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Application News

MALDI-TOF Mass Spectrometry Analysis MALDI-8030
Liquid Chromatograph Mass Spectrometer LCMS™-9030

Analysis of Oligonucleotide Therapeutics using MALDI-8030 and LCMS-9030

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User Benefits

- Confident characterization of oligonucleotides
- Elemental formula confirmation by ESI-QTOF
- Information of sequence by MALDI-ISD

■ Introduction

Molecular characterization of nucleic acids using mass spectrometry is of current growing interest, because oligonucleotide therapeutics are a promising medicine to cure some diseases by working at upper stream of action mechanism with fewer side effects. Whereas a high-resolution accurate ESI mass spectrometer enables an exact intact mass measurement, routine oligo sequence analysis is a still hurdle, using ESI instruments. The complete internal oligo sequence is rarely obtained using typical ESI-MS/MS technique. On the other hand, in-source decay (ISD) using MALDI-TOFMS was reported as a useful method to conduct sequence analysis[1)], although the instruments often employed do not have sufficient specification for exact mass measurement. Here, we report the characterization of oligonucleotide therapeutics using ESI-QTOF, LCMS-9030, and a dual polarity benchtop linear MALDI-TOFMS, MALDI-8030.

■ Measurement Conditions and Samples

- Oligonucleotide

Phosphorothioated oligonucleotides, differing in the structure of the sugar constituents were obtained from GeneDesign (Japan). The sequences of the oligonucleotides are shown in figure 1. All oligonucleotides were prepared at 10 pmol/μL in milliQ water.

- MALDI-TOFMS

3-HPA (3-hydroxypicolinic acid) and ammonium citrate were applied to ISD measurement as matrix and additives, respectively. Matrix solution and samples were layered on a stainless MALDI plate. ISD with negative ion detection was performed on a MALDI-8030, dual polarity benchtop linear MALDI-TOF.

- ESI-QTOF

An exact mass measurement in negative ion detection was conducted using a LCMS-9030. The solvent consisted of 50 mmol/L HFIP, 10 mmol/L DIPEA, and acetonitrile, was applied to the ESI at a flow rate of 0.2 mL/min. The MS range of the QTOF was set as *m/z* 500 to 3000. Deconvolution of ESI spectra was performed with ReSpect in LabSolutions Insight™.

MALDI-8030

LCMS-9030

■ Results

Two oligonucleotides shown in Fig.1 were subjected to an exact mass measurement using the LCMS-9030 in negative mode. Multiply-charged ions of the oligos distributed from $[M-4H]^{4-}$ to $[M-6H]^{6-}$ were observed (Fig. 2 and 3, insets). Exact masses of two oligos were successfully obtained by deconvolution of the ESI spectra. The results are shown in Fig.4. *m/z* 6711.6733 was obtained from the ESI-MS of the LNA-Oligo, and m/z 6431.7241 from the S-oligo. Since the theoretical masses of LNA- and S-oligo were 6711.6731 and 6431.7240 respectively, differences between the observed *m/z* and theoretical one were 0.03 ppm and 0.02 ppm respectively, which enabled the determination of the elemental formula of the two oligos.

Next, the MALDI-8030 was applied to conduct a negative mode ISD analysis of two oligos. A transition from MS measurement to ISD in the instrument is quick and easy by simply increasing laser irradiation. MALDI-ISD ladder sequence confirmation of the two oligos was shown in Fig.5 and 6. Fragment ions, denoted as a- and w-ions, were assigned by matching against the theoretical average masses. w-ions derived from almost the whole oligo sequences were found in the spectra. Only one or two ions derived from the 3'-terminal units were missed due to an overlap with the matrix signals. In the case of the S-oligo, with the exception of two 5'-terminal units, almost a complete series of a-ions were detected. However, in the case of LNA-oligo, the detected a-ions corresponded to those derived from the internal sequence, indicating that a-ions support confirmation of the sequence.

■ Conclusion

Exact mass measurements using the LCMS-9030 resulted in doubtless consistency between the theoretical and observed masses. ISD using the MALDI-8030 resulted in mainly an internal sequence information, which is difficult to obtain with any MS/MS technique. The MALDI-ISD and ESI-QTOF are a useful combination to characterize oligonucleotide therapeutics.

■ Reference

1) Shimizu H, Jinno F, Morohashi A, Yamazaki Y, Yamada M, Kondo T, Asahi S.
J Mass Spectrom. 2012 Aug;47(8):1015-22.

LNA-Oligo	G(L)^5(L)^5(L)^T(L)^5(L)^a^g^t^5(x)^t^g^5(x)^t^t^5(x)^G(L)^5(L)^A(L)^5(L)^5(L)
S-Oligo	g^5(x)^5(x)^t^5(x)^a^g^t^5(x)^t^g^5(x)^t^t^5(x)^g^5(x)^a^5(x)^5(x)

Legend N(L): LNA* (A, T, G) (small character): DNA
5(L): LNA*(5-mC DNA) 5(x): 5-methyl dC
*LNA: Locked Nucleic Acid ^ : Phosphorothioated

Fig. 1 Sequences of oligonucleotides

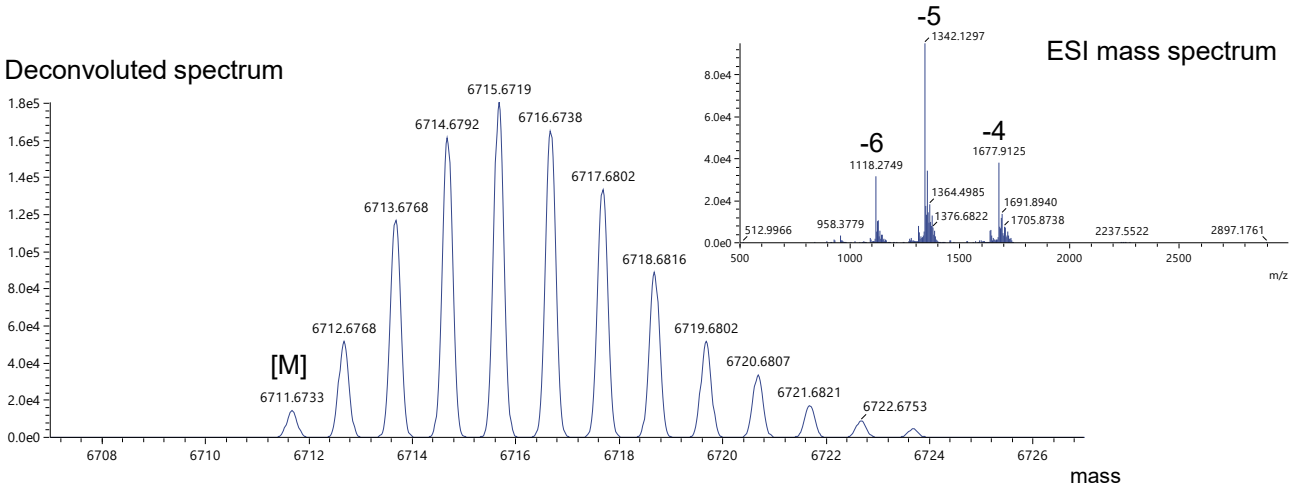

Fig. 2 Exact mass measurement of LNA-Oligo

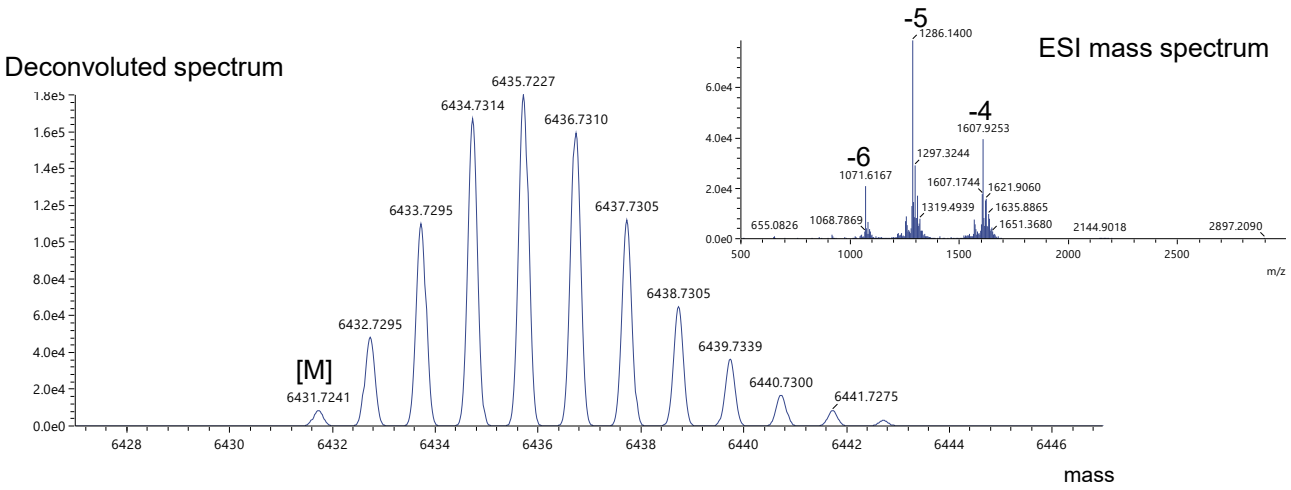

Fig. 3 Exact mass measurement of S-Oligo

LNA-Oligo	$C_{210}H_{264}N_{67}O_{112}P_{19}S_{19}$
calc.[M]	6711.6731
Obs.[M]	6711.6733
diff./ppm	0.03

S-Oligo	$C_{200}H_{264}N_{67}O_{102}P_{19}S_{19}$
calc.[M]	6431.7240
Obs.[M]	6431.7241
diff./ppm	0.02

Fig. 4 Accuracy in the exact mass measurement of the oligos.

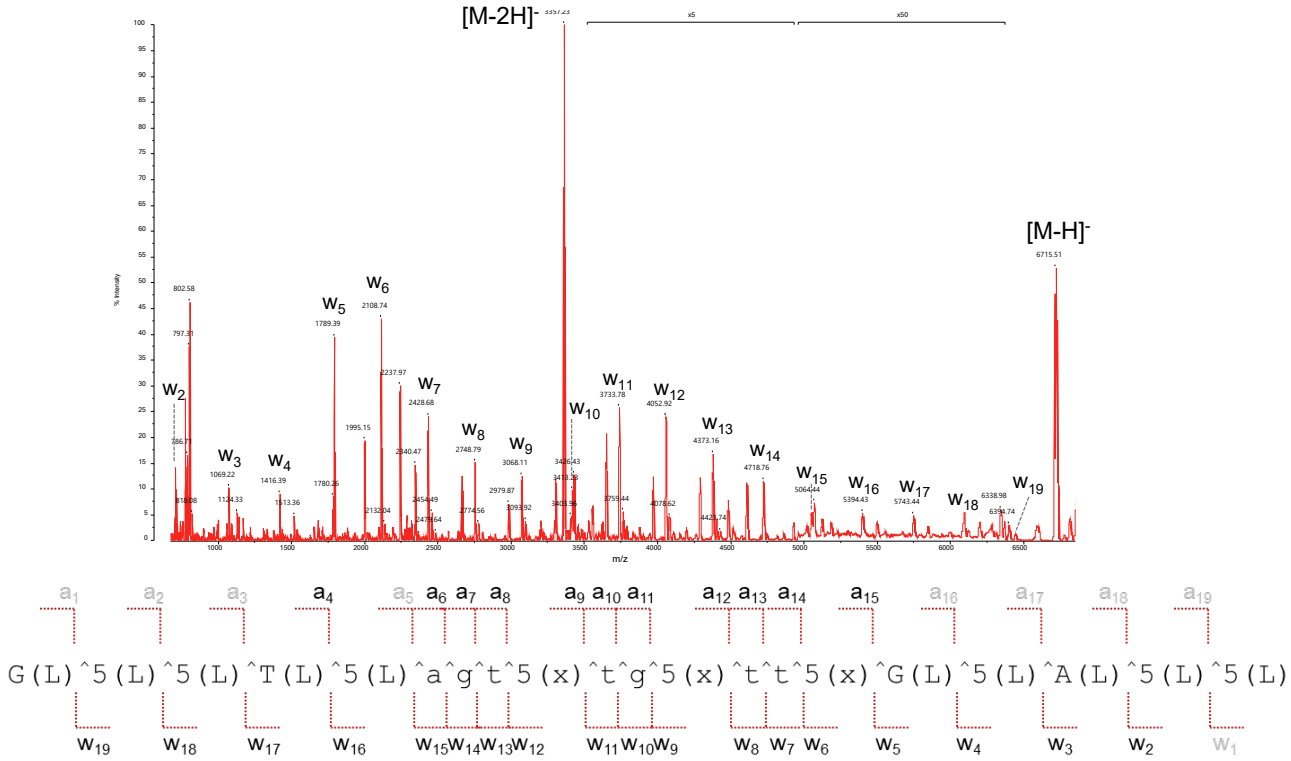

Fig. 5 ISD of LNA-Oligo, and assignment of a- and w-series ions.

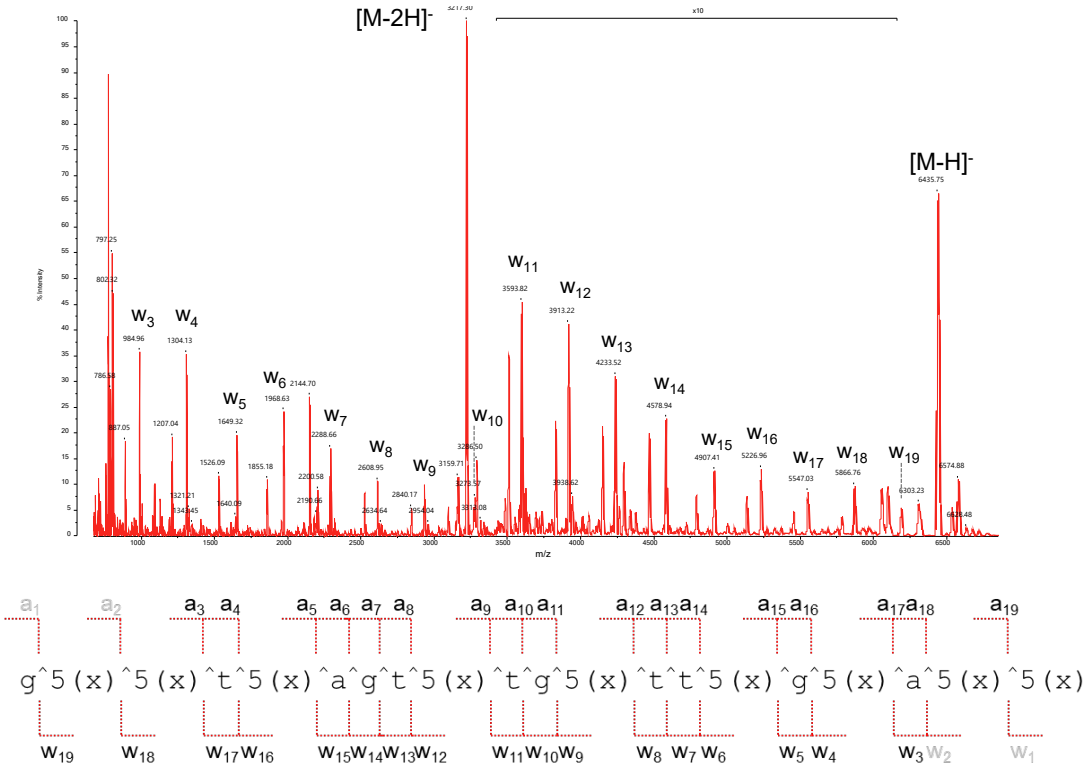

Fig. 6 ISD of S-Oligo, and assignment of a- and w-series ions.

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