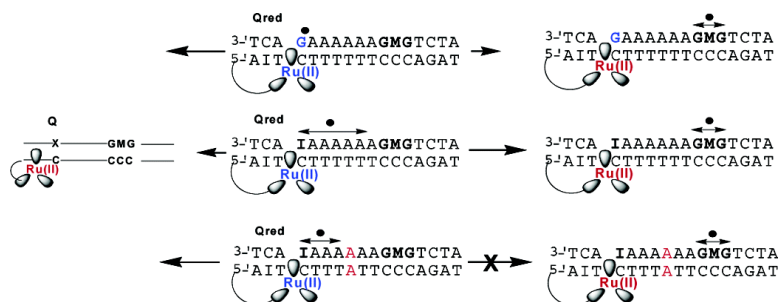## Communication

Rapid Radical Formation by DNA Charge Transport through Sequences Lacking Intervening Guanines

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Rapid Radical Formation by DNA Charge Transport through Sequences Lacking Intervening Guanines

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DNA charge transport (CT) has been the focus of considerable investigation given its potential importance to cellular mechanisms of oxidative damage, its application in DNA-based sensors, and the general interest in developing a mechanistic understanding of electron-transfer chemistry over long distance.[1,2] Oxidative damage mediated by DNA has been demonstrated over a distance of 200 Å.[3,4] Mechanistically, a mixture of tunneling and hopping has been proposed.[5-8]

We have prepared assemblies with pendant ruthenium intercalators containing the dipyridophenazine (dppz) ligand as oxidant to probe DNA CT both spectroscopically and biochemically.[3,9] Using the flash quench reaction[10] to generate the ruthenium(III) oxidant in situ, these assemblies provide a powerful system for characterizing long range DNA CT. An excited state complex, $*Ru^{2+}$, is oxidatively quenched by a nonintercalating electron acceptor (Q) to form Ru^{3+} and reduced quencher, Q^{red}. The Ru^{3+} complex can react with Q^{red} or oxidize an electron donor such as guanine or methylindole.[11] The resultant base radical can also react with Q^{red} or undergo further reactions to form irreversible oxidative products. In Ru-tethered assemblies containing methylindole as an artificial base, the formation of the methylindole radical cation has been characterized by transient absorption and EPR spectroscopies and the resultant irreversible damage has been monitored biochemically.[12] CT to form the methylindole radical cation occurs at the rate $\geq 10^7$ s^{-1} and is independent of distance over 17−37 Å. Here we present results on a series of Ru-modified DNA duplexes of varied sequence.

The DNA assemblies each include a 4-methylindole nucleoside (M) embedded between two G bases for greater stability as well as [Ru(bpy′)(dppz)(phen)]$^{2+}$ tethered to the 5′ end of the oligomer; this tethered complex intercalates[9] 2−3 base pairs from the end of the duplex. The Ru−M distance of 31 Å is held constant. Sequences contain either a G or inosine (I) at the hole injection site with the intervening sequence to the GMG oxidation site varied as all A's, all T's, or containing an intervening AA mismatch. Luminescence decays ($\tau_1 = 80$ ns, $\tau_2 = 280$ ns) and quenching yields (~85%) are equivalent in all duplexes, reflecting the minimal perturbation of the I substitution.

Figure 1 shows transient absorption data monitored at 600 nm after laser excitation at 470 nm for assemblies **Ru-I-A-M** and **Ru-I-A-M-mis**. For **Ru-I-A-M**, the data at 600 nm clearly show the rise of a positive signal consistent with the formation of the methylindole radical cation. Associated with the rise of this positive signal is a rapidly decaying component of the negative signal at 440 nm (*vide infra*). These rates are both $\geq 10^7$ s^{-1} and show that oxidation to form the methylindole radical cation 31 Å away is indistinguishable from Ru^{3+} reduction.[13] Importantly, in **Ru-I-A-M**, there are no guanines intervening or at the site of hole injection, yet CT is rapid.

Significantly, the transient at 600 nm for **Ru-I-A-M-mis**, which contains an AA mismatch intervening the Ru and methylindole,
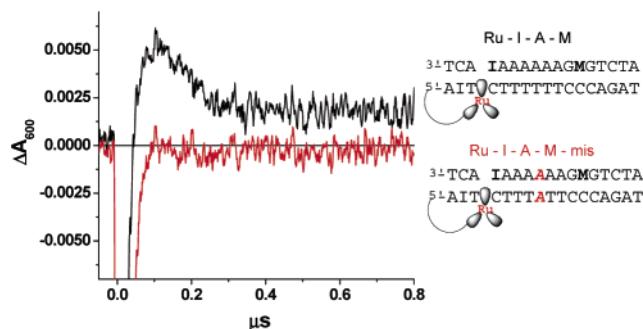

Figure 1. Time-resolved transient absorption traces for **Ru-I-A-M** and **Ru-I-A-M-mis** at 600 nm using instrumentation as previously described.[12] Samples contained 20 μM Ru-DNA, 300 μM [Ru(NH$_3$)$_6$]$^{3+}$, 10 mM NaCl, 10 mM potassium phosphate, pH = 7, λ_{exc} = 470 nm. A large initial negative spike is due to emission from $*Ru^{2+}$.
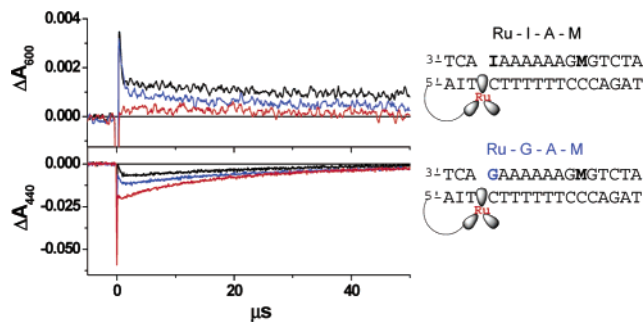

Figure 2. Time-resolved transient absorption traces for **Ru-I-A-M, Ru-G-A-M**, and **Ru-I-A-M-mis** measured at 600 nm (top) and 440 nm (bottom). Conditions are as in Figure 1.

indicates negligible formation of the methylindole radical cation. Notably, in this assembly, despite the lack of formation of product radical, the transient at 440 nm (Figure 2) is consistent with rapid hole injection. This observation of an attenuated yield in radical formation in the mismatch-containing assembly is wholly consistent with the diminished yields of CT products in mismatched DNAs observed by gel analysis[14] or by electrochemistry.[15] Therefore, the intervening mismatch clearly inhibits radical formation.[16]

Diminished radical formation is also evident with guanine substitution at the site of hole injection. In Figure 2, the transients on a longer time scale for **Ru-I-A-M** are compared to those for **Ru-G-A-M** as well as for **Ru-I-A-M-mis**. Here the expectation might have been that hole injection into the bridge would be less efficient for **Ru-I-A-M** than **Ru-G-A-M** because of the higher oxidation potential of I at the injection site.[17] We observe rapid formation of the radical in both cases. Nonetheless, the 600 nm signal is noticeably larger for **Ru-I-A-M** than that for **Ru-G-A-M** at long time (≥ 2 μs). Similar differences in radical yield are evident with T's intervening (data not shown). In fact, substitution at the injection site may have a more pronounced effect on radical yield
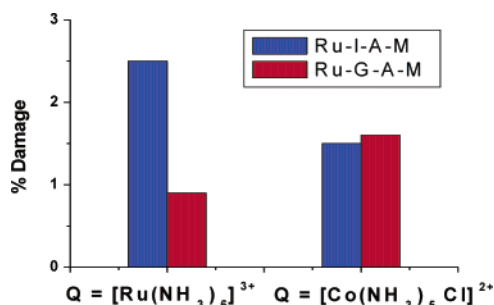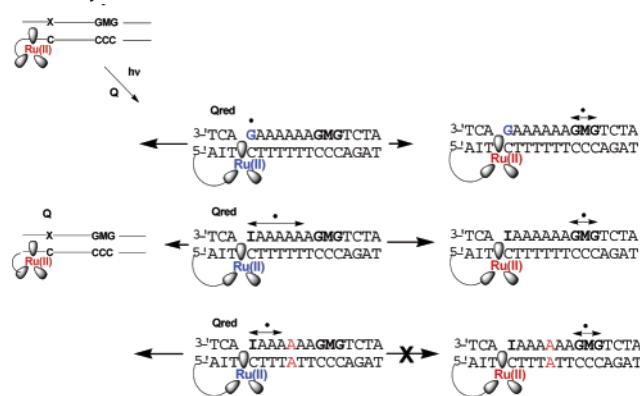
Figure 3. Quantitation of damage yields in assemblies **Ru-I-A-G** and **Ru-G-A-G** as a function of different quenchers after 5 min of irradiation at 470 nm. Samples contained 5 μM Ru-DNA, 125 μM quencher, 50 mM NaCl, and 5 mM sodium phosphate, pH 7.

Scheme 1. Proposed Model for Hole Injection and Subsequent Reactivity

than well-matched sequence variations in the intervening bridge. At 440 nm we also see a component with a slow rate (4×10^4 s^{-1}) of Ru^{2+} recovery, reflecting unreacted Ru^{3+}, and as expected, the amount of this signal correlates inversely with the extent of methylindole radical formation at 600 nm.

We can account for the differences seen in **Ru-I** versus **Ru-G** assemblies on the basis of the extent of radical delocalization and its effects on subsequent reaction with Q^{red}. To test this notion, DNA damage quantitation was carried out using two different quenchers on analogous assemblies to **Ru-I-A-M** and **Ru-G-A-M** where a GGG was substituted for GMG (Figure 3).[18] Using [Ru(NH$_3$)$_6$]$^{3+}$ as Q, more oxidative damage is observed at the GGG site in duplex **Ru-I-A-G** compared to duplex **Ru-G-A-G**, consistent with our spectroscopic measurements of radical yield. However, reaction of the DNA radical with the diffusive quencher at the site of hole injection can be minimized using a sacrificial quencher such as [Co(NH$_3$)$_5$Cl]$^{2+}$, which is unstable in its reduced form. Indeed, the damage yield at the GGG site with [Co(NH$_3$)$_5$Cl]$^{2+}$ is comparable for **Ru-I-A-G** and **Ru-G-A-G**.[19] Thus, the yield of oxidative damage in a remote DNA site is modulated sensitively by reactivity at the injection site.[20]

Scheme 1 illustrates our model to account for these data. After flash-quench, in assembly **Ru-G-A-M**, we expect hole injection to be centered on the guanine; for **Ru-I-A-M**, hole injection may be more delocalized since the -IAAAAAA- bridging bases all have similar oxidation potentials. For both DNA radicals, two pathways are then available: hole migration to form the lowest energy methylindole radical, or reaction with Q^{red}. For **Ru-I-A-M-mis**, CT

to form the methylindole radical is disrupted, so the hole remains localized proximal to the injection site, and only reaction with Q^{red} is available. Importantly, more efficient reaction with the reduced quencher is likely with the hole localized in the proximity of the original quenching. Thus, for **Ru-G-A-M**, reaction with Q^{red} is more favorable than for **Ru-I-A-M**, where the hole is more delocalized. This difference is necessarily tempered by the stability of Q^{red}; with the sacrificial cobalt quencher, reaction to form the lowest energy methylindole radical is equally favored for both assemblies. Given the possibility of reaction with Q^{red}, however, the presence of guanine at the injection site does not serve to facilitate CT but instead diminishes net product yield.

In summary, 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 with no intervening guanines. Radical yield was, however, strikingly sensitive to an intervening mismatch. Also critical is the sequence at the injection site since this sequence determines initial hole localization and hence the probability of hole propagation. Indeed, here the presence of a guanine site serves to increase hole localization and diminish CT through the base pair stack.

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