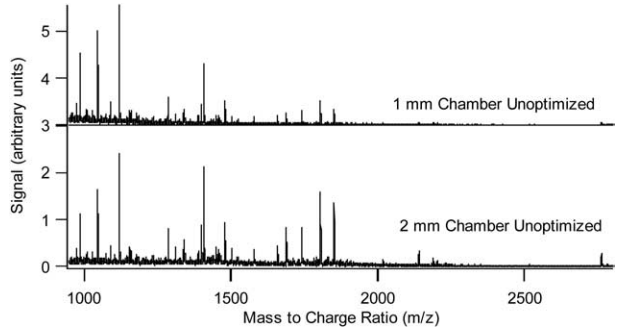

Figure 1. Comparison of average spectra generated for a 1 min acquisition from 5 fmol bovine catalase digest with a 1 and 2 mm heated laminar flow chamber installed with the AP MALDI source. The plume was misaligned by approximately 400 μm from the central axis of the two chambers. The potentials were 3000, 500, 500, and 100 V applied to the target plate, curtain plate, laminar flow chamber, and orifice, respectively. The chamber temperature was optimized to 200 °C and the curtain gas was set to approximately 1.2 L/min. The Q0 and base pressures were approximately 7 mTorr and 4.5×10^{-5} torr, respectively. The LIT was operated with trap fill times of 100 ms, resulting in approximately 92 summed measurements at 4000 Da/s scan speed.

Thermal degradation of samples crystallized on MALDI targets can occur. Directing a 150 °C gas along the outside of the laminar flow chamber emanating 1 mm behind its opening as shown in Figure 2a resulted in a target temperature of 65–70 °C after approximately 10–15 min. Under these conditions thermal degradation was observed for fibrinopeptide A ions, resulting in dehydration of the molecular ion (loss of 18 Da). The extent of degradation was proportional to the time that the target plate spent in the source, as shown in Figure 3 for a four peptide mixture. There was a 72% loss of signal for protonated fibrinopeptide A over the course of approximately 3 h. There was no evidence for thermal degradation of the other three peptides from the mixture. Limiting the target temperature to approximately 45 °C with a cool bath gas reduced the thermal degradation to approximately 20% over 3 h. These results are particularly important for the future incorporation of larger sample plates, capable of holding hundreds of sample spots. Analysis times for a single plate may be on the order of hours, so thermal degradation would be unacceptable. The rest of the experiments were conducted with the gas emanating at 1.2 L/min approximately 3 mm behind the front edge of the laminar flow chamber, giving it sufficient time to cool before reaching the target to keep its temperature below 45 °C.

The target plate potential was also important with the AP MALDI source. The optimum field corresponded to approximately 800–1000 V/mm between the target plate and the entrance of the heated chamber. With higher electric fields, there was no significant gain in performance but discharge became an issue. However, lowering the target plate potential to 0 V resulted in complete loss of signal with this source.

Vacuum MALDI Optimization

Optimization of the operational parameters for the vacuum MALDI source was trivial. The optics were similar to those used in the AP MALDI source, and the laser settings were optimized to the same values. Vacuum MALDI performance is relatively insensitive to the pressure within the target plate (0.5–2 torr) and Q0 regions (3–10 mTorr) provided that there is sufficient gas density for collisional cooling to occur [25]. Therefore, the region between the target plate and cone was pressurized to approximately 1 torr, giving approximately 7 mTorr and 4.5×10^{-5} torr for Q0 and the base

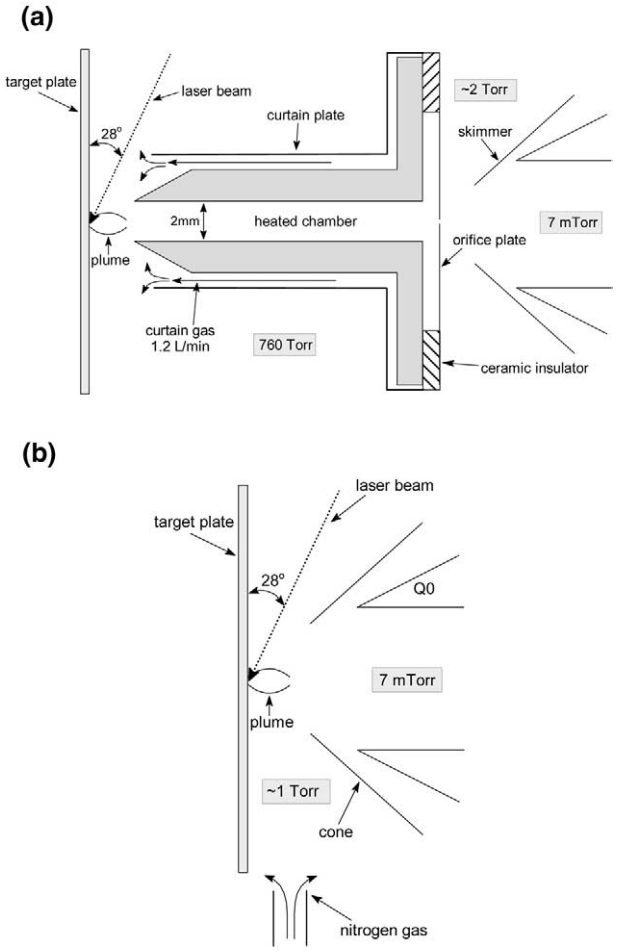

Figure 2. (**a**) Diagram showing the gas flows and pressures used for the AP MALDI source on the 4000 QTRAP. The sample plume was generated at atmospheric pressure with a small flow of counter-current gas. The plume was sampled through a heated laminar flow chamber with 2 mm i.d. Ions were sampled through a conductance-limiting orifice into a region maintained at approximately 2 torr. The central core of the gas expansion was sampled through a 2.6 mm aperture in a skimmer. The Q0 region was maintained at approximately 7 mTorr. (**b**) Diagram showing the gas flows and pressures used for the vacuum MALDI source on the 4000 QTRAP. The sample plume was generated in a region maintained at approximately 1 torr with the addition of a nitrogen gas flow. Since the ions were generated in an intermediate pressure region, there was no need to apply heat for declustering. Ions were sampled through a cone with a 4 mm aperture into the Q0 region (maintained at approximately 7 mTorr).

pressure, respectively, as shown in Figure 2b. These gas pressures were selected to match those used with the AP MALDI source while providing optimal vacuum MALDI performance. The only parameter that required optimization was the target plate potential. Figure 4 shows data acquired (1 min acquisitions) for 5 fmol of bovine catalase digest with various target plate potentials. Even if no potential was applied to the target plate, the signal was only attenuated by a factor of approximately 2–2.5 relative to the optimum situation. Application of any potential to the plate ranging from approximately 5 to 50 V resulted in similar performance. Vacuum MALDI performance was very robust as evidenced by such small losses with unoptimized source potentials. A key advantage of the vacuum MALDI source was that it did not require the same extensive tuning that the AP MALDI source needed.

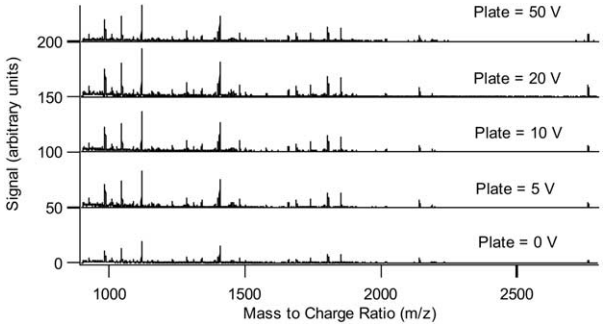

Figure 4. Effect of target plate potential on vacuum MALDI performance. The plate potential was adjusted between 0 and 50 V. The trap fill time was 100 ms and the scan speed was 4000 Da/s. The laser was operated at 10 Hz, corresponding to a single pulse per trap fill. Approximately 92 measurements were summed over a 1 min time period.

Vacuum MALDI versus AP MALDI

As described above, all of the pressures in this instrument were identical for the two MALDI sources from the cone/skimmer to the detector. Since the cone (vacuum MALDI) and skimmer (AP MALDI) were both grounded in these experiments, it was also possible to maintain all of the ion path potentials constant with the two ion sources. Therefore, any differences in performance were expected to be a result of differences in ion generation and sampling efficiency between AP MALDI and vacuum MALDI. Initial experiments were performed to compare the MS performance for protein digests with the two sources. Figure 5 shows data acquired for the average of a series of 1 min acquisitions (average of 30 sample spots taken over a 2 week period) with each source for a sample of 5 fmol bovine catalase digest. For each source, the trap operational parameters were 4000 Da/s scan speed and 100 ms trap fill time. Using the 10 Hz laser, this corresponded to on average 1 laser pulse per trap fill. It is important to note that it would have been possible to use more laser pulses per trap fill at this level prior to the onset of space charge issues with the LIT; however, it was avoided for this comparison since the main goal was to compare data from the two sources rather than to generate maximum ion currents prior to space charge. The data in Figure 5 show that AP MALDI intensities approached that of vacuum MALDI in the lower mass range of *m/z* 975–1200. However, in the higher mass range (*m/z* >1200) the intensities were higher with vacuum MALDI (factor of 2 gain for *m/z* 1852 and factor of 4 gain for *m/z* 2763). Averaging the count rates for all of the known peptide peaks in the two spectra, the vacuum MALDI performance exceeded that of AP MALDI by approximately 2.1X. Similar comparative results were obtained with 5 fmol samples of BSA digest and rabbit phosphorylase B digest (data not shown). It is interesting to note the qualitative similarity of the two spectra shown in Figure 5. These data support the work of Loboda and coworkers [12] showing that raising the pressure of the target

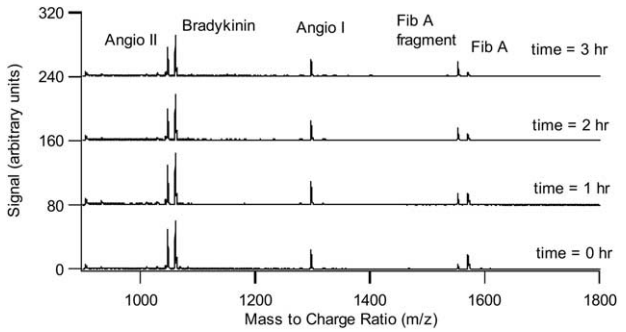

Figure 3. Thermal degradation data acquired using the AP MALDI source with a four peptide mixture (10 fmol each) containing angiotensin II (Angio II), bradykinin, angiotensin I (Angio I), and fibrinopeptide A (Fib A). The laminar flow chamber was heated to 200 °C and a hot curtain gas was used to heat the sample target plate. Thermal degradation of fibrinopeptide A was observed over time. For each trace, approximately 207 measurements were summed over a 1 min period with trap fill times of 2 ms. The scan speed was 4000 Da/s and Q0 trapping was enabled.

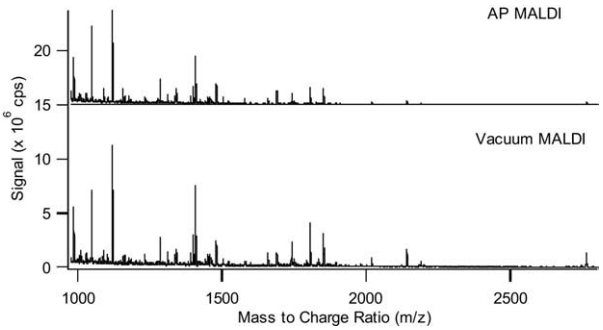

Figure 5. Comparison of the performance of vacuum MALDI and AP MALDI for 1 min acquisitions on a sample of 5 fmol bovine catalase digest. The spectra show the presence of peptides corresponding to *m/z* values of 985.6, 1046.5, 1119.6, 1155.6, 1285.6, 1407.6, 1479.7, 1688.9, 1804.9, and 1851.9, as well as others. Mass spectra were acquired over an *m/z* range of 900–2800. This range covered the most information rich region for the present protein digest, while preventing space charge due to lower *m/z* background resulting from the use of undiluted matrix.

region in vacuum MALDI allows the user to generate MALDI signals for small peptides with minimal fragmentation. The ability to operate vacuum MALDI as a "soft" ionization technique has also been demonstrated by Corr and colleagues for small molecule quantitation [26, 27].

Figure 6 shows a comparison of mass spectra acquired with the two sources for 500 amol bovine catalase digest. Each spectrum is the average of 30 acquisitions (1 min) with 4000 Da/s scan speed and 100 ms trap fill time. Figure 6 shows that both the background and the peptide intensities were once again higher with vacuum MALDI and the largest gains were achieved for vacuum MALDI in the higher *m/z* range. A comparison of the signal/background ratios was conducted for each of the peptides in the two spectra. Even though the signals were substantially higher with vacuum MALDI (approximately a factor of 2.5), the gain in signal/background ratios was much smaller (approximately a factor of 1.4). Similar results were also obtained using a four peptide mixture containing angiotensin II, bradykinin, angiotensin I, and fibrinopeptide A (data not shown). Even though the vacuum MALDI intensities at the 10 fmol level were approximately 2X greater than those for AP MALDI, the signal to noise ratios were similar.

One of the strengths of instruments designed with a QqLIT geometry is the ability to selectively fill the ion trap with fragment ions of a particular *m/z*. Since ion selection occurs prior to the collision cell (tandem in space), there is no need to first fill the trap with all ions and then isolate a particular precursor (tandem in time). This translates into strong performance for MS/MS of peptides and other compounds. Tables 1a and b show MASCOT search results for MS/MS acquired from a single sample spot containing 1 fmol of BSA digest with vacuum MALDI and AP MALDI. For these data, MS/MS were run on each spot until sample depletion was observed as evidenced by an inability to generate further MS/MS spectra. At the 1 fmol level, sample depletion required approximately 15–17 min using the 10 Hz laser. In total, it was possible to run 11 and 9 MS/MS for vacuum MALDI and AP MALDI, respectively, prior to sample depletion. The number of peptides successfully identified was 7 and 4 for vacuum and AP MALDI, respectively. The total MASCOT scores were 178 and 114, giving sequence coverage of 16 and 7% for vacuum and AP MALDI, respectively. The higher ion currents with vacuum MALDI translated into an improvement in the number of peptides that could be identified by MS/MS from a single sample spot. In addition, the higher ion currents for vacuum MALDI lead to a reduced MS/MS acquisition time for each peptide. The limitations to acquisition and identification of more MS/MS data were sample depletion from the target spot and limited signal/background for low intensity peptides. The importance of increased parent ion intensities is less apparent when conducting this comparison with larger amounts of sample because sample depletion is no longer an issue, so it is possible to carry out MS/MS on essentially all of the peaks in the mass spectra with either of the sources.

In all cases, the data generated with the AP MALDI source was of lower intensity than that generated using the vacuum MALDI source on the QqLIT instrument by approximately a factor of 2. However, it seems likely that future improvements such as pulsed focusing [24, 28], improved interface designs, and gas throughput increases may improve AP MALDI performance to the point that it is comparable with vacuum MALDI. In

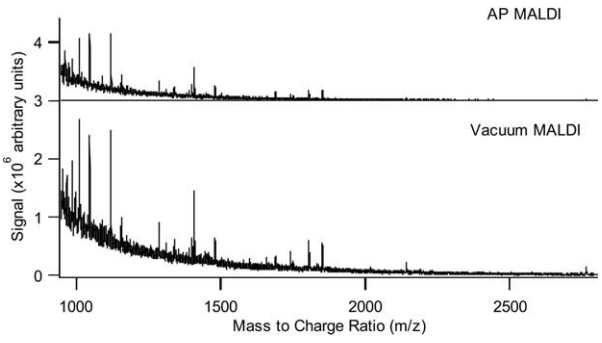

Figure 6. Comparison of the performance of vacuum MALDI and AP MALDI for 1 min acquisitions on a sample of 500 amol bovine catalase digest. The spectra show the presence of peptides corresponding to *m/z* values of 985.6, 1046.5, 1119.6, 1155.6, 1285.6, 1407.6, 1479.7, 1688.9, 1804.9, and 1851.9, as well as others.

Table 1a. MASCOT search data for MS/MS acquired from a single sample spot containing 1 fmol BSA digest using the vacuum MALDI ion source. Data were acquired over approximately a 15 min period

Peptide Ion (*m/z*)	MASCOT Score	MASCOT Rank
1249.6	26	1
1305.7	39	1
1420.7	44	1
1465.6	7	2
1479.8	22	1
1639.9	30	1
1881.9	18	1

Number of Peptides Identified = 7; Total Score = 178; Coverage = 16%

Table 1b. MASCOT search data for MS/MS acquired from a single sample spot containing 1 fmol BSA digest using the AP MALDI ion source. Data were acquired over approximately a 17 min period. MASCOT searches were conducted for singly charged ions allowing for 1 missed cleavage and carboxymethylation of the peptides from the tryptic digest. The peptide and MS/MS tolerances were 0.4 and 0.3 Da, respectively

Peptide Ion (*m/z*)	MASCOT Score	MASCOT Rank
1249.6	26	1
1305.7	28	1
1420.7	43	1
1639.9	17	1

Number of Peptides Identified = 4; Total Score = 114; Coverage = 7%

addition, AP MALDI affords rapid interchangeability between MALDI and electrospray for sequence coverage improvements. For these reasons and the low cost associated with AP MALDI, it presents an attractive alternative for proteomics work even though the performance is currently lower than vacuum MALDI.

Vacuum MALDI provides the benefits of improved performance and simplicity of use over current versions of the AP MALDI source. The source performance is extremely robust regardless of the optimization and there is no need for extensive tuning of the gas flows, potentials, and temperature to achieve optimal performance. Using the configuration described here (cone prior to Q0), MS spectra were qualitatively similar to those generated from AP MALDI (soft ionization source). The drawbacks include higher cost and the need to break vacuum to swap between MALDI and electrospray.

Conclusions

The results presented in this paper show a direct comparison between the performance of AP and vacuum MALDI on a 4000 QTRAP utilizing the same laser, optical fiber, lens configuration, and ion path conditions. The vacuum MALDI source provided highest sensitivity with approximately a 2-fold improvement in ion current compared to the AP MALDI source. The higher ion currents translated directly into improved MS/MS performance with improved sequence coverage for protein digests. With the AP MALDI source, it was important to carefully optimize parameters such as the plate potential, target plume position, temperature, and interface arrangement. There was also some evidence for thermal degradation of samples stored on a heated target plate over time and precautions were necessary to minimize these effects. For these AP MALDI experiments, the interface was optimized such that the bulk of the sample plume could be sampled through the heated laminar flow chamber into the instrument. It remains to be seen if gas throughput increases will translate into improved performance on this system if the bulk of the sample plume is already sampled through the heated chamber. However, regardless of the plume consumption efficiency, redesigning the AP MALDI source to further shorten the heated chamber should also improve the transport efficiency for ions from atmosphere°to°vacuum°[22].°The°QqLIT°geometry°of the 4000 QTRAP makes it possible to generate subfemtomole MS and MS/MS data for protein digests with either of the two MALDI sources. These results suggest that AP MALDI offers sufficient sensitivity for many biological applications; however, applications requiring the utmost sensitivity are best done with vacuum MALDI approaches on this instrument.

Acknowledgments

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