Colorimetric Redox Reactions to Determine Base Composition in Unknown Monomeric DNA Samples

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Introduction

Deoxyribonucleic acid (DNA) codes for proteins using a unique order of four major nitrogenous bases: adenine, thymine, cytosine, and guanine. In several biological applications, such as the determination of DNA stability[1], production of biologic-based drugs[2], and even taxonomic reorganization of species[3], it becomes necessary to understand the relative amounts of each nucleotide in a DNA sample, regardless of their sequence. However, most assays used to determine base composition use expensive equipment, harsh chemicals, and advanced computer software. In addition, the determination of base composition is an important educational tool in undergraduate laboratories, but these limitations are infeasible for many institutions. Therefore, our research focus is to develop an easier, safer, and faster base composition assay.

Colorimetry is a field of chemistry that uses changes in visible color and the absorbance of light to qualitatively and quantitatively analyze a mixture. Potassium permanganate ($KMnO_4$) is an ionic compound capable of reacting with thymine to create a *syn*-diol, reducing the manganese ion from a +7 oxidation state to a +4 oxidation state, thus changing the color from dark purple to bright yellow. Bicinchoninic acid (BCA) has been used along with copper sulfate ($CuSO_4$) for decades to change colors from teal to violet in the presence of proteins, but it has recently been used to react with abasic sites in monomeric DNA, followed by UV/Vis spectroscopy to quantify the number of sites using absorbance[4]. This experiment integrates both of these techniques to create a holistic base composition assay for monomeric DNA.

Objective

The objective of this experiment is to develop colorimetric assays that can qualitatively and quantitatively determine the presence and composition of nucleobases in a monomeric sample using redox reactions.

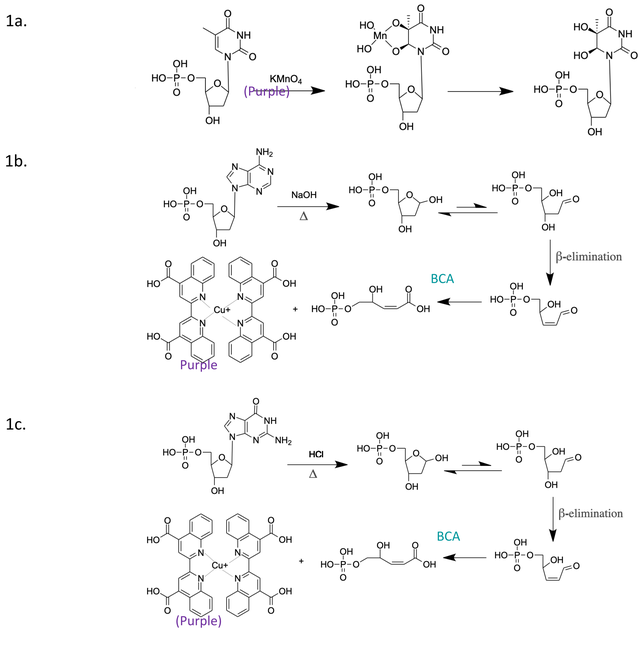

Figure 1. A) Scheme for the reaction of $KMnO_4$ with thymine, resulting in a yellow color. B) Scheme for the base-catalyzed cleavage of adenine, resulting in an abasic site and the subsequent reduction of copper, producing a purple color. C) Scheme for the acid-catalyzed cleavage of a purine (guanine used as an example), resulting in an abasic site and the subsequent reduction of copper to produce a purple color.

Method

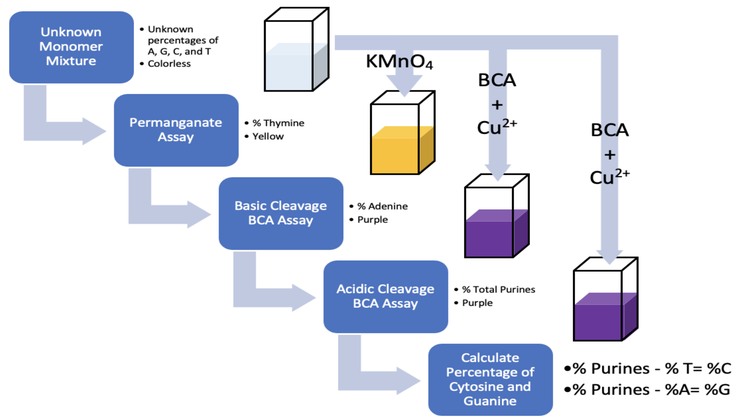

Figure 2. Flowchart denoting the order of reactions used in this experiment and the data collected at each step.

$KMnO_4$ Assay

1 mM DNA monomer solutions were incubated in a phosphate buffer (pH=7), followed by the addition of $KMnO_4$ (1 mM final concentration) Then, a picture was taken on a smartphone equipped with a colorimeter app and the G value was measured. Results displayed in figures 4a and 4b.

Basic Cleavage Assay

10 mM monomer solutions in varying ratios (0% purines/100% pyrimidines to 100% purines/0% pyrimidines in increments of 20%) were added to a 1.7-mL Eppendorf tube along with 100 µL sodium phosphate buffer (pH=7) and 20 µL 3M NaOH to a total volume of 1 mL. This was performed in triplicate, then each tube was incubated for 10 minutes at 90°C. After incubation, each solution was added to a cuvette, and 300 µL BCA was added to each cuvette. After a 5 minute incubation at room temperature, the absorbance at 562 nm was recorded. Results displayed in figures 4c and 4d.

Acidic cleavage Assay

Varying ratios of purines to pyrimidines (0% purines/100% pyrimidines to 100% purines/0% pyrimidines in increments of 20%) were added to a 1.7 mL Eppendorf tube along with 100 µL sodium phosphate buffer (pH=7) and 100 µL 1M HCl to a total volume of 1 mL. This was performed in triplicate, then each tube was incubated for 60 minutes at 90°C. After incubation, each solution was added to a cuvette, and 300 µL BCA was added to each cuvette. After a 5-minute incubation at room temperature, the absorbance at 562 nm was recorded. Results displayed in figures 4e and 4f.

Results

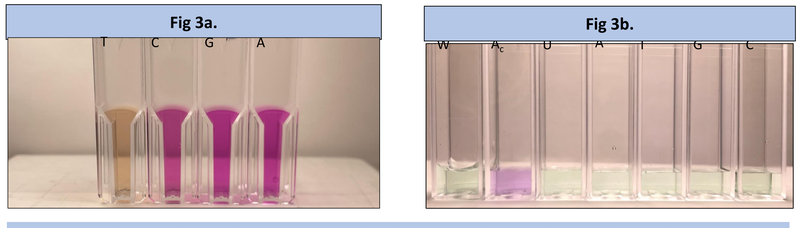

Figure 3. A) Specificity of $KMnO_4$ assay for thymine. B) Specificity of basic cleavage BCA assay for adenine

Results

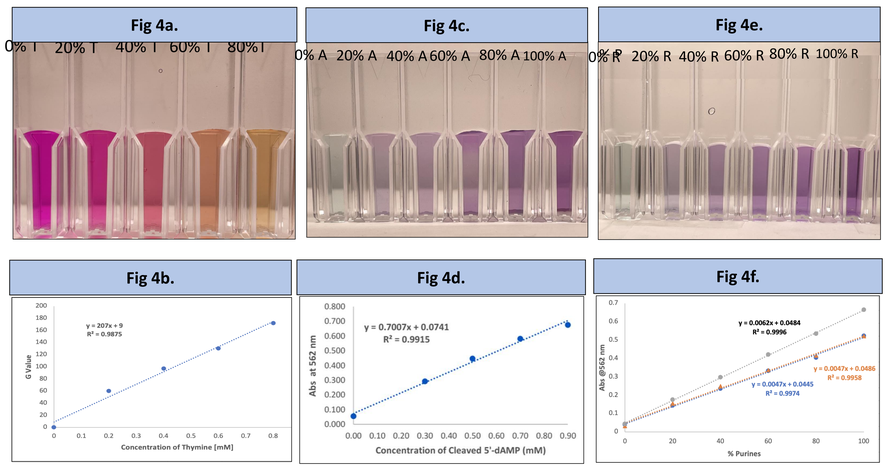

Figure 4. A) $KMnO_4$ assay at increasing concentrations of thymine (% T). B) Plot of G values with increasing concentrations of thymine. C) BCA assay after basic cleavage with increasing concentrations of adenine (% A). D) Plot of absorbance values at 562 nm with increasing amounts of adenine. E) BCA assay following acidic cleavage with increasing concentrations of total purines. F) Plot of absorbance values at 562 nm at increasing concentrations of purines at 50% A /50% G (blue) 25% A/ 75% G (orange), and 75% A/ 25% G (gray).

Discussion

The objective of this experiment was to develop a colorimetric assay for the qualitative and quantitative determination of base composition in an unknown monomeric DNA sample. The qualitative results were robust, offering a clear color change from purple to yellow using $KMnO_4$ for the determination of thymine and teal to purple using BCA for the determination of adenine and/or total purines. In addition, the quantitative results showed a linear trend with direct proportionality to specific DNA monomer concentration, with R^2= 0.9875 for the permanganate assay, R^2= 0.9915 for the base-catalyzed BCA assay, and R^2=0.9958, 0.9974, and 0.9996 for 25% A/75% G, 50% A/50% G, and 75% A/25% G acid-catalyzed BCA assays, respectively. Future research work includes applying the assay to unknown DNA monomer samples in a single-blind manner. The ultimate goal of this experiment is to incorporate it into an undergraduate organic chemistry laboratory.

Acknowledgements

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