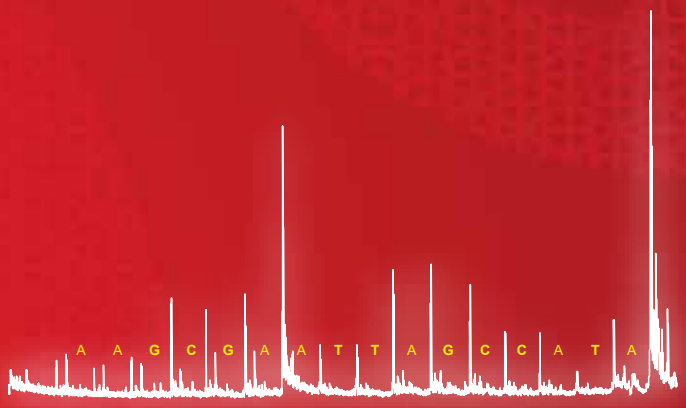
Access to your success

SHIMADZU

Oligonucleotide Mass Analysis and In-Source Decay Sequencing on a MALDI TOF Mass Spectrometer

AXIMA™

- Accurate mass determination of oligonucleotides is possible in both linear and reflectron modes when using MALDI TOF mass spectrometry
- No additional sample clean-up is employed
- In-source decay provides a rapid and simple method for the sequence determination of oligonucleotides
- No extra preparation or cost is incurred in order to generate sequence information
- Mass determination and in-source decay may be automated allowing unattended acquisition of large numbers of samples



Oligonucleotide Mass Analysis and In-Source Decay Sequencing on a MALDI TOF Mass Spectrometer

Introduction

Mass spectrometry has the capacity to analyze oligonucleotides more quickly and accurately than many other techniques. These important factors become critical in a high throughput environment, for example in the case of industrial primer synthesis where tens of thousands of samples may be generated each day. Here, it is vital that the product quality is of a high standard and that the correct sequence has been generated.

Limitations arise when the nature of the sample is considered. For example, electrospray experiments may be difficult due to the high salt content of synthesised primers that can suppress the ionization process, produce cationic salt adducts, and promote multiple charge states making spectral interpretation more complicated.

It has been previously demonstrated that MALDI TOF mass spectrometry can efficiently analyze large numbers of samples under fully automated conditions with accuracy sufficient to identify a primer by its expected intact mass. This methodology is not labour intensive and requires little or no sample clean-up prior to analysis. The resultant spectrum is usually uncomplicated and easy to interpret. This process has subsequently been accepted by numerous enterprises as an industry standard method of quality control.

Following on from these simple mass identification experiments, we have progressed to oligonucleotide sequencing using MALDI mass spectrometry. It has been well documented that this type of sample may be sequenced using sophisticated DNA sequencers or by enzymatic digestion followed by mass spectrometry. Here we will present a simple and speedy method of sequencing oligonucleotides that requires no further sample preparation and little expertise.

Principle - In-Source Decay (ISD)

- In typical MALDI spectra, the laser power employed is usually held at threshold level in order to achieve the highest resolution and hence mass accuracy possible.

- This threshold level of laser power usually helps to maintain the integrity of the sample and minimize fragmentation during analysis.

- However, when the laser power is increased by around 20-30%, fragmentation may be observed in the form of additional peaks at lower mass in the spectrum. In the case of oligonucleotide analysis, these peaks form a “ladder” from which sequence information may be ascertained.

- The mass difference between each fragment peak may be determined simply by subtracting one mass from the next - this delta mass corresponds to the base lost.

- Masses of the bases are

- dC = 289.2 Da
- dT = 304.2 Da
- dA = 313.2 Da
- dG = 329.2 Da

- The sequence (or partial sequence) may then be read off the spectrum.

- An added advantage of this type of sequence experiment is the fact that it can be carried out on the same sample spot as the intact mass analysis was acquired from without the need to re-prepare or enzymatically digest the sample. This obviously also has far lower cost implications.

Methods

3-hydroxypicolinic acid (3-HPA) was prepared in 50% aqueous acetonitrile at a concentration of 50 mg/ml. Diammonium hydrogen citrate (50 mg/ml in 50% aqueous acetonitrile) was added to the matrix in a 1:1 v/v ratio. The matrix solution (0.5 μl) was spotted onto a regular stainless steel 384 well MALDI target and 0.5 μl of oligonucleotide sample added. No sample clean-up was carried out in order to speed up the preparation time.

External calibration was achieved using two standard oligonucleotides, a 20-mer and a 40-mer of known sequence and mass (Figure 1).

Positive ion mass spectra were acquired using an AXIMA™ *Assurance* in linear mode or an AXIMA™ *Confidence* incorporating a curved field reflectron, in reflectron mode. The mass range acquired was set at 900-15,000 Da. Both mass spectrometers comprise a UV nitrogen laser (337nm), near ultra-high vacuum system with a novel laser beam focussing mechanism for increased ion extraction efficiency and increased sensitivity, an integrated transient recorder and optimized ion optics for high resolution accuracy. In the case of the AXIMA™ *Confidence*, a beam blanker was employed to remove interfering matrix ions and was set at 900 Da.

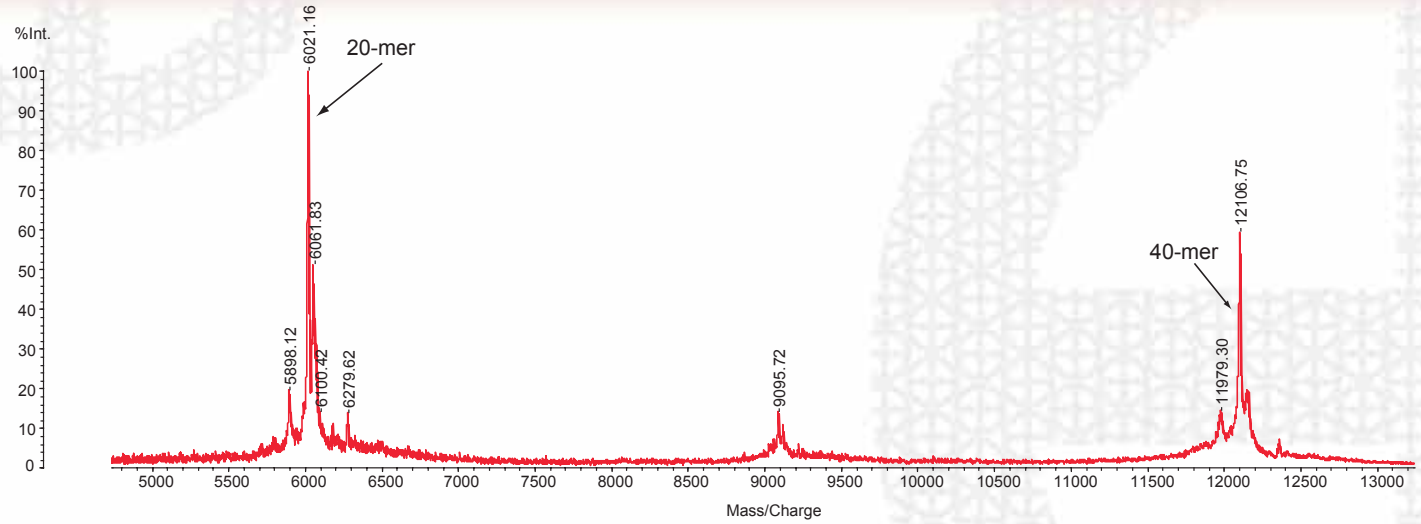


Figure 1. Typical positive ion linear mode external calibration spectrum using 20-mer and 40-mer

Results

A commercially prepared 20-mer was analyzed in positive ion linear mode identifying the primer average mass. This spectrum is displayed in Figure 2. The zoom view of the primer clearly shows the $(M+H)^+$ ion and two salt adducts corresponding to $(M+Na)^+$ and $(M+K)^+$.

The threshold level laser power used to generate this spectrum was increased by 20-30% in order to promote in-source decay of the parent ion. The resultant spectrum, shown in Figure 3, clearly shows a "ladder" of fragment ions the mass difference of which may be easily calculated to determine the loss of a particular base. The sequence of the oligonucleotide may then be simply read from the spectrum.

The sequence determined was then compared with the sequence provided with the sample.

Sequence provided :

5'-ATA CCG ATT AAG CGA AGT TT-3'

Sequence determined from Linear ISD spectrum :

ATA CCG ATT AAG CGA A

The sequence coverage was calculated as 80% of expected sequence.

This sample was also analyzed in positive ion reflectron mode using the AXIMA™ Confidence . The resultant spectrum is shown in Figure 4. It may be observed that there is a clear improvement in resolution of the parent primer ion. This resolution is to the baseline and calculated to approximately 20,000. This increase in resolution in turn allows more accurate mass assignment.

The in-source decay experiment was also carried out in positive ion reflectron mode and the results are displayed in Figure 5. The "ladder" sequence is again clearly identifiable and oligonucleotide sequence information obtained. However, the signal-to-noise ratio is reduced when compared to the same experiment performed in linear mode. This is due mainly to the fragile nature of the sample and the reduction in sensitivity imposed by the reflectron when analyzing higher masses. Nevertheless, the sequence coverage observed is equal to that found when operating in linear mode.

In addition, as the only alteration in acquisition conditions is the increase in laser power, this whole process may be simply automated. This process would allow determination of primer mass and provide key sequence information that may be used in a quality control environment.

Furthermore, ISD allows the determination of the actual sequence from an apparently correct intact mass that may be inadvertently passed as a successful synthesis, when actually a sequence difference via substitution of bases has occurred.

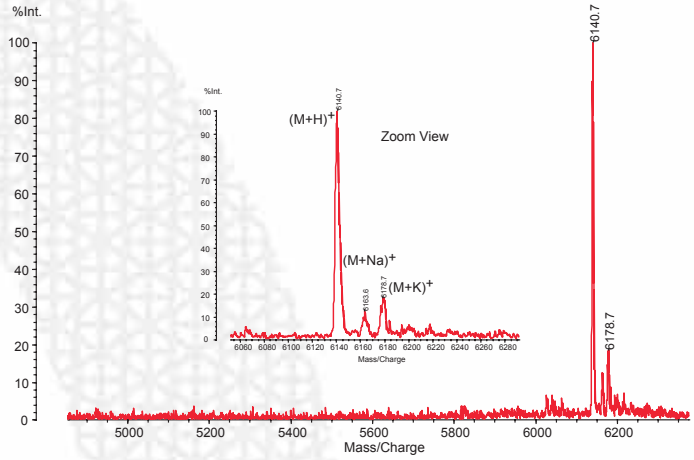


Figure 2. Positive ion linear mode spectrum of commercially available 20-mer

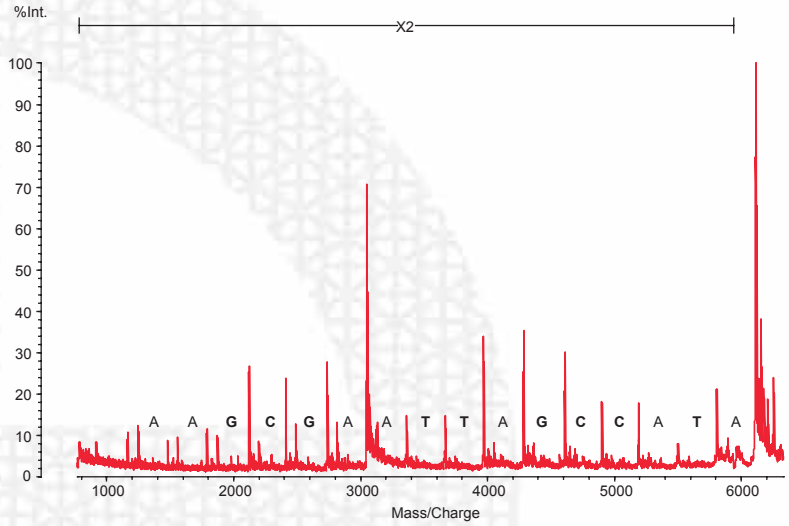


Figure 3. In-source decay spectrum (positive ion linear mode) of commercially available 20-mer

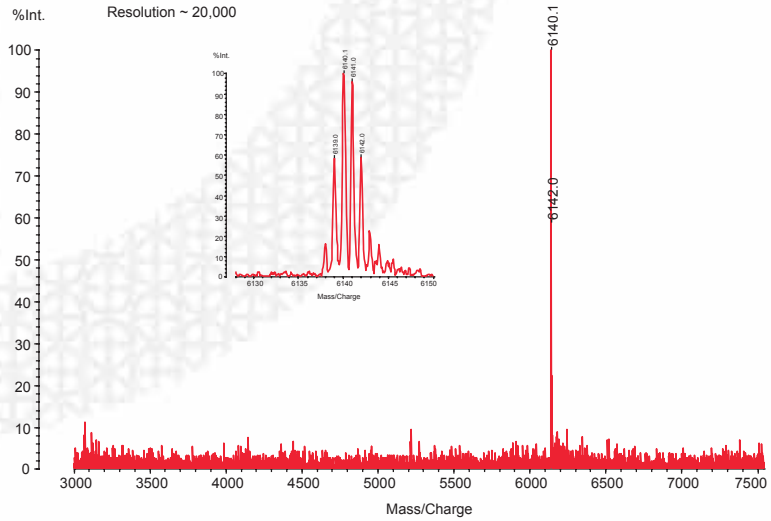


Figure 4. Positive ion reflectron mode spectrum of commercially available resolution

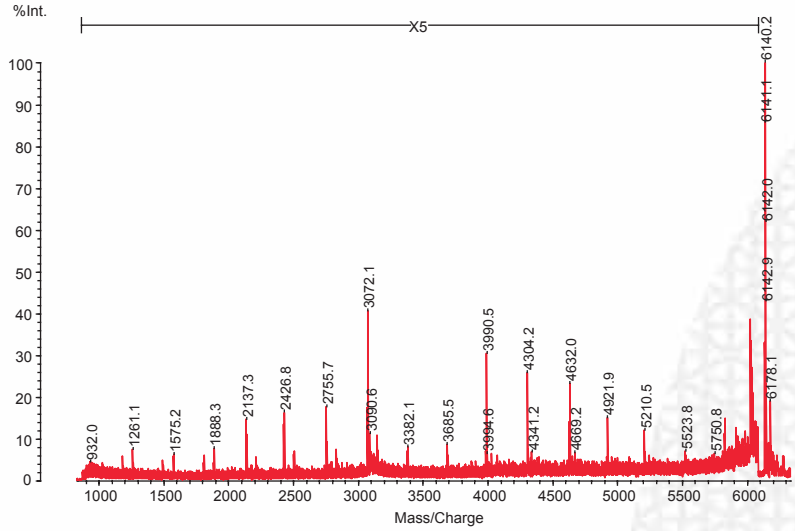


Figure 5. In-source decay spectrum (positive ion reflectron mode) of commercially available 20-mer

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