TITLE: Characterization of α-anomeric Damaged DNA in Complex with Endonuclease IV using Fluorescence Resonance Energy Transfer (FRET)

AUTHORS: Alexander Spring-Connell and Beatrice Edjah*

FACULTY SPONSOR: Markus Germann, Professor, Chemistry Department

Keywords: Alpha-anomeric, Endonuclease IV, FRET

Introduction:
In base excision repair (BER), “small” oxidative DNA base damage is repaired in a series of steps initiated with a damage specific glycosylase that locates and removes the damaged base leaving a toxic abasic site. A damage general endonuclease (Endo IV or APE1) then binds to the abasic site, introduces a ~67° kink in the DNA and nicks the phosphodiester backbone. Subsequently, DNA polymerases and ligases complete the repair. Intriguingly, Endo IV is also able to process α-anomeric damage (chiral inversion at the anomeric carbon), without requiring a damage specific glycosylase (a process conserved in the unrelated human APE1 endonuclease). We are investigating the molecular mechanism of how α-anomeric damaged DNA is processed by Endo IV. FRET is being utilized to determine the conformation of the α-anomeric DNA substrate in complex with EndoIV.

Methods:
The α-C6 linker DNA was synthesized using an Applied Biosystem 391 DNA Synthesizer. The DNA was purified following conjugation with acceptor dye, TAMRA. The complementary strand with donor dye fluorescein, was purchased from Integrated DNA Technologies. Duplexes were assembled to determine the Förster distance and quantum yield. Enzyme binding assay experiments were conducted using the duplex.

Results:
Previous experiments utilized DNA substrates with 5’ terminal guanines. It was later realized that guanosine has a quenching effect on fluorescein which hampered the interpretation of the data. A recent series of experiments using the new duplex substrates containing 5’ terminal thymines was therefore designed. These substrates revealed that when bound to EndoIV, TAMRA interacts slightly with the enzyme. This interaction must be taken into account for FRET distance calculations. Both sets of assays confirm binding of E261Q to the substrate.

Discussion:
After taking into account the quenching and TAMRA interaction effects, initial data reveals that both series of experiments show that EndoIV imposes a 67° kink on the DNA similar to what was observed in an abasic DNA substrate.