Structural Characterization of Normal and Modified Oligonucleotides by Matrix-assisted Laser Desorption Fourier Transform Mass Spectrometry

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Matrix-assisted UV laser desorption Fourier transform mass spectrometry (266 nm, nicotinic acid matrix) can be used for the detailed structural characterization of normal and modified oligonucleotides. The negative ion spectra for these compounds revealed abundant $(M - H)^-$ ions as well as fragment ions that provided the information necessary to determine oligomer sequence and to differentiate isomers. The nicotinic acid matrix was required for the production of $(M - H)^-$ ions for the oligonucleotide dimers, trimers, tetramers, and hexamers examined in this study. Elimination of the nicotinic acid matrix resulted in complete loss of the $(M - H)^-$ ions as well as most of the larger fragment ions for the oligomers. The primary fragmentation pathway was observed to be phosphate ester bond cleavage with the resulting charge retained on the 3′ end of the oligomer and enabled isomeric differentiation of compounds such as d(5′-CGCG-3′) and d(5′-CCGG-3′). Collision-induced dissociation experiments of the $(M - H)^-$ ions for these compounds confirmed the preferential loss of nucleotides from the 5′ end of the oligomers. The presence and location of modifications such as methyl and ethyl alkyl groups to the oligonucleotides could also be identified. *(J Am Soc Mass Spectrom 1991, 2, 402–412)*

Structural characterization of normal and modified oligonucleotides is necessary not only for determining oligonucleotide sequences but also for examining the nature of DNA modification induced by carcinogenic agents. Because oligonucleotides are thermally labile and nonvolatile, particle desorption techniques have been used to generate molecular ions from the underivatized oligonucleotides. Mass spectral analysis of small oligonucleotides has previously used either fast atom bombardment (FAB) [1–3] or plasma desorption (PD) [4–6] ionization techniques. While these two techniques have been successful in generating molecular ions for small oligonucleotides, the irreproducibility of molecular ion formation, poor sensitivities, and lack of complete structural information in many cases have limited their applications. The capabilities of laser desorption mass spectrometry (LDMS), initially demonstrated more than 10 years ago [7], for the soft ionization and structural characterization of small quantities (picomoles or less) of biological compounds suggest that this technique would be well suited for the detailed examination of normal and modified oligonucleotides. However, while biomolecules such as peptides and oligosaccharides with molecular masses up to 3000 u can be ionized intact in *some* cases by LD with addition to the sample of ionic dopants such as KBr [8, 9], this technique has not generally been successful for the soft ionization of oligonucleotides with molecular masses larger than 800 u. Laser desorption of oligonucleotides under these experimental conditions typically generates extensive fragmentation of the glycosidic bonds to produce the nucleic base ions, with virtually no fragment ions indicative of the sequence of the oligomer.

The development of matrix-assisted UV LDMS has made possible the ionization and detection of extremely large peptides (with molecular masses exceeding 100,000 u) [10, 11]. This technique has tremendous potential for the direct determination of molecular masses of large biomolecules such as peptides and oligonucleotides, although the lack of fragmentation limits the amount of structural information that can be obtained for these molecules. For example, one application of this matrix-assisted LDMS technique may be the rapid measurement of large oligonucleotide fragments from DNA, which would have important implications for the DNA sequencing requirements of the Human Genome Project [12]. However, while extremely large peptides have been examined by the matrix-assisted technique, the utility

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of this technique for the investigation of large oligonucleotides has not yet been as successful.

Matrix-assisted LD time-of-flight (TOF) experiments for small oligonucleotides (3–20 base units in length) using absorbing [13–15] and nonabsorbing matrices [16] have recently been reported. These TOF experiments indicate primarily $(M + H)^+$ or $(M - H)^-$ ions with virtually no fragment ions from the oligonucleotide, and provide molecular mass but almost no structural information for the oligonucleotides. Although the matrix-assisted ionization method has been developed and used almost exclusively with TOF mass spectrometers, research is currently in progress in our laboratory as well as in many other labs to demonstrate this technique with trapped ion mass spectrometers, such as Fourier transform mass spectrometry (FTMS). The potential capabilities of FTMS for wide mass range, high resolution measurements, and ion trapping experiments (for ion/molecule reactions and MS^n experiments) [17] suggest that this instrumental technique should be useful for the detailed structural characterization of ions generated by the matrix-assisted technique. For example, the examination of normal and modified oligonucleotides requires not only measurements of molecular masses but also detailed investigation of nucleic base sequences, determination of the 3′ ends for the oligomers, and identification of any adducts present. While the matrix-assisted LD technique provides a useful method for generating $(M + H)^+$ or $(M - H)^-$ ions from oligonucleotides for molecular mass determinations, the accurate mass measurements and multistage mass spectrometry capabilities of the FTMS can be used to probe the identities and fragmentation pathways of the ions and determine the sequences and modifications of oligonucleotides, including isomeric differentiation. This report outlines the application of matrix-assisted LD FTMS, initially demonstrated for small peptides [18], for the detailed structural characterization of normal and modified oligonucleotides.

Experimental

All chemicals were obtained either commercially or as gifts from biochemical research groups and were used without further purification. The nicotinic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI), the oligonucleotide dimers from Pharmacia LKB Biotechnology Inc. (Piscataway, NJ), the oligonucleotide alkyl phosphotriester dimers from Chemsyn Science Laboratories (Lenexa, KS), the oligonucleotide trimers from Dr. Bob Foote (Oak Ridge National Laboratory, Biology Division, Oak Ridge, TN), the oligonucleotide tetramer isomers of d(C, G, C, G) from Dr. K. Bruce Jacobson (Oak Ridge National Laboratory, Biology Division, Oak Ridge, TN) and the oligonucleotide tetramers d(TGCA, TG*CA) and hexamer d(GCTAGC) from Dr. John Essigmann (Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA).

The samples were prepared for the LD experiments by mixing 1 μL of an aqueous analyte solution (approximately 50–500 μmol/L) with a few microliters of an aqueous nicotinic acid matrix solution (approximately 50–250 mmol/L), based on experimental conditions outlined for the TOF experiments [10, 11]. A few microliters of this resulting solution was then dried onto the tip of a stainless steel solids probe used for the LD experiments. Nicotinic acid was found to be a particularly good matrix for the 266 nm LD FTMS experiments of oligonucleotides. Although this compound is somewhat volatile and evaporates in the vacuum system, matrix-assisted LD spectra can be acquired for at least 30 min before the matrix evaporates significantly. The detection limit of the technique was not examined in detail; in general, 10–100 pmole of analyte were placed on the probe tip (this amount certainly exceeds that required for the LD process).

All experiments were performed with an Extrel FTMS-2000 Fourier transform mass spectrometer (3 Tesla magnet) (Madison, WI) [17] equipped with a Quanta Ray DCR-11 Nd:YAG pulsed laser. The laser was operated in the Q-switched mode (donut-shaped beam, 8 ns pulse width) to generate radiation at 266 nm. The laser beam was focused to approximately 250 × 250 μ onto the tip of the probe, giving an estimated power density range of $1 \times 10^6 - 1 \times 10^7$ W/cm^2 for 266 nm. Increasing the laser power density above this value resulted in increased fragmentation, in accord with results obtained by other research groups [11]. The laser power was attenuated by adjusting the delay between the flashlamp and Q-switch firing. The laser was fired at 10 Hz for at least 5 min prior to the FTMS experiment to allow it to warm up and stabilize. For the FTMS experiments, the laser was fired only at the beam event. Even though the laser power varies somewhat at repetition rates slower than 10 Hz, this factor did not significantly influence the success of the matrix-assisted experiments. The FTMS experiment was initiated by firing the laser at the beam event to ionize the sample. A complete mass spectrum could be obtained from a single laser shot, although for most spectra in this report, 2 to 20 laser shots were signal averaged to provide better quality spectra. Immediately following the laser pulse, an ejection pulse (150 ms duration) was used to eliminate the $(M - H)^-$ ions of the matrix compound. This event was followed by broad band excitation and detection of the ion cyclotron signal in the FTMS source cell under medium resolution conditions (FWHM = 500–3000). The total experiment time was approximately 200 ms. Known fragment ions such as PO_3^- and the nucleic bases were used as internal calibrant ions for each spectrum. The pressure in the FTMS cell was approximately 6×10^{-8} torr for most experiments, although the abundance of the $(M - H)^-$

for the tetramers was enhanced when a static pressure of argon (4×10^{-6} torr) was added to the cell. Collision-induced dissociation (CID) experiments were performed by isolating the ion of interest and then accelerating (100–500 eV translational energy range) that ion into argon as a collision gas (static pressure of approximately 5×10^{-6} torr).

Even though both TOF and FTMS are pulsed techniques and can be interfaced with pulsed LD/ionization, there are several important differences between these two mass spectral techniques. First, the time scale for ion detection in the two techniques is dramatically different. Ion detection in FTMS occurs tens to hundreds of milliseconds after ion formation, whereas the TOF instruments detect ions on much shorter time scales (typically microseconds). More important is the fact that fragmentation of a large ion in a linear TOF instrument does not affect its detection because the fragment ions arrive at the detector at the same time that the undissociated parent would have arrived. Thus, more ion fragmentation is usually observed in the FTMS experiment as compared to linear TOF (no reflectron) or other conventional mass spectral techniques [19]. Second, because the FTMS is a trapped ion technique, it has a dynamic range of approximately 10^6 and can be selective to the kinetic energies of the laser-desorbed ions. The dynamic range implies that matrix ions must be ejected from the cell prior to ion detection to enable the analyte to be observed. For TOF experiments, the ions are accelerated by kilovolt potentials out of the source region, so ions with significant kinetic energies (10–100 eV) degrade the resolution of the spectra but are still detected. However, in the FTMS experiment, ions with kinetic energies greater than the trapping potentials of the FTMS cell (typically 1–5 volts) will be completely lost from the trap if they cannot be sufficiently decelerated. Third, ion detection by FTMS is accomplished by monitoring ion image current on the receiver plates, as opposed to ion detection by an electron multiplier in TOF. This difference implies that while FTMS cannot count single ions, it does not suffer from multiplier insensitivity to large ions. Recent reports have indicated that FTMS can be used to detect ions at $m/z > 30{,}000$ for cesium iodide clusters [20], however, the ionization and FTMS detection of large ions ($m/z > 10{,}000$) for biomolecules has not been as successful. [21].

Results and Discussion

Laser desorption without matrix-assistance can be used to generate $(M - H)^-$ ions from oligonucleotide dimers such as d(ApA) in some cases; however, the abundance of these ions is usually quite small with respect to the abundances of the fragment ions. Matrix-assisted LD of dinucleotides using the conditions outlined in the Experimental section dramatically increases the abundance of the $(M - H)^-$ ions while reducing the amount of the fragment ions. This technique allows oligonucleotide dimer isomers to be characterized and distinguished. For example, investigation of $d(A)_2$ and $d(G)_2$ with the matrix-assisted conditions revealed abundant $(M - H)^-$ ions as well as structurally informative fragment ions. For $d(A)_2$, an $(M - H)^-$ ion was observed at m/z 563 along with fragment ions corresponding to elimination of neutral adenine, $(M - H - AH)^-$, to form m/z 428, and cleavage of the phosphoester bond, (M – deoxyadenosine)$^-$, to give m/z 330. These two fragment ions were observed in roughly equal abundance. Examination of $d(G)_2$ revealed an $(M - H)^-$ ion at m/z 595 and similar fragmentation. The fragment ions corresponding to cleavage of the phosphate linkage provided sequence information for dinucleotides. The negative ion spectra obtained for two isomeric dimers, d(5′ – AG – 3′) and d(5′ – GA – 3′) are shown in Figure 1a and b. Although $(M - H)^-$ ions at m/z 579 are observed for each isomer, fragment ions are also observed that result from loss of the neutral nucleic bases as well as cleavage of the phosphate ester linkage. Note the preferential cleavage of the phosphate ester linkage with the charge retained on the 3′ end of the dimer. This particular fragmentation generates m/z 346 as the most abundant sequence ion from d(5′ – AG – 3′) (Figure 1a), whereas m/z 330 is the most abundant sequence ion from d(5′ – GA – 3′) (Figure 1b). Preferential fragmentation from the 3′ end of oligonucleotides has been observed in FAB [1–3, 22] and PD [23] spectra as well (these fragment ions have been designated as Y ions). Phosphate ester cleavage with charge retention on the 5′ end of the dimer (designated as X fragment ions [23]) also occurs, as indicated in Figure 1a, generating an ion at m/z 330, but in lower abundance than the Y fragment ion (m/z 346). The formation of Y fragment ions in greater abundance than X fragment ions was observed for most of the oligonucleotides examined in this study, and provided the information necessary to not only determine the sequence of the oligomers but also to identify the 3′ ends and differentiate isomers. This technique was found to be useful for the differentiation of other pyrimidine dimer isomers as well, such as d(5′ – TC – 3′) and d(5′ – CT – 3′). The sequence ions and abundances observed for several oligonucleotide dimers are summarized in Table 1. Although fragment ions corresponding to cleavage of nucleic bases were also readily observed in the negative ion spectra for these dimers, the abundant Y (and in some cases X) fragment ions listed in Table 1 were the most structurally informative for sequence determination and isomeric differentiation.

Because matrix-assisted LD FTMS provides structural characterization and isomeric differentiation of normal oligonucleotide dimers, it should be useful for the examination of modified dinucleotides as well. One example is the characterization of alkylated adducts which are formed when DNA reacts with alkylating agents such as methylnitrosourea. These

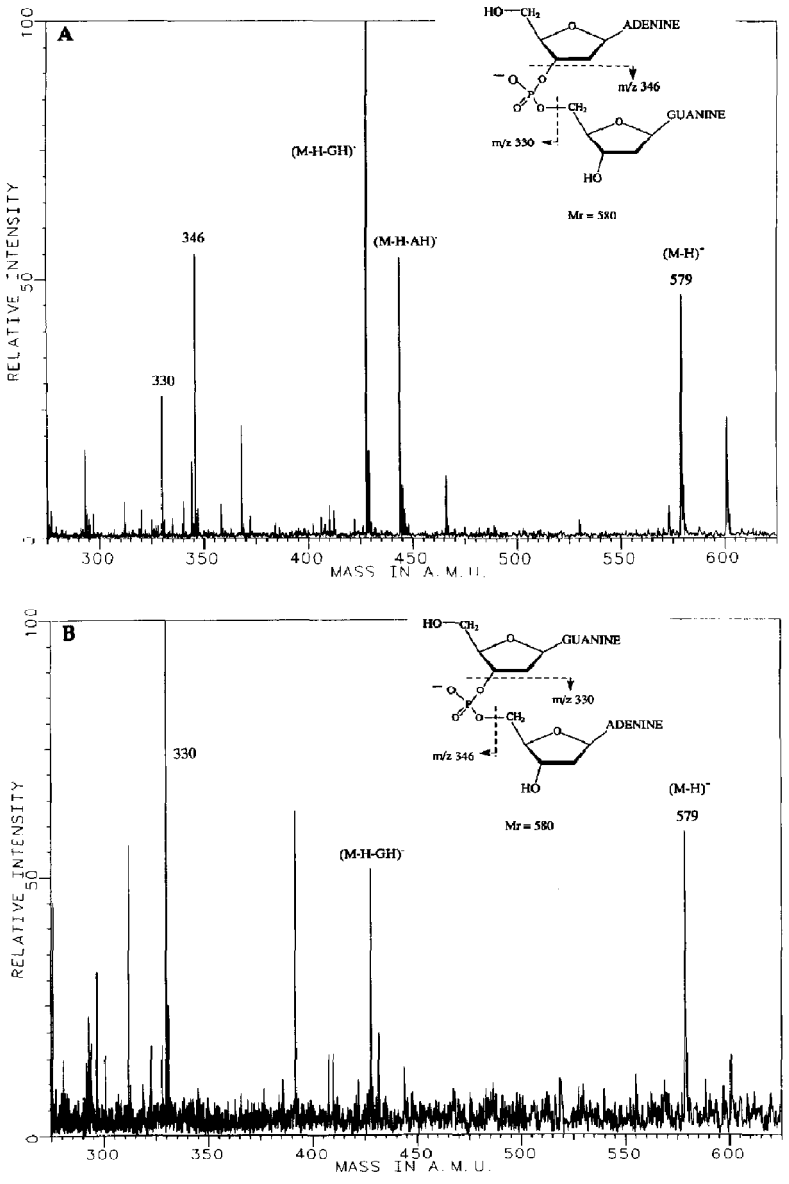
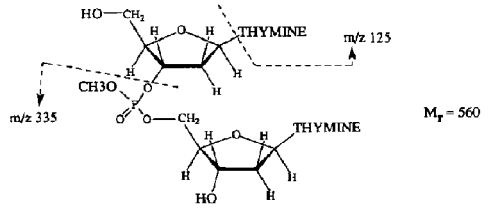


Figure 1. Negative ion matrix-assisted LD-FTMS spectra of two isomeric dinucleotides. (**a**) Spectrum of d(5′ – AG – 3′) illustrating $(M - H)^-$ at m/z 579 and fragment products corresponding to cleavage of glycosidic and phosphate-ester bonds. The ion at m/z 601 is due to sodium attachment to the dinucleotide. (**b**) Spectrum of d(5′ – GA – 3′) illustrating $(M - H)^-$ at m/z 579 and other fragment ions. Note that the fragment ions differ for these two compounds and provide isomeric differentiation.

reactions can result in alkylation of the phosphate linkage (forming alkyl phosphotriesters), the sugar ring, or the nucleic base of an oligonucleotide. Because biological activity and repair of alkyl adducts are dependent on the site and extent of oligonucleotide alkylation [24], modified oligonucleotide isomers need to be distinguished. Previous research has indicated that accurate mass measurements and CID experiments can be used to distinguish alkylation sites of the guanine nucleic base in methylated guanosines [25]. The combination of matrix-assisted LD with CID techniques should provide detailed structural information for oligonucleotide dimers d(TpT) containing alkylation of the phosphate linkage. Laser desorption of methyl thymidine phosphotriester dimer, shown below, generated a variety of negative ions including



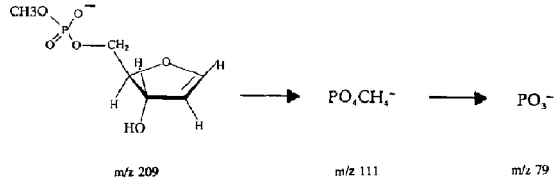
$(M - H)^-$ at m/z 559. Although $(M - CH_3)^-$ was observed, the most abundant fragment ions were due

Table 1. Sequence ions obtained by 266 nm laser desorption of oligonucleotide dimers

Dimer	$(M - H)^-$	Sequence ions (m/z)* Y	X
d(AA)	563	330 (100)	
d(GG)	595	346 (100)	
d(AG)	579	346 (55)	330 (27)
d(GA)	579	330 (100)	346 (—)
d(TC)	530	306 (13)	321 (6)
d(CT)	530	321 (24)	306 (5)

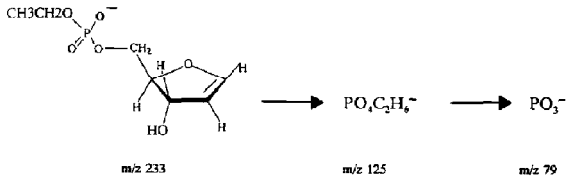
*Negative ion mass-to-charge ratio (normalized relative abundance).

to cleavage of the phosphate linkage to generate a Y fragment ion $(M - \text{thymidine})^-$ at m/z 335 and cleavage of the glycosidic bond to generate the thymine nucleic base ion $(T)^-$ at m/z 125, shown on the structure above. Isolation and CID of the m/z 335 ion yielded fragment ions at m/z 209 $(M - H - TH)^-$, 194 $(M - H - TH - CH_3)^-$, 125 $(T)^-$, 111 $(PO_4CH_4)^-$, and 79 $(PO_3)^-$. The ions at m/z 79 and 335 were used as internal calibrant ions and provided mass accuracies of millimass units for the other fragment ions (sufficient to determine their empirical formulas). The ion at m/z 111 was assumed to be $(PO_2OHOCH_3)^-$. This m/z 111 ion, along with the unmodified thymine at m/z 125, verified the site of methylation on the phosphate linkage of this oligonucleotide. Further evidence was provided by the collisional activation of the m/z 209 ion, shown below, which fragmented to ions



at m/z 111 and 79. The fragment ion at m/z 111 observed in the CID spectrum of the m/z 209 ion verified the phosphate methylation for this dinucleotide. Similar fragmentation for this compound has been reported with desorption chemical ionization [26].

Examination of a modified oligonucleotide dimer d(TpT) containing ethylation of the phosphate linkage revealed an $(M - H)^-$ ion at m/z 573 as well as abundant fragment ions at m/z 349 (Y ion generated by cleavage of the phosphate linkage) and m/z 125 (thymine). Isolation and CID of the m/z 349 ion, shown in Figure 2a, generated several fragment ions, including m/z 125, which was presumed to be the thymine nucleic base. Note that m/z 79 $(PO_3)^-$ is observed but m/z 111 $(PO_4CH_4)^-$ is not present for this compound. Close examination of the ion at nominal m/z 125 in Figure 2a revealed two ions, m/z 125.03565 ($C_5H_5N_2O_2^-$ of thymine) and 125.00092 $(PO_2OHOC_2H_5)^-$, as shown in Figure 2b. In this case, a resolution of greater than 3500 (FWHM) was needed to resolve these two isobaric fragment ions and verify the position of ethyl substitution on the phosphate linkage for this dimer. The identity of this m/z 125 ion was additionally confirmed by examination of the CID of the m/z 223 ion from the ethylated oligomer, shown below, which fragments to generate ions at m/z 125 ($PO_4C_2H_6^-$) and m/z 79 (PO_3^-).



In this case, accurate mass measurement confirmed that the m/z 125 ion corresponded to ethyl phosphate, $PO_4C_2H_6^-$. Similar alkylated phosphate ions were observed in the FAB spectra of isopropyl triester dinucleotides and could be used to verify the location of the alkyl modification [27].

The matrix-assisted FTMS technique demonstrated above for dinucleotides also provided molecular mass and sequence information for trinucleotides. For example, the negative ion matrix-assisted LD FTMS spectrum of d(GGT) is shown in Figure 3a. A molecular $(M - H)^-$ ion at m/z 899 was observed along with abundant Y fragment ions at m/z 650 and 321, which yielded the sequence information for the oligomer. Examination of d(GGT) under the same experimental conditions (i.e., laser wavelength, energy, sample concentration) *without* nicotinic acid as the matrix indicated complete loss of the $(M - H)^-$ ion as well as most of the sequence information. The only ion observed in this case was the m/z 321 ion, corresponding to the terminal thymidine phosphate residue of the oligomer, shown in Figure 3b. This result supports the enhancement effect of the matrix and helps to rule out other variables in the experimental procedure that may result in fragmentation differences. Note that the major fragment ions observed in Figure 3a result from fragmentation from the 3′ end (Y ions) and would distinguish d(5′ – GGT – 3′) from d(5′ – TGG – 3′). Laser desorption of the trimer d(TGT) yielded characteristic negative ions corresponding to $(M - H)^-$ at m/z 874 and fragment ions at m/z 650 and 321, which provided the sequence information for this trimer. Obviously, it is impossible to determine if these fragments are X or Y ions for this symmetric compound. The CID spectrum for the $(M - H)^-$ ion at m/z 874 for d(TGT), shown in Figure 4, reveals abundant fragment ions corresponding to cleavage of phosphate ester bonds. Collision-induced dissociation of $(M - H)^-$ at m/z 899 for d(GGT) verified that the fragment ions were predominantly Y ions, and revealed a fragmentation spectrum that was virtually identical to Figure 3a.

A series of oligonucleotide tetramers, including four d(C, G, C, G) isomers, were examined with this tech-

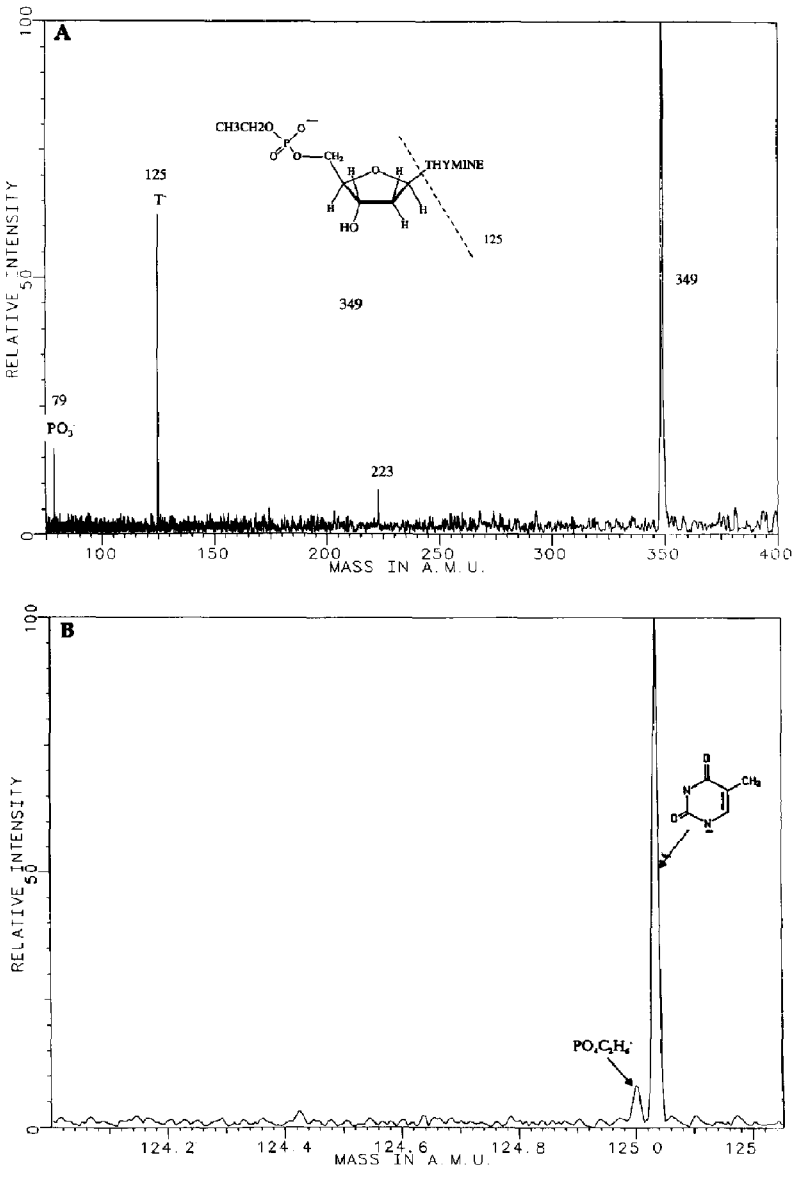


Figure 2. (a) Collision-induced dissociation spectrum of the m/z 349 ion from $d(T)_2$, ethyl triester. The ion at m/z 223 is generated by loss of neutral thymine (TH) from the parent ion at m/z 349. (b) High resolution spectrum of the ions at nominal m/z 125 in Figure 2a verifying the presence of two distinct species.

nique to ascertain the level of sequence information available from the fragment ions. This isomeric set included not only positional isomers such as d(5′ – CGCG – 3′) and d(5′ – CCGG – 3′), but also compounds differing only in the location of the 3′ end such as d(5′ – CGCG – 3′) and d(5′ – GCGC – 3′). Characteristic $(M - H)^-$ ions as well as abundant fragment ions were observed, as shown in Figure 5a and b for d(CGCG). One noticeable difference between the TOF [13–16] and FTMS spectra for oligonucleotide tetramers is the increased fragmentation of the oligonucleotides in the FTMS spectra, which provide detailed sequence information for these compounds. Factors that may account for the differences in fragmentation between TOF and FTMS (such as ion lifetimes or ion kinetic energies) have been outlined in the Experimental section. The identities of several fragment ions are listed in Figure 5b. Most of the fragment ions result from cleavage of either glycosidic or phosphate-ester bonds. Cleavage of phosphate-ester bonds generates structurally informative sequence ions and reveals Y ions (m/z 346, 635, 964), X ions (m/z 306, 635, 924), as well as other fragment ions for d(CGCG) (Figure 5b). Glycosidic bond cleavages result in losses of the neutral nucleic bases. Note that $(M - H - GH)^-$ at m/z 1022 is observed for

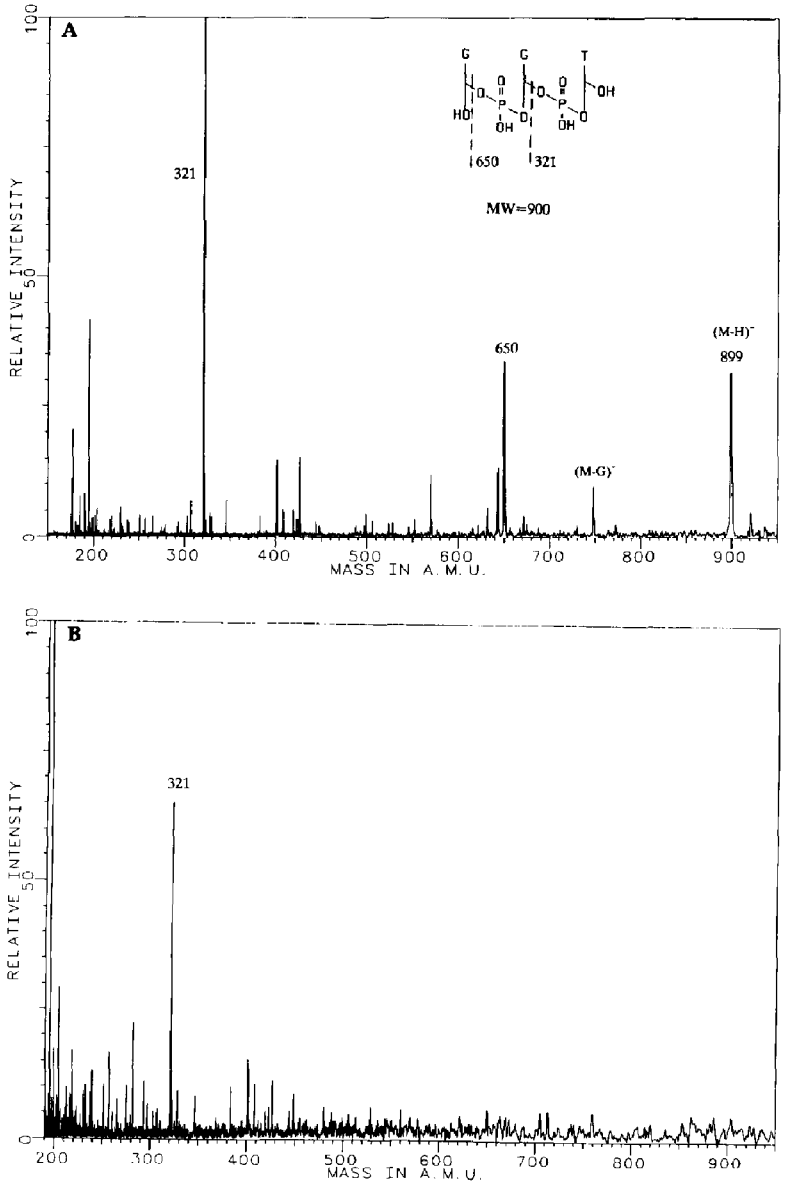


Figure 3. Negative ion matrix-assisted LD-FTMS spectrum of d(5′ – GGT – 3′). (**a**) 266 nm, nicotinic acid matrix used. (**b**) 266 nm, experimental parameters identical to (a) except nicotinic acid was *not* added as a matrix compound. Note the disappearance of the $(M - H)^-$ ion as well as the larger fragment ions.

d(CGCG) while $(M - H - CH)^-$ is completely absent. Although these particular fragment ions are not very abundant, they are structurally informative because the nucleic base elimination appears to originate from the 3′ end of the tetramer, as evident from inspection of Table 2. Combinations of both fragmentation pathways are observed in some cases, especially notable in the abundant ions at m/z 715 and 506. The m/z 715 ion is due to X fragmentation combined with loss of a nucleic base, as shown in Figure 5b, and has previously been reported by Gross and co-workers [22]. Loss of the 5′ deoxycytidine from this m/z 715 ion generates the fragment at m/z 506. Other fragment ions not specifically identified in Figure 5a result from sequential combinations of these two fragmentation pathways.

Even though many different fragment ions are observed in Figure 5a, examination of three spectral regions were sufficient to determine the abundant Y sequence ions (m/z 964, 635, 346) for this tetramer. These three regions outlined in Figure 5a can be used to identify the Y sequence ions for all the possible combinations of dC, dG, dA, and dT for the tetramers shown in Table 2. For example, the third step region

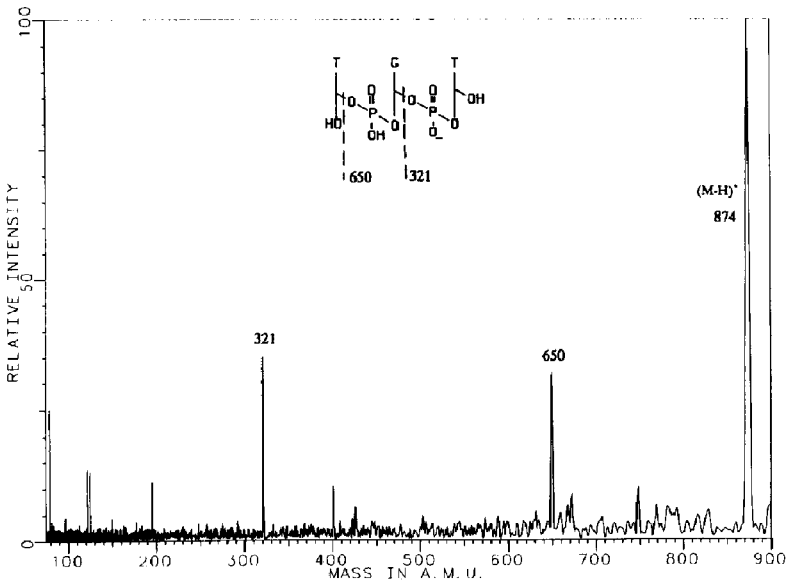


Figure 4. Collision-induced dissociation spectrum of $(M - H)^-$ from d(TGT). Note the abundant fragment ions at m/z 650 and 321 corresponding to phosphate-ester bond cleavage.

in Figure 5a corresponds to cleavage of the 5′ nucleoside from a tetramer and ranges from the smallest possible trimer, d(pCCC) at m/z 884, to the largest possible trimer, d(pGGG) at m/z 1004. In general, the most abundant ion observed in this mass region is one of the Y sequence ions from the tetramer, determined to be m/z 964 d(pGCG) for the tetramer d(CGCG). Sequence ions resulting from fragmentation of the 5′ end of the tetramers (X ions) were also observed in minor abundance but were more dominant when a cytosine residue was at the 3′ end of the oligomer.

Isomers differing in nucleic base sequence, for example d(CGCG) and d(CCGG), are easily distinguishable based on the observation of the abundant Y sequence ions, as shown in Table 2. Even though Y fragment ions at m/z 964 and 346 are observed for both of these compounds, the Y sequence ions in the m/z 600–700 region provide differentiation, yielding m/z 635 for d(CGCG) and m/z 675 for d(CCGG). For isomers that differ only in the location of the 3′ and 5′ ends, the combination of the abundant Y sequence ions along with identification of the nucleic base lost $(M - H - \text{base})^-$ provides unambiguous isomeric identification. For example, $(M - H - GH)^-$ is observed for d(5′ – CGCG – 3′) whereas $(M - H - CH)^-$ is observed for d(5′ – GCGC – 3′). Several other minor fragment ions are present in the spectra of these compounds and may provide additional structural information.

An oligonucleotide tetramer containing all four DNA bases, d(TGCA), was examined and its fragment ions are listed in Table 2. An $(M - H)^-$ ion at m/z 1172 was observed as well as Y sequence ions at m/z 948, 619, 330. For this tetramer, the $(M - H - \text{base})^-$ was very weak and could not be identified. Examination of a modified version of this tetramer, d(TG*CA) where G* is O^6 – methyl guanine, indicated an $(M - H)^-$ ion at m/z 1186 and characteristic fragment ions, as shown in Table 2. Note that the first two sequence ions (m/z 330 and 619) are identical to those observed for the tetramer d(TGCA); however, the third sequence ion at m/z 962 is 14 u higher than the analogous ion for d(TGCA), verifying that methyl adduction occurs at this position. Collision-induced dissociation techniques developed to distinguish methyl guanosine isomers [25] could be used to determine the exact site of methylation on the modified guanosine in this oligomer.

The largest oligonucleotide observed to date by matrix-assisted LD FTMS is the hexamer d(GCTAGC) with a molecular mass of 1791 u, shown in Figure 6. The negative ion spectrum revealed an $(M - H)^-$ ion at m/z 1790 as well as several fragment ions. The ions at $m/z > 1790$ correspond to multiple replacement of phosphate hydrogens by ammonium ions in the hexamer. These oligonucleotides often contain sodium or ammonium cations attached to the ionic phosphate oxygens and thus reveal a distribution of molecular ions. The most abundant fragment ions observed for this oligomer were due to cleavage of every second phosphate linkage from the m/z 1790 ion, forming m/z 1252 and 635. Examination of larger oligonucleotides failed to reveal molecular ions, implying that ion generation, ion trapping, and ion detection factors need to be further addressed and optimized to access higher mass regions. Studies are in progress to determine the optimal LD conditions for large oligonu-

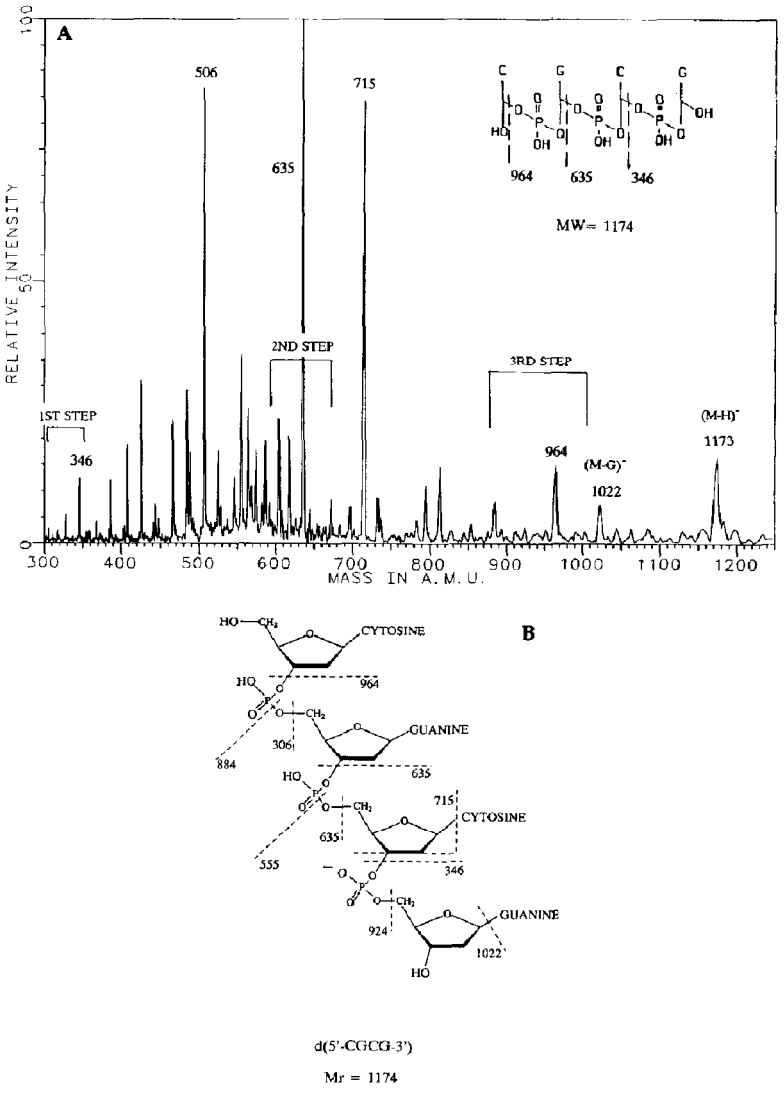


Figure 5. (a) Negative ion matrix-assisted LD-FTMS spectrum of d(5′ – CGCG – 3′). The three regions outlined as steps allow the Y fragment ions to be identified. (b) Schematic diagram of d(CGCG) indicating the fragmentation sites and mass-to-charge ratio values associated with different bond cleavages.

cleotides and to investigate instrumental restrictions and possible solutions for trapping large ions generated by the matrix-assisted LD technique.

Conclusions

Matrix-assisted UV LD FTMS can be used to characterize normal and modified oligonucleotide dimers, trimers, tetramers, and hexamers. The experimental conditions employed 266 nm LD with a nicotinic acid matrix, which was required for the production of $(M - H)^-$ ions for these oligomers. This ionization procedure revealed $(M - H)^-$ ions as well as fragment ions that provided detailed structural information for the oligomers. The capabilities of FTMS for accurate mass measurements, high resolution measurements, and multistage mass spectrometry (for example, CID) were exploited to investigate the ions thoroughly. In general, the predominant fragment ions observed for these compounds were due to phosphate ester bond cleavage with the charge retained on the 3′ end of the oligomer and provided isomeric differentiation. For oligonucleotides larger than trimers, many different types of fragment ions were observed and provided detailed structural information for determining sequences and distinguishing isomers. In addition to sequence information, the presence and location of modifications to the oligonucleotides, such as methyl and ethyl alkyl groups, could also be identified. The lack of observation of ions for oligonucleotides larger than hexamers suggests that current FTMS ion detection and/or ion trapping parameters need to be examined in detail. Further work is in progress to investigate the funda-

Table 2. Oligonucleotide tetramer sequence ions obtained by 266 nm laser desorption

Tetramer	$(M - H)^-$	$(M - H - base)^-$	Sequence ions m/z (relative abundance) Y	X
d(CGCG)	1173	$(M - H - GH)^-$	964 (15)	924 (3)
			635 (100)	635 (100)
			346 (13))	306 (3)
d(GCGC)	1173	$(M - H - CH)^-$	924 (4)	964 (6)
			635 (23)	635 (23)
			306 (29)	346 (47)
d(CCGG)	1173	$(M - H - GH)^-$	964 (10)	924 (3)
			675 (100)	595 (33)
			346 (69)	306 (15)
d(GGCC)	1173	$(M - H - CH)^-$	924 (3)	964 (3)
			595 (37)	675 (32)
			306 (48)	346 (33)
d(TGCA)	1172		948 (4)	939 (—)
			619 (26)	650 (4)
			330 (89)	321 (30)
d(TG*CA)	1186		962 (1)	953 (—)
			619 (7)	663 (2)
			330 (8)	321 (2)

G* = O^6 – methyl guanine.

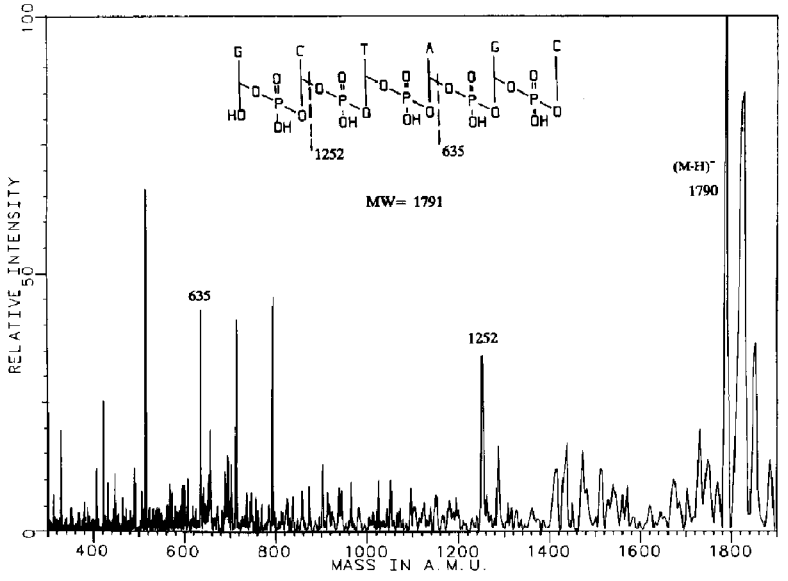


Figure 6. Negative ion matrix-assisted LD-FTMS spectrum of d(5′ – GCTAGC – 3′). The peaks at $m/z > 1790$ u are due to ammonium cations attached to the phosphate linkages of the original hexamer (oligonucleotides frequently contain ammonium or sodium cations on the ionic phosphate oxygens).

mental nature of the matrix-assisted LD process and to evaluate methods of enhancing the trapping and detection of large ions generated by this ionization technique.

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