Detection of Oligonucleotides by External Injection into an Ion Trap Storage/Reflectron Time-of-flight Device

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The detection of oligonucleotides has been studied using external injection of Matrix-assisted laser desorption/ionization (MALDI) produced ions into a quadrupole ion trap storage device. The ions stored in the trap are then detected by pulsed DC ejection into a reflectron time-of-flight mass spectrometer. It is shown that by using a low acceleration voltage from the probe tip and external injection into the trap that 3-mers to 9-mers can be stored for 20–50 ms and detected as intact ions. In comparison, direct MALDI inside the trap resulted in extensive fragmentation as a function of storage time. Using the external injection method with a He buffer gas in the trap yields a resolution of 250–400 in the ion trap storage/reflectron time-of-flight mass spectrometer. The resolution can be further enhanced to 400–800 using a bipolar extraction method. In addition, the trapping efficiency for the injection of externally produced ions into the trap can be enhanced using a dynamic trapping method. The MALDI mass spectra produced by this methodology result in excellent signal-to-noise ratio due to the capabilities of the trap for eliminating low mass matrix background from the spectra. © 1997 by John Wiley & Sons, Ltd.

The detection of DNA by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has become an area of intense interest.[1–25] The ability to detect DNA by mass spectrometry methods will in principle provide an enormous advantage for analysis of DNA in genetic screening and sequencing applications. In order for MALDI-MS to become practical for DNA applications though it requires: (a) the production and detection of large oligomers in the gas phase, (b) sufficient resolution for single base-pair (b.p.) detection and discrimination and (c) detection of oligomers to the low femtomol level. Most work on MALDI-MS of DNA has been performed with detection by time-of-flight (TOF) devices. The detection of large DNA oligomers > 500 b.p. by MALDI-TOFMS has been achieved[7] and even complicated mixtures of DNA fragments can be detected and analysed.[17,26] In terms of resolution, pulsed-delayed extraction in TOF mass spectrometry has been used to achieve a resolution in excess of 1000 for 31–35 mers[23–25] and more recently a resolution of 500 up to a 60-mer.[27] The sensitivity for detection of oligomers by MALDI-TOFMS can be in the low femtomol region for small oligomers, but as the size increases 100 femtomol sensitivity appears to be the more general limit. Nevertheless, new methodology is needed to extend the working range and limitations of present DNA detection.

An alternative method for detection of DNA by MALDI-MS may be ion trapping methods. Extensive work has been performed on detection of DNA in Fourier transform ion cyclotron resonance mass spectrometry traps[28,29] where a resolution in excess of 830 000 with a detection level in the low picomole range was achieved. A simple alternative though for detection of DNA is the quadrupole ion trap storage device. A number of groups have recently reported detection of proteins and peptides using MALDI-MS in an ion trap mass spectrometer (ITMS).[30–35] The ion trap presents several key advantages for detection of DNA in MALDI-MS experiments. In MALDI-TOF a major problem is the large number of matrix ions and neutrals that strike the detector and cause saturation of the detector response. This saturation can result in a loss of sensitivity and resolution for detection of large DNA species. However, with the use of the trap, the matrix can be effectively eliminated from the trap using resonant ejection techniques or can be prevented from reaching the trap if produced externally to the trap by adjusting the amplitude of the radio frequency of the trapping field as a function of time so that the matrix ions never reach the detector.[36] A second potential advantage of the trap is in terms of the sensitivity. The storage properties of the trap could allow a large number of MALDI pulses to be integrated in the trap as a function of time for detection of very low signals needed for real DNA applications. In addition, the trap may provide a means of obtaining improved resolution compared to TOF methods, where resolution is still limited for small oligomers by the energy and spatial distribution that results from the MALDI process.

In our work, we have examined the use of an IT/reflectron(re)TOF device[34,35,37] for detection of DNA. The IT/reTOF device, which has been described in detail previously uses a quadrupole ion trap as a front end storage device for a TOFMS detector. Thus, the ion trap serves as a means of storage and integration of ions

prior to detection using a TOFMS. In the experiments reported in this work, a method whereby MALDI is performed external to the trap and the ions guided through the endcap into the trap is used.[31,32] This method provides elimination of small molecule matrix background in the ITMS and has the potential for enhancing sensitivity. In addition, since the ions are generated external to the trap in a low DC voltage field the potential for fragmentation is reduced so that intact oligomers can be trapped and detected without significant fragmentation. The ability to eliminate fragmentation and detect intact DNA-ions is important in real DNA applications. Also, MALDI-produced ions are generally translationally hot which effectively reduces the resolution in TOFMS. However, in the IT/reTOF a buffer gas in the trap can be used to collisionally cool the hot ions so that improved resolution can be observed in the reTOF detector.[37]

In this work we report initial results on detection of small oligomers using the IT/reTOF device. It will be shown that by using external injection of MALDI-produced ions into the trap with a He buffer gas in the trap, intact ions can be observed with minimal fragmentation. In addition, using storage in the trap and a He buffer gas a resolution of 250–400 can be obtained. Furthermore, bipolar DC extraction can be used to improve this resolution to 400–800 ultimately limited by the post-acceleration detection. We also evaluate dynamic trapping as a means of enhancing the efficiency of trapping and for improving the S/N of the MALDI-produced ions.[31,32] It is also shown that these MALDI spectra can be observed with excellent S/N and almost total elimination of low mass matrix background using the storage properties of the trap.

EXPERIMENTAL

A schematic diagram of the external ion injection ion trap/reTOF MALDI system is shown in Fig. 1. The instrument consists of a reflectron time-of-flight mass spectrometer (Model D850, R. M. Jordan Co., Grass Valley, CA, USA) using a quadrupole ion trap (Model C1251, R. M. Jordan Co.) as the ion source region.[34,35,37] The ion trap is located in a vacuum chamber pumped by a 6 in. diffusion pump. This chamber is connected to the TOF tube by a 0.12 in. aperture such that even a high buffer gas pressure causes only a small increase in pressure inside the TOF

region. At a trap pressure of 1 mTorr, the pressure in the main chamber is 2×10^{-5} Torr while the TOF flight tube remains at 2×10^{-6} Torr. The frequency of the RF potential applied to the ion trap is 1.0 MHz.

Several modifications have been made to our original IT/reTOF MALDI experiment to optimize the design for efficient transmission and trapping of ions. In this work an external ion injection source is used where the probe tip is placed ~ 10 cm from the entrance endcap of the ion trap. The laser is introduced vertically relative to the probe axis. The probe tip is angled slightly (~ 20°) so that the laser is focused on the front surface of the probe tip. The design has been simulated using the SIMION 6.0 program (Lockheed Idaho Technologies Co., Idaho Falls, ID, USA) in both 2D and 3D matrix format and it has been shown that the angled surface introduces only negligible interference to the static electric field if the diameter of the front surface is sufficiently small (~ 2.0 mm dia.). The probe tip and L1 are both floated to the same positive voltage to provide sufficient initial kinetic energy to the ion so that it can penetrate into the ion trap. Two electronic lenses L1, L2 are then used to compress the ion beam generated from the surface. The Einzel lens collimates the ions towards the entrance aperture on the endcap of the ion trap. In other experiments, for comparison, MALDI was performed directly inside the ion trap as described in previous work.[34,35] Briefly, the MALDI probe tip is inserted into an aperture in the ring electrode while the laser enters through another aperture on the ring electrode in the counter position. The ions are generated directly in the ion trap.

A Quanta-Ray DCR-3 pulsed Nd:YAG laser (Spectra-Physics, Mountain View, CA, USA) was used as the laser source for MALDI. Both 355 nm and 266 nm were used in the experiments. Different buffer gases (He, Ar) were used in these studies to collisionally cool the ions entering the ion trap. In order to control the flow of buffer gas, the gas was stored inside a low pressure tank and transferred to the ion trap through stainless steel tubing (1/16 in. i.d.). The pressure of the buffer gas in the trap is controlled by tuning a needle valve. After the ions are stored in the ion trap for 10–20 ms, the RF voltage on the ring electrode is rapidly shut down and a DC negative pulse (~ 2 μs, −325 V) is applied to the entrance endcap electrode to extract ions out of the ion trap for detection by the
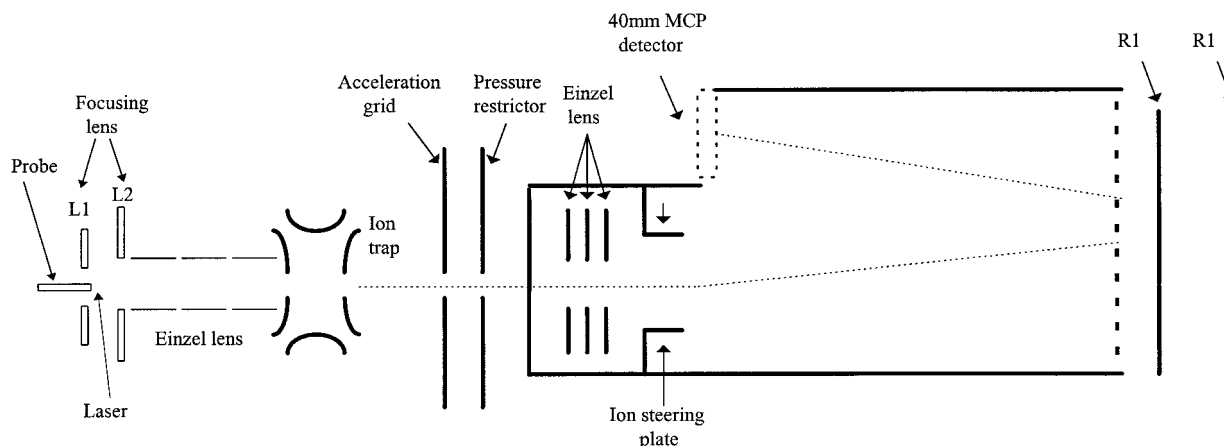

Figure 1. Schematic diagram of instrumental setup of the external ion injection IT/reTOF mass spectrometer.

reTOF detection system. The detector assembly consists of a Cu–Be conversion dynode in front of a dual microchannel plate (MCP) detector. The conversion dynode can be biased up to –5 kV in order to enhance detection of large species, but at the expense of resolution.

Dynamic trapping

The RF generator has been modified to perform dynamic trapping, where the RF voltage can be ramped up during the time that the ions enter the ion trap. The amplitude of the RF generator is controlled by an external control voltage which is generated from an in-house constructed control unit. This controller can use a 0–9 V output to ramp the RF generator from 0–5000 volts. The dynamic trapping method is controlled by a sequence of events as shown in Fig. 2. The controller receives two triggering signals to turn on/off its output. A BNC connector labelled 'oscillator' on the DCR-3 laser will output a pulse about 3 ms before the laser actually fires. A digital delay generator (DDG1) receives this pulse and after a given delay, generates another signal to start the controller. The controller will generate a control signal whose amplitude controls the amplitude of the RF voltage in a linear relationship. The relative turn-on time is adjusted by the digital delay generator. After the ions are stored for 10 ~ 20 ms, adjusted for performance, another digital delay generator, DDG2, sends a pulse to the RF generator to start the ion extraction and detection process. This pulse is also used to turn off the controller and the RF is also consequently turned off. The profile of the RF amplitude could be further controlled by
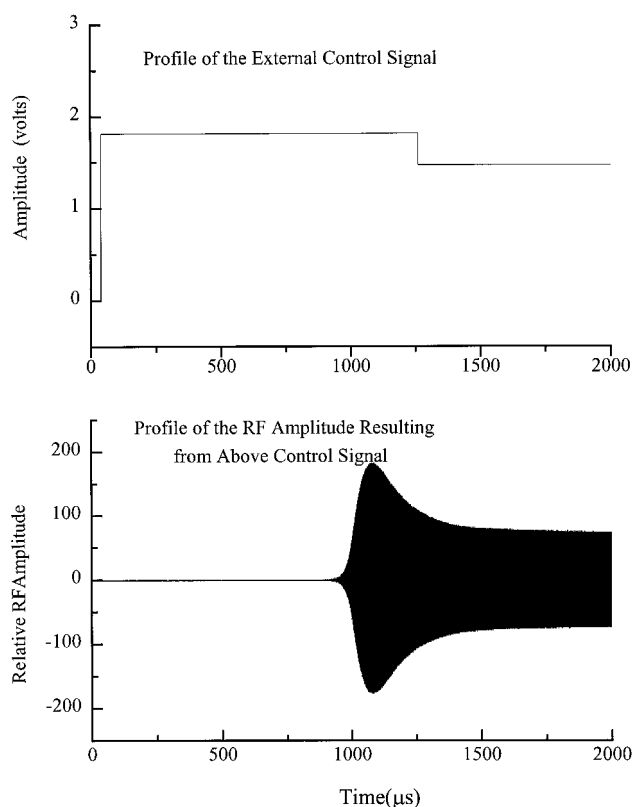
dropping the control signal voltage after the initial ramp-up. This feature is used to increase the trapping volume by reducing the RF.

Bipolar pulsing

Bipolar pulsing was performed by connecting a positive remote pulser and a negative remote pulser (Model CO1038, R. M. Jordan Co.) to the entrance and exit endcaps, respectively. The voltages of the two remote pulsers are controlled by two separate pulser power supplies. The two remote pulsers are triggered by the same triggering signal which provides the synchronization between the two pulsers. The positive and negative pulses are set at +300/–300 volts.

Data acquisition conditions and data processing

Each mass spectrum was collected by averaging 50 single shot mass spectra and the laser power is typically around 5×10^6 W/cm²/pulse. The mass spectra in the same figure use the same relative scale defined by the spectrum in which the absolute mass peak intensity is the strongest.

All data were acquired using a Lecroy 9350 AM 500 MHz digital oscilloscope (Lecroy Corp., Chestnut Ridge, NY, USA), and the binary data were transformed to ASCII format and graphed with Origin 2.94 (Microcal Software, Northhampton, MA,USA).

Chemicals and sample preparation

The matrices used in this work, 3-hydroxypicolinic acid (3-HPA), picolinic acid (PA), 2,5-dihydroxybenzoic acid (DHB) and ammonium citrate were purchased from Aldrich (Milwaukee, WI, USA). DNA oligomers were synthesized at the Biomedical Research Core Facilities at the University of Michigan without HPLC purification. The sequences are: 3 mer (AGT, TTT), 4 mer (AGCT), 5 mer (ATCGA), 6 mer (ATCGAT), 7 mer (ATCGATC) and 9 mer (ATCGATCGA).

The samples were dissolved in deionized water at a concentration of ~ 0.1 mM. The matrices were dissolved in water/acetonitrile (1:1 v/v). In the study comparing the effect of mixed matrices on the MALDI-MS signal of DNA, two mixture ratios were used (a) 3-HPA:PA = 4:1 (w/w) saturated solution (b) 3-HPA:PA = 1:4 (w/w) (200 mg/mL). Ammonium citrate (0.2 M) was used to enhance the signal intensity of the 7 mer and 9 mer. The sample solution, matrix solution and ammonium citrate were mixed at a ratio of 1:2:1. The final mixture was dropped on the probe tip, dried and delivered into the mass spectrometer.

RESULTS AND DISCUSSION

In initial work, MALDI-MS of oligonucleotides was performed directly in an ion trap as in previous work.[34,35] In Fig. 3 is shown the effect of storage time in the ion trap on the decay of a 3-mer oligonucleotide (5'-TTT-3') in the direct ion injection mode. In Fig. 3(a), at a relatively short trapping time of 100 μs, the peak associated with the molecular ion of the 3-mer oligonucleotide is observed as the base peak with a series of much less abundant fragment ion peaks resulting from base loss from the deoxyribose with or
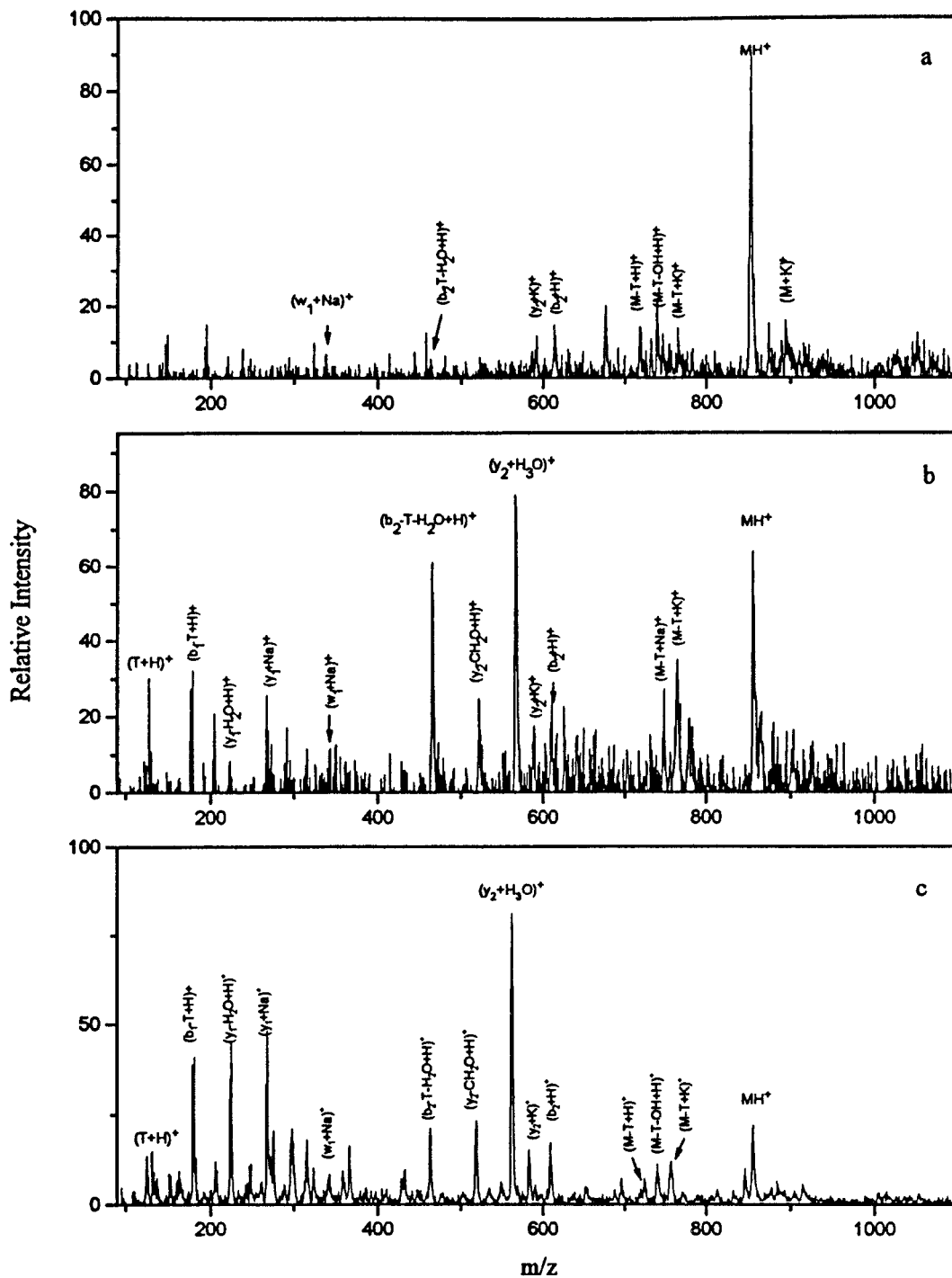

Figure 2. (a) The profile of dynamic trapping controlling voltage and (b) the profile of the RF output from the RF generator.

Figure 3. The time-dependent dissociation of oligonucleotides observed in the direct MALDI method in the IT/reTOF system for a storage time of (a) 100 μs, (b) 9 ms and (c) 39 ms. The matrix used here is 3-HPA:PA (4:1, w/w).

without the cleavage of the phosphodiester bonds of the oligonucleotide. As the storage time increased, additional fragment ions from the cleavage of the phosphodiester bonds were observed in the mass spectra. At 9 ms trapping time (Fig. 3(b)), additional fragment ions from cleavage of the backbone were observed. Although the molecular ion peak intensity is still intense, the base peak has been shifted to the fragment ion associated with backbone cleavage $((y_2 + H_3O)^+)$. As the trapping time was increased to 39 ms, the fragmentation pattern remained the same as the spectrum obtained at 9 ms trapping time; however, the signal intensity of the molecular ion was further

reduced. Other oligomers were examined and the molecular ion peaks of DNA oligomers larger than 5-mers were not observed due to extensive fragmentation in the trap. Several fragment ions corresponding to the notation of Fig. 3 were identified with characteristic cleavages in the molecules.[38,39]

MALDI-MS of oligonucleotide samples was subsequently performed external to the trap. The ions entered the trap through the entrance endcap and were trapped in the RF field. The resulting spectra are shown in Fig. 4(a)–(d) for 3–9 mers where these species are observed as intact ions with no significant fragmentation. It is apparent that external ion injection provides
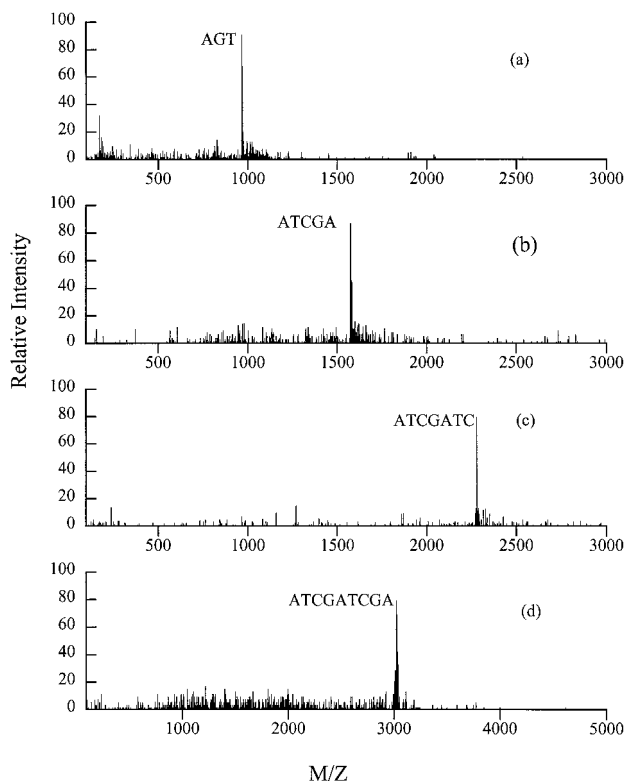
Figure 4. Mass spectra of 3, 5, 7 and 9 mer DNA samples obtained via MALDI followed by external ion injection into the ion trap. The matrix used for 3 and 5 mers is 3-HPA:PA (4:1, w/w). The matrix used for 7 and 9 mers is 3-HPA:PA (1:4, w/w).

a means of ion introduction into the trap following MALDI with little fragmentation, as opposed to MALDI directly inside the trap where extensive fragmentation is produced. In these spectra the ions were produced via MALDI from a probe tip at a potential of ~ 12 V and accelerated towards the trap as shown in Fig. 1. A set of Einzel lenses are used to focus the ions through the endcap of the trap where the ions are trapped and stored. A buffer gas of He at 2–5 mTorr was used to enhance trapping efficiency. The matrices used in the spectra were 3-HPA:PA (4:1) for the 3 and 5 mer samples and 3-HPA:PA (1:4) for the 7 and 9 mer samples. The laser wavelength used for MALDI was 266 nm.

In previous work it has been shown that large protein ions produced in the trap tend to fragment as a function of storage time in the trap.[34,35] It was shown that the highly excited protein ions produced by MALDI in the trap were accelerated by the RF field into the buffer gas and subsequently underwent extensive decay in the trap. The extent of decay was very dependent on the magnitude of the RF voltage. If the RF voltage was shut off during MALDI excitation and subsequently ramped up in about 200 µs then minimal fragmentation was observed. Thus, a key to long-lived fragmentation is that the large ions must be accelerated into a collision gas while in an excited state. Peptide ions that are not highly excited such as electrospray-produced ions tend to be very stable in the RF field inside the ion trap.[37] In the case of direct MALDI-MS in the trap, one might expect rapid fragmentation of oligonucleotide ions in the RF field since the oligonucleotide ions tend to undergo fragmentation much more readily than pep-

tide ions in the trap. A major advantage of producing ions external to the trap in the MALDI process is that the excited ions are accelerated only by a low DC voltage, so that fragmentation is minimal and relatively stable ions are focused into the trap. Indeed low energy collisions with the background gas further serve to collisionally relax and stabilize the MALDI-produced oligonucleotides as shown in previous work.[40]

Other factors that may be important in determining long-term fragmentation of oligonucleotides in the trap are the type of buffer gases in the trap. A heavier buffer gas such as Ar has been shown to be more efficient for trapping large ions compared to a lighter gas such as He.[33] In Fig. 5 are shown spectra of MALDI-produced ions external to the trap for a 4-mer using He and Ar as the buffer gas to enhance trapping of the ions at a pressure of 3 mTorr. The results show that for MALDI-produced oligonucleotide ions in the present external ion injection set-up Ar produces more fragmentation than He. Thus He buffer gas was used in the experiments described in this work. The pressure of Ar was reduced to check whether there was any change in the fragmentation. No significant difference in the fragmentation pattern was observed upon reducing the pressure of Ar inside the ion trap.

The degree of fragmentation generated in these experiments is also sensitive to the laser wavelength used and the matrix. In Fig. 6 are shown the mass spectra generated by MALDI of a 7-mer external to the trap with a 3-HPA:PA (1:4, w/w) matrix, using 355 nm (Fig. 6(a)) and 266 nm laser (Fig. 6(b)) radiation. The use of 266 nm laser radiation with the mixed matrix was found to introduce the minimal amount of fragmentation in all oligonucleotide samples tested in this work.
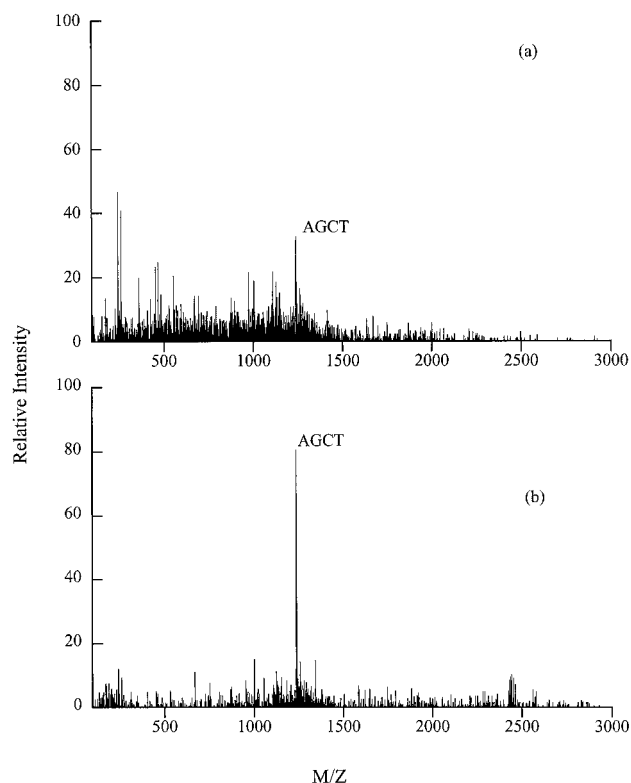

Figure 5. Comparison of the effects of different buffer gases using a 4-mer sample (a) Ar as the buffer gas, (b) He as the buffer gas. The matrix used here is 3-HPA:PA (4:1, w/w).
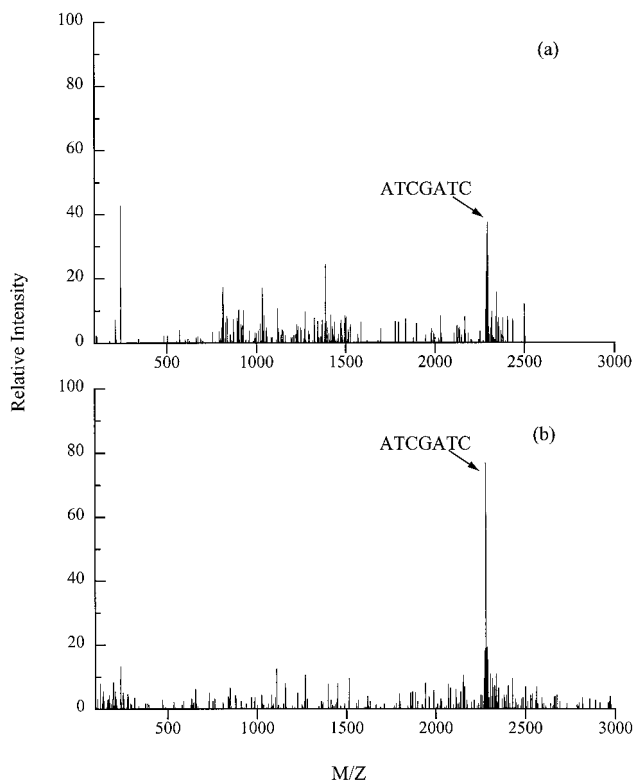
Figure 6. Comparison of the effects of different laser wavelengths using a 7-mer sample (a) 355 nm laser, (b) 266 nm laser radiation. The matrix used here is 3-HPA:PA (1:4, w/w).

Figure 7. Comparison of the effects of different matrix mixing ratios (a) 3-HPA:PA = 4:1 (w/w), (b) 3-HPA:PA = 1:4 (w/w).

3-HPA[4] and PA[6] have been reported as excellent matrices for DNA molecular ion generation. However, a mixture of these two matrices can reduce the threshold effect in the MALDI process and enlarge the window for optimal laser power. Two mixing ratios have been investigated and the results are shown in Fig. 7. In the case of DNA 9-mer sample, when the mixing ratio is 3-HPA:PA = 1:4 (w/w), the S/N is improved compared to a mass spectrum collected with a 4:1 mixed matrix. For DNA oligomers of < 5 mer in length, this effect is not significant. The result indicates that the mixing ratio is an important factor which influences the 'softness' of the ionization process. Adding ammonium citrate also improved the mass signal intensity as reported in earlier work.[41]

In addition to the processes controlling fragmentation, the processes affecting resolution in these experiments have also been investigated. The resolution in these experiments is typically between 250–400. The resolution is ultimately limited by the presence of the Cu–Be post-acceleration stage in the detector. Nevertheless, the observed resolution in the IT/reTOF detector for external injection of MALDI-produced oligonucleotide ions is influenced by several factors including buffer gas pressure, trapping time and extraction voltage. A tradeoff between buffer gas pressure and resolution was observed. An increase in buffer gas pressure inside the trap was found to increase the trapping efficiency, but at some point resulted in a decrease in resolution. The pressure was typically maintained at an estimated 3 mTorr of He in the trap as the optimal compromise between resolution and sensitivity.

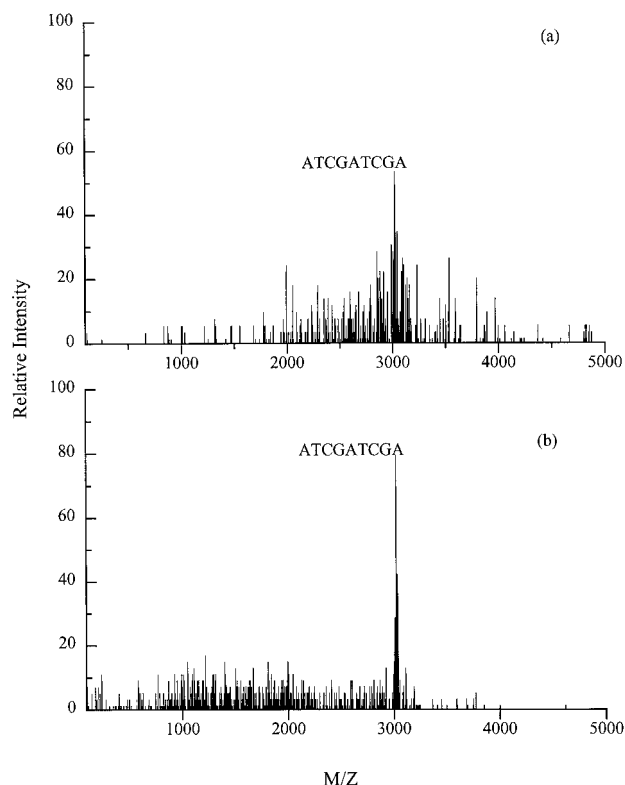The storage time of the oligonucleotide ions in the ion trap is an important variable in determining resolution. For 3- and 5-mers the longer trapping time (> 30 ms) improved resolution without reducing the signal intensity. However, for 7-mer and 9-mer samples such longer trapping times increase fragmentation due to collisions with the buffer gas. A storage time of 10–20 ms was found to provide trapping with minimal fragmentation without sacrificing resolution. The DC extraction voltage and duration applied to the ion trap affected the DNA signal. A –325 V, 2.2 μs DC pulse provided the best resolution when a 4-mer sample was used to optimize the system.

An important factor which influences resolution are space charge effects in the trap. A large number of low mass matrix ions are generated in the MALDI process. The ion saturation inside the trapping volume results in space charge that prevents the analyte ions from becoming tightly compacted to the center of the trap, thus reducing the resolution in the reTOF. The large number of matrix ions will also reduce the signal intensity of the analyte ions. The matrix ions result in space charge effects that expel the ions from the effective detection volume of the trap so that ions are not detected. In addition, the low mass matrix ions may saturate the MCP detector so that ion detection at higher mass will be adversely affected. The laser power, wavelength and matrix have been adjusted to enhance the target analyte signal at the expense of matrix background. The presence of a large amount of background low mass matrix ions is also a problem in traditional MALDI-TOF where these species saturate the detector. Although some of the ions can be eliminated with a deflector voltage, many species are small background molecules which cannot be easily eliminated. A key advantage of the external ion

trapping method is that the RF voltage can be set to raise the effective low mass cut-off of the trap so that the low mass ions are never stored in the trap.[36] The Einzel lenses can also be used as deflection plates to eliminate the ions. In the case of MALDI-TOF, the ions are accelerated to 20 kV or higher so that deflection of ions becomes more difficult. The use of 10–20 V acceleration of the MALDI-produced ions in the external injection method makes elimination of the ions very easy. In addition, the neutral ions never reach the detector in the IT/reTOF mass configuration on the same time scale as the stored ions. As shown in Fig. 4, background at low mass is relatively low in all the spectra due to the elimination of matrix ions in the MALDI spectra.

An important means of improving the resolution in the IT/reTOF MS is bipolar extraction.[42] Using simulations with the SIMION 6.0 program it has been shown that by simultaneously applying a negative voltage to extract ions from one endcap while applying a positive voltage to push out ions from the other endcap that both the signal intensity and resolution can be improved markedly. The comparison of mass spectra obtained in each pulsing scheme are shown in Fig. 8. The signals were collected from the same spot on the MALDI probe tip. In general, the bipolar extraction doubles the peak intensity and improves the resolution by 50–70%. The simulation of the bipolar extraction is shown in Fig. 9. In the unipolar pulsing mode, the entrance endcap is grounded and extraction endcap is set at –300 volts. In the bipolar extraction mode, the entrance endcap is set at +300 volts and the exit endcap is set at –300 volts. The ions are started at different positions around the center of the ion trap and at different angles (–25° ~ 45°) relative to the center

axis. The initial kinetic energy is set at 0.1 eV for all the ions. The ions have a molecular weight of 1000 Da and are singly charged. We calculate the time (T) and kinetic energy (E) at which the ions reach the first acceleration grid (GI) and statistically analyse the time and kinetic energy distribution. For the unipolar pulsing mode using 10 ions in the simulation, the standard deviation (σ) for T is ~102 ns and the σ for E is ~7 V. However, in the bipolar pulsing mode, the σ for T is ~33 ns and the σ for E is ~13 V. The results indicate that the bipolar pulsing mode reduces the spatial distribution by converting it to a larger energy distribution. The energy distribution can easily be compensated by the reflectron. This provides only a very simple description for the simulation of the extraction process. Many factors, such as the voltage of G1, the symmetry and synchronization of the bipolar pulsing, buffer gas pressure, etc., could be further optimized to provide a more complete simulation.

Another important consideration is the trapping efficiency of the MALDI produced ions. In the external injection method of injecting ions into the trap, the number of ions entering the trap is related to the phase of the RF voltage applied to the ring electrode of the trap. It is difficult to synchronize the RF phase with the ion arrival time because of the initial energy distribution in the MALDI process and since ions of different molecular weight travel at different speeds after the initial acceleration. A technique which can be used to enhance the trapping efficiency is dynamic trapping.[31,32] In this method the amplitude of the RF is initially set at 0 volts and the time at which the RF is turned on is adjusted relative to the time that the laser pulses. When the ions reach the ion trap, the RF voltage is low so that the ions can easily penetrate the potential barrier and are trapped. This is especially useful in the case of oligonucleotides since this trapping process results in reduced fragmentation. The rise and fall of the RF amplitude is termed 'matched dynamic trapping' and is useful for trapping excessively large ions. The use of dynamic trapping for improving the signal/noise ratio in these external injection experiments is shown in Fig. 10 for a 6-mer DNA sample produced by MALDI. The signal is increased by a factor of at least 2× under the same conditions where the dynamic trapping is applied. It has been reported in previous work[12] that the matched dynamic trapping method may improve the trapping efficiency for ions produced by external injection MALDI up to 40%.[31,32]

DNA molecules of up to 9-mers (~3000 *m/z*) produced by external injection MALDI have been trapped and detected using the IT/reTOF MS. Although larger oligomers have been detected by this method, the S/N for detection decreases rapidly. The upper practical mass limit of the current IT/reTOF is about 4000 u, where there is a significant deterioration in resolution and sensitivity for peptide samples, although larger samples have been detected. Further improvements in instrumentation will be needed to reach a higher mass range. These include improving the efficiency of trapping for larger species which may involve lower RF frequency on the ring electrode, higher acceleration voltage on the probe and improved focusing optics for guiding the ions from the probe into the trap. In addition, only –2000 V acceleration is applied to the
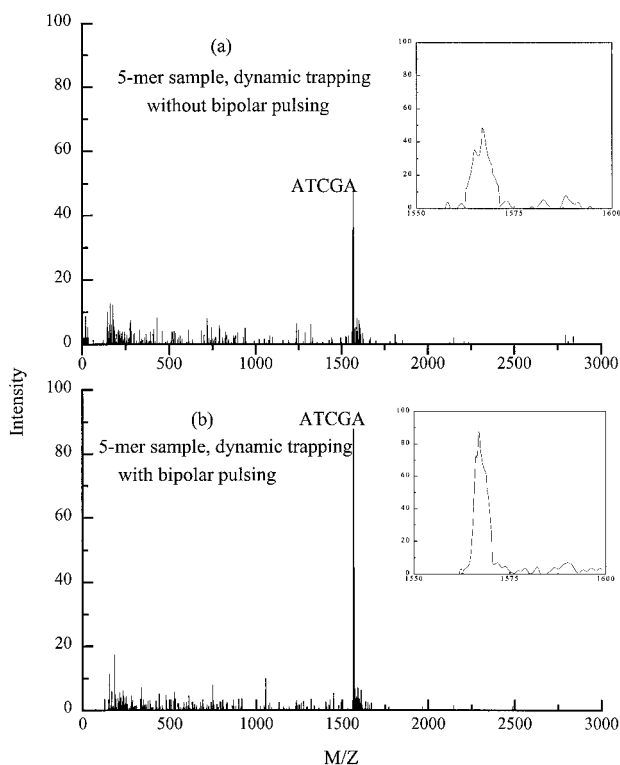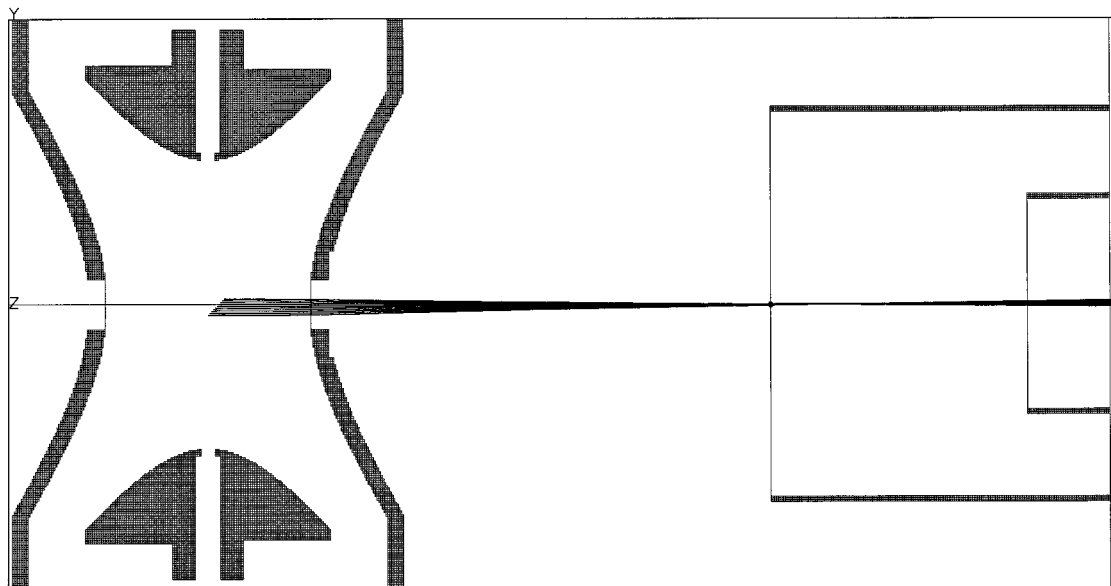

Figure 8. Comparison of the mass spectra with and without bipolar pulsing for a 5-mer sample (a) without bipolar pulsing, (b) with bipolar pulsing. The matrix used is 3-HPA:PA = (4:1, w/w).

SIMION

Figure 9. Simulation of the bipolar extraction process using SIMION 6.0. The voltages applied to the entrance/exit endcaps are $+300/-300$ V. The ions have molecular weight of 1000 Da and are singly charged. The initial kinetic energy for the ions are 0.1 eV and the initial angle relative to the center axis ranges from $-25°$ to $+45°$.
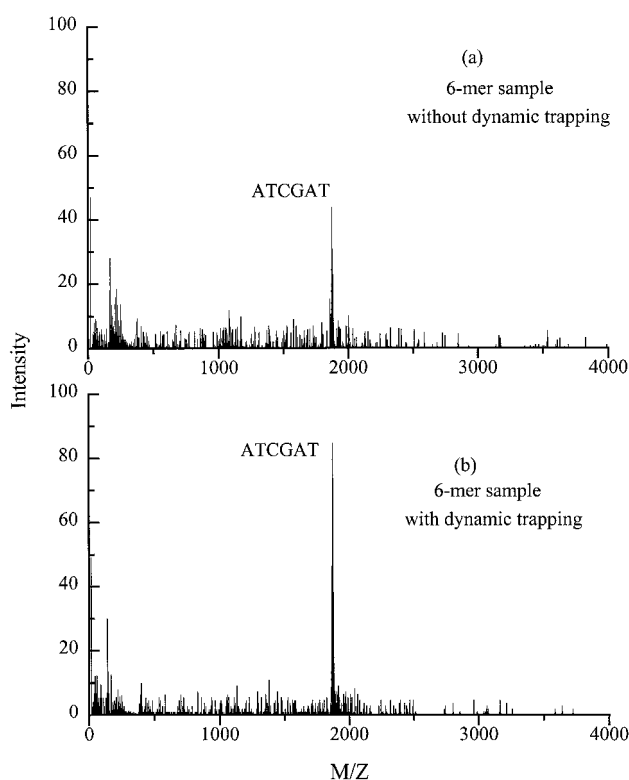

Figure 10. Comparison of the mass spectra (a) without dynamic trapping, (b) with dynamic trapping. The matrix used here is 3-HPA:PA (4:1, w/w).

ions entering the flight tube and higher voltages may be required for ions to efficiently reach the detector. Also the post-acceleration stage on the detector in a reTOF instrument has a maximum voltage limit of -5 kV which may not be sufficient for detecting increasingly larger ions. Modifications for extending the mass range are presently underway in our lab.

CONCLUSIONS

External ion injection-IT/reTOF has been used as a tool to detect DNA molecules up to a 9-mer sample. A comparison between the external and direct ion injection methods has demonstrated that the external ion injection is a much more effective method for trapping intact DNA molecular ions. The results show that the type of buffer gas, laser wavelength and matrix are important factors which can be varied to reduce the fragmentation of DNA molecules in the ion generation and storage processes. The trapping method has been used as a means to improve resolution for detection of DNA based upon the presence of buffer gas in the trap to relax the ions to the center of the trap to improve the spatial resolution and energy spread for TOF analysis. Also the trap has been used as a means to eliminate the large amount of matrix background, both neutral and ionic, in the spectra for enhancement of S/N and elimination of space charge for improvement of resolution. In addition, techniques such as dynamic trapping and bipolar pulsing have been used to improve the resolution and S/N.

Acknowledgments

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