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Mass Spectrometry of Laser-desorbed Oligonucleotides

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Various matrices were used for laser-desorption ionization of mixtures of small DNA segments. Parent negative ions of 34-mer as well as other smaller single-stranded oligomers were observed for selected organic acids and dye compounds. Both positive and negative parent ions were observed with very little fragmentation. Effects of various matrices and the potential applications to DNA sequencing are briefly discussed.

During the past decade, tremendous effort has been expended on applying mass spectrometry to biopolymer measurements for biological and medical applications.[1] However, there are two major difficulties: developing a reliable method to deliver these nonvolatile biopolymers to the mass spectrometer's ionization zone, and achieving ion production without significant fragmentation in either the desorption or ionization steps. Electrospray and laser-desorption ionization (LDI) have been considered to have good potential for overcoming these difficulties.[2] Hillenkamp and his co-workers[3] first reported the discovery that large polypeptide molecules could be put into the gas phase and ionized by a laser in the presence of a large excess of nicotinic acid molecules serving as a matrix. Since then, various research groups[4–15] have reported measurements of protein ions by the matrix-assisted laser-desorption ionization method. Observation of protein ions with molecular weights greater than 100 000 Da, without significant breakup have been reported.[7] However, multiply charged ions and polymer ions were also observed. Most matrix-assisted laser desorption and ionization of proteins has utilized ultraviolet (UV) laser beams. Characteristics of matrix-assisted UV desorption of protein in terms of velocity distribution and laser power density have been discussed both by Beavis and Chait,[16,17] and by Hillenkamp *et al.*[2]

Matrix-assisted UV desorption and ionization has also been applied to DNA segments. However, the success has been limited to very small segments. Spengler *et al.*[18] reported the observation of 8-mer single-stranded DNA ions with detection sensitivity of 10 to 100 pmol. Nelson *et al.*[19,20] obtained positive-ion DNA spectra by laser ablation and ionization from a frozen aqueous matrix with laser wavelength tuned to the electronic resonant excitation of sodium or copper atoms. They[19] detected $pd(A)_8$ single-stranded DNA oligomer. Recently, Currie and his co-workers observed negative parent ions ot 60-mers with mixed matrix materials.[21]

In this work, we report time-of-flight negative-ion mass spectra from matrix-assisted laser desorption of mixtures of single-stranded oligomers up to 34-mer by various laser wavelengths and different matrix materials. Positive-ion desorption spectra up to 20-mer were also observed. The effects of matrix-to-analyte ratio on the sensitivity and resolution of the time-of-flight mass spectrometer are studied. Potential applications and future development for DNA sequencing are also briefly discussed.

EXPERIMENTAL

A schematic diagram of the experimental apparatus is shown in Fig. 1. A Nd-YAG laser (Spectra Physics DCR1, Mountain View, CA, USA) capable of delivering four different wavelengths (i.e., 1064 nm, 532 nm, 355 nm, and 266 nm) was used for laser ablation. The maximum energies per laser pulse for 1064 nm, 532 nm, 355 nm, and 266 nm are 700 mJ, 400 mJ, 200 mJ, and 100 mJ, respectively and the corresponding pulse durations are 10 ns, 7 ns, 5 ns, and 5 ns, respectively. However, typical laser fluence used in this work was less than 200 mJ/cm^2 to prevent any possible production of plasma or fragmentation. No focusing was attempted. The dimensions of the time-of-flight (TOF) spectrometer are also shown in Fig. 1. An ion deflector was used to deflect electrons and small ions whenever it was necessary since the large number of desorbed electrons and small ions was often enough to saturate the electron multiplier. The pulse duration of the ion deflector was adjustable, ranging between 4 and 40 μs, and the maximum switching voltage was 5 kV. A conversion box was used to receive ions and emit secondary electrons. A Johnston Laboratories, Inc., electron multiplier (model MM-1, Cockeysville, MD, USA) was

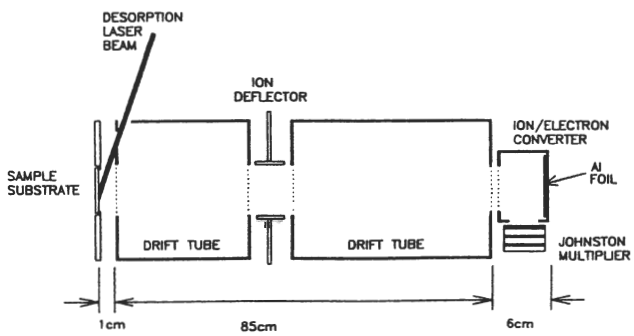

Figure 1. Schematic diagram of experimental setup.

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used to detect secondary electrons from an aluminum foil in the conversion box. The conversion box was installed to reduce the probability of saturation of the electron multiplier by scattered laser light, and to prevent the contamination of the first dynode of the Johnston multiplier by the organic and bio-organic samples used. Signals from the Johnston multiplier went through a preamplifier and subsequently to a fast digital oscilloscope (Yokagawa, model DL2140B, Atlanta, GA, USA) with a maximum sampling rate of 280 samples/s. TOF waveforms from each laser pulse were transferred to a computer for data storage and retrieval. The maximum resolution ($m/\Delta m$) of the TOF spectrometer is only about 200. However, the time-of-flight resolution of heavy DNA segments is somewhat lower. This is probably due to the matrix materials, since the initial velocity distribution of desorbed oligomers is expected to be similar to the velocity distribution of the matrix material.[22]

Oligomers used in this work were purchased from Oligos Etc., Inc. They were made synthetically. All the oligomers were extracted and precipitated by the manufacturer to remove most of the residual organics and salts. No further purification of commercial products was pursued. The samples were prepared by mixing an aqueous analyte solution with an aqueous matrix solution with selected mole ratios of matrix to analyte. About 20 pmol of analyte was put onto a stainless-steel plate with a spot size of ~6 mm. However, the laser-beam size was only 0.5 mm × 1 mm which is a factor of 50 less than sample spot size. Thus, the quantity of each oligomer needed for this investigation can be somewhat lower than 20 pmol. The data were taken within 10 min after the sample was introduced into the chamber and a vacuum of $\sim 10^{-7}$ Torr had been reached.

RESULTS AND DISCUSSION

A laser-desorption negative-ion mass spectrum of mixtures of 3, 4, 5, 8, 11, 15, 20, and 34-mer with 3-methylsalicylic acid and 3-hydroxy-4-methoxybenzaldehyde mixtures as matrixes is shown in Fig. 2. The wavelength of the laser was 355 nm and the laser fluence was 160 mJ/cm^2. Parent ions of each oligomer were observed. No obvious fragmented or dimer ions appeared. Signals in the low-mass region are due to the matrix compounds. Some of the minor peaks were from impurities in the samples. The mole ratio of matrix material to analyte was 15 000 to 1. Similar results were obtained with a 266 nm laser beam for a few different matrix materials which include sinapinic acid, caffeic acid, 3-methylsalicyclic acid, 3-hydroxy-4-methoxybenzaldehyde, and nicotinic acid. The negative-ion spectrum obtained using a 266 nm laser beam with 3-methylsalicyclic acid as matrix is shown in Fig. 3. Some minor peaks were observed, probably due to the strong absorption of DNA at 266 nm.

Several different chemicals have been used as matrix and various wavelengths of laser beams have been tried to obtain laser-desorption ionization spectra. Most laser-desorption ionization spectra have been obtained by using either UV or IR laser beams.[14,15] However, in this work, Rhodamine B dye was also used with a 532 nm laser beam for LDI. Parent negative ions were also observed without significant fragmentation.

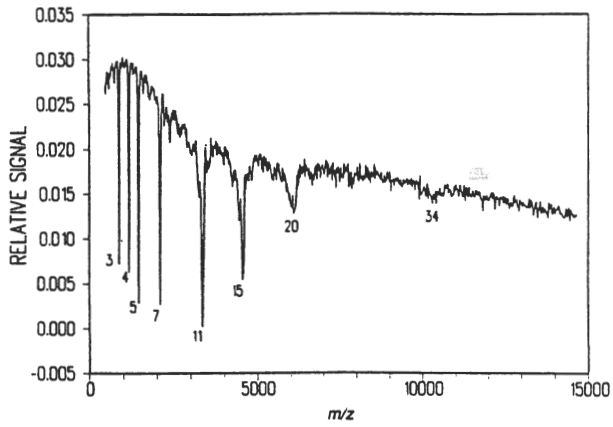

Figure 2. Negative-ion mass spectrum obtained from mixtures of oligonucleotides which include 3-mer (5′-AGT-3′), 4-mer (5′-AGTC-3′), 5-mer (5′-AGTCC-3′), 7-mer (5′-AGTCCTG-3′), 11-mer (5′-AGTCCTGAAGT-3′), 15-mer (5′-AGTCCTGAA-GTCCTG-3′), 20-mer (5′-AGTCCTGAAGTCCTGAAGTC-3′), and 34-mer (5′-AGTCCTGAAGTCCTGAAGTCAGTCCTGAA-GTCCT-3′). Each analyte loaded is about 20 pmol and the quantity laser desorbed is estimated as ~350 fmol. A mixture of 3-methylsalicylic acid and 3-hydroxy-4-methoxybenzaldehyde with a ratio of 2 to 1 was used as matrix material. The mole ratio of matrix to analyte is 15 000:1. The laser wavelength is 355 nm with laser fluence at 160 mJ/cm^2.

Experimental results are shown in Fig. 4. It was found that the 532 nm laser beam is more efficient in producing DNA ions than are UV beams (i.e., 355 nm and 266 nm). Although the absorption coefficient of Rhodamine dye is higher at 532 nm than at 355 nm or 266 nm, increasing the fluence of the UV laser bean did not produce more parent ions for the 15-mer. The increased yield of larger oligomer ions by 532 nm light should not be due just to the absorption efficiency. The possible dissociation of dye molecules by UV laser beams may reduce the desorption efficiency of larger oligomers. Since the negative-ion yield using heavy Rhodamine dye molecules as a matrix is less than when using the smaller 3-methylsalicylic acid, the size of matrix compound may not be an important factor for LDI. It is found that a matrix with a high vapor

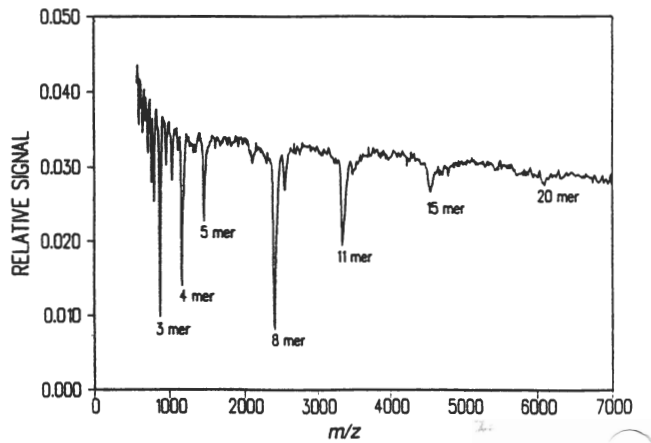

Figure 3. Negative-ion mass spectrum obtained from mixtures oligonucleotides which include 3-mer, 4-mer, 5-mer, 8-mer, 11-mer, 15-mer and 20-mer. Chemical structures for these oligomers are the same as for Fig. 2. The sequence of 8-mer is 5′-AGTCCTGA-3′. 3-methylsalicylic acid was used as the matrix, with matrix-to-analyte ratios of 10 000:1.

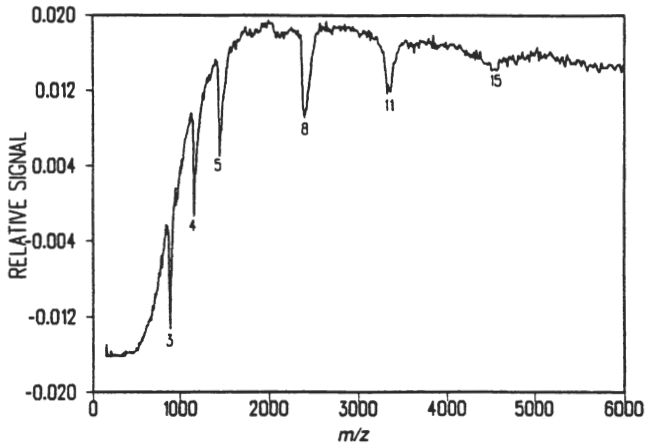

Figure 4. Negative-ion mass spectrum obtained from mixtures of oligonucleotides which include 3-mer, 4-mer, 5-mer, 8-mer (5'-AGTCCTGA-3'), 11-mer, 15-mer, and 20-mer. Chemical structures for these oligomers are the same as for those in Fig. 2. The matrix material was Rhodamine B. The laser wavelength was 532 nm and the laser fluence was 40 mJ/cm^2. The mole ratio of matrix to analyte was 4000:1. No 20-mer was observed.

pressure is, in general, more efficient for desorbing DNA ions. Although most matrix materials tested up to now are organic acids, some aldehydes and dye compounds such as 3-hydroxy-4-methoxybenzaldehyde and Rhodamine B were tried and found to be comparably efficient.

A positive-ion spectrum of 20-mer from laser desorption and ionization is shown in Fig. 5. No significant breakup was observed. When DNA salt was used for positive-ion spectra, fragmented ions were clearly observed (see Fig. 6). This may indicate that DNA molecules with sodium content tend to produce fragmentation from laser ablation. The presence of sodium ions may lead to the need for high laser fluence to desorb DNA such that bond breaking becomes possible. Proposing a detailed mechanism will definitely require more study.

Since the discovery of matrix-assisted UV desorption, a wide range of mole ratios of matrix compounds to analytes has been tried (from 100:1 to 10 000:1).

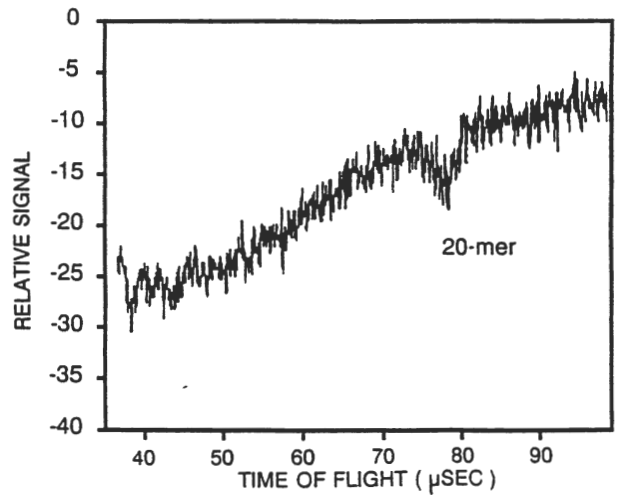

Figure 5. Positive-ion mass spectrom of 20-mer (5'-AGTCCTGAAGTCCTGAAGTC-3') with sinapinic acid + ammonium acetate (mass ratio 1:1) used as matrix material. The laser wavelength and fluence were 355 nm and 150 mJ/cm^2, respectively.

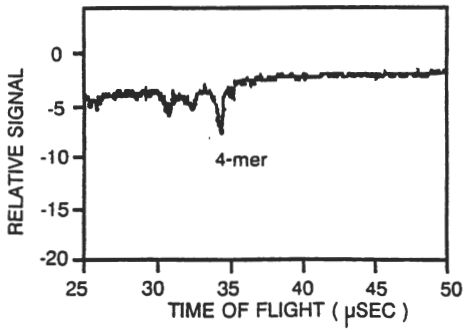

Figure 6. Positive-ion mass spectrum of d(A)$_4$ (sodium salt) with caffeic acid as matrix material. The laser wavelength used was 355 nm and the laser fluence was 80 mJ/cm^2. Mole ratio of matrix to analyte is 5000:1. Significant breakup was observed.

Figure 7 shows the resolution dependence of different oligomers on the matrix to analyte ratio. The resolution, $m/\Delta m$, tends to decrease as the ratio increases. However, for a selected matrix, a high ratio of matrix compound to analytes is more efficient for producing larger oligomer ions. Experimental results are shown in Fig. 8. Detailed mechanisms for matrix-assisted laser-induced ion production are still not well known. Experimental results by Pan and Cotter,[23] as well as Beavis and Chait,[17] indicate that the analyte ions have a velocity distribution similar to that of the matrix ions. Thus, the kinetic energy spread increases as molecular weight of analytes increases, such that peaks from larger oligomers get broader. Pan and Cotter[23] found that the kinetic energy distribution increases with higher mass but not linearly. The drop in velocity is partly due to a collision process. A lower matrix-to-analyte ratio may make it more difficult for the matrix ion plume to carry heavy ions. Thus, the resolution becomes better, but the detection sensitivity becomes lower.

In Fig. 2, it can be seen that overall detection efficiency stays roughly constant for oligomers up to 11-mer. Detection efficiency starts to drop by about a factor of 2 to 3 for each increase of 5-mer of DNA size. If this general trend can be extended to much larger oligomers, it would be extremely difficult to detect any single-stranded oligonucleotide longer than 100-mer for most commonly used matrix materials. Considering the gain of the electron multiplier and the transmission of

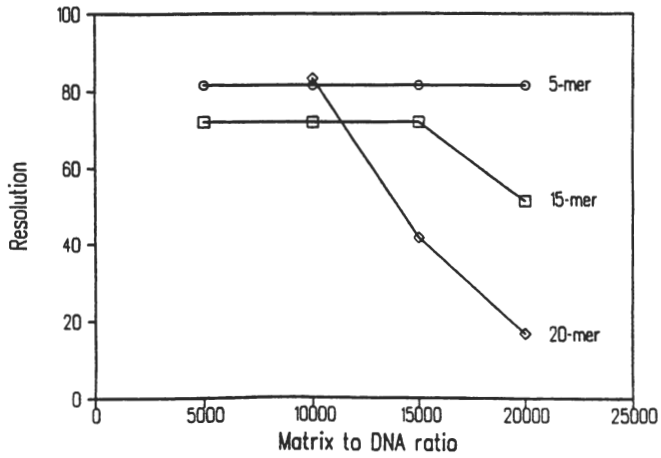

Figure 7. Resolution dependence vs the ratio of matrix to analyte

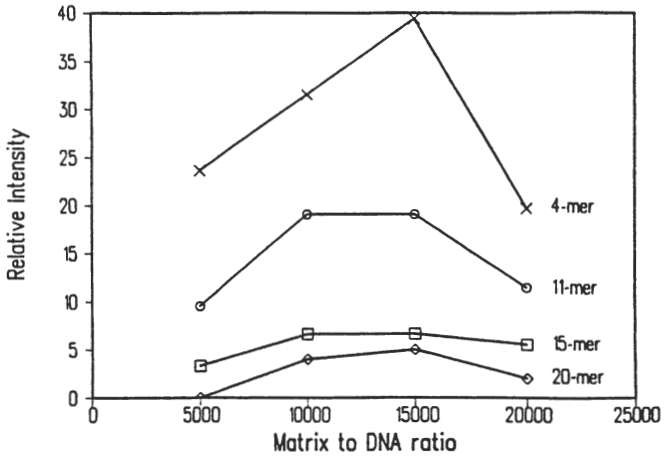

Figure 8. The relationship of the quantity of detected negative parent ions of oligonucleotides to the matrix-to-analytes mole ratio.

the TOF, the number of ions produced for the 20-mer is estimated to be between 10 and 100 ions per laser pulse. The signal observed for 34-mer ions is less than 10 ions per laser pulse. With the typical laser energy per pulse of 400 μJ used in this work, the number of desorbed matrix molecules is estimated at about 10^{11} to 10^{13} per laser pulse, assuming a significant fraction of the laser energy has contributed to vaporization of matrix material. Thus, the desorbed DNA molecules should be of the order of 10^7 to 10^9 per laser pulse. This indicates that the number of negative ions of large oligomers produced compared to the total number of desorbed neutral DNA molecules is less than 1 in 10^6. Recently, Romano and Levis[24,25] used gel electrophoresis for identification and observed the desorption of very large single-stranded DNA molecules (~1000-mer) with dye as the matrix. This may indicate that a significant amount of neutral oligonucleotide molecules is desorbed during the laser ablation without producing a significant quantity of fragmentation. Thus, we consider that there is a reasonable probability of success using post ionization of a neutral single-stranded DNA molecule after laser desorption to detect very large oligonucleotides and using a TOF mass spectrometer.

It has been suggested that laser-desorption TOF mass spectrometry can be an efficient way of performing DNA sequencing, by eliminating the need for gel electrophoresis. When an adequate ionization scheme can be found which does not cause much fragmentation, fast sequencing of large DNA segments (>300-mer) can become feasible.[26,27] Without post ionization, LDI can probably be used only for sequencing small DNA segments (<100-mer) unless an unusual matrix can be found which will increase ion production efficiency by several orders of magnitude. Using the standard Sanger's method for DNA sequencing, each oligomer obtained is about 5 to 50 femtomol, which implies that the detection sensitivity of LDI needs to be improved by at least a factor of 10 to assure its routine use for DNA sequencing. The use of post-desorption ionization can possibly provide much better detection sensitivity. We plan to pursue post-desorption ionization of DNA molecules in the near future.

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