Articles

Analysis of Broad-Range DNA Fragments with Yttrium Oxide or Ytterbium Oxide Nanoparticle/Polymer Sieving Matrix Using High-Performance Capillary Electrophoresis

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We have developed the yttrium oxide (YNP) or ytterbium oxide (YbNP) nanoparticle/polymer matrices for the size-dependent separation of DNA ranging from 100 bp to 9,000 bp. High separation efficiency (> 10^6 plates/m) and the baseline resolution for various DNA standards (100 bp, 500 bp, and 1 kbp DNA ladder) were obtained in 10 min with these matrices. The effects of concentrations of both polyethylene oxide (PEO) and nanoparticles were investigated and the highest performance was obtained at 0.02% PEO with 0.02% YNP or YbNP. Similar sieving power for both YNP and YbNP matrices was observed probably due to the similar sizes of nanoparticles, resulting in the formation of comparable sieving networks for DNA separation. For the reduction of electrosmotic flow, either dynamic or permanent coating of the capillary inner wall was compared and it turned out that PEO was superior to polyvinylpyrrolidone (PVP) or polyacrylamide (PAA) for better separation efficiency.

Key Words: Capillary, Nanoparticle, DNA, Yttrium oxide, Ytterbium oxide

Introduction

The construction of genetic map and detailed information of complete nucleotide sequence of human genomic DNA have great medical significance since small change in human DNA (mutation, polymorphism, etc.) would lead to considerable human diseases.[1-4] DNA diagnosis in conjunction with DNA sequencing, DNA polymorphism,[5] and forensic analysis[6] has been successfully performed by electrophoretic techniques.[7]

Capillary electrophoresis (CE)[8] has been proven to have numerous advantages including high speed separation, high separation efficiency, and enhanced resolution over slab-gel electrophoresis (SGE) and ion chromatography (IC).[9] CE is also feasible for simultaneous multiple operation by using capillary array[10] or parallel channels in a microchip.[11]

The development of separation medium is one of the most important factors for DNA separation by CE since migration behavior and resolution of DNA fragments are determined by a sieving matrix. A number of different polymers such as cellulose derivatives including methyl cellulose,[12] hydroxyethyl cellulose,[13] and hydroxypropylmethyl cellulose,[14] linear polyacrylamide,[15] polyethylene oxide,[16] polyvinyl pyrrolidone,[17] polyvinyl alcohol,[18] and poly-*N*,*N*-dimethylacrylamide (PDMA)[19] had been employed. Most of the polymers require the use of the coated capillary, which basically reduces both electrosmotic flow and the adsorption of DNA onto the capillary inner wall. Since these polymers require *in situ* synthesis of polymer layer on the capillary wall, the problems associated with capillary fouling, coating inhomogeneity, and limited lifetime have been issued. To circumvent these difficulties, an alternative dynamic coating protocol using mainly hydrogen bonding between the Si-OH group of the capillary inner wall and the water-soluble polymers such as PEO, PVP, and PDMA have been successfully applied.[16,17,19]

Recent developments on DNA separation matrix have focused on the combination of good separation efficiency with dynamic coating and low viscosity for high throughput analysis. For example, mannitol added poly(*N*-isopropylacrylamide) (PNIPAM),[20] gold nanoparticle (GNP),[21] and synthetic PEO[7] showed the potential for this purpose. However, it turned out that mannitol-added PNIPAM had a little dynamic coating ability for the reproducible DNA separation. In the case of GNP, the preparation of gold nanoparticle took long (4 > hr) and the cost was high for nanoparticle production. PEO also showed a disadvantage in that the capillary was not readily reusable.

In this paper, we have developed the DNA sieving matrix containing yttrium or ytterbium oxide nanoparticles mixed with PEO. Since these nanoparticles and PEO were commercially available, the preparation of the matrix was relatively cheap and took less than 0.5 hr. DNA fragments ranging from 100 bp to 9000 bp were employed and their separation was investigated with various concentrations of PEO and yttrium or ytterbium oxide nanoparticles. Also, either dynamic or permanent coating of the capillary inner wall with various polymers was compared and optimized.

Experimental

Chemicals. Bare fused silica capillaries with 75 μm i.d. and

360 μm o.d. were purchased from Polymicro Technologies (Phoenix, AZ, USA). A detection window was prepared by burning the polyimide coating with a hot sulfuric acid. Total lengths of the capillary were 30 cm with the effective lengths of 22 cm. The platinum electrode (0.5 mm ϕ) was obtained from Aldrich Co. (MO, USA).

Tris base, boric acid, and ethylenediaminetetraacetic acid (EDTA) (all from Aldrich Co.) were used for TBE buffer preparation. A monomeric dsDNA intercalating dye, ethidium bromide (EB) was purchased from Aldrich. Since EB is mutagenic and carcinogenic, a pair of lab-glove should be worn during treatment. Polyethylene oxide (M_r = 8,000,000), polyvinylpyrrolidone (M_r = 1,300,000), yttrium oxide (Y_2O_3) and ytterbium oxide (Yb_2O_3) nanoparticles were also obtained from Aldrich Co.

Standard dsDNA fragments, 100 bp ladder, 500 bp ladder, and 1kp ladder (Takara Bio Inc., Japan) with the concentration of 50-125 ng/mL were employed for the experiment. Deionzed water (Mili-Q reagent water system, MA, USA) was used throughout the experiment.

Capillary electrophoresis with nanoparticle. A high-voltage power supply (-30 kV, Spellman, NY, USA) was used for electrophoresis with the electric field strength of 100 V/cm - 300 V/cm. A 1.5 mW He-Ne laser (Edmund Scientific Co., NJ, USA) with 543.6 nm output was used for the excitation of DNA labeling dye, EB. Two RG610 optical filters were used to block scattered laser light. The fluorescence signal was collected with a 10X microscope objective (Nikon, Japan) into the photomultiplier module (H5784-02, Hamamatsu, Shinzuka, Japan) and transferred directly through a low-pass filter to an A/D interface board (National Instrument Co., TX, USA). The control of the high-voltage power supply and data collection at 7 Hz was performed by an in-house LabView program with an IBM compatible computer.

For the permanent coating of the capillary wall, Hjerten's method was employed.[22] Briefly, 0.004% of γ-methacryloxypropyltrimethoxysilane (γ-MAPS, Aldrich Co., Mo, USA) was introduced into the capillary for 1.5-2 h for the activation of surface silanol group. Then, acrylamide (3.5%) solution containing 1.0 mg/mL $K_2S_2O_8$ and *N,N,N',N'*-tetramethylethylenediamine (TEMED) was pushed into the capillary for 3 h. For dynamic coating of the capillary wall, the capillary was firstly flushed with 10 mM HCl for 20 min. Then, TBE containing PEO (0.05%) was introduced with the positive pressure on one side with the syringe and the negative pressure on the other side with the vacuum pump for 5-10 min. The capillary coated with this method could survive more than 4 weeks if stored in neutral water when not in use. It was found that migration time for each DNA at the given condition after coating showed error less than 2%.

A 1X TBE buffer (89 mM Tris, 89 mM borate, and 2 mM EDTA, pH 8.4) was filtered once with 0.25-μm membrane filter paper (Milipore Co., MA, USA). A stock solution of nanoparticle (0.1%, w/v) in 1X TBE prepared after ultrasonication for 30 min. Then, it was diluted for the concentration of 0.005% - 0.01% by 1X TBE containing PEO (0.05%, w/v, M_r 8,000,000). This solution was homogeneously mixed by vigorous stirring for 4 h. Then, it was degassed by vacuum. The TBE buffer containing nanoparticle was pushed into the capillary with the positive pressure for 1 - 2 min. Two glass vials for TBE buffer containing nanoparticle were placed on both ends of the capillary. The capillary was electrophoretically equilibrated by applying the voltage the same as the separation electric field for 10 min before sample injection. The injection for DNA sample was performed at 4 kV for 4 s. After each run, the capillary was flushed with water for 15-30 min, and then a new nanoparticle buffer was introduced.

Results and Discussion

Figure 1 shows the electropherograms of 500 bp DNA step ladder with different sieving conditions. When the capillary inner wall was covalently coated with the acrylamide polymer (see Hjerten's method at experimental section),[22] the electrosmotic flow was reduced enough to let DNA migrate toward detection window. This was good enough to produce DNA peak in electropherogram, however, no separation of 10 DNA fragments in 500 bp DNA step ladder was obtained as shown in Figure 1(a). This was expected since electrophoretic mobilities of 10 DNA fragments would be similar without sieving medium since their charge-to-mass ratio are the same. When yttrium nanoparticle (YNP) was added into the separation buffer, still no separation was observed, but the peak became more sharpened (Figure 1(b)). It is known that nanoparticles tend to be adsorbed onto the polymer layer of the capillary inner wall, which further reduces DNA interaction with the capillary wall, resulting in sharp peaks and shortened migration time. When the low concentration (0.02%) of polyethylene oxide (PEO) was added in the buffer, partial

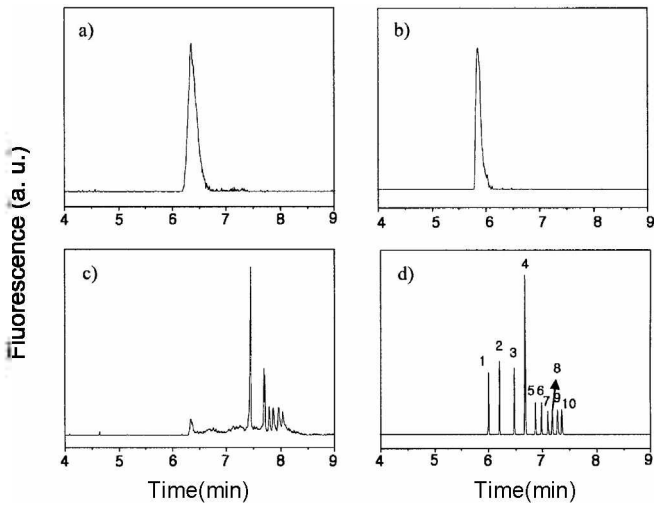

Figure 1. Separations of 500 bp DNA step ladder with (a) no sieving medium, capillary wall-coated by Hjerten's method, (b) 0.02% YNP only, capillary as in (a), (c) 0.02% PEO only, capillary wall-dynamically coated, (d) 0.02% YNP and 0.02% PEO, capillary as in (c). Electrophoresis conditions: DNA sample concentration, 60-125 ng/μL; PEO prepared in 1X TBE (89 mM Tris, 89 mM borate, and 2 mM EDTA (pH 8.4)) at 0.02%, 0.5 μg/mL EB; electrokinetic injection at 4 kV for 3s; separation at 5.4 kV, fused-silica capillary; 360 μm o.d., 75 μm i.d., 30 cm total length, and 22 cm effective length. Peak assignment; 1 = 500 bp, 2 = 1000 bp, 3 = 1500 bp, 4 = 2000 bp, 5 = 2500 bp, 6 = 3000 bp, 7 = 3500 bp, 8 = 4000 bp, 9 = 4500 bp, 10 = 5000 bp.

separation was obtained with better resolution for longer DNA sizes (Figure 1(c)). This concentration is far below PEO (M_r = 8,000,000)'s entanglement (overlap) threshold (c*) of 0.07%.[23] It should be noted that for the concentration above c*, the polymer begins to form "pores" to provide DNA fragments with size-dependant separation. With our low PEO concentration, it is known that instead of forming pores, DNA molecules drag the polymer along as they are encountered during migration (transient entanglement mechanism),[24] which was supported by dynamic formation and deformation of U-shape in DNA conformation.[23] When the drag force by DNA is not enough due to smaller mass of DNA, it is expected that the resolution suffers as shown in Figure 1(c) for relatively smaller size of DNA fragments. An excellent separation efficiency was obtained with the separation medium of the mixture of PEO and YNP as shown in Figure 1(d). All 10 different DNA fragments were baseline resolved in 10 min. According to Eisenberg's model,[25] nanoparticle and polymer have certain degree of interaction, causing the limited movement of both nanoparticle and polymer. Therefore, it leads to the formation of immobilized and restricted mobility regions around the nanoparticle, resulting in the formation of the effective network for DNA fragment separation.

Figure 2 represents the transmission electron microscopic (TEM) images of yttrium (YNP) and ytterbium (YbNP) nanoparticles. The average particle sizes are between 25 - 40 nm for both YNP and YbNP. The particle shape is not perfectly spherical, but their appearances are alike. As shown in Figure 1(d) and Figure 5(b), the separation of DNA 500 bp ladder was successful for both YNP and YbNP. From those results, it seems that similar separation efficiency and the resolution for both YNP and YbNP matrices could be attributed to their apparent sizes. In our previous work, somewhat improved resolution for DNA fragments was obtained with relatively smaller silica nanoparticle (~7 nm), however, the separation efficiency was deteriorated with α-alumina nanoparticle (~200 nm). The matrix containing GNP[21,23] showed better resolution for DNA *Hind*III digest and ΦX174 RF DNA *Hae*III digest. In that case, the particle size of GNP was around 56 nm, which is similar to those of our YNP and YbNP nanoparticles. Note that GNP is spherical,[26] but the

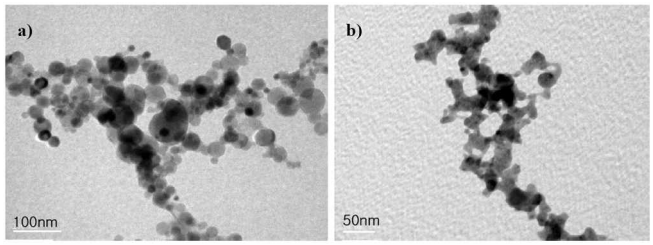

Figure 2. Transmission electron microscopy (TEM) image of nanoparticles. (a) YNP (average particle size, 25-30 nm), (b) YbNP (average particle size, < 40 nm). TEM acceleration voltage: 200 kV.

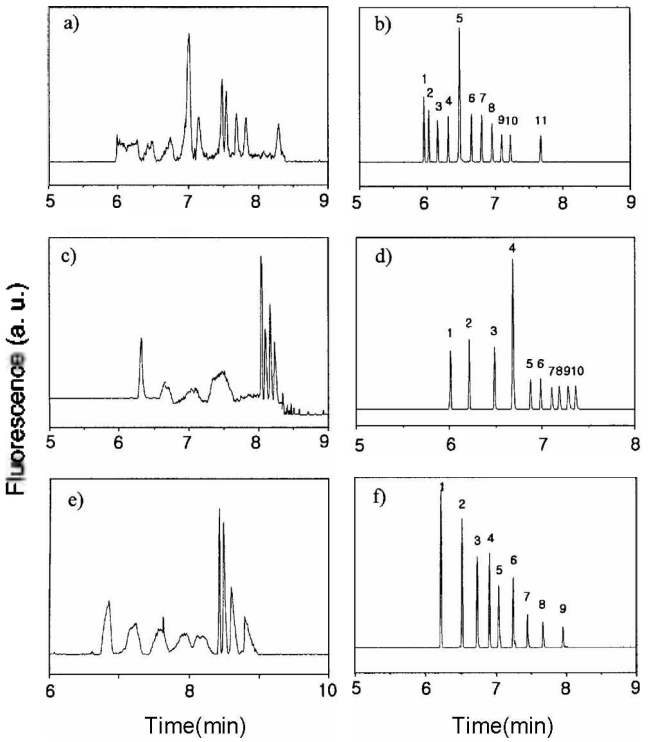

Figure 3. Separation of 100 bp DNA step ladder using (a) 0.10% PEO only and (b) 0.02% YNP and 0.10% PEO, peak assignment; 1 = 100 bp, 2 = 200 bp, 3 = 300 bp, 4 = 400 bp, 5 = 500 bp, 6 = 600 bp, 7 = 700 bp, 8 = 800 bp, 9 = 900 bp, 10 = 1000 bp, 11 = 1500 bp. Separation of 500 bp DNA ladder using (c) 0.02% PEO only and (d) 0.02% YNP and 0.02% PEO, peak assignment; the same as in Figure 1. Separation of 1 kbp DNA ladder using (e) 0.02% PEO only and (f) 0.02% YNP and 0.02% PEO, peak assignment; 1 = 1000 bp, 2 = 2000 bp, 3 = 3000 bp, 4 = 4000 bp, 5 = 5000 bp, 6 = 6000 bp, 7 = 7000 bp, 8 = 8000 bp, 9 = 9000 bp. Other conditions were the same as in Figure 1.

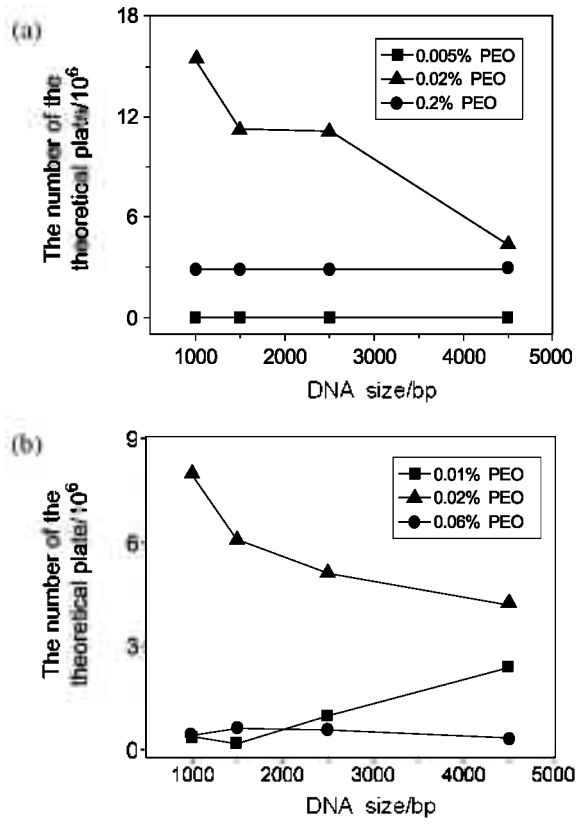

Figure 4. The number of the theoretical plates of 500 bp ladder DNA using (a) YNP (0.02%) and (b) YbNP (0.02%) with different PEO concentrations.

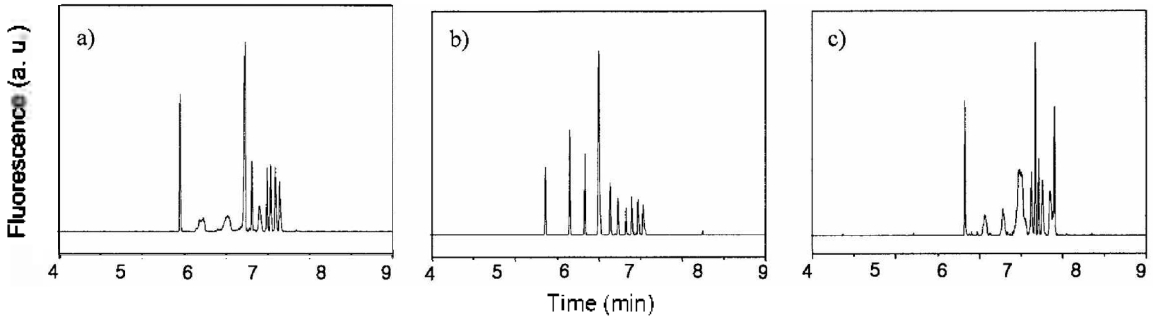

Figure 5. Separation of 500 bp DNA step ladder using (a) 0.005% YbNP, (b) 0.02% YbNP and (c) 0.04% YbNP. Electrophoresis conditions: DNA sample concentration, 60-125 ng/μL; PEO prepared in 1XTBE (89 mM Tris, 89 mM borate, and 2 mM EDTA (pH 8.4)) at 0.02%, 0.5 μg/mL EB; electrokinetic injection at 4 kV 3s; separation at 5.4 kV. fused-silica capillary: 360 μm o.d., 75 μm i.d., 30 cm total length, and 22 cm effective length. Peak assignment; the same as in Figure 1.

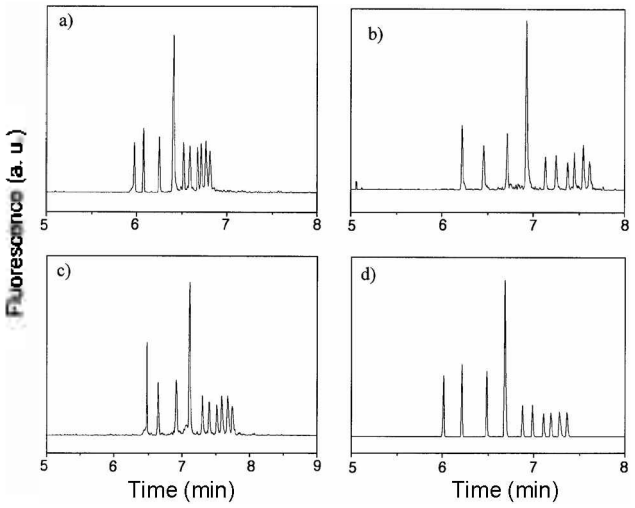

Figure 6. Separation of 500 bp DNA step ladder using (a) 5% PVP coating overnight, (b) 0.5% PEO coating overnight, (c) permanent coating, (d) 0.02% PEO dynamic coating. Electrophoresis conditions: DNA sample concentration, 60-125 ng/μL; PEO prepared in 1X TBE (89 mM Tris, 89 mM borate, and 2 mM EDTA (pH 8.5)) at 0.02%, 0.5 μg/mL EB; electrokinetic injection at 4 kV 3s; separation at 5.4 kV. fused-silica capillary: 360 μm o.d., 75 μm i.d., 30 cm total length, and 22 cm effective length. Peak assignment; the same as in Figure 1.

shape of YNP (Figure 2a), YbNP (Figure 2b), α-alumina[27] and silica nanoparticle[28] is somewhat random. Therefore, it seems that the formation of effective sieving network depends on the size of nanoparticles rather than the shape of them.

YNP/PEO separation matrices have been applied to several different DNA samples (100 bp, 500 bp, and 1 kbp DNA ladder) as shown in Figure 3. With the good combination of YNP and PEO, DNA samples ranging from 100 bp to 9 kbp were successfully separated in 8 min. When PEO concentration was varied with fixed nanoparticle concentration at 0.02%, the highest separation efficiency was obtained at 0.02% for both YNP and YbNP concentrations (Figure 4). When the PEO concentration was fixed at 0.02%, better separation efficiency was obtained with increasing YbNP concentrations (Figure 5). However, the resolution started being deteriorated at nanoparticle concentration larger than 0.04%. This loss of resolution may be caused by the formation of partial aggregation of nanoparticle and EB (for DNA fluorescence) in the bulk solution that weakens the interaction between DNA and nanoparticle.

Since the capillary inner wall coating is important for DNA separation, several coating reagents were tested (Figure 6). In terms of the ability to reduce the electroosmotic flow and the longevity of polymer coating, it is known that PVP provides better coating effect compared to that of PEO. However, the resolution suffers more with PVP than PEO as shown in Figure 6(a) and (d).

This may be caused by the higher adsorption of nanoparticles onto PVP, resulting in worse formation of DNA separation matrix. It seems that polyacrylamide (PAA) used in the permanent coating (Figure 6(c)) has similar nanoparticle stability when they were adsorbed onto PAA. Better separation efficiency was observed with PEO dynamic coating in 20 min (Figure 6(d)) rather than overnight (Figure 6(b)), facilitating easy preparation of DNA separation matrix.

Compared to other types of nanoparticles especially gold, either YNP or YbNP nanoparticles are cheaper and easier to prepare in the separation buffer. Since the viscosity of the nanoparticle containing sieving matrix is low (< 15cp), the potential for automation and multiplexing for DNA diagnosis is excellent. Application of this sieving matrix to genetic disease diagnosis with PCR products and lab-on-a-chip is under progress in our laboratory.

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