EXPLORING DNA DESTABILIZATION INDUCED BY THE THYMINE DIMER LESION USING SMALL MOLECULAR PROBES AND THERMODYNAMIC TECHNIQUES

Amy Rumora

DNA frequently forms mutations due to endogenous or environmental conditions. The Base Excision Repair pathway (BER) removes damaged bases from DNA with a series of enzymatic steps starting with the DNA glycosylase. The thymine dimer lesion is formed from the cycloaddition of two same strand, neighboring thymines. This disables them from base pairing with adenine and causes a kink in the duplex DNA (Husain et al. 1988). Despite the extensive research done on the thymine dimer mutation and its repair mechanism, little is known about the way in which the specific DNA glycosylase locates the DNA lesion in order to carry out the BER pathway. It is possible that the thymine dimer lesion causes thermodynamic and kinetic destabilization to the DNA strand in which it is located.

In order to study DNA destabilization caused by the thymine dimer, DNA containing the dimer was isolated and labeled with the radioactive isotope, $^{32}$P. DNA base modifying chemicals including Dimethyl Sulfate (DMS), Potassium Permanganate (KMnO4), and Diethyl pyrocarbonate (DEPC) were reacted with the DNA. Following the base modification, the sugar phosphate backbone of the DNA was cleaved using piperidine and observed using electrophoretic techniques and imaging with a phosphorimager. Data from this method has revealed significant reactivity of the bases around the thymine dimer. This suggests that destabilization of the duplex DNA may play a part in recognition of the lesion by a base excision repair enzyme.

A standard method for measuring destabilization induced by a DNA lesion involves thermodynamic techniques and measurements (Poklar et al. 1996). A temperature-controlled UV/vis spectrophotometer was used to illustrate considerable differences in the shape of the sigmoidal melting curves and thermodynamic parameters. Differential Scanning Calorimetry (DSC) is used to determine and confirm values obtained through spectrophotometry. Thermal melting of the B-form duplex strand and duplex DNA containing the lesion reveals differences in thermodynamic values.