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Increased Sequence Coverage of Thymine-rich Oligodeoxynucleotides by Infrared Multiphoton Dissociation Compared to Collision Induced Dissociation

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Abstract

Infrared multiphoton dissociation (IRMPD) of thymine-rich oligodeoxynucleotides in a linear ion trap mass spectrometer affords far more extensive fragmentation than conventional collision induced dissociation (CID). For oligodeoxynucleotides containing one non-thymine base, CID results primarily in cleavage on the 3' side of the non-thymine nucleobase, whereas IRMPD results in cleavages between all of the nucleobases and thus provides complete sequence coverage. Furthermore, for oligodeoxynucleotides containing a single non-thymine base, it is shown that the full series of diagnostic sequence ions observed in the IRMPD mass spectra arise from secondary dissociation of the two primary products formed from the initial cleavage site located next to the non-thymine base.

INTRODUCTION

Tandem mass spectrometry has proven to be a versatile strategy for sequencing nucleic acids in the gas phase.^{1–7} Collision induced dissociation has been the most popular method for activation and fragmentation of multi-deprotonated nucleic acids, with oligodeoxynucleotides containing up to a few dozen nucleobases being successfully sequenced.^{2–7} Upon collisional activation, oligodeoxynucleotides typically undergo loss of a nucleobase which may be followed by cleavage of the adjacent 3'-C-O phosphodiester bond of the deoxyribose to yield complementary a – B and w ions as shown in Scheme 1. These latter series of ions are the primary diagnostic ions used to sequence the oligodeoxynucleotide. More recently, alternative methods such as infrared multiphoton dissociation (IRMPD),^{1,8,9} electron capture dissociation (ECD),¹⁰ electron detachment dissociation (EDD)^{8,11} and electron transfer dissociation (ETD)¹² have also shown promise for sequencing oligodeoxynucleotides.

A common observation noted upon CID of deprotonated oligodeoxynucleotides is the low extent or absence of thymine base loss as well as the lack of cleavage on the 3' side of thymine-containing nucleotides, presumably due to the low proton affinity of thymine relative to the other bases. The reduced favorability of proton transfer from the 5'-phosphate group to thymine discourages base loss and subsequent 3'-phosphoester C-O bond cleavage. This phenomenon is especially prevalent for thymine-rich stretches, thus causing gaps in sequence coverage in the resulting mass spectra.^{4,5,13–15} When shorter d(T)_n strands dissociate, different types of fragment ions are often formed.⁶ For example, T-rich oligodeoxynucleotides were collisionally activated and successfully dissociated by using higher energy collision energies, producing a, b, c, d, and z ions.⁶ Recently, Yang and co-

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Supplemental Information: Three additional CID and IRMPD mass spectral figures are available as supplemental information.

workers applied IRMPD to completely dissociate dT₆, yielding diagnostic sequence ions and supporting the conclusion that thymine-rich oligodeoxynucleotides fragment by a different mechanism that does not involve neutral base loss.⁸ Additionally, as observed in other studies, when mixed base sequences were studied, there was no fragmentation seen on the 3' side of the thymine bases resulting in only partial sequence coverage.⁸ Two other studies have also shown that thymine cleavage may occur for oligodeoxynucleotides in high charge states,^{4,7} albeit in charge states that are not always easily produced by conventional ESI conditions.

In the current study, infrared multiphoton dissociation (IRMPD), an alternative dissociation technique to collision induced dissociation (CID), has been used to enhance the fragmentation of thymine-rich sequences without high charge states or multiple stages of MSⁿ. The compelling advantage of IRMPD in the context of activation and dissociation of oligodeoxynucleotides arises from the fact that primary fragment ions, such as the base loss ions typically created upon activation of oligodeoxynucleotides, may undergo rapid absorption of photons and thus secondary dissociation to yield a richer array of products. At low irradiation times, cleavage patterns are similar to those observed upon CID; however, as the irradiation time is increased, cleavage between thymine bases occurs, thus allowing complete sequencing of these oligodeoxynucleotides.

EXPERIMENTAL SECTION

Materials

Single strand oligodeoxynucleotides were purchased from Integrated DNA Technologies (Coralville, IA) and synthesized on the 1 μ mole scale and desalted by a proprietary technique similar to an oligodeoxynucleotide purified column (OPC) method. Stock solutions were prepared in water at approximately 1 mM and the exact concentration was determined using UV-Vis absorbance measurements at 260 nm and the extinction coefficients provided by the manufacturer. Each oligodeoxynucleotide was diluted to ~10 μ M in a solution of 50 mM ammonium acetate and 25% methanol directly before analysis by ESI-MS.

Mass Spectrometry

Analytical solutions were directly infused into a Thermo Fisher LTQ two-dimensional linear ion trap mass spectrometer (San Jose, CA) at 3 μ L/min using a Harvard Apparatus PHD 2000 syringe pump (Holliston, MA). Negative ions were produced using an ESI voltage of 3.5 kV and a heated capillary temperature of 90°C. Nitrogen sheath and auxiliary gas flows of 30 and 10 arbitrary units, respectively, were used to aid the desolvation of the ions. Spectra were acquired using an ionization time of 100 ms and summing 150 scans. This mass spectrometer was also equipped with a 48-5 Synrad 50 W continuous wave CO₂ laser (Mukilteo, WA) to enable IRMPD following the setup described by Gardner and Brodbelt.¹⁶ Typical CID conditions consisted of an isolation width of 4.0 m/z, a 0.25 *q* value, and an activation time of 30 msec. The normalized collision energy was adjusted so that there was survival of ~20% of the precursor, typically around 13–14% normalized collision energy was used. Typical IRMPD conditions include an isolation width of 4.0 m/z, a *q* value of 0.1, and the laser operating at full power (~50 W). The irradiation time was adjusted to vary the amount of fragmentation observed, typically between 0.5 – 1.5 ms.

RESULTS AND DISCUSSION

The CID and IRMPD behavior of a series of seven 14-mer oligodeoxynucleotides were evaluated in the present study. Five T-rich oligodeoxynucleotides, as well as one with a single T-rich stretch and one random mixed based sequence, were chosen to compare the

sequence coverage obtained by the two complementary activation methods. For each sequence, the $[ss]^{4-}$ ion was isolated and activated using either collision induced dissociation (CID) or infrared multiphoton dissociation (IRMPD). CID and IRMPD spectra were also acquired for the 3- charge state for each oligodeoxynucleotide (data shown in Supplemental Figures 1 and 2), and the fragmentation patterns and trends are similar to those described in more detail below for the 4- charge states. Examples of the CID spectra obtained for the mixed base oligodeoxynucleotide and one of the thymine-rich oligodeoxynucleotides (4- charge state) are shown in Figure 1. The fragmentation of the mixed base sequence shows the expected pattern in which cleavage occurs between nearly every base, resulting in formation of the characteristic w and a - B ions as well as some non-informative internal fragments (Figure 1a). However, the thymine-rich strand showed only one cleavage site next to the non-thymine base (Figure 1b). This latter fragmentation pattern which is devoid of diagnostic sequence ions is characteristic of the CID spectra obtained for thymine-rich oligodeoxynucleotides. As the collision energy was increased, no additional fragmentation occurred for either oligodeoxynucleotide.

Nucleic acids undergo extremely efficient absorption of IR photons (10.6 μm) due to the extensive phosphodiester backbone,^{1,9} and thus activation of the oligodeoxynucleotides with as little as 1.0 msec irradiation time with a 50 W laser affords significant dissociation. Representative IRMPD mass spectra acquired for the same T-rich oligodeoxynucleotide used in Figure 1B are shown in Figure 2, with the irradiation time ranging from 0.95 to 1.5 msec. At the shortest irradiation time, the fragmentation pattern was similar to the one obtained upon CID, in which only cleavage at the non-thymine base occurred, yielding the dominant $a_7 - C$ and w_7 ions. However, as the irradiation time was increased to 1.2 and then 1.5 msec (Figure 2b and 2c), cleavage occurred between every nucleobase, thus affording complete sequencing of the oligodeoxynucleotide. Moreover, fragments such as d - H_2O and y ions are seen in addition to the conventional w and a - B ions. The striking change in the IRMPD mass spectra as the irradiation time was increased by only 0.5 msec suggests that the initial $a_7 - C$ and w_7 ions undergo rapid photoabsorption and secondary dissociation to generate all the other product ions seen in Figure 2c.

The comparative CID and IRMPD results for the other oligodeoxynucleotides are summarized in Scheme 2, in which the IRMPD product ions were summed over an irradiation period ranging from 1.2 - 1.5 ms. For each oligodeoxynucleotide, CID or IRMPD of both the 3- and 4- charge states yielded similar patterns, and thus only the results for the 4- charge state are summarized in Scheme 2. Both mixed-base sequences and thymine-rich sequences were analyzed as well as thymine-rich sequences in which the location of the non-thymine base was varied. Regardless of the position of the non-thymine base, collisional activation promoted one cleavage directly adjacent to the non-thymine bases producing a pair of complementary w and a - B ions, whereas IRMPD resulted in significant cleavages along the entire DNA strand. The far greater sequence coverage observed upon IRMPD again reflects the significant impact of secondary activation which converts the initial product ions stemming from cleavage adjacent to the non-thymine bases into a full series of diagnostic sequence ions.

To further probe the fragmentation pathways of the T-rich oligodeoxynucleotides, MS^3 experiments were performed, as illustrated in Figure 3 for 5'-TTTTTTGTTTTTT-3' (4-). The first stage of CID produces the abundant w_7^{2-} and $[a_7 - G]^{2-}$ ions that are indicative of cleavage next to the non-thymine base. Each of these primary fragment ions was isolated and subjected to a second stage of CID, and the resulting MS^3 spectra are shown in Figures 3b and 3c. When these two smaller product ions containing only thymine bases are activated, they undergo cleavage between each nucleobase. The combination of the two MS^3 spectra mirror the IRMPD spectrum obtained for the original intact oligodeoxynucleotide

(see Supplementary Figure 3). After their formation during the first stage of CID, the resulting T-rich w and a – B ions can be readily dissociated in a second stage of CID because the fragmentation of these ions is not initiated by base loss, instead arising from cleavages along the backbone. Likewise, because IR irradiation causes activation of both the precursor ion and all ensuing fragment ions, the activation and dissociation of the complementary w and a-B ions readily proceeds during IRMPD (Supplementary Figure 3c).

Energy-variable CID and time-variable IRMPD data was collected for one mixed base oligodeoxynucleotide and one thymine-rich sequence to compare the trends in the product ions as a function of energy deposition. As expected, the energy-variable CID data for the mixed base sequence primarily shows base loss, w ions, and a – B ions as illustrated in Figure 4a. Even as the collision energy is increased substantially, the distribution of the fragment ions remains unchanged. The corresponding time-variable IRMPD data for this same sequence is shown in Figure 4b. At low irradiation times, the same products as observed upon CID are dominant. As the irradiation time is increased, more numerous a – B and w ions are observed that provide full sequence coverage. At very high irradiation times, other products such as c ions, internal fragment ions, and individual base ions are observed, and the abundance of base loss ions, w ions, and a – B ions decreases. The increase in the abundance of the internal and individual base ions mirrors the decay in the a – B and w ions, again reflecting the importance of rapid secondary dissociation during IRMPD.

Energy-variable CID data for the thymine-rich sequence is shown in Figure 4c and the two most abundant products are the w₇ and a₇ – C ions which are indicative of the primary cleavage that occurs next to the non-thymine base. As the collision energy is increased up to 20% normalized collision energy (0.047 V), there are no changes in ion abundances nor formation of new products. The time-variable IRMPD data (Figure 4d) shows the initial formation of these same ions at low irradiation times. As the irradiation time is increased above 1.0 msec, an array of other sequence ions become prominent as the two initial fragment ions undergo secondary dissociation and conversion into diagnostic w and a – B ions.

CONCLUSIONS

Thymine-rich DNA sequences have long posed a significant challenge for characterization by tandem mass spectrometry because the thymine bases do not readily cleave, thus restricting the formation of a complete series of a – B and w sequence ions. While for the most part CID only promotes cleavage next to non-thymine bases, IRMPD overcomes this limitation via extensive secondary dissociation of the primary a – B and w ions to generate a more complete fragmentation pattern. IRMPD is particularly efficient due to the strong absorptivity of the phosphodiester backbone of oligodeoxynucleotides, thus allowing complete sequence coverage after only 1.0 ms irradiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Collision induced dissociation (CID) of a) 5'-GGCTTAGGCATTGC-3' (4-) and b) 5'-TTTTTCTTTTTT-3' (4-). An asterisk (*) indicates an internal fragment. The star (★) represents the precursor ion.

Figure 2.

Infrared multiphoton dissociation (IRMPD) of 5'-TTTTTCTTTTTT-3' (4-) with an activation time of a) 0.95 b) 1.2 and c) 1.5 msec. An asterisk (*) indicates an internal fragment. ∇ indicates a loss of water from the nearest higher mass ion. The star (★) represents the precursor ion.

Figure 3. MS^3 of 5'-TTTTTTGT(TTTTTTTT)-3' (4-). a) CID of $[ss]^{4-}$, b) CID (MS^3) of the w_7^{2-} ion formed in the top spectrum, c) CID (MS^3) of the $[a_7-G]^{2-}$ ion formed in the top spectrum. ∇ indicates a loss of water from the nearest ion. The star (★) represents the precursor ion.

Figure 4.

a) Energy-variable CID of 5'-GGCTTAGGCATTGC-3' (4-). b) Time-variable IRMPD of 5'-GGCTTAGGCATTGC-3' (4-). c) Energy-variable CID of 5'-TTTTTCTTTTTT-3' (4-). d) Time-variable IRMPD of 5'-TTTTTCTTTTTT-3' (4-).

Scheme 1.

Typical CID fragmentation pattern resulting in complementary a-B and w ions.

CID	IRMPD
5'-TTTTTTC)TTTTTTT-3'	5'-T)T)T)T)T)C)T)T)T)T)T-3'
5'-TTTTTTA)TTTTTTT-3'	5'-T)T)T)T)T)A)T)T)T)T)T-3'
5'-TTTTTTTG)TTTTTTT-3'	5'-T)T)T)T)T)G)T)T)T)T)T-3'
5'-TTTG)TTTTTTTTTTT-3'	5'-T)T)TG)T)T)T)T)T)T)T)T-3'
5'-TG)TTTTTTTTTTTTT-3'	5'-T)G)T)T)T)T)T)T)T)T)T-3'
5'-G)G)C)T)TA)G)G)C)A)T)TG)C-3'	5'-G)G)C)T)TA)G)G)C)A)T)TG)C-3'
5'-G)C)G)TTTTG)C)C)G)C)G)G-3'	5'-G)C)G)T)T)T)TG)C)C)G)C)G)G-3'

Scheme 2.

Comparison of product ions observed with CID and IRMPD for seven different oligodeoxynucleotides.