ELSEVIER

Journal of Chromatography A, 744 (1996) 321–324

JOURNAL OF CHROMATOGRAPHY A

Capillary gel electrophoretic separation of DNA restriction fragments in a discontinuous buffer system

András Guttman[a,*], Éva Szökő[b]

[a]*Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, CA 92634, USA*

[b]*Semmelweis University of Medicine, Department of Pharmacodynamics, Nagyvárad tér 4, H-1089 Budapest, Hungary*

Abstract

Separation of single and double stranded DNA molecules by capillary gel electrophoresis has a rapidly growing interest. Similar to the polyacrylamide slab gel based separation methods, in capillary gel electrophoresis, the peak/band spacing usually decreases with the increasing size/length of the DNA molecule. Additionally, employing the regularly used Tris–borate buffer system, fronting peaks are often obtained. By the application of an electrolyte step gradient during capillary gel electrophoretic separation of dsDNA molecules, the apparent peak shape can be improved and the required analysis time decreased.

Keywords: Buffer composition; Capillary gel electrophoresis; DNA

1. Introduction

Recently, capillary gel electrophoresis (CGE) has been more and more extensively applied to the analysis of single and double stranded DNA molecules, such as in DNA sequencing [1], restriction fragment mapping [2] and polymerase chain reaction (PCR) product analysis [3]. This new methodology provides direct quantitation by on-column ultraviolet (UV) or laser-induced fluorescence (LIF) detection, high resolution, fast single sample analysis and allows the use of small sample volumes [4]. It has been demonstrated that the use of low viscosity polyacrylamide matrices enables easy replacement of the gel-buffer system in the capillary column after each run [5]. Employing the replaceable gel format also allows pre- or on-column modifications in the gel-buffer system. Such modifications have been reported earlier with the use of special intercalator additives to enhance separation performance [6]. Besides the use of special buffer additives, the buffer system itself can be modified, if necessary, during the separation.

It has been reported in polyacrylamide slab gel (PAGE) separations of DNA molecules that the application of an electrolyte step gradient during the separation enables the read length of DNA sequence data to be increased by 30–50% [7]. This was accomplished in a simple way, by the application of a different (high conductivity) buffer system in the anodic buffer reservoir for the separation. Applying the electric field, a buffer concentration boundary is formed, causing reduced vertical band spacing, and consequently higher read length [8]. However, using a high conductivity buffer system, even in only one of the reservoirs, the electric current and the concomitant Joule heat production increased significantly. This is also the case in capillary format, however, due to the much higher surface-to-volume ratio and

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00246-4

the efficient cooling systems, working up to a relatively high power (5 W) is allowed [9].

Various gradient separation modes of DNA molecules in capillary electrophoresis have been reported, such as temperature [10] and voltage [11] gradients. In this paper, an on-column transient electrolyte concentration step gradient-mediated focusing is discussed for DNA restriction fragment separations by CGE. The electrolyte step gradient can be applied at the beginning of the electrophoresis separation, or, if necessary, delayed in time, such as when the separation starts with the regular buffer system in both the anodic and cathodic reservoirs, then one of them is replaced sometime during the separation process. The effect of the electrolyte boundary on the apparent peak shape of the different size dsDNA restriction fragments is also discussed.

2. Experimental

The P/ACE 2100 capillary electrophoresis system with the eCAP dsDNA 1000 kit was used during the experiments (Beckman, Fullerton, CA, USA), in reversed polarity separation mode (anode on the detection side). The ΦX-174 HaeIII restriction fragment digest was obtained from Promega (Madison, WI, USA). Previously published standard dsDNA analysis conditions [12] were used throughout the study, unless noted otherwise. The high conductivity buffer was prepared by reconstitution of the lyophilized eCAP dsDNA 1000 gel in 1 *M* sodium acetate containing 1000 m*M* Tris–borate, 2 m*M* EDTA (TBE) buffer, pH 8.3. The capillary length was 30 cm to the detector (37 cm total) and a constant running voltage of 11.1 kV (300 V/cm) was applied during the separations. Ultraviolet (UV) detection of the peaks was accomplished at 254 nm. Capillary cartridge running temperature was maintained at 20±0.1°C, sample temperature was ambient until analyzed. The electrolyte boundary was applied from the anodic side of the CE system, using the regular TBE buffer containing 1 *M* sodium acetate (pH 8.3).

3. Results and discussion

It was shown by Mikkers et al. [13] and later by Vigh [14] that gaussian peak shapes are attained in electrophoretic separations when, at a given position (x) and time (t), the sample constituents (s) have the same mobility as the carrier constituents (b), thus:

$$\mathrm{E}^{\mathrm{S}}(x,t) = \mathrm{E}^{\mathrm{B}}/(1-B) \tag{1}$$

where E^{S} is the field strength in the sample zone, E^{B} is the field strength in the buffer zone, and B is expressed as follows:

$$\mathrm{B} = \frac{C_A^{\mathrm{S}}}{C_X^{\mathrm{B}}}\left(\frac{r_k - r_X}{r_X - r_Y}\right)\left(\frac{r_Y - r_k}{r_k}\right) \tag{2}$$

where C_A^S and C_X^B are the analyte and the buffer co-ion concentrations in the sample and the background electrolyte zones, respectively, r_k is the relative mobility of the analyte, r_X is the relative mobility of the buffer co-ion and r_Y is the relative mobility of the buffer counter-ion. When $B=0$, $\mathrm{E}^{\mathrm{S}}(x,t)=\mathrm{E}^{\mathrm{B}}$, a perfect mobility match exists and symmetrical sample peaks are obtained. If $B\neq 0$, fronting or tailing of the peaks can be observed. One way to improve the peak shape is to change the concentration or the mobility of the buffer co-ion, but manipulation with the buffer counter-ion is also possible. As suggested by Eq. 2, the relative mobility of the buffer counter-ion can be varied in order to attain $B=0$ or close to zero conditions, resulting in better apparent peak shapes.

Fig. 1 exhibits two CGE separation traces of the ΦX-174 HaeIII restriction fragment mixture. The upper trace (A) shows a separation when regular 100 m*M* Tris–borate–2 m*M* EDTA, pH 8.3 (TBE) buffer was used in the electrolyte reservoirs, both at the anodic and cathodic sides. Fronting characteristics of each peak can be observed, probably due to the mobility mismatch between the separation buffer co-ion (borate~$9\cdot10^{-5}$ cm^2/V s) and the dsDNA fragments ($15–20\cdot10^{-5}$ cm^2/V s).

When the electrolyte was replaced in the anodic reservoir for a 1 *M* sodium acetate-containing TBE buffer (pH 8.3), the resulting separation is shown in the lower trace (B) in Fig. 1. Applying these separation conditions, almost symmetrical peaks were achieved, although, a slight tailing can be observed for the peaks with the highest base pair numbers (>1000 bp). An approximately 10% decrease in the separation time window between the 72-mer and 1353-mer sample components was also

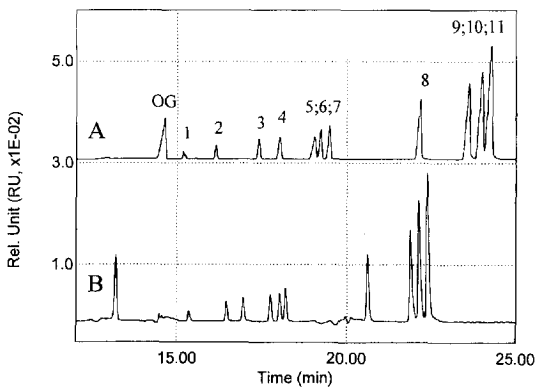

Fig. 1. Capillary gel electrophoresis separation of the ΦX-174 HaeIII restriction fragment mixture. The upper trace (A) shows the separation with regular TBE buffer in the buffer reservoirs, both at the anodic and cathodic sides. The lower trace (B) shows the separation of the same test mixture obtained with TBE buffer on the cathodic and 1 *M* sodium acetate-containing TBE buffer on the anodic side. Conditions: Capillary, 37 cm (30 cm to detector), 100 m*M* Tris–borate–2 m*M* EDTA buffer, pH 8.3 (TBE), $E = 300$ V/cm; Separation temperature, 20°C.

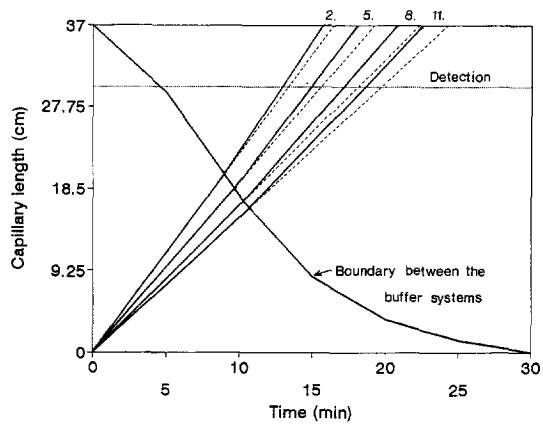

Fig. 2. Formation of the buffer boundary and migration of the sample components in the capillary by changing the composition of the anodic buffer (see Section 2. The solid lines indicate the migration of sample components (peaks 2, 5, 8 and 11) applying the transient electrolyte boundary. The dashed lines show the migration of the same peaks with the original TBE background electrolyte in both electrode reservoirs.

observed and the overall separation time decreased by the same rate.

The higher peak symmetry obtained can be explained by the effect of the high mobility sodium (counter-ion) entering the capillary from the anodic side, resulting in a favorable change in term *B* of Eq. 1. This change provides a better mobility match between the sample and the carrier constituents. When the front of each dsDNA fragment band runs into this high concentration and high mobility buffer zone, they experience a lower electric field strength and slow down. However, the back of the same analyte band is still in the lower conductivity zone, exposed to a higher potential drop. Therefore, their higher velocity causes compression of the peaks at the conductivity interface, resulting in peak sharpening and an apparent increase in efficiency. A similar buffer concentration effect has been used previously in on-column sample injection/preconcentration techniques [15].

Fig. 2 shows the migration of the buffer boundary and some of the sample components (peaks 2, 5, 8 and 11) in the gel-filled capillary column. It is important to note that the electric current increases remarkably, from 20 to 40 μA, when the high concentration Na^+ front enters the capillary, resulting in a somewhat faster migration of the sample components. This is probably due to the decrease in the buffer viscosity caused by the increased heat production, which is derived from the significantly higher electric current. As the mobility of the components slightly increase with the use of the high ionic strength buffer, the band spacing is decreased. There is also an observable increase in apparent peak efficiency, due to the better peak shapes attained. The average theoretical plate numbers of the separated eleven peaks have been increased from 276 000 to 390 000, in instances where TBE–TBE buffers were present in both reservoirs and where TBE–1 *M* sodium acetate/TBE buffers were present in the reservoirs, respectively.

Acknowledgments

The authors gratefully acknowledge Dr. Gyula Vigh for his stimulating discussions. The support of Drs. Kálmán Magyar and Nelson Cooke is also highly appreciated.

References

[1] J.Z. Zhang, Y. Fang, J.Y. Hou, H.J. Ren, R. Jiang, P. Roos and N.J. Dovichi, Anal. Chem., 67 (1995) 4589.

[2] D.N. Heiger, A.S. Cohen and B.L. Karger, J. Chromatogr., 516 (1990) 33.
[3] W. Lu, D.S. Han, J. Yuan and J.M. Andrieu, Nature, 368 (1994) 269.
[4] B.L. Karger, A.S. Cohen and A. Guttman, J. Chromatogr., 492 (1989) 585.
[5] A. Guttman, US Pat., 5 332 481.
[6] A. Guttman and N. Cooke, Anal. Chem., 63 (1991) 2038.
[7] M.D. Biggin, T.J. Gibson and G.F. Hong, Proc. Natl. Acad. Sci. U.S.A., 80 (1983) 3963.
[8] J.Y. Sheen and B. Seed, Biotechniques, 6 (1988) 942.
[9] R.J. Nelson, A. Paulus, A.S. Cohen, A. Guttman and B.L. Karger, J. Chromatogr., 480 (1989) 111.
[10] C. Gelfi, P.G. Righetti, L. Cremonesi and M. Ferrari, Electrophoresis, 15 (1994) 1506.
[11] A. Guttman, B. Wanders and N. Cooke, Anal. Chem., 64 (1992) 2348.
[12] eCAP dsDNA Kit, Care and Use instructions, Beckman Instruments, Inc., Fullerton, CA.
[13] F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, J. Chromatogr., 169 (1979) 1.
[14] Gy. Vigh, Personal communication.
[15] F. Foret, E. Szökö and B.L. Karger, Electrophoresis, 14 (1993) 417.