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Improved separation of double-stranded DNA fragments by capillary electrophoresis using poly(ethylene oxide) solution containing colloids

The analysis of double-stranded (ds) DNA fragments by capillary electrophoresis (CE) using poly(ethylene oxide) (PEO) solution containing gold nanoparticles (GNPs) is presented, focusing on evaluating size dependence of the GNPs and PEO on resolution and speed. To prevent the interaction of the capillary wall with DNA, the capillary was dynamically coated with polyvinylpyrrolidone. Using different PEO solutions containing GNPs ranging in diameter from 3.5 to 56 nm, we have achieved reproducible, rapid, and high-resolution DNA separations. The results indicate that the sizes of PEO and GNPs as well as the concentration of PEO affect resolution. The separation of DNA ranging in size from 8 to 2176 base pairs (bp) was accomplished in 5 min using 0.2% PEO (8 MDa) containing 56 nm GNPs. We have also demonstrated the separations of the DNA fragments ranging from 5 to 40 kbp using 0.05% PEO (2 MDa) containing 13 nm GNPs or 0.05% PEO (4 MDa) containing 32 nm GNPs. With very low viscosity ($<$ 15 cP), automatic replacement of the sieving matrices is easy, indicating a great potential for high-throughput DNA analysis using capillary array electrophoresis systems.

Keywords: Capillary electrophoresis / DNA separation / Gold nanoparticle / Poly(ethylene oxide)
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1 Introduction

Capillary array electrophoresis (CAE) in conjunction with laser-induced fluorescence (LIF) has been considerably assisted the rapid progress in Human Genome Project, which has driven the success of completing the draft sequence well ahead of schedule [1]. In addition, numerous techniques based on capillary electrophoresis (CE) and CAE have been demonstrated, which enable the analyses of a variety of genes and the resulting RNA molecules and the proteins; human and microbial identification by differentiating short tandem repeats, screening for human genetic defects, and so forth [2–5].

The success of CAE and CE for DNA separation is due in part to the use of entangled and uncross-linking polymer solutions [6–13]. Polymer solutions provide advantages over cross-linking gel, including easy preparation, low viscosity, and flexibility, thereby allowing automatic filling and replacement of sieving matrices in capillary arrays. Of considerable importance are poly(ethylene oxide) (PEO), polyvinylpyrrolidone (PVP), poly-*N*,*N*-dimethylacrylamide (PDMA), and hydroxyethylcellulose (HEC) that possess the self-coating ability, preventing any tedious coating process and problems associated with coating inhomegenity, capillary fouling, and limited shelf life. Because the nature of a polymer is essentially determined by the chemical composition and molecular architecture, copolymers provide desirable properties from different monomers, creating several attractive features such as high sieving ability, low viscosity, and dynamic coating ability, and have been tested for DNA separation [14, 15]. Alternatively, the application of a mixture of different sizes of PEO for the separation of DNA has been demonstrated [16]. By taking the advantages of good dynamic coating ability of PDMA and good separation ability of linear polyacrylamide (LPA), a mixture of the two polymers was used for DNA separation [17]. Moreover, polymer solutions prepared from two different polymers have also been tested for DNA separation in the presence of electroosmotic flow (EOF) [18].

Polymer solutions that provide high sieving ability and long DNA read length are generally highly viscous, resulting in a difficulty of automatically replacing sieving matrices. Thus, searching for low-viscosity polymer solutions with high sieving ability still remains an important issue

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Abbreviations: CAE, capillary array electrophoresis; **EtBr**, ethidium bromide; **GNPs**, gold nanoparticles; **HEC**, hydroxyethylcellulose; **PEO**, poly(ethylene oxide); **SNP**, single-nucleotide polymorphism; **TEM**, transmission electron microscopy

for high-throughput DNA analysis such as single-nucleotide polymorphisms (SNPs) that are often conducted using a CAE instrument [4]. Recently, adding additives to low-viscosity sieving matrices has proven effective and simple for DNA separation [19, 20]. DNA separation has also been conducted using PDMA (100 kDa) containing montmorillonite clay that functions like a dynamic cross-linking plate for the PDMA chains and effectively increases its apparent molecular mass [21].

In this paper, we investigated the use of PEO containing gold nanoparticles (GNPs) for the analysis of DNA by CE. We demonstrated high-resolution DNA separations using low-viscosity PEO solutions containing GNPs ranging in diameter from 3.5 to 56 nm. Using these solutions, filling of the capillary is easy and the separation is highly reproducible. Importantly, we have found that the relative size of PEO/GNPs plays a role in determining resolution.

2 Materials and methods

2.1 Chemicals

Sodium tetrachloroaurate(III) dihydrate was obtained from Sigma (St. Louis, MO, USA). Trisodium citrate, sodium borohydride, glycine, sodium hydroxide, PEO, and PVP were purchased from Aldrich (Milwaukee, WI, USA). Ethidium bromide (EtBr) was obtained from Molecular Probes (Eugene, OR, USA). DNA marker V (pBR 322/*Hae*III digest) and DNA marker VI (pBR 328/*Bgl*I and *Hin*fI digest) were purchased from Roche Diagnostics (Mannheim, Germany). KiloBase DNA marker was purchased from Pharmacia Biotech (Uppsala, Sweden), 5 kb DNA ladder from Gibco/BRL (Bethesda, MD, USA). The pH of glycine buffer was adjusted with NaOH to 9.0.

2.2 Apparatus

The basic design of the separation system has been previously described [22]. Briefly, a high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA) was used to drive electrophoresis. The entire detection system was enclosed in a black box with an high-voltage (HV) interlock. The high-voltage end of the separation system was housed in a plexiglass box for safety. A 4.0 mW He-Ne laser with 543.6 nm output from Uniphase (Mantense, CA, USA) was used for excitation. The emission light was collected with a $10\times$ objective (numeric aperture = 0.25). One RG 610 cutoff filter was used to block scattered light before the emitted light reached the phototube (Hamamatsu R928). The fluorescence signal was transferred directly through a 10 kΩ resistor to a 24-bit A/D interface at 10 Hz (Borwin, JMBS Developments, Le Fontanil, France) and stored in a PC. Capillaries (Polymicro Technologies, Phoenix, AZ, USA) with 75 μm ID and 365 μm OD were dynamically coated with 5.0% PVP overnight prior to use for DNA separations.

2.3 Synthesis of GNPs

Different sizes of GNPs were prepared according to reported methods [23–25]. Preparation of 3.5 nm GNPs: a 50 mL aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ and 2.5×10^{-4} M trisodium citrate was prepared in a conical flask. Next, 1.5 mL of ice-cold, freshly prepared 0.1 M $NaBH_4$ solution was added to the solution while stirring. The solution turned pink immediately after adding $NaBH_4$, indicating particle formation. Preparation of 13 nm GNPs: to a 50 mL sample of 4 mM trisodium citrate that was brought to a vigorous boil with stirring in a round-bottom flask fitted with a reflux condenser was rapidly added 0.25 mL of 200 mM $HAuCl_4$. The solution was boiled for another 3 min, during which time the solution changed color from pale yellow to purple and then changed to deep red. The solution was sit aside while cooling to room temperature. Preparation of 32 and 56 nm GNPs: to three aliquots each of 50 mL of 0.01% $HAuCl_4$ that was heated to boiling with reflux condensers were rapidly added 0.5 and 0.3 mL of 1% trisodium citrate, respectively. The solutions were boiled for another 8 min, during which time the solutions of 32 and 56 nm GNPs changed to pink and purple, respectively. The solutions were sit aside while cooling to room temperature.

2.4 Spectroscopic measurement and transmission electron microscopy (TEM)

A double-beam UV-vis spectrophotometer (Jasco V-570; Jasco, Tokyo, Japan) was used to measure the absorbance of the GNPs in aqueous, glycine, and polymer solutions and the polymer-GNP composites in water. The maximum wavelengths for the surface plasmon resonance (SPR) obtained from the measured UV-vis spectra (not shown) were 518, 519, 528, and 535 nm for the 3.5, 13, 32, and 56 nm GNPs, respectively, noting that the sizes of GNPs as expected. The GNPs in the presence and absence of PEO solutions as well as the polymer-GNP composites were imaged using an H7100 TEM (Hitachi High-Technologies, Tokyo, Japan) operating at 75 keV. TEM samples were prepared by dropping two 20 μL aliquots of the samples onto a holey carbon-coated copper grid. After deposition, any remaining solution was wicked away, and the grids were dried 1 h at room temperature. The TEM images (not shown) further confirm the sizes of the GNPs and show that the size distribution of the GNPs is less than 5.0%.

2.5 Polymer solutions containing GNPs

The prepared GNPs were diluted with 25 mM glycine buffer, pH 9.0, by a volume ratio of 3:10. Certain amounts of the polymer were gradually added into glycine buffer containing the GNPs in a beaker. During the addition of PEO, a magnetic stirring rod was used to produce a well-homogeneous suspension. After the addition was complete, the suspension was stirred for at least 1 h more. Finally, the polymer solution was degassed with a vacuum system in an ultrasonic tank. In this study, PEO ($\times$ MDa) means that the polymer solution was prepared from PEO with molecular weight (MW) $\times$ 000 000 Da, in which $\times$ represents 2, 4 or 8.

2.6 DNA separation by CE

Prior to use, capillaries were dynamically coated with 5.0% PVP overnight. Before conducting separations, PVP was flushed out with deionized water and then filled with PEO solution containing the GNPs by low pressure (syringe pushing). DNA was injected at the cathode end into the coated capillary filled with PEO solution containing the GNPs at 1 kV for 10 s and the separation was conducted at 15 kV. After each run, PEO solution was flushed out *via* low pressure.

3 Results and discussion

3.1 GNPs in polymer solution

Polymers, in addition to capping agents such as citrate, are commonly used to prevent aggregation and agglomeration of GNPs [26, 27]. By measuring UV-vis absorption and TEM of the GNPs in aqueous and polymer solutions, we confirmed the sizes of the GNPs and found that the GNPs are stable in three polymer solutions (PVP, HEC, and PEO) commonly used for DNA separation for more than three months. Among these three polymers, PVP provides the most stability for the GNPs, supported by no change in the absorption band of the GNPs around 520 nm in three months. Recently, we have demonstrated that aggregation and/or agglomeration of the GNPs takes place to various extents, depending on the concentration of PEO solution and the relative size of GNP/PEO (Huang *et al.*, submitted).

Attempts to conduct the separation of DNA either in a GNP-coated capillary using PEO or in a bare fused-silica column using PEO containing the GNPs was failed in terms of reproducibility. This is due in part to aggregation of the GNPs on the capillary wall, supported by an appearance of dark blue color on the detection window after several runs. To overcome this problem, we used 5.0% PVP to coat the capillary, which is a common strategy to suppress EOF and minimize DNA interactions with the capillary wall [13]. In this study, PVP also plays a role in minimizing the interaction between the GNPs and the capillary wall and stabilizing the GNPs.

3.2 Separation of small DNA fragments

Although at concentrations far greater than the entanglement threshold concentration (Φ^*) for a polymer both long and short polymer chains provide an equally good resolution for short DNA, low concentrations of polymer solutions prepared from long chains is superior for a wider range of DNA separation [28]. Thus, we performed the separation of DNA markers V and VI (8–2176 bp) using 0.2% PEO (8 MDa) in the absence and presence of 56 nm GNPs. Figure 1 shows the separation was complete in 5 min using 0.2% PEO (8 MDa) containing 0.3 $\times$ 56 nm GNPs, with the relative standard deviation (RSD) of the migration times less than 1.0%. It is important to point out that the solution was not viscous ($<$ 15 cP), suggesting that the GNPs did not cause marked changes in the viscosity of PEO. Compared to that using a mixture of PEO that is very viscous and has been used for DNA sequencing [29], the resolution is comparable while the analysis is faster. The differences suggest that the GNPs and citrate (capping agent) might play a role in improving resolution.

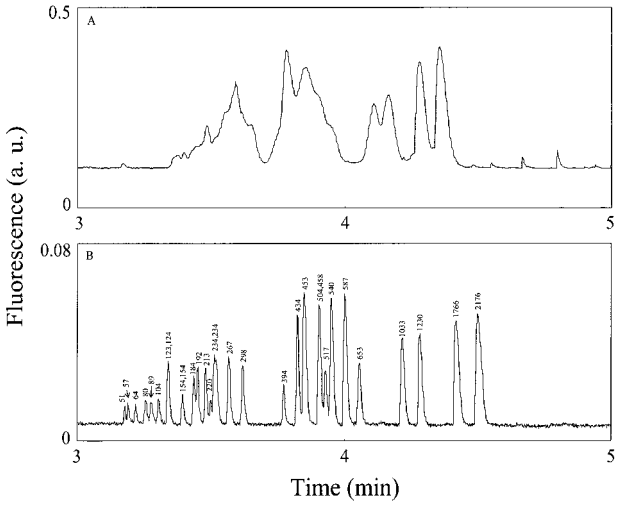

Figure 1. Separations of 10 μg/mL DNA markers V and VI using (A) 0.2% PEO (8 MDa) and (B) 0.2% PEO (8 MDa) containing 0.3 $\times$ 56 nm GNPs. Electrophoresis conditions: PEO was prepared in 25 mM glycine, pH 9.0, containing 0.5 μg/mL EtBr; electrokinetic injection at 1 kV for 10 s; separation at 15 kV in a 40 cm long (30 cm to the detector) fused-silica capillary with 365 μm OD and 75 μm ID.

Table 1. Effect of the GNPs on the separation efficiency and migration time for the DNA markers V and VI

PEO (Mda) (%)	GNPs (nm)	Resolved peak numbers		Migration time (min) (RSD%)		Resolution (bp/bp)				
		51 bp–298 bp[a)]	394 bp–653 bp[b)]	First peak[c)]	Last peak[d)]	80/89	213/220	517/540	1033/1270	1766/2176
8 (0.2)	No	4	2	3.34 (1.8)	4.33 (1.2)	0	0	0	0.8	1.1
8 (0.2)	32	14	8	3.06 (0.7)	4.18 (1.2)	0.7	0.4	0.7	1.7	1.4
8 (0.2)	56	16	8	3.18 (0.6)	4.47 (0.9)	0.8	1.1	1.2	1.9	1.7
4 (0.3)	No	11	4	3.68 (0.3)	4.96 (0.2)	0	0	0	0.5	0.8
4 (0.3)	32	15	8	3.38 (0.6)	4.95 (0.2)	1.0	0.6	0.6	1.5	1.5
4 (0.3)	56	15	7	3.53 (0.2)	5.22 (0.2)	1.2	0.6	0	1.3	1.5

Conditions were as in Fig. 1. a) Expected peaks: 18, b) Expected peaks: 9, c) 51 bp fragment, d) 2176 bp fragment

To explore the role that citrate played, we conducted the separations using 0.2% PEO (8 MDa) prepared in a buffer at pH 9.0 composed of 100 mM glycine and citrate at the concentration ranging from 70 μM to 1.2 mM (0.3 times the concentration of citrate used in preparing the 13 nm GNPs). With increasing citrate concentration, separation times became shorter (less than 4.5 min) and resolution for the DNA fragments smaller than 124 bp slightly improved (data not shown), suggesting that the interaction between citrate and DNA should take place (the effect of Joule heating was ignored as supported by stable and low currents, < 10 μA). To further support the role of the GNPs playing in improved resolution, the separations were separately carried out using 0.2% PEO (8 MDa) containing the 32 nm GNPs and using 0.3% PEO (4 MDa) in the absence and presence of $0.3 \times$ 32 nm and 56 nm GNPs, respectively. Please note that the viscosity values for these polymer solutions were similar, less than 15 cP. In terms of resolution and speed, 0.2% PEO (8 MDa) containing 56 nm GNPs is superior as presented in Table 1. It is interesting to note that the separation is slightly faster in the presence of the 32 nm GNPs than the 56 nm ones when using PEO (8 MDa). This is mainly due to the effect of citrate (different amounts of citrate used to prepare various sizes of GNPs). It should be pointed out that the resolution improvement is not impressive in the presence of $0.3 \times$ GNPs with the size smaller than 32 nm when using PEO (8 MDa) or PEO (4 MDa) solutions. From our previous result (Huang *et al.*, submitted), we have learned that the changes in color and plasmon absorption of GNPs are due to aggregation and/or agglomeration. Thus, we believe that the various morphologies of PEO matrices in the absence and presence of GNPs should also contribute to various separation results. In the end, we concluded that adding large GNPs to the long chains of PEO is superior for the separation of small sizes of dsDNA.

3.3 Separation of large DNA fragments

The separation of kbp DNA ladder (0.5–10 kbp) either using 0.2% (8 MDa), 0.3% (4 MDa), or 0.5% PEO (2 MDa) without the GNPs was successful. However, in the presence of $0.3 \times$ GNPs, the separation is faster and the peak profiles are sharper and more symmetric as shown in Table 2. A representative electropherogram depicted in Fig. 2 shows that 11 peaks were resolved and more symmetric in the presence of the 3.5 nm GNPs. Please note that we did not see size dependence of the GNPs (3.5–56 nm) on the electropherogram patterns but changes in the migration times when conducting the separation using PEO at the same concentration. This result and the fact that the concentrations of these three solutions were all above their Φ^* values that are 0.07, 0.12 and 0.2%, respectively, suggests that the siev-

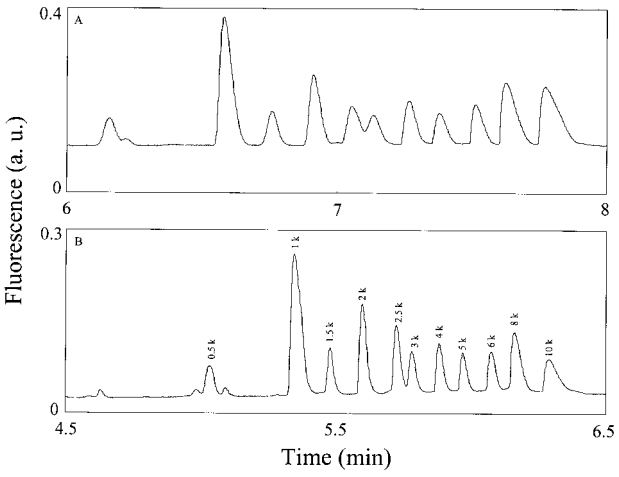

Figure 2. Separations of 10 μg/mL kbp DNA marker using (A) 0.5% PEO (2 MDa) and (B) 0.5% PEO (2 MDa) containing 0.3×3.5 nm GNPs. Other conditions were the same as in Fig. 1.

Table 2. Effect of the GNPs on the migration time and separation efficiency for the kbp DNA

PEO (MDa) (%)	GNPs (nm)	Migration time (min) (RSD%)			Resolution (kbp/kbp)					
		1.5 kbp	3 kbp	10 kbp	1.5/2.0	2.0/2.5	2.5/3.0	3.0/4.0	5.0/6.0	8.0/10.0
8 (0.2)	No	4.25 (1.6)	4.50 (1.8)	5.01 (1.6)	1.0	1.3	0.9	1.2	0.7	0.7
8 (0.2)	3.5	4.23 (0.7)	4.50 (0.9)	5.00 (0.8)	2.2	1.6	1.2	1.6	1.1	0.9
4 (0.3)	No	5.05 (1.4)	5.30 (1.5)	5.82 (1.7)	0.8	0.9	0.7	1.0	0.7	0.8
4 (0.3)	3.5	4.92 (0.4)	5.23 (0.4)	5.76 (0.3)	1.8	1.7	1.2	1.5	1.4	1.0
2 (0.5)	No	6.73 (0.9)	7.10 (1.0)	7.73 (0.9)	1.8	1.4	0.7	1.3	1.3	1.1
2 (0.5)	3.5	5.52 (1.1)	5.82 (1.2)	6.33 (1.1)	2.3	2.3	0.9	1.8	1.9	1.3

Conditions were as in Fig. 2.

ing mechanism is the main determinant for separation. A similar result has been found that the GNPs only slightly affected resolution when using 2.0% PEO (8 MDa) for the separation of DNA markers V and VI (data not shown).

The separation of very large DNA fragments has been demonstrated using HEC solutions at the concentrations below its Φ^* [30, 31]. The success of the separation stems from that DNA drags along the polymer molecules it encounters during migration, with a support of dynamic formation and deformation of U-shape in DNA conformation [32, 33]. Unlikely, we have demonstrated that the separation of DNA ranging in size from 5 to 40 kbp was successful using 1.5% HEC at high pH ($>$ 10.0) in the presence of EOF [34], but unsuccessful using PEO at the concentrations below its Φ^* in the absence of GNPs. Figure 3A shows that the eight fragments were completely resolved in the presence of 0.3×13 nm GNPs when using 0.05% PEO (2 MDa), while the separation was failed without the GNPs as shown in the inset. A similar result is presented in Fig. 3B when using 0.05% PEO (4 MDa) containing the 32 nm GNPs. Compared to Figs. 1 and 2, tailing of the peaks corresponding to the large DNA fragments is apparent. The differential conductivity from the background electrolytes and interaction with the PEO-GNPs of the DNA fragments are possible reasons for the peak tailing. In both cases, the separations were carried out at low electric fields in order to achieve a high resolving power [35], leading to long separation times. The PEO concentrations were both lower than their Φ^* values, revealing that improved resolution is not due to the sieving mechanism. The results suggest that the DNA fragments might interact (drag) with the PEO adsorbed on the GNPs, which is similar to the DNA separation by end-labeled free-solution electrophoresis [36, 37]. Unlikely, attachment of DNA onto beads is not required by using PEO containing the GNPs. Table 3 further compares the size dependence of GNPs on the migration time and resolution. The results clearly show that the relative size of

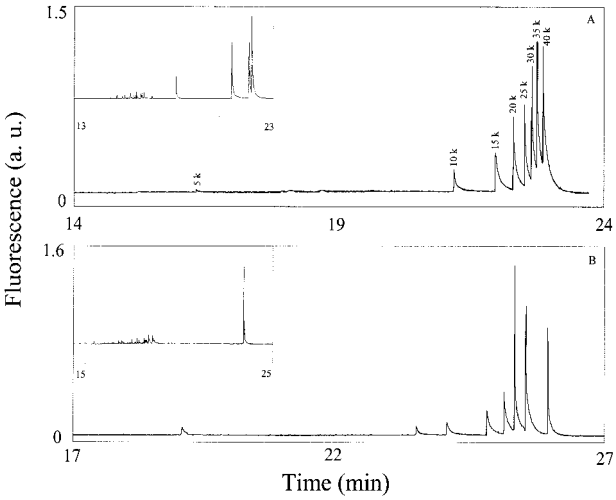

Figure 3. Separations of 10 μg/mL 5 kbp DNA ladder using (A) 0.05% PEO (2 MDa) containing 0.3×13 nm GNPs and (B) 0.05% PEO (4 MDa) containing 0.3×32 nm GNPs. The separations in the absence of the GNPs are shown in the two insets, respectively. Electrophoresis conditions: electrokinetic injection at 1 kV for 10 s and separation at 1 kV; other conditions were the same as in Fig. 1.

PEO/GNP is an important parameter. It is also worth noting the loss of resolution for the large DNA fragments ($>$30 kbp) in the case of 56 nm GNPs, no matter what size of PEO used. With large GNPs, PEO chains are probably not long enough to protrude into solution. In consequence, the interaction between PEO molecules and DNA reduced, leading to loss of resolution.

3.4 Size dependence of DNA mobility

To further explore the effect of the GNPs on DNA separation, some of the electrophoretic mobilities for the DNA fragments were plotted as a function of the 1/fragment size in Figs. 4 and 5. The plots of the mobility *versus*

Table 3. Comparison of the migration time and resolution for the 5 kbp DNA ladder fragments in the presence and absence of the GNPs

PEO (MDa) (%)	GNPs (nm)	Migration time (min) (RSD%)		Resolution (kbp/kbp)				
		5 kbp	40 kbp	10/15	20/25	25/30	30/35	35/40
2 (0.05)	No	–	21.90 (0.5)	–	–	–	–	–
2 (0.05)	13	16.41 (0.5)	22.40 (0.6)	3.8	0.6	0.8	0.6	0.6
2 (0.05)	32	17.04 (0.9)	23.93 (1.0)	5.8	2.2	3.7	–	–
4 (0.05)	No	–	23.55 (0.6)	–	–	–	–	–
4 (0.05)	13	18.11 (1.7)	24.88 (1.8)	2.9	1.7	–	–	–
4 (0.05)	32	18.99 (1.4)	25.48 (1.3)	2.9	1.5	1.0	1.3	2.7

Conditions were as in Fig. 3.

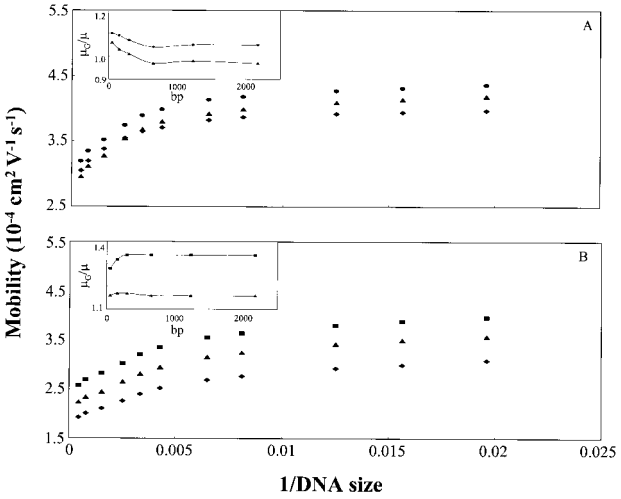

Figure 4. Plots of the electrophoretic mobilities for the DNA markers V and VI as a function of the fragment size using (A) 0.2% PEO (8 MDa) and (B) 0.5% PEO (2 MDa), respectively. (◆) no GNPs; (■) 3.5 nm; (●) 32 nm; and (▲) 56 nm GNPs. Two insets are the ratios of the mobilities for the DNA fragments in the presence of the GNPs (μ) to those in the absence of the GNPs (μ). Conditions were as the same in Fig. 1.

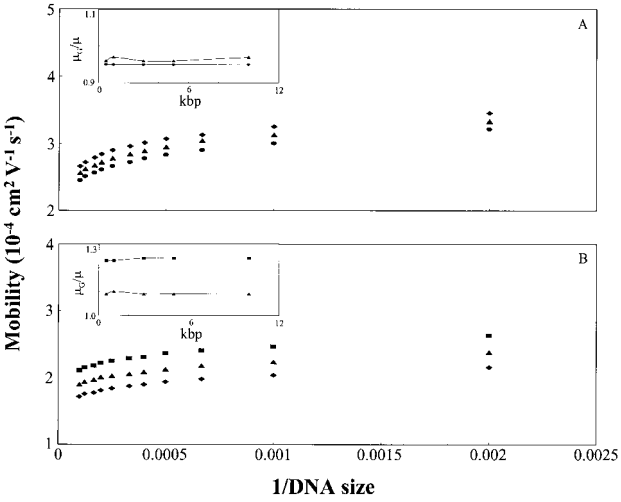

Figure 5. Plots of the electrophoretic mobilities for the kbp DNA fragments as a function of the fragment size using (A) 0.2% PEO (8 MDa) and (B) 0.5% PEO (2 MDa), respectively. Notations are the same as in Fig. 4; other conditions were the same as in Fig. 1.

1/fragment size shown in Fig. 4 reveal that the bias reptation model seems to apply to the DNA fragment larger than 300 bp. To clearly show the size effect of the GNPs on the migration mobility, the ratios of the mobility values (μ_G/μ) for the DNA fragments in the presence and absence of the GNPs are plotted as a function of the fragment size, which are shown in the insets. In the case of PEO (8 MDa) containing the 56 nm GNPs (MW is about 2400 MDa), the migration mobility for the large DNA fragments ($>$ 298 bp) decreased. Decreases in mobility were also found in the case of separating kbp DNA using PEO (8 MDa). This suggests that PEO (8 MDa) adsorbed on the GNPs might interact with the DNA fragments through its protruding part. On the other hand, when using PEO (2 MDa), the mobility for all the DNA fragments increases in the presence of the GNPs. With shorter chains, PEO adsorbed on the GNPs could not protrude to the solution to interact with DNA. As discussed above, citrate and the changes in the morphology of PEO matrices are likely important contributors for the increases in mobility. The changes in the morphology of the PEO matrices due to the aggregation of the GNPs have been demonstrated in the TEM images (Huang *et al.*, submitted). Although the trends are different when using different sizes of PEO, it clearly shows that the increases in the mobility are greater when using 3.5 nm GNPs (lighter and greater concentration of citrate used), mainly due to the effect of citrate on mobility.

4 Concluding remarks

The addition of GNPs is a promising approach to improvement of the separation performance of PEO matrix in DNA separation. The separation of small DNA fragments is successful using low-viscosity PEO solution containing the GNPs. In the presence of the GNPs, the separation is faster, reproducible and efficient. With low viscosity and high sieving ability, PEO solution containing the GNPs should be useful for SNPs using a CAE instrument. Using 0.05% PEO (4 MDa) containing 0.3×32 nm GNPs, the separation of 5 kb ladder is realistic. Because the GNPs are stable in a number of linear polymer solutions including HEC and PVP, our future effort will be made to investigate their applications in DNA separation. Advances in nanotechnology have made possible for the synthesis of a variety of nanomaterials for different purposes such as electronics, optics and biosensors. The success of this work simply opens the avenue to apply nanomaterials, especially those with suitable functional groups that recognize biomolecules of interest such as DNA and proteins, for the separation of biomolecules [38–41]. Now, we are applying this technique to the separation of proteins using GNPs with biofunctional groups such as carbohydrate.

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