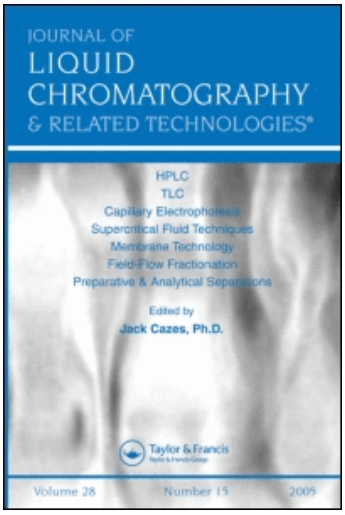

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SEPARATION OF DNA FRAGMENTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS

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ABSTRACT

High resolution separations of DNA fragments up to 10,000 base pairs have been performed using high-performance liquid chromatography, capillary gel electrophoresis, and capillary electrophoresis in entangled polymer solutions. These methods were compared with respect to their performance and efficiencies in the resolution of DNA fragments.

INTRODUCTION

A major application of slab gel electrophoresis is size-selective separation of double-stranded DNA fragments[1]. Slab gel electrophoresis is successfully employed in the restriction fragment length polymorphism (RFLP) analysis, polymerase chain reaction (PCR)

analysis, and Southern blotting, however, this technique is time-consuming, labor-intensive, and non-quantitative process.

High-performance liquid chromatography (HPLC)[2-4] and capillary electrophoresis (CE)[5-21] have been rapidly developing techniques offering the benefits of rapid separation and quantitation. In HPLC separations, microparticulate ion exchangers based on non-porous polymers have led to high resolving powers of DNA fragments[2-4]. DNA restriction fragments within the size range 100-12,000 base pairs can be separated by capillary gel electrophoresis (CGE) using capillaries filled with crosslinked polyacrylamide gel[7] and linear polyacrylamide[7-10]. Recently some researchers demonstrated the separation of DNA restriction fragments by electrophoretically sieving these fragments through a buffer containing hydrophilic polymer in uncoated or coated fused silica capillaries[14-21]. The aim of these research efforts is to develop new technology in the RFLP analysis[20], PCR analysis[17], and Southern blotting[9]. In this paper, we attempt to examine the performance and efficiencies of HPLC, CGE, and CE in entangled polymer solutions with respect to the resolving power of these new technologies in the separation of DNA fragments.

MATERIALS AND METHODS

A 1-kbp DNA ladder (1.0 μg/μL) was obtained from GIBCO BRL (Tokyo, Japan). The 1-kbp DNA ladder contained 23 fragments of 75, 134, 154, 201, 220, 298, 344, 396, 506, 517, 1018, 1636, 2036, 3054, 4072, 5090, 6108, 7126, 8144, 9162, 10180, 11198, and 12216 base pairs (bp) and was solubilized in Milli-Q water. The DNA sample was stored at -18 °C until use. Methylcellulose (4000 cps) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical-reagent or electrophoretic grade from Wako (Osaka, Japan).

An LC-800 HPLC system (Jasco, Tokyo, Japan) was used for the separation of DNA fragments. A TSKgel DEAE-NPR column purchased from TOSOH (Tokyo, Japan, 35 mm x 4.6 mm i.d.) is an anion exchanger based on a nonporous polymer support, having a particle diameter of 2.5 μm. Diethylaminoethyl groups are chemically bonded to the nonporous polymer support. Eluents for the column were (A)0.25 M sodium chloride in 20 mM tris(hydroxymethyl)aminomethane(Tris)-HCl buffer(pH 9.0) and (B)1 M sodium chloride in 20 mM Tris-HCl buffer(pH 9.0). Sample solution(10 μl) was injected into the column and chromatographed at a flow-rate of 1.0 ml/min. Column temperature was kept at 40 °C. DNA fragments were detected at 260 nm.

Capillary gel electrophoretic separations were carried out by using an Applied Biosystems Inc.(ABI) Model 270A capillary electrophoresis system. Polyimide coated fused silica capillaries(375 μm o.d., 100 μm i.d., GL Sciences, Tokyo, Japan) of 30 cm effective length and 50 cm total length were used. Polyacrylamide gel filled capillaries were prepared according to the literature[7]. Buffer was 50 mM Tris, 50 mM boric acid, and 2.5 mM EDTA, pH 8.0. Sample solution was introduced electrophoretically (1 s at 5 kV) into the capillary. Gel filled capillaries were run with buffer solution at 10 kV (200 V/cm, 9-11 μA) at 30 °C. DNA fragments were detected at 260 nm.

Capillary electrophoretic separations in entangled polymer solutions were carried out by using a Waters Quanta 4000 capillary electrophoresis system. Polyimide coated fused silica capillaries(375 μm o.d., 100 μm i.d., GL Sciences, Tokyo, Japan) of 42 cm effective length and 50 cm total length were used. Polyacrylamide-coated capillaries were prepared according to the literature[22]. Buffer was 50 mM Tris, 50 mM boric acid, 2.5 mM EDTA, and 0.5% methylcellulose, pH 8.0. Sample solution was introduced electrophoretically (10 s at 5 kV) into the capillary. Capillary electrophoresis in entangled polymer solutions was

performed using polyacrylamide-coated capillaries with buffer solution at 10 kV (200 V/cm, 20 μA). DNA fragments were detected at 254 nm.

RESULTS AND DISCUSSION

A 1-kbp DNA ladder contains DNA fragments in their base pair (bp) range from 1000 to 12 000 (ca. 1000, ca. 2000, ... ca. 12 000 bp). The mixture also contains the additional fragments, ranging from 75 to 1636 bp arise from enzymatic digestion of the cloning vector which was used in the preparation of the sample. The 1-kbp DNA ladder sample is adequate as a model substrate to demonstrate the resolving power of HPLC, CGE, and CE in entangled polymer solutions, because it consists of 23 species including a broader base pair range of DNA fragments.

HPLC Separation of DNA Fragments

Figure 1 shows the separation of the 1-kbp DNA ladder by HPLC. We chose the non-porous ion-exchange column, because this type of the column is the most suitable for the complete and rapid separation of DNA fragments[2] and polynucleotides[13]. The separation was performed by utilizing a gradient elution technique at 40 °C, gradient and column temperature were optimized by some experiments with an aid of HPLC computer simulations. The fragments were identified according to the literature[4]. The peak at the retention time of 8.2 min would be a DNA fragment of 75 bp, but it could not be identified due to very low detectability.

The mixture of DNA fragments ranging from 134 to 1636 bp was separated almost completely as shown in Fig. 1. Some peaks of DNA fragments ranging from 2036 to 8144 bp appeared, but were poorly

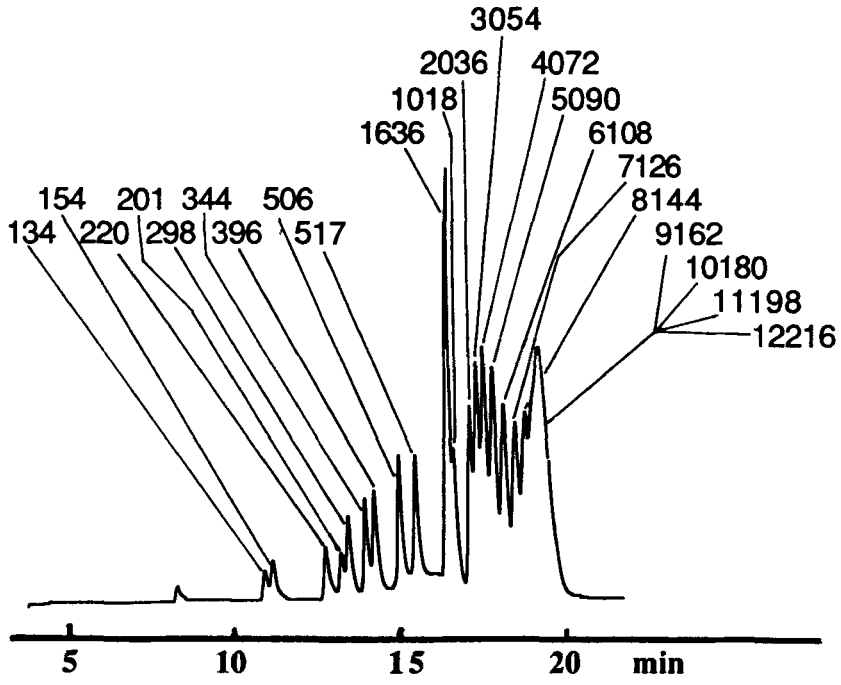

FIGURE 1 HPLC separation of a 1-kbp DNA ladder. Column; TSKgel DEAE-NPR(35 mm x 4.6 mm i.d.). Eluents; (A)0.25 M sodium chloride in 20 mM Tris-HCl buffer(pH 9.0) and (B)1 M sodium chloride in 20 mM Tris-HCl buffer(pH 9.0). Gradient program; 0-3 min from 27 to 34% B, 3-60 min from 34 to 100% B at 40 °C. Flow rate 1.0 ml/min. Detection; 260 nm.

resolved. Large fragments around 10 kbp in length could not be resolved. The separation time was ca. 20 min. The resolution of DNA fragments as shown in Fig. 1 was slightly poorer than those of similar separations reported[2-4], because we used shorter column (35 mm) than others used. Plate number was achieved to be 1-5 x 10^5 plates per meter. Resolution, Rs, was calculated to be in the range of 0.2-1.2. The reproducibility of the retention time was in the range of 1-2% relative standard deviation (R.S.D.) (n=5).

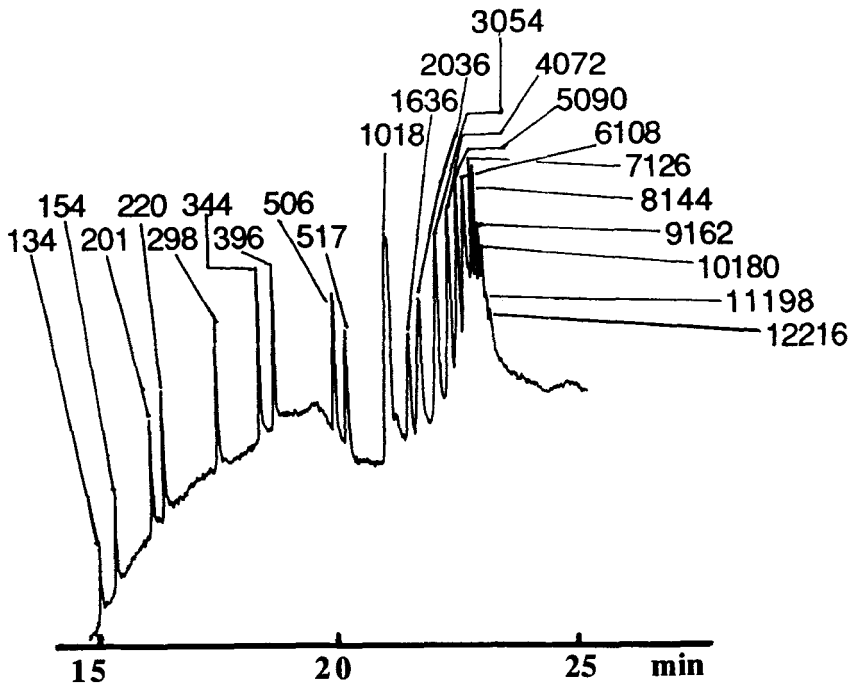

FIGURE 2 CGE separation of a 1-kbp DNA ladder. Capillary; 100 μm i.d., 375 μm o.d., length; 50 cm, effective length; 30 cm. Running buffer; 50 mM Tris, 50 mM boric acid, and 2.5 mM EDTA, pH 8.0. Gel contained 3% T and 5% C. Field; 200 V/cm, current; 13 μA. Injection; 5 kV for 1 s. Detection; 260 nm.

CGE Separation of DNA Fragments

Figure 2 shows electropherogram of the 1-kbp DNA ladder sample separated by CGE with crosslinked polyacrylamide gel (3% T and 5% C) filled capillary at 200 V/cm. The fragments were identified by their sizes in base pairs, the assignments agreeing with the reported separation of the 1-kbp DNA ladder[7]. The peak for DNA of 75 bp could not be identified due to very low detectability. Figure 2 clearly

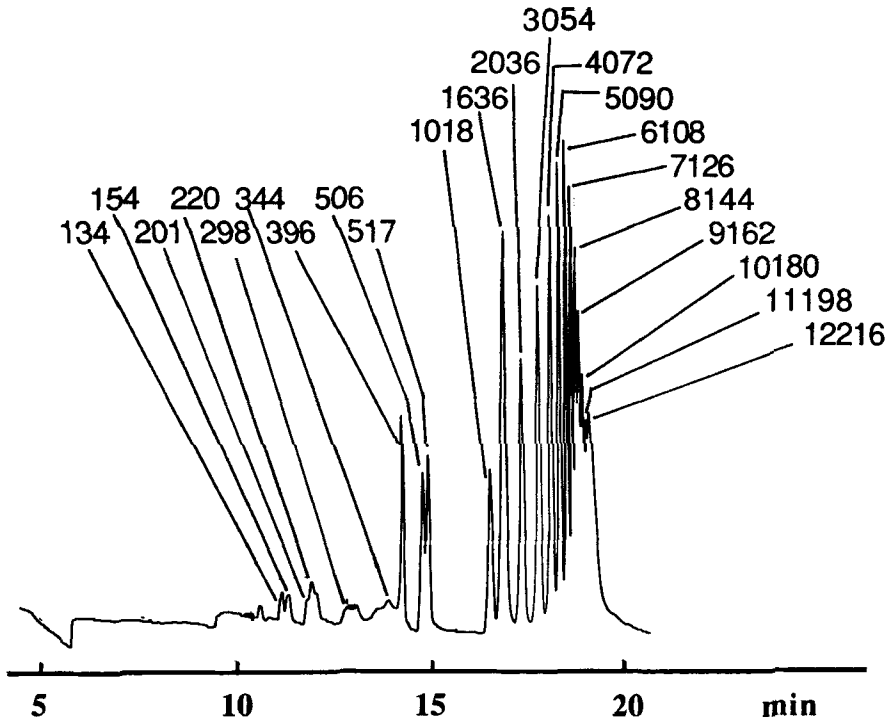

FIGURE 3 CE separation of a 1-kbp DNA ladder. Polyacrylamide-coated capillary; 100 μm i.d., 375 μm o.d., length; 50 cm, effective length; 42 cm. Running buffer; 50 mM Tris, 50 mM boric acid, 2.5 mM EDTA, and 0.5% methylcellulose, pH 8.0. Field; 200 V/cm, current; 20 μA. Injection; 5 kV for 10 s. Detection; 254 nm.

demonstrates that DNA fragments ranging from 134 to 4072 bp are baseline resolved and larger fragments ranging from 5090 to 12 216 bp are almost resolved under the conditions given, and yet the separation was completed in less than 23 min. The resolution of larger fragments achieved using high crosslinked polyacrylamide was slightly poorer than that achieved using low and zero crosslinked polyacrylamide[7], because

the pores of high crosslinked polyacrylamide were generally too small for larger fragments to migrate efficiently. However, it is noteworthy that two fragments of 506 and 517 bp, which differ by only 11 bp and are usually not separated by slab gel electrophoresis, are baseline resolved. Plate number of each peak was estimated to be ca. 1–5 x 10^6 per meter. Resolution, Rs, of each band was in the range of 0.5–2.0. The reproducibility of the migration time was in the range of 2–4% R.S.D. (n=5).

CE Separation of DNA Fragments in Entangled Polymer Solutions

Figure 3 shows the separation of the 1-kbp DNA ladder performed in the linear polyacrylamide-coated capillary, using 0.5 % methylcellulose at 200 V/cm. The fragments were identified by their sizes in base pairs, the assignments agreeing with the reported separation of the 1-kbp DNA ladder[7]. The peak for DNA of 75 bp could not be identified due to very low detectability. Figure 3 demonstrates that the separation of individual ladder fragments is excellent. DNA fragments ranging from 134 to 8144 bp are baseline resolved and larger fragments ranging from 9162 to 12 216 bp are almost completely resolved under the conditions given, and yet the separation was completed in less than 18 min. The separation provides results very similar to those previously reported[14-21]. Two fragments which are usually not separable on a slab gel, the 506 and 517 bp fragments, were baseline resolved. Plate number of each peak was estimated to be ca. 0.8–3 x 10^6 per meter. Resolution, Rs, of each band was in the range of 0.8–2.0. The reproducinbility of the migration time was in the range of 1–2% R.S.D. (n=5).

Comparison of the Resolving Power of These Techniques

HPLC offers rapid separation and quantitation of DNA fragments as shown in Fig. 1. However, although ion-exchange HPLC packings appear very suitable for the complete separation of DNA fragments up to 1000 bp in size, the resolution of larger fragments was found to decrease significantly with increasing fragment size. For example, the analysis time of HPLC in Fig. 1 was comparable to those of CGE (Fig. 2) and CE in entangled polymer solutions (Fig. 3). However, HPLC separation of larger fragments showed poor resolution, whereas each component was resolved completely in CGE and CE. As described above, plate number for CGE and CE in entangled polymer solutions was found in the hundreds of thousand. The efficiency measured for HPLC of Fig. 1 exhibited lower throughout, e.g. tens of thousand plates per meter. These results clearly illustrate that resolving power of CGE and CE is much higher than that of HPLC in the separation of larger fragments, whereas a difference in resolving power of both techniques is not obviously distinguishable in the separation of DNA fragments up to 1000 bp.

We have found that CGE and CE in entangled polymer solutions were well suited for the rapid separation of a wide base pair range of DNA fragments. The resolving power and the speed of separation of both techniques were almost comparable as shown in Figs. 2 and 3. However, the use of polyacrylamide-coated capillaries and polymer solutions as molecular sieving medium allows one to avoid difficulties associated with the use of gel-filled capillaries[5], such as bubble formation during polymerization and electrophoresis. Bubble formation within the gel leads to reduced resolution, a drop in current, and breakdown of the gel structure.

In conclusion, our results suggest that HPLC, CGE, and CE in entangled polymer solutions are equally sufficient for the separation of

DNA fragments up to 1000 bp, whereas for larger fragments CGE and CE are superior to HPLC with the respect to resolving power and efficiency. The advantages offered by CGE and CE suggest that these technique may be a preferred alternative to slab gel electrophoresis and are applicable to the rapid and high-precision restriction mapping of double-stranded DNA fragments.

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REFERENCES

1. D. Rickwood, B.D. Hames (Editors), *Gel Electrophoresis of Nucleic Acids - A Practical Approach*, IRL Press, Oxford, 2nd ed., 1990.

2. M.A. Strege, A.L. Lagu, *J. Chromatogr.*, **555**: 109-124(1991).

3. M. Merion, W. Warren, C. Stacey, M.E. Dwyer, *BioTechniques*, **6**: 246-251(1988).

4. Y. Kato, Y. Yamasaki, A. Onaka, T. Kitamura, T. Hashimoto, T. Murotsu, S. Fukushige, K. Matsubara, *J. Chromatogr.*, **478**: 264-268(1989).

5. Y. Baba, M. Tsuhako, *Trends Anal. Chem.*, **11**: 280-287(1992) and references therein.

6. A.S. Cohen, D.R. Najarian, A. Paulus, A. Guttman, J.A. Smith, B.L. Karger, *Proc. Natl. Acad. Sci. U.S.A.*, **85**: 9660–9963(1988).

7. D.N. Heiger, A.S. Cohen, B.L. Karger, *J. Chromatogr.*, **516**: 33–48(1990).

8. A. Guttman, N. Cooke, *J. Chromatogr.*, **559**: 285–294(1991).

9. J.W. Chen, A.S. Cohen, B.L. Karger, *J. Chromatogr.*, **559**: 295–305(1991).

10. A. Guttman, N. Cooke, *Anal. Chem.*, **63**: 2038–2042(1991).

11. A. Guttman, A.S. Cohen, D.N. Heiger, B.L. Karger, *Anal. Chem.*, **62**: 137–141(1990).

12. Y. Baba, T. Matsuura, K. Wakamoto, Y. Morita, Y. Nishitsu, M. Tsuhako, *Anal. Chem.*, **64**: 1221–1225(1992).

13. Y. Baba, T. Matsuura, K. Wakamoto, M. Tsuhako, *J. Chromatogr.*, **558**: 273–284(1991).

14. M. Zhu, D.L. Hanse, S. Burd, F. Gannen, *J. Chromatogr.*, **480**: 311–319(1989).

15. A.M. Chin, J.C. Colburn, *Am. Biotechnol. Lab.*, **7**(10A): 16–18(1989).

16. M. Strege, A. Lagu, *Anal. Chem.*, **63**: 1233–1236(1991).

17. H.E. Schwartz, K. Ulfelder, F.J. Sunzeri, M.P. Busch, R.G. Brownlee, *J. Chromatogr.*, **559**: 267–283(1991).

18. P.D. Grossman, D.S. Soane, *J. Chromatogr.*, **559**: 257–266(1991).

19. P. Bocek, A. Chrambach, *Electrophoresis*, **12**: 1059–1061(1991).

20. K. Ulfelder, H.E. Schwartz, J.H. Hall, F.J. Sunzeri, *Anal. Biochem.*, **200**: 260–267(1992).

21. Y. Baba, M. Tsuhako, S. Enomoto, A.M. Chin, R.S. Dubrow, *J. High Resolut. Chromatogr.*, **14**: 204–206(1991).

22. S. Hjerten, *J. Chromatogr.*, **347**: 191–198(1985).