

MALDI-PSD and ESI-MS/MS for the Structure Determination of d(GTATTAT) and its Four Photoproducts

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Introduction

We recently reported a study of d(TATTAT) and its four photoproducts and showed that tandem mass spectrometry together with ^1H NMR are useful for the characterization of these materials in which two adjoining bases are linked[1]. We were only able to assign the major fragments as either w and [a - base] ions or d and [z - base] ions, owing to the symmetry of d(TATTAT). Electrospray-ionization ion-trap tandem mass spectrometry and MALDI-TOF with post source decay were used to study unsymmetric oligonucleotide d(GTATTAT) to resolve these mechanistic ambiguities and to extend the method.

Methods

The deoxyoligonucleotide d(GTATTAT) was synthesized by traditional phosphoramidite chemistry. After irradiation under UV-C light for two hours, the reaction mixture was separated by HPLC with a C-18 column under a gradient of 6-12% CH_3CN in 50 mM triethylammonium acetate buffer (pH = 6.8). The Dewar isomer was generated by irradiating the (6-4) isomer with light from a 450-watt, medium-pressure mercury arc lamp for 2 hours. The sample was separated by 30 mm from the light source, and the light was filtered through Pyrex and Mylar.

The samples were dissolved in a 50% $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ solution at a concentration of 10 pmol/uL. MALDI-PSD experiments were done on a Perspective Biosystems Voyager RPDE. A 2:1:2 molar ratio of 2,4,6-trihydroxyacetophenone(2,4,6-THAP): 2,3,4-THAP: ammonium citrate was used as the matrix. ESI-MS/MS experiments were done on a Finnigan LCQ, and 50% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ was used as the spray solvent. Both MALDI-PSD and ESI-MS/MS were carried out in the negative ion mode. In ESI-MS/MS the doubly-charged ion (m/z 1053.5) was selected as the precursor ion, while in MALDI-PSD the singly-charged ion (m/z 2108) was selected as the precursor ion.

Results and Discussion

The HPLC trace shows that the four major photoproducts d(GTAT[c, s]TAT), d(GTAT[6-4]TAT), d(GT[]ATTAT), and d(GTATT[]AT) can cleanly separated from each other and from the starting material. The photoproduct identities were determined by ^1H -NMR. Additionally, the UV spectrum of the (6-4) product had an absorption maximum near 325 nm. The two TA* photoproducts were further characterized by an acid-hydrolysis fluorescence assay.

ESI-MS/MS of the starting material d(GTATTAT) (Figure 1) shows that the major fragmentation ions are w and [a - base] series ions. Cleavages at the 3' side of Ade and Gua are favorable so w_4^- and w_6^{2-} are very abundant. The w_1 ion was also detected but with lower abundance. The [a₆ - Ade] ion was formed as both singly and doubly charged with high abundance and the [a₃ - Ade] ion was also very abundant. Because cleavages at the 3' side of Thy are not favorable, [w₂/a₅ - Thy]⁻, [w₃/a₄ - Thy]⁻, and [w₅/a₂ - Thy]⁻ were not detected.

In the ESI-MS/MS of the (6-4) photoproduct(Figure 1), new ions of medium abundance at m/z 1474 and m/z 634 were detected. These ions were assigned as a₅ and w₂ ions. Both ions are formed by cleavage at the 3' side of the (6-4) photoproduct. These two ion species were also detected in the ESI-MS/MS of d(GTAT[Dewar]TAT), although their abundances are much lower than those of the (6-4) photoproduct. However, in the ESI-MS/MS of d(GTAT[c, s] TAT), no a₅ and w₂ ions were detected. Thus, ESI-MS/MS distinguishes these three isomers. Unfortunately, there is no difference in the ESI-MS/MS of the starting material and that of the [c,s] isomer. Therefore, MS³ experiments of these two isomers were done. The triply charged precursor ion was selected for the MS/MS experiment, and the w₄ ion for MS³. Comparison of the MS³ of the two isomers shows that the w₃ (m/z 938) is formed in the fragmentation of the starting material, whereas it is not seen in the spectrum of the [c,s] isomer.

ESI-MS/MS also allowed us to distinguish the two TA* photoproducts (Figure 2). For d(GT[]ATTAT) the a_3 ion was abundant whereas the $[a_3 - Ade]$ ion was not detected. Similarly, for d(GTATT[]AT), the a_6 ion was very abundant whereas the $[a_6 - Ade]$ ion was not observed. Note that a_3 and a_6 were not seen for the starting material, the (6-4), the [c, s], or the Dewar isomer.

MALDI-PSD was also used to distinguish the photoisomers. In the spectrum of the starting material, w_4 , w_6 , and $[a_6 - Ade]$ ions are very abundant. The $[a_3 - Ade]$ and w_2 ions were not detected because MALDI-PSD is not sufficiently sensitive at low mass. In the spectrum of the (6-4) photoproduct, the a_5 ion is very abundant. Like the ESI-MS/MS data, the MALDI-PSD of the [c,s] isomer is similar to that of the starting material, whereas the Dewar isomer again shows medium abundance of the a_5 ion. In the MALDI-PSD of the two TA* photoproducts, the a_6 ion is very abundant in the d(GTATT[]AT) spectrum, yet the corresponding a_3 ion could not be seen for d(GT[]ATTAT). Again, this is most likely due to a lack of adequate sensitivity at low mass.

A phosphodiesterase partial digestion combined with MALDI-TOF(a Ladder method) was also tried to map the photoadduct site. The digestion of the photoadducts stop at the 3'-side of the photo-modification site.

Conclusions

The major fragmentation ion series in ESI-MS/MS and MALDI-PSD for the deoxyoligonucleotide d(GTATTAT) and its photoisomers are w and $[a - base]$. The d and $[z - base]$ ions are not seen. MALDI-PSD and ESI-MS/MS can be used to distinguish d(GT[]ATTAT), d(GTATT[]AT), d(GTAT[Dewar]TAT), d(GTAT[6-4]TAT) from each other and from their starting material. Neither technique can be used to distinguish [c,s] isomer from the starting material. However, ESI-MS³ can distinguish these two isomers. The Ladder method gives us another method to determine the site of the photoproduct.

Acknowledgments

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[1] D. Vollmer, X. Zhao, J.-S. Taylor, M.L. Gross. Int. J. Mass Spectrom. Ion Processes 165/166(1997)487.

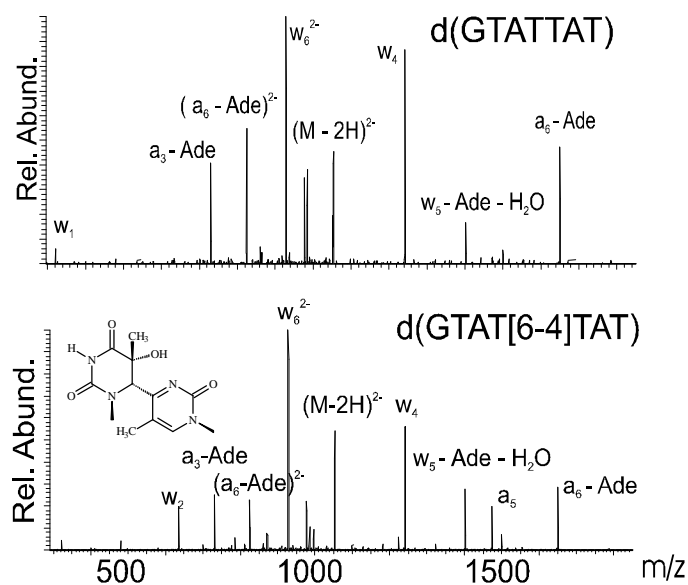


Fig.1 ESI-MS/MS of d(GTATTAT) and d(GTAT[6-4]TAT)

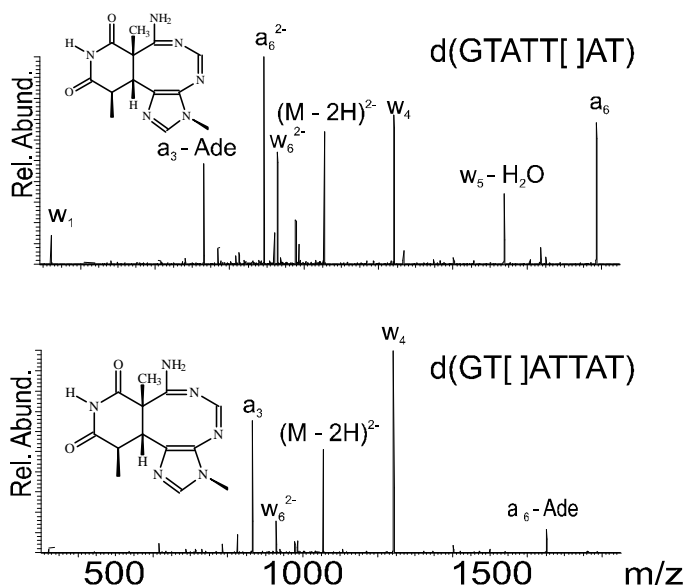


Fig.2 ESI-MS/MS of the two TA* photoproducts of d(GTATTAT)