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Polyethyleneimine-Coated Capillaries for the Separation of DNA by Capillary Electrophoresis

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A new capillary electrophoresis of DNA fragments has been achieved with the cooperative effects of the electrophoretic and electroosmotic mobilities realized via the polyethyleneimine (PEI) coating of the capillary walls. As a result, seven of the 11 different-sized DNA fragments have produced resolved CE peaks. Our results demonstrate that analyses of DNA constituents can be performed under a wide range of conditions by the appropriate tuning of the coating and filler within the capillary.

Key Words: Capillary electrophoresis, DNA, Polyethyleneimine

Introduction

Currently, the microscopic understanding of DNA structure and function is a major problem of widespread interest. Recently, capillary electrophoresis (CE) has arisen as an effective and accessible method for the fast resolution of the microscopic constituents of complex mixtures.

Molecular sieving with diluted solutions of entangled linear polymers such as polyacryamide (PAA)[1−3] cellulose derivatives[4−6], polyvinyl amine (PVA)[7−9] or polyethylene glycol (PEG)[9,10] have been developed for CE separations. Due to the advantages of shorter analysis times, easily refreshed buffer systems, and stability under high electric fields compared to gel-filled capillaries, this separation procedure has recently played an important role in DNA analysis.

Applications of CE to the analysis of fragmented DNA have been achieved in bare silica capillaries, with PAA coatings introduced by Hjerten[11] being the most commonly used modification method. However, no attempt had been made so far to separate DNA in a capillary coated with a positively charged polymer, because the opposite charge of DNA would cause adsorption on the wall. On the other hand, in the last few years, the interaction of DNA with oppositely charged polymers has attracted great interest due to the importance of such systems for gene delivery. Among the several cationic polymers used for this purpose, the ability of polyethyleneimine (PEI) to act as an efficient vector for gene and oligonucleotide transfer

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into mammalian cells has been reported[12]. Thus, the use of PEI coating[13] in the capillary electrophoretic separation of DNA fragments appears to be called for.

In this work, we demonstrate DNA separation in PEI-coated capillaries, as a result of which, seven out of 11 different-sized DNA fragments produced fully resolved CE peaks.

Experimental

Chemicals

Two different hydroxyethylcellulose (HEC) samples were used. An HEC sample with a manufacturer-determined number average molecular mass of $M_n \cong 438{,}000$ g/mol was obtained from Polyscience Inc. (Warrington, England). HEC type Cellosize-WP-40; 75-125 mPa x s for a 2% w/w aqueous solution, 20 °C and PEI (molecular mass range 6.10^5-1.10^6) were purchased from Fluka (Fluka AG, Buchs, Switzerland). The buffer used in all experiments with DNA was 89 mM Tris, 89 mM borate, and 5 mM EDTA with a pH of 8.2. All buffer reagents were purchased from Merck (Darmstadt, Germany). The ΦX174 DNA/ *Hae* III digest containing 11 fragments of 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078 and 1353 bp was obtained from Sigma (St. Louis, MO, USA).

Equipment

Separations were carried out with a commercial CE injection system (Prince Technologies BV, Emmen, Netherlands) in combination with an on-column variable wavelength UV Visible detector (Lambda 1000, Bishoff, Leonberg, Germany). The wavelength was set at 260 nm. The fused silica capillaries used for separation experiments were 75 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA). The total and effective lengths of capillaries are indicated in the figures. The injection was carried out at the cathodic side using controlled pressure for a fixed time.

Coating Procedure

The coating procedure was as described before[13]. The procedure involves the following steps.

The fused silica capillary was first etched by flushing the capillary with a solution of 1 M sodium hydroxide for 30 min at 1.10^{-1} MPa and with water for 15 min at the same pressure. Then the capillary was flushed with a solution of 10% PEI in water at $1.5.10^{-1}$ MPa for 10 min and the PEI solution was left in the capillary for 1 h. Next, the polymer solution was pressed out of the capillary with air at $1.5.10^{-1}$ MPa. Finally, the capillary was rinsed with water for 15 min.

Automated capillary rinsing, sample introduction, and execution of the electrophoretic runs were controlled by a personal computer. Data processing was carried out with commercial CE software (Prince Technologies BV, Emmen, Netherlands). A washing step of 2 min with 0.1 M HCl and 2 min with buffer between runs was applied.

Results and Discussion

When DNA separation is performed in a PAA-coated capillary, electroosmotic flow (EOF) is mostly suppressed and DNA fragments migrate with their electrophoretic mobility. Molecular size separation is achieved

from small fragments to larger fragments. On the other hand, in the bare silica column, the separation order is reversed due to EOF.

The pH dependence of electroosmotic flow was reported before for capillaries coated by the same procedure[13]. Eight different buffers were used covering a pH range from 3 to 10.4. In this pH range, the electroosmotic flow was always in the anodal direction. This is due to the protonation of the amine groups of the PEI adsorbed on the capillary wall, which changed the sign of the surface charge. Thus, when the DNA sample is injected from the negative side of the capillary, the electroosmotic mobility (EOM) and electrophoretic mobilities of DNA fragments are in the same direction.

Fragments migrate in order, from smaller to larger, as in PAA-coated capillaries. Figure 1 shows the separation electropherogram of DNA fragments containing 11 base pairs in two different concentrations of HEC, medium viscosity. The number average molecular mass of this sample was determined in our laboratory by measuring the viscosity of the HEC solutions at $M_n \cong 100{,}000$ g/mol. At 0.5% concentration of HEC, the small fragments ranging in size from 73 to 603 bp (except for the pair 271/281) completely separated and evidently the last three larger fragments also came together, but with a visible resolution structure overriding this large peak. Increasing the concentration of HEC to 0.8% improves the resolution of the small fragments, but the resolution structure of the last peak is lost. Thus, the increase in the concentration of the sieving medium selectively improves the separation of small fragments because of the decrease in the pore size. Figure 2 shows the separation of the same sample in HEC with an average molecular weight of 438,000. Here again the resolution of small fragments increases with increasing concentration of polymer in the buffer. At 0.35% concentration of HEC, the resolution of seven small fragments is quite good, but the three larger fragments come together. At concentrations below 0.35% HEC, these three peaks are still unresolved.

In a capillary column coated with a positively charged polymer, the irreversible surface adsorption, and therefore lack of mobility, of a negatively charged polymer such as DNA could be expected. Thus, surprisingly, Figures 1 and 2 show the clear resolution of at least seven of the 11 DNA fragments from the coated capillary. Cellulose derivatives on silica capillaries are known to form a dynamic coating. The sieving polymer that we used in a similar vein could have been expected to prevent, by its own coating, surface adsorption. However, our observation that no changes occur in EOM, across TBE buffers containing HEC in the molecular weights and concentrations used in Figures 1 and 2 and in TBE buffers with no HEC, indicates that HEC does not influence the surface composition. The EOM of the column is $3.2.10^{-4}$ $cm^2V^{-1}s^{-1}$ in all buffers. The mobility increases that could be expected from viscosity differentials caused by different HEC concentrations are suppressed by the weak dependence of the viscosity on HEC concentration, especially at low concentrations ($< 2\%$), compared with other cellulose derivatives[14].

In conclusion, the invariance of the EOM in the buffers that we have used shows the lack of interaction between HEC and PEI. By contrast, DNA does interact with PEI. The electrophoretic mobilities obtained with DNA fragments are lower than those obtained with DNA fragments in buffers containing HEC in the same concentrations but in silica columns[15]. The decrease in the DNA fragment mobilities reflects the interaction with the walls. However, the invariance of the column EOM in the following injections and the reproducible DNA separation results show that there is no irreversible adsorption. The slowing down of the DNA fragments is apparently due to a complex formation-type interaction between the DNA and the PEI on the walls. A similar effect was observed in previous studies with phenols and inorganic ions in PEI columns[16,17]. Specifically, in large base pairs, this interaction is greater, so that the electrophoretic mobilities approach zero and the fragments sweep together under the effect of the EOF. A DNA fragment can have

multiple conformations with PEI. For all fragments, the interaction is both electrostatic and hydrophobic. However, for large fragments, the hydrophobic interaction is stronger, causing the peaks to be broader, due to the multiple conformations.

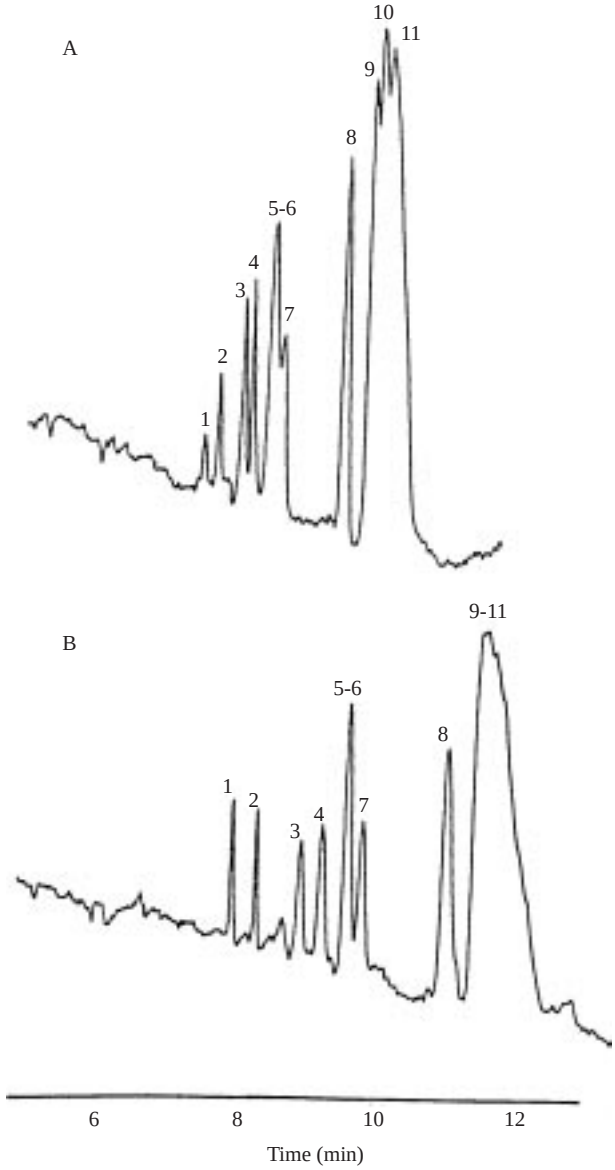

Figure 1. Capillary electrophoresis of ϕX174/ *Hae* III restriction fragments in solutions of (A) 0.5% and (B) 0.8% HEC (medium viscosity), in 89 mM Tris, 89 mM Boric acid, 5 mM EDTA, pH: 8.2. 10% PEI coated capillary, 75.2 cm (effective length 63.3 cm) x 75 μm I.D. UV detection at 260nm. Run voltage –28kV. Peaks: 1 = 72 bp, 2 = 118 bp, 3 = 194 bp, 4 = 234 bp, 5 = 271 bp, 6 = 281 bp, 7 = 310 bp, 8 = 603 bp, 9 = 872 bp, 10 = 1078 bp, 11 = 1353 bp.

HEC is necessary to achieve separation. The coating alone does not cause any separation effect. When DNA fragments are injected to the TBE buffer, a single peak is observed, as seen in Figure 3.

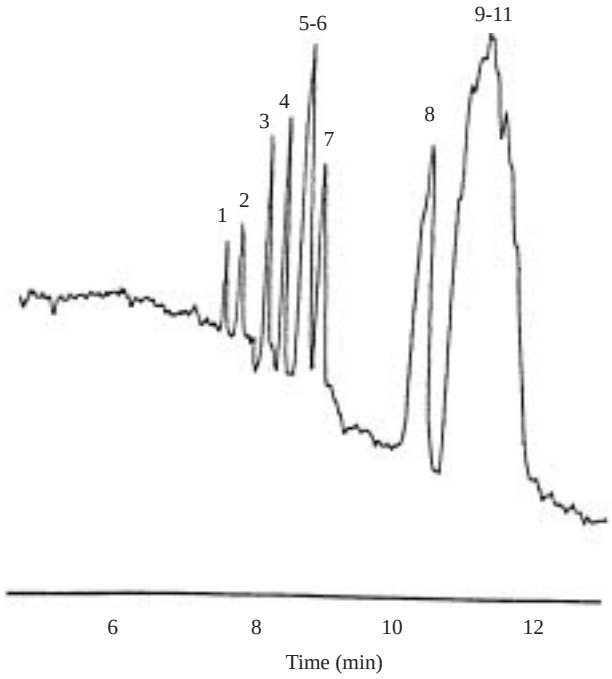

Figure 2. Capillary electrophoresis of ϕX174/ *Hae* III restriction fragments in a solution of 0.35% HEC ($M_n \cong$ 438,000) in 89 mM Tris, 89 mM Boric acid and 5 mM EDTA, pH: 8.2. Other conditions and peak identifications are the same as in Figure 1.

Figure 3. Capillary electrophoresis of ϕX174/ *Hae* III restriction fragments in a 89 mM Tris, 89 mM Boric acid and 5 mM EDTA buffer (0% HEC), pH: 8.2. Other conditions and peak identifications are the same as in Figure 1.

When PAA and silica columns are compared, the retention time in the silica column is long and the resolution is good, since the EOM and the electrophoretic mobility are in opposite directions. In PAA-coated columns, since the DNA fragments arrive under their electrophoretic mobilities alone, the retention time is shorter and the resolution not as good. However, in the PEI column, a resolution is achieved in spite of the EOM and the electrophoretic mobilities being in the same direction.

The efficient separation of proteins in the PEI-coated capillaries has been reported before[18]. In this study, we observed an interaction between proteins and PEI and the migration selectivity of proteins changed with the addition of PEI to the buffer. Recently, the interaction between DNA and cationic surfactants by CE has been reported[19]. For the same reason, the study of the interaction between short DNA fragments and the oppositely charged cationic polymer PEI, using coated capillaries, following the changes of electrophoretic mobility because of the interaction, should give interesting results in the construction of non-viral genetic delivery systems.

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