Location of Abasic Sites in Oligodeoxynucleotides by Tandem Mass Spectrometry and by a Chemical Cleavage Initiated by an Unusual Reaction of the ODN with MALDI Matrix

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We describe two approaches employing electrospray ionization (ESI) tandem mass spectrometry (MS/MS) and matrix assisted laser desorption/ionization (MALDI) post-source decay (PSD) for determining the location of an abasic site in modified oligodeoxynucleotides (ODNs). With MS/MS, we found both complementary fragment ions (a_n' and w_n') produced at the abasic site were predominant in the mass spectra and allowed the location to be determined. Under MALDI conditions, most ODNs carrying an abasic site are singly charged, and PSD gives predominately w_n' ions at the abasic sites, revealing their location. We also describe another approach for identifying and locating abasic sites in model ODNs; namely, an "in situ" derivatization coupled with MALDI mass spectrometry (MS). In general, an ODN n-mer containing an abasic site at the m-th position from the 5'-terminus can react with the matrix component, anthranilic acid, to form a Schiff base. The adduct upon MALDI breaks into 3' and 5' fragments (w_{n-m}, b_m, a_m, d_{m-1}) at the abasic site, revealing its location. ESI MS methods are also applicable for detecting the hydrazone derivatives of abasic sites, and the fragmentation of hydrazones shows the location of the abasic site. (J Am Soc Mass Spectrom 2002, 13, 1418–1426) © 2002 American Society for Mass Spectrometry

Abasic sites in DNA and oligodeoxynucleotides (ODNs) result from the cleavage of the N-glycosidic bond of a nucleoside. Most methods of detection are based on the propensity of the aldehyde function of the deoxyribose at the abasic site to undergo strand breakage under alkaline conditions or condensation with various nucleophiles, as was recently reviewed [1]. Thus far, mass spectrometry has played a minor role in locating abasic sites. Two recent reports show that the use of enzymes to release small oligodeoxynucleotides followed by simple mass measurement [2, 3] can be an effective approach to locating abasic sites. Others used enzymes to produce abasic sites and made use of the ease of alkaline cleavage at those sites to produce small ODNs, which can be mass-measured by matrix-assisted laser desorption ionization (MALDI) mass spectrometry [4]. Another recent development of a physical method for detecting abasic sites on DNA is atomic force microscopy [5]. Despite these advances, there remains a need to develop alternative methods that make fuller use of the capabilities of mass spectrometry and that can be used for ODNs either taken as models in studies of DNA damage or as fragments released from damaged DNA.

One approach would be to implement tandem mass spectrometry, which is now emerging as an effective tool for structural analysis and quantification of modified ODNs [6–16]. Vouros and co-workers [11], in an early example, showed that the combination of electrospray ionization (ESI) coupled with tandem mass spectrometry (MS/MS) can determine the molecular mass and the sequence of short, modified ODNs. That group later investigated the chemo selectivity of a carcinogenic diol metabolite on reaction in vitro with an ODN dodecamer [12]. We recently demonstrated that ESI MS/MS and MALDI PSD are successful strategies to distinguish both normal and UV photo-damaged ODNs containing 4–8 nucleotides [13, 14]. Vouros and co-workers [15] also reported a similar method to identify and locate an aflatoxin B_1 modified guanine in three isomeric ODN 9-mers. The latter work of Vouros [15] and that of Sheil's groups [16] demonstrated the simple and informative decomposition reactions of abasic sites produced after loss of a base in a mass spectrometric fragmentation. To our knowledge, no one has systematically studied the fragmentation of ODNs bearing

Published online October 24, 2002

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1044-0305/02/$20.00
PII S1044-0305(02)00701-8

Received June 30, 2002
Revised August 30, 2002
Accepted September 2, 2002

"natural" abasic sites (i.e., sites in which the nucleobase reacts in solution and is replaced with an OH group).

We continue to be motivated to develop such methods because abasic sites are biologically important lesions [17, 18]. They play key roles as intermediates in DNA repair of damaged or incorrect bases. For example, one of our interests is the hypothesis that estrogen metabolites react with DNA to give depurinating adducts, forming abasic sites that lead to mutations and ultimately to breast cancer [18, 19]. We report here the use of tandem mass spectrometry to understand the fragmentation of ODNs containing authentic abasic sites under low energy collision-induced dissociation (CID) and MALDI PSD conditions. We also describe another approach in which MS/MS and an unusual chemical derivatization using the MALDI matrix prove to be useful for determining the structure of ODNs that possess abasic sites.

Experimental

Materials

Water was pretreated with a Milli-Q (Millipore, Bedford, MA) ultra pure water filtration system before its use as solvent. The matrix materials, 3-methoxy-4-hydroxycinnamic acid, anthranilic acid (AA), and nicotinic acid (NA) were obtained from Aldrich Chemical Co. (Milwaukee, WI) and purified by recrystallization from water. Decolorizing charcoal was used to remove impurities during the recrystallization. The 6-aza-2-thiothymine (6-ATT), 2',4',6'-trihydroxyacetophnone (2,4,6-THAP), 2',3',4'-trihydroxyacetophnone (2,3,4-THAP), and ammonium citrate were also obtained from Aldrich Chemical Co. and used without further purification. To minimize production of metal-ion adducts, a few beads of cation-exchange resin in the NH_4^+ form were usually added to the oligonucleotide analyte prior to the mass spectrometric analysis. They were prepared from chromatography beads (AG50W-X8, 100–200 mesh; Bio-Ad, Melville, NY) in the H^+ form by using a literature procedure [20]. The *Escherichia coli* uracil DNA glycosylases (UDG) was obtained from Life Technologies Inc. (Rockville, MD).

The ODNs in Table 1 (X = uracil) and those used as standards for MALDI calibration, [d(T5), d(T10), d(T18)] were synthesized at the Nucleic Acid Chemistry Laboratory, Washington University School of Medicine.

The modified ODNs (Table 1, X = abasic site) were prepared by treating the unmodified ODNs (Table 1, where X is uracil) with uracil DNA glycosylase (UDG) to remove uracil, leaving behind an abasic site [21, 22]. For T3U (Table 1, X = abasic site), 15 μL of 5'-d(TTUTTT) (0.65 μg/μL) was mixed with 2 μL of 10× UDG buffer (50 mM Tris, pH 7.5, 50 mM NaCl, 5 mM EDTA). *Escherichia coli* UDG (3 units) was then added, and the incubation continued at 37 °C for 45 min. Following treatment with UDG, the modified ODN was purified and desalted by ethanol precipitation. The purified ODN was then resuspended in ultra pure water to give a final concentration of 40 μM. All the other modified ODNs (Table 1) were prepared by the same method.

Table 1. Sequences and abbreviations of ODNs used in this study

Oligo Name	Sequence
T3X[a]	5'-d(TTX TTT)
T4X	5'-d(TTT XTT TT)
T5X	5'-d(TTT TXT TT)
T6X	5'-d(TTT TTX TTT TTT)
TC3X	5'-d(TCX TCT)
CT4X	5'-d(CTC XTC TT)
CC5X	5'-d(CCT CXT CT)
TC6X	5'-d(TCC TCX TCT TCC)
T2X5U	5'-d(TXT TUT)
CT3X5U	5'-d(TCX TUC)
T49X	5'-d(TTT XTT TTX TTT)
CT9X1	5'-d(CTT TCC TCX TCT TCC CTT)
CT9X2	5'-d(CTC CTT TCX TCT TCC CTT)

[a]X is either uracil or an abasic site.

The 2,4-dinitrophenyl hydrazone (2,4-DNP) derivatives of the abasic ODNs were prepared by a modified procedure. ODNs containing an abasic site (5 μL, 40 pmol/μL) were mixed with 5 μL 2,4-DNP (0.2 M) and reacted at room temperature for 5 min. The solvent was then evaporated in vacuum, and the modified ODN was resuspended in 20 μL of ultra pure water. The 4-nitrophenyl hydrazone (4-NP) and anthranilic acid derivatives of the abasic ODNs were prepared by similar procedures, except that the AA derivative was resuspended in 20 μL 95:5 (vol:vol) ethanol/H_2O. For MALDI analysis, abasic ODNs were simply mixed with the AA/NA matrix, and the Schiff bases were formed directly as the solvent evaporated.

Instrumentation

ESI MS/MS experiments were performed on a Finnigan LCQ ion-trap mass spectrometer (Finnigan MAT, San Jose, CA), operated in the negative-ion mode. A 5-μL aliquot of a 10-μM solution of the ODNs was injected for each run. The carrier solution (|:|CH_3CN/H_2O/0.1% formic acid) was infused at a flow rate of 5 μL/min into the electrospray source, to which was applied a voltage of 4.0 kV. The capillary temperature was 100 °C. The vacuum chamber was operated at a pressure of 1 mtorr with helium. For MS/MS experiments, the collision energy was adjusted so that the relative intensity of the peak corresponding to the precursor ion was reduced to 10–20% of its intensity in the absence of collisional activation. Helium was used as the collision gas for the ESI MS/MS experiments, and the collision energy was 20–40% of the maximum tickling voltage (maximum amplitude of 5 V).

MALDI-TOF spectra were acquired on a Voyager-DE RP (PerSeptive Biosystems, Framingham, MA) mass spectrometer equipped a 1.2-m flight tube and a

nitrogen laser (337 nm). MALDI mass spectra were obtained in the negative-ion mode at an accelerating voltage of 25 kV. The pulse delay time was 100 ns. For molecular weight determination, the matrix solution was either saturated 6-ATT [23] or a 2:1:2 molar mixture of 2,4,6-THAP, 2,3,4-THAP, and ammonium citrate (2,4,6-THAP: 0.05 M) [24]. For MALDI PSD experiments, 3-methoxy- 4-hyroxycinnamic acid (as a saturated solution) was used as matrix. Samples were prepared by mixing 1 μL of analyte and 2 μL of matrix solution on the MALDI plate and air-drying the spot. A molecular weight determination required approximately 40 laser shots. Solutions of 0.5 μL of 1 μM d(T5), d(T10), d(T18), or d(T20) were used to calibrate the *m/z* scale of the mass spectrometer. MALDI PSD spectra were obtained using a higher laser power and averaged by accumulating data from 100–200 laser shots. The raw data were acquired with a Tektronix 520A digital oscilloscope and processed with GRAMS 386 software (Thermo Galactic Industries, Salem, NH), which was provided with the mass spectrometer computer system.

Results and Discussion

Molecular Weight and Stability Determination

Introduction of an authentic abasic site into a synthetic ODN can be challenging because the abasic site is unstable under certain conditions. For this reason, various synthetic models such as cyclic tetrahydrofuran, propanediol, and deoxynitribitol are used to mimic the abasic site [25, 26]. Although these analogs are stable and easier to handle, they are not models for studying the reactivity of a "natural" abasic site. To determine the sequence of ODNs that possess such sites, we designed two series of T-rich and CT-rich ODNs (Table 1) which contain deoxyuracil at different positions (X = uracil). We chose pyrimidine-rich ODNs because our long-range goal is to introduce limited numbers of purines into model ODNs and use them as reaction centers for carcinogens. The chosen ODNs were treated with uracil DNA glycosylase (UDG), which selectively excises uracil from DNA by hydrolyzing the N-glycosidic bonds between uracil and the sugar moiety, leaving behind an abasic site in which an OH replaces the nucleobase [21, 22].

We assessed the purity of the abasic ODNs (Table 1, X = abasic site) by using both MALDI and ESI mass spectrometry in the negative-ion mode. The results for 5'-d(CTCXTCTT) (CT4X, X = abasic site), obtained by using 2:1:2 molar mixture of 2,4,6-THAP, 2,3,4-THAP, and ammonium citrate as the matrix are typical. The simple spectrum (not presented) shows a peak at *m/z*2218 (expected *m/z* is 2217) for the deprotonated ODN containing an abasic site, a doubly deprotonated molecule at *m/z* 1108 (15% RA), and a fragment ion (7% RA) of *m/z* 1219 (a w_4 fragment according to the nomenclature of McLuckey et al. [27]). The production of the w_4 fragment, formed by cleavage at the abasic site, is consistent with the idea that DNA is weakened at that site. The gentler ESI introduction does not produce a detectable w_4 ion. By using both MALDI and ESI, we confirmed the molecular weight (to within $\pm$0.5 u for ESI and $\pm$1.0 u for MALDI) for all the ODNs containing an abasic site (Table 1). We found the abasic species to be relatively stable for these experiments, in agreement with Shishkina and Johnson [28].

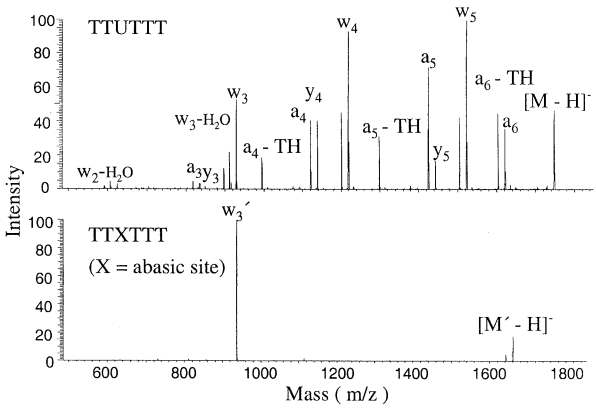


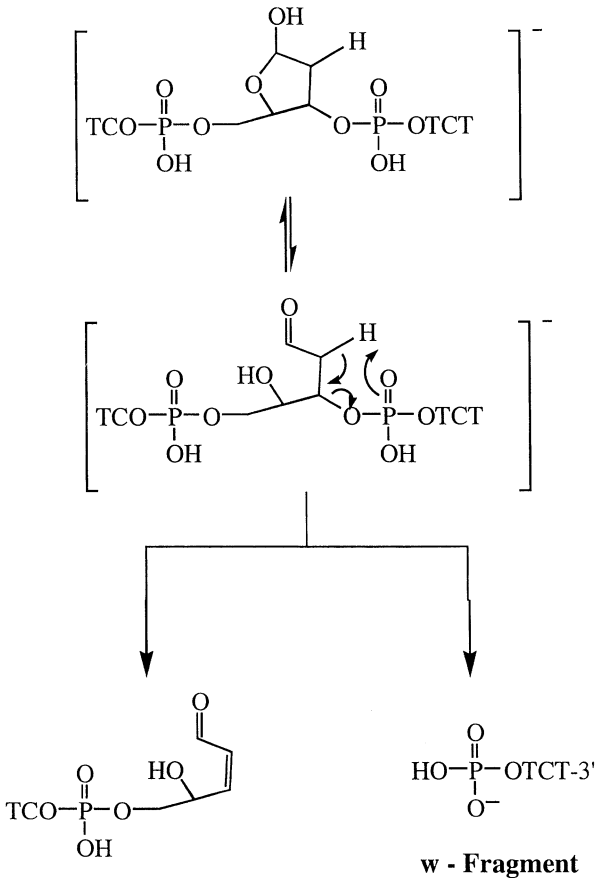
Figure 1. Product-ion spectra (MS/MS) of ESI-produced ions from T3X (Table 1) without (top) and with (bottom) an abasic site.

Location of the Abasic Sites in Model ODNs by ESI MS/MS

T-rich ODNs. We chose a series of T-rich ODNs as a first step to study the differences in the product-ion spectra (MS/MS) of ESI-produced ions of normal and abasic ODNs. ESI of the unmodified T3X (Table 1, X = uracil) produced an abundant molecular ion at *m/z* 1747 (spectrum not shown). Collision-induced decomposition of the ESI-produced $[M - H]^-$ precursor causes, expectedly, extensive decomposition, leading to a complicated product-ion spectrum (Figure 1). The product-ion spectrum exhibits w, a, [a − base], y, and [w − H_2O] (or x) anion series.

The product-ion spectrum of the $[M - H]^-$ from T3X (Table 1, X = abasic site) is considerably simpler than that of the corresponding unmodified ODN, as was noted for abasic sites produced in mass spectrometric fragmentation [15, 16]. The precursor containing the abasic site gives only the w_3' ion at the abasic site, revealing its location (Figure 1) (we use the prime notation for the fragments as a convenience to distinguish the abasic ODNs). This observation is also in accord with the fragmentation mechanism we recently proposed for the formation of [a_n − base] and w ions [29, 30]. That is, the presence of an abasic site obviates the base-loss step that initiates the fragmentation of *unmodified* ODNs and apparently reduces the activation energy (Scheme **1**). We did not investigate the mechanism further because it is likely to be analogous to that for formation of [a_n − base] and w ions in normal ODNs.

To test the method further, we designed two iso-



Scheme 1. Fragmentation mechanism for the formation of w-ions.

meric ODN 8-mers, T4X and T5X, that contain abasic sites at the fourth and fifth position from the 5'-end (Table 1, X = abasic site). The full-scan mass spectra of the two isomers are identical (spectra not shown). The most abundant product ion is of *m/z* 1130, which is doubly deprotonated. The collision-induced dissociation of the *m/z* 1130 ion of the two isomers is distinctive. T4X gives only the w_4' (*m/z* 1233) and a_4' (*m/z* 1027), whereas that of T5X shows exclusively w_3' (*m/z* 929) and a_5' (*m/z* 1331); both sets of fragmentation occur at the abasic site. More confirmation comes from Figure 2, which shows the ESI product-ion spectra of a larger T-rich ODN, T6X (Table 1, X = abasic site). Note again the w_6', a_6', and $[a_6' - H_2O]^-$ that are produced in reactions at the abasic site.

CT-rich ODNs. We also chose to study a series of CT-rich ODNs. For the abasic CT4X (Table 1, X = abasic site), the dominant w_4' and a_4' in the spectrum clearly indicate the abasic position (Figure 3). Similar results were obtained for CC5X, and a larger ODN, TC6X (Figure 4). The top panel of Figure 3 shows the CAD spectrum of the $[M - 2H]^{2-}$ of an unmodified CT4X (Table 1, X = uracil). The prominent ions in the spectrum are the $[M - 2H - CH]^{2-}$ (CH is neutral cytosine) and some members of the w-ion series. The favored w

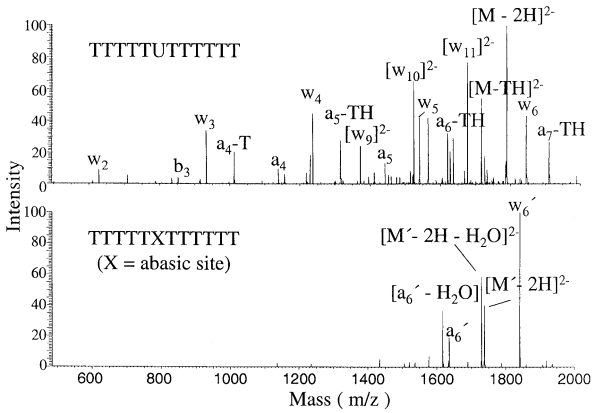


Figure 2. Product-ion spectra (MS/MS) of T6X (Table 1) introduced by ESI. Top spectrum is of the ODN without an abasic site; bottom is of the ODN with an abasic site.

ions are from cleavage of the ODN backbone near the cytosine.

Model ODNs containing two abasic sites. To evaluate whether MS/MS can locate multiple abasic sites in ODNs, we chose T2X5U and CT3X5U (Table 1, X = uracil) as test compounds. We found that UDG only selectively removed the 5'-uracil bases from these ODNs, leaving the 3'-uracil base intact, an observation that is consistent with results in the literature [31–33]. For example, collisional activation of the $[M - H]^-$ (*m/z* 1639) of T2X5U produced only the w_4' of *m/z* 1219, indicating removal of only the second uracil base from the 5'-end.

When we treated T49X (Table 1, X = uracil), containing two mid-chain uracil bases, using the same reaction conditions as those for T2X5U and CT3X5U and submitted the product to ESI analysis, we found the $[M - 2H]^{2-}$ of *m/z* 1684, indicating that UDG treatment nearly quantitatively removed both uracils. For the ESI MS/MS analysis of T49X, the ion of *m/z* 1684 gave four product ions of *m/z* 1676, 1027, 1009, and 929, which are the $[M - H_2O - H]^-$, a_4, $[a_4 - H_2O]^-$, and w_3,

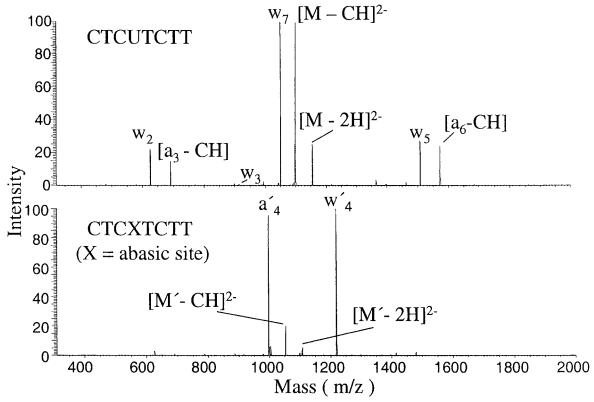


Figure 3. Product-ion spectra of ESI-produced $[M - 2H]^{2-}$ of CT4X (Table 1). Top spectrum is of the ODN without an abasic site; bottom is of the ODN with an abasic site.

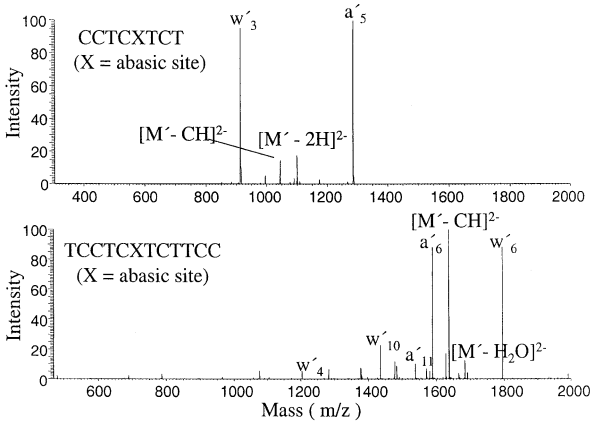


Figure 4. Product-ion spectrum of ESI-produced $[M - 2H]^{2-}$ of CC5X (top) and TC6X (bottom) (Table 1), both of which contain an abasic site.

respectively. The w_3, a_4 and $[a_4 - H_2O]^-$ locate the abasic sites at the fourth and ninth positions from the 5'-end.

Location of the Abasic Sites in Model ODNs by MALDI PSD

T-rich ODNs. We submitted the ODNs containing abasic sites to MALDI PSD so as to compare the results with those from ESI MS/MS. The PSD spectrum of the $[M - H]^-$ of T3X (Table 1, X = abasic site) is similar to that from low-energy collisional activation (Figure 1); the w_3' formed by cleavage at the abasic site is the only detectable product ion (Figure 5). PSD of MALDI-produced $[M - H]^-$ ions from 5'-d(TTUTTT), however, is complicated, showing w, y, a, $[a - base]^-$ series (Figure 5).

The PSD spectra of MALDI-produced [M − H] anion of TC3X, T4X, and T5X (Table 1, X = abasic site) that contain abasic sites also gave simple, yet distinctive fragmentation patterns (spectra not shown): w_3' for TC3X, w_4' (*m/z* 1235) for T4X, and w_3' (*m/z* 930) for T5X. T6X (Table 1, X = abasic site) gave predominant formation of w_6'. That these w_n' are important indicators of abasic sites for T-rich abasic ODNs is made clear by PSD of the unmodified isomeric T4X and T5X (Table 1, X = uracil); these spectra are complicated and similar to that of T3X (X = uracil) (spectra not shown).

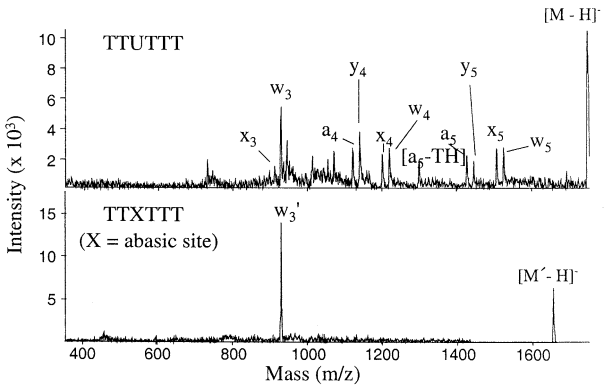


Figure 5. Product-ion spectra (PSD mode) of MALDI-produced ions from T3X (Table 1). The top spectrum is of the ODN without an abasic site; the bottom is of that with an abasic site.

CT-rich ODNs. To evaluate the possibility of identifying abasic sites in CT-rich ODNs by MALDI PSD, we chose again the CT-rich ODNs described previously. For TC3X (Table 1, X = abasic site), the prominent fragment is w_3' (*m/z* 915). For the unmodified TC3X (Table 1, X = uracil), the dominant product ions are w_4, $[a_5 - CH]^-$, where CH is neutral cytosine, and the internal fragment (*m/z* 771) ion.

Two larger CT-rich ODNs, CT4X and CCC5X, in which abasic sites were introduced (Table 1, X = abasic site) showed exclusive formation of w_4' (*m/z* 1219) and w_3' (*m/z* 915), respectively (spectra not shown). PSD of the unmodified CT4X and CC5X (Table 1, X = uracil) gave the expected and distinctive $[a_n - CH]^-$ and w ions from fragmentation near the cytidines. The product ions of *m/z* 771 and 1365 in both spectra and of *m/z* 1653 in that of CC5X are $[pUTC - CH]^-$, $[pTCUTC - CH]^-$, and $[pCTCUTC]^-$, respectively. The formation of internal ions is not yet understood. Other CT-rich ODNs, including, TC6X, T2X5U, and CT3X5U (Table 1, X = uracil), gave similar results.

Abasic Sites in ODNs React with MALDI Matrix

We reasoned that the reactivity of an aldehyde group of the abasic site with primary amines to give a Schiff base [34, 35] might also be the basis for a MALDI MS method. For example, anthranilic acid (AA) should react with the open-chain aldehyde at an abasic site (Table 1). We chose this matrix material because, when mixed with nicotinic acid and diammonium citrate (2:1:0.003), it gives an effective matrix for the MALDI of ODNs [36]. The MALDI mass spectrum of T3X (X = abasic site) in this matrix showed an intense peak with a 119 Da increase in mass relative to the T3X molecular ion peak (Figure 6). This peak was assigned to be the anthranilic-acid derivative (Schiff base) of T3X (Scheme **2**). The removal of water during MALDI sample spot preparation shifted the equilibrium to the right (step 2, Scheme **2**), favoring conversion to product.

The presence of a Schiff base promotes a series of facile fragmentations at the abasic site upon MALDI analysis. The most abundant fragment ion is w_3 (*m/z* 929) (Figure 6). The complementary a_3 (*m/z* 842) is also seen but at much lower abundance (Figure 6). We propose (Scheme **2**) a β-elimination mechanism for generating w_3 and a_3 [37]. The adjacent phosphate group deprotonates C2 of the abasic deoxyribose, and subsequent electron-pair movement cleaves the 3-phosphodiester bond to give w_3 and a_3. The a_3 can also undergo proton transfer to give intermediate **2** and then fragment to d_2 (*m/z* 625) by a δ-elimination mechanism (Scheme **2**). The δ-elimination, which can only occur

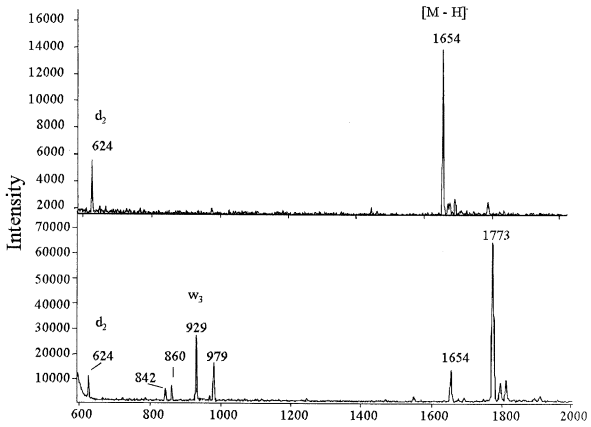


Figure 6. MALDI mass spectra of T3X (Table 1, X = abasic site) by using as matrices: 6-aza-2-thiothymine (top) and a 2:1:0.003 molar ratio anthranilic acid, nicotinic acid, and diammonium citrate (bottom).

following the β-elimination, is referred to as a β,δ-elimination [37]. The peak at *m/z* 860 represents a b_3 ion from T3X (Figure 6). We propose that the *m/z* 979 peak represents a double derivative involving both a Schiff base and an ester (e_3, Scheme **2**). These new products, hereafter denoted as e-ions in the spectrum, may also serve as indicators of abasic sites in modified ODNs. We do not yet know the mechanism for formation of the e-ion. One possibility is an S_N2 displacement of the phosphate by the AA. This displacement is made possible by the nearby Schiff's base. Another possibility is by a strand break to cleave the phosphodiester bond, followed by a 1,2-addition of the AA to the double bond conjugated to the Schiff's base.

To establish more convincingly the generality of the method, we selected T4X and T5X (Table 1, X = abasic site), which are isomeric ODNs containing abasic sites at different positions. Both the MALDI mass spectra of T4X and T5X revealed an ion at *m/z* 2381, which represents the Schiff's base with the matrix AA, confirming the existence of the abasic sites (spectra not shown). The fragment ions promoted by the Schiff's base from T4X and T5X are quite distinct (Table 2). The most abundant fragments of both ODNs are w' ions. The w_4' from T4X clearly indicates that the abasic site is located at the fourth position from the 5'-terminus, whereas w_3' from T5X indicates that the abasic site is at the fifth position. We also detected other ions, including a, b, d, e (Table 2), but they were of generally lower abundance.

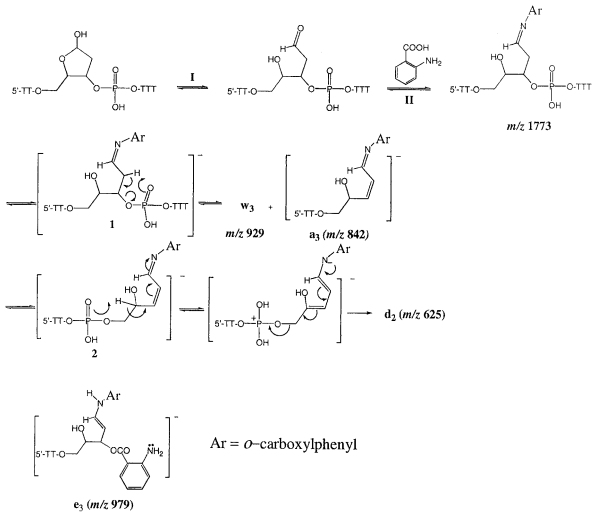
We also chose to use a series of CT-rich abasic ODNs in our studies (Table 1) because these single-stranded ODNs containing one or a small number of purines should also be useful models in research. The mass spectrum of CT4X (Table 1, X = abasic site) shows that ions of *m/z* 2217 and 2336, which represent the molecular ions of CT4X and its Schiff-base adduct with the matrix AA, respectively. The peaks observed in the lower mass range of the spectrum represent fragment ions generated at the abasic site. The nature of the fragments is consistent with the fragmentation scheme we proposed in Scheme **2**. The fragment ions, especially w_4 (*m/z* 1219), frame the abasic site in CT4X (Figure 7). Neither these fragment ions nor the Schiff base-matrix adduct were formed when another matrix, THAP, was used.

To examine whether the proposed fragmentation applies to larger CT-rich ODNs, we investigated two isomeric 18-mers, namely CT9X1 and CT9X2 (Table 1, X = abasic site). The MALDI spectrum of CT9X1 exhibits two peaks at *m/z* 5185 and 5303, representing the $[M - H]^-$ of the unreacted ODN and its derivative with the matrix AA, respectively (Figure 8). To calibrate the mass scale, d(T10) (giving an *m/z* 2979 ion) was added as an internal standard. The peak for the ODN CT9X1 (Table 1, X = uracil) that had not reacted with the UDG was also seen at *m/z* 5278, indicating incomplete enzyme reaction. The fragment ions induced by the Schiff-base derivative are e_9 at *m/z* 2745, w_9 at *m/z* 2694, b_9 at *m/z* 2625, and d_8 at *m/z* 2390 (Figure 8). These ions clearly indicate the location of the abasic site in CT9X1. We also found that CT9X2 gave the same fragment ions as CT9X1 because they are isomers and have the abasic site at the same position in their sequence. Not surprisingly, the relative abundances of the fragment ions from CT9X1 and CT9X2 are very similar (data not shown).

Similar results were obtained for all the other abasic ODNs in Table 1, and the MALDI MS data are summarized in Table 2.

Assay of Schiff-Base and Other Derivatives of Abasic ODNs by ESI MS

To evaluate whether ESI MS can also be used to characterize the Schiff-base derivatives of abasic ODNs, we chose a 12-mer ODN, TC6X (Table 1) as an example.



Scheme 2. Fragmentation scheme for the formation of w-, a-, and d-ions.

Table 2. The fragment ions produced upon activation of the $[M - H]^-$ ions of Schiff-base derivatives of various ODNs

ODNs	$[M - H]^-$	AA derivative	w_{n-m}	b_m	a_m	D_{m-1}	e_m
T3X	1654	1773	w_3 (929)	b_3 (860)	a_3 (842)	d_2 (625)	e_3 (979)
T4X	2262	2381	w_4 (1233)	b_4 (1165)	a_4 (1147)	d_3 (927)	e_4 (1283)
T5X	2262	2381	w_3 (929)	b_5 (1468)	a_5 (1450)	d_4 (1233)	e_5 (1588)
T6X	3479	3598	w_6 (1841)	b_4 (1773)	a_4 (1756)	d_5 (1537)	e_6 (1892)
TC3X	1624	1743	w_3 (915)	b_3 (846)	a_3 (828)	d_2 (611)	e_3 (965)
CT4X	2217	2336	w_4 (1219)	b_4 (1135)	a_4 (1117)	d_3 (900)	e_4 (1254)
CC5X	2202	2321	w_3 (915)	b_5 (1425)	a_5 (1407)	d_4 (1189)	e_5 (1543)
TC6X	3388	3507	w_6 (1797)	b_6 (1728)	a_6 (1710)	d_5 (1493)	e_6 (1847)
CT9X1	5185	5303	w_9 (2694)	b_9 (2625)	a_9 (2608)	d_8 (2390)	e_9 (2744)
CT9X2	5185	5303	w_9 (2694)	b_9 (2625)	a_9 (2608)	d_8 (2390)	e_9 (2744)

ESI of the unmodified TC6X (Table 1, X = uracil) produced a molecular ion, $[M - H]^-$, at *m/z* 3389. Treatment of TC6X with AA, as described in the experimental section, followed by analysis by ESI MS showed surprisingly that less than 10% of the original TC6X was detected as a Schiff-base adduct (spectrum not shown). This observation suggests that the derivative readily undergoes hydrolysis in the ESI carrier solution 1:1 (vol:vol) acetonitrile/water with 0.1% formic acid. Therefore, we adjusted the carrier solution by decreasing the amount of water. When we used 95:5 (vol:vol) acetonitrile/water as the carrier solution, the peaks representing $[M - H]^-$ and the $[M - 2H + Na]^-$ for the Schiff's base (*m/z* 3508 and 3530) with the AA matrix were of 100% and 30%, respectively, and that for the starting ODN dropped to 30% RA. There were no low mass fragment ions, suggesting that the Schiff-base adduct remains intact during ionization, in accord with the more gentle nature of ESI with respect to MALDI.

Aldehydes also react with hydrazines to give analogous adducts. For example, 2,4-dinitrophenylhydrazine is a common reagent used to prepare the corresponding 2,4-DNP derivatives of aldehydes. Unlike Schiff-base derivatives, the products of these reactions are generally quite stable. Thus, 2,4-DNP should also react with the abasic site of the TC6X (Table 1, X = abasic site) to form a hydrazone. Indeed, in the ESI mass spectrum of the unreacted ODN, the base peak corresponded to the $[M - H]^-$ of this material, whereas after reaction, a new ion of *m/z* 3569 representing the hydrazone derivative of TC6X was most abundant and that of the unreacted ODN was approximately 20% RA. There was no significant difference between the relative intensities of the *m/z* 3389 and *m/z* 3569 peaks when different ratios of acetonitrile/water were used as the carrier solution. This is consistent with the higher stability of the 2,4-DNP derivatives of abasic ODNs compared to Schiff bases. Similar results were obtained when 4-nitrophenylhydrazine was used as the derivatizing reagent.

Conclusions

Both ESI MS/MS and MALDI PSD produce appropriate information that locates the abasic sites in single-stranded ODNs. Under ESI-MS/MS conditions, the collision of the doubly charged ODNs containing an abasic site yields complementary a_n' and w_n' ions from fragmentation at those sites. Under MALDI conditions, most ODNs carrying an abasic site are singly charged, and PSD gives predominately the w' ions.

ESI MS/MS can also identify two abasic sites in ODNs. The MALDI PSD approach, however, is unable to do this because ODNs containing two abasic sites decompose during the longer ion flight through the ion reflector required for PSD. Another limitation is that the activation of large ODNs is not effective. One means to overcome this problem is to use an enzyme-generated ladder and MALDI, a method we recently demonstrated [4].

Other problems, such as unusual analyte/matrix

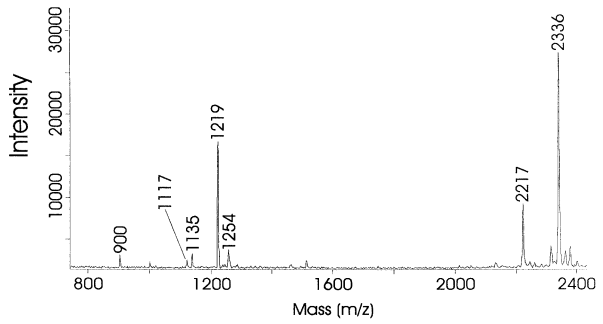


Figure 7. MALDI mass spectra of CT4X (Table 1, X = abasic site) by using as matrix a 2:1:0.003 molar ratio anthranilic acid, nicotinic acid, and diammonium citrate.

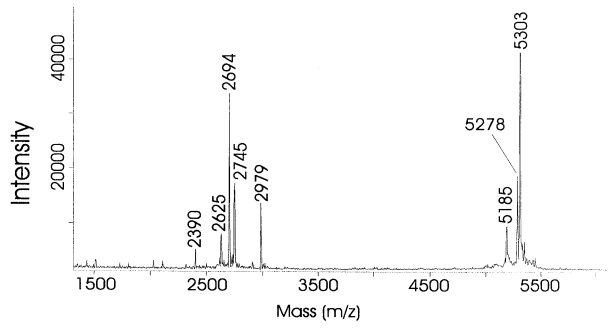


Figure 8. MALDI mass spectra of CT9X1 (Table 1, X = abasic site) obtained by using as matrix a 2:1:0.003 molar ratio anthranilic acid, nicotinic acid, and diammonium citrate.

reactions, can be turned to advantage to yield another method whereby the abasic site is derivatized in situ during preparation of the MALDI sample. This "in situ" derivatization coupled with MALDI MS is a rapid and specific approach for identifying and locating abasic sites in model ODNs. In general, an ODN n-mer containing an abasic site at the m-th position from the 5'-terminus can react with an appropriate matrix component to form a Schiff base. The adduct will fragment to give $w_{n-m'}$ $b_{m'}$ $a_{m'}$ $d_{m-1'}$ and e_m upon MALDI, and the $w_{n-m'}$ ion will probably be most abundant. One may readily interpret these fragment ions, especially $w_{n-m'}$ to establish the location of an abasic site.

Acknowledgments

The research reported in this article was supported by the NIH (grant no. P01CA49210) and by the National Centers for Research Resources of the NIH (grant no. P41RR00954).

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