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Application News

MALDI-TOF Mass Spectrometer: MALDI-8030

Sequence Analysis of Antisense Nucleic Acid Using a MALDI-8030 Benchtop MALDI-TOF Mass Spectrometer

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User Benefits

- MALDI-ISD enables simple and reliable sequencing of antisense nucleic acid.
- There is no need to interpret complicated MS/MS spectra, which saves labor and time.
- Nucleic acid therapeutics can be analyzed using a simple and affordable benchtop instrument.

■ Introduction

Nucleic acid therapeutics have recently attracted particular attention as a new pharmaceutical modality. Unlike biopharmaceuticals, nucleic acid therapeutics can be produced by cost-effective chemical synthesis. The development and standardization of analytical methods for quality control are still in progress, with efforts being made in collaboration with industry and academia.

Mass spectrometry (MS) has now become an established analytical tool for analyzing biopolymers, such as nucleic acids, proteins, and glycans, thanks to the development of various soft ionization methods and instrument performance improvements. Although tandem mass spectrometry (MS/MS) is generally used to sequence nucleic acids, collision-induced dissociation (CID), the technique typically used for conventional ion cleavage, has been problematic because of the difficulty in producing fragments that suggest particularly internal sequences.

Matrix-assisted laser desorption/ionization (MALDI) is used as a technique for ionizing various biopolymers, including nucleic acids. MALDI can softly ionize a sample without causing unwanted fragmentation. However, by using a specific MALDI matrix with increased laser irradiation, it is possible to cause ionization and fragmentation at the same time. Then the resulting in-source decay (ISD) fragment ions can be used for structural analysis. MALDI-ISD fragmentation of nucleic acids provides successive fragment ions by simple cleavages and is therefore highly useful for simple sequencing of nucleic acids.

This Application News describes an example of sequencing antisense nucleic acids by ISD fragmentation using a Shimadzu MALDI-8030 benchtop linear MALDI-TOF mass spectrometer.

■ Model Nucleic Acid Therapeutics Samples

In this study we analyzed three synthetic antisense nucleic acids ("model therapeutics") which replicate the nucleic acid therapeutics mipomersen, nusinersen and inotersen (Table 1). Each sample was dissolved in water to a concentration of 0.1 mg/mL (about 13 to 14 pmol/μL) and then 0.5 μL of the sample solution was measured with a MALDI-TOF MS system.

Table 1 3 Model Nucleic Acid Therapeutics Used in this Study

Name	Chemical Formula	Mw	Note
Mipomersen	C230H324N67O122P19S19	7177.2	ASO, PS (full), 2'-MOE, 20 mer
5'-G*-mC*-mC*-mU*-mC*-dA-dG-dT-dmC-dT-dG-dmC-dT-dT-dmC-G*-mC*-A*-mC*-mC*-3'			
Nusinersen	C234H340N61O128P17S17	7127.2	ASO, PS (full), 2'-MOE, 18 mer
5'-T*-mC*-A*-mC*-T*-T*-T*-mC*-A*-T*-A*-A*-T*-G*-mC*-T*-G*-G*-3'			
Inotersen	C230H318N69O121P19S19	7183.1	ASO, PS (full), 2'-MOE, 20 mer
5'-T*-mC*-T*-T*-G*-dG-dT-dT-dA-dmC-dA-dT-dG-dA-dA-A*-T*-mC*-mC*-mC*-3'			

* = 2'-O-(2-methoxyethyl) m = 5-methyl d = 2'-deoxy

■ Mass Spectrometry

0.5 μL of the sample solution was deposited on a MALDI target plate, mixed with a 0.5 μL matrix solution on the plate, and left to dry. Mass spectra were obtained using the MALDI-8030 system (Fig. 1).

Matrix solution: 40 mg/mL THAP or HPA in 50 % acetonitrile with 40 mM ammonium citrate dibasic

THAP: 2',4',6'-Trihydroxyacetophenone monohydrate

HPA: 2-Hydroxypicolinic acid

Fig. 1 MALDI-8030 MALDI-TOF Mass Spectrometer

■ Mass Spectrum of Model Nucleic Acid Therapeutics

HPA and THAP are typical MALDI matrices for ionizing nucleic acids. These matrices successfully ionized model nucleic acid therapeutics as deprotonated form $[M-H]^-$ in negative-ion mode. The formation of unwanted cation adducts was suppressed by adding ammonium citrate to the matrix solution (Fig. 2).

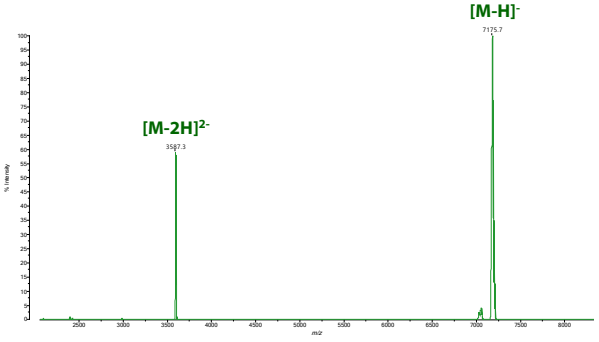

Fig. 2 Negative-Ion MALDI Mass Spectrum of Mipomersen Using a THAP as a Matrix

■ Characteristics of MALDI-ISD Fragments of Oligonucleotides

MALDI-ISD of oligonucleotides produces two specific types of consecutive fragment ions, either a-series fragment ions from the 5′-terminus side or w-series ions from the 3′-terminus side, which are formed by simple cleavage at the phosphodiester bond (Fig. 3). This simplicity offers simpler sequencing than using electrospray ionization (ESI) followed by MS/MS using CID, which generally produces various types of cleavages that depend on the precursor charge-state ($[M-nH]^{n-}$).

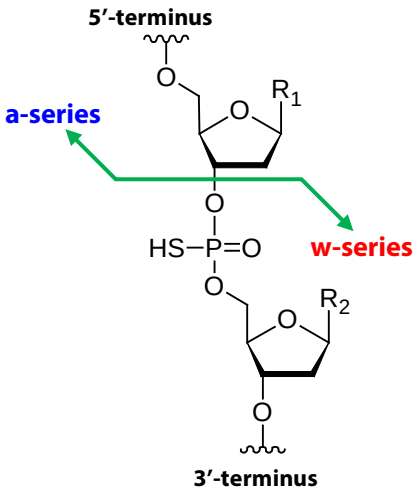

Fig. 3 Ion Fragmentation Nomenclature of Oligonucleotides Observed from MALDI-ISD

■ Characteristics of MALDI-ISD Fragments of Model Nucleic Acid Therapeutics

Fig. 4 shows negative-ion MALDI-ISD mass spectra of model nucleic acid therapeutics (mipomersen, nusinersen, and inotersen) using HPA as a matrix. The results show that HPA is more suitable than THAP for ISD ion measurements (Fig. 2 and 4). Simple and consecutive ISD ions confirmed the sequences of model nucleic acid therapeutics, even those with various types of modifications such as phosphorothioation.

Because the MALDI-8030 is a linear TOF mass spectrometer, it does not support MS/MS measurements. Nevertheless, nucleic acid sequencing is possible by using MALDI-ISD measurements. This technique would be applicable not only for synthetic nucleic acids but also for their unknown impurities isolated by HPLC.

■ Conclusion

Because MALDI preferentially produces singly-charged ions, mass confirmation of synthetic nucleic acids can be achieved easily without interpreting complicated mass spectra. Furthermore, the ability to perform MALDI-ISD measurements with an instrument lacking MS/MS capability means the internal sequence of nucleic acids can be confirmed using a simple benchtop-type linear MALDI-TOF MS system. That also enables the sequencing of model nucleic acid therapeutics that contain various chemical modifications based on the successive ISD fragment ions originating from a simple cleavage.

■ Acknowledgements

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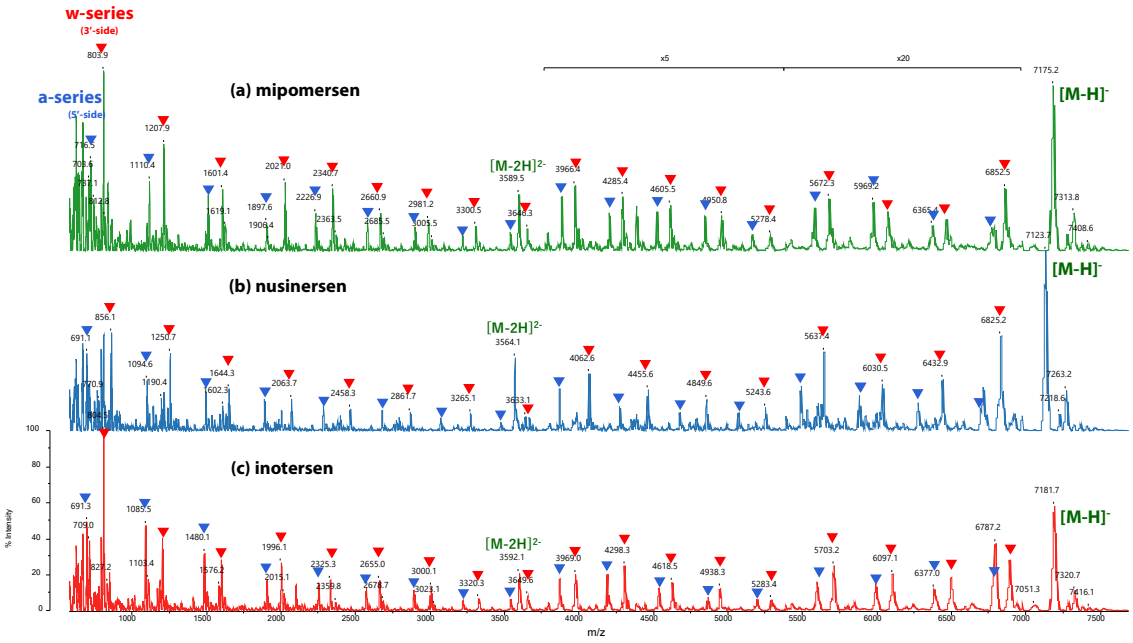

Fig. 4 Negative-Ion MALDI-ISD Mass Spectra of Model Nucleic Acid Therapeutics Using HPA as a Matrix
(a) mipomersen, (b) nusinersen, and (c) inotersen

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