ELSEVIER

Journal of Chromatography A, 802 (1998) 179–184

JOURNAL OF
CHROMATOGRAPHY A

Enhanced separation of DNA sequencing products by capillary electrophoresis using a stepwise gradient of electric field strength

Hideko Inoue[a], Mitsutomo Tsuhako[a], Yoshinobu Baba[b,*]

[a]*Department of Chemistry, Kobe Pharmaceutical University, Motoyamakita, Higashinada, Kobe 658, Japan*
[b]*Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, The University of Tokushima, Shomachi, Tokushima 770, Japan*

Abstract

The effect of the electric field strength gradient on the separation of DNA sequencing fragments was investigated. We demonstrate that the stepwise gradient of electric field improves the separation of DNA sequencing fragments more than 500 bases in size and diminishes the analysis time for DNA sequencing of lager DNA fragments. The use of the electric field strength gradient induces an increase in the theoretical plate number as predicted by the theoretical formulation discussed in this paper. © 1998 Elsevier Science B.V.

Keywords: Electric field strength gradients; DNA

1. Introduction

Rapid progress in the Human Genome Project has stimulated investigations for the development of new technologies for high-throughput DNA sequencing [1–4], including capillary electrophoresis (CE), capillary array electrophoresis, mass spectrometry, single-molecule detection, scanning tunneling microscopy and sequencing by hybridization on a DNA chip. Of these, capillary electrophoretic technologies [5–19], including array techniques [8,9] and microfabricated techniques [18,19], provide the most successful and promising technologies for high-throughput DNA sequencing.

More recently, much attention has been focused on the long reading for DNA sequencing using CE [10–17] as well as high-speed DNA sequencing [5–9]. Early reports of DNA sequencing generated 350 bases of sequence in less than 30 min; these systems were operated at high electric field more than 300 V/cm and at around room temperature [5–9,13]. With a 300 cm long capillary with a low electric field strength of 70 V/cm 680 bases could be separated [12], but it took 10 h. There have been reports of separation of fragments over 500 bases in length in 2 h by using non-crosslinked polyacrylamide with a high electric field strength of 200 V/cm at room temperature [10,11]. The use of high temperatures was effective for improving the separation rate compared with room temperature operation. Fragments up to 640 bases are separated in less than 2 h using 5% T non-crosslinked polyacrylamide at 60°C and at an electric field of 150 V/cm [15] [T=(g acrylamide+g N,N′-methylenebisacrylamide)/100 ml solution]. More recently, DNA sequencing for more than 1000 bases was achieved within 80 min by using low concentrations (2% T) of high-molecular-mass linear polyacrylamide, elevated column temperature (50°C), and moderately high electric field (150 V/cm) [16].

*Corresponding author.

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PII S0021-9673(97)01098-4

Further improvement in the separation of DNA sequencing products will be expected by use of field strength gradients, because a field strength gradient method provided an enhanced resolution of double-stranded DNA molecules in CE [20]. However, no attempts have been reported on the application of stepwise gradient of electric field for the improvement of resolution of the DNA sequencing products. In this paper, we demonstrate a simple stepwise gradient of an electric field strength improves the separation of large DNA sequencing fragments more than 600 bases.

2. Experimental

Unless otherwise stated, chemicals used were reagent or electrophoretic grade from Wako (Osaka, Japan). An Amplitaq FS CycleSequencing Kit from Perkin-Elmer Applied Biosystems Division (Foster City, CA, USA) and M13mp18 single-stranded DNA from Takara (Kyoto, Japan) were stored at −20°C until use. A DNA sequencing reaction product was obtained by using M13mp18 DNA template and the Amplitaq FS Cycle Sequencing Kit with the GeneAmpPCR system 2400 (Perkin-Elmer Applied Biosystems Division). The sequencing product was recovered from the reaction mixture with ammonium acetate–ethanol. DNA was dried under a vacuum and resuspended in 4 μl of deionized formamide. The sample was denaturated at 90°C for 150 s before electrokinetic injection, 2–5 kV for 10–40 s.

All DNA separations were performed on a linear polyacrylamide-filled capillary, in which a linear polyacrylamide was chemically bound to the inner surface of the capillary, prepared according to the literature [21–23]. CE was carried out with a P/ACE System 2050 equipped with a laser-induced fluorescence (LIF) detector (Beckman Instruments, Fullerton, CA, USA). Fluorescence is excited with a 3 mW beam from an argon ion laser (λ=488 nm) and detected at 560 nm.

3. Results and discussion

First, the effect of electric field gradient on the electrophoretic velocity and the theoretical plate number is formulated [20]. The electric velocity (v) of the DNA molecule can be expressed as Eq. (1), when a constant electric field (E) is applied in the CE of DNA without an electroosmotic flow (EOF) by use of inner-surface coated capillary.

$$v = \mu E \tag{1}$$

where μ is the electrophoretic mobility of the DNA molecule at the given field strength.

Eq. (1) should be modified when a stepwise gradient electric field (E') is applied, because the applied electric field is a function of time (t). The electrophoretic mobility of the DNA molecule (μ') is a function of the electric field, so we can write as follows:

$$v' = \mu' E' \tag{2}$$

Eq. (2) states that the actual velocity, v', of a DNA molecule is influenced by the field strength in use at a given time and by the mobility, which is also a function of the field strength. Thus, when a stepwise gradient electric field is applied, the electrophoretic acceleration (a) can be expressed as the change in electrophoretic velocity.

$$a = \mathrm{d}v'/\mathrm{d}t = \mathrm{d}(\mu' E')/\mathrm{d}t \tag{3}$$

where dv' and dt are the electrophoretic velocity and the time increments, respectively.

The basic equation of calculating theoretical plate numbers (N) in CE under a constant electric field is as Eq. (4) [24].

$$N = (\mu E l)/2D \tag{4}$$

where l is an effective length of capillary and D is the diffusion coefficient of the solute.

Combination of Eqs. (1) and (4) gives,

$$N = (vl)/2D \tag{5}$$

The electrophoretic velocity in Eq. (5) should be replaced by the electrophoretic velocity (v') under a stepwise gradient electric field. Therefore, the change of N' in time due to the stepwise gradient electric field is as Eq. (6).

$$\frac{\mathrm{d}N'}{\mathrm{d}t} = \frac{\mathrm{d}v'}{\mathrm{d}t} \cdot \frac{l}{2D} \tag{6}$$

Combination of Eqs. (3) and (6) finally gives the

relationship between the plate number under the stepwise gradient electric field and the electrophoretic acceleration[1].

$$\frac{dN'}{dt} = \frac{al}{2D} \quad (7)$$

Thus we conclude that the change in the theoretical plate number is a linear function of the acceleration and then the change in resolution is proportional to the square root of the acceleration, $dR_s/dt \sim d(a^{1/2})/dt$, when a linear field strength gradient is used.

Fig. 1 compares the separations of the DNA sequencing product using different constant field strengths. The separations were performed by using a 4% T linear-polyacrylamide filled capillary at 30°C and at the constant electric fields of 125 (A), 100 (B) and 74 (C) V/cm, respectively. The DNA sequencing fragments up to 500 bases are resolved at 125 and 100 V/cm as shown in Fig. 1A and B. Several peaks up to 640 bases appear, but any peaks greater than 650 bases are not resolved. On the contrary, many peaks greater than 800 bases are observed at a lower electric field of 74 V/cm, as shown in Fig. 1C, compared with the higher electric fields as shown in Fig. 1A and B. The lower electric field is effective to analyze the longer DNA fragments, but longer analysis time is required.

We have several selections for the gradient shapes, including linear, convex, concave and stepwise gradient of electric field. Some linear gradient techniques were successfully applied to the separation of ΦX174 DNA restriction fragments, consisting of 11 DNA fragments [20]. In some instances the linear gradient does not give sufficient separation between very closely related compounds, such as DNA sequencing products. During continuous ramping, the time that the DNA molecules are exposed to the optimal field for their separation may be too short. In this case a stepwise field strength gradient is better because closely related molecules are exposed to their optimal separation field strength for a longer period of time, resulting in better resolution.

Fig. 2 shows the separation of a DNA sequencing product employing a stepwise voltage gradient method. The method consisted of two consecutive steps, 74 V/cm from 0 to 100 min and 100 V/cm from 100 min to 180 min for Fig. 2A and 74 V/cm from 0 to 80 min and 100 V/cm from 80 to 140 min for Fig. 2B. Other conditions are the same as those in Fig. 1. The resolving power for the DNA sequencing fragments greater than 500 bases is much higher than those obtained using the constant electric field of 100 and 125 V/cm as shown in Fig. 1A and B. The separation of larger DNA sequencing fragments as in Fig. 2 is almost comparable to the separation at 74 V/cm as in Fig. 1C, but the analysis time is significantly diminished by using the stepwise gradient of electric field. Additionally, the bands of larger DNA fragments as shown in Fig. 2 are much sharper than those as shown in Fig. 1 as expected from Eq. (7).

The relationship between migration time and DNA base number is plotted from the data of Figs. 1 and 2 as well as data obtained by other experiments for the stepwise gradient of 74 V/cm from 0 to 100 min and 125 V/cm from 100 min to 180 min and 74 V/cm from 0 to 80 min and 125 V/cm from 80 to 140 min as shown in Fig. 3. Comparison of the plot at 74 V/cm with that of stepwise gradient indicates the stepwise gradient is effective for the decrease in the analysis time.

A simple stepwise gradient method was applied to the separation of DNA sequencing products in order to increase the resolving power in capillary electrophoretic separation. The use of stepwise gradient techniques showed that the resolving power can be optimized for a given DNA chain length range, especially for the separation of longer DNA fragments, and separation time can be significantly reduced. Further investigation on the conditions of stepwise change in the electric field will achieve a longer reading of DNA sequences greater than 1000 bases in a single capillary run.

Acknowledgements

This work was supported by a Grant-in-Aid for Creative Basic Research (Human Genome Program), Cancer Research, and Scientific Research on Priority Area from Japan Ministry of Education, Science and Culture.

[1] Eq. (7) is slightly different from Eq. 9 in Ref. [20], $dN'/dt = d/dt[a(l/2D)]$, because Eq. 9 in Ref. [20] has a small misprint [25].

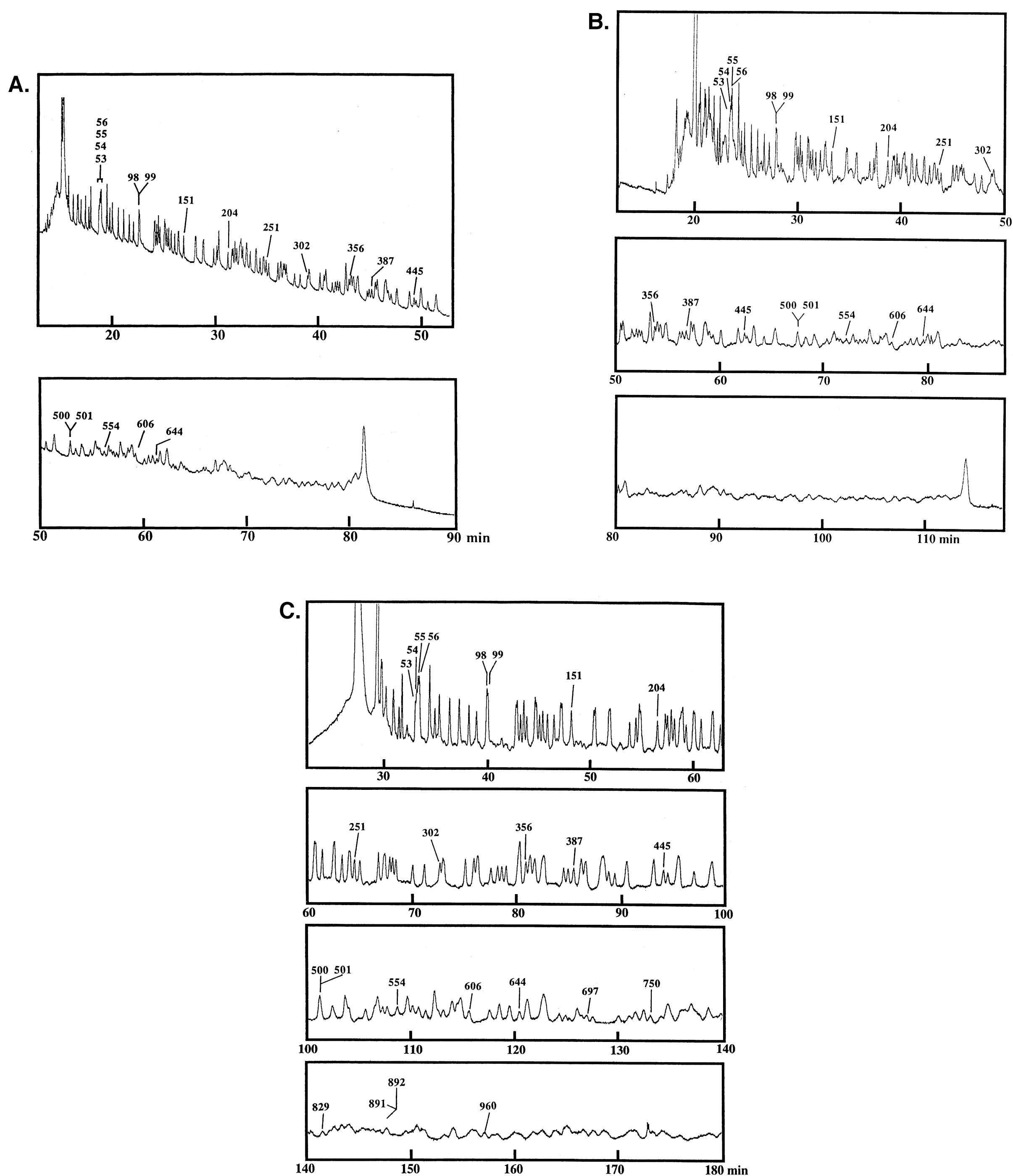

Fig. 1. Separations of FAM-labeled (Perkin-Elmer Applied Biosystems Division) C-termination sequencing products of M13mp18 by CE under a constant electric field strength. Capillary, 27 cm (effective length 20 cm)×100 μm I.D.×360 μm O.D.; separation matrix, 4% T linear polyacrylamide; running buffer, 0.1 *M* Tris–borate and 7 *M* urea; temperature, 30°C; sample injection, 5.4 kV for 10 s; electric field strength, (A) 125, (B) 100, (C) 74 V/cm; current, (A) 11.5 (B) 8.9 (C) 5.9 μA.

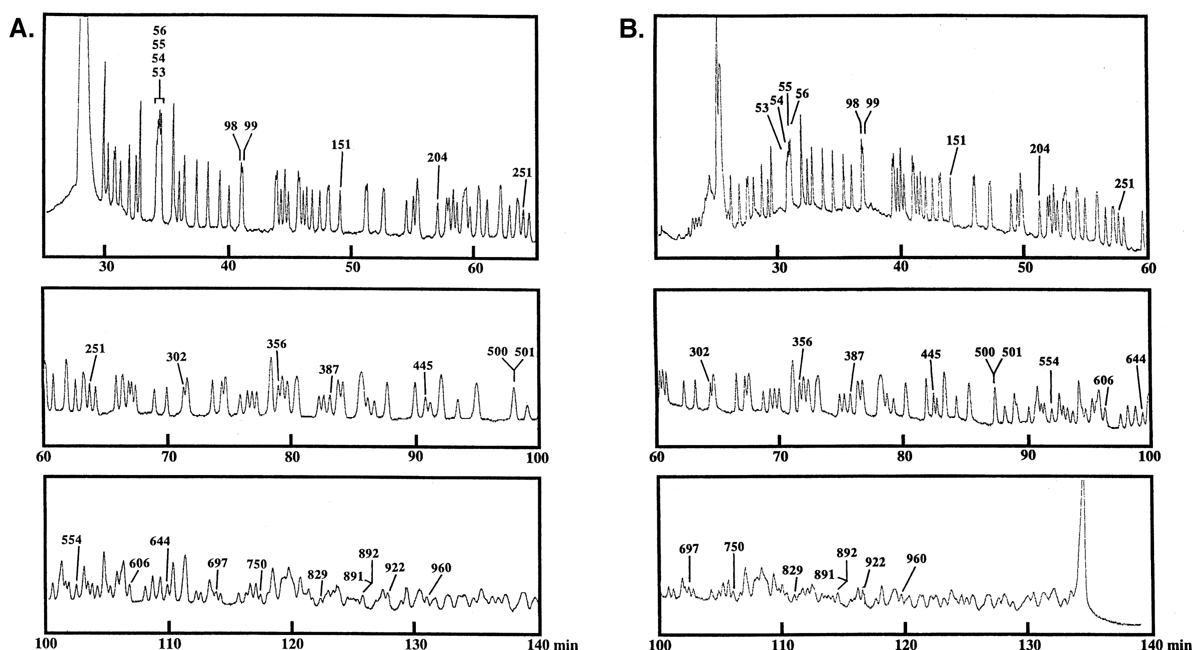

Fig. 2. Separation of FAM-labeled C-termination sequencing products of M13mp18 by CE under a stepwise change in the electric field strength. Stepwise electric field, (A) 74 V/cm (6.0 μA) from 0 to 100 min and 100 V/cm (8.1 μA) from 100 min to 180 min, (B) 74 V/cm (5.9 μA) from 0 to 80 min and 100 V/cm (8.1 μA) from 80 to 140 min. Other conditions as in Fig. 1.

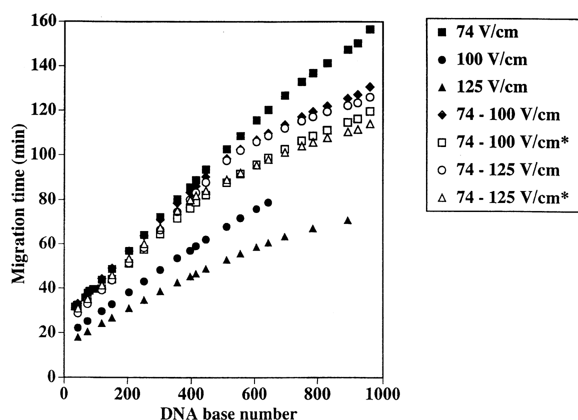

Fig. 3. Plots of the migration time of DNA sequencing product vs. their base number under the constant and the stepwise gradient electric fields. Constant electric fields, 74, 100 and 125 V/cm. Stepwise gradient electric fields, 74–100 V/cm: 74 V/cm from 0 to 100 min and 100 V/cm from 100 min to 180 min, 74–100 V/cm*: 74 V/cm from 0 to 80 min and 100 V/cm from 80 to 140 min, 74–125 V/cm: 74 V/cm from 0 to 100 min and 125 V/cm from 100 min to 180 min, 74–125 V/cm*: 74 V/cm from 0 to 80 min and 125 V/cm from 80 to 140 min.

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