A New Colorimetric Assay of Apurinic/Apyrimidinic (Abasic) Sites of Deoxyribonucleic acid (DNA) Using Bicinchoninic Acid [1]

Isaiah O. Stephens and Dr. Elizabeth M. Thomas,

Department of Biology and Chemistry, Morehead State Univeristy, Morehead, KY 40351

Introduction

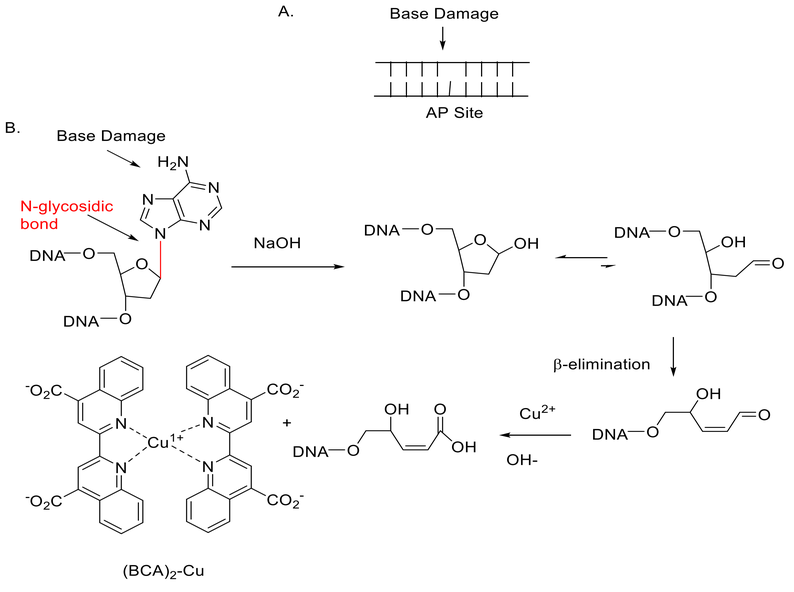
Apurinic/apyrimidinic (AP or abasic) sites are DNA lesions that result from the loss of a nucleobase by hydrolysis of the *N*-glycosyl bond. It is estimated that AP sites are the most frequent lesions in cells with about 10,000-50,000 times per day per cell under typical aerobic conditions [2]. The formation of AP sites is caused by environmental and cancer therapeutic genotoxins such as alkylating agents, oxidizing agents, ionizing radiation, and ultraviolet radiation.

The treatment of DNA nucleotides with sodium hydroxide, a chemical reagent that produces adenosine-specific depurination, creates an abasic site [3]. The resultant adenosine-specific depurinated DNA can then be observed and quantified using the colorimetric reagent solution bicinchoninic acid, BCA, which is commercially available (Scheme 1).

A rapid, colorimetric method for assay of abasic sites within DNA would be beneficial within undergraduate teaching laboratories to introduce students to DNA structures and how DNA lesions are produced structurally.

Objective

The objective of this laboratory experiment is to quantify abasic sites in DNA mononucleotides using a bicinchoninic (BCA) colorimetric assay. When there is a base damage, it leads to an AP site within double stranded DNA (Scheme 1A). Then upon base damage, the *N*-glycosidic bond hydrolyzes and then the AP site is colorimetrically detected when Cu^{2+} reduces to Cu^{1+} which chelates to form chromogen $(BCA)_2$-Cu complex (Scheme 1B).



Scheme 1. Mechanism of AP site formation and colorimetric detection.

Method

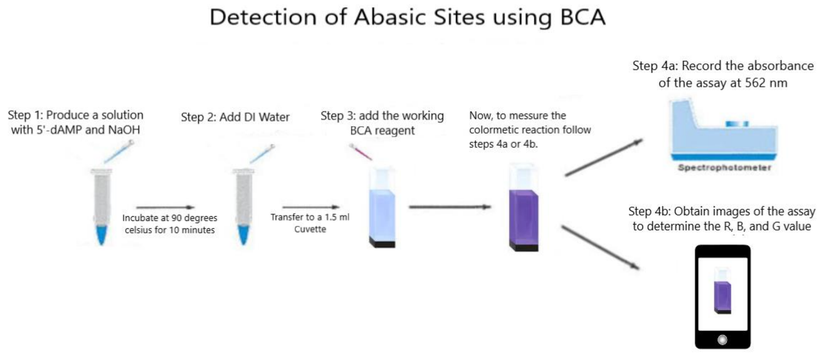


Figure 1. Illustration of AP site assay using BCA and two methods of measurement.

A 10 mM 5′-monophosphodeoxyadenosine (5'dAMP) is mixed in equal volume with 3.0 M sodium hydroxide. The solution is then heated at 90°C for 10 minutes to induce abasic sites in the 5'dAMP [4]. Solutions are then transferred to cuvettes and deionized water is added to ensure constant final volume of 1.0 ml. The colorimetric reagent, BCA 50:1 (Reagent A:Reagent B) is added to a sample; samples containing abasic sites undergo color change from clear to violet. The intensity of the violet color is used to determine the concentration of abasic sites present in the sample. Two different methods are used to quantify the concentration of abasic sites (Figure 1). In Figure 1, step 4a depicts a traditional method of obtaining absorbance at 562 nm (spectrometer) where higher absorbance indicates higher solution. In Figure 1, step 4b uses a smartphone to quantify the absorption by obtaining R, B, and G values from an image of the assay.

Results

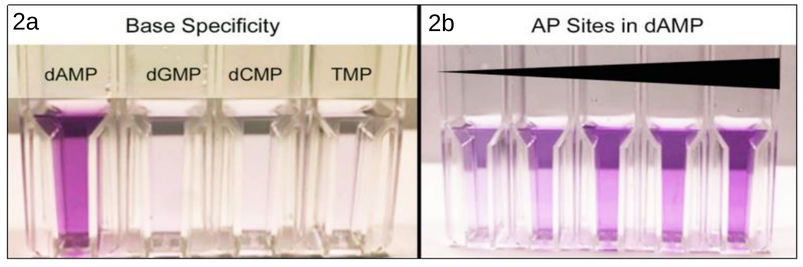


Figure 2. A)Specificity of NaOH cleavage B) Assays with increasing amount of abasic sites

The procedure was evaluated on all four DNA nucleotides (5′-monophosphodeoxy guanosine (5'-dGMP), 5′-monophosphodeoxyadenosine (5'-dAMP), 5′-monophosphodeoxycytidine (5'-dCMP), 5′-monophosphodeoxythymidine (5'-dTMP)) and showed specificity generating abasic sites only in the 5'dAMP sample. A linear correlation between the concentration of abasic sites and the absorbance was observed with a higher concentration of abasic sites displaying a higher absorbance when BCA was added.

Results

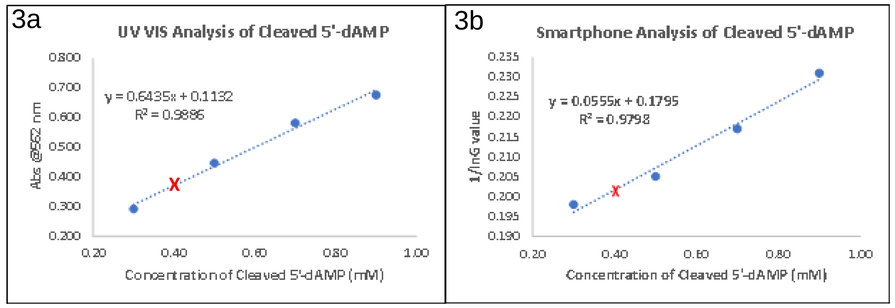


Figure 3. A) Absorbance at 562 nm vs Concentration of Cleaved 5'dAMP B) 1/ln(G) value vs Concentration of 5'dAMP

The absorbance at 562 nm was plotted verses the concentration of cleaved 5'dAMP to generate a linear trend line with an R^2 value > 0.98. The G value for the solution color of each assay is obtained using images like those in figure 2. These G values are then used to create a graph plotting 1/ln(G) vs Concentration of cleaved 5'-dAMP yielding an R^2 value >0.97. Both methods illustrate a linear correlation which can be used to quantify the concentration of an unknown sample depicted by the red x on graphs 3a and 3b (Figure 3).

Discussion

Herein, describes is a novel colorimetric assay that can selectively detect AP/abasic sites from cleaved 5'-dAMP. The assay is successful in producing graphs with a strong linear correlation for both proposed methods. Using the smartphone method does requires proper lighting for images and consistency when acquiring the images. With these considerations the assay has shown to be quite robust and was also incorporated into an undergraduate education lab to expand students' knowledge of DNA structure, common DNA lesions, colorimetric assays, and basic hands-on laboratory skills.

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