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Atmospheric Pressure MALDI/Ion Trap Mass Spectrometry

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A new sample ionization technique, atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI), was coupled with a commercial ion trap mass spectrometer. This configuration enables the application-specific selection of external atmospheric ionization sources: the electrospray/APCI (commercially available) and AP MALDI (built in-house), which can be readily interchanged within minutes. The detection limit of the novel AP MALDI/ion trap is 10−50 fmol of analyte deposited on the target surface for a four-component mixture of peptides with 800−1700 molecular weight. The possibility of peptide structural analysis by MS/MS and MS^3 experiments for AP MALDI-generated ions was demonstrated for the first time.

A new atmospheric pressure ionization technique, atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI), has recently been invented and coupled to an orthogonal acceleration time-of-flight mass spectrometer.[1−3] The advantages of this technique include the following: sample handling under normal atmospheric pressure conditions, softer analyte ionization compared with conventional vacuum MALDI, and increased resolution of individual components of complex analyte mixtures.[1−3] Another attractive feature of AP MALDI is that it is an external ion source with respect to the mass analyzer. As a result, any type of MS instrument capable of analyzing atmospheric pressure ions may be coupled with this source with only minor modifications. In particular, any electrospray (ESI) instrument may be easily converted to AP MALDI mode by the replacement of its external ESI source, thus making it possible to create an "all-in-one" instrument capable of several different atmospheric pressure ionization techniques (i.e., ESI, APCI, and AP MALDI).

The atmospheric pressure ions produced by any external ion source must be transferred to a vacuum chamber for mass analysis. The better the efficiency of ion transfer, the higher the sensitivity of the technique. This statement is valid for any source of atmospheric pressure ions (ESI, APCI) and is particularly important in the case of AP MALDI. In its present embodiment,[1−3] ions are produced only during 1−5-ns bursts. As a result, the production of an atmospheric pressure ion current is ∼1 order of magnitude weaker than a typical ESI source.[2] Ion loss in atmospheric pressure interfaces (API) is a major disadvantage of AP MALDI compared with the classical vacuum technique. To a certain extent, these losses may be compensated by the consumption of the majority of the deposited sample for AP MALDI MS analysis, which is not typical in the conventional vacuum technique. A sensitivity of 60−100 fmol for a peptide mixture (1−2.4 kDa) has been demonstrated for an AP MALDI source coupled with a Mariner orthogonal time-of -flight instrument (PE Biosystems, Framingham, MA).[2,3] Improvements to the performance of the API seem to be the primary way to increase the sensitivity of all atmospheric pressure ionization techniques, in particular, AP MALDI. Several modifications to API construction have been introduced in the last several years. For example, a rf-only ion guide enables focusing of charged ions while pumping away neutrals. The recent invention of the ion funnel[4−6] enables an increase in ion transmission in the region between the inlet orifice and skimmer. Thus, progress in the API performance will enhance the sensitivity of AP MALDI to levels comparable with those of vacuum MALDI.

Mass analyzer selection is also an important factor of the AP MALDI configuration. As mentioned above, AP MALDI is a rather weak ion source. Therefore, the possibility of a contribution from every ion to the final spectrum is important in the successful application of the AP MALDI technique. Mass filters, which include magnetic sector and quadrupole instruments, scan the spectrum, transmitting the ions in a relatively narrow m/z range. As a result, the duty cycle of these devices is low and the accumulation of a spectrum takes a long time. The ion current of the AP MALDI source varies during an experiment when the irradiated target spot is exhausted, requiring adjustment of the target position. Because of this, mass filters are not good candidates as mass analyzers of AP MALDI ions. Instead, orthogonal acceleration time-of-flight, ion trap, and FT ICR instruments, which have better duty cycles than those of mass

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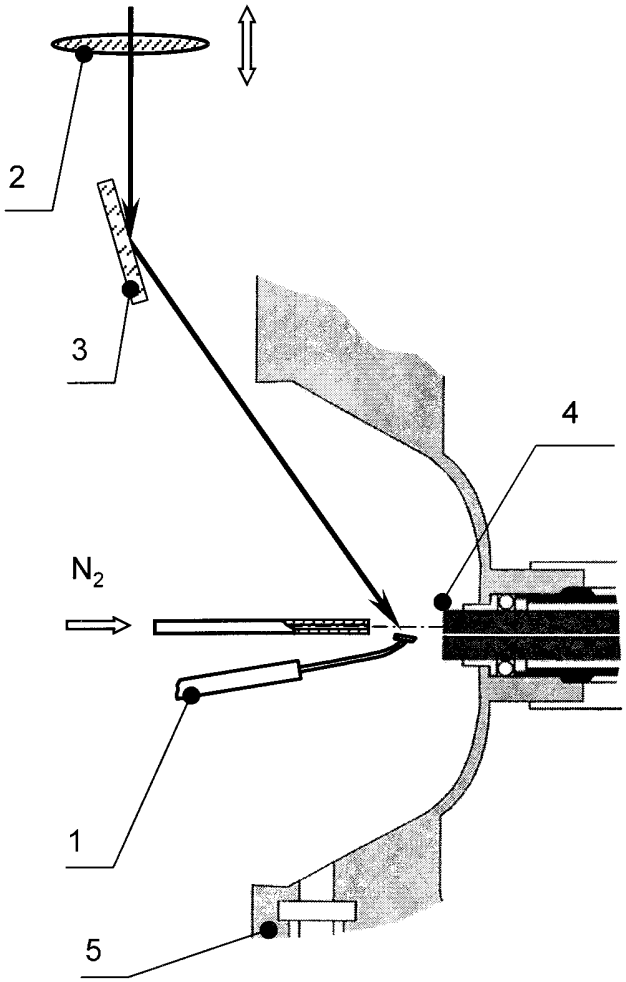

Figure 1. Schematic of the AP MALDI source: 1, target holder; 2, quartz lens; 3, mirror; 4, stainless steel capillary inlet; 5, inlet flange of the atmospheric pressure interface of Finnigan LCQ Classic.

filters, are appropriate choices for AP MALDI mass analyzers. While time-of-flight instruments have the advantage of an unlimited m/z range, ion traps and FT ICR instruments have MS^n capabilities, which is of importance in the analysis of biological samples. The focus of the current investigation is the preliminary study of the AP MALDI/ion trap MS technique.

EXPERIMENTAL SECTION

A Finnigan "LCQ-classic" electrospray ion trap mass spectrometer (Finnigan MAT, San Jose, CA) was equipped with a pneumatically assisted AP MALDI ion source built in-house (Figure 1). The source, described previously,[2,3] was constructed without essential modifications. This ion source was equipped with an adapter, which enables the replacement of the standard ESI source of an LCQ instrument in less than 5 min. Approximately 1.4−1.5 μL of 1:1 (v/v) of analyte/matrix solution was deposited on a replaceable stainless steel target (1.5 × 2.5 mm) and allowed to dry for ∼30 min.

Sensitivity measurements were made by ablating approximately 2−50 fmol of a peptide mixture applied to a target tip. For MS/MS and MS^3 experiments, 20−50 and 200−500 fmol of the peptide mixture were used to obtain spectra, respectively. In all other experiments, approximately 3−5 pmol of analyte was applied to the target tip. For all peptide spectra shown, the matrix used was α-cyano-4-hydroxycinnamic acid (Hewlett-Packard, Palo Alto, CA). The peptides were supplied by Sigma (St. Louis, MO). An ESI potential of 2.7 kV was applied between the target tip and LCQ inlet transport capillary, and the temperature of the transport capillary was 180 °C. The laser (Continuum Inc., Santa Clara, CA) was tuned to generate 355-nm UV pulses at 10 Hz with 1 mJ of energy and was focused by a system of two mirrors and a quartz lens (F = 500 mm) onto the surface of the target plate. The size of the laser spot was adjusted to optimize the analyte ion current by varying the lens-to-target distance. A stream of dry nitrogen was applied through a stainless steel capillary to assist in the transport of ions toward the inlet orifice of the instrument and to provide a moisture-free environment in the region around the AP MALDI target. The optimum nitrogen flow was 10 arbitrary units (∼0.1 $L \cdot s^{-1}$).

AP MALDI mass spectra were recorded in the spectrum acquisition mode of the Finnigan LCQ classic. Because the ion current generated by the AP MALDI source is more than 1 order of magnitude weaker compared with the ESI source, the LCQ instrument automatic gain control (AGC) option was not useful for this configuration. Even with the AGC on, the ion trapping time was found to coincide with a maximum specified value of 500 ms, but the spectrum quality was diminished due to increased noise level. Thus, the ion trapping time was set manually to 200−400 ms. The shorter trapping time decreases the detection sensitivity, while times above 400 ms decrease the spectrum resolution if analyte deposition exceeds the 500−800-fmol level. This observation may be due to the space charge effect inside the ion trap. It should be mentioned that the ion accumulation period covers several laser pulses and the instrument scan time is short compared with the ion accumulation phase. Thus, the duty cycle of the AP MALDI/ion trap instrument is close to 100% even without synchronization of the laser pulses with the trapping cycle.

Once data acquisition commenced, the laser was switched on and the target was scanned slowly in the $x-y$ plane, exposing the entire target surface to laser irradiation. Acquisition ceased when either a satisfactory spectrum was recorded or when the majority of sample had been ablated and no analyte peaks could be detected. Spectrum accumulation typically took 1−2 min for targets with 2−50 fmol of analyte material, but spectra with excellent signal-to-noise ratio can be recorded in several seconds for targets with 500 fmol of analyte or higher. After the spectrum is recorded, data interpretation is accomplished with the LCQ software. Further experiments can be conducted utilizing the MS^n capabilities of the ion trap.

RESULTS AND DISCUSSION

Sensitivity of Detection. The determination of the sensitivity of the AP MALDI source coupled to an ion trap mass spectrometer was a major consideration in this study. Therefore, achieving high sensitivity for the AP MALDI source was paramount. As described previously,[2] the sensitivity of the AP MALDI source depended on the following factors: the geometry of the target tip as well as its position relative to the inlet, the laser beam energy density on the target surface, the voltage applied to the target tip, the nitrogen gas flow rate, and the position of the gas nozzle. In addition, sensitivity could be improved with optimization of source param-

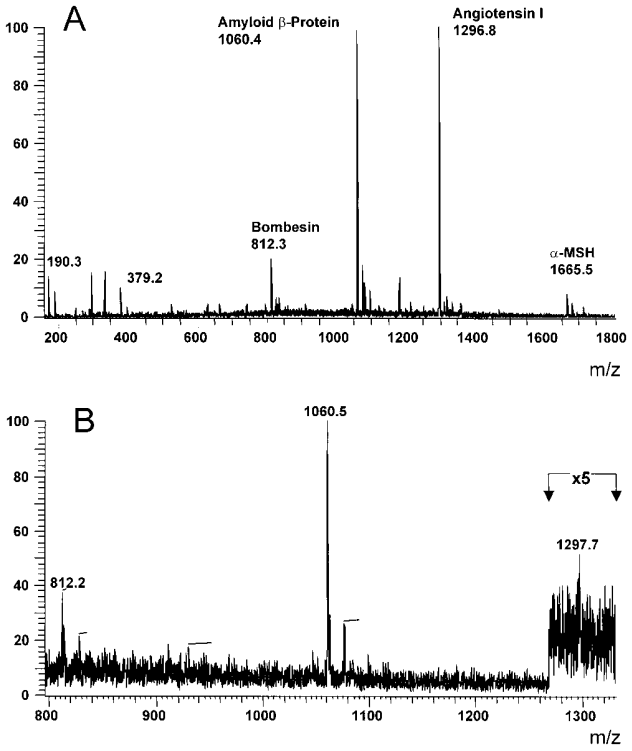

Figure 2. AP MALDI spectrum of the four-peptide mixture, obtained by deposition of 0.75 μL of analyte solution and 0.75 μL of matrix (α-cyano-4-hydroxycinnamic acid, HP matrix solution: (A) 50 and (B) 5 pg/μL of each peptide.

eters and experimental conditions as well as utilization of a more sensitive mass analyzer.

A standard solution of four peptides in the range of 800−1700 Da was prepared in order to evaluate the sensitivity of the AP MALDI source. This peptide mixture contained 50 pg/μL of bombesin fragment 8−14 (FW 812.0), amyloid β-protein fragment 25−35 (FW 1060.3), angiotensin I (FW 1296.5), and α-MSH (FW 1664.9), i.e., 30−60 fmol/μL. The AP MALDI spectrum in Figure 2A demonstrates the sensitivity achieved for 20−50 fmol/peptide. Further dilution of this peptide mixture to 5 pg/μL (2−5 fmol for 0.75 μL deposited) yielded the spectrum in Figure 2B. In this spectrum, the amyloid β-protein fragment 25−35 peak has a signal-to-noise ratio of >10, while the other three peptide peaks are not discernible from noise.

MS/MS and MS^n. The MS/MS and MS^n capabilities of the ion trap can provide sequential and structural information important in the analysis of small biological samples. MS/MS was performed on the MH$^+$ peak of angiotensin I (m/z 1297.5) and the resulting spectrum (Figure 3) displays b-, y-, and a-type ions,[7] as well as some internal fragmentation. Figures 2 and 3 were acquired using the same sample target, indicating that 20−50 fmol of the peptide mixture was sufficient to acquire both MS and MS/MS spectra.

The MS^3 spectrum was obtained from the peptide mixture containing 300−600 fmol of each peptide. The b_9 fragment of angiotensin I at m/z 1165.6 was selected for MS^3 analysis (Figure 4). The resulting spectrum contains b and a ions and a few internal fragments. It also contains y ions that result from the close

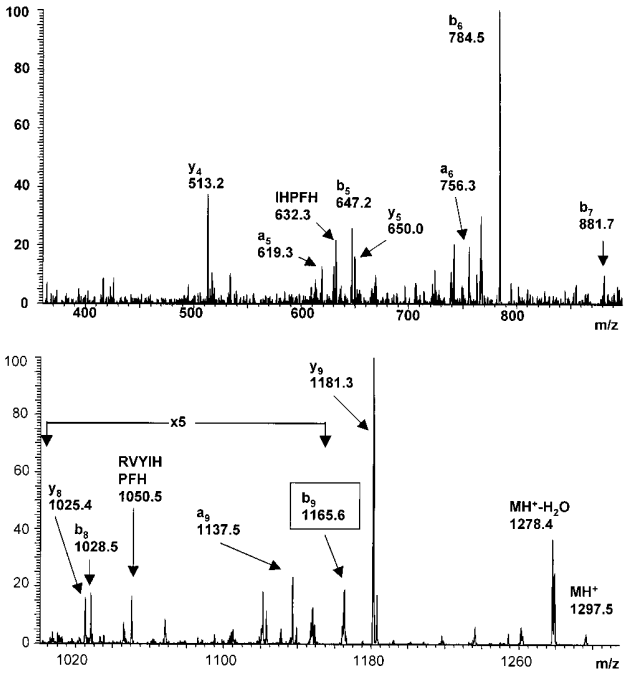

Figure 3. MS/MS spectrum of four-peptide mixture (50 pg/μL of each peptide) at m/z 1297.5.

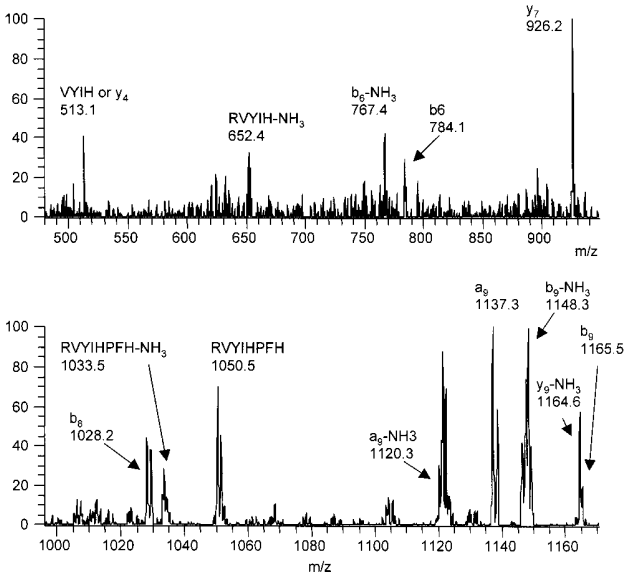

Figure 4. MS^3 spectrum of four peptide mixture (0.5 ng/μL of each peptide) at m/z 1297.5 → 1165.5 →. The peak at m/z 1165.5 was collected within a range of ±1.5 Da. Thus, MS^3 was performed on both the b_9 and the $y_9 - NH_3$ ions.

proximity of b_9 (m/z 1165.5) and $y_9 - NH_3$ (m/z 1164.6) ions that were both collected for fragmentation within the selected mass range of ±1.5 Da.

Negative Ion Detection. Covalent modifications to primary sequences can have an effect on protein function.[8] AP MALDI may be implemented in the study of such peptide modifications. A sulfated peptide, Tyr[$(SO_3H)^{27}$]-cholecystokinin amide fragment 26−33 (FW 1143.3), was analyzed to determine whether the intact molecular ion could be observed. Attempts to obtain the molecular

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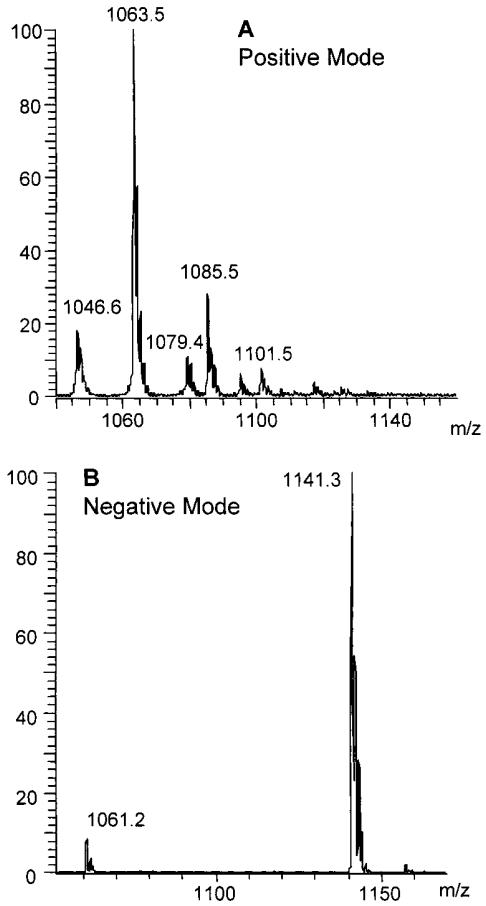

Figure 5. AP MALDI spectra of Tyr[$(SO_3H)^{27}$]-Ccholecystokinin amide fragment 26−33. (A) Positive mode displays only desulfated ion at *m*/*z* 1063.5. (B) Negative mode displays both the deprotinated molecular ion at *m*/*z* 1141.3 and the desulfated ion at *m*/*z* 1061.2.

peak in the positive mode failed (Figure 5A), as only the desulfated peptide (*m*/*z* 1063.5) peak could be determined. Both ESI and classical vacuum MALDI produce very similar spectra, both containing no molecular ion peaks.[9] The degree of the sulfotyrosine lability may be dependent on both the peptide sequence and the ionization conditions.[10] The observation is that the positively charged molecular ion of this particular sulfated peptide is not stable and fragments lose the sulfate group readily. Both ESI and vacuum MALDI negative mode spectra show a strong molecular peak of the deprotonated sulfated peptide with a minor desulfated ion peak.[9] To record the molecular peak of this analyte using the AP MALDI technique, the LCQ instrument was switched to negative mode. Figure 5B represents the first negative AP MALDI spectrum recorded with a strong deprotonated sulfated peptide ion peak at *m*/*z* 1141.3 and a weaker desulfated ion peak at *m*/*z* 1061.2.

Analyte/Matrix Cluster Ion Production. The occurrence of analyte/matrix cluster production under atmospheric pressure MALDI conditions was described previously.[1−3] Such analyte/cluster ion formation may complicate spectral interpretation and lead to a decrease in sensitivity. Cluster ions are more prevalent

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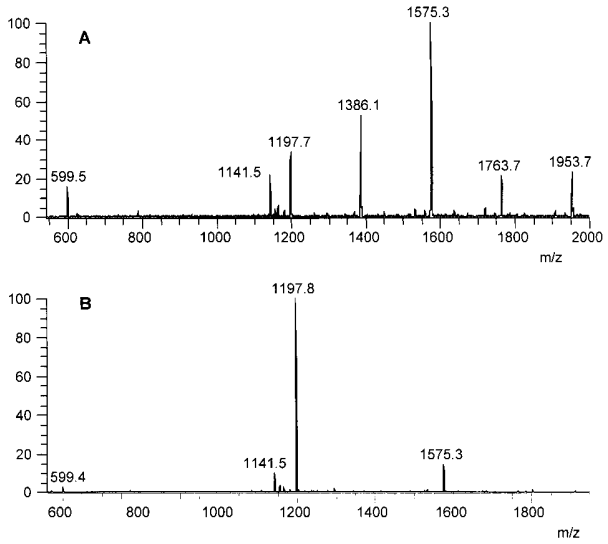

Figure 6. AP MALDI spectrum of 5 pmol/μL arginine-rich peptide, VRKRTLRRL, with 1 pmol/μL of gramicidin S. (A) Successive addition of matrix ions to the arginine-rich peptide with analyte/matrix clusters at *m*/*z* 1386.1 (MH^+ + 189 Da), 1575.3 (MH^+ + 2(189) Da), 1763.7 (MH^+ + 3(189) Da), and 1953.7 (MH^++ 4(189) Da. The peak at *m*/*z* 599.5 is the doubly charged peak for the arginine-rich peptide. (B) Declustering of the peptide when the octapole region ion source CID is raised by 50 V.

for heavier analyte ions such as proteins.[2] Because the range of our LCQ classic instrument is limited to *m*/*z* 2000, only peptide spectra with analyte masses less than 2000 Da were attempted. These spectra contain no analyte/matrix cluster peaks with the experimental conditions described above. On the other hand, the phenomenon of analyte/matrix cluster formation seems to be dependent on the chemical nature of the analyte.[2] For example, analyte/matrix cluster ion peaks are more intense for bradykinin compared with either angiotensin or LH−RH.[2] A possible explanation is that bradykinin contains two arginine residues, while both angiotensin and LH−RH contain only one. To further support this observation, the AP MALDI spectrum of an arginine-rich peptide, VRKRTLRRL (FW 1196.8), was recorded. This peptide contains four arginine residues and thus was expected to display analyte/matrix cluster formation. A mixture containing 3.5 pmol of the peptide and 0.7 pmol of gramicidin S (FW 1140.5) was applied to the target surface for analysis. Figure 6A shows successive addition of matrix ions (addition of 189 Da) to the arginine-rich peptide, producing analyte/matrix clusters at *m*/*z* 1386.1 (MH^+ + 1 matrix ion), 1575.3 (MH^+ + 2 matrix ions), 1763.7 (MH^+ + 3 matrix ions), and 1953.7 (MH^++ 4 matrix ions). In addition, *m*/*z* 599.5 is the doubly charged peak for the arginine-rich peptide. Both the extensive analyte/matrix cluster formation and the appearance of a strong doubly charged analyte peak might be attributed to the high percentage of arginine present in the peptide sequence. Still, the practical question remains: how to improve the AP MALDI spectrum simplicity for this particular peptide? The LCQ instrument provides an additional possibility in this respect. It contains two transport rf-only octapoles inside the atmospheric pressure interface. The octapole ion source CID feature of the LCQ instrument can be used for tunable ion heating by the application of a potential difference between the skimmer and the first octapole, which enables fragmentation of analyte

cluster ions yielding molecular ions and neutral matrix molecules. Figure 6B demonstrates the effective declustering of the peptide that results when the potential difference between the skimmer and the first octapole is raised by 50 V. An intense MH^+ peak (m/z 1197.8) and minor doubly charged (m/z 599.4) and $MH^+ +$ 2 matrix (m/z 1575.3) peaks are present in this spectrum.

CONCLUSIONS

A new atmospheric pressure ionization technique, AP MALDI, has been successfully coupled with a commercial ion trap, the LCQ classic instrument manufactured by Finnigan MAT. This provides the advantage of utilizing the same instrument for a variety of applications, such as electrospray, atmospheric pressure chemical ionization, and AP MALDI. Replacement of the external ionization source of this instrument is fast and simple.

The applicability of the AP MALDI/ion trap combination for the analysis of peptide mixtures was tested for the four-component mixture of peptides with masses in the range of 800−1700 Da. The sensitivity of this technique was evaluated by a sequential dilution of the peptide mixture. The preliminary results demonstrate that 20−50 fmol of analyte material deposited on the AP MALDI target surface produce mass spectra with a quality sufficient for the identification of the mixture components. MS/MS and MS^3 spectra for MALDI ions generated under atmospheric pressure conditions were recorded for the first time. This provides a powerful tool for the acquisition of structural information for AP MALDI-generated ions.

The analyte chemical nature dependence of a previously reported phenomenon, analyte/matrix cluster formation under AP MALDI ionization conditions, has been studied in some detail. Cluster formation has been shown to increase drastically with an increase in the number of arginine residues present in a given peptide. The LCQ instrument provides the additional benefit of effectively declustering AP MALDI-generated ions by use of the tunable electrical field ion heating by increasing the potential between the skimmer and the first octapole to induce octapole ion source CID.

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