Chapter 6

Summary and Perspectives

A large body of past experiments has provided a strong foundation for the work presented here. Beginning with the first demonstration of DNA-mediated charge transport (CT) between metallointercalators in 1993,[1] strong evidence began to accumulate that the π stack of the DNA helix can serve as a medium for the conduction of charge. Through the development of new chemical systems and ingenious experimental devices, and the systematic modification of experimental parameters, the factors that affect the efficiency of DNA-mediated CT have come into focus.[2] It is now known that mismatches, lesions, and bulges intervening between the CT donor and the acceptor decrease the efficiency of the reaction. In addition, proteins that bend the DNA duplex disrupt CT, while proteins that fill gaps in the base stack with aromatic residues restore it. The efficiency of charge injection into DNA depends strongly on the driving force of the reaction and on the extent of electronic coupling between the CT trigger and the DNA base stack. Conversely, these factors also govern the efficiency of back electron transfer (BET), which decreases the yield of permanent products formed by DNA-mediated CT. We now undertand the factors affecting the efficiency of DNA-mediated CT at such a level that we can engineer chemical systems in which CT is intentionally facilitated or hindered.

We know considerably less about the factors affecting the rate of DNA-mediated CT. From the first fluorescence quenching experiments, in which an instrument with a response time of 10 ns was incapable of resolving the quenching event, it was apparent that this process is rapid. Ultrafast transient absorption experiments have shown that CT can occur over a distance of several base pairs at rates of $\sim 10^{11}$ s^{-1}. In studies involving ethidium as an electron acceptor, no dependence of the rate on distance was observed, but the distances that could be studied were limited by poor coupling of ethidium to the base stack.[3] In a related study, the absorbance of the excited charge donor, 2-aminopurine (Ap), was monitored over time in several DNA assemblies. With increasing distance between Ap and the charge acceptor, the lifetime of *Ap increased dramatically. The strong distance dependence observed in this system does not agree with the shallow distance dependence observed in the ethidium system or in guanine oxidation experiments.[4] Such discrepancies highlight our lack of knowledge regarding the kinetics of DNA-mediated CT. This is therefore an area

rich with possibilities for future research.

We have developed a rhenium-based vibrationally-active metallointercalor for use as a photooxidant in time-resolved studies of DNA-mediated CT. The photophysical characteristics and redox potential of $[Re(CO)_3(dppz)(py'\text{-}OR)]^+$ are similar to those of other Re photooxidants. Electrochemical and biochemical experiments show that the Re complex is a sufficiently strong oxidant from the excited state to oxidize guanine. Results from time-resolved infrared (TRIR) experiments agree. In the presence of DNA that does not contain guanine, a vibrational band due to the metal-to-ligand charge transfer (MLCT) excited state is observed. In solution with DNA that contains only guanine and cytosine, several observations suggest that quenching of the MLCT state occurs at an ultrafast time scale. These include the disappearance of the MLCT band, a relative decrease in the intensity of the other transient bands formed, and lower emission intensity. The formation of a transient band in this sample near 1700 cm^{-1} is also consistent with the formation of the guanine radical. These results show that excitation of these Re photooxidants can trigger long-range oxidation and that fast vibrational spectroscopy is a viable method by which to probe DNA-mediated CT.

The physical and photochemical characteristics of $[Re(CO)_3(dppz)(py'\text{-}OR)]^+$ are similar to those of other metal complex photooxidants. DNA binding leads to hypochromicity of the metal complex absorption and raises the melting temperature of DNA, indicating that the complex binds by intercalation. In guanine oxidation experiments, the photooxidation efficiency of the Re complex was between that of $[Rh(phi)_2(bpy')]^{3+}$ and that of $[Ir(ppy)_2(dppz')]^+$. When these complexes are bound to DNA, excitation of the metal complexes results in formation of their reduced states. This is consistent with the ability of the complexes to oxidize guanine. The lifetime of each reduced species, which corresponds to the rate of BET, is correlated to the yield of guanine damage, suggesting that BET is a major limiting factor in the formation of damage. These results verify our knowledge about the factors that affect the efficiency of DNA-mediated CT: the stronger binding strength and higher driving force for oxidation by the Rh complex result in a high yield of guanine damage, and the shorter excited state lifetime and more facile BET observed in the Ir

system result in a low yield of guanine damage.

Time-resolved spectroscopic experiments conducted in the presence of redox-active proteins are promising. Evidence has been observed for the DNA-mediated oxidation of p53 using the flash-quench technique. Similarly, a weak signal observed at long time by TA spectroscopy upon the addition of SoxR to solution is consistent with oxidation of this protein. The results of experiments on EndoIII are not as straightforward, although it is clear that the addition of protein does affect the system. These preliminary results support biological models for the use of these proteins in DNA-mediated lesion detection and transcriptional activation. In future expeirments, refinement of the experimental parameters and more sensitive instrumentation may enable measurements of the rates of DNA-mediated protein oxidation.

Much is yet to be learned about the factors affecting the kinetics of DNA-mediated CT. In light of the successful use of TRIR spectroscopy to study the oxidation of guanine, time-revolved vibrational spectroscopy will be an invaluable tool for understanding how DNA-mediated CT systems evolve over time. Similarly, the preliminary evidence for the DNA-mediated oxidation of p53, SoxR, and EndoIII will provide a solid foundation for further spectroscopic experiments involving redox-active proteins. Just as our work builds on that of others who have come before us, the experimental results presented here will provide a basis for future explorations of DNA-mediated CT.

References

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