TUNING THE MOBILITY OF FLUORESCENT, DNA-TEMPLATED, SILVER NANOCLUSTERS FOR ELECTROPHORETIC SEPARATIONS IN MICROCHANNELS

J.T. Del Bonis-O'Donnell, D. Fygenson, S. Pennathur
University of California Santa Barbara, USA

ABSTRACT

We demonstrate that the electrophoretic mobility of DNA-templated fluorescent nanoclusters (AgDNAs) can be finely tuned via DNA sequence without altering the fluorescence spectra, allowing these novel fluorophores to be used as fluorescent mobility markers for electrokinetic-based separation assays using lab on chip devices. Starting with hairpin DNA templates containing varying lengths of poly-dT overhangs, we show that spectrally identical clusters form that can easily be separated electrophoretically. Using short overhangs produces a poly-disperse pair of clusters. We use Alexafluor488 tagged DNA to look in detail at the polydispersity of initial single stranded DNA and compare to the resulting fluorescent clusters.

KEYWORDS: microfluidics, separation, fluorophores, silver DNA nanoclusters

INTRODUCTION

In the last 20 years, electrokinetic-based microfluidic separation devices have emerged as powerful analytical tools for the separation and identification of various analytes [1][2], many of which rely on fluorescence detection owing to its sensitivity and straightforward detection. A particularly powerful separation assay relies on the ability to detect untagged analytes using intermediate fluorescent markers that vary in their electrophoretic mobility [3]. However, fluorescence detection is limited to commercially available fluorophores, where little flexibility or control is provided over their electrokinetic properties. Silver-DNA nanoclusters (AgDNA), a new class of novel fluorophores, are hybrid macromolecules in which a silver superatom is stabilized in aqueous solution by segments of single stranded DNA [4]. AgDNA fluoresce at wavelengths that can be tuned across the visible spectrum and into the infrared by varying the DNA sequence. Their small size, spectral tuning and biocompatibility have lead to a variety of novel uses in biological imaging, genetic analysis and chemical detection [5]. However, systematically tuning the physical properties of AgDNA has remained a challenge due to the unpredictable sensitivity of sequence on cluster formation. In this work, we develop an approach for creating a set of spectrally identical AgDNA fluorophores with tunable electrophoretic mobilities for future separation assays. We also use microchip CE to study the conformational heterogeneity of the AgDNA and the single stranded DNA used to make them.

EXPERIMENTAL

Starting with a hairpin DNA, modifications were made to its sequence that both preserved and altered the secondary structure. While some changes produced clusters that differed both in mobility and spectra, we were able to systematically alter the mobility of one red cluster by appending poly-dT repeats to the 3’ end of the hairpin sequence to create overhangs in the stem (Figure 1a). AgDNA nanoclusters were prepared by mixing DNA oligos (IDT DNA, Inc) in

Figure 1: (a) Schematic depicting the DNA sequences and predicted secondary structures used to synthesize fluorescent AgDNA nanoclusters. (b) Normalized fluorescence emission under 260 nm excitation for the hairpin 12C-hp10, the overhang poly-dT variants, and a DNA sequence containing only 10 thymine after addition of silver and reduction.
a solution of sodium acetate (pH 4.8) and silver nitrate and incubating for at least 20 min. The solution was then reduced using freshly prepared sodium borohydride to a final concentration of 25 µM DNA, 10 mM sodium acetate, 200 µM silver nitrate and 100 µM sodium borohydride. Bright red fluorescence was observed within 10 minutes of reduction and measured using a Tecan infinite Pro200 plate reader (Figure 1b). Peak excitation and emission wavelengths were 577 nm and 655 nm respectively.

Microchip capillary electrophoresis (CE) was performed using borosilicate microchannels 20 µm deep by 50 µm wide (Dolomite Ltd, UK) arranged in a simple cross channel geometry with a 30 mm long separation channel [6]. The chip was mounted using a custom chuck to an inverted microscope (Olympus IX71). Electric fields up to 78.8 kV/m were applied using a high voltage sequencer connected to the chip reservoirs using platinum wire (LabSmith HVS448 6000D-LC). Fluorescence was detected using a 20x objective (Olympus), EMCCD camera (Andor) and a mercury bulb light source. Before use, channels were flushed sequentially with deionized water, NaOH, HCl and running buffer (10mM sodium acetate pH 4.8 and 1% PVP (w/w)). PVP was used to suppress electroosmotic flow and also served as an efficient sieving matrix for DNA. Samples containing AgDNA were mixed with a solution of sodium acetate and PVP prior to loading into a reservoir for separation.

RESULTS AND DISCUSSION

We found that appending poly-dT tail to the 3’ end of the stem region of the initial hairpin sequence did not affect the spectra or the yield of the resulting red emitting AgDNA, providing a convenient handle for increasing the size of AgDNA. Electrophoretic separations performed on chip showed that AgDNA with overhang lengths that differed by 10 or more bases were rapidly separated in under 20 seconds, demonstrating their tuned electrophoretic mobility differences.

Our microchip CE also revealed that the hairpin and the hairpin containing only a 5 base overhang both produced two elution peaks indicating the presence of two distinct clusters with indiscernible fluorescence emission. This suggests that a particular silver cluster is stabilized by two different conformations of the same DNA sequence. A long poly-dT overhang, however, appears to disrupt one of the conformations. To further explore the role of conformation on AgDNA cluster formation, an Alexafluor tagged DNA (12C-hp10-T10-AF488) was separated and detected before and after reduction with sodium borohydride. The initial DNA strand contained several distinct conformational populations, while the AgDNA cluster afterwards appeared to correspond to only a single conformation (Figure 3).

![Figure 2: Electropherograms of samples containing mixtures of AgDNA containing poly-dT overhangs. (a) The 12C-hp10 strand produces two distinct clusters while the strands containing overhangs eluted at later times with single elution peaks for lengths of poly-dT >10 bases. (b) An overhang of only 5 bases produces two distinct species and overlaps significantly with the 10 base overhang cluster.](attachment:figure2.png)
CONCLUSION

We demonstrate an approach to tuning the mobility of a novel AgDNA fluorophore without altering fluorescence emission or yield. Our microchip based CE separations reveal distinct conformations of optically similar clusters, previously unresolved by fluorescence measurement techniques. We also find that single stranded DNA exist in a heterogeneous mixture of conformations and that a particular conformation is preferred in the formation of the AgDNA nanoclusters.

ACKNOWLEDGEMENTS

Supported by the Institute for Collaborative Biotechnologies through grant W911NF-09-0001 and W911NF-12-1-0031 from the U.S. Army Research Office. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

REFERENCES


CONTACT

Prof. Sumita Pennathur, email: sumita@engineering.ucsb.edu