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DNA-Driven Focusing for Protein−DNA Binding Assays Using Capillary Electrophoresis

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A DNA-driven focusing technique is reported for protein−DNA binding assays using capillary electrophoresis. A fluorescent DNA aptamer of 84 nucleotides (RT12) was used to bind to a specific protein, human immunodeficiency virus type 1 reverse transcriptase. The aptamer−protein complexes were effectively focused, separated by capillary electrophoresis, and detected by laser-induced fluorescence (LIF). With this DNA-driven focusing, the separation efficiency of the aptamer−protein complex reached 5 million theoretical plates/m, and the sensitivity for the detection of this complex was improved by 70−120-fold. The DNA-driven focusing technique was further applied to protein−DNA binding assays and to enhance the detection of DNA adducts. DNA adducts present in short oligonucleotides or genomic DNA were recognized by and bound to specific antibodies, and the complexes were focused electrophoretically and detected by LIF. The results demonstrate that the DNA-driven focusing can improve separation, sensitivity, and speed of analysis. The focusing is tolerant to high-salt medium, which is usually necessary to support physiological protein−DNA binding. This technique may be applied to nucleic acid analysis, aptamer affinity analysis, immunoassays for DNA damage, and DNA/RNA based binding assays.

Capillary electrophoresis (CE) generally has high efficiency and high speed for analytical separation. Application of CE to protein analysis, however, often suffers from protein adsorption and peak broadening.[1−3] The objective of this work is to improve protein binding assays by focusing proteins after their binding to highly charged molecules. We report here a novel DNA-driven focusing approach, using aptamer binding to protein and antibody binding to DNA damage as examples.

Aptamers are specific nucleic acid molecules selected by systematic evolution of ligands by exponential enrichment (SELEX) from combinatorial libraries of random oligonucleotide sequences.[4−11] The selection is based on the ability of the specific nucleic acids to recognize ligand molecules and the subsequent amplification of the bound nucleic acid sequences through polymerase chain reaction. Thus, the sequences exhibiting high affinity and specificity to target ligands are selected with increasing cycles of SELEX. The resulting affinity expands over several orders of magnitude with dissociation constants as low as 1 pM.[4] The specificity of aptamers results from the key molecular interactions between aptamer−ligand complex, including the precise stacking of flat moieties, specific hydrogen bonding, and molecular shape complementarity (e.g., encapsulation).[12] Aptamers have been demonstrated in a wide range of analytical applications.[13−25] The recent developments in the use of expanded libraries containing modified bases and riboses,[26] the automated SELEX,[27,28] and the capillary electrophoresis-based SELEX (CE-SELEX)[15,29] further provide the possibility to produce aptamers in high quality and large number. Encouraged by the rapid development of aptamers, we further demonstrate that an aptamer can serve as a focusing engine to focus proteins and to improve protein analysis by CE.

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We took advantage of the two unique properties of aptamers, highly negative charges and high affinity, to improve protein analysis. Diverse DNA molecules, which naturally possess a high density of negative charges, can be focused by field amplification stacking,[30] pH-mediated stacking,[31] and transient anion isotachophoresis (t-ITP).[32−34] Affinity binding of proteins to aptamers results in the modification of the proteins to negative charges, thereby promoting proteins (after binding to aptamers) to be focused under the same conditions as focusing of nucleic acids themselves. In this case, aptamers serve as a focusing engine to drive proteins to be focused electrophoretically. This focusing strategy is therefore termed DNA-driven focusing.

To demonstrate DNA-driven focusing, we first examined the focusing of the reverse transcriptase (RTase) protein of human immunodeficiency virus 1 (HIV-1) using a specific aptamer for this protein. HIV-1 RTase is a key diagnostic and therapeutic target of HIV-1.[35] The aptamer affinity analysis of HIV-1 RTase is greatly improved using DNA-driven focusing. With this focusing strategy, the affinity complex formation, and the CE separation of the aptamer−protein complex are carried out under the optimal conditions for each component. The sensitivity is enhanced by 70−120-fold.

We further examined the application of DNA-driven focusing to protein−DNA binding assays using DNA adducts as target analytes. DNA adducts of benzo[*a*]pyrene diol epoxide (BPDE) present in short oligonucleotides and in genomic DNA are recognized by and bound to adduct-specific antibody. The complex is focused, separated from the excess antibody, and quantified as a measure of the DNA adducts. The results demonstrate that DNA-driven focusing can improve sensitivity, separation efficiency, and speed of protein−DNA binding assays that involved from short oligonucleotides to genomic DNA. Protein−DNA binding assays are essential to studies of DNA-associated biological events, such as gene regulation, transcription, replication, repair, and rearrangement of DNA.[36−39]

EXPERIMENTAL SECTION

Reagents. A single-stranded DNA aptamer for HIV-1 reverse transcriptase, RT12,[35] with a sequence of 5′-ATC TAC TGG ATT AGC GAT ACT CGA TTA GGT CCC CTG CCG CTA AAC CAT ACC GCG GTA ACT TGA GCA AAA TCA CCA CTG CAG GGG-3′ was synthesized, labeled with 5′-carboxyfluorescein (FAM), and purified at the University Core DNA Services, University of Calgary, Canada. Recombinant HIV-1 reverse transcriptase was obtained from Worthington Biochemicals (Lakewood, NJ).

The 16- and 90-mer oligodeoxynucleotides, each containing a single BPDE adduct, were synthesized, labeled with tetramethylrohdamine (TMR), and purified as described previously.[40,41] Genomic DNA samples containing BPDE adducts were obtained from A549 cells that were incubated with BPDE, as described previously.[42] Primary antibody (mouse monoclonal anti-BPDE-dG antibody, 8E11) was purchased from BD Pharmingen (San Diego, CA). Goat anti-mouse secondary antibody fragment (Fab) with Alexa Fluor 546 label was purchased from Molecular Probes (Eugene, OR). Fused-silica capillaries (20-μm i.d., 150-μm o.d.) were obtained from Polymicro Technologies (Phoenix, AZ). Solvents and other biochemicals were supplied by Sigma (Oakville, ON, Canada) and Fisher Scientific (Nepean, ON, Canada).

CE-LIF. Laboratory-built CE systems with laser-induced fluorescence (LIF)[42,43] and with laser-induced fluorescence polarization (LIFP)[39,44,45] detectors were used. The TMR-labeled and Alexa Fluor 546-labeled fluorescent species were excited with a 543.5-nm helium−neon green laser of 5 mW (Melles Griot, Irvine, CA), and the fluorescence was detected at 580 nm with a photomultiplier tube (PMT) (R1477, Hamamatsu Photonics). The 5′-FAM-labeled aptamer and its protein complexes were excited with a 488-nm argon ion laser of 5 mW (model 2214-65, Uniphase, San Jose, CA). Fluorescence intensities of vertical and horizontal polarizations were split with a polarizing beam splitter (Melles Griot, Nepean, Canada) and separately detected with two PMTs (R1477, Hamamatsu Photonics). Fluorescence polarization was used as supporting information for differentiating large species from small species.

Samples were electrokinetically injected into the capillary by applying an injection voltage of 10 kV for 10 s unless otherwise stated. Uncoated capillaries (14−30-cm length, 20-μm i.d., 150-μm o.d.) were used for separation that was carried out at room temperature. A positive separation voltage of 15−20 kV was applied to the injection end of the capillary, and the detection end was grounded. After each analysis, the capillary was washed with 0.02 M NaOH for 7 min, followed by water for 2 min and running buffer for 7 min by applying 15−20 kV. All capillary electrophoresis data was analyzed using Igor Pro software (version 3.1, WaveMetrics Inc., Lake Oswego, OR).

The sample and running buffers were modified from conventional CE buffer of 1 × TG, pH 8.3 (25 mM Tris, 192 mM glycine) by adjusting the pH to below 8.3 using acetic acid titration or to pH above 8.3 using Tris titration. The final buffer was appropriately diluted by Ultrapure water to maintain a consistent specific conductivity between the buffers. These buffers are referred to as 1 × TGA or 1 × TG, respectively. TGA buffers of varying pH were prepared by adjusting the pH of 1 × TGA with either additional acetic acid or Tris.

Aptamer-Driven Focusing of HIV-1 RTase Complexes of Aptamer. Aptamer RT12 is fluorescently labeled with FAM to

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allow for fluorescence detection. HIV-1 RTase protein (5 nM) and RT12 aptamer (11 nM) were mixed in a buffer of 2 × TGA, pH 7.5 (14 mM Tris, 108 mM glycine, and 10 mM acetic acid) and 200 μg/mL human IgG and incubated for 10 min at room temperature. The addition of human IgG was to stabilize the protein (HIV-1 RTase) and its complexes with RT12. No complex of IgG with RT12 or HIV-1 RTase was observed. The sample mixture was analyzed using CE-LIFP, and the fluorescent species were the RT12 and its complex with HIV-1 RTase. Optimum CE running buffer for the aptamer-driven focusing was 1 × TG at pH 8.5 (30 mM Tris and 160 mM glycine). For comparison, 2 × TGA (pH 7.5), 1 × TGA (pH 8.2), and 1 × TG (pH 9.2) buffers were also tested, which were not suitable for aptamer-driven focusing.

Focusing of Antibody Complex of BPDE−DNA Adducts. Mouse monoclonal anti-BPDE antibody 8E11 was used as a primary antibody to bind with BPDE−DNA adducts in DNA. Alexa Fluor 546-labeled fragment of goat anti-mouse IgG was used as a secondary antibody to allow for fluorescence detection. DNA samples containing BPDE adducts were denatured by heating at 95 °C for 5 min followed by chilling on ice for 15 min to prevent DNA from reannealing. A denatured DNA sample (80 μg/mL DNA) was incubated with 2.0 μg/mL primary antibody, 2.0 μg/mL secondary antibody, and 30.0 μg/mL human IgG in either 2 × TGA or 1 × TGA buffer at pH 7.5 (7 mM Tris, 54 mM glycine, and 5 mM acetic acid) for 30 min at room temperature. The addition of human IgG was to stabilize the antibodies and their complexes.[46] The sample mixture was analyzed using CE-LIF, and the fluorescent species were the secondary antibody, the complex of the secondary antibody and the primary antibody, and the complex of DNA with the primary antibody that bound to the secondary antibody. To optimize the focusing conditions, CE running buffers of 1 × TG of varied pH (from 8.3 to 9.2) were tested. The optimum DNA-driven focusing was achieved with a CE running buffer of 1 × TG, pH 8.5.

Fast Analysis of BPDE−DNA Adducts. A denatured DNA sample, primary antibody, and secondary antibody were prepared in 1 × TGA at pH 7.5 and incubated at room temperature for 30 min, as described above. A short capillary (16-cm total length, 14 cm to the detection window) was used for CE separation. The sample was electrokinetically injected into the uncoated fused-silica capillary (i.d. 20 μm, o.d. 150 μm) by applying 10 kV for 3 s. Separation was carried out at 18 kV (1120 V/cm) using 1 × TG, pH 8.5 (30 mM Tris and 160 mM glycine) as the running buffer. The complex corresponding to the DNA adducts migrated out at 24 s.

Focusing of antibody using short oligonucleotides. Two fluorescently labeled single-stranded oligonucleotides, 16 and 90 mer, each containing a single BPDE adduct, were used to bind with mouse monoclonal anti-BPDE antibody (8E11). The mixtures of antibody and oligonucleotides in either 1 × TGA, pH 7.5 or 1 × TG, pH 8.5 were incubated for 30 min at room temperature, and then subject to CE-LIF analysis. Two CE running buffers, 1 × TGA, pH 7.5, and 1 × TG, pH 8.5, were used for comparison.

In a separate experiment, a fluorescently labeled secondary antibody was tested for focusing under the same CE conditions as described for the antibody complex with the short oligonucleotides.

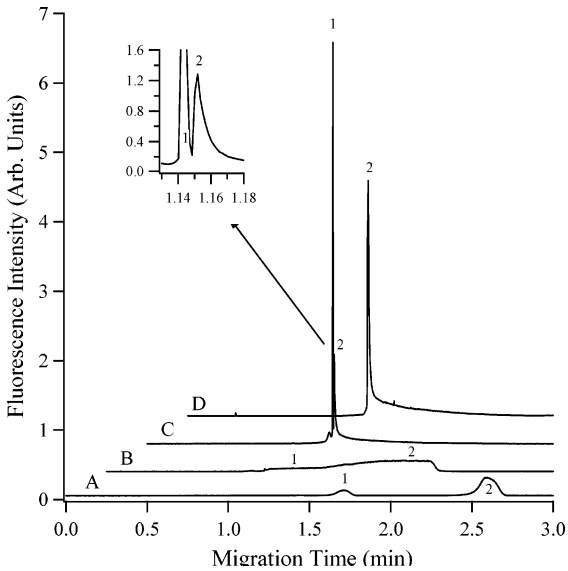

Figure 1. Electropherograms from CE-LIFP analysis of a mixture containing 5 nM HIV-1 RTase, 11 nM aptamer (RT-12), and 200 μg/mL human IgG in 2 × TGA (pH 7.5) buffer. The human IgG was added to stabilize the aptamer−protein complex. The mixture was incubated at room temperature for 30 min prior to CE-LIF analysis. Running buffers were (A) 2 × TGA, pH 7.5, (B) 1 × TGA, pH 8.2, (C) 1 × TG, pH 8.5, and (D) 1 × TG, pH 9.2. The samples were electrokinetically injected into the uncoated fused-silica capillary (30-cm length, 20-μm i.d., 150-μm o.d.) by applying 10 kV for 10 s. Electrophoretic separation was carried out at 20 kV. Fluorescence (λ_{ex} = 488 nm and λ_{em} = 515 nm) from the 5′-carboxyfluorescien-labeled aptamer (peak 2) and its complex with the protein (peak 1) was detected using a fluorescence polarization detector. Only vertically polarized fluorescence was shown.

RESULTS AND DISCUSSION

We demonstrate the DNA-driven focusing using two protein−DNA binding systems. The first uses an aptamer to focus a protein, RTase, and the second involves antibody binding to DNA adducts.

Figure 1 demonstrates that a DNA aptamer (RT12) enables focusing and determination of a specific protein (HIV-1 RTase). These electropherograms were obtained from CE-LIF analysis of a mixture of RT12 aptamer and HIV-1 RTase protein under different CE conditions that facilitate either focusing (Figure 1C) or no focusing (Figure 1A and 1B). With an appropriate focusing (Figure 1C), both the free (unbound) aptamer (peak 2) and the aptamer−protein complex (peak 1) are focused to narrow peaks and are well separated from each other (see inset of Figure 1). Without appropriate focusing (Figure 1A and B), however, the two peaks are much dispersed. To achieve the focusing of the aptamer and its protein complex, we use 2 × Tris−glycine acetate (2 × TGA, pH 7.5) as the sample buffer and 1 × Tris−glycine (1 × TG, pH 8.5) as the running buffer. An anion of much higher electrophoretic mobility (acetate, $\mu = -42.7 \times 10^{-5}$ cm^2 V^{-1} s^{-1})[47] in the sample and an anion of lower electrophoretic mobility (glycine, $\mu = -1.9 \times 10^{-5}$ cm^2 V^{-1} s^{-1} at pH 8.5, estimated from the reported data[47]) in the running buffer lead to conditions for DNA-driven focusing of a protein−aptamer complex that has a mobility between acetate and glycine. This process is similar to t-ITP[48,49] for DNA.

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No focusing was observed when the sample buffer and the running buffer were the same (1 × TGA, pH 7.5) (Figure 1A). Simply changing pH (7.5−8.5) of the TGA buffers for the sample and CE separation did not improve focusing, suggesting that the use of an anion of higher electrophoretic mobility in the sample is essential to the focusing of aptamer and its specific protein complex. Further increase in pH of the running buffer to 9.2 caused dissociation of the aptamer−protein complex as only the free aptamer was observed (Figure 1D).

LIFP measurements provided support for the identities of the protein−aptamer complex and the unbound aptamer peaks in the capillary electropherograms. Polarization values (*P*) for aptamer and its complex were 0.080 and 0.126, respectively, consistent with their molecular size.

Under the optimal focusing conditions (Figure 1C), the peak intensity of the aptamer−protein complex increased by 70−120-fold over the nonfocusing conditions (Figure 1A and 1B). The limit of detection for HIV-1 RTase is below 1 pM, estimated from the signal-to-noise ratio of 3. As a result of the focusing, the separation efficiency of the complex approached to 5×10^6 theoretical plates/m (Figure 1C), an improvement of 3 orders of magnitude over the conventional approach (no focusing condition) (Figure 1A).

The principle and benefit of the DNA-driven focusing were further applied to developing CE-LIF immunoassays for DNA damage. To selectively determine trace levels of damaged bases within a large DNA molecule of excess normal bases, a specific primary antibody was used to recognize and bind to the specific damage in the DNA molecule. A fluorescently labeled secondary antibody was used to bind with the primary antibody, so that the fluorescent ternary complex of the two antibodies with the DNA damage can be detected with highly sensitive LIF.[42] Figure 2 shows electropherograms from CE-LIF analysis of samples containing BPDE−DNA adducts, primary antibody, and secondary antibody. A significant focusing of the DNA−antibody complex (peak 2) results in an increase in the peak intensity by over 30-fold and the corresponding separation efficiency by 2 orders of magnitude (4.5×10^6 theoretical plates/m) (Figure 2B). Without focusing, the peak of the DNA adduct with antibody complex (peak 2) is much broader (Figure 2A and C), and the separation efficiencies are below 1×10^4 theoretical plates/m.

To optimize the DNA-driven focusing for the detection of DNA adducts, we also examined the use of 1 × TG running buffers of varied pH (from 8. 3 to 9.2) and the constant sample buffer (1 × TGA, pH 7.5). The separation efficiency of the DNA adduct complex with the two antibodies are above 3×10^6 theoretical plates/m, suggesting an efficient focusing of the complex. These results indicate that the DNA-driven focusing is not governed by the pH of the running buffer, although the DNA adduct complex with two antibodies can be dissociated at higher pH. The relative intensity of the DNA adduct complex with two antibodies is only 12% when the CE buffer pH is 9.2 as compared to that obtained under the optimum pH (pH 8.5). The optimum running buffer is 1 × TG at pH 8.5 (Figure 2B).

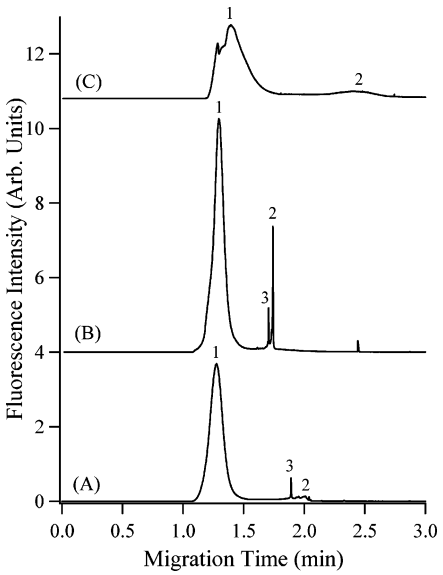

Figure 2. Electropherograms from CE-LIF analysis of BPDE−DNA adducts, showing focusing of the complex (peak 2) between the DNA and antibodies under the DNA-driven focusing conditions (B). Denatured genomic DNA (80 μg/mL) containing BPDE adducts, a primary mouse monoclonal antibody (2 μg/mL), a fluorescently labeled secondary antibody fragment (Fab) (2 μg/mL) and nonspecific human IgG (30 μg/mL) were incubated at room temperature for 30 min in (A) 1 × TG, pH8.5, (B) 1 × TGA, pH 7.5, and (C) 1 × TGA, pH 7.5. Corresponding CE running buffers were (A) 1 × TG, pH8.5, (B) 1 × TG, pH 8.5, and (C) 1 × TGA, pH 7.5. The samples were electrokinetically injected into the uncoated fused-silica capillary (30-cm length, 20-μm i.d., 150-μm o.d.) by applying 10 kV for 10 s. Electrophoretic separation was carried out at 20 kV. Laser-induced fluorescence (λ_{ex} = 543.5 nm and λ_{em} = 580 nm) was detected from the secondary antibody fragment that was labeled with Alexa Fluor 546. Peak 1 represents the secondary antibody and its complex with the primary antibody, which overlapped. Peak 2 is the immunocomplex of BPDE−DNA adducts with the primary antibody that bound to the secondary antibody. Peak 3 is an impurity from the secondary antibody.

To examine the effect of various sizes (and charges) of DNA on the DNA-driven focusing, we tested the use of shorter oligonucleotides, 16 and 90 mer. These oligonucleotides were synthesized with a single BPDE lesion in the middle of the chain and were fluorescently labeled at the 5′ end with TMR. Their complexes with mouse monoclonal antibody against BPDE (MAb 8E11) were monitored to test their effect on the focusing behavior of the protein (antibody). The number of theoretical plates was determined as a measure of focusing. Figure 3 shows that the extent of focusing of DNA-antibody complex increases with the length of oligonucleotide, from 16 to 90 mer. For the complex of one antibody and one 90 mer, the number of theoretical plates is 2×10^5, representing a 12- or 18-fold improvement over the nonfocusing conditions. It is interesting that even a short 16-mer oligonucleotide (4.8 kDa) is able to drive a much larger IgG antibody (156 kDa) to be focused. These results demonstrate that DNA makes the major contribution to the unique focusing of the DNA−protein complex.

Table 1 compares the number of theoretical plates for antibody (Ab) alone, Ab(90 mer) complex, and Ab−genomic DNA complex under the three sets of conditions. With an appropriate focusing, the separation efficiencies for the Ab(90 mer) complex and Ab−genomic DNA complex are significantly improved. In contrast,

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Table 1. Comparison of Separation Efficiencies (Theoretical Plates per Meter) for a Primary Antibody, Complex of the Antibody with BPDE−90 Mer DNA, and the Tertiary Complex of Secondary Antibody (2°Ab) with the Primary Antibody (1°Ab) and the BPDE Adducts in Genomic DNA[a]

sample buffer	1 × TGA, pH 7.5	1 × TG, pH 8.5	1 × TGA, pH 7.5
CE running buffer	1 × TGA, pH 7.5	1 × TG, pH 8.5	1 × TG, pH 8.5
antibody	6.9×10^2	2.1×10^3	3.8×10^3
antibody-90 mer	1.5×10^4	1.0×10^4	1.8×10^5
2°Ab−1°Ab-genomic DNA	1.2×10^3	9.2×10^3	4.5×10^6

[a] The focusing of antibody was negligible whereas the focusing for the complex of antibody with DNA is significant, indicating the DNA-driven focusing.

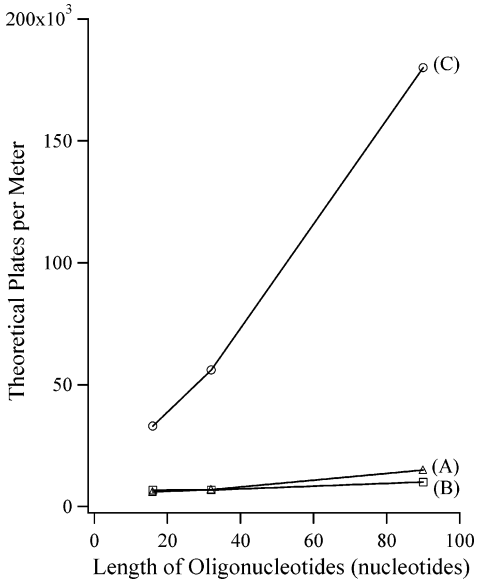

Figure 3. Focusing of antibody complex using BPDE−16 mer and BPDE−90 mer oligonucleotides. (A) Both sample buffer and CE running buffer were 1 × TGA, pH 7.5. No clear focusing. (B) Both sample buffer and CE running buffer were 1 × TG, pH 8.5. No clear focusing. (C) Sample buffer was 1 × TGA, pH 7.5 and CE running buffer was 1 × TG, pH 8.5. As a result of the DNA-driven focusing, separation efficiency of the antibody-DNA complex increases with the length of DNA. The complex containing 32 bases results from the binding of the antibody to two 16 mer, $Ab(16\ mer)_2$ (1:2 stoichiometry). Other conditions for CE-LIF analysis were the same as described in Figure 2.

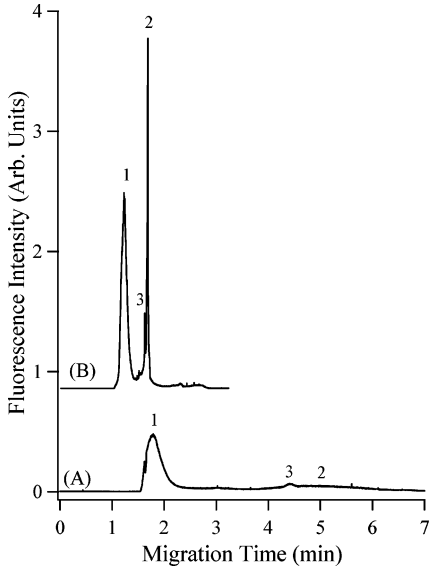

Figure 4. Electropherograms from CE-LIF analysis of BPDE−DNA adducts, showing the separation and focusing of the antibody complex with DNA adduct. The salt content in the sample was twice that shown in Figure 2. The CE-LIF analysis was carried out under the following conditions: (A) Both sample and CE running buffers were 2 × TGA, pH 7.5; (B) sample was prepared in 2 × TGA, pH 7.5, and the CE running buffer was 1 × TG, pH8.5. Peak 1 represents the secondary antibody and its complex with the primary antibody, which overlapped. Peak 2 is the immunocomplex of BPDE−DNA adducts with the primary antibody that bound to the secondary antibody. Peak 3 is an impurity from the secondary antibody.

the unbound antibody displays poor separation efficiency (<4000 theoretical plates/m) under the tested conditions. Only a slight improvement (3−6-fold) in separation efficiency is achieved with the use of a running buffer of higher pH (1 × TG at pH 8.5). This is mainly due to the reduced adsorption of antibody on the capillary wall and not to the focusing. These results further suggest that DNA plays an important role in the DNA-driven focusing of the proteins.

The DNA-driven focusing can tolerate high-salt content in the sample. Thus, it overcomes the common problem of peak distortion and broadening in CE when biological samples have higher conductivity than the running buffer. This benefit can be seen from the DNA-driven focusing of the aptamer−protein complex (Figure 1C as compared to Figure 1B). It is further demonstrated from the focusing of a large DNA adduct with antibody complex in the sample buffer of high-salt content (Figure 4). With a higher salt content in the sample than that in the running buffer, the large DNA adduct complex is more efficiently focused (Figure 4B). The peak intensity of the DNA adduct complex (peak 2) is increased by 70-fold, and the separation efficiency is 6.2×10^5 theoretical plates/m. In addition, the complex signal (relative to fluorescently labeled secondary antibody, peak 1) is 3-fold higher than that from focusing under the same conductivity (Figure 2B). The improvement seen in Figure 4B over that in Figure 2B is partially because the buffer containing high-salt content at near physiological pH promotes the complex formation of the DNA adduct with the antibodies. As a result of the improved sensitivity, we are able to achieve a detection limit of 1 pM or 3×10^{-21} mol for BPDE−DNA adducts. Focusing of the protein−DNA complex at high-salt content is a unique advantage. Since most noncovalent binding under the physiological conditions occur in the high-salt environment, this technique is particularly useful for the study of protein−DNA binding under biological relevant conditions.

In addition to the improvement in separation efficiency and sensitivity, the DNA-driven focusing also speeds up analysis. Because of the improved separation efficiency, a short capillary

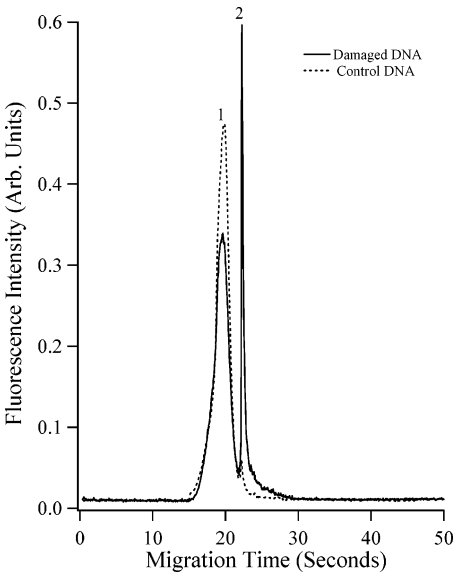

Figure 5. Fast analysis of BPDE−DNA adducts by using capillary electrophoresis immunoassay combined with laser-induced fluorescence detection. The capillary used was 16 cm long with a detection window 14 cm away from injection (positive) end. The samples were electrokinetically injected by applying 10 kV for 3 s and separated by applying 18 kV. The sample buffer was 1 × TGA at pH 7.5, and the CE running buffer was 1 × TG at pH 8.5. Other CE-LIF conditions and peak representation were the same as shown in Figure 2. The dashed trace represents the analysis of a DNA sample from the control cells in which no BPDE−DNA adduct is detectable. The solid trace represents the analysis of cellular DNA of treated cells, and peak 2 represents the BPDE−DNA adducts present in the sample.

(16 cm) is sufficient for the separation. Figure 5 shows rapid analysis of BPDE−DNA adducts within 30 s. To our knowledge, this is the fastest assay for DNA damage. This is only possible because of the improved separation efficiency due to the effective focusing of the antibody complex with the DNA adducts. The corresponding antibody−DNA adduct peak (peak 2) has the column efficiency of 4.4×10^5 theoretical plates/m. The fast analysis (30 s) greatly improves the throughput of CE analysis of DNA damage, which is a bottleneck of the current DNA damage assays.

In summary, we have demonstrated that the DNA-driven focusing technique greatly enhanced the sensitivity of immunoassays of DNA adduct (30−70-fold) and CE analysis of proteins using affinity aptamers (70−120-fold). Separation efficiency, as determined by the number of theoretical plates, was improved by as much as 3 orders of magnitude. The dramatically improved separation efficiency enabled rapid analysis (30 s) using short capillaries. The DNA-driven focusing can tolerate high-salt content in the sample, which is particular useful for the analysis of biological samples. Both small oligonucleotides (16 mer, 90 mer, and aptamer) and large DNA (cellular DNA) are able to facilitate electrophoretic focusing of proteins.

In the DNA-driven focusing strategy, DNA is the essential binding partner and functions as an engine to drive the whole complex to be focused. The key to the focusing is to establish electrophoresis conditions under which the mobility of the DNA−protein complex is between those of the electrolytes (anions) in the sample and in the running buffer. This can be readily achieved by using different anions in the sample and in the running buffer, which have different mobilities. TG is an appropriate running buffer because glycine has a very low electrophoretic mobility. At pH 8.5, the electrophoretic mobility of glycine is ~20-fold lower than that of acetate. To achieve the focusing, the anion of higher electrophoretic mobility (e.g., acetate) was used in the sample buffer (TGA). In additional to TGA, the use of phosphate, Tris-HCl, and Tris-acetate as sample buffers was also successful to achieve DNA-driven focusing (data not shown).

The DNA-driven focusing is different from the dynamic pH junction technique.[50] Focusing cannot occur if the running buffer and the sample buffer have the same anion of higher electrophoretic mobility. While the pH of the CE running buffer is critical to the pH junction technique, it does not significantly affect the DNA-driven focusing. The DNA-driven focusing can be achieved at the same pH for both the sample buffer and the running buffer.

The DNA-driven focusing technique described here is applicable to detection of various DNA damage and DNA−protein binding assays. DNA aptamers are particularly useful to facilitate DNA−protein binding assays. Aptamers of high affinity to a variety of target compounds can be obtained by the SELEX process. Automated and improved aptamer selection processes have potential to produce the desired aptamers at faster speed.[15,28] Thus, the DNA-driven focusing using aptamers has potential applications to a wide range of compounds.

The principle of the DNA-driven focusing technique is not limited to the use of DNA and aptamer as the focusing engine. Other molecules (e.g., antibodies and antigens) can be modified with highly charged moieties (charge modulators).[51−53] Affinity electrophoresis assays making use of charge modulators may also benefit from the DNA-driven focusing strategy. Improvement in separation efficiency, sensitivity, and speed of analysis make the technique potentially useful for a range of bioanalytical applications.

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