Analysis of oligonucleotides using JMS-S3000 "SpiralTOF™-plus 2.0"

Product used : Mass spectrometer (MS)

Introduction

A nucleotide is a compound in which a phosphate group is bound to a nucleoside consisting of a base and a sugar and is a building block of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acid drugs are attracting attention as therapeutic agents for diseases that have been difficult to treat, and as of October 2022, 16 nucleic acid drugs have been approved in Japan, U.S.A., or EU[1]. In recent years, synthesized oligonucleotides have been utilized as nucleic acid medicines. Molecular weight confirmation of synthesized oligonucleotides is important for the quality control of pharmaceuticals. In this report, we used the synthesized oligonucleotide (Table 1) as a sample and measured it with a MALDI-TOFMS.

Table1) The base sequence of synthesized oligonucleotide and *m/z* value.

Sequence	Lowest *m/z* value of $[M+H]^+$	Average *m/z* value of $[M+H]^+$
5'-CGCTAAGTACGCAATGGGCC-3'	6125.0710	6127.9928

Measurement conditions

The matrix 3-HPA (3-Hydroxypicolinic acid) was prepared to 20 mg/mL 50% AcN (20 mM diammonium citrate aqueous solution). A 50 µM aqueous solution of the synthesized oligonucleotide was used as a sample. After mixing the matrix solution and the sample solution at 5:1 (v/v), they were spotted on a target plate, dried in air, and measured in Linear TOF mode (positive ion mode) and SpiralTOF mode (positive ion mode).

Results and summary

Fig. 1 shows the measurement results in Liner and Spiral TOF modes. In both modes, protonated molecules of oligonucleotides were observed as the most intense peaks, and sodium ion adducts were also observed. In the Linear TOF mode, it is difficult to resolve the isotope peaks due to the low mass resolution, but in the SpiralTOF mode, a mass resolution of about 40,000 was obtained, and the isotope pattern was clearly observed. Comparing the simulated isotope pattern (Fig. 2a) and the measured mass spectrum (Fig. 2b), the isotopic peak patterns are well agreed. The mass error of the monoisotopic peak was confirmed to be -6.4 mDa (-1.0 ppm) by the external standard method.

As described above, JMS-S3000 "SpiralTOF™-plus 2.0" was shown to be a very effective analytical tool for nucleic acid analysis.

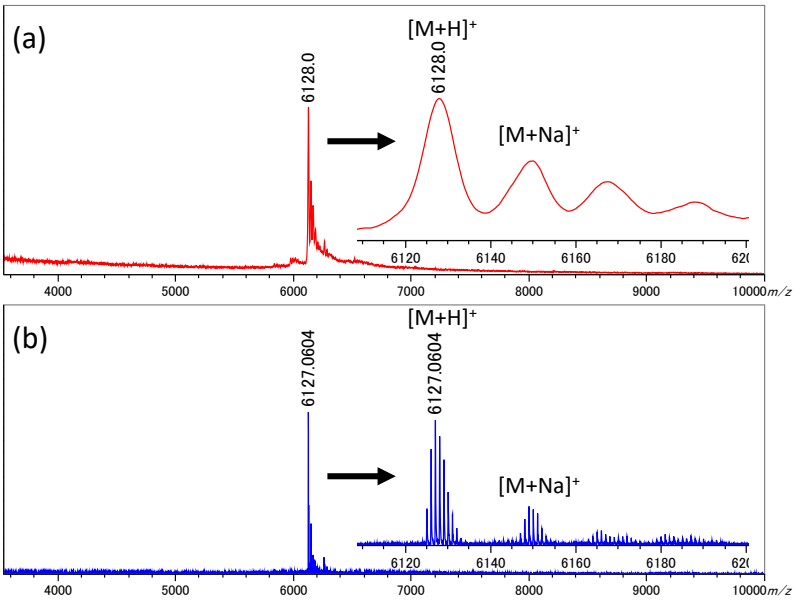

Fig. 1 Mass spectra of LinearTOF mode(b) and SpiralTOF mode(a) .

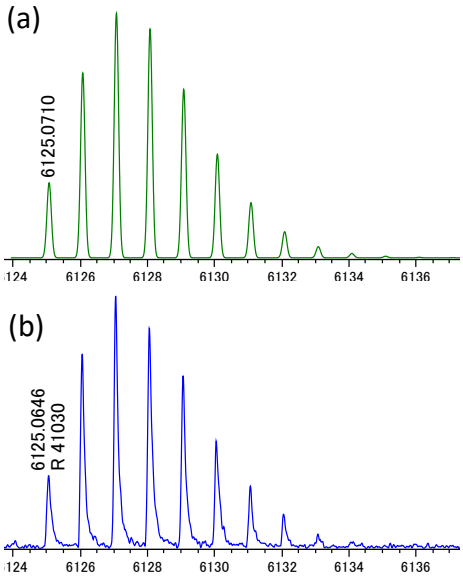

Fig. 2 Comparison of theoretical isotope pattern(a) and obtained isotope pattern of $[M+H]^+$(b).

Reference

1) Web site of the division of molecular target and gene therapy products, national institute of health sciences, Japan (https://www.nihs.go.jp/mtgt/pdf/section2-1.pdf)

Acknowlegement

Sample courtesy of Prof. Masumi Taki, the University of Electro-Communications

JEOL Ltd.
3-1-2 Musashino Akishima Tokyo 196-8558 Japan Sales Division Tel. +81-3-6262-3560 Fax. +81-3-6262-3577
www.jeol.com ISO 9001 • ISO 14001 Certified
▼ Local office
• AUSTRALIA & NEW ZEALAND • BELGIUM • BRAZIL • CANADA • CHINA • EGYPT • FRANCE • GERMANY • GREAT BRITAIN & IRELAND • ITALY • KOREA • MALAYSIA • MEXICO • RUSSIA • SCANDINAVIA • SINGAPORE • TAIWAN • THE NETHERLANDS • USA