Characterizing DNA Secondary Structure of Hairpin-Containing Base Excision Repair Intermediates

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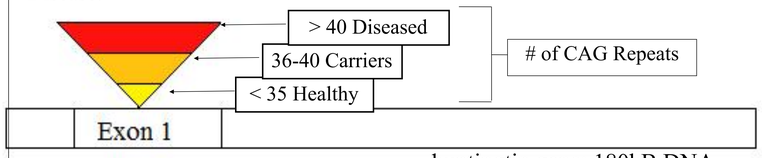
ABSTRACT

Regions of repetitive DNA, in particular trinucleotide repeats, are common throughout the human genome and are of interest as a source of genomic instability.[1] DNA strands that have trinucleotide repeats, such as $(CAG)_n$ repeats can expand to yield a longer repeat tract. While the exact mechanism of expansion is still unknown, expansion is hypothesized to result from the ability of $(CAG)_n$ repeat DNA to form non-canonical secondary structures, including stem-loop hairpins. Hairpins are "hotspots" for DNA damage because the unpaired guanine in the loop of the hairpin is subject to oxidation, leading to an 8-oxo- 7,8 dihydroguanine lesion, which must be repaired.[2] This project is focused on the Base Excision Repair (BER) pathway, which is responsible for repairing these oxidized base lesions. Within mixed sequence DNA, the enzymes involved in BER remove damaged bases and replace them to restore the integrity of the DNA sequence. DNA Ligase 1 typically completes the repair event, creating a new phosphodiester bond at the nick site in the DNA.[3] However, $(CAG)_n$ repeat DNA leads to hairpin-containing intermediates which contain extra nucleotides. If DNA Ligase 1 is able to ligate these hairpin-containing strands, the repeat tract will be expanded. My project will be using chemical probes that react with solvent exposed bases to sequence and characterize the secondary structure of proposed hairpin-containing intermediates of the Base Excision Repair pathway.

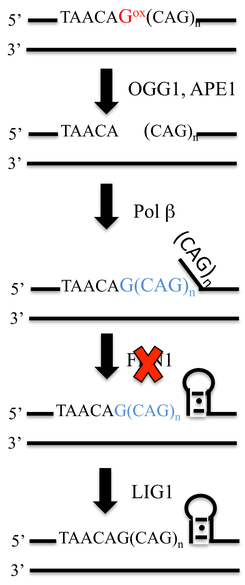
INTRODUCTION

Trinucleotide repeats in DNA, such as $(CAG)_n$ repeats, have been shown to be linked to genetic instability and neurodegenerative disorders. [1] These microsatellite regions are linked to genetic instability because of their ability to expand. For example, expansion of a $(CAG)_n$ tract within Exon 1 of the Huntingtin gene is responsible for the neurodegenerative disorder Huntington's Disease.[1]



huntingtin gene; 180kB DNA

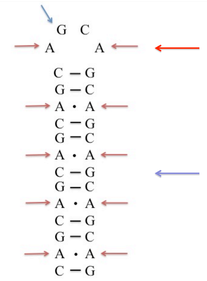
Long Patch BER (repeat sequence)



Toxic Oxidation Cycle

The removal of 8-oxoG, part of essential genomic maintenance, proceeds via the base excision repair (BER) pathway.[4] In the proposed "toxic oxidation cycle" model, the ability for $(CAG)_n$ repeat DNA to adopt non-canonical conformations, such as a hairpin conformation, contributes to error in the base excision repair pathway.[4] In the proposed toxic oxidation cycle, a flap that contains repeats, created after the insertion of extra nucleotides by Polβ, has the ability to form a hairpin structure. FEN1, an enzyme that is part of BER that cleaves flaps, is unable to cleave a hairpin structure. If LIG1 completes the repair cycle and seals the DNA backbone, the extra nucleotides have been incorporated into the genomic DNA and the repeat tract has expanded.

The DNA substrates in this project contain repeat sequences a varying number of nucleobases away from the nick site depending on the substrate. The chemical probing analysis allows the determination of whether or not a hairpin is forming as part of the secondary structure. With chemical probing, the DNA is sequenced and the varying reactivity indicates whether the DNA substrate formed a hairpin or not.



Indicates solvent exposed Adenines upon formation of a hairpin

Indicates the most solvent exposed Guanine upon formation of a hairpin

As shown in the CAG_{10} hairpin, if a hairpin forms in the secondary structure, the Adenine bases will be solvent exposed because there will be no base pairing. In addition, the solvent exposed guanine in the loop of the hairpin is especially reactive upon chemical probing.

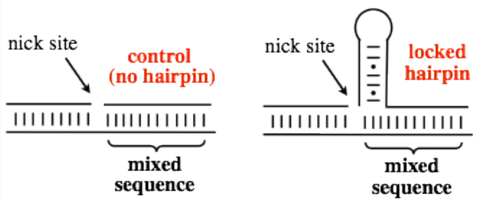
PROJECT GOALS

My goal is to characterize the sequence and secondary structure of DNA substrates. Upon characterization, the oligonucleotides will be used to study the mechanism of DNA Ligase 1 on hairpin-containing substrates in order to determine what kind of non-canonical DNA structures this enzyme can act on. The structural information which I hope to gain from these experiments will be valuable for future studies where the substrate specificity of DNA Ligase I and other enzymes in the BER pathway are evaluated. A more complete understanding of the mechanism that results in Trinucleotide repeat expansion could provide insight for future strategies to prevent genomic instability.

EXPERIMENT & RESULTS

I. DNA Substrates

The DNA substrates were synthesized using phosphoramidite chemistry and purified using reverse-phase HPLC in the Delaney Lab. A control substrate containing a well-matched duplex and a nick site was synthesized. Further, a set of DNA constructs containing a nick site and a $(CAG)_{10}$ repeat hairpin were synthesized. In the $(CAG)_{10}$ containing substrates, the hairpin is located a set number of nucleotides away from the nick site, and surrounded by well-matched mixed sequence to "lock" the structure in place. The position of the hairpin is varied from 0-15 nucleotides downstream of the nick site.



To enable visualization on a gel, DNA substrates were 5'-end ^{32}P labeled following the lab protocol.

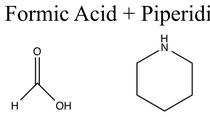
II. Chemical Probing Analysis

Maxam-Gilbert reactions, diethylpyrocarbonate reactions, and peroxynitrite reactions were all carried out on the DNA Substrates.

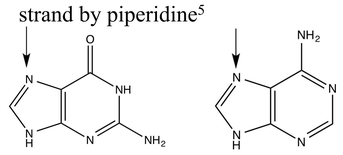
A. Maxam Gilbert Reaction

Maxam Gilbert reactions cleave single stranded DNA at specific bases within the sequence. When formic acid and piperidine are combined with the singled stranded DNA strand, the strand is cleaved at the purine bases, adenine and guanine. When hydrazine is combined with the DNA, the strand is cleaved at the pyrimidine bases, cytosine and thymine.

Adenine/ Guanine Cleavage:
Formic Acid + Piperidine

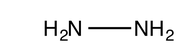


Formic acid protonates N7 of Adenine and Guanine, which is subsequently cleaved from the DNA strand by piperidine[5]

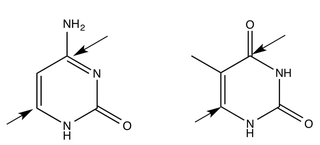


Guanine **Adenine**

Cytosine/ Thymine Cleavage:
Hydrazine

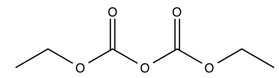


Hydrazine attacks Cytosine and Thymine nucleobases at C4 and C6. Piperidine then cleaves the DNA at these modified bases.[5]



Cytosine **Thymine**

B. Diethylpyrocarbonate



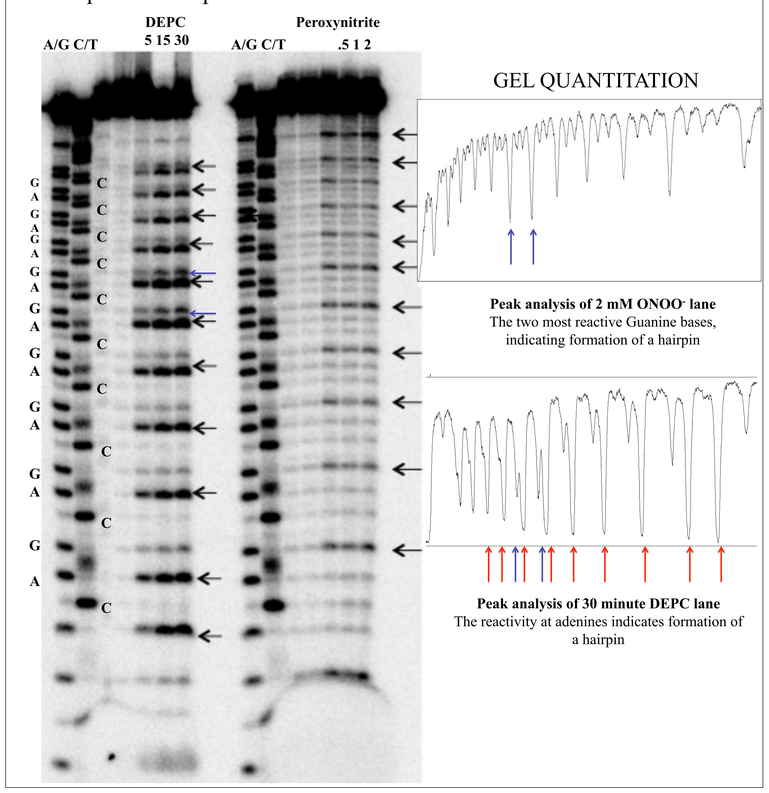
Diethylpyrocarbonate, better known as DEPC, is a DNA chemical probe that reacts with solvent exposed Adenines and, to a lesser extent, Guanines. The actual chemistry of the reaction is not very well known. DEPC was reacted with the DNA substrates for 5, 15, or 30 minutes on a heat block and then quenched. The 30 minute lane on a gel will indicate the most reactivity at adenine bases.

C. Peroxynitrite

Peroxynitrite reacts with CO2 to form a nitrogen dioxide radical that alters double stranded DNA. It is not peroxynitrite which reacts with DNA, but rather one of the reactive radical products.[6] Different concentrations of peroxynitrite were reacted with the double stranded DNA substrate, including .5 mM, 1 mM, and 2 mM. The 2 mM $ONOO^-$ shows the highest reactivity at guanine bases.

III. Results of Chemical Probings

The gel below is a representative result of the chemical probing reactions. The substrate examined in this experiment was "9n" construct ($(CAG)_{10}$ repeat hairpin located 9 base pairs downstream from the nick site). In addition, I quantitated each lane within the gel, which indicates the extent of reactivity at each base. The quantitation revealed more reactivity at the adenine bases in addition to more pronounced reactivity at the guanine in the loop of the hairpin.



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