

Application News

No. B67

MALDI-TOF Mass Spectrometry

Synthesis Confirmation for Nucleic Acid Medicines - Rapid Sequence Confirmation Using a MALDI-TOF Mass Spectrometer -

Medicines utilizing nucleic acids such as DNA and RNA that control genetic information are called "nucleic acid medicines". These nucleic acid medicines allow targeting of molecules such as messenger RNA (mRNA) and micro RNA (miRNA) which cannot be targeted with traditional low-molecular-weight drugs and antibody medicines, and are expected to be innovative next-generation pharmaceuticals for the treatment of genetic disorders which have been difficult to treat so far.

The functions of nucleic acid medicines are various: small interfering RNA (siRNA) to control protein synthesis by coupling with mRNA, miRNA to strengthen miRNA functions, aptamers that bind to a protein to inhibit its functions, and ribozymes to directly cleave the target RNA are but a few. Their basic structure is a chain comprising a few dozen (deoxy)nucleotides including adenine, thymine, guanine, cytosine, and uracil, which are components of DNA and RNA.

Such nucleic acid medicines can be chemically synthesized without the need to culture cells as in antibody medicines. The obtained molecules are medium-sized having a molecular weight ranging from several thousands to tens of thousands. The confirmation whether or not synthesized nucleic acid medicines are arranged in the intended base sequence is a critical quality characteristic to ensure the action of pharmaceuticals.

This article reports an example of determining a molecular weight and base sequence of synthetic nucleic acid using MALDI-TOF mass spectrometry.

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■ Pretreatment of Synthetic Nucleic Acid

Approx. 200 μ L slurry of cation exchange resin (Dowex 50w 100-200 mesh, Dow Chemical) was activated in 1N hydrochloric acid and washed with water. The resin was added to an empty microspin column (BioRad) and centrifuged to remove the solution components.

Then 10 μ L of sample solution (base sequence: 5'-CTGAGACACTGAAGGTAGGA-3', 100 pmol/ μ L) was applied to the column and centrifuged. The sample solution that passed through the cation exchange resin was collected.

1 μ L of the collected solution was loaded on the MALDI target plate, and the matrix solution (0.5 μ L) was overlaid and dried, and then analyzed.

■ MALDI-TOF Mass Spectrometer

For the analysis, a benchtop MALDI-TOF mass spectrometer "MALDI-8020" (Fig. 1) was used. This instrument maintains the performance of AXIMA Assurance while achieving significant downsizing. A 200 Hz solid laser is employed for the ionization laser and a fully automated ion source cleaning device and an oil-free diaphragm pump are incorporated.



Fig. 1 Appearance of Benchtop MALDI-TOF MS "MALDI-8020"

■ Mass Spectrometry

As the result of mass spectrometry of the sample, a singly-charged ion was detected at m/z 6214.5.

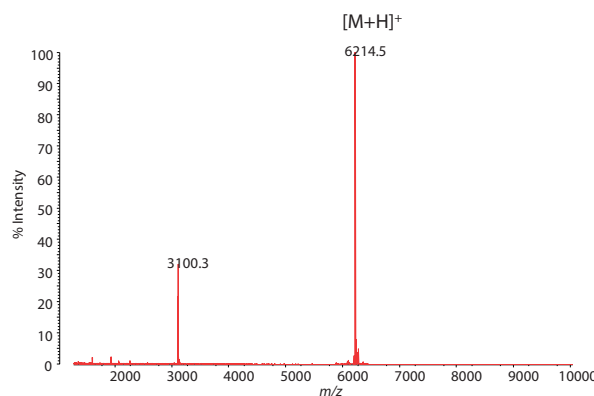


Fig. 2 MALDI-TOF Mass Spectrum of Synthetic Nucleic Acid
Measurement mode: Positive Linear

Next, in-source decay (ISD) analysis of the sample was performed. As the result, the ISD spectrum of the synthetic nucleic acid was obtained as shown in Fig. 3. In the ISD process for nucleic acid measurement in the positive ion mode, cleavage at the w series occurs in preference to others so a very simple spectrum can be obtained.

By assigning the fragment ions of this ISD spectrum, it is possible to obtain the sequence information of the nucleic acid easily.

As demonstrated above, the benchtop, positive linear mode-only MALDI-TOF mass spectrometer "MALDI-8020", can confirm the molecular weight and sequences of synthetic nucleic acids easily.

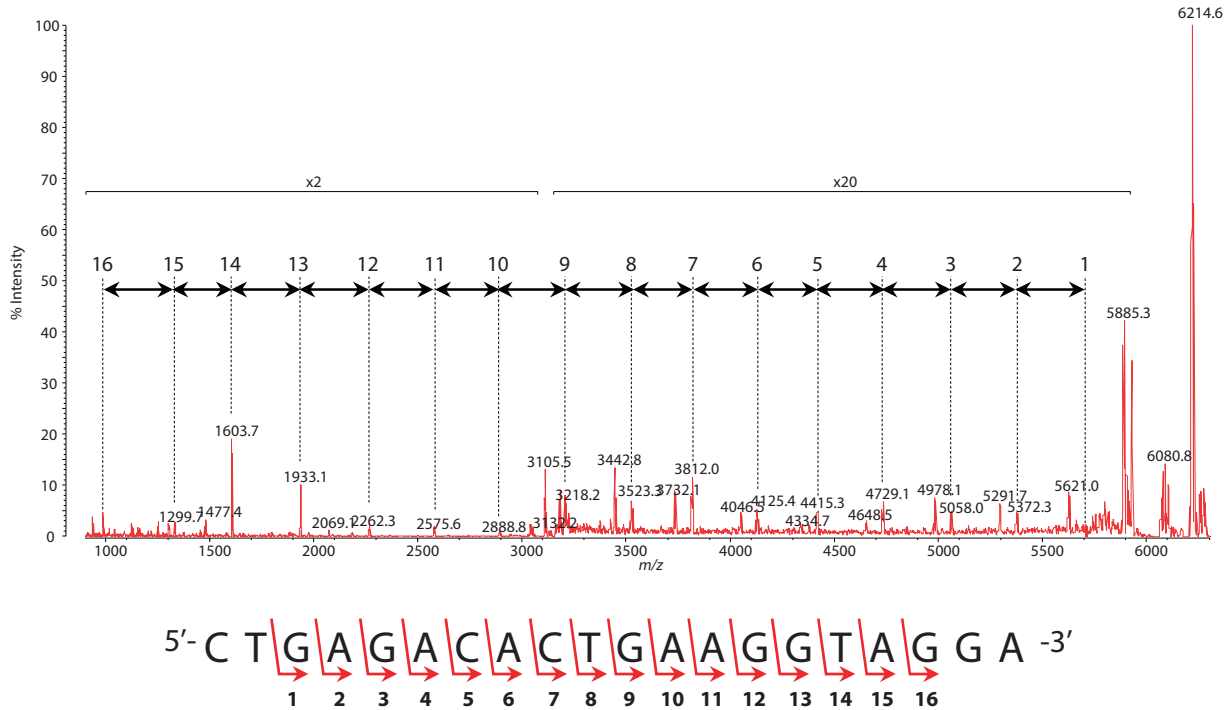


Fig. 3 MALDI-ISD-TOF Mass Spectrum of Synthetic Nucleic Acid and Relegated Cleavage Ions
Measurement mode: Positive Linear

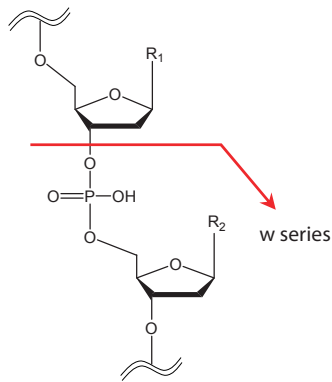


Fig. 4 Cleavage Site in Synthetic Nucleic Acid in Positive Mode ISD