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Morpholino antisense oligonucleotides analyses using a compact matrix-assisted laser-desorption/ionization digital-ion-trap mass spectrometer (MALDI-DIT-MS)

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Abstract:

- Morpholino antisense oligonucleotides were analyzed with a compact matrix-assisted laser/desorption ionization digital-ion-trap mass spectrometer (MALDI-DIT-MS).
- Under the optimal conditions, $[M+H]^+$ of the model analytes were detected with higher sensitivity and the entire sequences were analyzed.

1. Introduction

- As the practical application of oligonucleotide therapeutics rapidly advances, the development of simple and rapid analytical techniques is desired.
- At last year's ASMS, we reported on the analysis of antisense oligonucleotides as single-stranded DNAs and siRNAs using a compact matrix-assisted laser-desorption/ionization digital-ion-trap mass spectrometer (MALDI-DIT-MS).[1)]
- On the other hand, there have been few reports on the analyses of morpholino antisense oligonucleotides. One reason for this may be that collision-induced dissociation (CID) is difficult to obtain their sequence information.
- Recently, sequencing of morpholino antisense oligonucleotides by electron capture dissociation (ECD) combined with LC-MS was reported.[2)]
- Herein, we report morpholino antisense oligonucleotides analyses using MALDI-DIT-MS.

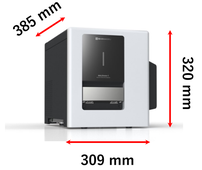
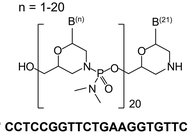


Fig. 1 MALDImini™-1 (MALDI-DIT-MS)

2. Methods

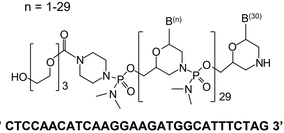
- MALDImini™-1 (Shimadzu Corporation) was used as MALDI-DIT-MS (Fig. 1).
- Viltolarsen (MedChemExpress, CO., Ltd) and eteplirsen (MedChemExpress, CO., Ltd) were used as model analytes of morpholino oligonucleotides (Fig. 2). Analyte solution was prepared as a 20 pmol/μL aqueous solution.
- Several compounds were evaluated as MALDI matrices. Matrix solutions were prepared as a 40 mg/mL acetonitrile/water (50/50, v/v) solution containing 70 mM ammonium citrate dibasic.
- The analyte solution and the matrix solution were mixed at 1:1 (v/v), and 1 μL of the mixture solution was dropped onto the sample plate, to be measured by MALDI-DIT-MS.

Viltolarsen



5' CCTCCGGTTCTGAAGGTGTTC 3'
(MW 6924.82)

Eteplirsen



5' CTCCAACATCAAGGAAGATGGCATTTCTAG 3'
(MW 10300.59)

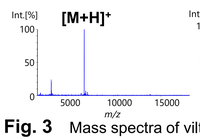
Fig. 2 Structure of viltolarsen and eteplirsen (model analytes)

3. Results

3-1. MW analyses

- Under the optimal conditions for MW analysis, $[M+H]^+$ of analytes were observed with high sensitivity using MALDI-DIT-MS (Fig. 3).
- Particularly, $[M+H]^+$ was observed with higher sensitivity and uniformity of peak detection while suppressing fragmentation by a combination matrix 3-hydroxypiclinic acid (3-HPA)/2, 4, 6-trihydroxyacetophenone (THAP) (3-HPA/THAP).

Viltolarsen



Eteplirsen

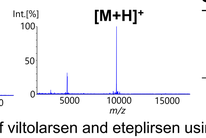


Fig. 3 Mass spectra of viltolarsen and eteplirsen using 3-HPA/THAP as a matrix.

● **Instrumental Conditions**

Dynode voltage (V)	7000
Detector voltage (V)	1300
RF delay (μs)	25

3-2. Sequence analyses

- Under the optimal conditions for sequence analysis, many fragment ion peaks ranging from low to high mass regions were observed for the viltolarsen and eteplirsen (Fig. 4).
- As a result, the entire sequence of them were analyzed.
- At this time, characteristic fragment ion species derived from cleavage specific to MALDI-DIT-MS were detected.

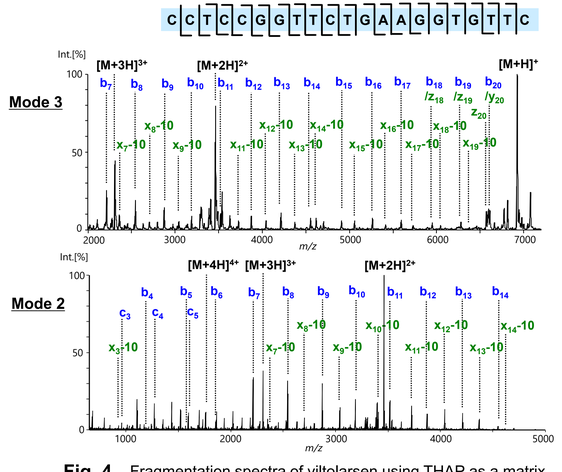
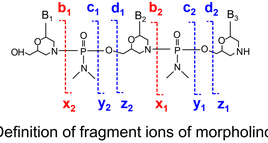


Fig. 4 Fragmentation spectra of viltolarsen using THAP as a matrix.

● **Instrumental Conditions**

Dynode voltage (V)	8000
Detector voltage (V)	1800
RF delay (μs)	17-18



Definition of fragment ions of morpholino antisense oligonucleotides[2)]

3-3. Fragmentation scheme

- We estimated the formation mechanism of the fragment ions and summarized the cleavage scheme (Fig 5).

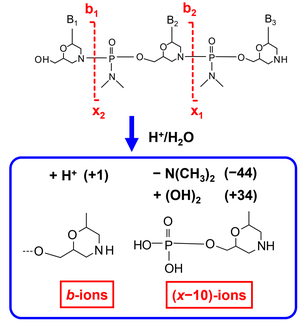


Fig. 5 Hypothesis on the mechanism of *b*-ions and (*x*−10)-ions formation.

4. Conclusions

- MALDI-DIT-MS enabled easy and rapid MW analysis and sequence analysis of morpholino oligonucleotides.
- Combined with last year's results, it was confirmed that a single compact MALDI-DIT-MS could perform MW analysis, sequence analysis including modification sites, and terminal modifications, for antisense oligonucleotides, including morpholino oligonucleotides, and siRNAs.

Reference

1) ASMS (2023) ThP571, Fukuyama *et al.*
2) *Anal. Chem.*(2023) ,95 16352–16358, Karasawa *et al.*

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