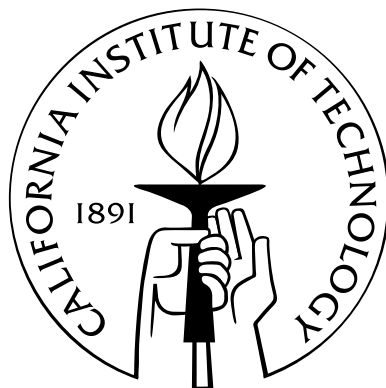


Investigating DNA-Mediated Charge Transport by Time-Resolved Spectroscopy

Thesis by
Eric Daniel Olmon

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Abstract

In all organisms, oxidation threatens the integrity of the genome. Numerous studies have suggested that DNA-mediated charge transport (CT) may play an important role in the sequestration, detection, and repair of oxidative damage. To fully understand the mechanism of DNA-mediated CT, it is necessary to characterize transient intermediates that arise during the reaction and to determine the lifetimes of these intermediates. Time-resolved spectroscopy is the most appropriate experimental method for such observations. Each intermediate has a characteristic spectrum. By observing time-dependent changes in the absorption spectrum of the sample, it is therefore possible to determine what species are present at a particular time and how long it exists in solution. Experiments presented here involve the use of time-resolved spectroscopy to better understand the process of DNA-mediated CT.

The study of DNA-mediated CT requires a robust and consistent method for triggering the CT reaction. The metal complexes that have traditionally been used for this purpose provide several advantages over organic phototriggers: they are synthetically versatile, they are stable in solution, they exhibit rich photophysics, and many are strong photooxidants. However, the spectroscopic features used to follow the photochemical processes triggered by these probes are generally broad optical bands. These can be difficult to resolve in samples that contain several absorbing species. For this reason, we have developed a Re photooxidant bearing a set of vibrationally active carbonyl ligands that can be covalently tethered to DNA. Unlike many absorption bands in the visible range, the vibrational absorption bands of these ligands are narrow, well-resolved, and specific. Such probes can be used to follow the complex photophysical pathways observed in biochemical systems with good precision, making them useful for the study of DNA-mediated CT.

Specifically, the complex $[\text{Re}(\text{CO})_3(\text{dppz})(\text{py-OR})]^+$ (dppz = dipyrido[3,2-*a*:2',3'-*c*]-phenazine; py'-OR = 4-functionalized pyridine) offers IR sensitivity and can oxidize DNA directly from the excited state. The behavior of several covalent and noncovalent Re-DNA constructs was monitored by time-resolved IR (TRIR) and UV/visible spectroscopies, as well as biochemical methods, confirming the ability of the complex to trigger long-range oxidation of DNA. Optical excitation of the complex leads to population of metal-to-ligand charge transfer excited states and at least two distinct intraligand charge transfer excited states. Several experimental observations are consistent with charge injection by excited Re^* . These include similarity between TRIR spectra and the spectrum of reduced Re observed by spectroelectrochemistry, the appearance of a guanine radical signal in TRIR spectra, and the eventual formation of permanent guanine oxidation products. The majority of reactivity occurs on the ultrafast time scale, although processes dependent on slower conformational motions of DNA, such as the accumulation of oxidative damage at guanine, are also observed.

The photooxidation activity of this Re complex was compared directly to that of other metallointercalators that have been used previously in our laboratory to oxidize DNA. The complexes $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ (phi = 9,10-phenanthrenequinone diimine; bpy' = 4-methyl-4'-(butyric acid)-2,2'-bipyridine), $[\text{Ir}(\text{ppy})_2(\text{dppz}')]^+$ (ppy = 2-phenylpyridine; dppz' = 6-(dipyrido[3,2-*a*:2',3'-*c*]phenazin-11-yl)hex-5-ynoic acid), and $[\text{Re}(\text{CO})_3(\text{dppz})(\text{py}'\text{-OH})]^+$ (py'-OH = 3-(pyridin-4-yl)-propanoic acid) were each covalently tethered to DNA. Biochemical studies show that upon irradiation, the three complexes oxidize guanine by long-range DNA-mediated CT with the efficiency: $\text{Rh} > \text{Re} > \text{Ir}$. Comparison of spectra obtained by spectroelectrochemistry after bulk reduction of the free metal complexes with those obtained by transient absorption (TA) spectroscopy of the conjugates suggests that excitation of the conjugates at 355 nm results in the formation of the reduced metal states. Electrochemical experiments and kinetic analysis of the TA decays verify that the primary factors responsible for the trend observed in the guanine oxidation yield of the three complexes are the thermodynamic driving force for CT, variations in the efficiency of back electron transfer, and coupling to DNA.

The ability of redox-active DNA-binding proteins to act as hole sinks in DNA-mediated CT systems was also studied by time-resolved spectroscopy. Such experiments are designed to provide support for the utilization of DNA-mediated CT in biological systems. In studies involving the cell cycle regulator p53, photoexcitation results in the formation of a weak transient band at 405 nm. This band, which is not observed in samples lacking the protein, resembles the primary spectral feature of the tyrosine cation radical. Although the signal is weak and reproducibility is inconsistent, these results suggest that photolysis of the sample leads to DNA-mediated oxidation of tyrosine in p53. Similar experiments were conducted on the transcriptional activator SoxR. Here, the presence of dithionite, required in solution to keep the protein reduced, complicates the photochemistry of the system considerably. Regardless, a weak absorbance at 418 nm that develops following photolysis at 355 nm provides evidence for the DNA-mediated oxidation of the protein. The behavior of the base excision repair protein endonuclease III was also observed in the presence of DNA and metal complex oxidants. In flash-quench studies, addition of the protein results in the formation of a strong negative signal at 410 nm in TA traces. In studies involving direct photooxidation by Rh, Ir, and Re complexes, no new transients are detected upon the addition of protein, but changes in the intensities of the resultant TA spectra and in the steady-state absorbance spectra following photolysis indicate that DNA-mediated oxidation of the protein may be taking place.

The experiments described here comprise several new developments in the story of DNA-mediated CT. First, proof of concept has been given for a valuable new vibrationally-active Re probe. Further modifications on the characteristics of this complex and further study by time-resolved vibrational spectroscopy will allow us to observe DNA-mediated CT with high spectral resolution. Second, comparison between this Re probe and established photooxidants shows that the Re complex is a strong photooxidant in its own right and that this complex can be added to our growing toolbox of CT phototriggers. Third, time-resolved studies involving redox-active proteins have provided preliminary direct evidence for the ability of these proteins to serve as CT probes themselves. Further refinement of the experimental methods used in these experiments will allow us to observe such processes

with greater sensitivity, increasing our knowledge of the mechanism and applications of DNA-mediated CT.

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