CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

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Project Number

S0416

Project Title

Braking the Double Helix: Effects of UV Radiation on Super-Coiled DNA

Abstract

Objectives/Goals

UV radiation has enough energy to nick and break the phosphate backbone of DNA. Last year I proved that natural levels of solar UV radiation were sufficient to nick or break the DNA. This year I asked if the breakage was occuring randomly or at specific sites.

Methods/Materials

I used a super-coiled plasmid for this project because, on an agarose gel, the broken parts of the DNA move at different speeds. The band that has moved the farthest is the super-coiled DNA, in the middle is the linear DNA (broken), and the band that has moved the least, is the open circle DNA (nicked). To identify the super-coiled band on the gel, I performed an Ethidium Bromide (EtBr) test. In this test I added a range of EtBr concentrations (for a final concentration of 0.1 µg/mL to 5 mg/mL) to the super-coiled DNA. As the EtBr intercalates into the DNA, the plasmid is forced into an open-circle. As more EtBr is added, the plasmid is forced to positively super-coil. To find the linear DNA, I used DNA digested with EcoR1. After adding the EtBr to the DNA, I let it sit for 15 minutes at room temperature then loaded it into the gel. I ran it as described under Gel Electrophoresis. To test the sequence specificity of the breakage, I took, pUC19, and put it under a UV sterilization lamp. Replicate DNA samples were exposed for up to 1 hr. I digested the exposed DNA with three restriction enzymes, Bam H1, EcoR1, and Hind III and then ran the digested DNA on a 1.2% agarose gel.

Results

I found distinct bands on the gel, below the super-coiled (migrated faster), which suggests that some parts of the backbone are more susceptible to breakage than others. I was surprised to find that in the digested DNA, some open circle remained, increasing in amount with the amount of UV exposure. I interpreted that as the enzyme reached the nick in the DNA, and broke off because it was unable to go beyond the nick.

Conclusions/Discussion

I concluded that the DNA is more susceptible to brakage on the phosphate backbone.

Summary Statement

Whether DNA breaks at certain points on the phosphate backbone

Help Received

Used lab equipment at my mother's lab.