FINAL REPORT

Title: Investigations of Single Stranded DNA Aptamers as a New Tool for Chemical Separations (DE-FG02-88ER13931)

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Research Publications

Hemmingsen, S. L. and McGown, L. B., "Phase-Resolved Fluorescence Spectral and Lifetime Characterization of Commercial Humic Substances", **Appl. Spectrosc.**, **51**, 921-929 (1997).

Kinkennon, A. E. and McGown, L. B., "Fluorescence Lifetime Characterization of Bacteria using Total Lifetime Distribution Analysis with the Maximum Entropy Method", **J. Fluorescence**, **7**, 201-210 (1997).

Simon, L. D., Abramo, K. H., Sell, J. K. and McGown, L. B., "Oxazole Yellow Dye Interactions with Short DNA Oligomers of Homogeneous Base Composition and their Hybrids", **Biospectroscopy**, **4**, 27-35 (1998).

Kotia, R. B., Li, L., McGown, L. B., "Separation of nontarget compounds by DNA aptamers", **Anal. Chem.**, **72**, 827-831 (2000).

Doctoral Dissertations Supported Wholly or in Part by DOE During this Project Period

- D1. "Fluorescence Lifetime Characterization of Bacteria using Total Lifetime Distribution Analysis", Amy E. Kinkennon, 1997.
- D2. "Fluorescence Probe Studies of Humic Substance Standard Materials", Jarafshan Mobed Mistry, 1998.
- D3. "Spectroscopic and Thermal Investigations of Short, Single-Stranded Nucleic Acid Ligands", Kimberly Harris Abramo, 1998.
- D4. "DNA Aptamers: A Novel Approach to Chemical Separation", Ruchi Bhatnagar Kotia, Duke University, 2000.

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SUMMARY OF RESEARCH RESULTS

Separation of Enantiomers

Although separation of enantiomers is unrelated to the goals of this DOE project, the results are presented here in order to support the hypothesis of this proposal that DNA aptamers are effective stationary phases for non-target compounds. The important difference between this work and previous demonstrations of enantiomers for chemical sensing and separations is that the latter used an RNA aptamer that was selected through the arduous combinatorial process specifically for D-trp over L-trp. In contrast, we used a DNA aptamer that was selected for binding to thrombin without any consideration towards enantiomeric selectivity in the selection process. Our preliminary work demonstrates that the same short, single strand of DNA can be used for separations of non-target compounds as disparate as amino acid enantiomers and PAHs. Thus, we needn't perform the selection process to identify an aptamer for each specific separation and can, in fact, bypass the lengthy and arduous process of aptamer selection altogether. Moreover, our work indicates that an aptamer can be used for separation and analysis of several components in a mixture rather than restricting its use to isolation of a single target compound.

Two related aptamers were used in our work: a 15-mer (5'-GGTTGGTGTGGTTGG-3') which was selected for thrombin affinity and a 20-mer (5'-GGTTTTGGTTTTGGTTTTGG-3') in which the loops connecting the G-quartet were extended from three to four bases and contained only Ts. The G-quartet structure of **1** has been well-described in the literature and that of **2** was inferred from comparison of its circular dichroism spectrum to that of **1**.

Excellent, baseline separation of D-trp and L-trp was achieved. When the relative amounts of the enantiomers in the mixture was changed the peaks in the electropherogram changed accordingly. When the mixtures were injected onto a bare column or a column coated with linker but no aptamer, a single, poorly defined peak was detected, indicating that the separation is due to interactions between the enantiomers and the aptamer. L-trp migrated slower than D-trp, and its peak is broader, which is consistent with batch fluorescence studies in which aptamer **1** decreased the fluorescence emission of L-trp but not of D-trp, indicating more interaction with the L enantiomer.

Separation of PAHs

Although separations were reproducible, they are the best results we were able to obtain. In general, separations were incomplete and imprecise due to column instability. We therefore tried a different linker for aptamer immobilization for the PAH studies. Also, aptamer **1** was used instead of aptamer **2** in the PAH separations because thermal melt experiments showed that the conformation of **1** is significantly more stable than **2**. CD spectra and thermal melts also showed

that the conformation of **1** remained stable in mixed solvents containing as much as 50% or more of an organic solvent such as ethanol, methanol or acetonitrile in the aqueous run buffer. This was of critical importance for separation of PAHs since most are essentially insoluble in water and would not enter the column in the absence of the organic solvent.

Separations of mixtures of naphthalene and phenanthrene, and of naphthalene and benzo[a]pyrene (B[a]P) were performed. There was a lack of resolution on bare columns and on columns coated with linker but no aptamer. Although the peaks were not as well resolved as those in the enantiomer separation, the results are qualitatively reproducible despite fluctuations in retention time. In all trials, phenanthrene migrated faster than naphthalene, while B[a]P, which is known to interact strongly with double-stranded DNA, migrated slower than naphthalene.

The results of these studies demonstrate the potential of aptamers as a new class of stationary phase reagent for chemical separation with broad applicability, well beyond simple isolation of the aptamer's target compound.

Interactions of Metals with DNA Aptamers

We performed fundamental studies of the interactions of G-quartet-forming aptamers with lanthanide and other metal ions, directed towards the use of aptamers for separations and analysis of metal ions. The DNA aptamers used in this study include **1** and **2** (see above) and a third aptamer, d(GGTTGGTTTGGTTGG), referred to as **3**. Aptamer **3** is identical to **1** except that the middle G is replaced by T in the upper loop, so that G is included only in the G-tetrads and the loops are composed entirely of T.

The G-quartet structure, which is present in aptamers that bind to thrombin, is known to be stabilized by some metal ions, particularly K^+ . It is of interest to determine whether stabilization is common to all metal ions and whether the interactions are sufficiently different among the lanthanide metals to permit their chromatographic separation using aptamer reagents. The interactions were studied using circular dichroism spectroscopy and thermal melt experiments, energy transfer, and NMR spectroscopy. The NMR experiments were designed and interpreted with the help of Professor Tom Wenzel of the Department of Chemistry at Bates College and performed at Duke.

Results show that the G-quartet forming aptamers exhibit a range of interactions among the different lanthanide metals. Lanthanide ions tend to destabilize the G-quartet conformation, particularly that of **1**, but do not directly disrupt the hydrogen bonding in the G-tetrads. Destabilization is therefore attributed to selective association of the lanthanide ions with the thymines in the bridges of the G-quartet structure. In the absence of K^+ , lanthanides destabilize the alternate aptamer conformation.

The lanthanide ions have a greater effect on the ^{31}P NMR than on the ^1H NMR of the aptamers, which indicates that they are in closer contact with the oxygen atoms in the phosphate groups than with the oxygen or nitrogen atoms of the DNA bases. This is similar to ds-DNA, in which Tb^{3+} is known to bind to the phosphate groups. In contrast, K^+ is situated between the tetrad planes and coordinated to the 8 guanines in the tetrad.

Aptamer 3 showed the most discrimination among the lanthanides as judged by the range of T_m values. This is consistent with the NMR results which show that the metals are associated with the thymines. Since 3 has more thymines than 1, it is reasonable that the larger number of possible sites for association with the metal ions would increase the diversity of interactions among the metals. Aptamer 2 shows the smallest range of T_m values, probably due to the relative instability of the G-quartet conformation in this structure.

Of the metals with f electrons, the degree of destabilization of the G-quartet structures of 1 and 3 are directly correlated with the size of the ion ($\text{Eu}^{3+} > \text{Tb}^{3+} > \text{Lu}^{3+}$). La^{3+} , which has no f electrons, destabilized the conformations more than Eu^{3+} and Tb^{3+} but less than Lu^{3+} . Clearly, the electronic structure of the metal ions plays an important role in the interactions with the aptamers since the p-block metals (Al^{3+} , Ga^{3+} and In^{3+}) did not affect the conformation of the aptamers.

In summary, our results indicate that there are substantial differences among the interactions of metals with the G-quartet forming aptamers which could form the basis of metal ion separations using aptamer-coated capillaries. Aptamer 3 appears to provide the best discrimination among metals, suggesting that it is a good candidate for the stationary phase.

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