ELSEVIER

Journal of Chromatography A, 931 (2001) 163–173

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Effect of glycerol-induced DNA conformational change on the separation of DNA fragments by capillary electrophoresis

Dehai Liang, Liguo Song, Zijian Chen, Benjamin Chu*

Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, USA

Received 18 May 2001; received in revised form 7 August 2001; accepted 7 August 2001

Abstract

The effect on DNA separation of adding glycerol to the running buffer was studied using linear polyacrylamide (LPA) or poly(ethylene oxide) (PEO) as separation medium. For both LPA and PEO, it was found that the addition of 25% (v/v) glycerol to the running buffer enhanced the separation of large double-stranded DNA fragments and increased the migration time. The two buffers used, 1×TBE (Tris–boric acid–EDTA) and 1×TTE (Tris–*N*-tris(hydroxymethyl)methyl-3-amino-propanesulfonic acid (TAPS)–EDTA), showed similar improvement, but the effect on the 1×TBE buffer was more amplified. The difference in buffer properties, such as viscosity, conductance, and pH, had little effect on the separation. We attribute the improvements made in the separation to the ability of glycerol to induce a conformational change in DNA as demonstrated by dynamic light scattering results. The presence of glycerol can increase the electrostatic interactions between the phosphate groups, decrease the hydration sphere of the polynucleotides, and compete with water to form hydrogen bonds with the side group of bases. These interactions increase the DNA contour length and reduce the effective charge over weight ratio, which can explain the experimental data. The complex formed by boric acid and glycerol had a stronger effect on the DNA conformation change than glycerol itself. This enhancement was also observed in DNA sequencing analysis.

Keywords: Buffer composition; DNA; Glycerol

1. Introduction

Capillary electrophoresis (CE) using polymer solutions as separation media has been proven to be a powerful method for DNA analysis. Many hydrophilic polymers have been tested and developed for this purpose with varying degrees of success, e.g. linear polyacrylamide (LPA) [1,2], agarose [3], cellulose and its derivatives [4,5], poly(*N*,*N*-dimethylacrylamide) (PDMA) [6], poly(vinylpyrrolidone) [7], and poly(ethylene oxide) (PEO) [8,9]. Generally, a high sieving ability is the prerequisite for a successful separation medium. But dynamic coating ability and adjustable viscosity (or low viscosity) are also important since they make the separation media easier to handle and more suitable for automation. $PEO_{99}PPO_{69}PEO_{99}$ [PPO, poly(propylene oxide)] [10,11] and poly(*N*-isopropylacrylamide)–g-PEO [12,13], which possess both of these properties, have been developed in our laboratory.

Testing and developing a new polymer matrix usually takes time and effort since many parameters

*Corresponding author. Tel.: +1-631-632-7928; fax: +1-631-632-6518.

E-mail address: bchu@notes.cc.sunysb.edu (B. Chu).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01189-X

involving the molecular architecture and topology, the electric field and solution conditions are involved. Enhancing DNA separation by manipulating existing polymers with additives seems to be a good choice, e.g. the addition of ethidium bromide to the running buffer not only improves the detectability of double-stranded (ds) DNA, but also enhances the resolution; 5–7 *M* urea maintains the DNA in the denatured state and thus plays an important role in DNA sequencing analysis. Recently, organic solvents containing hydroxyl groups, such as glycerol [14,15], methanol [16], and mannitol [17], have been tested as new additives in the separation of dsDNA, with cellulose or its derivatives being used as separation medium. Cheng et al. [14,15] reported that the addition of glycerol (<7.5%) to the running buffer improved the separation of dsDNA using (hydroxypropyl)methylcellulose (HPMC) in 1×TBE (Tris–boric acid–EDTA) buffer as the separation medium. The authors attributed the improvement to the presence of borate in the buffer solution, in which the borate acts as a central linkage to form dimeric 1:2 borate–didiol complexes with both glycerol and HPMC, allowing an entangled solution with different pore sizes to form chemically [15].

Recent studies [18] showed that glycerol had the ability to reduce the thermal stability of dsDNA by interacting with the polynucleotide solvation sites (i.e., by replacing water) and by modifying the electrostatic interactions between the polynucleotide and its surrounding atmosphere of counterions. Such interactions inevitably change the DNA conformation. However, its effect on DNA separation was ignored. Moreover, the addition of glycerol to the running buffer also changed the buffer dielectric constant, viscosity, and conductance, of which the effect on DNA separation has not been clarified.

In this study, dynamic light scattering was used to confirm the DNA conformational change caused by glycerol. To determine its effect on DNA separation, cellulose or its derivatives could not be used as the separation medium, because the interactions among the polymer, boric acid, and glycerol might conceal the DNA conformational effect [15]. Thus, LPA and PEO, which are widely used and do not form a complex with boric acid and glycerol, were chosen as the separation media in this work. To determine whether or not the complex formed by boric acid and glycerol has an additional effect on the DNA conformation change, two running buffers, 1×TBE and 1×TTE [Tris–*N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS)–EDTA], were used for the separation of dsDNA. This approach also helps our understanding of the effect on DNA separation caused by the change in buffer properties. The DNA conformational effect on single-stranded (ss) DNA separation was also studied.

2. Experimental

2.1. Materials

Acrylamide, glycerol, Tris, boric acid, TAPS, EDTA, urea and ethidium bromide were purchased from Sigma (St. Louis, MO, USA). All chemicals were electrophoresis grade and no further purification was performed. PEOs with average M_r (molecular mass determined by viscosity measurements) of $8 \cdot 10^6$ and 600 000 were purchased from Aldrich (Milwaukee, WI, USA). LPA of molecular mass $2.2 \cdot 10^6$ was synthesized in the laboratory. The detailed procedure is reported elsewhere [19].

2.2. Solutions

1×TTE buffer (pH 8.2, 50 m*M* Tris, 50 m*M* TAPS and 2 m*M* EDTA), 1×TBE buffer (pH 8.3, 89 m*M* tris, 89 m*M* boric acid and 2 m*M* EDTA) and each with 25% (v/v) glycerol were prepared for dsDNA separation. 1×TTE+5 *M* urea and the same buffer with 25% (v/v) glycerol were prepared for ssDNA separation. LPA and PEO solutions were prepared by dissolving the polymers in the above buffers to different known concentrations and stored at room temperature for a few days before use.

2.3. DNA sample

pBR322/HaeIII digest was purchased from Sigma and diluted to 10 μg/ml with deionized water. Calf thymus DNA solution [mixture of DNA fragments with an average size less than 2000 base pairs (bp)] was purchased from GIBCO BRL (Grand Island, NY, USA) and diluted with the desired buffer to $1 \cdot 10^{-4}$ g/ml. The single dye-labeled DNA sequenc-

ing reactions were performed using carboxyfluorescein (FAM)-labeled −21 M13 primer on a pGEM-3Zf(+) template, following the instructions of the ABI PRISM Dye Primer (−21 M13 forward) Cycle Sequencing Ready Reaction Kit (PE Biosystems/Perkin-Elmer, Foster City, CA, USA). The temperature cycling protocol, which was carried out using the GeneAmp polymerase chain reaction (PCR) System 2400 (PE Biosystems/Perkin-Elmer), used 15 cycles of 10 s at 95°C, 5 s at 55°C and 1 min at 70°C, followed by 15 cycles of 10 s at 95°C and 1 min at 70°C. The reaction products were purified by ethanol precipitation and resuspended in 20 μl deionized formamide.

2.4. CE

A laboratory-built capillary electrophoresis system with laser-induced fluorescence detection was used to perform the DNA separation. A detailed description has been given elsewhere [20]. When LPA was used as separation medium, a certain length [13 cm for dsDNA separation and 48 cm for ssDNA separation] fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was coated by polymerization of acrylamide monomer inside the column according to the Hjerten method [17]. When PEO was used as separation medium, the capillary was flushed with 1 *M* HCl for 10 min. A detection window of 1.5 mm width was opened at the predetermined length from the cathodic end by stripping the polyimide coating off the capillary with a razor blade. Both cathode and anode reservoirs (1.6 ml volume) were filled with the desired buffer. The polymer solution was injected into the capillary tubing using a 50-μl syringe. The capillary tubing was then assembled onto the holder and a pre-run at a constant electric field strength of 200 V/cm was used to stabilize the current and also to introduce the fluorescent dye into the separation medium (for dsDNA). The DNA sample was injected into the capillary electrokinetically. The voltage was generated using a PCI-MIO-16E-4 multifunction I/O board (National Instruments, Austin, TX, USA) together with a high-voltage power supply FC30P04 (Glassman High Voltage, Whitehouse Station, NJ, USA). The fluorescence was detected using a Hamamatsu R928 photomultiplier tube (Rockaway, NJ, USA) and data were acquired by the same I/O board and processed by a Pentium personal computer.

2.5. Dynamic light scattering (DLS)

A laboratory-built laser light scattering spectrometer with the laser (DPSS, Coherent, 200 mW) operating at a wavelength of 532 nm was used to perform dynamic light scattering (DLS) at an angle of 30°. The intensity–intensity time correlation function was measured using a Brookhaven BI-9000 digital correlator. CONTIN analysis was used to analyze the DLS results.

3. Results and discussion

The influence of the glycerol concentration was investigated and it was found that 25% (v/v) glycerol was the optimal concentration where reproducible results were obtained and the most obvious effect on DNA separation was observed. Fig. 1a shows the electropherogram of the pBR322/Hae III digest using 2.5% (w/v) LPA in 1×TBE buffer without glycerol. All the fragments larger than 20 bp in the pBR322/Hae III digest were clearly separated within 12 min with the 10 cm effective length column at 150 V/cm. The doublet of 123 and 124 bp was baseline separated as shown in the inset of Fig. 1a. Adding 25% (v/v) glycerol to the 1×TBE running buffer resulted in three kinds of effects, as seen in Fig. 1b: (1) it ruined the separation of the 123 and 124 bp pair as shown in the inset; (2) it enhanced the separation of larger dsDNA fragments; and (3) it prolonged the migration time. Another separation medium, 2% (w/v) PEO with $M_r = 8 \cdot 10^6$, produced nearly identical results, as shown in Fig. 2a and b. To clearly understand the effect of glycerol, the electropherogram data in Figs. 1 and 2 were fitted using Peakfit 4 (SPSS, San Rafael, CA, USA) software. The 123 and 124 bp pair was not included in the fitting data due to the poor separation in the buffer with 25% (v/v) of added glycerol. All the peak fittings exhibited $r^2 > 0.99$. The resolution (R_s) was calculated according to the equation:

$$R_s = 2(t_1 - t_2)/(w_1 + w_2) \quad (1)$$

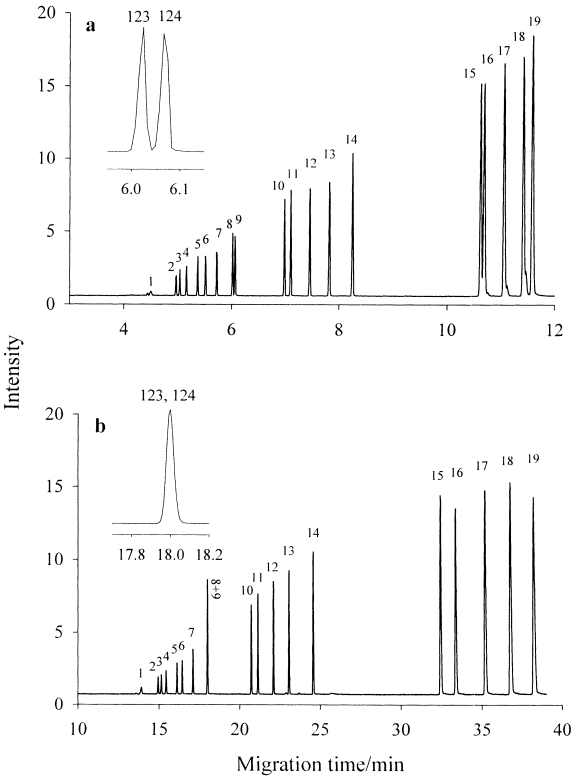


Fig. 1. Separation of pBR322/HaeIII digest using 2.5% LPA in (a) 1×TBE buffer and (b) 1×TBE buffer with 25% (v/v) glycerol. The insets show the separation of the doublet of 123 and 124 bp. Conditions: 10 cm effective capillary length with 100 μm I.D.; 3 μg/ml ethidium bromide; DNA sample injected electrokinetically into the column at 50 V/cm for 3 s; 150 V/cm running electric field strength. The DNA peak identification is as follows: (1) 21, (2) 51, (3) 57, (4) 64, (5) 80, (6) 89, (7) 104, (8) 123, (9) 124, (10) 184, (11) 192, (12) 213, (13) 234, (14) 267, (15) 434, (16) 458, (17) 504, (18) 540, (19) 587.

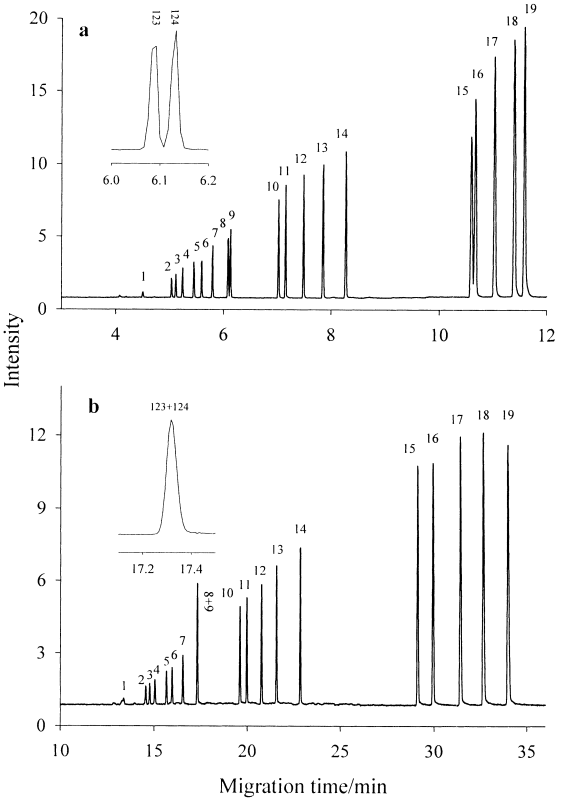


Fig. 2. Separation of pBR322/HaeIII digest using 2% PEO in (a) 1×TBE buffer and (b) 1×TBE buffer with 25% (v/v) glycerol. The insets show the separation of the doublet of 123 and 124 bp. Peak identification and other conditions as in Fig. 1.

where w_1 and w_2 are the temporal peak widths of DNA fragments 1 and 2, respectively, t_1 and t_2 are the migration times of DNA fragments 1 and 2, respectively, and $t_1 > t_2$. Since R_s is directly related to the size difference of the two fragments $(n_1 - n_2)$, $R_s/(n_1 - n_2)$ is used here for a proper comparison of different fragment pairs. Data for the change in resolution as well as the mobility at different DNA sizes are shown in Fig. 3. The addition of 25% (w/v) glycerol to the 1×TBE buffer significantly decreased the mobility of the dsDNA fragments in all sizes, as seen in Fig. 3a. The retardation factor (r), which is defined as the ratio of DNA mobility in running buffer to that in the same buffer but with 25% (v/v) glycerol, was calculated according to the equation:

$$r = u_1/u_2 \quad (2)$$

where u_1 and u_2 are the DNA mobility in the buffer without and with glycerol, respectively. The data are shown in the inset of Fig. 3a. The retardation factor is about 3.0 for DNA fragments below 400 bp. For DNA larger than 400 bp, the retardation factor increased with increasing DNA size. From the change in the resolution, as shown in Fig. 3b, the addition of glycerol enhanced the separation of dsDNA fragments with sizes larger than 120 bp. To clearly understand the result for the resolution, the selectivity (S) and the efficiency (N) were also calculated according to the equations:

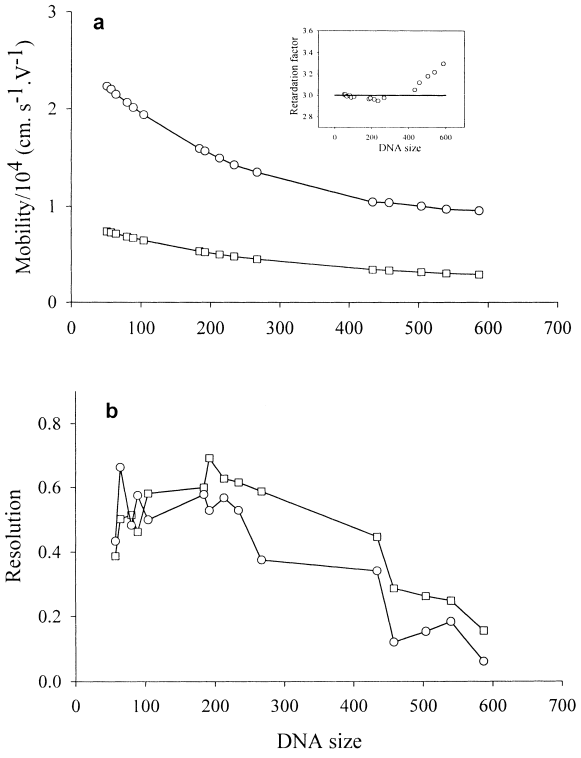


Fig. 3. Plot of (a) mobility and (b) resolution versus size of DNA fragments in 1×TBE buffer (○) and 1×TBE buffer with 25% (v/v) glycerol (□). All the data were calculated from the fitting of the electropherograms in Fig. 1. The inset shows the change in the retardation factor at different DNA size.

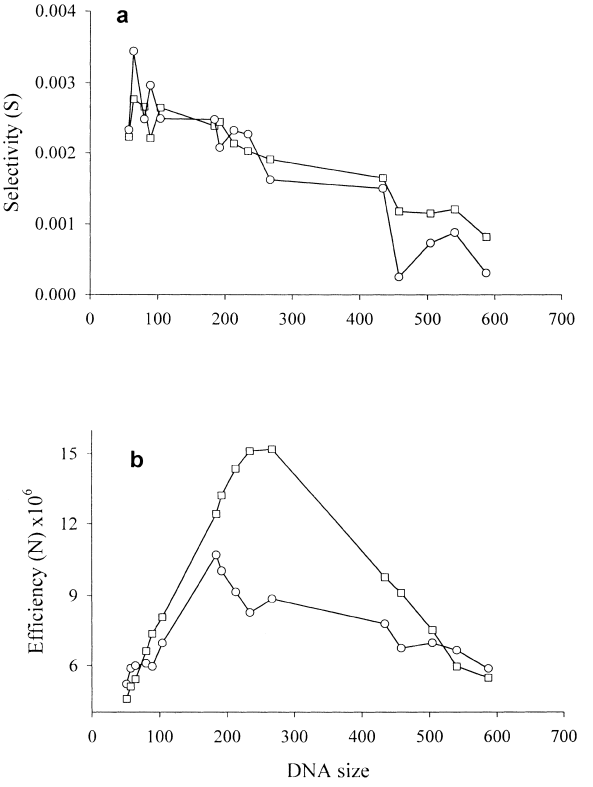


Fig. 4. Plot of (a) selectivity and (b) efficiency versus size of DNA fragments in 1×TBE buffer (○) and 1×TBE buffer with 25% (v/v) glycerol (□). All the data were calculated from the fitting of the electropherograms in Fig. 1.

$$S = 2(t_1 - t_2)/(t_1 + t_2) \quad (3)$$

where t_1 and t_2 are the migration times of two fragments and $t_1 > t_2$;

$$N = 5.54(t/w_{1/2})^2 \quad (4)$$

where t and $w_{1/2}$ are the migration time and full peak width at half maximum height, respectively.

The calculated results for the selectivity and efficiency are shown in Fig. 4. The addition of 25% (v/v) glycerol to the 1×TBE buffer increased the selectivity for DNA fragments larger than 250 bp (Fig. 4a) and also increased the efficiency for DNA fragments between 100 and 500 bp (Fig. 4b). However, DNA fragments larger than 500 bp appear to have lower efficiency. The resolution shown in Fig. 3b is the compromise result for the selectivity and the efficiency. When 2% (w/v) PEO was used as the separation medium, a similar trend for the selectivity, efficiency and resolution was observed (data not shown).

By changing the buffer from 1×TBE to 1×TTE, the separation of pBR322/HaeIII digest showed different results, as shown in Fig. 5. Fig. 5a shows the electropherogram of pBR322/HaeIII digest using 2.5% (w/v) LPA in 1×TTE buffer. It has almost the same separation as that in 1×TBE, as seen in Fig. 1a. The addition of 25% (v/v) glycerol to the 1× TTE buffer also deteriorated the separation of the doublet of 123 and 124 bp (shown in the inset in Fig. 5), improved the separation of 434 and 458 bp and increased the migration time, as seen in Fig. 5b, which were the same effects as in 1×TBE buffer. However, the doublet of 123 and 124 bp was half height separated here, which is different from the ‘‘no separation at all’’ results in Fig. 1b. The

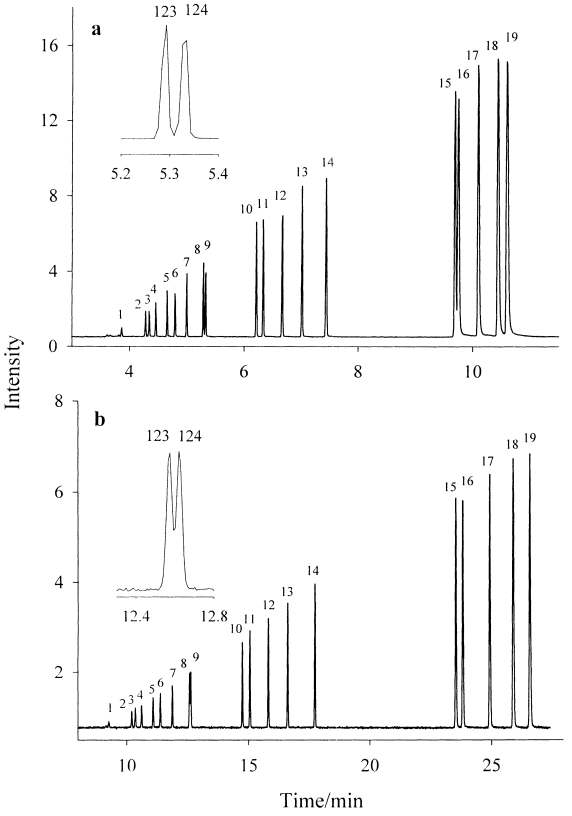


Fig. 5. Separation of pBR322/HaeIII digest using 2.5% LPA in (a) 1×TTE buffer and (b) 1×TTE buffer with 25% (v/v) glycerol. The insets show the separation of the doublet of 123 and 124 bp. Peak identification and other conditions as in Fig. 1.

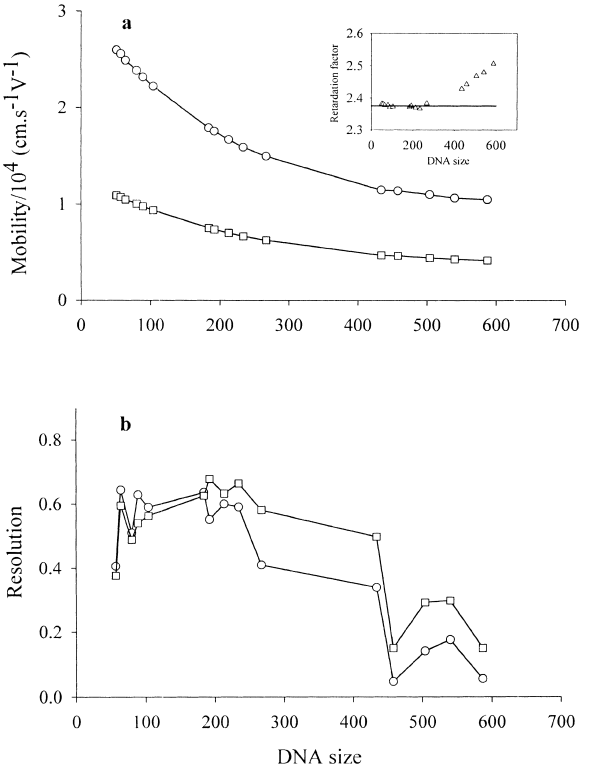


Fig. 6. Plot of (a) mobility and (b) resolution versus size of DNA fragments in 1×TTE buffer (○) and 1×TTE buffer with 25% (v/v) glycerol (□). All the data were calculated from the fitting of the electropherograms in Fig. 4. The inset shows the change in the retardation factor at different DNA size.

improvement for 434 and 458 bp was also less than in 1×TBE buffer and so was the decrease in the migration time. All the peaks except 123 and 124 bp in Fig. 5a and b were also fitted and the change in the mobility and the resolution is shown in Fig. 6. The addition of 25% (v/v) glycerol to the 1×TTE buffer also greatly decreased the mobility of DNA fragments, as seen in Fig. 6a. The retardation factor is about 2.37 for DNA fragments below 400 bp and increased with increasing fragment size after 400 bp, as seen in the inset in Fig. 6a. The addition of glycerol to the 1×TTE buffer also enhanced the separation of DNA fragments larger than 200 bp, as shown in Fig. 6b, which is longer than that in Fig. 3b, where it was 120 bp. The calculated data for the selectivity and efficiency are shown in Fig. 7. The addition of 25% (v/v) glycerol to the 1×TTE buffer improved the selectivity for DNA sizes larger than 250 bp, almost the same as that in 1×TBE buffer, as shown in Fig. 4a. However, the improvement in the efficiency was different when compared with the results in Fig. 4b, where the starting point was about 200 bp instead of 100 bp and the DNA fragments with sizes larger than 500 bp were not deteriorated even though they were also decreased. Not only for LPA, but 2% (w/v) PEO in 1×TTE showed similar results (data not shown). From a comparison of the results obtained using 1×TBE and 1×TTE, we can draw the conclusion that the addition of glycerol to the 1×TTE buffer had almost the same, but a weaker, effect as that in 1×TBE.

Without glycerol, 1×TBE and 1×TTE buffer

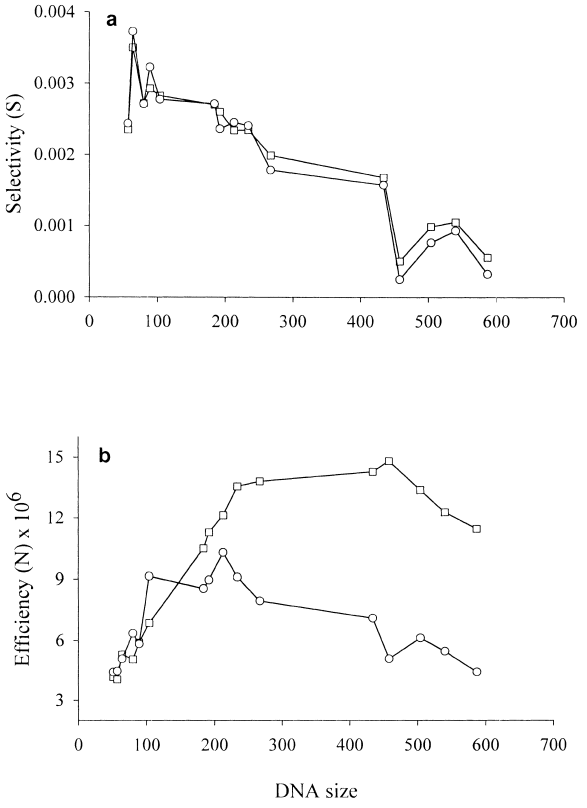
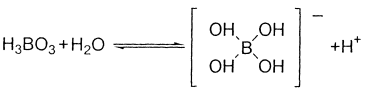
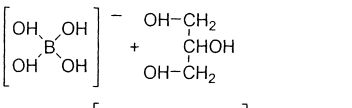


Fig. 7. Plot of (a) selectivity and (b) efficiency versus the size of DNA fragments in 1×TTE buffer (○) and 1×TTE buffer with 25% (v/v) glycerol (□). All the data were calculated from the fitting of the electropherograms in Fig. 5.

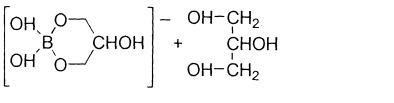
exhibited almost no differences in dsDNA separation, as shown in Figs. 1a and 5a. However, the performance changed when 25% (v/v) glycerol was added, as shown in Figs. 1b and 5b. The main difference between the two buffers is that the boric acid in the 1×TBE buffer can form a complex with the glycerol molecules, which is well established in the literature [21,22]. The reactions are as follows:



(5)



(6)



(7)

Complex formation between boric acid and glycerol releases a proton, which will increase the ionic strength and decrease the buffer pH. The complex may also increase the buffer viscosity due to its enlarged size. Experimental data showed that the addition of 25% (v/v) glycerol to the 1×TBE buffer reduced the buffer pH from 8.3 to 7.4, while the pH was maintained at 8.2 in 1×TTE buffer. The buffer viscosity increased by a factor of 2.19 in 1×TBE buffer, while it increased by a factor of 2.03 in 1×TTE buffer. Another influence was on the current, which was usually between 11.2 and 11.6 μA for both the 1×TBE and 1×TTE buffers on a 10 cm effective length capillary with 100 μm I.D. at 150 V/cm. By adding 25% (v/v) glycerol, the current increased slightly to about 12.4 μA in 1×TBE buffer, but decreased drastically to about 3.2 μA in 1×TTE buffer. A comparison is shown in Table 1.

Table 1
Changes in buffer properties caused by glycerol

	Buffer			
	1×TBE	1×TBE with 25% glycerol	1×TTE	1×TTE with 25% glycerol
pH	8.3	7.4	8.2	8.2
Viscosity (cP)	0.95	2.08	0.98	1.99
Current (μA)	11.2–11.6	12.3–12.5	11.2–11.6	3.1–3.3

The mobility of DNA fragments has a direct relationship with the medium mesh size which is determined by the polymer concentration [23,24]. However, the DNA fragments showed very different mobility in 1×TBE buffer and 1×TTE buffer at the same polymer concentrations when 25% (v/v) glycerol was added, which is shown as the retardation factor in the insets in Figs. 3a and 6a. The change in viscosity on adding glycerol to 1×TBE (by a factor of 2.19) and 1×TTE (by a factor of 2.03) cannot explain the large difference in the retardation factors. The current in 1×TBE with glycerol is much higher than that in 1×TTE with glycerol and thus it should have a lower migration time taking the higher Joule heating effect into account. However, this is not the case, as shown in Figs. 1b and 5b. The change in buffer pH from 8.3 to 7.4 may also have no effect on the DNA mobility since it is well known that the pK of DNA is about 2.2.

In brief, the glycerol-induced changes in buffer properties, such as viscosity, conductance, and pH, have little effect on DNA separation with repect to mobility and resolution. Theoretically, DNA mobility is mainly affected by the mesh size and the charge over weight ratio of the DNA fragments. LPA and PEO showed similar electrophoresis results, suggesting that glycerol did not interact or interacted only slightly with the polymer chains. Thus, we assume that the addition of glycerol has little effect on the mesh size. However, it has the ability to change the DNA conformation, which will influence the DNA charge over hydrodynamic weight ratio.

To confirm that our assumption is correct, DLS experiments were performed using two calf thymus DNA (<2000 bp) samples prepared in 1×TBE buffer and 1×TBE buffer with 25% glycerol. The hydrodynamic radius (R_h) of the DNA was measured, as shown in Fig. 8. At 25°C, the mixture of DNA (<2000 bp) in 1×TBE buffer had a wide size distribution with an average R_h of about 48.2 nm. When 25% glycerol was added, the average R_h was increased to 88.2 nm and the size distribution also became narrower. It is well established that the mobility of a DNA fragment is proportional to its charge over size ratio. The increase in DNA size caused by glycerol can explain the electrophoresis results shown in Figs. 1 and 3. However, the retardation factor was 3.0, as shown in Fig. 3a,

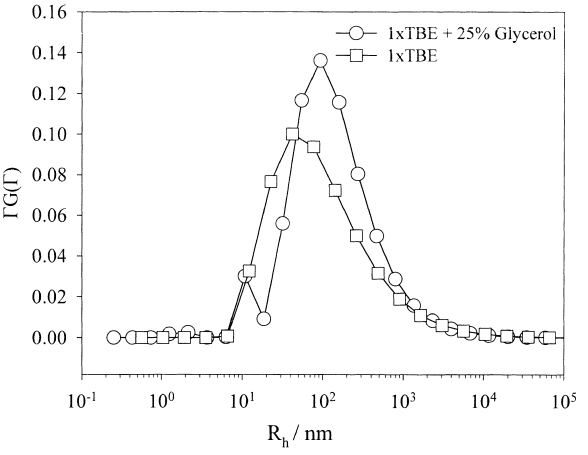


Fig. 8. Plot of the intensity distribution function $\Gamma G(\Gamma)$ vs. hydrodynamic radius R_h of $1 \cdot 10^{-4}$ g/ml calf thymus DNA (<2000 bp) at 25°C and scattering angle 30°. Γ is the characteristic line width and $G(\Gamma)$ is the normalized characteristic line width distribution.

which is larger than the increase in the average R_h (by a factor of ~1.8), suggesting the existence of other effects that can give rise to a further decrease in mobility. We attribute the further decrease as being due to a reduction in the charge on the DNA fragments.

The electrophoresis and DLS results can be explained by the theory of Vecchio et al. [18], where glycerol generally destabilizes the DNA double helix by affecting the forces that stabilize the double helix, such as electrostatic interactions and hydrogen bonds. Firstly, the replacement of the water molecules by glycerol changes the environment in the vicinity of the DNA chains and affects the hydration sphere of both the polynucleotide and its counterions. With glycerol having a larger size and being more hydrophobic than water, the DNA chain will inevitably adjust its conformation to accommodate the environmental change. Secondly, the addition of glycerol reduces the dielectric constant of the buffer and thus enhances the electrostatic interactions between the phosphate groups. The repulsion between the negative charges of the phosphate groups enlarges the distance between them. Moreover, the glycerol molecules will compete with water in the formation of hydrogen bonds with the side groups of the bases, resulting in a protrusion into the groove of

the double helix. In brief, the presence of glycerol changes the DNA conformation by influencing the stabilizing forces on the double helix. The interactions effectively increase the DNA contour length and decrease the effective charge to size ratio, and thus increase the DNA migration time.

The increased contour length and decreased effective charge make the DNA chains less sensitive to the electric field. However, small size DNAs can still migrate through the polymer network without or with little orientation along the direction of the electric field, resulting in little change in the mobility. This change will not bring any improvement to the selectivity, the efficiency, and the retardation factor for different DNA fragments. On the other hand, larger DNA chains have to orientate along the direction of the electric field to pass through the polymer matrix. The larger size DNA will take a longer time than smaller size DNA to complete the orientation, resulting in an improved selectivity and increased retardation factor. The slower orientation also results in a more uniform DNA conformation and increases the efficiency. However, the prolonged migration time also broadens the bandwidth, especially for DNA fragments larger than 500 bp, which show a decrease in efficiency.

The complex formed by boric acid and glycerol also has a polyol group as the glycerol, but with a larger size and molecular mass, as shown in reactions (6) and (7). It can also induce the DNA molecules to change conformation as glycerol does, but to a greater extent due to its larger size, i.e. the complex has a greater ability than glycerol to change the DNA conformation in the way discussed above. This effect could explain why glycerol had a stronger effect in 1×TBE buffer than in 1×TTE buffer.

The glycerol-induced DNA conformation change also had an effect on the intercalating behavior of ethidium bromide. It is well known that ethidium bromide is bound to the DNA molecule through intercalation of the ethidium ion between two adjacent base pairs, resulting in an unwinding of the DNA double helix at the location of the binding, a decrease in the charge density and a larger separation of the phosphate group [25,26]. As far as electrophoresis is concerned, the addition of ethidium bromide to the running buffer improved the detectability of dsDNA, enhanced the resolution and increased the migration time. Without ethidium bromide (or other intercalating dye), no separation medium has been reported to be able to separate the doublet of 123 and 124 bp in pBR322/HaeIII digest. Pariat et al. [1] reported the ethidium bromide effect when LPA was used as the separation medium: the doublet of 123 and 124 bp in pBR322/Hae III digest was base-line separated and the separation of 434 and 458 bp deteriorated. However, glycerol induced a DNA conformational change and counteracted the effect of ethidium bromide, at least in the separation of 123/124 and 434/458, as seen in Fig. 1b.

The separation of ssDNA is also enhanced by the addition of 25% (v/v) glycerol to the running buffer. Fig. 9 shows the separation of a C-terminated pGEM-3Zf(+) sequencing sample using 2.5% (w/v) LPA in 1×TTE buffer with 5 *M* urea (top plot in each panel) as well as in the same buffer with 25% (v/v) added glycerol (bottom plot in each panel). As for the DNA fragments below 104 base, the addition of glycerol to the running buffer had almost no effect on the resolution, but drastically increased the migration time, as seen from panel A in Fig. 9. Enhancement of the separation became obvious when the DNA fragments reached 200 bases. As seen in panel B, the doublet of 438 and 439 base had a much better resolution in the running buffer with 25% (v/v) glycerol added than without. Enhancement of the separation remained effective for larger DNA fragments. The addition of glycerol to the running buffer did not increase the total read length, as seen in panel C, but the total run time increased by a factor of about 2.4. A mixture of 1.5% (w/v) PEO with $M_r = 8 \cdot 10^6$ and 1.4% (w/v) PEO with $M_r = 0.6 \cdot 10^6$ [8] was also used as a separation medium to test the effect of glycerol on ssDNA separation. The separation of a C-terminated pGEM-3Zf(+) sequencing sample showed almost the same results as 2.5% (w/v) LPA (Fig. 9) (data not shown). ssDNA is more flexible than dsDNA and is in a random coil conformation when denatured. The increased electrostatic interactions between phosphate groups help to maintain a uniform conformation of ssDNA chains. This kind of reaction enhances ssDNA separation, but increases the migration time, which is almost the same as for dsDNA. It is worth noting that any separation medium, besides LPA and PEO, can give an improved result with added glycerol, since the

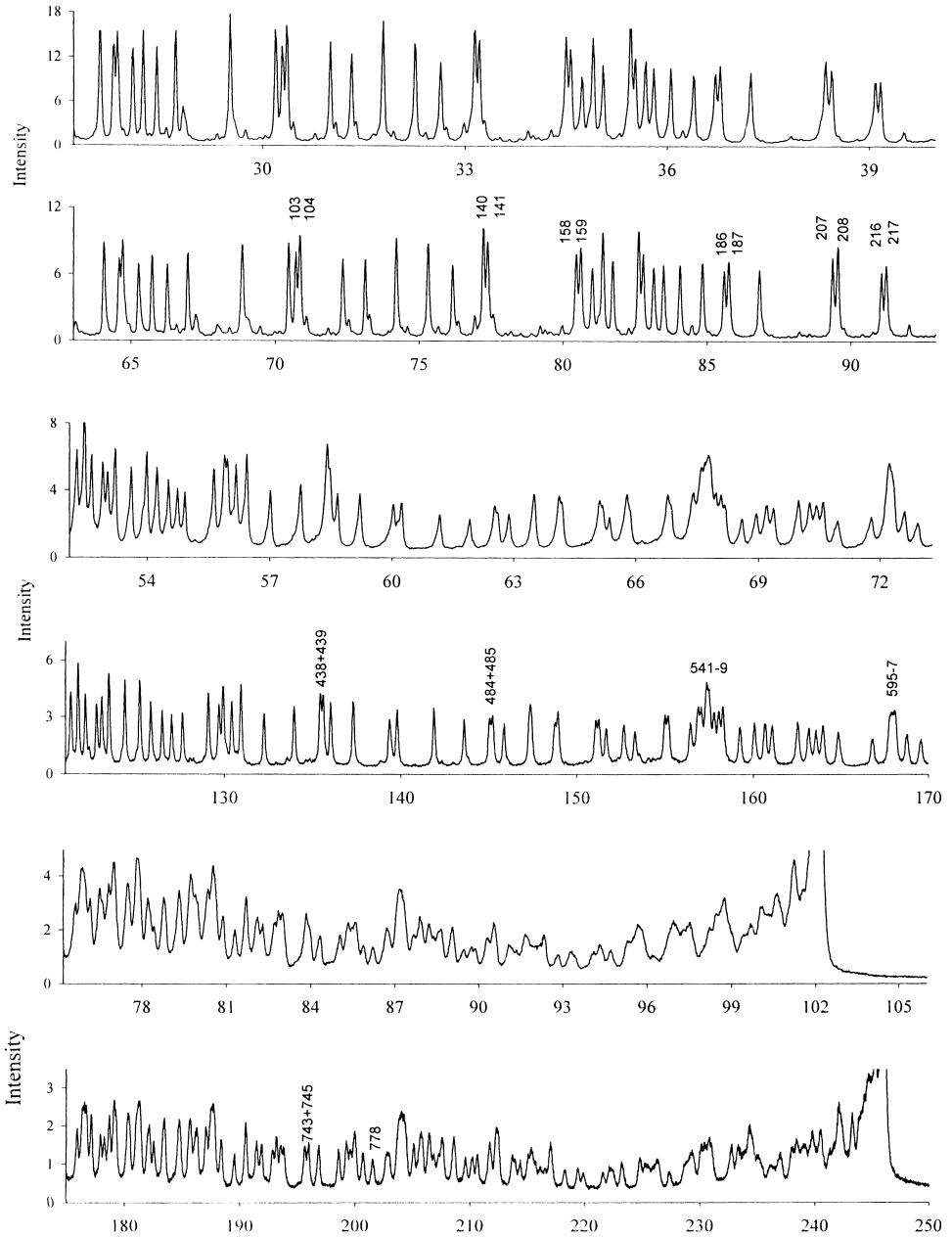


Fig. 9. Comparison of the separation of a C-terminated single dye labeled DNA sequencing sample using FAM-labeled −21 M13 primer on a pGEM-3Zf(+) template in 1×TTE buffer+5 *M* urea with (bottom plot in each panel) and without 25% (v/v) added glycerol (top plot in each panel). The three panels show the separation of different sized DNA fragments. The total effective capillary length was 40 cm with 100 μm I.D. The DNA sample was injected electrokinetically into the column at 300 V/cm for 5 s. The separation electric field strength was 150 V/cm.

enhancement of DNA separation results from the change in DNA conformation. It has little to do with the separation medium.

4. Concluding remarks

The addition of glycerol to the running buffer changes the DNA chain conformation by influencing the forces stabilizing the DNA double helix. DNA chains modified by glycerol have a larger contour length, a decreased effective charge over weight ratio, and a more uniform conformation. The separation of both dsDNA and ssDNA fragments is enhanced. This method is not applicable to high-speed separation due to the loss of migration time. However, it has the potential to be used in mutant studies or supercoiled DNA studies due to its ability to change the DNA conformation.

Acknowledgements

B. Chu gratefully acknowledges support of this research by the National Human Genome Research Institute (6R01HG0138606).

References

[1] Y.F. Pariat, J. Berka, D.N. Heiger, T. Schmitt, M. Vilenchik, A.S. Cohen, F. Foret, B.L. Karger, J. Chromatogr. A 652 (1993) 57.
[2] H. Zhou, A.W. Miller, Z. Sosic, B. Buhholz, A.E. Barron, L. Kotler, B.L. Karger, Anal. Chem. 72 (2000) 1045.
[3] P. Bocek, A. Chrambach, Electrophoresis 13 (1992) 31.
[4] M. Strege, A. Lagu, Anal. Chem. 63 (1991) 1233.
[5] O. Muller, M. Minarik, F. Foret, Electrophoresis 19 (1998) 1436.
[6] M.A. Quesada, H.S. Dhadwal, D. Fisk, F.W. Studier, Electrophoresis 19 (1998) 1415.
[7] Q. Gao, E.S. Yeung, Anal. Chem. 70 (1998) 1382.
[8] N. Iki, E.S. Yeung, J. Chromatogr. A 731 (1996) 273.
[9] Y. Kim, E.S. Yeung, J. Chromatogr. A 781 (1997) 315.
[10] C. Wu, T. Liu, B. Chu, Electrophoresis 19 (1998) 231.
[11] D. Liang, B. Chu, Electrophoresis 19 (1998) 2447.
[12] D. Liang, L. Song, S. Zhou, V.S. Zaitsev, B. Chu, Electrophoresis 19 (1999) 2856.
[13] D. Liang, S. Zhou, L. Song, V.S. Zaitsev, B. Chu, Macromolecules 32 (1999) 6236.
[14] J. Cheng, T. Kasuga, K.R. Mitchelson, E.R.T. Lightly, N.D. Watson, W.J. Martin, D. Atkinson, J. Chromatogr. A 677 (1994) 169.
[15] J. Cheng, K.R. Mitchelson, Anal. Chem. 66 (1994) 4210.
[16] J.J. Lee, K.J.J. Lee, Biochem. Mol. Biol. 31 (1998) 384.
[17] F. Han, B.H. Huynh, Y. Ma, B. Lin, Anal. Chem. 71 (1999) 2385.
[18] P.D. Vecchio, D. Esposito, L. Ricchi, G. Barone, Int. J. Biol. Macromol. 24 (1999) 361.
[19] L. Song, D. Liang, J. Kielezcawa, J. Liang, E. Tjoe, D. Fang, B. Chu, Electrophoresis 22 (2001) 729.
[20] C. Wu, M.A. Quesada, D.K. Schneider, R. Farinato, F.W. Studier, B. Chu, Electrophoresis 17 (1996) 1103.
[21] J.T. Smith, W. Nashabeh, Z. El Rassi, Anal. Chem. 66 (1994) 1119.
[22] A.C. Zittle, Adv. Enzymol. 12 (1951) 493.
[23] D. Broseta, L. Leibler, A. Lapp, C. Strazielle, Europhys. Lett. 2 (1986) 733.
[24] C. Heller, Electrophoresis 20 (1999) 1962.
[25] J.B. LePecq, C.J.J. Paoletti, Mol. Biol. 27 (1967) 87.
[26] P.G. Wu, B.S. Fujimoto, L. Song, J.M. Schurr, Biophys. Chem. 41 (1991) 217.