SEQUENCE DEPENDENT MOBILITY OF SHORT OLIGONUCLEOTIDES IN FREE-SOLUTION ELECTROPHORESIS

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INTRODUCTION

Since completion of the human genome project, many additional organisms have been sequenced and ~1.8 million human genetic Single Nucleotide Polymorphisms (SNPs) have been identified and documented by the SNP consortium, http://snp.cshl.org. While the technology workhorses for these efforts have been gel-based and capillary electrophoresis DNA sequencers, there is increased interest in the development of SNP technologies that utilize mass spectrometry (MS) for SNP analysis (e.g. http://www.sequenom.com). Thus, methods to amplify, manipulate and sequence DNA segments that are less than 20 to 30 bases in length and are compatible with MS systems are being developed.

In free solution, polyelectrolytes that are much longer than their persistence length have an electrophoretic mobility that is independent of fragment length [1]. This characteristic makes it difficult to separate long DNA in free solution and is the reason that both gel and capillary electrophoresis systems use additional polymers to provide a molecular sieve that allows separation of these molecules based on length. Short DNA fragments, however, show an increase in mobility with length which becomes constant for lengths more than several hundred bases [2]. Sieving matrices are therefore unnecessary in separations used for genetic identification of short DNA segments. Use of fluorescence detection does, however, require the attachment of dye molecules that can affect the mobility. Thus, electrophoretic separations of oligonucleotide-dye complexes actually utilize a strategy known as end-labeled free solution electrophoresis or ELFSE, which has been shown to be quite useful for intermediate-length DNA fragments [3].

Another trend in electrophoresis technology development is the utilization of microfluidic channels on a chip. There are a number of practical advantages to using microfluidic chips in lieu of standard capillaries. First is size. The chips used in this experiment are only 1.6 cm wide and 9.5 cm long. Because of the size, the buffer and sample volumes are reduced and it is possible to place many sample flow paths on a single chip. As an example of this multiplexed approach, the research group of Mathies has developed a number of assays utilizing helped to pioneer chip-based capillary electrophoresis systems [4]. The use of small channels facilitates the application of higher field strengths yielding good separation performance. Since the channels on a microfluidic chip can be manufactured with dimensions smaller than those found on most standard capillaries, the surface to volume ratio is higher resulting in greater heat dissipation and less joule heating at high applied fields. The ability to apply higher field strengths results in separation completion on the order of minutes instead of hours.

In the present work, free-solution electrophoresis of 15-mers of a single base and the corresponding, hybridized double strands are presented. This work was motivated by previous studies that showed multiple-peak separations for various multi-base ssDNA sequences. Sequence dependent conformation changes have been observed in other systems and our goal was to assess the effects of sequence on the formation of multiple peaks in high-field free-solution electrophoresis of short ssDNA.

MATERIALS AND METHODS

The microfluidic chip used in this work contains 2 intersecting channels: an injection channel and a separation channel that is 8 cm in length. Both have a cross-section with a semi-circular/rectangular shape: a width of 50μm and a depth of 20μm. The DNA fragments were labeled by attaching Texas Red (Ex. 580 nm, Em. 600 nm; Molecular Probes, Inc.) to one end of the strand to allow detection by laser-induced fluorescence. Because the size of these fluorescent labels are on the order of two to three deoxyribonucleotides, we expect the electrophoretic mobility to be influenced by changes in the frictional drag especially with shorter length DNA strands.

The electro-osmotic flow (EOF) was minimized not by the standard method of coating the channel walls with a polymer, but by using a high ionic strength buffer, 10X Tris-borate-EDTA (TBE) at pH 8.3. A neutral dye, Rhodamine B (Sigma-Aldrich), was used to measure electro-osmotic flow (EOF) in the channel.

The following single stranded DNA oligomers of RP grade were used as received: $(dA)_{15}$, $(dT)_{15}$, $(dC)_{15}$, $(dG)_{15}$. All oligomers were

labeled with Texas Red using either a C6 or C7 linker with the label on the 5' end of the oligomer expect for $(dA)_{15}$, which to facilitate hybridization with the labeled $(dT)_{15}$, was labeled on the 3' end. Additionally, $(dA)_{15}$ and $(dG)_{15}$ were also labeled with Cy3 (Ex. 548 nm, Em. 562 nm; Amersham Biosciences). In addition to the labeled oligomers, an unlabeled $(dC)_{15}$ was synthesized for hybridization to the labeled $(dG)_{15}$.

Capillary electrophoresis was performed using the Microfluidic Tool Kit from Micralyne (Edmonton, Alberta Canada). A more detailed description and schematic of the system and chip are provided in reference [5]. This system consists of two high voltage power supplies that supply a potential difference across the injection and separation channels of a microfluidic chip. The optical system for obtaining the fluorescence signal within the channels contains a 3-axis positioner (Newport Optics) that allows one to focus a 40X objective (NA 0.55) anywhere along the injection or separation channels, but was located 7cm from the channel intersection for the data presented here. This objective collects the fluorescence for either an eyepiece or a photomultiplier tube (Hamamatsu) for detection. The excitation light source is a Nd:YAG (532 nm) diode pumped solid state laser.

Both ssDNA and dsDNA were prepared by dissolving a given amount of freeze-dried oligomer into milli-Q water and mixing with the buffer to obtain final concentrations of approximately 5-10 μM. The sample solutions were placed in the sample reservoir while buffer was placed in the other three reservoirs. The DNA was injected into the separation channel by electrophoretically migrating the DNA sample reservoir to the sample waste reservoir by applying a field strength of 2500V/cm across the injection channel for 20s. The electrophoretic separation was then accomplished by applying a field strength of 588V/cm across the separation channel.

RESULTS AND CONCLUSIONS

The results of single-stranded $(dA)_{15}$ and of $(dT)_{15}$ and the double-stranded complement at a field strength of 588V/cm are shown in Figure 1 with the $(dT)_{15}$ observed to migrate slightly slower than $(dA)_{15}$. Two peaks are clearly present for the single stranded molecules. A number of physical mechanisms have been eliminated as the source of this "peak-splitting" and the current hypothesis is that dye-oligo interactions are bi-stable, forming either a conformation in which the oligo collapses around the dye, or another conformation in which the dye is tethered from the oligo with little interaction.

Results for the other two oligomers, $(dC)_{15}$ and $(dG)_{15}$ are shown in Figure 2. Unlike poly(dT) and poly(dA), only $(dC)_{15}$ shows two bands. A decrease in the fluorescence intensity of about 60% is observed for the strand containing only guanine. This decrease indicates quenching of the fluorophore, Texas Red®, by guanine which is due to an electron transfer from the ground state guanine to the excited state of the fluorophore. For this electron transfer to occur the fluorophore must be interacting with guanine. Previous fluorescence life time studies have shown the existence of a ground state complex that results from a strong hydrophobic interaction between the dye and the rings of the nucleotide bases in polar solvents. Therefore, the observation of quenching during electrophoretic migration suggests that, in this case, the dye is not tethered behind the strand but is actually interacting with the bases within the strand, suggesting that the collapsed conformation is the predominant form.

If the peak splitting is indeed the result of different secondary structures of ssDNA with the fluorophore, Texas Red®, then, a single band should be observed when these secondary structures are eliminated upon hybridization of the complement strands. This is indeed observed in Figures 1 and 2 for $ds(T\text{-}A)_{15}$ and $ds(G\text{-}C)_{15}$, respectively.

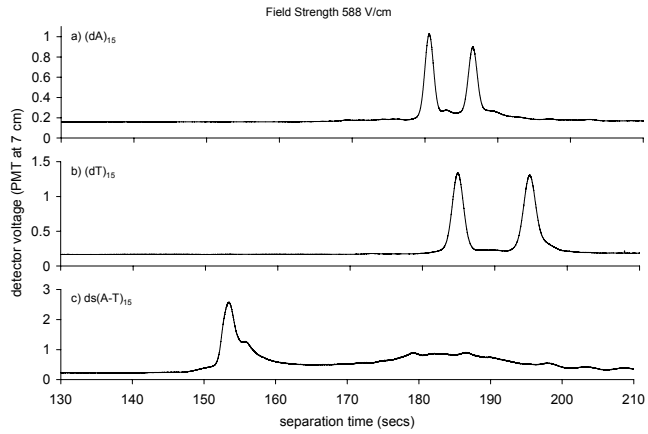

Figure 1. High-Field Free-Solution Electrophoresis of $(dA)_{15}$, $(dT)_{15}$ and $ds(T\text{-}A)_{15}$.

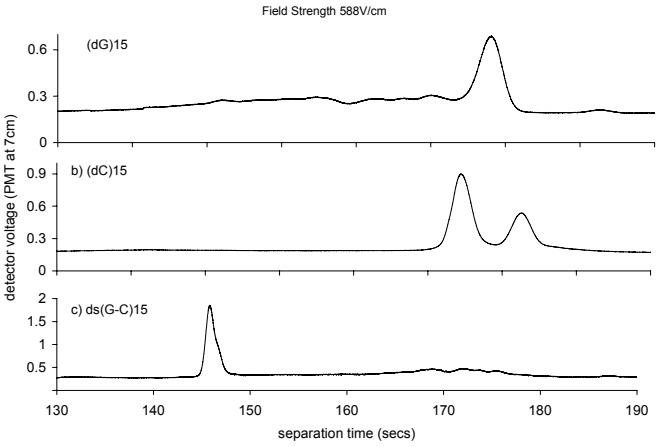

Figure 2. High-Field Free-Solution Electrophoresis of $(dC)_{15}$, $(dG)_{15}$ and $ds(G\text{-}C)_{15}$.

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