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Capillary electrophoretic analysis of DNA restriction fragments and PCR products for polymorphism and mutation studies in cystic fibrosis and Gaucher's disease*

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Abstract: Two methods are described for the analysis of DNA restriction fragments and PCR products in studies on polymorphism and mutation in cystic fibrosis and Gaucher's disease, based on capillary electrophoresis. In one CE system, a Beckman kit for producing a chemical gel (polymerized within the capillary) is used for single-stranded DNA fragments from 10 to 300 bases in size. Its performance was demonstrated on the separation of a mixture of polydeoxyadenylic acids $p(dA)_{40-60}$ at 30°C. Electrokinetic injection was used (5–7 kV for 5–20 s), the applied field being 300 V cm^{-1} for an effective length of 7, 20 or 30 cm and 100 μm i.d., with Tris-borate buffer containing urea. Typical electropherograms are presented for the analysis of CF mutation ΔF508 in PCR products from homozygous and heterozygous individuals, illustrating the resolution of two complementary single strands (95b and 95b) of a DNA fragment. DNA fragments differing in size by only one base could also be resolved, as shown for the 105b and 106b fragments obtained from a heterozygote for 3905 insT CF mutation, with a run time of ca 45 min. If discrimination were only required between fragments differing by two or more bases, run times could be reduced by 6 when using a capillary length of only 7 cm × 100 μm i.d. A second CE system based on a kit for producing a physical gel (dissolution of polyacrylamide in buffer prior to filling the capillary) gave high resolution for double-stranded DNA fragments from 100 to 1500 base pairs under similar CE conditions, but with 175 V cm^{-1} at 20°C. This was shown for the DNA standard Phi-X 174 when DNA fragments differing in size by 5 base pairs could be resolved within the region 100–200 bp.

Keywords: *PCR products; DNA restriction fragments; capillary electrophoresis; cystic fibrosis; Gaucher's disease.*

Introduction

In the molecular biology field, the standard method for the separation of DNA species employs slab gel electrophoresis. However, today capillary electrophoresis (CE) appears to be an attractive alternative approach [1–3]. In this paper, this fast, reproducible and entirely automated technique was employed for polymorphism and mutation studies in cystic fibrosis (CF) and Gaucher's disease.

The analysis of PCR products by CE are, for the most part, based on the sieving power of polyacrylamide gel [4, 6]. The gels are prepared by two different procedures involving either polymerization of polyacrylamide within the capillary (chemical gel) or dissolution of polyacrylamide as additive in the buffer (physical gel). These two types of gel, available as Beckman Kit ss DNA 100 for the chemical gel and as Kit ds DNA 1000 for the physical gel, were tested.

Experimental

Reagents

Kit ss DNA 100 and Kit ds DNA 1000 were purchased from Beckman (Gagny, France). The Centricon 10 filters were obtained from Grace (Division Amicon, Epernon, France).

Apparatus

CE was performed with an automated P/ACE 2000 (Beckman Instruments, Gagny, France) controlled by a Beckman System Gold software.

Patients

The study included one homozygote and one heterozygote for the major mutation ΔF508 in

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CF, one homozygote for 3905 insT CF mutation, one heterozygote for 84 insG mutation in Gaucher's disease, three heterozygotes for extragenic polymorphisms in CF and two control subjects for CF mutations.

ΔF508 is located in exon 10 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene [7]. The size of the normal PCR fragment is 98 bp. If the phenylalanine codon is deleted at position 508 the size of the PCR fragment is 95 bp. 3905 insT is located in exon 20 of the CFTR gene. Primers have been chosen to give a 105 bp fragment for the normal product or 106 bp fragment if the mutation is present. 84 insG is located in exon 2 of the glucocerebrosidase gene [8]. Primers have been chosen to generate a 75 bp fragment. If the mutation is present, a BSaB1 site is created giving two fragments of 18 and 57 bp.

Sample preparation

DNA was extracted from the leucocytes of patients and then amplified by PCR (30 cycles). The maximum quantity of DNA obtained was 1 μg. The sample (PCR products or restriction fragments of PCR) was diluted 10 times and then centrifuged using Centricon-10 filters to concentrate and to remove superfluous reaction components from PCR and digestion. This procedure was repeated three times before capillary electrophoretic analysis. Single-stranded DNA was obtained by heating at 95°C for 5 min just before injection.

Capillary electrophoresis

In Kit ss DNA 100, the polyacrylamide gel was bound to the wall of the capillary (chemical gel) and used in a tris-borate buffer containing urea. The markers and test mixture were the polydeoxyadenylic acids $p(dA)_{40-60}$ and the polydeoxythymidilic acids $p(dT)_{20/40}$.

In Kit ds DNA 1000, the gel was a polyacrylamide dissolved in a tris borate buffer. The gel matrix is liquid and replaceable (physical gel). The base-pair DNA ladder was Phi-X 174 RF DNA Hae III digest and the internal standard was Orange G dye.

Kit ss DNA 100 was used for the separation of single-stranded (ss) DNA fragments from 10 to 300 bases in size and Kit ds DNA 1000 to separate double-stranded (ds) DNA fragments from 100 to 1500 base pairs (bp). The capillary inserted in a cassette, was filled with the running buffer and equilibrated at a set temperature (30°C for the chemical gel, 20°C for the physical gel). The injections were all made electrokinetically at negative polarity. They were performed at 5–7 kV for 5–20 s. The applied electric field was 300 V cm^{-1} for the chemical gel and 175 V cm^{-1} for the physical gel. The capillary dimensions wer 7, 20 or 30 cm in effective length (from the injection end to the detector) × 100 μm i.d. The runs lasted from 5 to 45 min. Detection was accomplished on line by UV absorption at 254 nm.

Results and Discussion

Capillary electrophoretic analysis of ss DNA fragments

The $p(dA)_{40-60}$ mixture represents a good test and reference for the analysis of small fragments. The baseline resolution of this 40–60 bases standard on chemical-gel filled capillary is shown in Fig. 1. Typical electropherograms of PCR products from homozygous and heterozygous individuals for the major CF mutation (ΔF508) are given in Fig. 2. It can be seen that the separation of two complementary single strands (95-b, 95-b) of a DNA fragment was achieved. The CE separation of DNA fragments with a size difference of one base and with sizes slightly higher than 100 bases, such as the 105-b and 106-b fragments obtained from a heterozygote for 3905 insT CF mutation, is shown in Fig. 3. If the difference in size of the analysed fragments corresponds to only one base, a capillary length of 37 cm is needed to achieve their separation, but when the difference is higher than two bases, a capillary length of 7 cm is

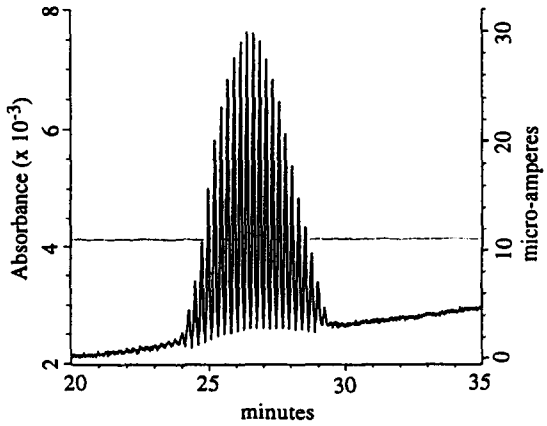

Figure 1
Separation of polydeoxyadenylic acids mixture $p(dA)_{40-60}$ on a chemical-gel filled capillary. Running conditions: effective length, 30 cm; applied electric field, 300 V cm^{-1} injection at 7 kV for 10 s; UV detection at 254 nm.

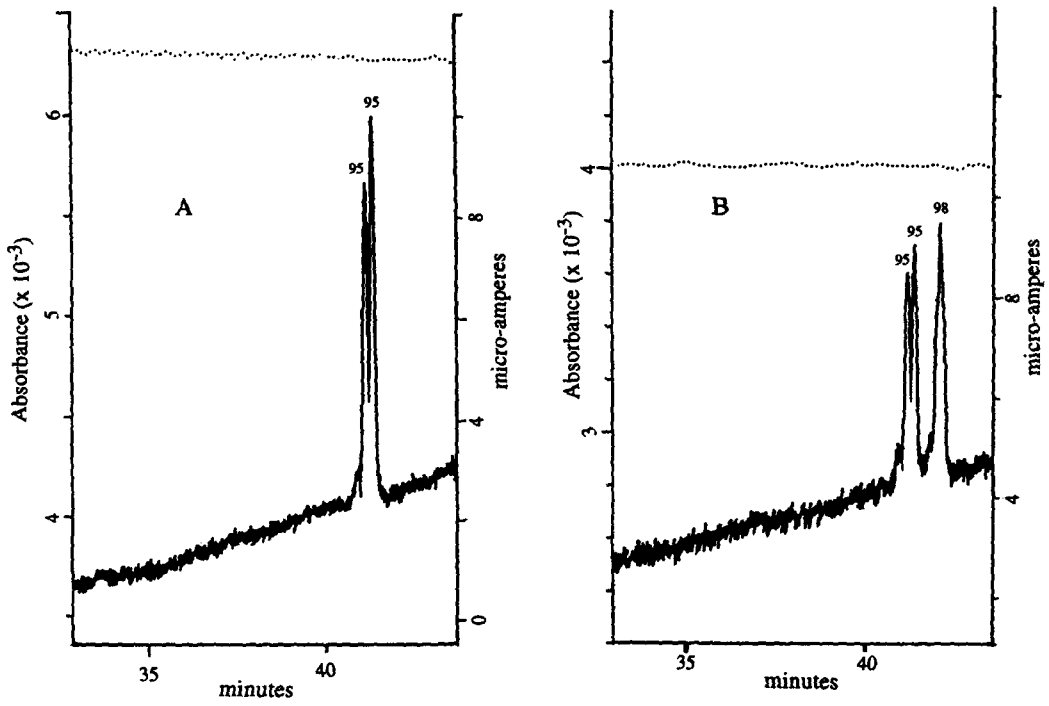

Figure 2
Typical electropherograms obtained for the analysis of the major CF mutation ΔF508 in PCR products from: (A) homozygous individual (95-b peak); and from (B) heterozygous individual (95-b and 98-b peaks). The separation of two complementary single strands (95-b, 95-b) can be observed in (A) and (B). Analytical conditions as in Fig. 1.

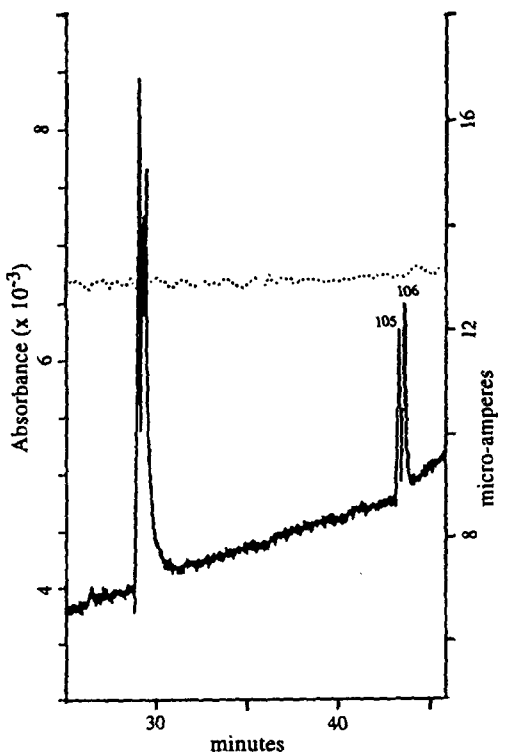

Figure 3
Separation of 105-b and 106-b DNA fragments obtained from a heterozygote for 3905 insT CF mutation. Analytical conditions as in Fig. 1.

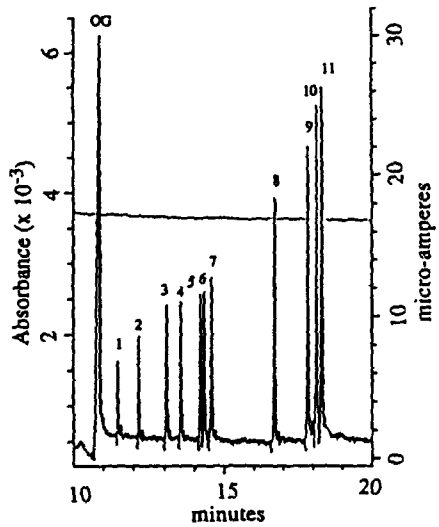

Figure 4
Electropherogram of the Phi-X 174 DNA standard on a physical-gel filled capillary. Peaks: OG = Orange G; 1, 72; 2, 118; 3, 194; 4, 234; 5, 271; 6, 281; 7, 301; 8, 603; 9, 872; 10, 1078; 11, 1353 base pairs. Running conditions: effective length, 30 cm; applied electric field, 175 V cm^{-1}; injection at 5 kV for 10 s; UV detection at 254 nm.

sufficient and thus the run time is reduced by a factor of 6. Furthermore, in CE the separation of fragments which are small but very distinct in size, such as fragments 18-b, 57-b and 75-b obtained from a heterozygote for 84 insG mutation, can be easily achieved in a single run compared to slab-gel electrophoresis.

Capillary electrophoretic analysis of ds DNA fragments

Figure 4 shows the high resolution separation of the standard Phi-X 174 on a physical-gel filled capillary. Efficient separations of ds DNA fragments ranging from 300 to 955 bp were achieved for three extragenic polymorphism studies in CF. Peak capacity was excellent; within the region 100–200 bp, DNA fragments with a size difference of 5 bp were resolved and for a fragment increment of 100 bp, the difference in fragment size which could be resolved increases of 10 bp.

It should be noted that successful operation requires careful attention to sample handling and pretreatment.

Conclusion

Using chemical- and physical-gel filled capillaries, rapid, reproducible and high resolution separations of DNA fragments and PCR products were obtained. The results of this study show that CE has great potential for clinical applications in the molecular biology field. Moreover, it may be noted that CE is an entirely automated instrumental technique.

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