

**OXIDATIVE DNA DAMAGE BY
LONG-RANGE CHARGE TRANSPORT**

Thesis by
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ABSTRACT

Ever since the elucidation of the double helical structure of DNA, it has been proposed that the stack of base pairs within the double helix may mediate charge transport (CT) reactions. In fact, CT through DNA can result in chemistry at a distance, yielding oxidative DNA damage at a site remote from the bound oxidant. DNA CT chemistry depends upon coupling within the stacked base pair array, and this chemistry is remarkably sensitive to sequence-dependent DNA structure and dynamics. Using a variety of octahedral transition metal complexes, DNA CT has been probed to explore mechanistic considerations and biological possibilities.

Interactions with DNA by a family of ruthenium(II) complexes bearing the dipyridophenazine (dppz) ligand or its derivatives have been examined. An intercalative binding mode has been established based on luminescence enhancements in the presence of DNA, excited state quenching, fluorescence polarization values and enantioselectivity. Oxidative damage to DNA by these complexes using the flash/quench method has also been examined. A direct correlation between the amount of guanine oxidation obtained via DNA CT and the strength of intercalative binding was observed. These results support the importance of close association and intercalation for DNA-mediated CT. Electronic access to the DNA base pairs, provided by intercalation of the oxidant, is a prerequisite for efficient CT through the DNA π -stack.

Using polypyridyl ruthenium complexes, a reductive flash/quench scheme in DNA has also been explored. The flash/quench scheme previously utilized in DNA studies involves an oxidative quencher and allows for examination of electronic hole transport through DNA. In contrast, a reductive flash/quench technique would allow for

direct observation of electron transport through the base stack. In our studies, p-methoxydimethylaniline and potassium iodide have proven to be effective reductive quenchers of dipyridophenazine complexes of ruthenium. However, by transient absorption spectroscopy, high performance liquid chromatography, gel electrophoresis, and electron paramagnetic resonance we are unable to observe any DNA reduction products with the ruthenium complexes examined. Rates of back electron transfer may in fact be faster than trapping of the anion radical, thus hindering observation of long-range damage.

The oxidative flash/quench technique was applied in probing DNA CT in a range of DNA assemblies containing a tethered ruthenium intercalator and methylindole (M), a low potential nucleobase analog, where radical formation at a distance as a function of DNA sequence could be examined both by laser spectroscopy and biochemical methods. Hole injection and subsequent formation of the methylindole radical cation were observed at a distance of over 30 Å at rates $\geq 10^7$ s⁻¹ in assemblies containing no guanine bases intervening the ruthenium intercalator and GMG oxidation site. Radical yield was, however, strikingly sensitive to an intervening base mismatch; no significant methylindole radical formation was evident with an intervening AA mismatch. Also critical is the sequence at the injection site; this sequence determines initial hole localization and hence the probability of hole propagation. With guanine rather than inosine near the site of hole injection, decreased yields of radicals and long-range oxidative damage are observed. The presence of the low energy guanine site in this case serves to localize the hole and increase the probability of back reaction at the injection site therefore diminishing CT through the base pair stack.

DNA assemblies containing a pendant dppz complex of Ru(II) along with two oxidative traps, a site containing the nucleoside analog methylindole (5'-GMG-3') and a 5'-GGG-3' site, were constructed to explore charge equilibration across the base pair stack. In these assemblies the base radicals form with a rate of $\geq 10^7$ s⁻¹. Interestingly, the rate of base radical formation does not change upon the addition of a second radical trap, the 5'-GGG-3' site; however the yield of methylindole oxidation is significantly lower. This observation indicates that the 5'-GGG-3' site is effective in competing for the migrating charge and provides a second trapping site. Importantly, switching the orientation of the two trapping sites does not affect the yield of oxidized products at either site. Therefore, in DNA both forward and reverse charge transport occur so as to provide equilibration across the duplex on a time scale that is fast compared to trapping at a particular site. Further evidence of charge equilibration results from incorporating an intervening base-stacking perturbation and monitoring the fate of the injected charge. These experiments underscore the dynamic nature of DNA charge transport and reveal the importance of considering radical propagation in both directions along the DNA duplex.

DNA conjugates containing adjacent duplex and guanine quadruplex assemblies have been designed to explore CT into quadruplex architectures. The quadruplex assemblies have been characterized structurally using circular dichroism and by assaying for chemical protection. Using an intercalating rhodium photooxidant, noncovalently bound or tethered to the duplex end, oxidizing radicals are found to be trapped in the folded quadruplex. Damage is observed almost exclusively at the external tetrads of the quadruplex. Little damage of the center tetrad is observed, due most likely to lowered

efficiency of radical trapping within the quadruplex core. This pattern of damage is distinct from that observed for repetitive G sequences within duplex DNA. The data indicate, furthermore, that in the conjugates examined, the guanine quadruplex provides a more effective trap than a 5'-GG-3' guanine doublet within duplex DNA. Additionally, within these assemblies, sufficient base-base overlap must exist at the duplex/quadruplex junction to allow for charge migration. This funneling of damage to the quadruplex, as well as the unique pattern of damage within the quadruplex, requires consideration with respect to the analysis of oxidative DNA damage within the cell.

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