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Novel elution strategy for monitoring DNA counter-migration in the presence of electroosmotic flow

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Abstract

The migration behavior of native (i.e., unlabelled) DNA in the presence of electroosmotic flow (EOF) was investigated in bare fused-silica capillaries. Employing a novel elution strategy, the influence of EOF on the net mobility of DNA was assessed by collecting the DNA that migrated anodically (i.e., against EOF) and out of the capillary inlet. Various conditions of pH and buffer-zone continuity were employed to characterize this phenomenon. Tris acid (TA, pH 5.14) and Tris base (TB, pH 9.36) were used as buffers in continuous systems, in which the capillary and the inlet reservoir contain the same buffer, and discontinuous systems, in which the capillary contains either TA or TB, and the inlet reservoir contains water. DNA that was ejected into the inlet vial was subsequently analyzed by capillary electrophoresis–laser-induced fluorescence. Both ΦX174/*Hae*III DNA and the β-actin product of single-cell reverse transcriptase-polymerase chain reaction were used as DNA samples in this study. The mechanism of elution was found to depend on bulk flow, in the case of continuous solutions. However, with the discontinuous system, a localized decrease in EOF generated in the capillary tip appeared to impact elution. These findings serve to introduce an alternative approach for characterizing the mobility of highly charged species.

Keywords: Electroosmotic flow; DNA

1. Introduction

The onset of the genomics era sparked a surge in high-resolution DNA separations, culminating with the recent completion of the first sequencing of the human genome [1]. A key player in this endeavor was capillary electrophoresis (CE), which has emerged as the new "work-horse" method for many applications. Consequently, there has been an explosion in the number and variety of ways to perform CE-based DNA separations. Despite the wide variation in specific technique, most methods have two common features: (1) a polymeric separation medium to provide size-based distinction of DNA [2,3] and (2) suppression of electroosmotic flow (EOF) [2,3].

A sieving matrix is usually necessary to separate DNA, since its mobility in free solution is independent of molecular weight for fragments greater than 170 base pairs (bp) [4]. If a sieving medium is not used, some other means of distinguishing DNA fragments must generally be employed. One such method involves the addition of an uncharged tail to the DNA molecules to provide a change in the charge-to-mass ratio of the fragments [5,6]. Both the addition of streptavidin [5] and poly(ethylene glycol) [6] have been used in this manner to yield a high-resolution separation of DNA. Wei and Yeung used a monomeric surfactant to separate double-stranded (ds) DNA fragments [7]. The monomers form rodlike micelles in solution, which function as a dynamic polymer medium for size-based distinction. There have also been reports in which the mechanism of DNA migration in free solution has been studied. Olivera et al. first examined the mobility of native and denatured T4 DNA in 1964 [8]. Work by Stellwagen and co-workers has encompassed comprehensive studies of DNA migration in the absence of sieving polymers [4,9–12]. However, since all of these authors were primarily concerned with the mechanistic aspect, they had little need for actual separation [4,8–12].

While a sieving medium permits distinction of different sized fragments, EOF suppression is normally incorpo-

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rated to improve resolution and to decrease analysis time, in both polymer and free-solution CE. Nucleic acids, in general, are highly negatively charged molecules, with a charge of −2 per bp [8]. The result is an extremely large electrophoretic mobility in most buffers and polymer solutions, which generally precludes elution by EOF as in capillary zone electrophoresis (CZE). If the polarity is reversed so that DNA migrates electrophoretically, the opposition of EOF severely retards migration and could impair resolution [2,3].

A few groups nonetheless have utilized EOF in the analysis of DNA. Chang's group has shown the separation of double-stranded (ds) DNA fragments in the presence of EOF [13–22]. In their approach, DNA samples are electrophoretically introduced at the anode into a bare capillary containing only a Tris-borate buffer. Upon commencement of electrophoresis, EOF causes poly(ethylene oxide) solution at the anode to be drawn into the capillary to provide the separation matrix. Size-based resolution of DNA results with the largest fragments eluting first. Barron et al. have studied various dilute and semidilute polymers as media, with EOF, for the separation of ΦX174/*Hae*III and λ/*Hind*III fragments [23]. In this work, the capillary was first filled with polymer solution, and then DNA samples (spiked with a neutral marker to monitor EOF) were pressure-injected at the anode. Migration commenced in the cathodic direction, with the polymers providing size-based distinction. Iki et al. have performed the equivalent of CZE of various dsDNA markers, with Tris–acetate–EDTA and Tris–borate–EDTA buffers [24]. They demonstrated that at a pH > 8, EOF is sufficient for elution of POPO-3-labelled DNA fragments from 72 bp to 23 kbp. The mechanism of separation is reportedly based on the excess of positive charge in the diffuse layer, which reduces the effective charge of those DNA molecules most able to freely access it.

Although most of these studies have investigated dsDNA, EOF has similar effects on single-stranded (ss) DNA [4] and RNA [25]. We have shown previously that the net migration of RNA, from a dilute Tris buffer (pH 8.5) into an aliquot of water, is against EOF [25]. This characteristic enabled the novel manipulation of RNA from a cell lysate sample (in a capillary) into a small aliquot of water. The RNA was ejected from the capillary and subsequently analyzed, while the proteins, which remained in the capillary due to EOF, were immediately separated by CZE. Thus, the selective mobility of nucleic acid, under the influence of EOF, is a property that can be controlled and used to direct analyte movement as desired.

In this paper, we further investigate the migration of DNA under various conditions of EOF, including pH and buffer-zone continuity. A ΦX174/*Hae*III DNA standard in the presence of 140 mM NaCl, and the β-actin product of single-cell reverse transcriptase-polymerase chain reaction (RT-PCR), were used as samples in this study. This characterization of DNA elution employed counter-migration and ejection of the DNA from the capillary inlet. This novel approach is shown to be a viable alternative for studying the mobility of highly charged nucleic acids.

2. Experimental

2.1. Chemicals and reagents

Hydroxypropylmethylcellulose (HPMC), molecular mass 10 000, was obtained from Aldrich (Milwaukee, WI, USA). Ethylenediaminetetraacetic acid disodium salt (EDTA) was purchased from Invitrogen (Gaithersburg, MD, USA). Tris(hydroxymethyl)aminomethane (Tris base, TB), tris(hydroxymethyl)aminomethane hydrochloride (Tris acid, TA), and mannitol were from Sigma (St. Louis, MO, USA). Poly(vinylpyrrolidone) (PVP), with a molecular mass of 1 000 000, came from Polysciences (Warrington, PA, USA). Ethidium bromide and BODIPY-TMR cadavarine IA (BODIPY-TMR) were from Molecular Probes (Eugene, OR, USA). Molecular biology grade water (i.e., RNase/DNase-free), used during RT-PCR preparation, was purchased from Biowhittaker (Walkersville, MD, USA). Gene-specific primers for human β-actin (sense: 5′-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3′; anti-sense: 5′-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′) were obtained from Clontech (Palo Alto, CA, USA). A ΦX174/*Hae*III DNA standard, consisting of 11 double-stranded fragments (72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, and 1353 bp), and all other RT-PCR reagents were purchased from Promega (Madison, WI, USA). Deionized (DI) water (resistance >18 MΩ) was obtained from a Milli-Q system (Bedford, MA, USA) and autoclaved prior to use. All other chemicals were purchased from Fisher Scientific (Fairlawn, NJ, USA).

2.2. Cell preparation

A human breast carcinoma cell line (MCF-7), obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) was used for the single-cell RT-PCR experiments. The cells were cultured in Minimum Essential Eagle medium (ATCC) supplemented with 10% fetal bovine serum, 0.18 μg/mL streptomycin, 0.18 IU/mL penicillin, and 10 μg/mL bovine insulin (all from Invitrogen). The cells were grown in an incubator kept at 37 °C and 5% CO_2, and were isolated for RT-PCR using a previously described method [26].

2.3. RT-PCR protocol

A single-tube RT-PCR master mixture was prepared similarly to that described previously [26], by combining the following in a sterile, 200 μL vial: (a) 27.5 μL of molecular biology grade water; (b) 10 μL of AMV/*Tfl* 5× reaction buffer; (c) 2.5 μL of each human β-actin primer (20 μM each primer); (d) 1 μL of PCR nucleotide mixture (10 mM each

nucleotide); (e) 4 μL $MgSO_4$ (25 mM); (f) 2 μL of RNasin ribonuclease inhibitor (40 IU/μL); (g) 1 μL *Tfl* DNA polymerase (5 IU/μL); and (h) 0.5 μL of AMV reverse transcriptase (10 IU/μL).

For each single-cell RT-PCR reaction, 5 μL of the master mix was placed into a 30 μL vial constructed from a sterile, 200 μL pipette tip [26]. An intact cell was then delivered to the mixture and the vial was sealed [26]. The vial was placed into dry ice for 1 min and subsequently thawed at room temperature. The reaction solution was then mixed, wrapped in a 6 cm × 4.5 cm piece of aluminum foil to prevent evaporation and ensure efficient heat transfer, and placed on ice until all reaction vials had been prepared.

RT-PCR was accomplished with a GeneAmp System 2400 thermal cycler (Perkin-Elmer, Foster City, CA). Reverse transcription was achieved at 48 °C for 45 min, followed by denaturation of the cDNA at 94 °C for 2 min. PCR was performed directly following reverse transcription using the following cycling profile: 35 cycles of 94 °C for 1 min (denaturation), 60°C for 1 min (annealing), 68 °C for 2 min (extension); and 1 cycle of 68 °C for 7 min (final extension).

2.4. DNA migration

The migration behavior of native (i.e., unlabelled) DNA was assessed by monitoring its counter-migration to EOF through subsequent collection outside of the capillary. This approach was chosen to maintain consistency with our previous observations of RNA [25]. Fig. 1 shows a schematic of this procedure, which was performed using a sample containing 100 μg/mL ΦX174/*Hae*III DNA in 140 mM NaCl (sample pH: 6.87), or the product mixture of single-cell RT-PCR. Two capillaries were used: an ejection capillary for migration studies, and an analysis capillary for CE. The ejection capillary was 40 cm × 23 μm i.d. (Polymicro Technologies, Phoenix, AZ, USA) and filled with either 5 mM TB (pH 9.36) or 5 mM TA (pH 5.14); unadjusted pH for both solutions. An aliquot of the DNA standard was hydrodynamically injected by elevating the inlet 8 cm relative to the outlet for 1 min, resulting in a 0.8 nL injection volume. The RT-PCR product was injected for 2 min, resulting in a 1.6 nL injection volume. (Different injection times are due to the low concentration of DNA resulting from RT-PCR.) The capillary inlet was then placed into a vial containing 10 μL of solution. A potential of +7.8 kV was applied to the inlet reservoir for 2 min to allow DNA migration from the capillary (the outlet reservoir, containing buffer, was grounded). Between each step, the capillary tip was carefully immersed for 2 s each in 100 mM NaOH, followed by water, to remove any DNA adsorbed to the external surface. The electrophoretically collected DNA samples (referred to as "ejected" samples) were mixed with a pipet and placed on ice until analysis by CE. For comparison, DNA samples that were not subjected to the ejection step prior to analysis by CE with laser-induced fluorescence (CE–LIF), are referred to as "original" samples.

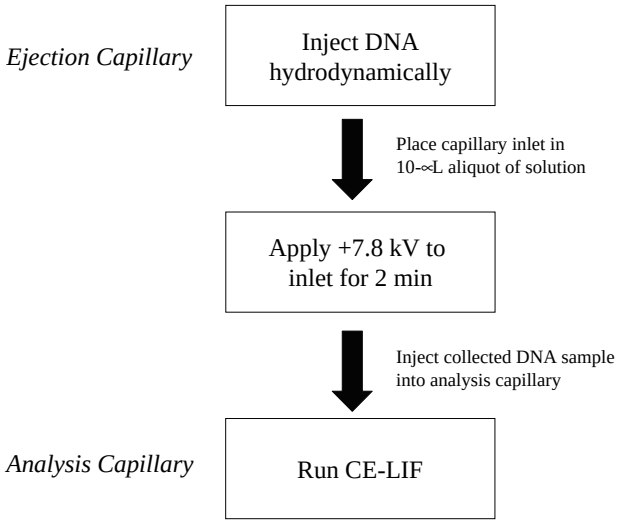

Fig. 1. Schematic illustrating protocol for monitoring DNA migration.

2.5. Buffer systems

Two types of buffer systems were used to investigate DNA migration in the presence of EOF: continuous and discontinuous. For continuous solutions, the capillary, inlet vial, and outlet vial all contained the same buffer, and are designated as either TA/TA or TB/TB. For discontinuous solutions, the capillary and outlet vial contained either TA or TB, however, the inlet vial was filled with autoclaved water. These systems are referred to as TA/H_2O or TB/H_2O.

2.6. CE–LIF

The CE–LIF system used for separation and detection of DNA, which has been described previously, is a laboratory-built instrument using a 543 nm green helium–neon laser for excitation of fluorescence [26]. The laser was focused on the detection window using a 1 cm focal length lens. Fluorescence was collected through a 10× microscope objective (Melles Griot, Irvine, CA, USA), passed through an RG-610 filter (Schott Scientific Glass, Parkersburg, WV, USA), and imaged onto a photomultiplier tube (R928; Hamamatsu, Bridgewater, NJ, USA). Data were digitized using a DT 2804 data board. Electropherograms were generated using ChromPerfect software (Justice Innovations, Mountain View, CA, USA), converted to ASCII, and replotted using Excel (Microsoft, Seattle, WA, USA). Separations were effected using 40 cm (25 cm effective length) × 50 μm i.d. fused-silica capillaries (Polymicro). The sieving matrix for separation of nucleic acids consisted of 1% HPMC, 0.5% PVP, 6% mannitol, and 0.5 μg/mL ethidium bromide dissolved in TBE buffer (100 mM TB–100 mM boric acid–2 mM EDTA, pH 8.3). All samples were electrokinetically injected for 3, 10, or 15 s at the cathode. CE was commenced by applying a potential of +7.8 kV to the outlet (the inlet was grounded).

2.7. EOF determination

Bulk EOF was determined for the TB/TB and TA/TA systems. The experiments were performed on the same CE–LIF system used for DNA samples, under normal polarity, using the buffer instead of the sieving matrix. A 2.5 μg/mL solution of BODIPY-TMR in dimethyl sulfoxide (DMSO), the neutral marker, was injected hydrodynamically by elevating the inlet 10 cm relative to the outlet for 10 s. A potential of +7.8 kV was applied to the inlet (the outlet was grounded) to initiate CE.

2.8. Calculations

The Poiseuille equation was used to calculate the volumes of hydrodynamic injections. Volumes derived electrokinetically were estimated using the average analyte velocities from individual electropherograms. Enhancement factors for individual peaks were assessed according to Eq. (1),

$$\text{enhancement} = \frac{(S/N_e)(\text{dilution factor})(t_o)}{(S/N_o)(t_e)} \quad (1)$$

where S/N_o and S/N_e are the signal to noise ratios (peak-to-peak) for a particular peak in the electropherograms derived from either the original or the ejected samples, respectively, and t_o and t_e the electrokinetic injection times for the original and ejected samples, respectively. Dilution factors were determined by dividing the collection volume (e.g., 10 μL) by the sample volume that was originally introduced hydrodynamically into the ejection capillary.

3. Results

3.1. ΦX174/HaeIII DNA standard

3.1.1. Discontinuous solutions

Characterization of selective DNA migration was initially performed using a 100 μg/mL DNA standard/140 mM NaCl solution. The addition of NaCl was necessary to maintain consistency with our previous RNA work [25], which involved high-salt lysate samples. Two discontinuous buffer systems, 5 mM TB/H_2O (pH 9.36) and 5 mM TA/H_2O (pH 5.14), were investigated in bare capillaries using the procedure outlined in Fig. 1. Approximately 0.8 nL of the DNA/salt solution was hydrodynamically introduced into the capillary containing either TA or TB, then permitted to migrate for 2 min with the inlet of the capillary immersed in DI water (pH ~5). If the net mobility of negatively charged DNA under the given conditions opposes EOF, then the DNA will elute into the water. Such a long ejection time was purposely chosen to ensure that all of the DNA initially present in the injection plug would have sufficient time to exit the capillary. This aqueous DNA sample was subsequently analyzed using CE–LIF. To provide a basis for comparison, the original DNA/NaCl sample was directly injected into the CE–LIF system.

Representative electropherograms of the DNA standard are shown in Fig. 2. This DNA sample contains 11 fragments of known size, as indicated in Section 2 and on the plot. The remaining peaks are most likely the result of DNA degradation or impurities. Fig. 2A is the result of a direct injection

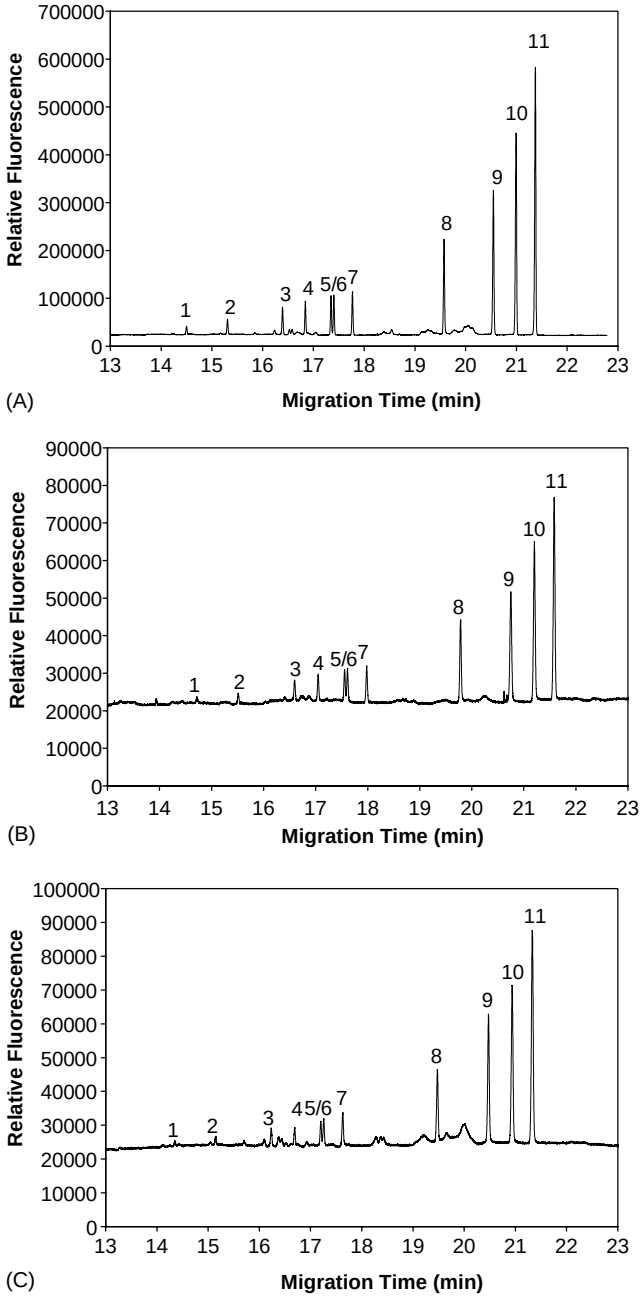

Fig. 2. Electropherograms of 100 μg/mL DNA ΦX174/*Hae*III DNA/140 mM NaCl solution using discontinuous buffer systems. (A) Original sample directly injected into analysis capillary; electrokinetic injection for 3 s at −7.8 kV. (B) Ejected DNA collected in water (TA/H_2O buffer system); electrokinetic injection for 10 s at −7.8 kV. (C) Ejected DNA collected in water (TB/H_2O buffer system); electrokinetic injection for 15 s at −7.8 kV. Other conditions given in Section 2.

of the DNA sample; Fig. 2B is the plot for DNA subjected to TA/H_2O (pH 5.14). The relatively low pH, hence low EOF, of this buffer results in efficient electrophoretic ejection of the DNA into water, as expected. Interestingly, the TB/H_2O system (pH 9.36) also permits migration of DNA against EOF (Fig. 2C) into water, which is consistent with our observations of RNA at an alkaline pH of 8.5 [25]. Our proposed explanation for this seemingly anomalous elution at high pH can be found in Section 4.

3.1.2. Signal enhancement

At first glance, a comparison of the electropherograms in Fig. 2 reveals a decrease in absolute signal between that from the original sample and those of the ejected samples. Upon closer examination, and taking into account the dilution of the collected DNA, it is revealed that signal enhancements actually exist, as previously reported with RNA [25]. Both TB/H_2O and TA/H_2O generated similar improvements, of 220- and 380-fold, respectively. We believe that the enhancement from the ejected DNA samples is primarily caused by improved electrokinetic injection efficiency, resulting from removal of competing salt ions. EOF present in the TB/H_2O and TA/H_2O capillaries, partially alleviated the migration of smaller ions from the capillary. Any ions that did co-migrate with the DNA, were sufficiently diluted in the collection vial, thus, the net result was “desalting”.

Run-to-run reproducibility for all peaks, taking into account hydrodynamic injection, ejection, and subsequent CE injection, varied from 2 to 15% ($n = 3$) for both buffers. However, most of the uncertainty actually results from the electrokinetic injection for CE, as the average R.S.D. for the ejection step ranged from 1 to 4% ($n = 3$), for all peaks. The reproducibility in enhancement was also good, which was assessed by quantitating the R.S.D. between individual fragments; values of 11% ($n = 11$) and 7% ($n = 11$) were obtained for DNA subjected to TB/H_2O and TA/H_2O, respectively.

3.1.3. Influence of EOF

Bulk EOF was measured for continuous systems (i.e., normal CE) using TA/TA or TB/TB. The EOF of TB ($(8.1 \pm 0.2) \times 10^{-4}$ cm^2 V^{-1} s^{-1}) was about twice that of TA ($(3.9 \pm 0.1) \times 10^{-4}$ cm^2 V^{-1} s^{-1}), despite the large difference in pH (i.e., 9.36 versus 5.14); $n = 3$ for each EOF measurement. This difference was sufficient to influence the migration direction when using continuous buffer systems. Electropherograms resulting from collected (ejected) DNA for TA/TA and TB/TB buffers are shown in Fig. 3. For TA, successful ejection occurred when the inlet reservoir was changed from water to TA, as shown by subsequent CE–LIF analysis (Fig. 3A). However, for the TB system, DNA ejection did not occur when TB was in the collection vial (Fig. 3B), which at pH 9.36, is consistent with the results shown by Iki et al. [24].

The situation changes with discontinuous solutions (Fig. 2), that is, in both cases, TA/H_2O and TB/H_2O, DNA migrated against EOF and ejected into the water. These observations indicate that the difference in *bulk* EOF for TA and TB no longer plays a significant role in the migration of DNA. Rather, we believe the migration of DNA under these conditions is determined by the *local* EOF inside the capillary inlet.

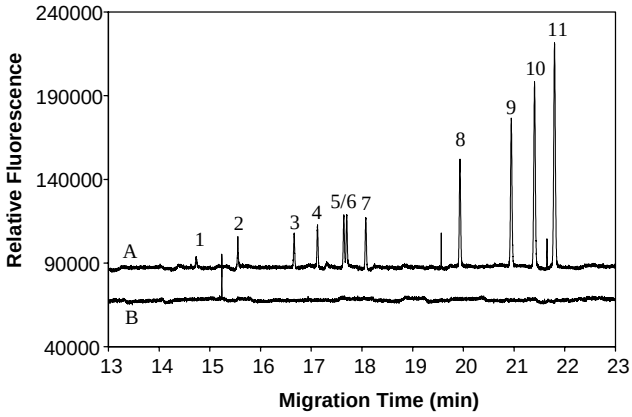

Fig. 3. Electropherograms of 100 μg/mL ΦX174/*Hae*III DNA/140 mM NaCl solution using continuous buffer systems. (A) Ejected DNA collected in TA (TA/TA buffer system); electrokinetic injection for 10 s at −7.8 kV. (B) Ejected DNA collected in TB (TB/TB buffer system); electrokinetic injection for 15 s at −7.8 kV.

3.2. RT-PCR mixture

DNA products of single-cell RT-PCR were also used in this work to study the effect of EOF on DNA migration. RT-PCR reaction mixtures are ideal samples for this type of study for two reasons. First, they provide two types of DNA to use during exploration of the elution process, the ds DNA product, which is similar to the DNA standard, and primer–dimers, which result from random annealing of the single-stranded oligonucleotide primers. Second, the samples also contain salt concentrations of approximately 70 mM, which allows confirmation of the desalting effect on a “real” sample.

An 838 bp product was created using gene specific primers for β-actin, and subjected to the procedure outlined in Fig. 1. Fig. 4A gives a representative electropherogram of a direct injection of the β-actin RT-PCR product mixture. The electrophoretic profile is consistent with previous single-cell RT-PCR experiments [26]. Two distinct peak sets are observed, a group of peaks between 13.80 and 14.61 min, resulting from the formation of primer–dimers and a peak at 20.33 min, corresponding to the 838 bp DNA product. The average S/N ratio for the DNA product in the direct injection is 92 ± 2 ($n = 2$). This limited sample size for statistical treatment results from a lack of reproducibility inherent to single-cell RT-PCR performed using a heterogeneous population of cells. Representative electropherograms of the ejected RT-PCR products using TA/H_2O and TB/H_2O are shown in Fig. 4B and C, respectively. As

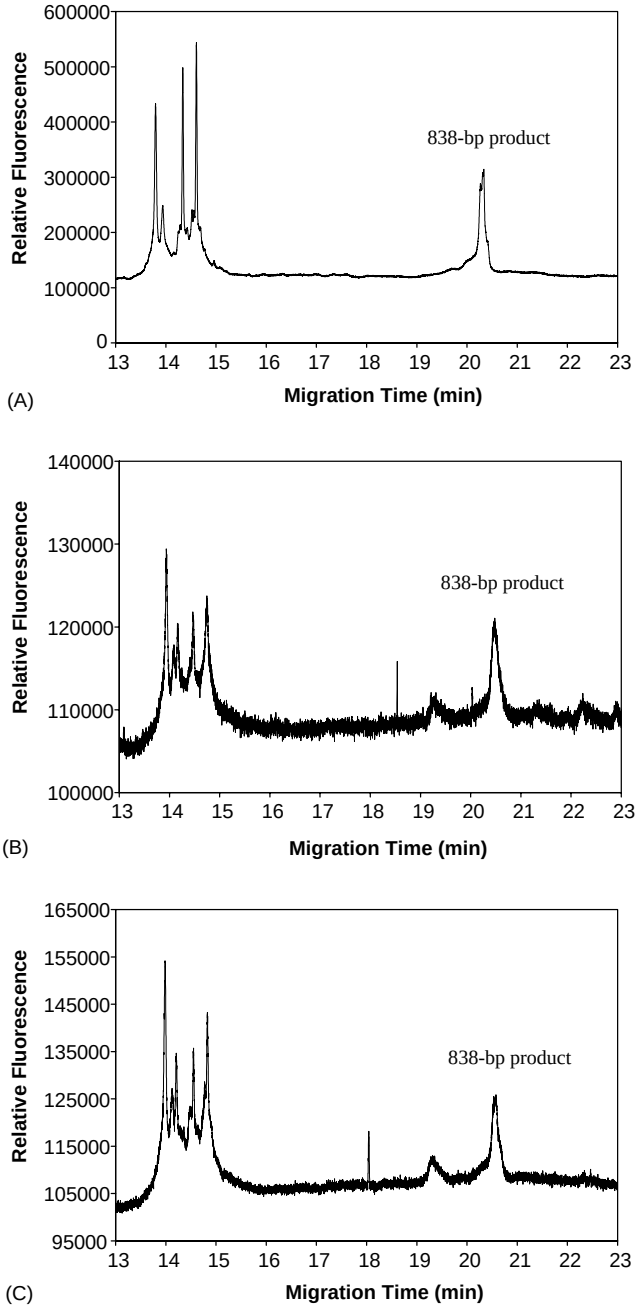

Fig. 4. Electropherograms of β-actin RT-PCR products of single MCF-7 cells using discontinuous buffer systems. (A) Original sample directly injected into analysis capillary; electrokinetic injection for 10 s at −7.8 kV. (B) Ejected RT-PCR product collected in water (TA/H_2O buffer system); electrokinetic injection for 10 s at −7.8 kV. (C) Ejected RT-PCR product collected in water (TB/H_2O buffer system); electrokinetic injection for 15 s at −7.8 kV.

is evident from these plots, both systems generated product peaks with similar S/N ratios. Within error, the ratios are not significantly different, with the TB/H_2O system giving a S/N of 6 ± 3 and the TA/H_2O system giving a S/N of 8 ± 3, which correspond to net improvements of 290 and 360, respectively. Consistent with the migration of ΦX174 DNA shown in Fig. 2B and C, the RT-PCR products also eject into water using both the acidic and the alkaline buffers. It is interesting to note that the primer–dimers also migrated anodically and were collected, as would be expected from previous work involving ss DNA samples [4].

4. Discussion

DNA manipulation within the realm of CE can adopt many forms, including improved separation and mechanistic studies. The goal of our work was to investigate the migration of DNA in free solution, under the influence of EOF, through observation of DNA ejection. Following hydrodynamic injection of a homogeneous sample into a capillary containing an aqueous buffer, a positive potential is applied to the inlet. Since the buffer in the bare fused-silica capillary does not suppress EOF, ions and other small molecules are swept toward the outlet (cathode). Under certain conditions, nucleic acids can be controlled to elute toward the inlet (anode), and out of the capillary into a small aliquot of solution. This ejected sample is subsequently analyzed on a separate CE–LIF system to evaluate DNA migration. If the net migration occurred in the direction of opposing EOF, a signal would be observed.

With the continuous buffer systems of TA/TA and TB/TB, this is the equivalent of normal CE. When an acidic buffer is used (i.e., TA/TA, pH 5.14), net DNA mobility opposes EOF and DNA is ejected from the capillary. That is, the bulk EOF is insufficient to pull DNA in the cathodic direction. However, the alkaline system TB/TB (pH 9.36), exhibits a larger EOF, which is strong enough to prevent anodic migration. Furthermore, for the continuous systems, the localized EOF at the capillary tip is equal to bulk EOF, once the voltage is applied and TB or TA enters the capillary. Thus DNA will be swept with EOF toward the cathode and will not elute out of the capillary inlet. These observations are consistent with the work of Iki et al., which demonstrated DNA migration in the cathodic direction, with EOF, at pH > 8 [24].

The proposed mechanism for migration with the use of discontinuous buffers, however, warrants further explanation. For TA/H_2O, DNA elution opposes EOF, as with TA/TA. The similar pH of TA (5.14) and our DI water (pH ∼5) results in a local EOF likely similar to that observed with TA/TA. Since this EOF is still low, DNA elutes toward the anode.

With TB/H_2O, the observation is strikingly different, which we believe is a direct effect of the change in EOF in the tip of the capillary, rather than bulk EOF. Because DNA elutes out of the capillary, in contrast to TB/TB, the *local* EOF must be small enough such that DNA overcomes the cathodic bulk flow, and migrates anodically. This behavior is not simply the result of a slight lowering in overall EOF, because the EOF measured by the neutral marker changes by no more than 2% for TB/H_2O compared to TB/TB (data

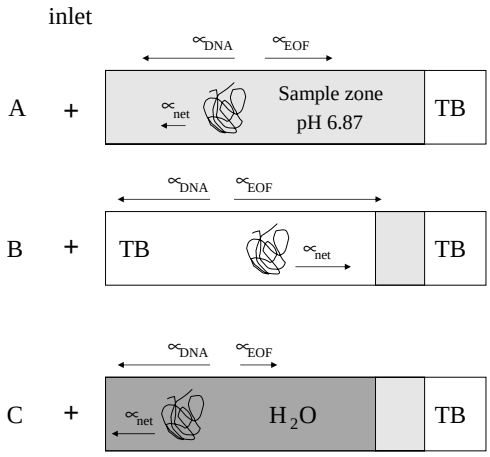

Fig. 5. Schematic depicting an expanded view of the capillary inlet. (A) Initial mobility of DNA upon commencement of electrophoresis. (B) TB/TB buffer system results in net cathodic DNA migration. (C) TB/H_2O buffer system results in net anodic DNA migration.

not shown). Such a minor difference would not account for the complete reversal of migration direction. Furthermore, our previous results with a discontinuous system (TA/H_2O; adjusted pH 8.5), revealed similar results, in that RNA from a high-salt cell lysate also eluted against EOF [25]. Although bulk EOF was not measured in that study, the pH of 8.5 would be expected to give similar results to TB/TB.

The proposed localized change in EOF, in the vicinity of the injected DNA, is thought to occur due to a pH change at the tip of the capillary. This process is depicted schematically in Fig. 5. The DNA solution is injected as a plug into high pH buffer (i.e., TB). As the pH of the sample zone is <8, once the voltage is applied, DNA likely begins to move against EOF (Fig. 5A). If bulk flow siphons TB from the vial into the inlet region of the capillary, the local pH, hence local EOF increases. The net mobility of the DNA changes direction and migrates cathodically, as in normal CE (Fig. 5B). The situation changes in the TB/H_2O system. Once H_2O envelops the sample zone, the net mobility of the DNA, now in a lower pH region, continues to elute anodically, out of the capillary (Fig. 5C). Although the entrance of H_2O is not sufficient to change bulk flow properties along the length of the capillary, it is substantial enough to affect the microenvironment, thus permitting DNA to migrate against strong electroosmosis. There is probably also a change in ionic strength generated by infusing water. Although this would probably lead to a slight increase in EOF [27], according to our results, said increase is clearly overshadowed by the dominant influence of pH on localized flow. Finally, it is worth noting that this decrease in EOF occurs very rapidly, with complete nucleic acid ejection occurring previously in as little as 10 s for RNA, and partial ejection of all but the largest fragment (28S) evident after 5 s [25]. Because the present work aimed to perform a more thorough characterization of DNA migration, a relatively long sampling time of 2 min was chosen to ensure complete ejection of all DNA species in the capillary.

5. Concluding remarks

The migration behavior of unlabelled DNA in the presence of EOF was demonstrated in bare fused-silica capillaries, using a novel elution strategy. Similar results were seen for different types of DNA samples—ΦX174/*Hae*III DNA and the β-actin product of single-cell RT-PCR. The mechanism of elution can be dictated by either bulk flow or localized EOF, depending on the specific conditions. For continuous solutions, pH, hence bulk EOF, was shown to be the significant determinant in whether or not DNA migrated against EOF and ejected from the capillary. At an acidic pH, DNA that was injected into the TA/TA system opposed EOF, whereas that injected into an alkaline pH (TB/TB) did not elute from the capillary inlet. However, with discontinuous solutions, in which water was placed in the inlet vial rather than buffer, localized EOF present inside the capillary tip caused DNA to migrate anodically, regardless of overall pH.

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