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Separation of Nontarget Compounds by DNA Aptamers

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The ability of DNA aptamers to separate nontarget compounds is demonstrated. Two G-quartet forming aptamers, a 15-mer and a 20-mer, were covalently linked to fused silica capillary columns to serve as stationary-phase reagents in capillary electrochromatography. Separations of binary mixtures of amino acids (D-trp and D-tyr), enantiomers (D-trp and L-trp), and polycyclic aromatic hydrocarbons were achieved. Aptamers offer several attractive features for stationary-phase reagents, including ease of synthesis and of attachment to surfaces and modification of their binding properties through minor changes in sequence.

Aptamers are oligonucleotides, often single stranded, which are selected through combinatorial libraries to bind with high affinity to target molecules or macromolecules. An outgrowth of pharmaceutical and medicinal research, applications of aptamers have been primarily limited to specific measurement or inhibition of their designated target molecules for therapeutic purposes. Analytical applications of these highly selective binding reagents are relatively unexplored.[1] Isolation of target compounds from complex samples and chemical sensing of target compounds are straightforward applications of aptamers. Recent work in chemical analysis has, quite reasonably, involved the binding of aptamers to their target compounds.[2,3] However, the structural motifs of aptamers, that provide very specific binding of designated target molecules, may also show more general ranges of selectivity and recognition for more diverse groups of molecules, which could be used to generate new methods for chemical separations.

Aptamers offer important features that, in combination, set these compounds apart from other stationary-phase reagents for chemical separations. They are reproducibly and accurately synthesized in a short time by automated processes, and custom synthesis is available at low cost from commercial vendors. They are easily modified through chemical methods to increase stability and to facilitate covalent attachment to surfaces. Their relatively small size should reduce steric hindrance to increase surface coverage, and their conformational stability serves to maintain their selectivity upon attachment to a surface. Perhaps most importantly, the ease with which their sequence can be modified provides a simple means for changing their structure and conformation in order to alter substantially their binding selectivity.

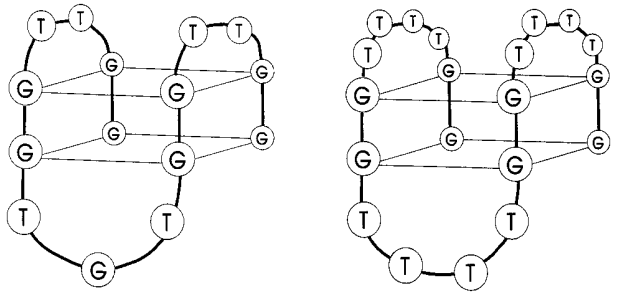

Figure 1. Structure of aptamer **1** (left) and inferred structure of aptamer **2** (right).

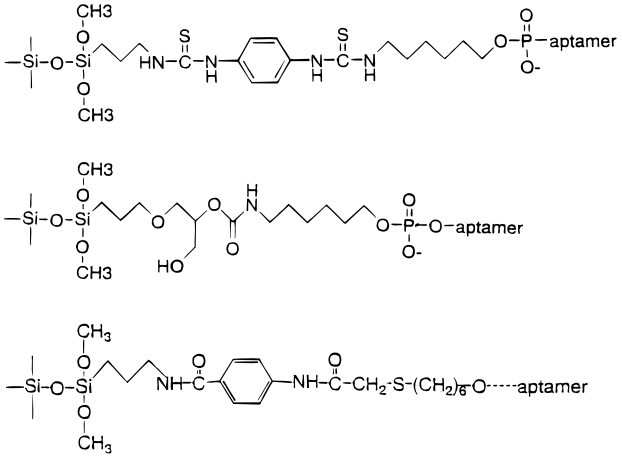

Figure 2. Structures of (top to bottom) linker 1[7], linker 2,[2] and linker 3.[8]

We report here the first demonstration of separations of nontarget compounds using aptamers. DNA aptamers that form the G-quartet structure were covalently attached to fused-silica capillaries and used in capillary electrochromatography (CEC) to separate binary mixtures of amino acids (D-trp and D-tyr), enantiomers (D-trp and L-trp), and polycyclic aromatic hydrocarbons (naphthalene and phenanthrene, benzo[*a*]pyrene, or benzo[ghi]perylene). Two related aptamers were used: a G-quartet-forming 15-mer (5′-GGTTGGTGTGGTTGG-3′), referred to as **1**, which was selected through combinatorial methodology for

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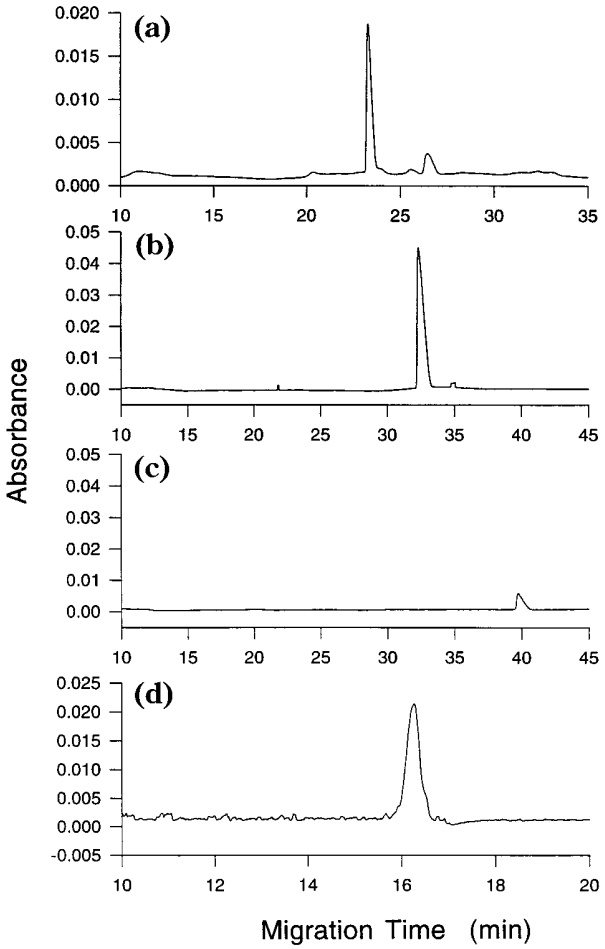

Figure 3. (a–c) CEC of amino acids using a capillary coated with aptamer **2**: (a) 2 mM D-trp and 1.5 mM D-tyr; (b) 4 mM D-trp; (c) 3 mM D-tyr. (d) CEC of the same mixture as in (a) but performed using a bare capillary. EOF values were typically around 0.77 mm/s.

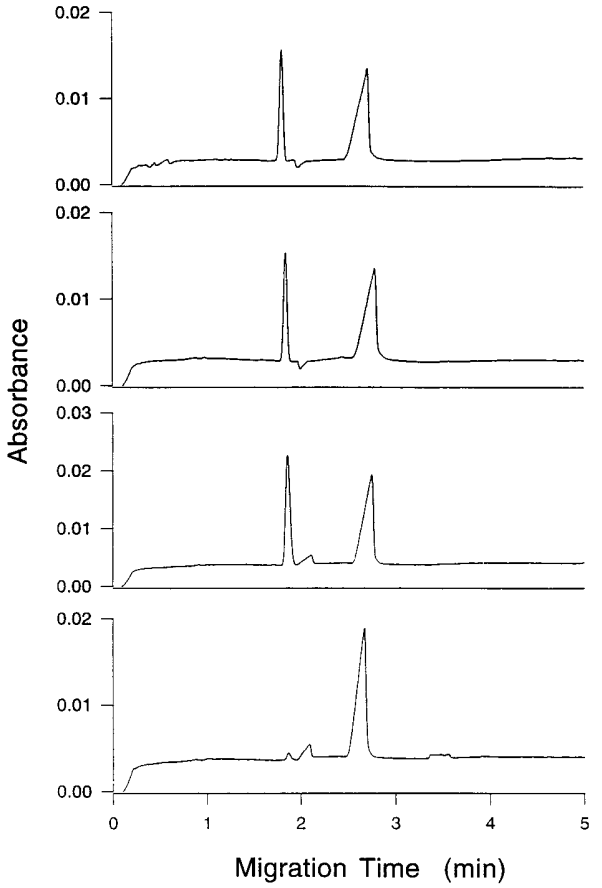

Figure 4. CEC of enantiomers using a capillary coated with aptamer **2.** From top to bottom: 0.5 mM D-trp and 0.5 mM L-trp; repeat run of same mixture; 0.5 mM D-trp and 0.1 mM L-trp; 0.1 mM D-trp and 0.5 mM L-trp. The origin of the small, asymmetric peak right after 2 min is unknown.

binding affinity to thrombin[4] and a related 20-mer (5′-GGTTTTG-GTTTTGGTTTTGG-3′), referred to as **2**, in which the loops connecting the G-quartet were extended to four bases and contained only Ts. The three-dimensional structure of **1** has been well-described in the literature[5,6] and that of **2** was inferred from comparison of its circular dichroism spectrum to that of **1**. These structures are shown in Figure 1. For both aptamers, formation of the G-quartet requires a cation such as K^+,[5] which was therefore included in the CEC run buffer.

EXPERIMENTAL SECTION

The 5′-modified aptamers were custom synthesized by Midland Certified (Midland, Texas) and used as received. Three different linkers (Figure 2) were used to attach the aptamers to the glass capillary surface. Linker 1 was taken from the literature[7] but modified in our work to omit a sequence of 15 thymines at the aptamer attachment end. Linker 2[2] and linker 3[8] were taken directly from the literature.

The separations were performed using a Beckman P/ACE capillary electrophoresis instrument. For D-trp and D-tyr, and for the PAH mobile phase composition profiles, the dimensions of the fused-silica capillary columns were 375-μm outer diameter, 75-μm inner diameter, 47-cm total length, and 40 cm to the UV–vis absorption detector window; for the other PAH experiments the total length was 28 cm and the distance to the detector was 21 cm. In one PAH experiment, a narrower capillary, with an inner diameter of 50 μm, was used.

Samples were prepared in solutions that were the same as the mobile phase except they did not contain KCl. For separation of D-trp and D-tyr, the mobile phase was 50 mM TRIS amine buffer, pH 7.2, with 5 mM KCl. CE conditions were 15 kV, forward polarity, 5-s pressure injection of sample, $T = 16$ °C. Absorbance was monitored at 280 nm. Conditions were the same for the amino acid enantiomers except that the buffer was at pH 10.0 and the sample-injection time was 2 s. For the PAH separations, the mobile phase contained various concentrations of acetonitrile or methanol in 25 mM TRIS buffer, pH 7.3, with 1 mM KCl. CE conditions

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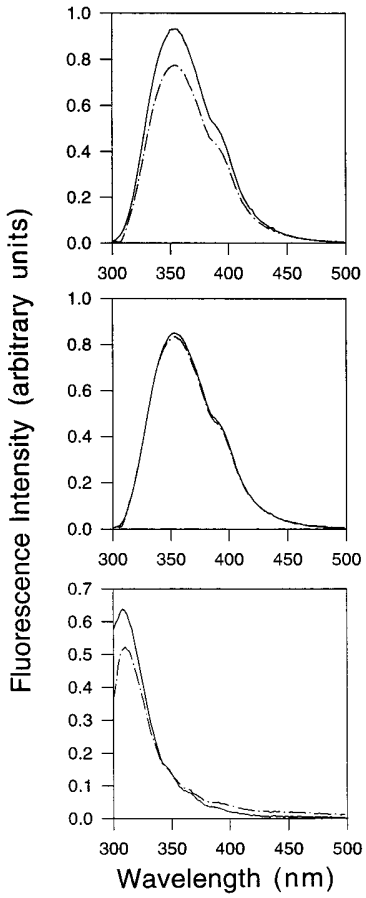

Figure 5. Fluorescence emission spectra of (a) L-trp, (b) D-trp, and (c) D-tyr, in the absence (—) and presence (-·-) of aptamer **2**. The samples contain 1 μM each of amino acid and aptamer in phosphate-buffered saline at pH 7.4.

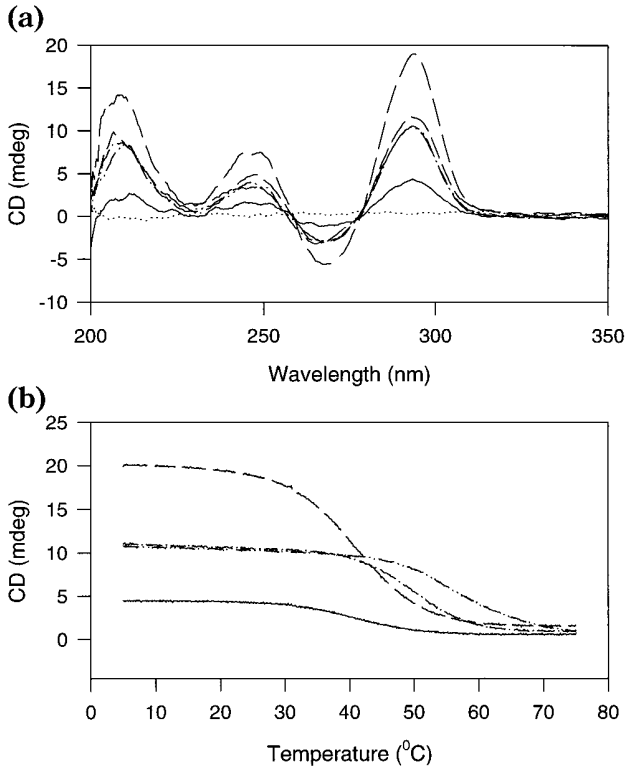

Figure 6. (a) Circular dichroism spectra of aptamer **1** in 0% ACN (—), 1% ACN (— —), 5% ACN (-—-), 30% ACN (-·-), 70% ACN (-···). A blank containing buffer only is also shown (···). (b) Thermal melts of aptamer **1** in 0% ACN (—), T_m = 40.9 °C; 1% ACN (— —), T_m = 41.3 °C; 30% ACN (-·-), T_m = 50 °C; 70% ACN (-···), T_m = 55.8 °C.

were 15 kV, forward polarity, 1-s pressure injection of sample, T = 15 °C. Absorbance was monitored at either 254 or 280 nm.

RESULTS AND DISCUSSION

Separation of Amino Acids and their Enantiomers. A mixture of D-trp and D-tyr was separated on a capillary that was coated with **2** using linker 1. As seen in Figure 3a, baseline separation was achieved. Figure 3 parts b and c show separate injections of D-trp and D-tyr, respectively, and Figure 3 part d shows the results for the mixture run through a bare capillary under the same conditions, which gave no separation. The retention time for the amino acids increases with each successive run due to degradation of the stationary phase over time. The attachment method utilizes phenylene diisothiocyanate (PDC) in the coupling reaction. Since PDC is known to be unstable in aqueous media, it can be assumed that, over a period of time, the aptamer becomes detached from the capillary surface due to decomposition of PDC in the aqueous mobile phase. This decreases the negative charge of the capillary surface, resulting in decreased electroosmotic flow and longer migration times.

Baseline separation of D-trp and L-trp was achieved using a capillary that was coated with **2** using linker 2 (Figure 4). When the relative amounts of the enantiomers in the mixture were changed, the peaks in the electrochromatogram changed accordingly, although, for reasons not yet understood, not in proportion to the concentration decreases. When the mixtures were injected onto a bare capillary or a capillary coated with linker but no aptamer, a single, poorly defined peak was detected, indicating that the separation is indeed due to interactions between the enantiomers and the aptamer. When pressure was used instead of electrical potential to drive the separation under otherwise identical experimental conditions on the same coated capillary, no separation was achieved, indicating that the applied potential serves in some way to facilitate selective interactions between the aptamer and the enantiomers. The mechanism of this effect has not yet been studied.

The CEC results for the amino acids are consistent with the results of batch fluorescence studies (Figure 5), in which **2** decreased the fluorescence emission of L-trp and D-tyr but not of D-trp, suggesting greater interaction between the aptamer and L-trp and D-tyr as compared with D-trp. Thus, both D-tyr and L-trp migrate slower than D-trp, which explains the retention orders in Figures 3 and 4.

Separation of PAHs. Since PAHs have very low aqueous solubility, it is desirable to perform the PAH separations using mixed aqueous/organic mobile phases. Toward this end, it was necessary first to study the effects of organic solvents on the G-quartet conformation. Aptamer **1** was used instead of **2** in these studies because thermal melt experiments showed that the conformation of **1** is significantly more stable than that of **2**. Thermal melts were performed using absorption CD to monitor the G-quartet conformation in mixtures of buffer with various concentrations of acetonitrile or methanol. The results for aceto-

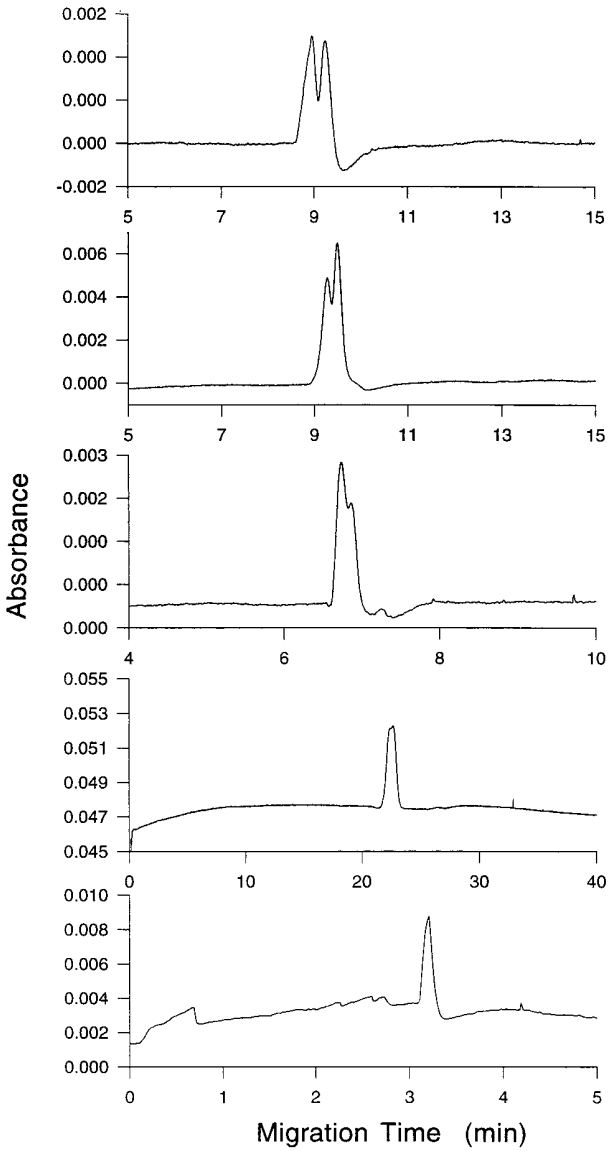

Figure 7. Separation of mixtures of naphthalene and BaP using a 75-μm i.d. capillary. From top to bottom: 0.5 mM naphthalene and 0.05 mM BaP using a capillary coated with aptamer **1**; 0.3 mM naphthalene and 0.05 mM BaP using a capillary coated with aptamer **1**; 0.5 mM naphthalene and 0.03 mM BaP using a capillary coated with aptamer **1**; 0.5 mM naphthalene and 0.05 mM BaP using a capillary coated with linker but no aptamer; 0.5 mM naphthalene and 0.05 mM BaP using a bare capillary. When separation occurred, naphthalene eluted first. EOF values were typically around 0.32 mm/s.

nitrile are shown in Figure 6. Surprisingly, the G-quartet conformation not only remains intact in the presence of either organic solvent up to as high as 70% v/v but also is stabilized by acetonitrile as indicated by an increase in melting temperature of the G-quartet conformation from 41 °C in the absence of ACN to 56 °C in 70% (v/v) ACN.

CEC separations were repeated 2−4 times for each PAH mixture. In all cases, elution order of the components in a mixture was the same for all replicates of that mixture. Figure 7 shows CEC runs of a mixture of naphthalene and benzo[*a*]pyrene (BaP) on a capillary coated with **1** using linker 1 and on a bare capillary and a capillary coated with linker but no aptamer. The mobile

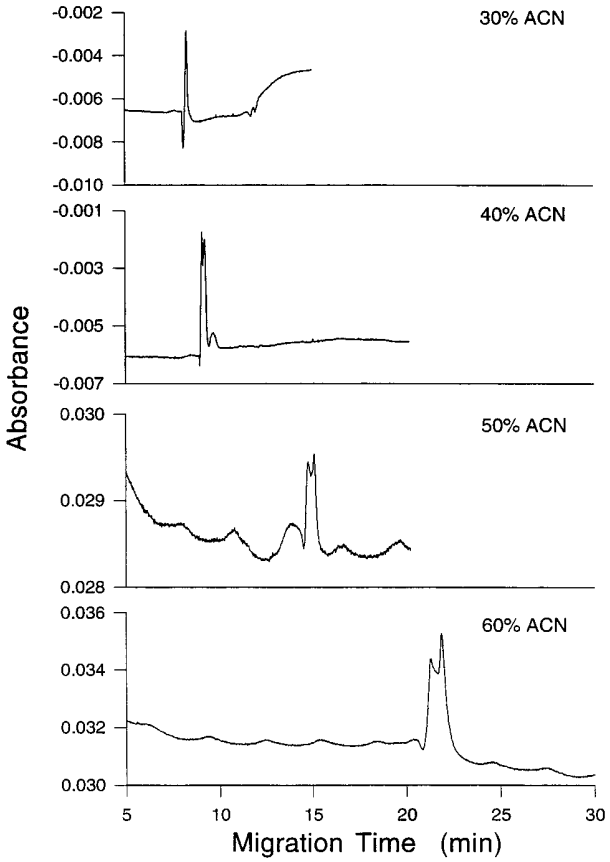

Figure 8. Separation of mixtures of naphthalene and BgP using a 75-μm i.d. capillary coated with aptamer **1**. From top to bottom: 0.02 mM naphthalene and 0.028 mM BgP; 0.08 mM naphthalene and 0.032 mM BgP; 0.01 mM of both; 0.001 mM naphthalene and 0.005 mM BgP. EOF values ranged from 0.89 mm/s for 30% ACN to 0.65 mm/s for 60% ACN.

phase contained 50% acetonitrile (v/v) in buffer and was used to prepare the PAH samples as well. The results show that the aptamer can provide at least partial separation between two PAHs, while no separation is achieved in the absence of aptamer. It is interesting that BaP, which is known to interact strongly with double stranded DNA, migrated slower than naphthalene on the aptamer-coated capillary.

The results for a mixture of naphthalene and benzo(ghi)-perylene (BgP) using various concentrations of ACN in the mobile phase are shown in Figure 8. In these experiments, linker 3 was used to attach **1** to the capillary surface in an attempt to improve surface coverage, since it was reported that linker 3 provided an estimated 40% surface coverage for attachment of DNA to silicon wafers.[8] Increasing the concentration of ACN from 30 to 60% (v/v) increased resolution of the two peaks, although baseline separation was not achieved. Increasing the ACN concentration to 70% ACN (not shown) degraded the coated capillary, rendering it useless.

Methanol also was tested for PAH separations. Since addition of methanol to the mobile phase decreases the electroosmotic flow, the voltage was increased to 20 kV in order to achieve reasonably short migration times. Figure 9 shows the effects of added methanol on the resolution of a naphthalene−BgP mixture. In contrast to ACN, separation improved with decreasing MeOH,

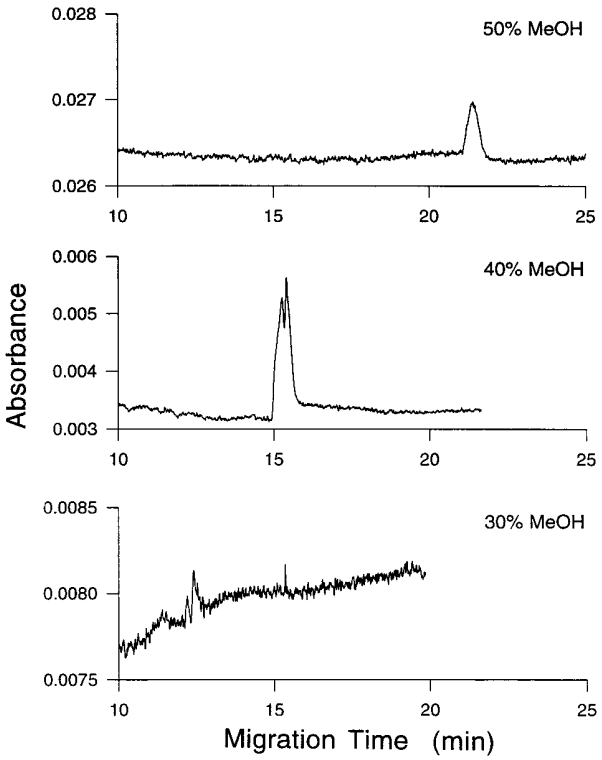

Figure 9. Separation of mixtures of naphthalene and BgP using a 75-μm i.d. capillary coated with aptamer **1**. From top to bottom: 0.01 mM naphthalene and 0.005 mM BgP; 0.001 mM of both; 0.001 mM of both. EOF values ranged from 0.49 mm/s (top) to 0.56 mm/s (bottom).

and baseline resolution was achieved at 30% (v/v) MeOH. Similarly, for a mixture of naphthalene and BaP, improvement in resolution was achieved by decreasing MeOH from 50 to 20% (v/v) (Figure 10a-d).

To evaluate the role of the G-quartet conformation in the separation, the naphthalene−BaP separation was performed in the absence of KCl in the mobile phase (Figure 10 part e). There was a drop in the baseline around 8 min at which time the sample is passing the detector but no distinct or resolved peaks were observed. It is possible that the PAH signal is obscured by the variable baseline. Nevertheless, it is clear from the differences between the run with KCl and the run without KCl, which were reproduced in replicate runs, that the K^+-dependent G-quartet structure in this aptamer plays a role in the separation of these PAHs.

We also evaluated the effect of capillary diameter on the separation. Decreased diameter should increase interactions between the PAHs and the aptamers on the capillary wall. The results in Figure 10 part f show that decreasing the internal diameter of the capillary from 75 to 50 μm improved the resolution of a naphthalene−BaP mixture. Unfortunately, this improvement was accompanied by a decrease in absorbance due to the decreased optical path, which decreases the sensitivity of the analysis. We are currently investigating the use of fluorescence detection in order to improve sensitivity and detectability.

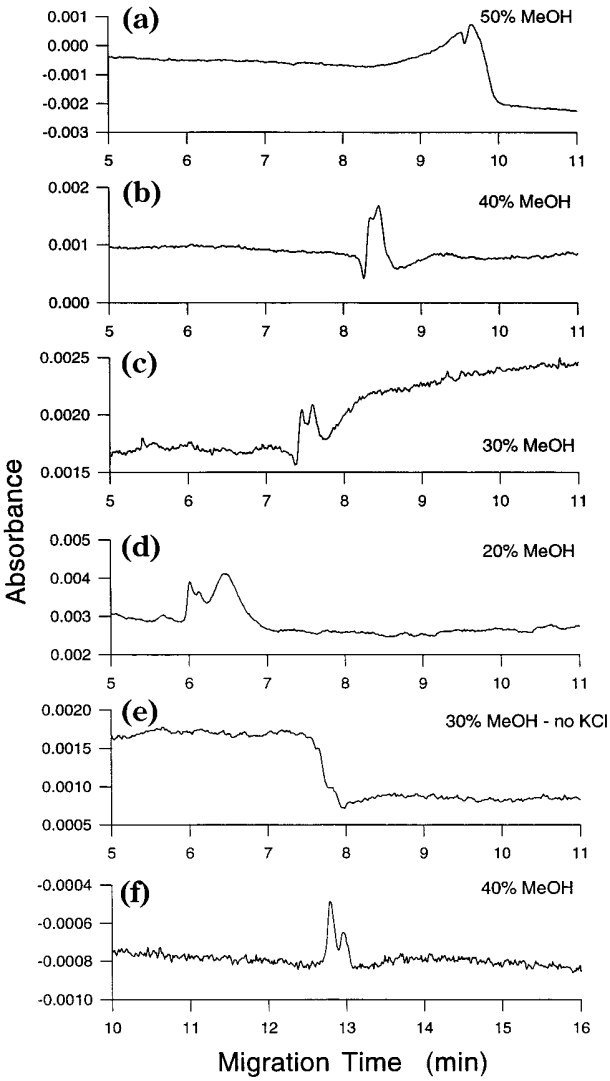

Figure 10. Separation of a mixture of naphthalene and BaP using a 75-μm i.d. (a−e) or 50-μm i.d (f) capillary coated with aptamer **1**. (a) and (b) 0.001 mM of both; (c) and (d) 0.005 mM of both; (e) 0.005 mM of both with no KCl in run buffer; (f) 0.01 mM of both. EOF values ranged from 0.69 mm/s for (a) to 1.12 mm/s for (d).

These studies demonstrate the ability of G-quartet-forming aptamers to separate nontarget compounds such as amino acids and amino acid enantiomers and to achieve partial resolution of PAHs. Further studies are underway to improve resolution through modification of experimental conditions and aptamer sequence.

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