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Tailoring the gas-phase dissociation and determining the relative energy of activation for dissociation of 7-deaza purine modified oligonucleotides containing a repeating motif

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Abstract

7-Deaza purine modified oligonucleotides with a repeating motif have been sequenced by slow heating methods illustrating the ability to alter the preferred unimolecular decomposition pathway. The modified oligonucleotides limited the number of product ions per repeat unit resulting in an increased signal-to-noise ratio for the tandem mass spectrometry data. Loss of 7-deaza purine nucleobases or 7-deaza purine related product ions were not observed. The results illustrate the importance of the N7 position of purine nucleobases for low energy gas-phase decomposition. FRAGMENT results showed that for the 3^- charge state of the 16-mer oligonucleotides, 5′-$(AATG)_4$-3′ and 5′-$(c^7Ac^7ATG)_4$-3′, that the 7-deaza deoxyadenosine modified sequence had a 38% greater relative energy of activation for unimolecular decomposition when compared to the unmodified sequence. For large DNA molecules that have purine-rich repeat units, the multiple sequence sites per repeat can result in a substantial loss of sequence information over large areas due to a reduced signal-to-noise ratio of the product ion spectrum. The incorporation of 7-deaza purines can limit the number of product ions during gas phase sequencing while ensuring sufficient sequence coverage to localize mutations or polymorphisms housed within a specific repeat unit. (Int J Mass Spectrom 219 (2002) 139–150)

Keywords: Genetic analysis; Electrospray ionization; Gas-phase dissociation

1. Introduction

DNA sequencing continues to be a central theme of genetic research as recently emphasized by the rough draft sequencing of the human genome [1,2]. Consequently, a resequencing era is fast approaching where genetic analysis, such as gene mapping, will become increasingly important. Electrospray ionization (ESI) coupled to mass spectrometry [3] has the potential to become a new strategy for several areas of genetic analysis that include gene mapping, characterization of complex genetic diseases, HLA typing, and human identification in cases of disaster, paternity suits and forensic science.

The ability of ESI to transfer large molecules intact into the gas phase as multiply charged ions permits the mass spectral inquiry of relatively large segments of DNA (e.g., polymerase chain reaction (PCR) products [4] amplified from genomic DNA [5–9]). An important class of DNA sequences highly amenable to ESI-MS characterization are short tandem repeats (STRs). STRs are highly polymorphic

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repeating sequences of DNA that have traditionally been used for gene mapping [10–12] and identification [13] purposes, but recent research has linked loss of heterozygosity in STRs to specific cancers [14,15].

The repeat pattern of STRs typically consists of a 2–7 nucleotide repeat unit that is duplicated 4–20 times. The identification of STRs with multiple repeat units, incomplete units, and single nucleotide polymorphisms (SNPs) has resulted in the classification of STRs into six main categories: (1) simple, (2) simple with non-consensus alleles, (3) compound, (4) compound with nonconsensus alleles, (5) complex, and (6) hypervariable, which can house several repeat units with intervening SNPs [16]. The current methodology for STR identification relies on laborious electrophoretic analysis of PCR products since the problem of nonspecific annealing, due to the repetitive nature of the sequence, has proved troublesome for DNA chip analysis except under extremely stringent conditions [17]. STR genotyping by ESI-MS has recently been shown [18,19] and may prove to be an effective alternative in a high throughput manner [20,21].

Another class of repetitive DNA amenable to ESI-MS classification is that associated with trinucleotide repeat diseases (e.g., Huntington's disease, Machado–Joseph disease) [22], which like STRs are comprised of repeat units that are duplicated numerous times. These neurodegenerative diseases are signified by dynamic instabilities that culminate in an increased number of repeats when passed from parent to offspring. The overall length of the repeating sequence has been used to verify the disease and also as an indicator for the age of onset and the severity of conditions [22].

Polymorphic base-pair substitutions, deletions, and insertions within the trinucleotide repeats have been implicated in mechanisms that stabilize the expansion of trinucleotide repeats [23]. Determination of the exact location and type of polymorphism within the repeat sequence could be instrumental in determining the mechanism of stabilization. For characterization by mass spectrometry, such determinations must be accomplished by gas-phase sequencing of PCR products using multistage mass spectrometry (MS/MS). Likewise several complex and hypervariable STRs will require MS/MS to accurately determine alleles for genotyping.

A critical issue that needs to be addressed when sequencing relatively large DNA in the gas-phase is the difficulty associated with purine-rich sequences. Purine-rich strands can be problematic during low-energy excitation MS/MS because the relatively large proton affinities of purines, as shown for nucleosides [24] and mononucleotides [25], can result in excessive neutral base loss [26,27] or multiple dissociation pathways that equate to a poor signal-to-noise (S/N) in the MS/MS spectrum. For PCR products derived from STR loci, a poor S/N could leave large segments of the oligonucleotide unsequenced due to product ions peak intensities that fall below the mass spectral noise level.

Herein, 7-deaza purine analogs are used to reduce the number of fragmentation pathways by channeling dissociation during the gas-phase sequencing of oligonucleotides using an ESI-Fourier transform ion cyclotron resonance mass spectrometer [28] (FT-ICR-MS) with sustained off resonance irradiation (SORI) [29] and infrared multiphoton dissociation (IRMPD) [30]. Modified nucleotides have previously been shown to alter the fragmentation pathways under low energy conditions [31–35]. Tang et al. used modified 2′-fluorocytidine analogs to stabilize oligonucleotides [31] as did Griffey et al. with 2′-*O*-methylation [34]. 7-Deaza purines were originally used in gel electrophoresis to reduce compression bands during DNA analysis [36]. Later, the modified purines prevented depurination (i.e., loss of the nucleobase without subsequent sequence ion formation) during desorption and ionization in matrix-assisted laser desorption ionization mass spectrometry [37–39].

Results presented here show that 7-deaza purines alter the threshold of dissociation at the modified sites and eliminate cleavage of the N-glycosidic bond during the slow heating methods of SORI and IRMPD. This allows a method to direct the fragmentation pathway and limit the number of product ions per repeat, thus, increasing the S/N in the MS/MS spectrum.

Additionally, these experimental results emphasize the critical role of the N7 site of purines in cleavage of the N-glycosidic bond and subsequent sequence ion formation. The ability of 7-deaza purines to induce desired changes in the gas-phase sequencing using ESI-MS will be essential for their use in locating polymorphisms within repeating DNA sequences. Also important is the compatibility of 7-deaza purines with the PCR [40,41], an essential step in STR characterization, for which we have recently documented [21].

2. Experimental

All spectra were obtained in the negative-ion mode with a modified ESI-FT-ICR mass spectrometer (IonSpec, Irvine, CA) using a 4.7 Tesla superconducting magnet (Cryomagnetics, Inc., Oak Ridge, TN) with a 128 mm bore. The magnet has a ±0.001% central field homogeneity over a cylindrical region 2-in. in diameter and 4 in. in length. The ESI source (Analytica of Branford, Inc., Branford, CT) was modified to accept a heated metal capillary [42] and our dual micro-electrospray source [43–46]. The ESI emitter tips are pulled from 50 μm i.d. fused-silica capillary (Polymicro Technologies, Inc., Pheonix, AZ) in the flame of a Bunsen burn to ca. 10 μm or less [47]. The dual emitters are remotely coupled to the ESI potential and precisely positioned by a spring-loaded XYZ micro-manipulator (Newport 460A series) and the analytes are infused at a flow rate of 3.3 nL/s. Ions were externally accumulated in the hexapole for 2 s prior to being injected and trapped in a 2.38-in. i.d. cylindrical cell centered in the homogeneous region of the magnet. The broadband spectra (512-k data points) were acquired at a sampling rate of 500 kHz using an analog-to-digital conversion (ADC) with 12 bits of accuracy. Mass resolving power of the product ion spectra were approximately 30,000 (FWHM).

Fragmentation of the oligonucleotides was accomplished by IRMPD or SORI using nitrogen as a collision gas. All SORI experiments were conducted using a 1000 Hz below resonance excitation waveform unless otherwise noted using nitrogen as the collisional target. The IRMPD experiments used a continuous, 25-W carbon dioxide laser (Synrad Inc., Mukilteo, WA) with the output power verified by a Power Wizard power meter (Synrad Inc.). Laser irradiation power and times for the 4^- charge state of the 5′-$(AATG)_4$-3′ repeat ranged from 2 W (1–6 s), 3 W (0.25–1 s), 4 W (0.25–1 s), 5 W (0.1–0.4 s) and 6 W (0.1–0.4 s). For the 4^- charge state of the 5′-$(c^7Ac^7ATG)_4$-3′, the parameters were 2 W (1–6 s), 3 W (1–2 s), 4 W (0.5–1.5 s), 5 W (0.25–1 s) and 6 W (0.15–0.3 s).

Focused radiation for gaseous multiphoton energy transfer (FRAGMENT) [48,49] experiments were conducted on the 3^- charge state of the 5′-$(AATG)_4$-3′ and 5′-$(c^7Ac^7ATG)_4$-3′ oligonucleotides. The oligonucleotides were electrosprayed using the dual electrospray source to alternately characterize each oligonucleotide to ensure minimal variations in instrumental conditions due to pressure in the ICR cell. FRAGMENT laser irradiation power and times for the 3^- charge state of the 5′-$(AATG)_4$-3′ oligonucleotide ranged from 2 W (1–10 s), 2.5 W (1–4 s), 3 W (0.5–2 s), 3.5 W (0.25–1 s), and 4 W (0.25–1 s). For the 3^- charge state of the 5′-$(c^7Ac^7ATG)_4$-3′ oligonucleotide, the parameters were 2 W (1–10 s), 2.5 W (1.5–8 s), 3 W (0.5–5 s), 3.5 W (0.5–1.5 s), and 4 W (0.5–1.5 s). Three spectra were taken at each power and time setting with the average plotted to determine the first-order rate constant for unimolecular dissociation.

The oligonucleotide sequences are related to known STR and variable number of tandem repeat sequences within the human genome. The 7-deaza purine modified 5′-$(c^7Ac^7ATG)_4$-3′, 5-$(CAc^7G)_5$-C-3′, 5′-$(CTc^7G)_5$-C-3′ and unmodified 5′-$(AATG)_4$-3′, 5-$(CAG)_5$-C-3′ and 5′-$(CTG)_5$-C-3′ 16-mer oligonucleotides were purchased from Midland Certified Reagent Co. (Midland, TX). All oligonucleotides were purified by microdialysis [50–52] against 10 mM ammonium acetate and electrosprayed at 2 mM from a solution of 20% isopropanol, 20% 100 mM piperidine and imidazole in 10 mM ammonium acetate, and 60% acetonitrile. All other reagents were obtained from Sigma at the highest available purity and used as received.

3. Results and discussion

SORI and IRMPD of oligonucleotides yield the commonly observed sequence ions a_n-BH and w_n as defined by McLuckey et al. nomenclature [53] that result from cleavage of the N-glycosidic bond (1′-C–N bond between the sugar and nucleobase), the 3′-C–O sugar to phosphate bond, and proton rearrangements. Though several reports have been published that help elucidate sequence ion formation [54–59] in the gas-phase, the exact mechanism(s) has (have) yet to be determined for oligonucleotides. Recent reports using deuterated singly-charged 4-mers and doubly-charged 7-mers indicate a transient charging of the nucleobase by deuterium transfer, or at least a partial transfer, prior to loss of the neutral base and then a subsequent cleavage of the 3′-C–O bond [60,61]. What has been shown for purines, and further illustrated here, is the importance of the N7 site of purines with regards to N-glycosidic bond stability and neutral base loss [37–39]. The N7 position is the most basic site in deoxyguanosine mononucleotides and the third most in deoxyadenosine mononucleotides [25], though this may change when dealing with oligonucleotides.

Shown in Fig. 1 are deoxyguanosine and deoxyadenosine nucleotides and their 7-deaza analogs in which the nitrogen at the N7 position of the nucleobase is replaced by a vinylic carbon. The 7-deaza modification prevents the hydrogen bonding or transient protonation at the N7 position that is believed to be the reason for destabilization of the N-glycosidic bond in unmodified purines [38,60,61]. The effects of 7-deaza purines on gas-phase sequencing of repeat sequences is examined using 16-mer oligonucleotides fragmented by SORI and IRMPD. The FRAGMENT method is also employed to determine a relative differences in the energy of activation for gas-phase dissociation between the 5′-(c^{7}Ac^{7}ATG)$_4$-3′ and the unmodified 5′-(AATG)$_4$-3′ oligonucleotides.

3.1. SORI-CID

Fig. 2(A) and (B) show the MS/MS results using SORI-collision-induced dissociation (CID) on the isolated 4$^-$ charge states of the 5′-(AATG)$_4$-3′ and

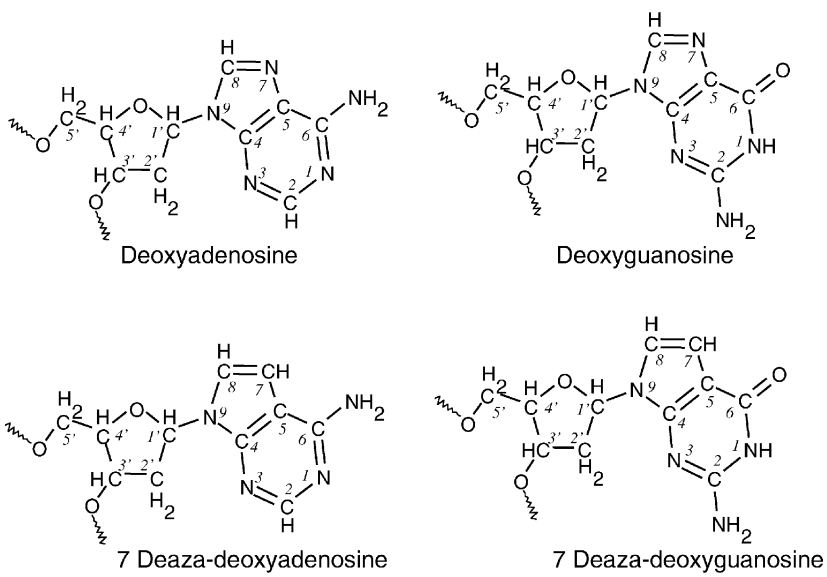

Fig. 1. Structure of purine nucleotides, deoxyadenosine and deoxyguanosine, and their 7-deaza purine analogs. The 7-deaza purines replace the N7 nitrogen with a vinylic carbon. Sequence ion formation for purine oligonucleotides is hypothesized to be associated with hydrogen bonding or transient protonation to the N7 site of the nucleobase.

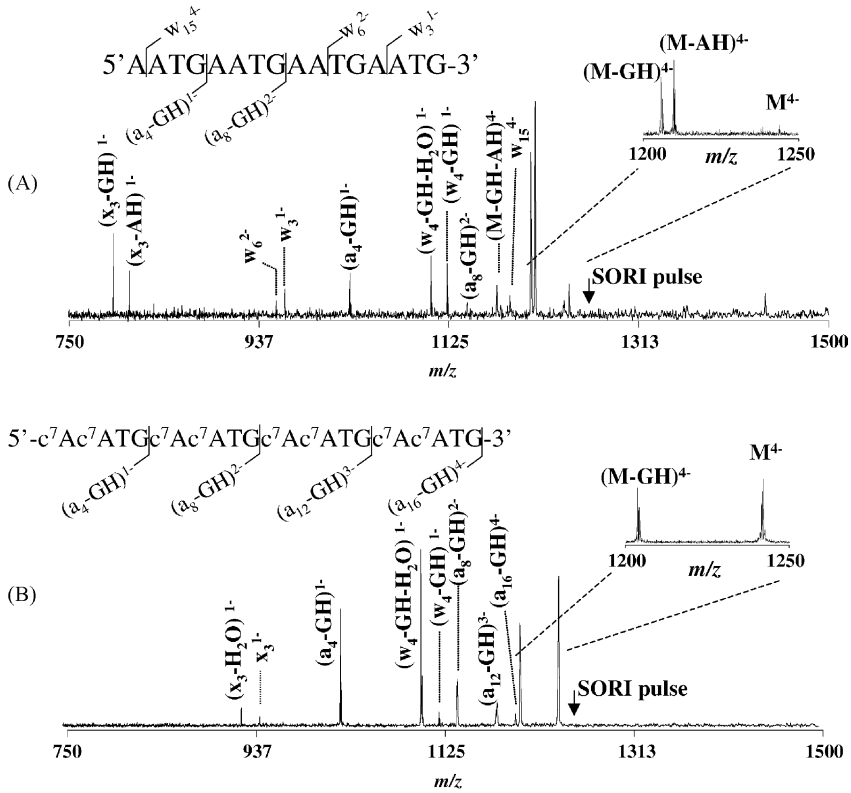

Fig. 2. MS/MS spectra using SORI-CID for fragmentation of (A) the 5′-$(AATG)_4$-3′ sequence and (B) the 5′-$(c^7Ac^7ATG)_4$-3′ oligonucleotide. Product ions in spectrum (A) are obtained from both deoxyadenosine and deoxyguanosine sites within the unmodified sequence with the expansion spectrum clearly showing loss of neutral guanine and adenine. The spectrum of the 7-deaza modified sequence (B) reveals only product ions associated with the deoxyguanosine sites illustrating the stabilizing effect at 7-deaza deoxyadenosine sites.

the 7-deaza 5-$(c^7Ac^7ATG)_4$-3 oligonucleotides, respectively. In Fig. 2(A), observed product ions are derived from deoxyguanosine and deoxyadenosine sites along the unmodified 16-mer sequence. Loss of neutral thymine or deoxythymidine related sequence ions were not observed in any of the experiments accomplished here and are typically not observed when using slow heating methods due to the low proton affinity of deoxythymidine [24,25]. Shown in the inset spectrum of Fig. 2(A) is the result of depurination of both neutral guanine and adenine with $(M\text{-}AH)^{4-}$ being the dominant product ion.

SORI-CID of the 7-deaza deoxyadenosine modified strand, shown in Fig. 2(B), resulted in sequence ion formation associated only with the deoxyguanosine sites. In the inset expansion spectrum of Fig. 2(B), the absence of 7-deaza adenine depurination is clearly evident, thus, indicating an increased energy of activation for unimolecular dissociation at the modified nucleotide sites. As should be expected when channeling fragmentation to fewer sites, an improvement in the S/N is also evident for the MS/MS spectrum of the modified strand (Fig. 2(B)), when compared to the unmodified strand (Fig. 2(A)).

The lack of the complementary w_n ions (w_4: $m/z = 1276$, w_8: $m/z = 1266$ and w_{12}: $m/z = 1264$) in Fig. 2(B) was caused by the proximity of their cyclotron frequency to the SORI 1000 Hz below resonance excitation frequency ($m/z = 1265$). For the w_8 and w_{12} ions, which fall very near to on-resonance

excitation, their orbital radius would reach the physical limit of the cell. The w_4 ions at 487 Hz off-resonance underwent subsequent fragmentation as illustrated in Fig. 2(B) (also in Fig. 2(A)) by the observance of the $(w_4\text{-GH})^{1-}$ and $(w_4\text{-GH-H}_2\text{O})^{1-}$ ions and possibly the tentatively labeled x_n series ions. The w_4, w_8, and w_{12} ions where observed when the SORI excitation frequency was changed to 2000 Hz below resonance of the precursor ion (data not shown).

Fig. 3(A) summarizes the SORI-CID results for the 5′-$(CAG)_5$-C-3′ 16-mer and the 7-deaza deoxyguanosine modified 5′-$(CAc^7G)_5$-C-3′ 16-mer. Furthermore, Fig. 3(B) compares the SORI-CID results for the 5-$(CTG)_5$-C-3′ 16-mer to the 7-deaza deoxyguanosine modified 5′-$(CTc^7G)_5$-C-3′ 16-mer. Product ions not observed whose cyclotron frequency was within 550 Hz of the SORI excitation waveform are indicated by "∗" and those outside of the excited and detected *m/z* range are indicated by "#". For the 5′-$(CAG)_5$-C-3′ and 5′-$(CAc^7G)_5$-C-3′ set, the unmodified sequence resulted in product ions derived from both purine sites as well as a deoxycytidine related sequence ion, w_{15}. Under the conditions used, the product ion formation at purine sites was preferred over the deoxycytidine sites. The 7-deaza deoxyguanosine modified sequence, 5′-$(CAc^7G)_5$-C-3′, produced sequence ions for deoxyadenosine and deoxycytidine sites without the observance of 7-deaza deoxyguanosine related product ions. There was a clear shifting of fragmentation to the deoxycytidine sites while maintaining sequence information at the deoxyadenosine sites. Limiting product ions to deoxyadenosine sites may be possible by attenuating the excitation energy. It would be tempting to define a preference for neutral base loss from this data of purines > cytosine, but other reports have demonstrated conflicting hierarchies that

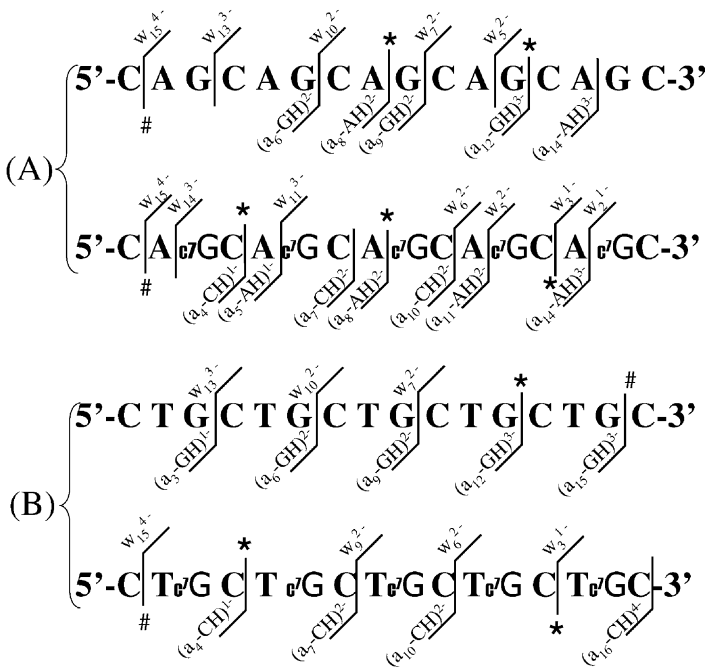

Fig. 3. 16-mer sequences showing the results of SORI-CID at 1000 Hz below resonance of the 4^- charge state for the (A) CAG and CAc^7G, and (B) CTG and CTc^7G repeats. In the 7-deaza deoxyguanosine modified oligonucleotides, there are no product ions associated with fragmentation at the modified nucleotide sites. The associated fragmentation pathway for 7-deaza deoxyguanosine has been eliminated resulting in a channeling of fragmentation to other sites. Product ions not observed whose cyclotron frequency was within 550 Hz of the SORI excitation waveform are indicated by "∗" and those outside of the excited and detected *m/z* range are indicated by "#".

probably depended on charge-state, sequence, and conformation in the gas-phase environment [24,26,57,59,62].

The 5′-(CTG)$_5$-C-3′ and 5′-(CTc^{7}G)$_5$-C-3′ repeat sequences in Fig. 3(B) show a similar shift in the modified sequence away from the 7-deaza deoxyguanosine sites to deoxycytidine related sequence ions. All MS/MS spectra for both the CAc^{7}G and CTc^{7}G repeat sequences exhibited a total absence of 7-deaza deoxyguanosine related product ions. The elimination of depurination at 7-deaza deoxyadenosine and 7-deaza deoxyguanosine sites would suggest a common dissociation pathway that relies on interactions with the N7 position.

3.2. IRMPD

Fig. 4 is the IRMPD spectrum obtained from the isolated 4$^-$ charge state of the 5′-(AATG)$_4$-3′ oligonucleotide using a laser power of 5 W for 400 ms. This unmodified 16-mer produced at least one-product ion for 10 of the 12 expected sequence ions in the *m/z* range examined. Depurination is also observed by the loss of neutral guanine and adenine [i.e. (M-GH)$^{4-}$

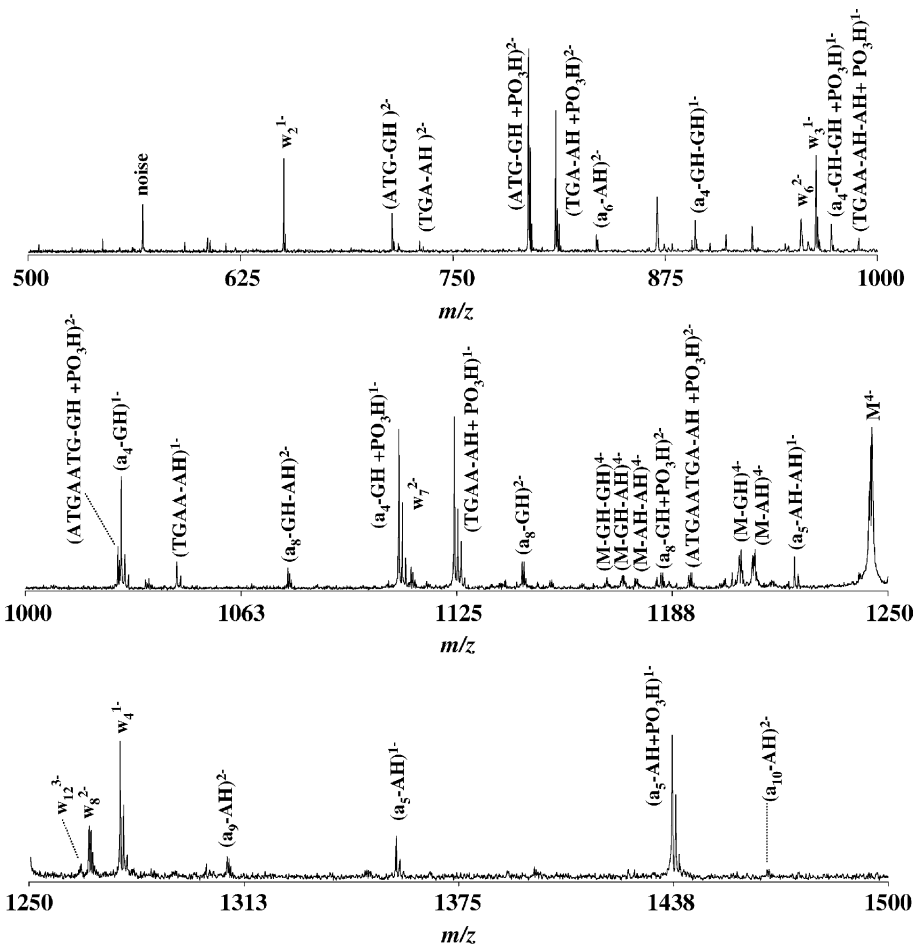

Fig. 4. The IRMPD spectrum of the 5′-(c^{7}Ac^{7}ATG)$_4$-3′ oligonucleotide obtained from the isolated 4$^-$ charge state. In the 1000–1250 *m/z* range, successive depurination is observed that includes both guanine and adenine nucleobases as well as for some of the sequence related ions. The peak intensities for loss of neutral guanine and adenine are approximately equivalent.

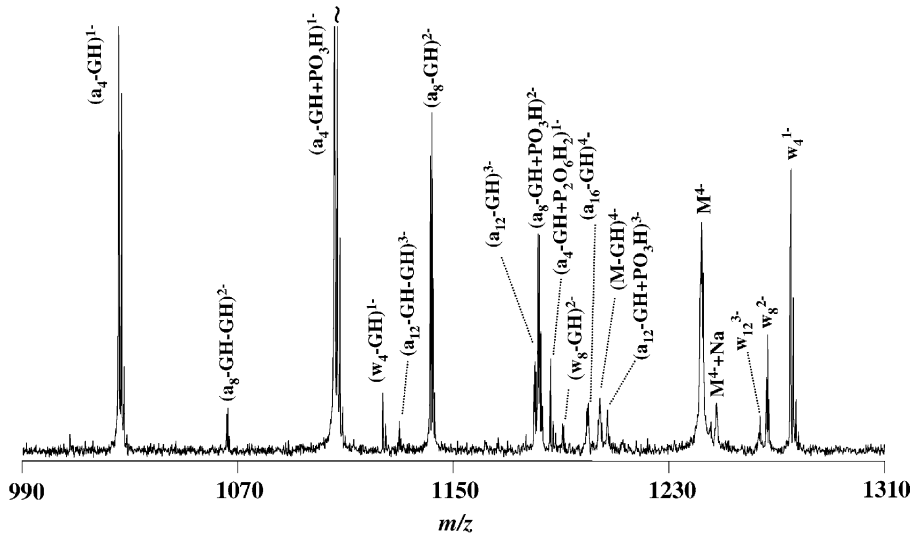

Fig. 5. The IRMPD spectrum of the isolated 4^- charge state of the 5′-(c^7Ac^7ATG)$_4$-3′ oligonucleotide. Sequence ions relating to deoxyguanosine sites were observed along with successive loss of neutral guanine, but the 7-deaza deoxyadenosine sites were resistant to fragmentation. The one sequence site per repeat would be sufficient to locate SNPs within large strands of DNA.

and (M-AH)$^{4-}$], accurately determined by the $\Delta m/z$ of the monoisotopic peaks from that of the molecular ion. The ease at which depurination occurs during slow heating methods is also evident by the multiple loss of neutral guanine and adenine for some of the sequence ions and the production of internal fragments. This is also a result of the product ions being coaxial and within the irradiation pattern of the infrared beam.

The missing purine related sequence ions of Fig. 4 were most likely produced, but their abundance was not sufficiently large enough to rise above the threshold of the noise. Moreover, we observed a significant amount of product ions that appear to involve phosphate group migration for which a mechanism was proposed by Hettich and Stemmler [63]. Initially, multiple sequence sites and numerous product ions may seem advantageous, but a high purine content for larger oligonucleotides and PCR amplicons would be detrimental. The numerous sequence sites would distribute the total ion intensity over many product ions greatly reducing the S/N for sequence ions in the MS/MS spectrum. This could result in large areas of the sequence not represented or prevention of a subsequent stage of mass spectrometry.

Fig. 5 shows the IRMPD spectrum for the 5′-(c^7Ac^7ATG)$_4$-3′ oligonucleotide resulting from a laser power of 5 W for 500 ms on the isolated 4^- charge state. The only product ions observed are those related to deoxyguanosine sites as well as the successive lose of neutral guanine [i.e. (a_8-GH-GH)$^{2-}$ and (a_{12}-GH-GH)$^{2-}$]. All seven sequence ions expected for this oligonucleotide were observed. Importantly, there is a definite absence of any loss of 7-deaza nucleobase or any 7-deaza associated product ions. This fact was valid for all MS/MS spectra acquired in these studies.

3.3. Relative energy of activation

The FRAGMENT method, as shown by Marshall and co-workers [48,49] for biological molecules and originated by Dunbar [64] for smaller molecules is used here to determine a relative energy of activation for gas-phase dissociation under low energy conditions for the isolated 3^- charge states of the 5′-(AATG)$_4$-3′ and the 7-deaza 5′-(c^7Ac^7ATG)$_4$-3′ oligonucleotides. The absorption of 10.6 μm (∼1.2 eV) photons allows an accumulation of internal energy, which is

statistically distributed according to RRKM theory [65,66], within the oligonucleotides until the threshold for dissociation is reached. With slow heating methods, this is expected to be the lowest energy dissociation pathway.

The first step of the FRAGMENT method relies on determining the first order rate constant for unimolecular dissociation in the gas-phase. The rate constant at a specific laser power is determined from the slope of the line obtained by plotting the natural log of the relative mass spectral peak intensity for the molecular ion with respect to laser irradiation time. Fig. 6(A) and (B) show the plots of experimental data to determine the rate constants for the 5′-(AATG)$_4$-3′ and the 7-deaza 5′-(c^{7}Ac^{7}ATG)$_4$-3′ oligonucleotides, respectively. The rate constants and laser intensities are applied to Eq. (1), as proposed by Dunbar [64], to determine the energy of activation.

$$E_a = qh\upsilon \frac{d \ln k}{d \ln I_{total}} \quad (1)$$

where E_a is the energy of activation for unimolecular dissociation, q the vibrational partition function, h Plank's constant, υ the laser frequency, I_{total} the total laser intensity in the IR-absorbing wavelength region and k is the first order rate constant. The value for the vibrational partition function was selected as an average of 1.05 for the ions internal temperature range expected, as previously detailed by Marshall and co-workers [48,49]. It should be noted that the E_a in Eq. (1) is not an absolute and must be used only as a relative value.

Fig. 7 is the plot of the natural log of the dissociation rate constants for each respective laser power vs. the natural log of the laser intensity in watts per area. The

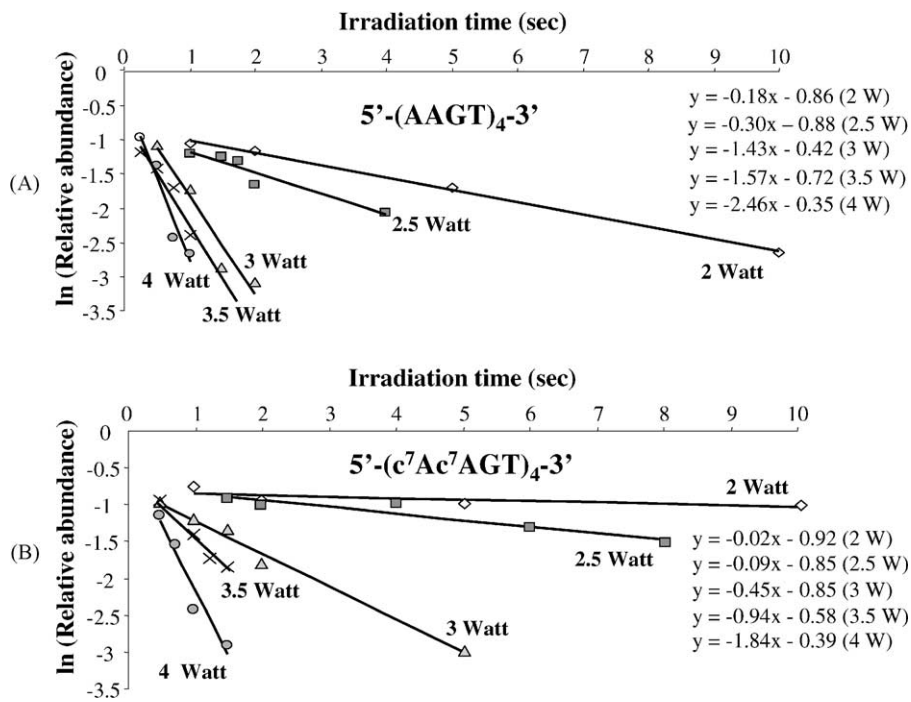

Fig. 6. Plot of the first-order rate law to determine the rate constants for unimolecular dissociation at specified laser powers for the 3$^-$ charge state of the AATG 16-mer (A), and the 3$^-$ charge state of the c^{7}Ac^{7}ATG 16-mer (B). The difference in rate constants between the two oligonucleotides is clearly evident by the different slopes at each laser power. The equations listed in (A) and (B) correspond to the linear least squares regression analysis results for each laser intensity; the laser intensity is stated in the parenthesis after each equation.

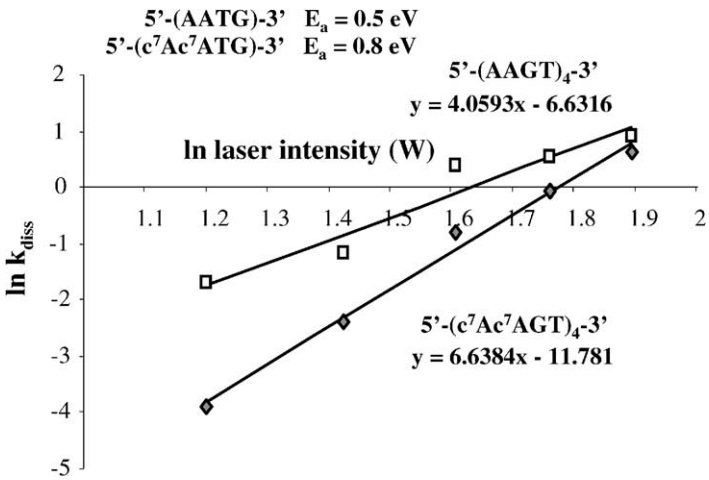

Fig. 7. The dissociation rate constant for the laser powers shown in Fig. 6(A) and (B) are used to determine a relative energy of activation for the 3^- charge state of the AATG 16-mer and c^7Ac^7ATG 16-mer. The AATG sequence was found to have 38% lower activation energy than the 7-deaza modified sequence, which indicates a change from deoxyadenosine to deoxyguanosine as the lowest pathway for dissociation.

E_a, calculated from the slope of the line, was 0.5 eV for the AATG 16-mer and 0.8 eV for the 7-deaza c^7Ac^7ATG sequence. Since there are several bonds that can absorb near the laser wavelength and the partition function is assumed for a single absorption bond, the E_a is probably underestimated. Additionally, the laser is not the only source of radiation; the total radiation is the sum of the laser and that emitting from the cell walls (the temperature of the ICR cell was about 25 °C [67,68]).

The experimentally determined E_a for the AATG 16-mer is 38% lower than that of the 7-deaza c^7Ac^7ATG sequence illustrating an increased stability for the 3^- charge state of the 7-deaza c^7Ac^7ATG sequence. A similar relative difference in energy of activation (∼24%) was observed between depurination from deoxyadenosine oligonucleotides (∼1.0 eV) and deoxyguanosine oligonucleotides (∼1.3 eV) by Klassen et al. using black body infrared dissociation (BIRD) [62], though not the identical nucleotide systems used here. The close correlation between the relative energy of activations as determined by BIRD and FRAGMENT has been shown previously for identical protein systems [48,49]. The effect of charge state on the relative E_a of each oligonucleotide has yet to be determined and is the focus of future research.

The relative difference in activation energy observed here might be due to conformational changes within the 7-deaza sequence that relates to the elimination of hydrogen bonding at the N7 site or changes in the gas-phase basicity of certain sites of the nucleobases. However, we believe this increase is the direct result of switching from the loss of neutral adenine to the loss of neutral guanine as the lowest energy pathway for dissociation due to the 7-deaza nucleotides. Thus, by selectively choosing the incorporation of 7-deaza purines, it is possible to limit the number of product ions and increase the S/N of the MS/MS spectrum. Importantly, restricting the number of product ions to even one per repeat unit is sufficient to localize an SNP within the repeating sequence.

4. Conclusions

The incorporation of 7-deaza purines in oligonucleotides and PCR amplicons provides for a method to restrict fragmentation during MS/MS using the low energy activation methods that result in sequence ions. The modified 7-deaza DNA produces MS/MS spectra with less complexity and increased signal-to-noise while maintaining sufficient sequence information to

localize interruptions to a specific repeat or identify alleles. Since 7-deaza purines are compatible with the PCR phase, additional procedures are not required. Prior knowledge of problematic sequences (i.e., specific STR loci of high purine content) and the use of 7-deaza purines could permit the routine, high throughput characterization of STRs by ESI-MS^n.

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