Abstract

Guanine-rich regions in the genome play crucial roles in regulating gene expression and maintaining chromosomal stability, but are susceptible to oxidative damage, particularly at guanine residues. In addition, guanine-rich sequences can associate into G-quadruplexes, non-canonical structures that can act as steric hindrances in key cellular processes. Both processes are implicated in the onset of many diseases.

In this dissertation, we investigated the structural changes induced by the oxidation of G-rich sequences. We focused on the incorporation of oxidative lesions and their effects on G-quadruplex formation and the structural equilibrium between double-stranded DNA and G-quadruplexes. Employing a combination of NMR, UV and CD spectroscopy, MD and DFT optimization, the work provides a comprehensive analysis of the structural and thermodynamic properties of oxidized G-rich sequences alone and in combination with a complementary C-rich strand.

We synthesized and analyzed a series of oligodeoxyribonucleotides with strategically positioned 8-oxoguanine residues. NMR spectroscopy revealed that 8-oxoguanine residues form stable quartets within G-quadruplex cores, characterized by a unique hydrogen-bonding scheme. These quartets exhibit a larger central cavity compared to canonical G-quartets, confirmed by DFT-optimized models and cation localization studies.

In two-stranded constructs, the position of 8-oxoguanine and abasic residues modulates the equilibrium between double-stranded DNA and G-quadruplexes. Despite the ability of spare G-tracts to rescue damaged sequences and facilitate G-quadruplex folding, most observed G-quadruplexes are kinetically trapped states, with double-stranded DNA as the preferred equilibrium structure. Constructs containing oxidative lesions exhibited varying degrees of structural polymorphism, influenced by lesion position.

This work provides novel insights into the structural and functional consequences of oxidative damage in G-rich genomic regions. The findings underscore the significance of oxidized lesions in influencing DNA stability and G-quadruplex formation. These results have implications for understanding the molecular basis of oxidative stress, mutagenesis, and the potential targeting of G-quadruplex structures in therapeutic strategies.