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Short communication

High-speed electrophoretic separation of DNA fragments using a short capillary

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Abstract

Capillary electrophoresis using a replaceable gel buffer was applied to the separation of DNA fragments. A short effective length capillary (1–2 cm) at low electric field allowed the separation of a 20–1000 bp ladder in 1 min. Although similar separation speed was achieved with a longer capillary at high field, the resolution of larger fragments was degraded. The short effective length capillaries were able to separate the wildtype and mutant PCR products of the TGF-β_1 gene in under 45 s.

Keywords: DNA

1. Introduction

Capillary electrophoresis (CE) is a versatile tool for separating nucleic acids in molecular biology [1]. As compared to slab gel electrophoresis, CE has the advantages of automation, small sample requirement, fast and efficient separation, real-time detection and negligible buffer waste. However, the major drawback of CE is low sample throughput because samples are analyzed sequentially. This problem is resolved by using a capillary array [2,3]. Since conventional CE systems are equipped with a single capillary, a practical way to increase throughput is to minimize the analysis time per sample. This has been accomplished by using a range of an effective length capillary as short as 7 cm and field strength as high as 2000 V/cm for the separations of small ions, drugs and proteins [4–7].

Recently, we were able to achieve fast separation of the wild-type and mutant PCR products of the TGF-β_1 gene in 60 s using a 7-cm effective length capillary and 560 V/cm [8]. However, high field strength degraded the resolution of large DNA fragments [9] and may cause migration anomalies [10]. Alternatively, one can use a shorter capillary length and a lower field strength; We did not study this procedure previously because the shortest effective length was limited to 7 cm for the Beckman unit. In the present study, the separation of DNA fragments using capillaries with an effective length of 1 or 2 cm was investigated. The experiments were realized because a home-built CE system was used.

2. Experimental

2.1. Apparatus

All experiments were performed with a home-built CE–LIF system similar to that used previously [11].

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The electric field for CE separation was provided by a Glassman high-voltage power supply (Whitehouse Station, NJ, USA). The capillary was mounted on an x–y translational stage for precise movement. The argon ion laser beam (ILT, Salt Lake City, UT, USA) was focused onto the capillary with a biconvex lens. Fluorescence was collected at 90° from the excitation beam with a 10× microscopy objective. After passing through a 488-nm interference filter, the fluorescence was detected by a photomultiplier tube (PMT, Oriel, Stratford, CT, USA). The PMT current was monitored by a picoammeter (Keithley, Cleveland, OH, USA). The output from the picoammeter was fed to a Beckman 406 data acquisition module. The data sampling rate was 20 Hz with a rise time of 0.1 s.

DNA separations were performed using a 50 μm I.D. DB-17 capillary varying in length (J&W, Folsom, CA, USA). The effective lengths were 1, 2 or 7 cm (10–15 cm total lengths). The capillary was cleaned occasionally by flushing with the gel buffer. The samples were injected electrokinetically, typically for 5 s, at 0.01 kV/cm for the 1 or 2 cm separation capillary and at 0.04 kV/cm for the 7 cm separation capillary.

2.2. Materials

A replaceable DNA separation buffer was obtained from Sigma (St. Louis, MO, USA), which was diluted to 60% in water before use. The running buffer contained 1 μ*M* of YO-PRO-1 (Molecular Probe, Eugene, OR, USA). The 20-bp and 100-bp ladders were obtained from Gensura (Del Mar, CA, USA). The injected concentrations of the 20-bp and 100-bp ladders were 0.5 and 0.25 ng/μl, respectively. The PCR procedure for the TGF-β_1 genotyping assay was described previously [8]. The PCR samples were diluted 25- to 100-fold with deionized water before CE injection.

3. Results and discussion

A short separation capillary can be used for fast analysis instead of using a long capillary with high field. A 7-cm capillary at 556 V/cm (Fig. 1a) allowed separation of the 20-bp ladder in 1.1 min.

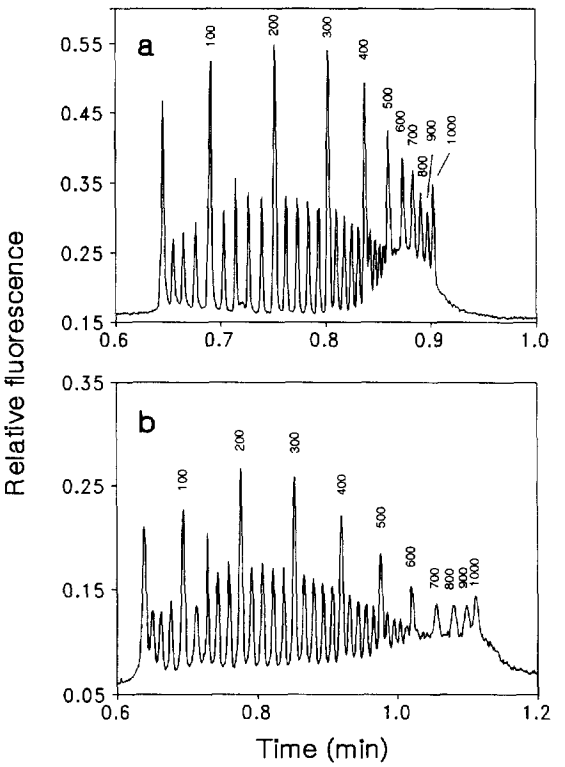

Fig. 1. Separations of a 20–1000 bp ladder using (a) a 7-cm effective length capillary at 556 V/cm; and (b) a 2-cm effective length capillary at 185 V/cm.

However, a 2-cm effective length capillary at 185 V/cm offered both a similar speed and a better resolution for the larger DNA fragments (Fig. 1b). This is because larger fragments migrate according to biased reptation, their mobilities are less dependent on molecular sizes at high fields [9]. In addition, column heating degraded the DNA separations at higher fields, as our home-built system does not have capillary cooling. As an example of fast genotyping using a short column, a 2-cm effective length capillary was applied to the separation of the wild-type and mutant TGF-β_1 PCR products that was fortified with the 20 bp-ladder. The two PCR products were separated in about 45 s at 260 V/cm (Fig. 2). A 1-cm effective length capillary and 185 V/cm was also able to separate the two PCR products in 35 s (Fig. 3). However, the peak resolution was not as good as in Fig. 2 but it is expected to improve once the injection and separation are optimized. In conclu-

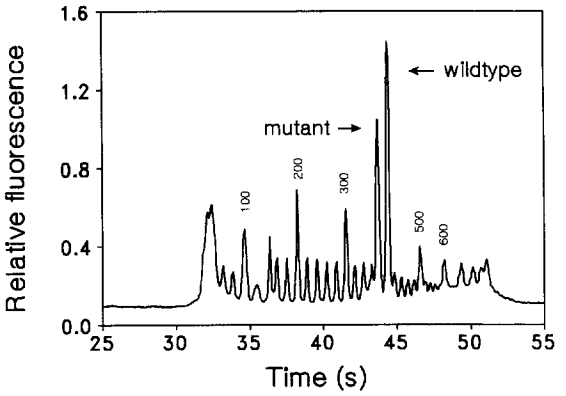

Fig. 2. Separations of the wildtype and mutant TGF-β_1 PCR products fortified with the 20–1000 bp ladder. A 2-cm effective length capillary and 260 V/cm were used.

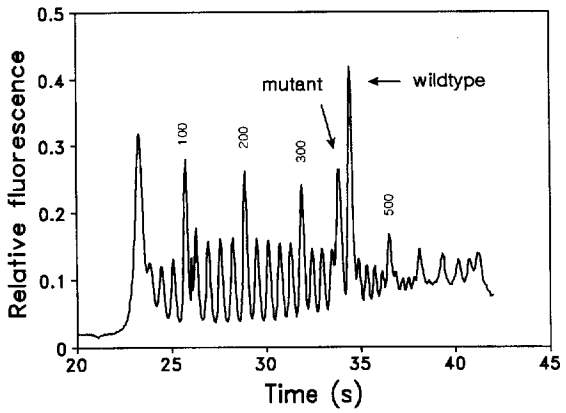

Fig. 3. Separations of the wildtype and mutant TGF-β_1 PCR products fortified with the 20–1000 bp ladder. A 1-cm effective length capillary and 185 V/cm were used.

sion, fast DNA separation was achieved with 1–2 cm effective length capillaries. Low field extends the size separation range, which is useful if analysis of large fragments is required.

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References

[1] P.G. Righetti (Ed.), Capillary Electrophoresis in Analytical Biotechnology, CRC Press, Boca Raton, FL, 1996.
[2] K. Ueno, E.S. Yeung, Anal. Chem. 66 (1994) 1424.
[3] R.A. Mathies, X.C. Huang, Nature 359 (1992) 167.
[4] R. Lausch, T. Scheper, O.W. Reif, J. Schlosser, J. Fleischer, R. Freitag, J. Chromatogr. A 654 (1993) 190.
[5] S. Hjerten, L. Valtcheva, K. Elenbring, J. Liao, Electrophoresis 16 (1995) 584.
[6] K.D. Altria, M.A. Kelly, B.J. Clark, Chromatographia 43 (1996) 153.
[7] L. Bert, F. Robert, L. Denoroy, B. Renaud, Electrophoresis 17 (1996) 523.
[8] K.C. Chan, G.M. Muschik, H.J. Issaq, K.J. Garvey, P.L. Generlette, Anal. Biochem. 243 (1996) 133.
[9] G.W. Slater, P. Mayer, P.D. Grossman, Electrophoresis 16 (1995) 75.
[10] J. Berka, Y.F. Pariat, O. Muller, K. Hebenbrock, D.N. Heiger, F. Foret, B.L. Karger, Electrophoresis 16 (1995) 377.
[11] K.C. Chan, G.M. Janini, G.M. Muschik, H.J. Issaq, J. Liq. Chromatogr. 16 (1993) 1877.