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Effect of sieving polymer concentration on separation of 100 bp DNA Ladder by capillary gel electrophoresis

T Nakazumi[1] and Y Hara[1]*

[1]Research Institute for Sustainable Chemistry, ISC, National Institute of Advanced Industrial Science and Technology, AIST, Central 5-2, 1-1-1 Higashi, Tsukuba 305-8565, Japan; E-Mail: nakazumi.t@aist.go.jp

*Correspondence: y-hara@aist.go.jp; Tel.: +81-29-81-6236

Abstract. We studied the effect of sieving polymer concentration on separation of a 100 bp DNA Ladder by capillary gel electrophoresis (CGE) using hydroxyethyl cellulose (HEC) with a molecular size of 1000 k. For measurement purposes, we selected a fused silica capillary with total length of 15 cm and effective length of 7.5 cm; this was applied to compact CGE equipment for a Point-Care-Testing (POCT) system. Measurement results of the 100 bp DNA Ladder sample indicated that small DNA separation was significantly affected by HEC sieving polymer concentration. This was due to the level of entanglement between small DNA molecules and the sieving polymer chain significantly influencing migration time, mobility, and resolution length of the CGE process. We concluded that 1.0 w/v % HEC sieving polymer concentration was optimal for CGE separation of DNA ≥ 1000 bp in the 100 bp DNA Ladder (100–1500 bp) when using the short-length capillary.

1. Introduction

Capillary gel electrophoresis (CGE) is a very useful tool for nucleic acid separation [1-4] and is especially suitable for separation of small DNAs produced by the polymerase chain reaction (PCR). For separation of PCR products by the CGE method, polymer solutions filled in capillary are needed to serve as the sieving polymer [5, 6]. Furthermore, in order to increase the accuracy of CGE separation analysis, many different aspects need to be considered, such as the type of sieving polymer solution, buffer solution, and migration process, among others [12-22]. There have been many previous studies regarding different sieving polymer solutions, such as poly(acrylamide) [7,8], poly(ethylene oxide) (PEO) [9], cellulose and its derivatives [10,11], and poly(vinyl pyrrolidine) (PVP) [12]. Linear polymer chains are basically used as the sieving polymer solution for separation of small DNAs during the CGE process; the linear polymer solution can be easily changed to the new sieving polymer solution with each CGE measurement. During the CGE separation process, DNA samples are entangled with the sieving polymer chain. Samples with different lengths interact differently with the sieving polymer chain and the migration time of the different DNA samples therefore also differs. Migration time is thus influenced by the level of entanglement between the sieving polymer chain and DNA samples [13, 14]. In this study, to prevent electroosmotic flow (EOF) during the CGE DNA separation process, a linear polyacrylamide chain was selected as the coating polymer for the fused silica capillary wall [15, 16]. The direction of EOF in the fused silica capillary is opposite to the direction of flow of DNA samples [17-19]. If conducting CGE without polymer coating on the capillary wall, DNA sample peaks cannot be detected because samples cannot be inserted into the capillary due to the prevention of EOF in the opposite direction to DNA samples. Because of this,

there have been many discussion of how to manage EOF by using coating polymers for the capillary wall; examples include cross-linked polyacrylamide [20], poly(N-acryloyl aminoethoxyethanol) [21], and poly(N-acryloyl aminopropanol) [22], among others.

In this study, we investigated the effect of sieving polymer concentration on separation of a 100 bp DNA Ladder. Hydroxyethyl cellulose (HEC), with a molecular size of 1000 k, was selected as the sieving polymer chain. The capillary had total length of 15 cm and effective length of 7.5 cm; these dimensions were selected to apply a short-size capillary to a compact CGE system that we are now developing. The compact CGE equipment is expected to be applicable for a Point-Care-Testing (POCT) system. Compared to slab gel electrophoresis (SGE), the CGE method is faster and requires smaller volume of DNA samples, with high-resolution in real time for analysis of PCR products. To develop the compact CGE system for POCT, in this investigation, we studied the influence of sieving polymer concentration on the separation process when using the short-size capillary to increase separation accuracy. Through measurement of small DNA samples, we show that the results of DNA separation were significantly affected by the sieving polymer concentration, due to entanglement between DNA molecules and sieving polymer chains.

2. Materials and Methods

2.1. Procedure for coating of short-size fused silica capillary

Firstly, the capillary was washed by using 1 N NaOH (15 min), water (15 min), and methanol (15 min). Next, an aqueous solution totaling 20 ml (containing 3-Methacryloxypropyltrimethoxysilane (Shin-Etsu chemical) (80 μL) and methanol (1 ml), with the addition of one drop of acetic acid) was sucked up into the capillary for 2 h at room temperature. Through this process, the silane coupling agent was covalently bonded to the glass wall of the fused-silica capillary. After washing with methanol and water, the monomer solutions were sucked up into the fused silica capillary for 2 h at room temperature. The monomer aqueous solutions were prepared by dissolving the acrylamide monomer (0.7 g), ammonium persulphate (APS) (20 mg), and N,N,N',N'-tetramethylethylenediamine (TEMED) (20 μL) in 20 ml aqueous solution. Before use, the monomer solutions were degassed for 30 min using nitrogen. The fused silica capillaries were then rinsed with water.

2.2. Self-constructed instrument for capillary gel electrophoresis (CGE)

All CGE measurements were performed with a self-constructed instrument composed of a high-voltage power supply (HJPQ-10P3, Matsusada) and a microscope with epi-illumination (IX73, Olympus, Tokyo, Japan) (Fig. 1). To detect the conjugate of SYBR Green II and DNA, a mercury lamp was used through the optical filter (U-FBWA, Olympus, Tokyo, Japan). By passing through the filter, a wavelength of 460–495 nm was utilized as the excitation light source. Fluorescence originating in the DNA samples was collected using a × 60 objective lens (UPlanFLN, Olympus) and the fluorescence signal was detected by a photomultiplier tube (PMT) (H8249-101, Hamamatsu Photonics, Hamamatsu, Japan). In addition, the signal received at the PMT was converted to a digital signal using National Instrument NI USB-6341. LabVIEW software (National Instrument) was used to control the applied voltage and acquire digital data. Fused silica capillaries with 75 μm circles (Polymicro Technologies, Phoenix, AZ, USA) were cut to 15 cm. As noted above, the effective length of the 15 cm capillary was 7.5 cm. To inject the DNA samples into the capillary, 1.5 kV was applied for 1 s; separation was then performed at 100 V/cm.

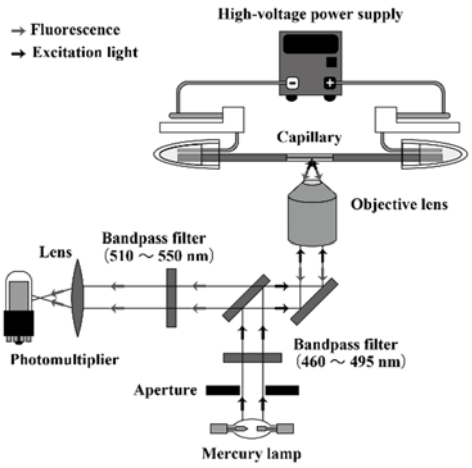

Figure 1. Schematic illustration of the capillary gel electrophoresis (CGE) instrument

2.3. CGE chemicals.

To prepare the running buffer for this experiment, 5 × TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1.0 mM EDTA, pH 8.3) (Nippon Gene) was diluted to 0.5 × with ultrapure water. The sieving polymer selected was hydroxyethyl cellulose (HEC), with a molecular size of 1000 k. The composition of the HEC polymer solution was adjusted to 0.5%, 0.8% and 1.0 w/v %, including 0.5 × TBE buffer and 2 × SYBR Green II (TAKARA BIO). The 100 bp DNA Ladder (TAKARA BIO), comprised of 11 double-stranded fragments with 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, and 1500 bp, was selected as the measurement target. The 130 μg/mL 100 bp DNA Ladder was diluted 10 times and was used in the CGE injection sample.

3. Results and Discussion

In this study, the fused silica capillary was coated with the polyacrylamide linear polymer chain, coupled with the silane coupling agent. The acrylamide linear polymer chain was synthesized after washing the capillary and chemically bonded to the silane coupling agent. When we measured small DNA samples using the capillary without coating, we did not detect the DNA peaks; this is because the DNA samples do not insert into the capillary due to EOF with opposite direction to that of the DNA samples. The capillary coating procedure is therefore very important for analysis of small DNAs by CGE.

Fig. 2 shows CGE measurement results for the 100 bp DNA Ladder using different concentrations of the sieving polymer chain (HEC, 1000 k). At > 1.0 w/v % HEC polymer solution, it was difficult to insert the polymer solution due to its high viscosity. In this study, we therefore measured CGE using concentrations ≤ 1.0 w/v %. As shown in Fig. 2, the fluorescence intensity at 1.0 w/v % was lower than at 0.5 w/v % and 0.8 w/v %. We considered this to be related to the insertion process of small DNAs. When the sieving polymer concentration increased, the mesh density of the sieving polymer chain increased. The introduction amount of small DNA samples therefore decreased and fluorescence intensity also decreased, as fluorescence intensity is determined by the amount of small DNA samples introduced.

To aid in evaluation of the electropherograms in Fig. 2, Fig. 3 summarizes the relationship between migration time and HEC concentration during the CGE process. As shown, migration time increased with an increase in sieving polymer concentration at all lengths of the small DNA samples. As the sieving polymer concentration increased, entanglement between the sieving polymer chain and DNA samples increased. With high concentrations of sieving polymer, DNA sample detection time was therefore longer than with low polymer concentrations. In addition, Fig. 4 shows the relationship between mobility and concentration of the HEC solution. As shown, when HEC concentration

decreased, mobility increased, because this is determined by the velocity of DNA samples in the capillary filled with HEC. As the HEC polymer concentration increases, electrophoresis of the DNA samples is inhibited. We therefore considered the mobility of DNA samples to be significantly affected by the concentration of the sieving polymer chain under constant applied voltage conditions.

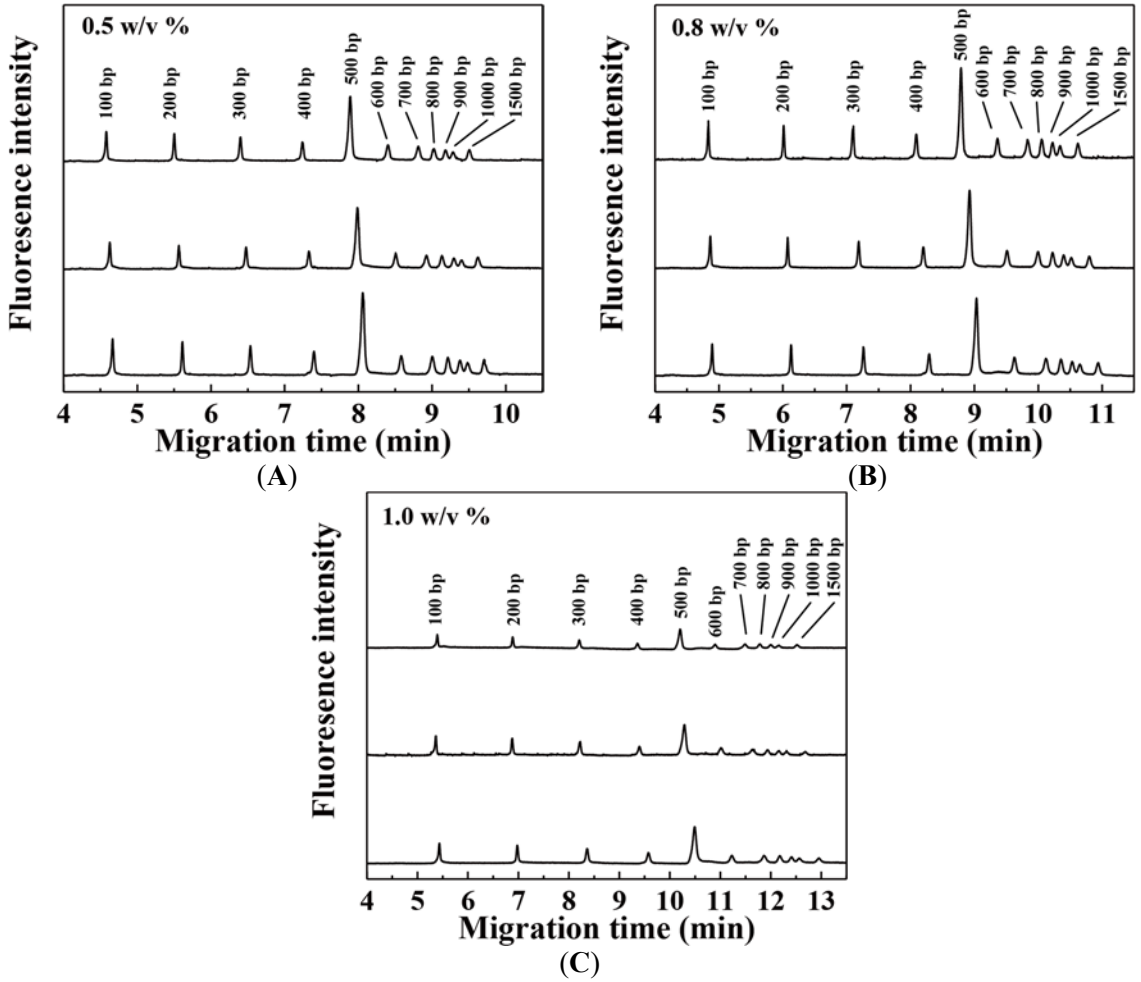

Figure 2. Separation of 100 bp DNA Ladder using different sieving polymer concentrations; (A) 0.5 w/v %, (B) 0.8 w/v %, and (C) 1.0 w/v %

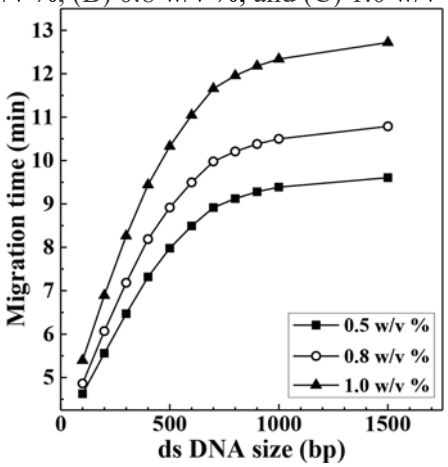

Figure 3. Relationship between migration time and concentration of HEC (as the sieving polymer) during CGE

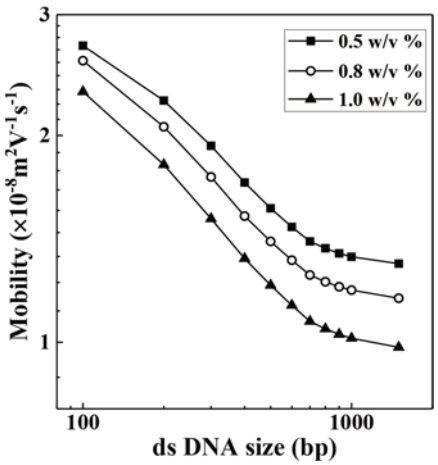

Figure 4. Relationship between mobility and concentration of HEC (as the sieving polymer) during CGE

Figure 5 shows the resolution length results of the 100 bp DNA Ladder samples. Resolution (Rs) was calculated using the following equation:

$$Rs = 1.18\ \Delta t / (w_{0.5}1 + w_{0.5}2) \quad (1)$$

Δt is the difference in migration time of the adjacent two peaks in the electropherograms. $w_{0.5}$ represents full width at half maximum of the peak in the graph. The resolution length (RSL) was determined by the following equation:

$$RSL = \Delta n / Rs \quad (2)$$

where Δn is the DNA length-difference of adjacent peaks in the graph, and Rs is the resolution value. As shown in Figure 5, the resolution length of the 100 bp DNA Ladder was almost the same ≤ 900 bp. At values ≥ 1000 bp, the difference in the resolution length increased. When the sieving polymer concentration was 1.0 w/v %, the RSL value was notably lower than at other concentrations ≥ 1000 bp. Lower RSL values indicate higher separation performance. Under these CGE conditions, for clear separation of the 1000–1500 bp DNA samples with the short-length capillary, we therefore conclude that 1.0 w/v % HEC (1000 k) sieving polymer concentration is optimal.

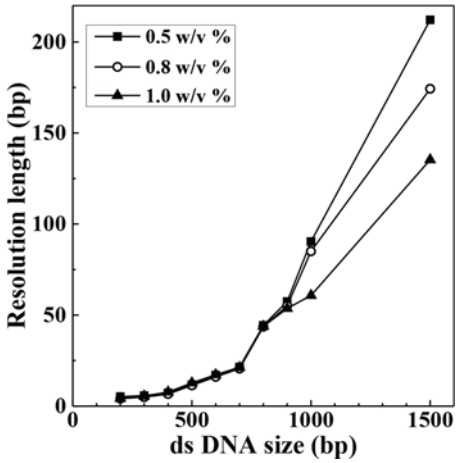

Figure 5. Relationship between resolution length and concentration of HEC (as the sieving polymer) during CGE

4. Conclusions

In this study, we investigated the influence of the concentration of sieving polymer (HEC, molecular size 1000 k) on separation of a 100 bp DNA Ladder sample with a short-length capillary (total length 15 cm and effective length 7.5 cm). When the concentration of sieving polymer increased, migration time increased. In addition, mobility decreased as the sieving polymer concentration increased. This clearly indicates that the strength of entanglement between DNA samples and the sieving polymer was determined by sieving polymer concentration. This is because resistance to DNA flow increased in the capillary as sieving polymer concentration increased. In addition, the resolution length was almost the same for DNA length ≤ 900 bp. On the other hand, under conditions ≥ 1000 bp in the 100 bp DNA Ladder sample, the resolution length at 1.0 w/v % was lower than at other concentrations. The optimal sieving polymer concentration for separation of the DNA ≥ 1000 bp in the 100 bp DNA Ladder sample (100–1500 bp) with the short-length capillary was thus 1.0 w/v %.

Acknowledgments

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