SHIMADZU

# Application News

MALDI-TOF Mass Spectrometry No.B33

## RNA Sequence Analysis using the Acid-Hydrolysis Method

Oligonucleotides with various types of sequences, with antisense effect as well as RNA interference effects, have been synthesized in the course of nucleic acid drug development. Although quality control of synthetic oligonucleotides is essential, no standard method has been established for base sequencing of oligonucleotides with relatively few bases, in the order of 20-30 bases. Thus, there is need for a simple, yet highly reliable sequence analysis method.
Here we investigated RNA sequencing analysis using the acid-hydrolysis method.

We conducted investigation of acid-hydrolysis of a 21-base synthetic siRNA and 2'-O-methylated siRNA. A mixed solution of a low molecular weight matrix (3-hydroxypicolininc acid: 3HPA) and acid (trifluoroacetic acid: TFA) was added to the sample solution, and the mass spectrum was acquired by MALDI-TOF-MS. As a result, we were able to identify the entire 19-mer sequence except for 2 bases at the 3'-terminal (Fig. 1), and were also able to verify that this is also effective for the 2'-O-methylation modified RNA (Fig. 2).

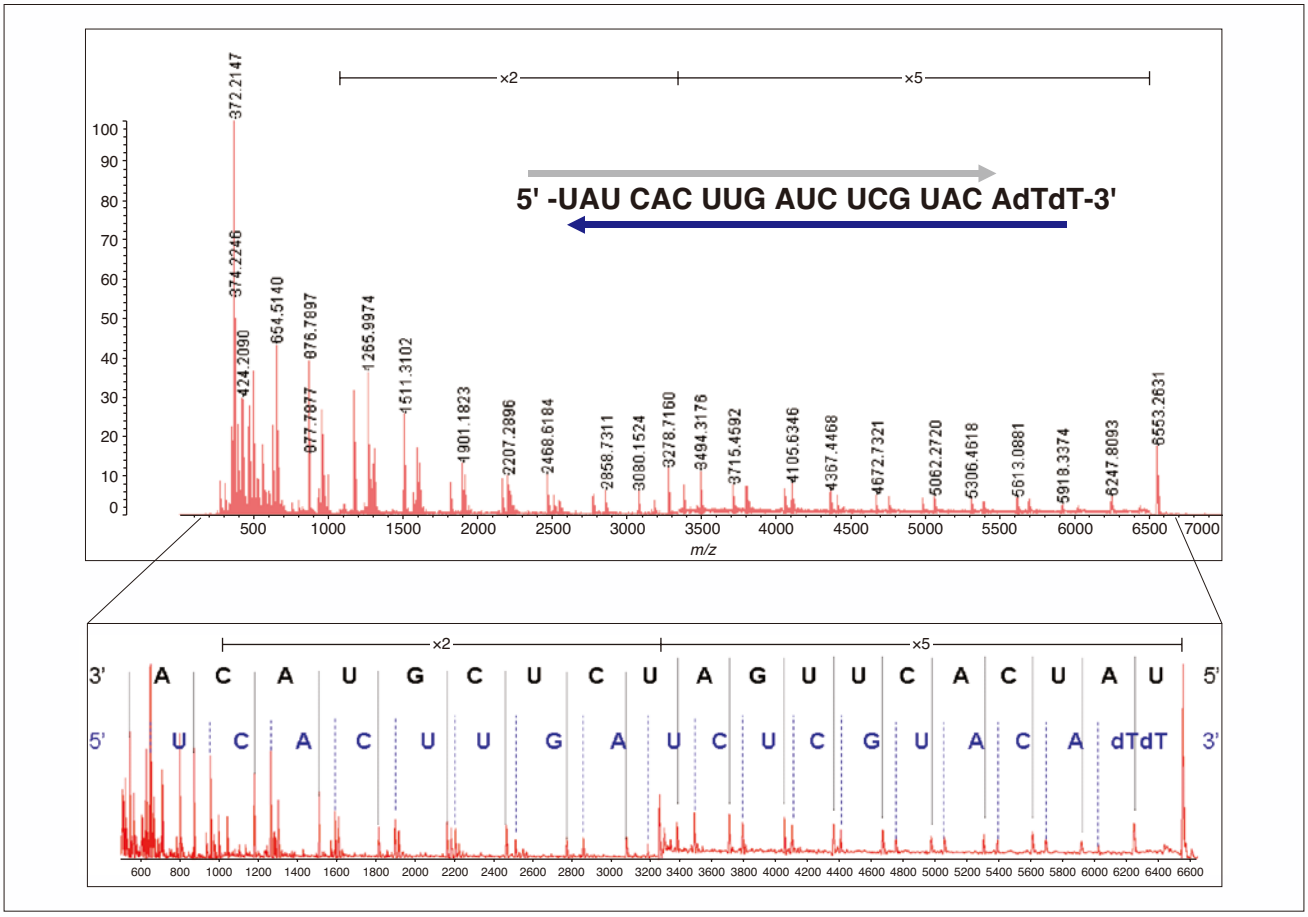


**Fig.1 Mass Spectra of siRNA (21-mer) after Acid Hydrolysis**

### ■ Measurement Conditions

Instrument : AXIMA *Assurance*
Measurement conditions : Positive/linear mode
Sample : siRNA 21 mer 5'-UAU CAC UUG AUC UCG UAC AdTdT-3' (SIGMA)
Matrix : 50 mg/mL 3HPA in 2.5 % TFA aq. + 10 mg/mL diammonium hydrogen citrate

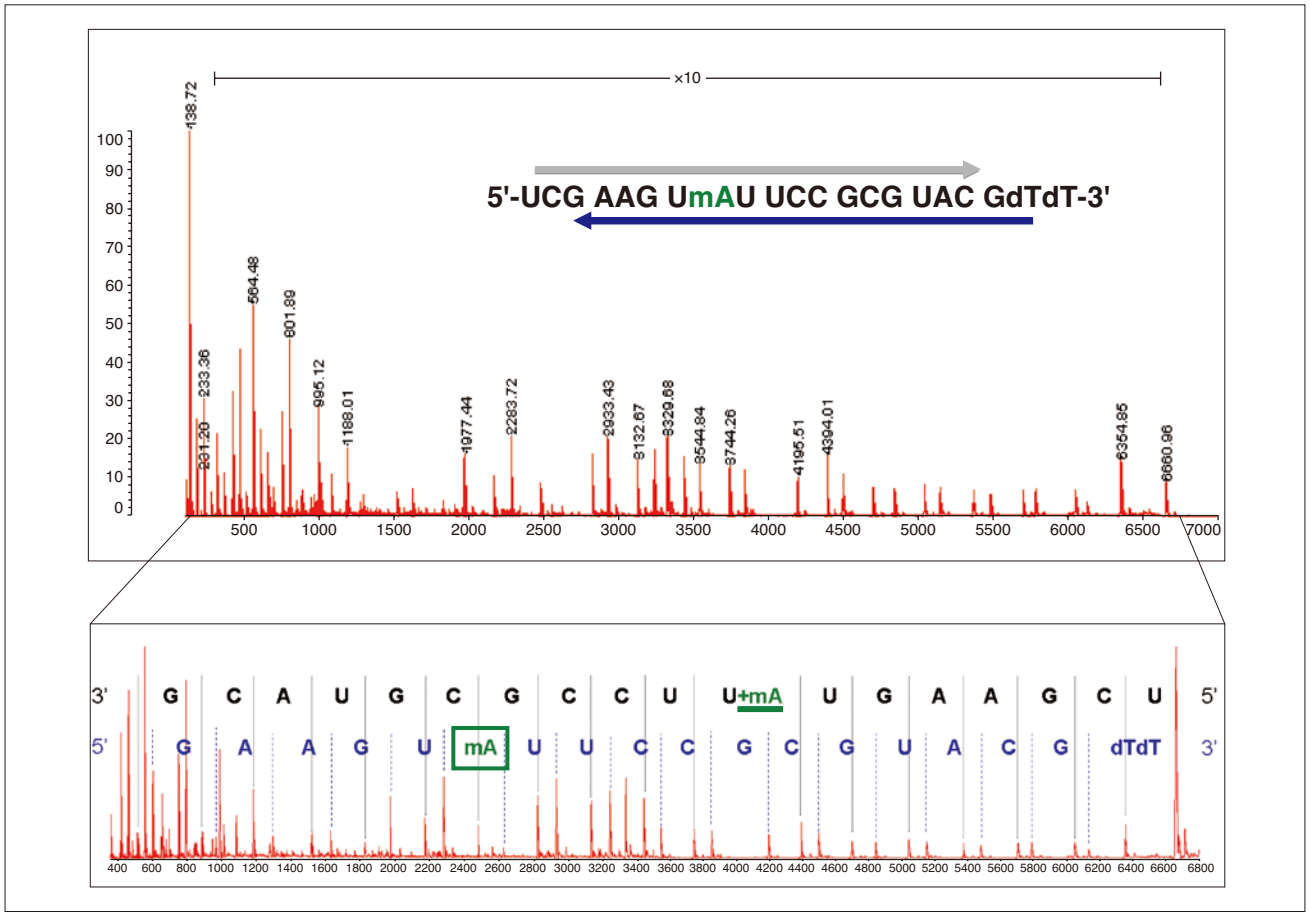


**Fig. 2 Mass Spectra of siRNA (mA: Containing 2'-O-methyl adenosine) after Acid Hydrolysis**

Ladder-shaped peaks due to acid hydrolysis were detected as shown in both Fig. 1 and Fig. 2, and RNA base sequencing was possible by reading the mass differences between peaks. Highly accurate base sequence information was obtained because the sequence ladder was detected one base at a time from both the 3'-terminal and 5'-terminal.

In addition, the optimum concentration of TFA was investigated for determining the best analysis conditions (Fig.3). As a result, the best spectrum information was obtained using a final concentration of 2.5 %.

The combination of MALDI-TOF-MS and use of the acid-hydrolysis was confirmed to be a powerful technique providing fast and easy base sequencing of RNA sequences of approximately 20 bases.

The AXIMA *Assurance* was used for measurement here. The same measurement can be performed with the AXIMA *Confidence* and AXIMA *Performance*.

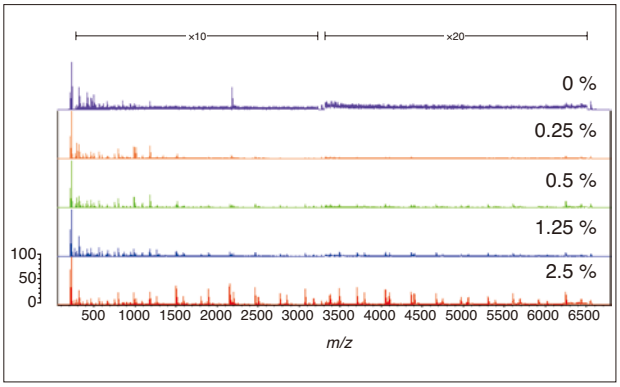


**Fig. 3 Verification of Best TFA Concentration**

[References]
Anal. Chem., 2009, 81, 3173-3179.

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