Fast analysis of oligonucleotides by nano-liquid chromatography-nano-electrospray mass spectrometry allowing determination of base-composition.

GHENT UNIVERSITY

A. Willems[1], D. Deforce[2], W. Lambert[3], E. Van den Eeckhout[2], C. Van Peteghem[3] and J. Van Bocxlaer[1]

[1]Laboratory of Medical Biochemistry, [2]Laboratory of Pharmaceutical Biotechnology, [3]Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium

1. Introduction

Polymerase chain reaction (PCR) technology is widely used in DNA sequencing and DNA analysis. In that respect, the availability of PCR primers and synthetic oligonucleotides is very important. These oligonucleotides have to be very pure and a defect in length or sequence is not tolerated. Therefore, each oligonucleotide should be tested after synthesis. The quality control method used for this purpose must be rapid, low cost and reliable, and use minimal labor (1). Mass spectrometry is able to detect length and sequence variations based on a difference in mass. Electrospray ionization mass spectrometry (ESI-MS) has become one of the most important mass spectrometric techniques for the analysis of oligonucleotides. However, the major difficulties arise from the adduction of non-volatile cations such as sodium or potassium to the polynucleotide backbone. Replacement of sodium ions with ammonium has been shown to considerably reduce sodium adduct formation in ESI-MS (2). We developed a nano-liquid chromatography (LC)-nano-ESI-MS method combined with column switching for the on-line removal of salt ions.

2. Experimental

Oligonucleotide: 5'-CCCTGGGCTCTGTAAAGAATAGTG-3' (Perkin Elmer) (MW = 7392.8, concentration = 25 pmol/µl). Before analysis, the samples were diluted 1 to 1000.

LC Conditions:

• Columns:

Pre-column: 300 µm, C18 Pepmap® (LC Packings)

Nano-column: 75 µm*150 mm, 3 µm, C18 Pepmap® (LC Packings)

• Gradient:

The pre-column was loaded with 0.4 M 1,1,1-3,3,3-hexafluoroisopropanol (HFIP) in water, pH 7.0 (including 10 mM ammonium acetate) at a flow rate of 12 µl/min.

The gradient consisted of 0.4 M HFIP in water (pH 7.0) (solvent A) and 0.4 M HFIP in 50/50 methanol/water (pH 7.0) (solvent B) and was started from 100% A to 100% B at a flow rate of 125 nl/min.

• Autosampler: Famos (LC Packings)

• Column switching: Switchos (LC Packings)

• Pump: Ultimate (LC Packings)

MS Conditions:

• Mass Spectrometer: Micromass Q-TOF hybrid mass spectrometer

• Ion Source: nano-electrospray source (Z-spray®) in negative ion mode

• Capillary voltage: 2200 V

• Cone voltage: 50 V

3. Results and discussion

In a first set of experiments, the samples were diluted in HPLC water and the pre-column was loaded with 0.4 M HFIP in water (pH 7.0). In this way, the oligonucleotide was captured at the top of the pre-column and impurities were removed. After 4 minutes the micro-trapping column was back flushed using the separation column gradient and the oligonucleotide was transferred to the analytical nano-column. In this system, the retention time of the oligonucleotide was around 8.5 minutes. The resulting mass spectrum contained an envelope of peaks, which corresponded to the multiple charge states. But also sodium and potassium adducts were observed (Figure 1). The deconvulted spectrum showed the molecular mass 7393 of the oligonucleotide and also salt adducts (Figure 2). These bound cations reduced sensitivity for the analyte. Reduction of the amount of adducts is necessary to obtain high quality spectra.

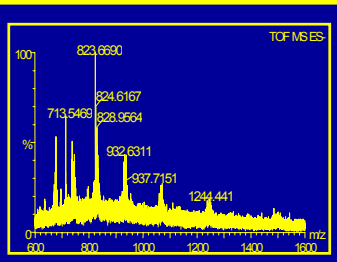

Figure 1: Spectrum of the oligonucleotide sample.

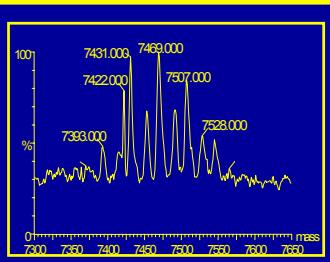

Figure 2: Deconvulted spectrum of the oligonucleotide sample (with the salt ions).

The strategy used for reducing the amount of sodium adducts involved the replacement of sodium ions by ammonium ions, which are less tightly bound to the oligonucleotide when ionized. Therefore, the samples were diluted in ammonium acetate (pH 7.0). Disruption of sodium adducts was observed, resulting in improved mass spectra (Figure 3). An end concentration of 0.3 M ammonium acetate gave the best results. But the potassium adducts were still visible (Figure 4).

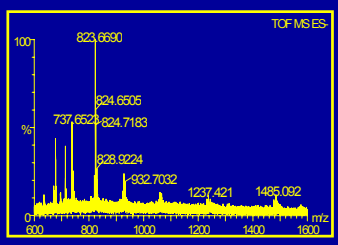

Figure 3: Spectrum of the oligonucleotide sample diluted in ammonium acetate.

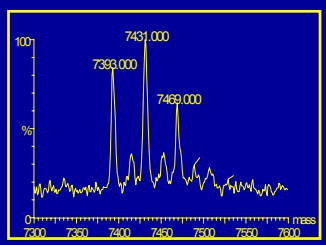

Figure 4: Deconvulted spectrum of the oligonucleotide sample diluted in ammonium acetate.

Addition of 10 mM ammonium acetate pH 7.0 to the solvent, used for loading the pre-column, gave a little reduction of the potassium ions. However, if, in addition, the loading time was changed from 4 to 8 minutes, a big reduction of the potassium adducts was achieved (Figures 5 and 6).

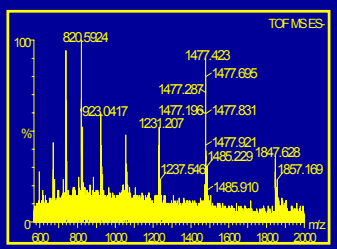

Figure 5: Spectrum of the oligonucleotide sample. No salt ions were observed.

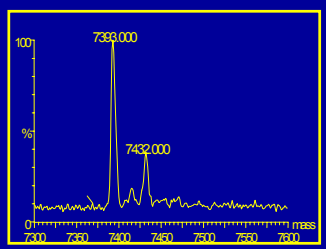

Figure 6: Deconvulted spectrum of the oligonucleotide sample.

4. Conclusion

It is concluded that the nano-LC-nano-ESI-MS method with column switching can remove salt ions, deleterious for mass spectrometric oligonucleotide length and sequence analysis. The procedure is rapid and fully automated, so it is ideally suited for the quality control of oligonucleotides.

5. Acknowledgements

We wish to thank Ing. S. Vande Casteele for all her help with the analysis of the samples. This work was supported by grant GOA99-120501-99 (Eigen OnderZoeksFonds).

6. References

(1) Ball, R.W., and Packman, L.C. Analytical Biochemistry, 246, 185-194 (1997).

(2) Potier, N., Van Dorsselaer, A., Cordier, Y., Roch, O., and Bischoff R. Nucleic Acids Research, 22 (19), 3895-3903 (1994).