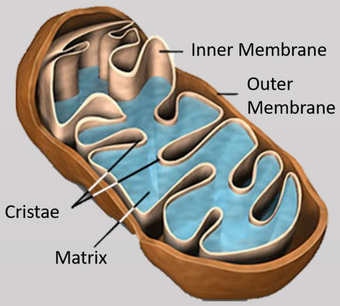

Separation, Quantification, and Visualization of DNA

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Introduction

Mitochondria are involved in numerous essential cellular pathways.[1] Mitochondria contain their own DNA genome (mtDNA), which is distinct from nuclear DNA. Scientists wish to understand the mitochondrial genome as a whole as well as how mitochondrial transcription is affected through changes in the proteins involved in regulation. The direct output of mitochondrial transcription is mitochondrial RNA (mtRNA), so separation and quantification of this mtRNA would be highly beneficial to this understanding. Ion-pair reversed-phase high performance liquid chromatography (IP RP HPLC) has been reported to be an effective way to analyze DNA and RNA oligonucleotides (Model 1).[2,3] IP RP HPLC can analyze oligonucleotide samples in under an hour as opposed to agarose gel electrophoresis followed by ethidium bromide detection or P-32 labelling which both take up to 4-5 hours to fully analyze samples.[4,5] We report a novel IP RP HPLC method which separated ssDNA samples of 54 nt and 58 nt with good resolution. This method also quantified ssDNA samples of 54 nt and 58 nt upwards of 5000 ng. This method will be further optimized to separate similarly sized RNA samples. The ultimate goal is to separate mixtures of nucleotides generated from in-vitro transcription reactions. We also report a method for imaging DNA molecules immobilized on mica in air using atomic force microscopy (AFM).

Methods and Materials

Diethyl pyrocarbonate (DEPC)-treated water was used for all oligonucleotide sample preparation. Samples were obtained lyophilized, and dissolved in sufficient DEPC-treated water such that the final concentration was 100 ng/µL.

Ion-pair reversed-phase high performance liquid chromatography (IP RP HPLC) was performed on a Shimadzu LC-2010HT Liquid Chromatography System with an Xbridge Oligonucleotide BEH C18 (130 A, 2.5 µm, 4.6 x 50 mm) column. Quantification of peaks was performed through integration of peak areas.

- Buffer A: 0.1 M TEAA in DEPC-treated water (pH 7.0)
- Buffer B: 0.1 M TEAA with 25% (v/v) ACN in DEPC-treated water (pH 7.0).

The gradient conditions used were: 38-39% B from 0 to 45 minutes, to 40% B from 45 to 55 minutes, to 100% B from 55 to 60 minutes, followed by a 10-minute re-equilibration to initial conditions. Flow rate was 0.5 mL/min. Column temperature was kept at 50 °C. Oligonucleotide samples were kept at 4 °C prior to analysis to prevent sample degradation.

Mica discs were epoxied to magnetic pucks and freshly cleaved with tape creating a near atomically flat surface. 50 µL of DNA was pipetted onto freshly cleaved mica discs and dried under nitrogen.

AFM imaging of DNA oligos was performed on an Asylum Research Cypher Scanning Probe Microscope. The microscope was operated in air tapping mode using cantilever oscillation frequencies between 850 and 2500 kHz. The lever used was a chromium/gold coated silicon lever with a 7 nm silicon tip and a spring constant of 85 N/m.

Results

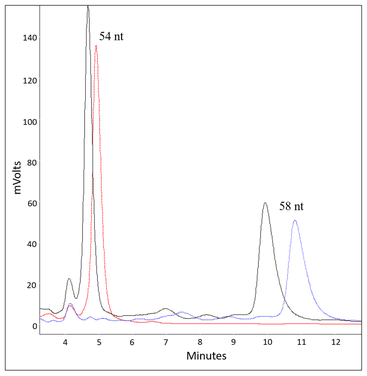


Figure 1. IP RP HPLC analysis of ssDNA samples. Injections consisting of 1000 ng of 54 nt DNA sample (red), 1000 ng of 58 nt DNA sample (blue), and an injection consisting of 1000 ng each 54 nt and 58 nt (black)

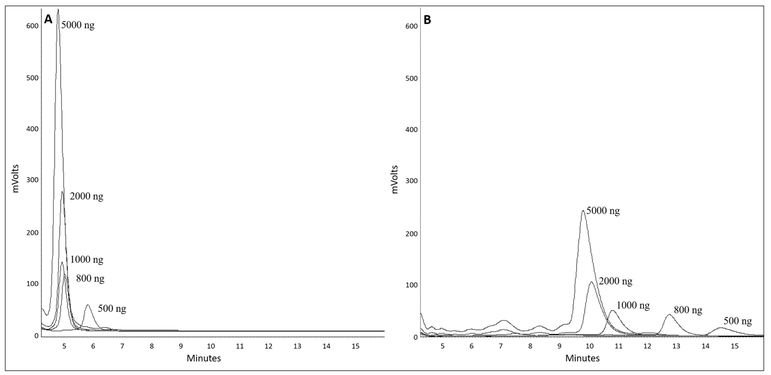


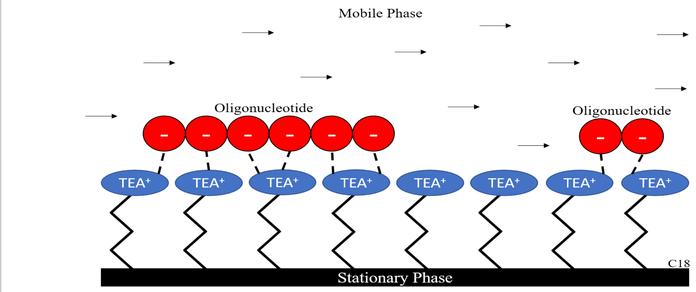
Figure 2. Overlay of IP RP HPLC chromatograms. (**A**) IP RP HPLC chromatograms of 54 nt DNA sample with varying injection sizes from 500 ng to 5000 ng. (**B**) IP RP HPLC chromatograms of 58 nt DNA sample with varying injection sizes from 500 ng to 5000 ng. Integrated peak areas for each DNA sample yielded an linear response (R^2 = 0.9999) with respect to injected mass of DNA.

Table 1. Quantification results from varying injections of 54 nt DNA samples after IP RP HPLC analysis. Quantification results determined through integration of peak areas. Correlation coefficient (R^2) for the data is 0.9999.

DNA injected (ng)	Integrated Peak Area (mVs)	Calculated DNA amount (ng)
500	1338083	489.9
800	2214286	803.1
1000	2741980	991.6
2000	5625227	2022.0
5000	13940253	4993.5

Table 2. Quantification results from varying injections of 58 nt DNA samples after IP RP HPLC analysis. Quantification results determined through integration of peak areas. Correlation coefficient (R^2) for the data is 0.9999.

DNA injected (ng)	Integrated Peak Area (mVs)	Calculated DNA amount (ng)
500	960860	513.0
800	1688220	827.5
1000	2037595	978.6
2000	4332044	1970.7
5000	11361591	5010.4



Model 1. Representative figure for analysis of oligonucleotides by IP RP HPLC. The mobile phase, TEAA, contains the ion-pairing reagent TEA^+ (blue circles) which adsorbs to the non-polar stationary phase forming a positively charged pseudo ion-exchange layer. This layer interacts with the polar bases of the oligonucleotides (red circles) allowing for separation to occur

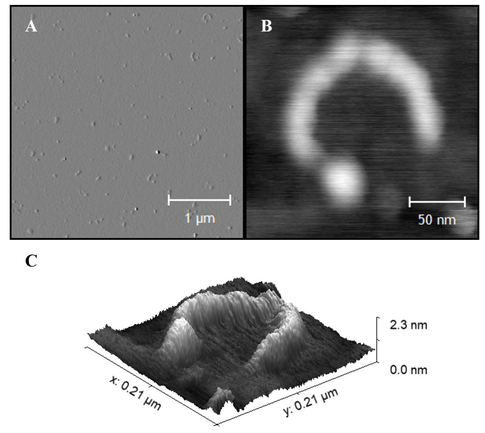


Figure 3. AFM images of DNA molecules immobilized on mica pucks in air. All images were obtained in AC/Tapping Mode. (**A**) Initial image, 5 µm^2. (**B**) Zoomed image, 210 nm^2. (**C**) 3D representation of zoomed image.

Future Directions

Going forward we hope to optimize the current method and apply it to separate RNA samples of similar sizes to the DNA samples tested. **The ultimate goal is to utilize this method and IP RP HPLC to separate and quantify RNA transcripts of unknown sizes generated via in-vitro transcription reactions.** We also want to improve AFM image quality, and shift the focus to imaging DNA samples and DNA-protein samples in liquid. From there, our aim is to **determine force constants of protein-DNA interactions** using functionalized lever tips.

Acknowledgements

Linfield Chemistry Department, Linfield College Student Faculty Collaborative Research,, National Science Foundation (MRI-DMR #1827971), Murdock Charitable Trust (Natural Sciences Grant #2015313), Linfield Physics Department, Dr. Jennifer Heath, Joseph Simpson, Wendell L. Foote Fund

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