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Hydrogen Attachment/Abstraction Dissociation (HAD) MS/MS for Single-Stranded DNAs

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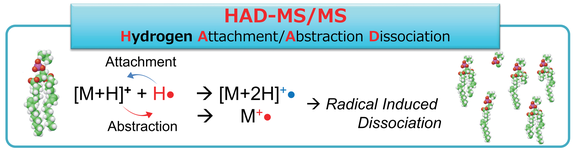
Overview

- ✓ Hydrogen attachment/abstraction dissociation (HAD) MS/MS, we have developed, specifically cleaves highly reactive binding sites for hydrogen radical (H•).
- ✓ We analyzed nucleic acids by HAD-MS/MS. Nucleic acids have recently been applied to pharmaceuticals and there is a growing need for analysis of them.
- ✓ HAD dissociated the ribose-phosphate backbone almost sequentially, while retaining the bases and chemical modifications of the nucleic acids.

1. Introduction

Hydrogen attachment/abstraction dissociation (HAD) MS/MS, we have developed, specifically cleaves highly reactive binding sites for hydrogen radical (H•). Although HAD belongs to the same radical-induced dissociation as ETD/ECD, HAD is applicable to 1+ and negative ions because it uses neutral species of hydrogen radicals. We have shown usefulness of HAD for analysis of proteins/peptides with post-translational modification (PTM)[1] and for C=C binding sites analysis of fatty acids[2].

As a new application, we analyzed nucleic acids by HAD-MS/MS. Nucleic acids have recently been applied to pharmaceuticals and there is a growing need for analysis of them.



Acknowledgments

Consideration for the hypothesis of HAD fragmentation scheme (Fig.4) was advised by Dr. Daiki Asakawa, National Institute of Advanced Industrial Science and Technology (AIST).

References

1) H. Takahashi *et al.*, *Anal. Chem.* 2016, 88 (7), 3810-3816.
2) H. Takahashi *et al.*, *J. Mass Spectrom. Soc. Jpn.* 2017, 65 (6), 268-273.
3) McLuckey, S.A. *et al.*, *J. Am. Soc. Mass Spectrom.* 1992, 3, 60-70.
4) A. Nyakas *et al.*, *J. Am. Soc. Mass Spectrom.* 2013, 24 (2), 249-256.

2. Materials and Methods

HAD-MS/MS was performed using LCMS-IT-TOF™ (Shimadzu Corporation, Japan) modified for HAD-MS/MS experiments. Hydrogen radicals were generated by passing H_2 gas through a heated tungsten capillary (length of 50 mm, inner diameter of 1 mm). HAD reaction time was 2 sec. Peaks were assigned manually based on the nomenclature for oligonucleotide fragment ions proposed by McLuckey *et al.*[3] with help of OMA[4] oligonucleotide mass calculator.

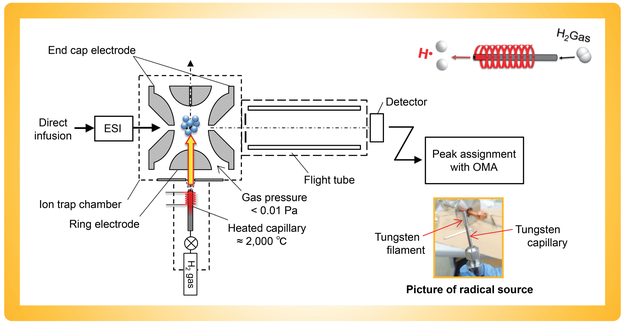


Fig.1 Schematic diagram for ESI-HAD-MS/MS using LCMS-IT-TOF.

Samples were a single-stranded DNA (mentioned in section 3-1) and mipomersen which is oligonucleotide therapeutics. The structure of mipomersen is shown in Fig.2. Generally, oligonucleotide therapeutics are chemically modified to enhance their stability or functionality in vivo. Because of the chemical modifications, structural analysis is considered to be more difficult.

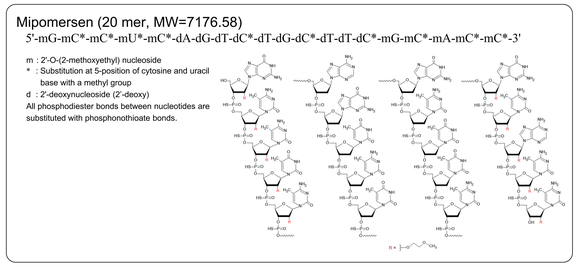


Fig.2 Structure of mipomersen.

3. Results and Discussion

3-1. Single-Stranded DNA

The synthesized 20 mer ssDNA as bellow was analyzed (Fig.3). It has no chemical modification and the same length as mipomersen.

20 mer ssDNA : 5'-TGTGCGTGTGTAGTGTGTCT-3', MW=6201.1

Both CID and HAD dissociated the ribose-phosphate backbone almost sequentially and preferred to cause a/w ions. CID lost bases of a-ions (i.e. [a-B] ions), whereas HAD retained them. The possible HAD fragmentation scheme of a/w ions is shown in Fig.4.

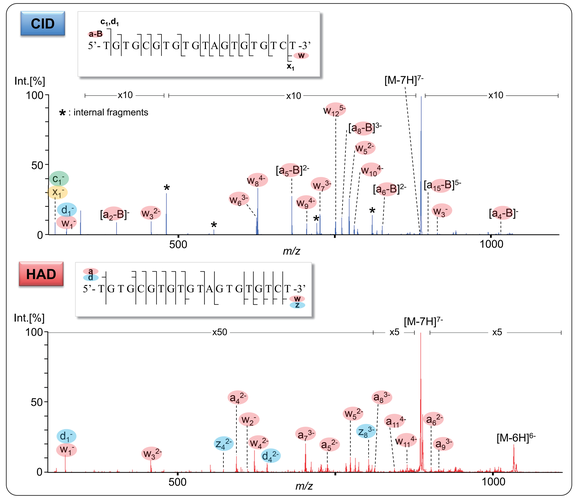


Fig.3 ESI-CID or HAD-MS/MS spectrum of 7- charged 20 mer ssDNA (20 pmol/μL). The solvent was 50% acetonitrile in water with 0.05% of formic acid.

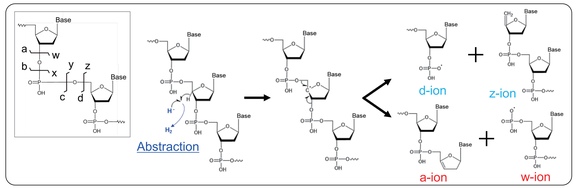


Fig.4 Hypothesis of HAD fragmentation scheme of a/w and d/z ions.

3-2. Mipomersen

Mipomersen, the oligonucleotide therapeutics, was analyzed (Fig.5). Both CID and HAD dissociated the ribose-phosphate backbone almost sequentially, while retaining the chemical modifications. CID preferred to cause [a-B]/w, b/x, c/y ions and internal fragments of which intensities were high. On the other hand, HAD preferred to cause a/w and d/z ions. The possible HAD fragmentation scheme of a/w and d/z ions is shown in Fig.4.

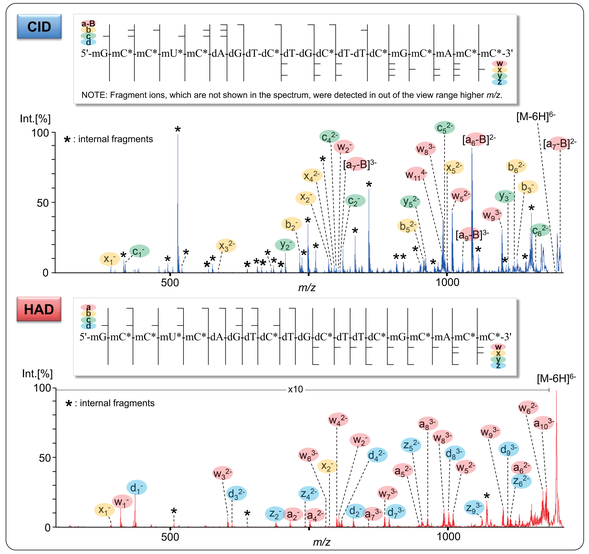


Fig.5 ESI-CID or HAD-MS/MS spectrum of 6- charged mipomersen (0.25 mg/mL ≈ 35 pmol/μL). The solvent was 50% acetonitrile in water with 0.05% of formic acid.

4. Conclusions

- ✓ HAD-MS/MS is useful for the structural analysis of oligonucleotide therapeutics.
- ✓ HAD dissociated the ribose-phosphate backbone almost sequentially and didn't tend to generate [a-B] ions or intense internal fragments.
- ✓ Future works; We will try to analyze the structure of impurities (especially analogues) contained in oligonucleotide therapeutics.

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