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Application of capillary zone electrophoresis-electrospray mass spectrometry for the characterization of oligonucleotides.

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1. Introduction

Synthetic oligonucleotides e.g. used as PCR primers have to be of high purity 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). Electrospray mass spectrometry (ESI-MS) has become a very important analytical tool for the characterization of these oligonucleotides based on a difference in mass. However, the major difficulties arise from the adduction of non-volatile cations such as sodium or potassium to the polyanionic backbone, resulting in highly complex spectra and decreased sensitivity. Removal of these metal ions can be accomplished by multiple precipitation, addition of organic bases e.g. piperidine and imidazole, addition of *trans*-1,2-diaminocyclohexane-*N,N,N,N*-tetraacetic acid (CDTA) or triethylamine or on-line microdialysis (2-4). A lot of these procedures involve the replacement of sodium ions with ammonium, which has been shown to considerably reduce sodium adduct formation in ESI-MS. Most of the approaches for the reduction of cation adduction are time-consuming, require relatively large amounts of sample or are off-line. In that respect, we developed an on-line capillary zone electrophoresis (CZE)-negative ESI-MS method using a Q-TOF mass analyzer for the analysis of oligonucleotides including concomitant removal of salt ions. The advantage of our method is that little sample is necessary and that the metal ions are exchanged for ammonium ions during separation in the ammonium carbonate buffer, thus eliminating the need for any sample preparation steps.

2. Experimental

Oligonucleotides:

The oligonucleotide samples (Applied Biosystems) applied in this study are displayed in Table 1, together with their expected average and observed molecular mass. The concentration of the oligonucleotides was between 125 and 180 pmol/µl. Oligonucleotide 3 was used as model oligonucleotide for the development of the method. The synthesized oligo's were not purified or desalted before analysis.

CZE-ESI-MS Conditions:

The CZE system (PRINCE Lauerlabs, Emmen, The Netherlands) was coupled to the Q-TOF mass spectrometer (Waters Corporation, Manchester, UK) with a fused silica capillary of 0.8m x 50µm i.d., which was inserted in the triaxial nano-electrospray source (Z-spray®). After injection, performed by applying 100 mbar pressure during 1 min, the samples were preconcentrated on the capillary using sample stacking. Electrophoresis was performed using a constant voltage of 14 kV, during which a constant pressure of 60 mbar was applied. The run buffer consisted of 25 mM ammonium carbonate (pH 9.7). Negative ESI was performed using an ionization voltage of –3.0 kV. The cone voltage was set at 36 V. The source temperature was kept at 80°C. The sheath flow consisted of 80/20 isopropanol/water (0.7 µl/min).

3. Results and discussion

In a first set of experiments, only 25 mM ammonium carbonate (pH 9.7) was used as run buffer. Using this buffer system, there were still sodium and potassium adducts visible, as can be seen in the deconvoluted spectrum of the oligonucleotide sample 3 (Figure 1). In an attempt to improve sensitivity, the concentration of the buffer was increased to 50 mM. This resulted in a little reduction of the metal adducts, but also gave an increase in the migration time of the model oligonucleotide from about 24 to 30 min, together with some signal suppression. Therefore, addition of other components was tested for the further reduction of the metal ions. According to Greig and Griffey (2), the use of piperidine resulted in a substantial decrease in sodium adduction, while the co-addition of imidazole improved the ESI-MS sensitivity. Limbach and coworkers (3) reported that the addition of the chelator CDTA can be used for the reduction of magnesium and sodium adduct ions. Addition of 2.5 mM piperidine and imidazole to the initial buffer system gave a reduction of the sodium adducts, yet no reduction of potassium adducts was observed (Figure 2). However, if 0.2 mM CDTA was added to the buffer system, a marked reduction of the potassium adducts, together with a smaller reduction of the sodium adducts was observed, resulting in a deconvoluted spectrum with very little adducts (Figure 3).

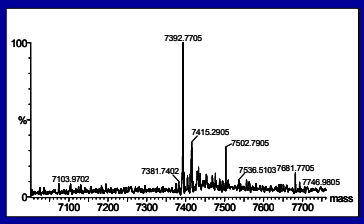

Figure 1: Deconvoluted spectrum using 25 mM ammonium carbonate (pH 9.7) as run buffer.

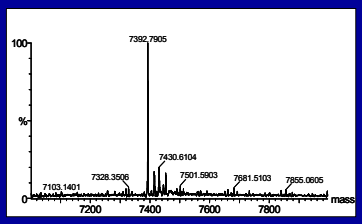

Figure 2: Deconvoluted spectrum after addition of piperidine and imidazole to the run buffer.

In a next step, the addition of 5% 2-20 mM ammonium carbonate buffer (pH 9.7) to the sheath flow was investigated for further improvement of the signal. Addition of 5% 5 mM ammonium carbonate buffer (pH 9.7) to the sheath flow gave the best improvement of the signal (Figure 4).

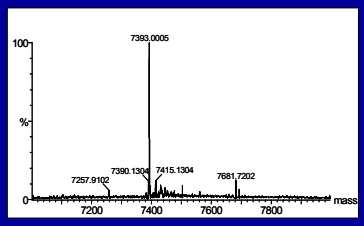

Figure 3: Deconvoluted spectrum after addition of CDTA to the run buffer.

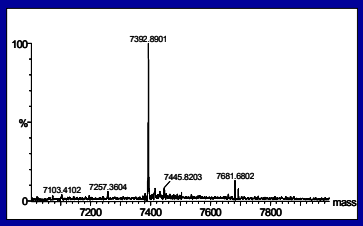

Figure 4: Deconvoluted spectrum after addition of CDTA to the run buffer and additon of 5% 5 mM buffer to the sheath flow.

Finally, our method was tested with 7 other oligonucleotides, which are listed in Table 1. Figure 5 shows the deconvoluted spectrum of oligonucleotide sample 6. There were no sodium or potassium adducts observed in this spectrum. As can be seen from Table 1, the maximum errors obtained on the mass determination of these short oligonucleotides are less than 35 ppm. For the mass range used in this study, this corresponds to an error of about 0.3 Da. These results are better than those of Deforce et al. (4), who also analyzed short oligo's using a CZE-ESI-Q-TOF-MS system (maximum errors of about 100 ppm, corresponding to an error of about 0.8 Da).

No.	Base composition	Expected average MM	Observed MM	Error (ppm)
1	5'-CCA CCA TGC CAC CTC CT-3'	5011.3092	5011.1401	-33.7
2	5'-GGT GCT CCA GGT GCC CAT-3'	5491.6027	5491.4902	-20.5
3	5'-CCC TGG GCT CTG TAA AGA ATA GTG-3'	7392.8587	7392.8901	4.2
4	5'-ATC AGA GCT TAA ACT GGG AAG CTG-3'	7425.8973	7425.6704	-30.6
5	5'-AAT AAG CTT CCA CCA TGC CAC CTC CT-3'	7795.1307	7795.1008	-3.9
6	5'-ATT GTC GAC GGT GCT CCA GGT GCC CA-3'	7963.2024	7963.2803	9.8
7	5'-ATT GTC GAC GCT CTT CAT CCT CGT TCT CA-3'	8745.7164	8745.7402	2.7
8	5'-ATT GTC GAC CAC AGC TGA GAC CTT CCA GCC-3'	9111.9673	9111.9004	-7.3

Table 1: Oligonucleotides used in this study.

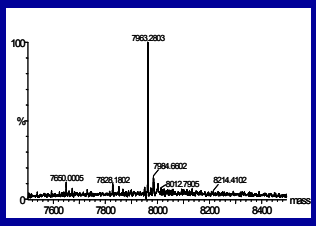

Figure 5: Deconvoluted spectrum of oligonucleotide sample 6.

4. Conclusion

It is concluded that the CZE-ESI-MS method can remove salt ions of oligonucleotide samples, deleterious for mass spectrometric oligonucleotide length and sequence analysis. The procedure is rapid, inexpensive, uses minimal labor and little sample, thus is ideally suited for the quality control of oligonucleotides.

5. Acknowledgements

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6. References

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