APPLICATION

MALDI

An optimised sample preparation for the MALDI-ToF-MS of oligonucleotides

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Nowadays the size of DNA fragments is determined routinely using gel electrophoresis. This method however is time consuming and inaccurate (±10%)[1]. In large part due to extensive sequencing projects (e.g. human genome project) there is a need for an alternativ method that is fast, accurate and suitable for automation.

MALDI-ToF-MS was introduced by Karas and Hillenkamp[2] and by Tanaka et al.[3] in 1988. Since then MALDI-ToF-MS developed thanks to its sensitivity, flexibility and speed into an alternative method to classical gel electrophoresis. Currently this method is used for the analysis of natural and synthetic oligonucleotides e.g. for the quality checking of primers or PCR (Polymerase Chain Reaktion) products and even for sequencing[4]. Usually oligonuclotides are measured as negative ions with 3-hydroxypicolinic acid as matrix[5]. Due to their many phosphate groups, oligonucleotides tend to form heterogeneous adducts, especially with alkali ions which results in low resolution (R = M/ΔM) and poor signal/noise ratio. In figure 1 the spectrum of $d(T)_{65}$-oligonucleotides is shown.

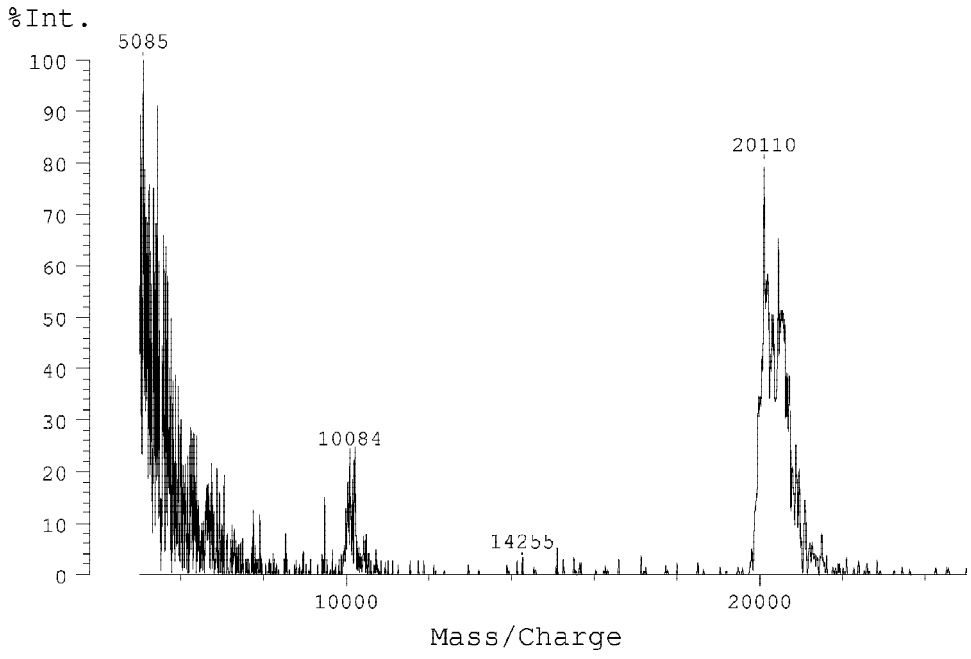

Figure 1: Negative ion spectrum of $d(T)_{65}$-oligonucleotides with 3-hydroxypicolinic acid as matrix

[1] Weber K. and Osborn M.: J. Biol. Chem. 244: 4406 (1969)
[2] Karas M., Hillenkamp F.: Anal. Chem. 60: 2299-2301 (1988)
[3] Tanaka K., et al.: Rapid Commun. Mass Spectrom. 2:151-153 (1988)
[4] Taranenko N.I.: Rapid Commun. Mass Spectrom. 11: 386-392 (1997)
[5] Wu K.J.:Rapid Commun. Mass Spectrom. 7: 142-146 (1993)

In this spectrum there is a broad, unresolved peak due to the many different adducts formed by the oligonucleotides. This effect can be minimised by replacing the metal ions by ammonium ions[6]. One way to do this is to add an ion exchange-resin directly onto the sample slide[7]. This method does help to optimise the measurement of oligonucleotides but it has a poor reproducibility and is difficult to automate. Another possibility is the addition of a surplus of ammonium ions in the form of ammonium hydrogen citrate onto the sample slide directly[8].

Ion exchange by the addition of a surplus of ammonium hydrogen citrate

Experimental

The following solutions were applied to the sample slide, mixed and dried in a stream of air.

- 0.5 µl HPA (40 mg/mg in acetonitrile/H_20 (50/50))
- 0.5 µl oligonucleotide solution (a few pmol)
- 0.5 µl ammonium hydrogen citrate (0,3M)

In figure 2 two spectra are compared which demonstrate the effect of the addition of ammonium hydrogen citrate.

As the comparison of the two spectra shows, with the addition of ammonium hydrogen citrate the resolution increased from R = 35 to R = 47 whereas the signal/noise ratio remained the same.

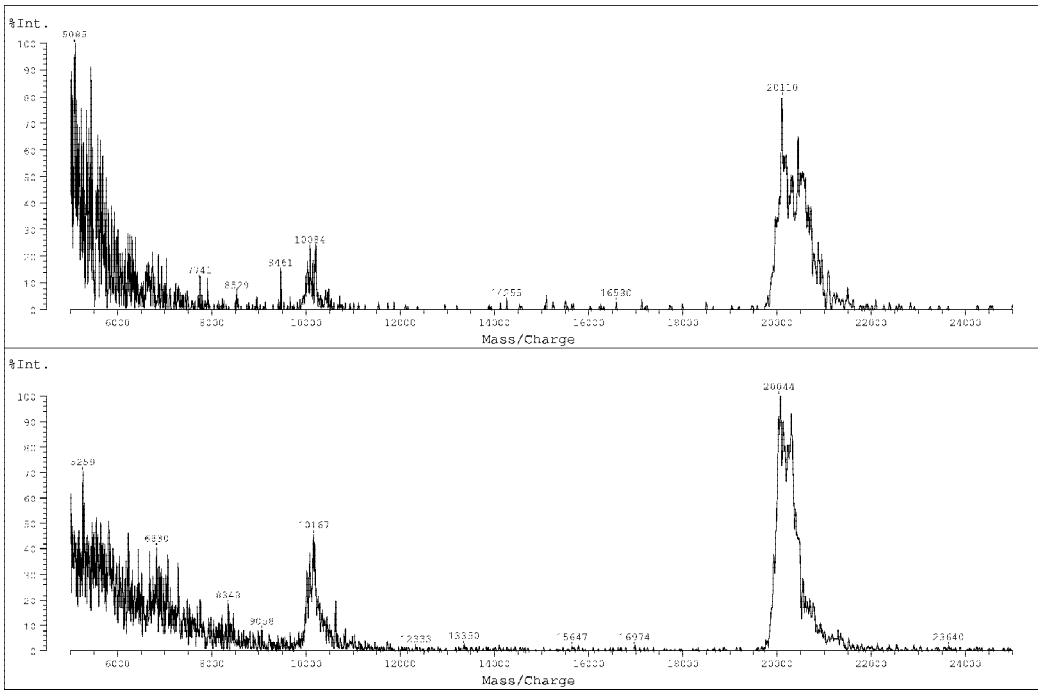

Figure 2: Negative ion spectra of $d(T)_{65}$-oligonucleotides with 3-hydroxypicolinic acid as matrix without and with addition of ammonium hydrogen citrate.

[6] Nordhoff E. et.al.: Rapid Commun. Mass Spectrom 6: 771-776 (1992)
[7] Bahr U., Karas M., Hillenkamp F.: J. Anal. Chem. 348: 783-791 (1994)
[8] Zhu Y.F.: Rapid Commun. Mass Spectrom. 10: 1591-1596 (1996)

“on probe purification”

A further method to optimize the measurement of oligonucleotides is the “on probe purification” described by Liu et al.[9]
By a pre-coating of the sample slide with nitrocellulose[10] (NC) the problems due to the presence of salt, buffer and other contaminants normally contained in DNA samples are reduced resulting in a better signal/noise ratio.

Experimental
The following solutions were applied to the sample slide and dried in a stream of air after every step.

- 2 µl NC solution (16 mg NC/ml acetone)
- 0.5 µl HPA
- 0.5 µl oligonucleotide solution (a few pmol)
- 0.5 µl HPA

In figure 3 two spectra are compared which show the effect of the pre-coating of the sample slide with nitrocellulose.

A comparison of the two spectra shows that the resolution has hardly been influenced by the pre-coating of the sample slide, however the signal/noise ratio was significantly improved.

Because of the results shown the two methods have been combined to optimize the resolution and the signal/noise ratio in one step.

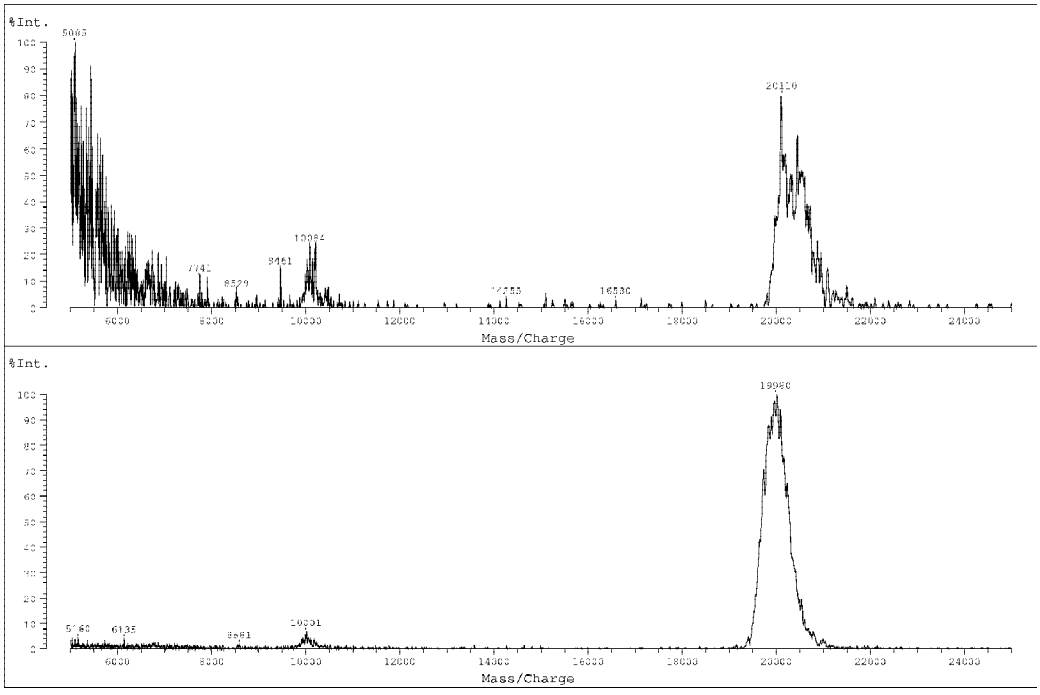

Figure 3: Negative ion spectra of $d(T)_{65}$-oligonucleotides with 3-hydroxypicolinic acid as matrix without and with nitrocellulose pre-coating of the sample slide.

[9] Liu Y.H. et.al.: Anal. Chem. 67: (No. 19) 3482-3490 (1995)
[10] Schleicher & Schüell: Protean BA85 (0,45µm)

Optimization of MALDI-ToF-MS of oligonucleotides by pre-coating the sample slide with nitrocellulose and the addition of ammonium hydrogen-citrate.

Experimental

The following solutions were applied to the sample slide and dried in a stream of air after every step.

- 2 µl NC solution (16 mg NC/ml acetone)
- 0.5 µl matrix
- 0.5 µl oligonucleotide solution (a few pmol)
- 0.5 µl matrix

For this method the matrix is prepared as follows: HPA (80 mg/ml in acetonitril/H_2O (50:50)) is mixed proportionally (50:50) with an ammonium hydrogen citrat solution (0.3M).

In figure 4 four spectra are compared showing the effect of the various optimization steps.

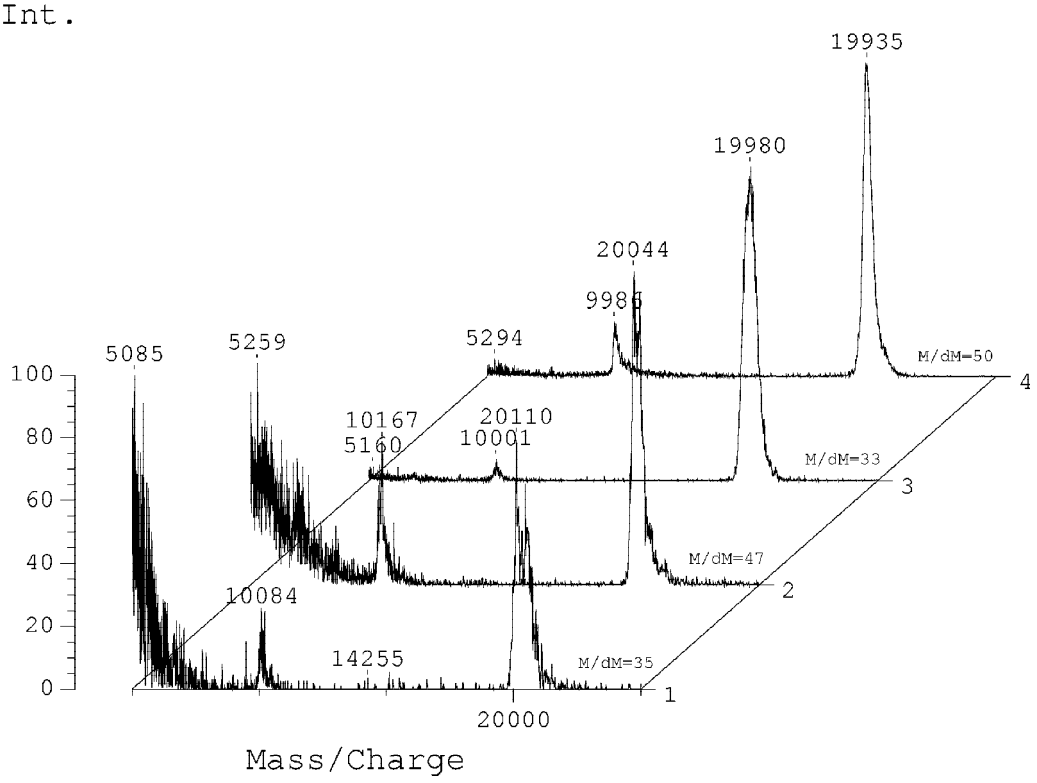

Figure 4: Negative ion spectra of $d(T)_{65}$-oligonucleotides with 3-hydroxypicolinic acid as matrix:

1. Without addition of ammonium hydrogen citrate or pre-coating of the sample slide with nitrocellulose
2. With addition of ammoniumhydrogencitrate
3. With pre-coating of the sample slide with nitrocellulose
4. Combination of the two methods

Summary

By means of the spectra shown it has been demonstrated that MALDI-ToF-MS is a real alternative method for the measurement of oligonucleotides. Resolution and signal/noise ratio were optimized with the help of a simple method. The complete sample pre-treatment was carried out on the sample holder. This holds the possibility for automation which will further increase the productivity of this already fast method.

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