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Journal of Chromatography A, 853 (1999) 355–358

JOURNAL OF CHROMATOGRAPHY A

Short communication

Application of capillary electrophoresis at low pH to oligonucleotides quality control

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Abstract

A method for oligonucleotides analysis by using capillary electrophoresis at low pH in free solution is described. It may be considered an alternative to classical analytical techniques which use basic buffers and require the presence of sieving media to separate oligonucleotides as a function of their length. On the contrary, at low pH oligo nucleotides can be separated only depending on their base composition. A large set of samples consisting of 72 synthetic oligonucleotides bearing a 5′-alkylamine moiety and designed for HLA genotyping were analysed. The quality of these synthetic oligos was easily assessed, and a single base difference in oligonucleotides of equal sequence was detected. The results suggest the application of this method to the emerging field of mutation detection and single nucleotide polymorfism analysis.

Keywords: Oligonucleotides

1. Introduction

Polymerase chain reaction (PCR) technology is widely diffused in DNA sequencing and DNA genotyping by sizing [1,2]. Therefore the availability of PCR primers and synthetic oligonucleotides (oligos) is of prime importance. The use of oligonucleotides is further expanding after the introduction of the DNA chip technology [3–5] that is based on a microarray of oligonucleotides chemically bound to a flat surface and hybridizated with a complementary sequence. This technology requires the availability of huge numbers of oligonucleotides in very tiny amounts. All these applications require high purity oligonucleotides and quality control and characterization are mandatory.

At present high-performance capillary electrophoresis (HPCE) and HPLC [6] are the main techniques for oligos separation: specifically capillary gel electrophoresis (CGE) has become an important tool because of its high resolving power [7–9]. HPLC may be a complementary approach and it has been recently proved as well-suited for coupling to mass spectrometry [10,11].

In a previous paper [12] we described a method for oligos separation in free acidic solutions at low pH without the need of any sieving media. This method relies on the chemical properties of oligos, since at low pH (pH<5) the four nucleobases (adenine, cytosine, guanine and thymine) are posi-

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00451-3

tively charged at an extent related to their pK_a [13]. Consequently at low pH the electrophoretic migration depends only on the differential charge carried by oligonucleotide of different composition.

We extended this study first to a set of standard oligonucleotides (as reported in our paper [14]), and in this work to a set of 72 aminated oligos analyzed using phosphate buffer pH 2.5. These oligos consist mainly of pairs with equal sequence differing by a single base at the 3′-end and were designed for the preparation of a microarray for HLA typing. HLA class I molecules are polymorphic cell surface glycoproteins, which are involved in cytotoxic T-cell recognition during the immune response. Therefore, HLA class I genes play an important role in transplantation, and an accurate assignment of the allelic types can determine graft survival. Several molecular typing methods have been proposed to facilitate and further improve resolution of HLA polymorphism [15]. We are currently pursuing the application of the DNA microarray technology to this field and these 72 oligonucleotides have been selected for allele specific extension on microarrays. In addition, these oligos are suitable to be applied in minisequencing technique for DNA genotyping.

By this technique [17] a DNA polymerase is used to extend specifically the 3′-end of a primer which anneals adjacently to a variable dideoxy-nucleoside triphosphate complementary to the nucleotide at the variable site. The separation of the products resulting from minisequencing in HPCE analysis at low pH in free solution may allow to identify the exact base added by DNA polymerase. Consequently, this approach can be applied for mutation detection and single nucleotide polymorphism analysis [18], as recently described using mass spectrometry analysis [19].

2. Materials and methods

2.1. Instrumentation

All electrophoretic separations were performed on a BioFocus 3000 system (Bio–Rad Labs., Hercules, CA, USA) working in reversed polarity. Separations were monitored on-column at 260 nm. The liquid cooling system controlled the temperature of the capillary column (20°C). CE analysis was performed in coated fused-silica capillary tubing (J&W, DB-Wax I.D. 50 μm) with a column length of 50 cm.

2.2. Reagents

The oligonucleotides were purchased from Genset (Paris, France) and were simply desalted by the manufacturer.

2.3. Procedures

All the oligonucleotides tested were diluted with water to 4 pmol/μl and stored at +4°C. They were injected by pressure into the capillary column (137.8 MPa.s) and electrophoresed at a constant voltage (10 kV) at 20°C unless otherwise stated in figure legends.

The buffer solutions were prepared titrating phosphoric acid with sodium hydroxide buffer pH 2.5 and then diluting to the working concentration. The solutions were filtered through a 0.45 μm pore size filter and stored at +4°C.

3. Results and discussion

The purpose of this paper is to confirm the potential of HPCE at low pH for the analysis of aminated (5′ alkylamino modified) oligonucleotides as well as for the separation of oligonucleotides with identical sequence but differing for a single base at their 3′ end.

We analysed 72 aminated oligonucleotides 19–21 mer in length with $0.28<R<3.5$, where R is the ratio A+C/G+T [12]. This parameter has been successfully applied to correlate migration time and oligonucleotide composition [12,16]. Using 50 mM phosphate pH 2.5, we have been able to characterize the quality of these synthetic products discriminating impure oligonucleotide. Among the whole set of aminated oligonucleotides, ten resulted of poor quality and Fig. 1 shows an example of a faulty synthesis of one oligonucleotide. Therefore this procedure permits a rapid quality control of the oligonucleotides, which is an important requisite for extension in microarray.

In addition, this analytical approach resulted powerful in the separation of oligonucleotide couples differing for a single base, e.g. with the same sequence except for the 3′ end base. Apart from

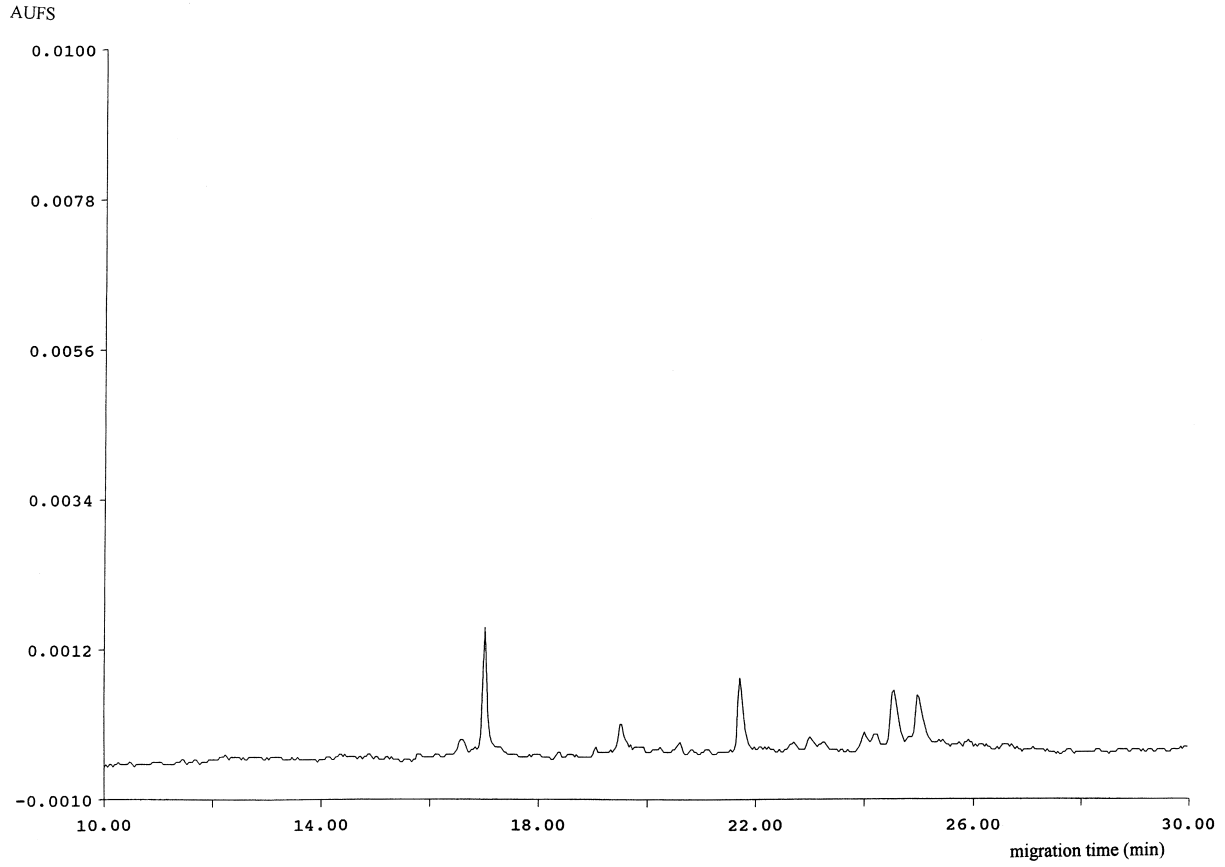

Fig. 1. Electropherogram of an impure oligonucleotide analysed in 50 m*M* phosphate buffer pH 2.5. Sequence: 5′-(a)GGCCGGAG-TATTGGGACCA-3′.

those of poor quality, tested oligonucleotide pairs yielded well separated peaks. Note in Fig. 2 the separation of two T/G terminated oligonucleotides in the same electrophoretic run.

Moreover, multiplexed analysis was feasible in the case of couples with significantly different *R* (i.e. composition). Indeed, all possible sequences scrambling were detectable under the described conditions, at least for 19–21mer oligonucleotides of various composition.

4. Conclusion

The described method allows a fast, inexpensive and automated analysis of as well as the separation of oligonucleotides differing for one base at 3′ end.

The robustness of this procedure in terms of both quality control test and separation of a single base differing oligonucleotides was assessed in the analysis of 72 modified oligos.

Based on the obtained results, this technique represents a valuable alternative for mutation detection and for single nucleotide polymorphism analysis using ‘‘minisequencing’’ protocols.

Acknowledgements

We wish to thank the Italian TELETHON foundation for partial financial support. We thank also CNR target project ‘‘Biotecnologie’’ for partial financial support.

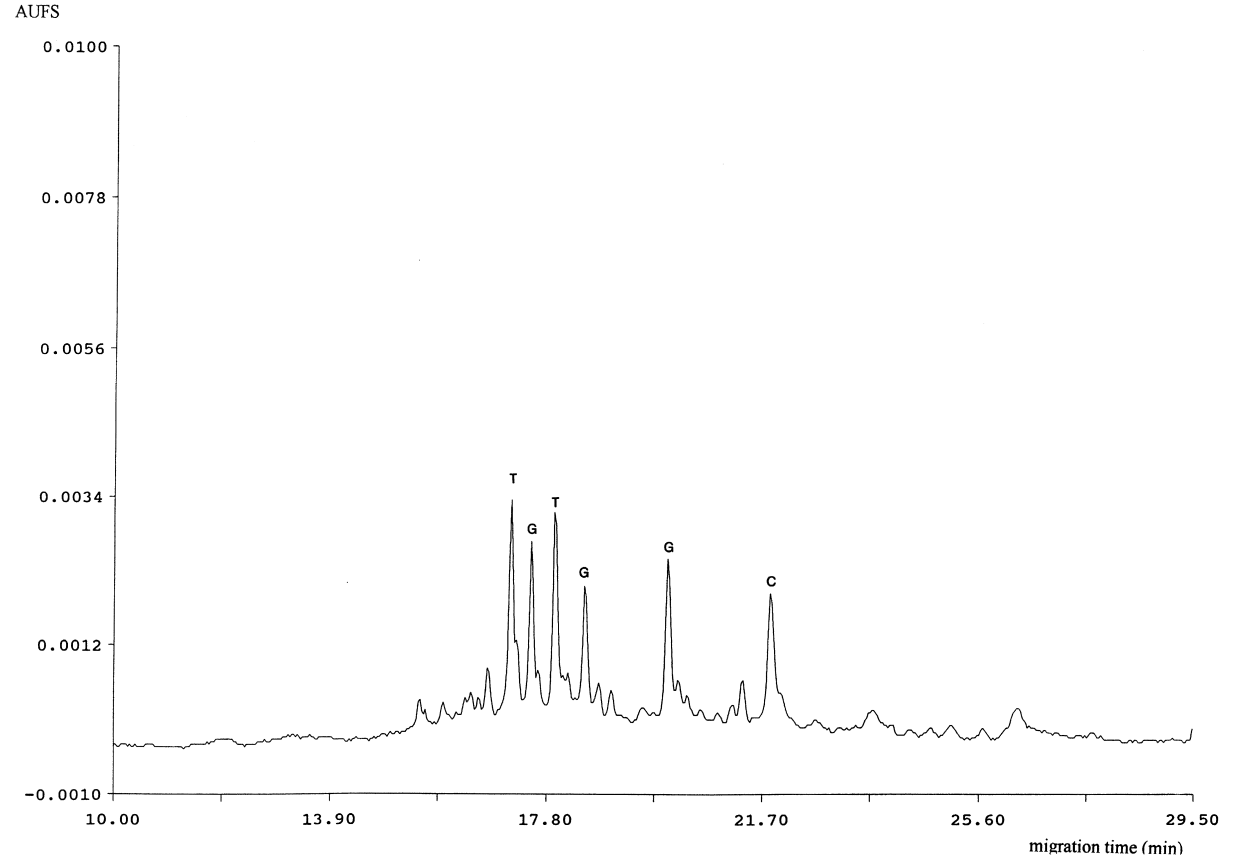

Fig. 2. Electropherogram of three couples of 19-20mer oligonucleotides analysed in 50 m*M* phosphate buffer pH 2.5. Sequence: 5′-(a)GTTGAGAGCCTACCTGGA**T**-3′; 5′-(a)GTTGAGAGCCTACCTGGA**G**-3′; 5′-(a)CGGAATGTGAAGGCCCA**G**-3′; 5′-(a)CGGAATGTGAAGGCCCA**C**-3′; 5′-(a)CCCATGTGGCGGAGCAGT**T**-3′; 5′-(a)CCCATGTGGCGGAGCAGT**G**-3′. Note the presence of small peaks as the result of impurity products during the synthesis.

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