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Improved Resolution in the Detection of Oligonucleotides up to 60-mers in Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry Using Pulsed–Delayed Extraction with a Simple High Voltage Transistor Switch

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Pulsed–delayed extraction using a simple high voltage transistor switch together with various sample purification approaches were used to enhance the resolution in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) of oligonucleotides up to 60 bases long. This switch can provide a 0–3 kV voltage pulse with a 75 ns fall time. A resolution of 500–900 was typically observed for samples from 5-mers to 60-mers using pulsed–delayed extraction (PDE) with the switch described herein. The resolution deteriorated to <100 for oligonucleotides of ≥ 65-mers. With the TOF acceleration region configuration used in this work, the resolution was found not to vary significantly over a delay range of 2–5 μs. As the DNA size increased to over 35-mer, HPLC purification was required to retain the enhancement in resolution provided by PDE MALDI-MS. © 1997 by John Wiley & Sons, Ltd.

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There has been rapid progress in the detection of oligonucleotides by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)[1] over the past several years.[2–22] MALDI-MS used in conjunction with the time-of-flight (TOF) mass analyzer has the potential to be a rapid and cost-effective replacement for gel electrophoresis in applications such as DNA screening and sequencing. There are three basic requirements that must be met for the use of MALDI-MS as a practical method in DNA sequencing and detection.[7] These include the capacity to detect oligonucleotides at least 300 b.p. long and, more importantly, the ability to detect mixtures of large numbers of oligonucleotides of mixed base composition over a broad mass range without significant suppression effects. A second important requirement is detection sensitivity into the low femtomole region over a broad mass range for both small and large oligonucleotides. The third key issue is the ability to achieve single base pair resolution for oligonucleotides as large as 300 b.p.

At present, the first of the above requirements has been demonstrated by various groups.[7,17] The detection of DNA greater than 500 b.p. long has been demonstrated by Chen and co-workers,[7] while the detection of complicated mixtures of enzyme digests of DNA with bases ranging from 9 b.p. to over 600 b.p. in length using polymer film substrates has been demonstrated by Liu and co-workers.[17,30] A major obstacle though for application of MALDI-MS to the detection of DNA is the relatively poor mass resolution observed in TOF experiments. This low mass resolution results from a variety of factors, including the initial kinetic energy distribution of ions formed in the MALDI process, metastable decay of DNA ions in the ion source and the formation of adducts between DNA ions and counter ions such as potassium ions, sodium ions, iron ions or with the matrix or other impurities. The formation of adducts can limit the mass resolution and sensitivity since it spreads the signal for a particular oligonucleotide over several mass units. In addition, the impurities may affect the matrix and analyte crystal formation and in turn the desorption efficiency.[10,31–33]

Several approaches have been employed to reduce the contribution of one or more of these factors to enhance the mass spectral resolution. Polymer substrates[12] and ion exchange beads[34] have been used for on-probe tip purification of DNA samples while the use of various ammonium salts has also been evaluated for their ability to enhance the signal quality and reduce Na^+ and K^+ adduct formation by exchange with NH_4^+ ions.[19,20] In terms of the initial kinetic energy distribution, pulsed delayed extraction (PDE) has been found to be very effective for small oligonucleotides.[23–29] In conventional MALDI instruments, the ions formed in the ion source are extracted immediately by a DC potential, while in the PDE method, a short time delay is introduced between the laser ionization and ion extraction events. The region between the repeller and the extraction grid is field-free during this time. Following the delay, a pulsed voltage is applied to the repeller or extraction grid and the ions are drawn out into the acceleration region. By adjusting the delay

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time and pulsed voltage, ions with the same *m/z* reach the detector at the same time.

Much improved resolution for oligonucleotides analysis has recently been demonstrated using the PDE method.[23–29] Reilly and co-workers[24] reported the observation of improved resolution over conventional DC extraction using space–velocity correlation focusing on small DNA oligomers up to a 31-mer. However, it was also observed that the resolution degraded considerably for large oligonucleotides. Juhasz and co-workers[25] demonstrated mass resolution of over 1000 on DNA fragments up to a 31-mer and the sequence information of a 11-mer by fast fragmentation and delayed extraction mass detection. In more recent work, Li and co-workers[35] demonstrated a resolution of 1300 for a 35-mer using PDE with mass accuracy of < 100 ppm. Thus recent work using PDE demonstrates the potential of MALDI-TOFMS for detection of small oligonucleotides with high resolution.

In order to perform PDE, a high voltage pulser that provides a short fall time is required. There have been a variety of commercial pulsers used to provide this voltage drop.[23,26] In the present work, a relatively simple home-built high voltage transistor switch was developed to provide a voltage drop of ~ 3000 V in less than 100 ns. This switch consists of two transistors as the active components in a relatively simple circuit that is convenient to use and inexpensive to construct. It has been evaluated for the measurement of DNA fragments for enhanced mass resolution on synthetic DNA oligomers of up to 70-mers for samples purified under different conditions.

EXPERIMENTAL

Instrumentation

The TOF mass spectrometer used in this work was a modified Wiley–McLaren design with a field-free drift length of 1 m. Two deflection plates ~ 0.7 m in length inside the drift tube were used for the deflection of matrix and other low mass ions to reduce possible detector saturation, although neutral species could not be eliminated. Nevertheless, the signals were found to be enhanced greatly by the use of the ion deflector. A two stage ion source (with spacings between the first and second acceleration region of 0.70 cm and 1.84 cm, respectively) modified for high voltage acceleration up to ± 20 kV (R. M. Jordan Co., Grass Valley, CA, USA) was used in this work. The laser source was a DCR11 Nd:YAG laser system (Spectrophysics, Mt. View, CA, USA) which provided 355 nm radiation with a pulse width of 5–6 ns for MALDI. The laser power was typically between 10^6–10^7 W/cm^2. The detector was an 18 mm triple microchannel plate (MCP) detector configuration (R. M. Jordan Co.). The typical background pressure in the TOFMS ion source during operation was 0.5–2 $\times$ 10^{-7} Torr using a Turbo-V 250 (Varian Associates, Inc., Lexington, Massachusetts, USA) molecular pump. The data were recorded using a Lecroy 9350M (500 MHz/s) oscilloscope and were subsequently transferred to an IBM 586 PC for analysis.

In order to provide a high-voltage pulse for pulsed-delayed extraction, a high voltage switch was developed and evaluated for its effectiveness in the enhancement of mass resolution of DNA oligomers. The mass spectra obtained using this switch showed enhancement of the resolution by 10 to 50 fold over the use of TOF without PDE. This circuit (see Fig. 1) can provide a 0–3 kV pulsed voltage in less than 100 ns (see Fig. 2). The mechanism of its operation can be outlined briefly as follows: the pulsed voltage and extraction grid voltage were first applied to the switch. When a logic level trigger pulse (2–4 V pulse) was applied to the switch unit from the pulse input, transistor Q1 and Q2 (NTE 389) conduct and bring the voltage at point 'A' to a zero value in less than 100 nanoseconds. The coupling effect of the capacitor reduces the extraction grid voltage value by an equivalent amount. After the narrow pulse width of the trigger pulse, the system resets itself to initial conditions in a few hundred microseconds and is ready for the next cycle. The maximum switch voltage is

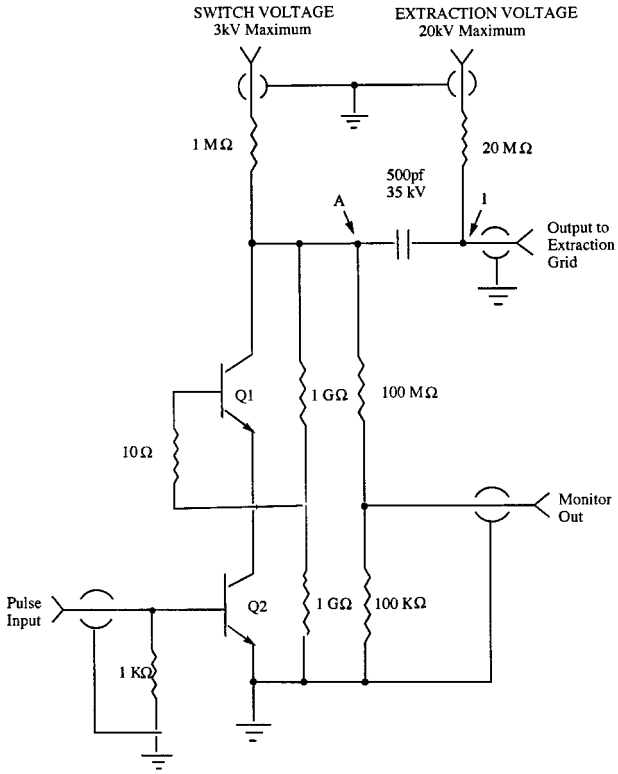

Figure 1. Schematic of the High Voltage Transistor Switch.

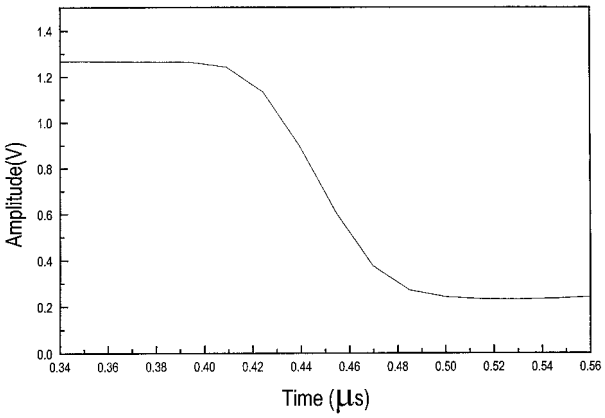

Figure 2. Pulse fall time of the High Voltage Transistor Switch (~ 75 ns) as measured at the monitoring point in Fig. 1.

limited by the highest voltage that the two transistors Q1 and Q2 can withstand, which is 1.5 kV for each of the transistors used in this circuit. The pulsed voltage employed can be varied (0–3 kV) by adjusting the input voltage provided by a high voltage DC power supply (Spellman High Voltage Electronics Corporation, Hauppauge, NY, USA). The distance from point 1 to the vacuum chamber input should be as short as possible. In our system this distance is approximately 15 cm. The delay/pulse generator used in this experiment was a DG535 digital delay/pulse generator (Stanford Research System, Inc. Sunnyvale, CA, USA). It has four independent delay outputs from 0 to 1000 s with a resolution of 5 ps and an output pulse level of 0–4 V DC. This pulse generator was triggered by the Q-switch output pulse of the laser power supply and subsequently served as the trigger for the high voltage switch which applied the pulsed voltage on the extraction grid of the ion source.

Sample preparation

The matrix used was a saturated solution of 3-hydroxypicolinic acid and picolinic acid (molar ratio 4:1) (both matrices were purchased from Aldrich Co., Milwaukee, WI, USA, and were used without further purification) in 36% acetonitrile (HPLC grade) and deionized water. The substrate solution was prepared by dissolving approximately 20 mg of solid Millipore Immobilon-Nitrocellulose pure membrane (Millipore Co., Bedford, MA, USA) in 1 ml of acetone (HPLC grade).

The oligonucleotides used in this work were purchased from the DNA Synthetic Facility (Human Genome Center, Medical School, The University of Michigan) and were dissolved in deionized water to provide a concentration of 100 pmol/μl and were stored at –4 °C. All the oligonucleotide sample solutions were used directly without further purification. DNA samples of a length of 35 b.p. and greater were HPLC purified and their solutions were similarly prepared. The mixture sample was prepared by mixing 1 μL of each component solution mentioned above.

Sample loading

The procedure of loading the sample onto the stainless probe tip is as follows: 2–3 μL nitrocellulose solution was applied to the probe tip surface and allowed to dry to form a very thin membrane. Subsequently, 1 μL of DNA solution was loaded onto the nitrocellulose layer to form a 2–3 mm^2 spot and was stirred using a micropipette tip while drying. In the case of oligonucleotide samples 20–70-mers, 1 μL of 0.2 M diammonium citrate was added to the surface following the DNA sample solution. Once the analyte layer was dry, 2 μL of matrix (3HPA/PA) solution was applied to the tip to cover the sample spot and the probe surface was dried under a stream of air. The crystals of the matrix were crushed using the wall of a micropipette tip.

PDE experimental conditions

All the spectra shown herein were obtained in the positive mode as the average of 20 laser shots. The voltages on the acceleration plates during the PDE experiments were: +15 kV on the repeller, +15 kV on the extraction grid, and a +3 kV potential drop was applied on the extraction grid. No post-acceleration voltage was applied to the detector. A deflector was used to eliminate matrix and other low mass ions. The use of the deflector increased the signal/noise ratio by 2–4 times in this work. The delay time over a range of 0.5–7 μs was optimized in the PDE experiments.

Table 1. Summary of PDE MALDI-TOFMS data

DNA oligomer	Delay time(μs)	Pulsed voltage (kV)	Parent ion (*m/z*)	Mass resolution	Figure
22-mer	4.10	3.0	6798.26	770	3
31-mer	2.77	3.0	9536.41	800	3
35-mer	4.10	3.0	10 832.96	830	4
40-mer	3.96	3.0	12 356.46	700	4
45-mer	4.50	3.0	14 286.17	650	4
50-mer	4.68	3.0	15 433.72	618	4
55-mer	4.70	3.0	16 943.45	630	5
60-mer	4.90	3.0	18 585.71	520	5
65-mer	4.56	3.0	20 087.99	<100	5
Mixture	3.70	3.0		600–800	6

RESULTS AND DISCUSSION

Mixed-base oligonucleotide samples between 5-mers to 70-mers were analyzed by PDE MALDI-TOFMS using the TOF configuration and the high voltage transistor pulse circuit described above. These samples were studied under a variety of conditions. In Table 1 is listed the resolution achieved for each oligonucleotide and the PDE delay time used to obtain these resolutions. Using this particular methodology, a resolution of typically 600–800 was obtained over a broad mass range with the resolution declining to ~500 as the DNA-mer size reached a 60-mer. This resolution should still be clearly sufficient to achieve single base resolution up to a 60-mer. An important point illustrated in Table 1 is that the resolution is achieved over a relatively wide range of delay times and it was found not to be strongly dependent on the delay time between ~2.5–5.0 μs used in this work. A number of mixed-base oligonucleotide samples in this size range but of different base composition were also examined during the course of this work.

An example of the PDE MALDI-TOFMS obtained for oligonucleotide samples of a 22-mer and 31-mer are shown in Fig. 3. These MALDI-MS spectra were obtained using the sample preparation described herein and the oligonucleotides were not high-performance liquid chromatography (HPLC) purified. The quality of the mass spectra in terms of resolution and S/N ratio appears similar to that obtained in previous work using PDE.[23–29] In addition, several peaks corresponding to Na^+, K^+ and NH_4^+ adducts were observed as in previous work. We do not observe significant Fe^{2+} adducts as described in the work of Reilly,[24] probably because of the presence of the nitrocellulose polymer substrate which prevents the interaction of the stainless steel probe tip with the MALDI process. In comparison, we found that the resolution and the quality of the spectra for oligonucleotide samples of 35-mer and larger began to deteriorate significantly using our sample preparation. However, if the samples were HPLC purified, then the MALDI-MS spectra obtained were similar in resolution and S/N ratio to the 22- and

31-mer spectra. The MALDI-MS of a 35-, 40-, 45- and 50-mer purified by HPLC are shown in Fig. 4. In each case, a resolution of 600–800 was observed and adduct peaks of Na^+, K^+ and NH_4^+ were also observed. Apparently, as the size of these synthesized *n*-mers increases, there are sufficient impurities present that can interfere with the matrix/sample crystallization process resulting in a deterioration of the mass spectral quality. The use of HPLC provides sufficient purification so that the resolution of the mass spectra using the PDE MALDI-TOFMS is retained. As the DNA size was extended to 55 and 60 nucleotides as shown in Fig. 5, the resolution decreases and the background on which the sharp DNA peak is situated also increases. Nevertheless the resolution still remains above 500. As the oligonucleotide size increases to a 65-mer and 70-mer, the resolution deteriorated to less than 100, as shown in Fig. 5, when a 65-mer was analyzed by PDE MALDI.

There are several factors that will affect the resolution in MALDI-MS of DNA. The most significant of these factors is the initial kinetic energy distribution of the ions formed in the MALDI process. The PDE method was used to investigate whether the kinetic energy distribution could be corrected for increasingly larger oligonucleotides and improved resolution achieved. The experimental pulsed extraction conditions are important to the performance of PDE MALDI-MS. One important parameter is the fall time of the pulsed voltage. The fall time of our home-built voltage switch is ~75 ns (see Fig. 2) as opposed to 20–30 ns used in other reported work.[23,26,28] The slower fall time of our pulsed voltage may account for the slightly lower resolution performance compared to previous work. The delay time and pulsed voltage amplitude are also important factors in PDE MALDI-MS. The maximum pulsed voltage of 3000 V was generally used in this work. In addition, using the ion source configuration described previously, a resolution of typically 600–800 could be achieved over the delay time of 2–5 μs for any oligonucleotide in the size range studied. It appears that using this particular configuration, the resolution for oligonucleotides is not very sensitive to the ion extraction pulse delay time. The particular delay time chosen in the range between 2–5 μs does not significantly affect the resolution in our experiments from 5-mers to 60-mers and the delays in Figs 3–6 were chosen to optimize the signal. This result may provide an advantage for the detection of mixtures of a large number of oligonucleotides of mixed base composition over a broad mass range without significant resolution degradation. This is demonstrated in Fig. 6 for an oligonucleotide mixture ranging from 5- to 35-mers, where the resolution remains between 600–800 over the entire mass range. The resolution over this range is more than sufficient to easily resolve the difference between a 30- and 31-mer with baseline resolution.

It should be noted that several other TOF acceleration region configurations were also studied for these

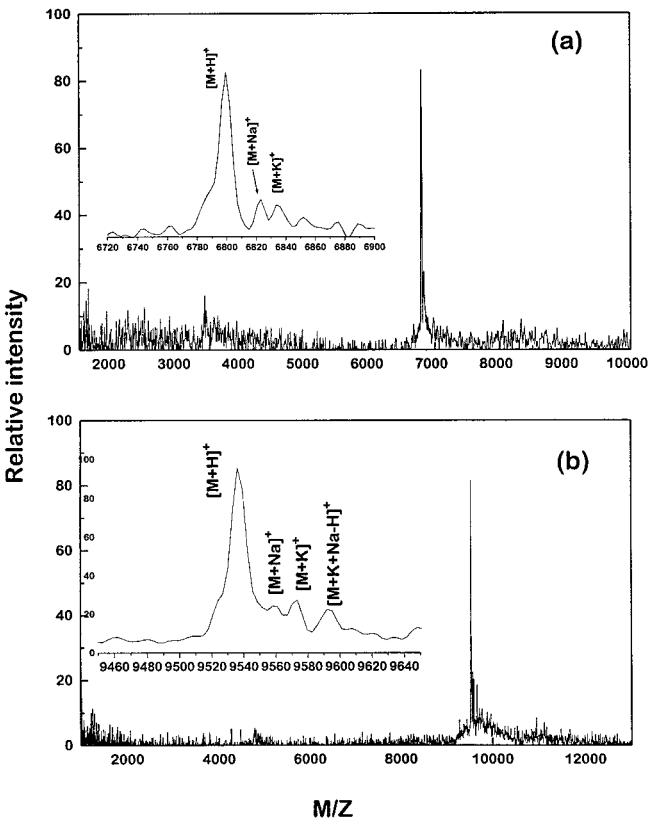

Figure 3. Positive ion PDE MALDI mass spectra of oligonucleotides: (a) 22-mer using a 4.10 μs delay time and 3.0 kV pulsed extraction potential; (b) 31-mer using a 2.77 μs delay time and 3.0 kV pulsed extraction potential.

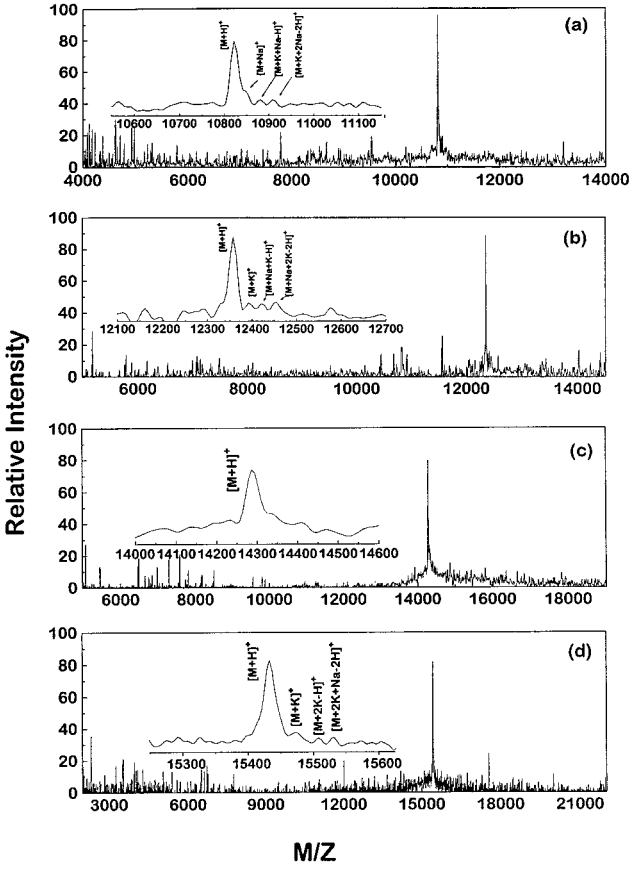

Figure 4. Positive ion PDE MALDI mass spectra of oligonucleotides: (a) 35-mer using a 4.10 μs delay time and 3.0 kV pulsed extraction potential; (b) 40-mer using a 3.96 μs delay time and 3.0 kV pulsed extraction potential; (c) 45-mer using a 4.50 μs delay time and 3.0 kV pulsed extraction potential; (d) 50-mer using a 4.68 μs delay time and 3.0 kV pulsed extraction potential.

effects on the PDE MALDI mass spectra. One configuration involved a two step acceleration region where the distances between the acceleration grids were 12.5 mm. We did not observe significant PDE resolution enhancement using these widely spaced plates. The other source tested was a three step acceleration source with 4 mm between adjacent grids as described by Li and co-workers.[28] A resolution comparable to that observed in Figs 3–5 was observed, although much shorter delay times (500–2000 ns) were required to maximize the resolution compared to the delay times used in our standard ion source. In this three region source, the resolution was generally found to be much more sensitive to delay time relative to the configuration used to achieve the spectra of Figs 3–6. This may be a disadvantage for the analysis of mixtures of *n*-mers over a broad mass range. The detection of the same mixture shown in Fig. 6 using this three step acceleration ion source configuration was not comparable in resolution over the entire mass range.

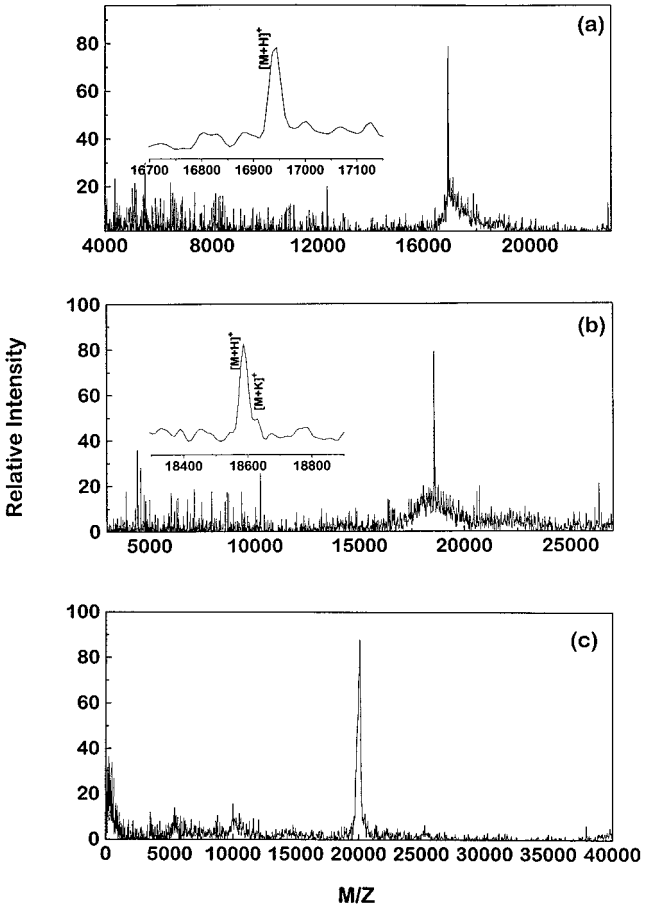

Figure 5. Positive ion PDE MALDI mass spectra of oligonucleotides: (a) 55-mer using a 4.70 μs delay time and 3.0 kV pulsed extraction potential; (b) 60-mer using a 4.90 μs delay time and 3.0 kV pulsed extraction potential; (c) 65-mer using a 4.56 μs delay time and 3.0 kV pulsed extraction potential.

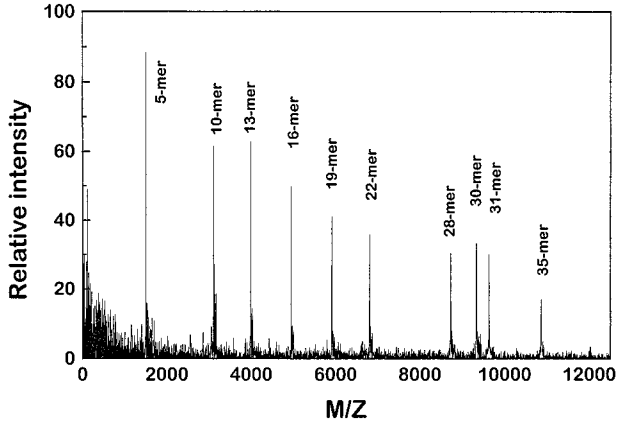

Figure 6. Positive ion PDE MALDI mass spectrum of a mixture of 5-mer, 10-mer, 13-mer, 16-mer, 19-mer, 22-mer, 28-mer, 30-mer, 31-mer and 35-mer using a 3.70 μs delay time and 3.0 kV pulsed potential. Resolution: 600–800 full width at half maximum.

The second important aspect of improving resolution and signal quality in PDE MALDI-MS is the elimination of interfering background and adduct ions. The samples used in this experiment are synthetic single-stranded oligonucleotides. On-probe tip purification was performed using an nitrocellulose (NC) membrane as the substrate and ammonium salts were used as co-matrix to improve the signal quality. The nitrocellulose film substrate has been used previously to enhance MALDI-MS detection of DNA samples.[17,30] The use of the nitrocellulose film on-probe tip purification through ion exchange allows for the partial elimination of the deleterious effects of salts and other contaminants present in these DNA samples. These contaminants are the major factors limiting the ion yield and mass resolution in the MALDI process. Further, the use of the nitrocellullose film substrate improves the shot-to-shot and sample-to-sample reproducibility of the ion yield due to the more homogeneous nature of the matrix/analyte crystals formed over the nitrocellulose surface. In addition, in mixtures of oligonucleotides the NC film also eliminates some of the suppression effects observed without the presence of the film. In the present study the NC film substrate was used effectively up to a 22-mer, while it was used together with diammonium citrate for analytes up to a 31-mer to enhance the signal and resolution. Oligonucleotides up to a 31-mer could be detected successfully without further purification and with high resolution using this strategy with PDE MALDI-TOFMS.

In the case of oligonucleotides greater than 35 bases long, the resolution was found to deteriorate even with the use of PDE. As the size of the synthesized oligomer increases, the percentage of the target DNA component decreases relative to impurities that include oligonucleotides one or two base units shorter or longer in length. The synthetic 40-mer used in this work has a yield in synthesis of ~ 70%. In addition, as the size of the oligonucleotide increases, the formation of adduct ions appears to be more severe. The presence of these various impurities degrades both the resolution and the signal quality. In order to achieve sufficient sample purity to improve the resolution and signal in these experiments, reverse phase HPLC was used to purify samples longer than 35-mer. The effect of such purification is demonstrated in Figs 4 and 5 where enhanced resolution was again achieved by PDE MALDI-TOFMS. This improved resolution is achieved by the use of the HPLC purified samples together with nitrocellulose as substrate and diammonium citrate as co-matrix. The HPLC purification is sufficient to provide enhanced resolution in the PDE MALDI-

TOFMS experiments, but it can not eliminate the majority of adduct ions. For more complete elimination of multiple adduct ions, ion-exchange HPLC may be a promising method. Studies are now underway in our laboratory to test the effect of such purification on increasingly larger oligonucleotides.

As the oligonucleotide sample size increased above a 65-mer, the resolution decreased to less than 100 as shown in Fig. 5 for a 65-mer in PDE MALDI. Several 65-mers and 70-mers of different base composition were studied by PDE MALDI and similar results were obtained. HPLC analysis proved the sample to be 99% oligomer of the specified sample size and analysis by capillary gel electrophoresis also yielded only one peak for the 65-mer. There may be a number of effects involved in the deterioration of the resolution including the large number of adduct peaks formed in the larger oligonucleotides. However, an increasing rate of metastable decay induced by the MALDI process in these large oligonucleotides may well be responsible for the loss of resolution. Further work using a reflectron energy analyzer will be needed to verify the extent of this effect.

CONCLUSION

In this work, pulsed–delayed extraction using a simple high voltage transistor switch together with various sample purification approaches were used to enhance the resolution in MALDI-TOFMS of oligonucleotides up to 60 bases in length. A resolution of 500–800 for oligonucleotides ranging from a 5-mer to a 60-mer was typically observed. In this size range and with this resolution, oligonucleotides differing in length by one base are separated. The resolution deteriorated to < 100 for oligonucleotides of 65-mer and larger. With the TOF acceleration region configuration used in this work, the resolution was found not to vary significantly over a delay range of 2–5 μs. Thus, a mixture of oligonucleotides could be analyzed without significant loss of resolution over the mass range studied. As the DNA size increased to over 35-mer, HPLC purification was required to observe the enhancement in resolution by PDE MALDI-MS.

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REFERENCES

1. M. Karas and F. Hillenkamp, *Anal. Chem.* **60**, 2999 (1988).
2. M. Karas and U. Bahr, *Trends Anal. Chem.* **9**, 3211 (1990).
3. R. W. Nelson, R. M. Thomas and P. Williams, *Rapid Commun. Mass Spectrom.* **4**, 348 (1990).
4. K. J. Wu, T. A. Shaler and C. H. Becker, *Anal. Chem.* **66**, 1637 (1994).
5. E. Nordhoff, M. Karas, R. Cramer, S. Hahner, F. Hillenkamp, F. Kirpekar, A. Lezius, J. Muth and J. W. Engels, *J. Mass Spectrom.* **30**, 99 (1995).
6. K. Tang, S. L. Allman and C. H. Chen, *Rapid Commun. Mass Spectrom.* **6**, 365 (1992).
7. K. Tang, N. I. Taranenko, S. L. Allman, L. Y. Cháng and C. H. Chen, *Rapid Commun. Mass Spectrom.* **8** 727 (1994).
8. K. Tang, N. I. Taranenko, S. L. Allman, C. H. Chen, L. Y. Cháng and K. B. Jacobson, *Rapid Commun. Mass Spectrom.* **8**, 673 (1994).
9. S. Mouradian, D. R. Rank and L. M. Smith, *Rapid Commun. Mass Spectrom.* **10**, 1475 (1996).
10. J. Bai, X. Liang, Y. H. Liu, Y. Zhu and D. M. Lubman, *Rapid Commun. Mass Spectrom.* **10**, 839 (1996).
11. Y. H. Liu, J. Bai, Y. Zhu, X. Liang, D. Siemieniak, P. J. Venta and D. M. Lubman, *Rapid Commun. Mass Spectrom.* **9**, 735 (1995).
12. J. Bai, Y. H. Liu and D. M. Lubman, *Rapid Commun. Mass Spectrom.* **8**, 687 (1994).
13. T. A. Shaler, Y. Tan, J. N. Wickham, K. J. Wu and C. H. Becker, *Rapid Commun. Mass Spectrom.* **9**, 942 (1995).
14. G. B. Hurst, M. J. Doktycz, A. A. Vass and M. V. Buchanan, *Rapid Commun. Mass Spectrom.* **10**, 377 (1996).
15. M. C. Fitzgerald, G. R. Parr and L. M. Smith, *Anal. Chem.* **65**, 3204 (1993).
16. K. J. Wu, A. Steding and C. H. Becker, *Rapid Commun. Mass Spectrom.* **7**, 142 (1993).
17. Y. H. Liu, J. Bai, X. Liang and D. M. Lubman, *Anal. Chem.* **67**, 3482 (1995).
18. A. Ingendoh, M. Karas, F. Hillenkamp and U. Giessmann, *Int. J. Mass Spectrom. Ion Processes*, **131**, 345 (1994).
19. S. Cheng, T. W. D. Chan, *Rapid Commun. Mass Spectrom.* **10**, 907 (1996).
20. Y. F. Zhu, N. I. Taranenko, S. L. Allman, S. A. Martin, L. Haff and C. H. Chen, *Rapid Commun. Mass Spectrom.* **10**, 1591 (1996).
21. W. C. Wiley and I. H. McLaren, *Rev. Sci. Instrum.* **26**, 1150 (1955).
22. W. H. Benner, D. Horn, J. Katz and J. Jaklevic, *Rapid Commun. Mass Spectrom.* **9**, 537 (1995).
23. M. L. Vestal, P. Juhasz and S. A. Martin, *Rapid Commun. Mass Spectrom.* **9**, 1044 (1995).
24. N. P. Christian, S. M. Colby, L. Giver, C. T. Houston, R. J. Arnold, A. D. Ellington and J. P. Reilly, *Rapid Commun. Mass Spectrom.* **9**, 1061 (1995).
25. P. Juhasz, M. T. Roskey, I. P. Smirnov, L. A. Haff, M. L. Vestal and S. A. Martin, *Anal. Chem.* **68**, 941 (1996).
26. R. S. Brown and J. J. Lennon, *Anal. Chem.* **67**, 1998 (1995).
27. S. M. Colby and J. P. Reilly, *Anal. Chem.* **68**, 1419 (1996).
28. R. M. Whittal and L. Li, *Anal. Chem.* **67**, 1950 (1995).
29. S. M. Colby, T. B. King and J. P. Reilly, *Rapid Commun. Mass Spectrom.* **8**, 865 (1994).
30. J. Bai, Y. H. Liu, T. C. Cain and David M. Lubman, *Anal. Chem.* **66**, 3423 (1994).
31. K. Tang, S. L. Allman, R. B. Jones and C. H. Chen, *Rapid Commun. Mass Spectrom.* **7**, 435 (1993).
32. N. I. Taranenko, K. Tang, S. L. Allman, L. Y. Cháng and C. H. Chen, *Rapid Commun. Mass Spectrom.* **8**, 1001 (1994).
33. S. L. Cohen and B. T. Chait, *Anal. Chem.* **68**, 31 (1996).
34. E. Nordhoff, A. Ingendoh, R. Cramer, A. Overberg, B. Stahl, M. Karas, F. Hillenkamp and B. Crain, *Rapid Commun. Mass Spectrom.* **6**, 771 (1992).
35. Y. Dai, R. M. Whittal and L. Li, *Rapid Commun. Mass Spectrom.* **10**, 1792 (1996).