CHAPTER 6

The Importance of Intercalation in Long-range Guanine Oxidation in DNA Duplexes.

Adapted and Modified from Delaney, S.; Pascaly, M.; Bhattacharya, P. K.; Han, K; Barton J. K. (2001) *Inorganic Chemistry,* 41, 1966-1974 to include the work contributed by PKB.

Abstract

The role of intercalation in long-range charge transport has been explored. Towards that goal, oxidative damage to DNA through DNA-mediated charge transport was compared directly for two DNA-tethered ruthenium complexes. One contains the dppz ligand (dppz = dipyridophenazine) that binds avidly by intercalation, and the other contains only bpy ligands (bpy = 2,2'-bipyridine), that, while bound covalently, can only associate with the base pairs through groove binding. Long range oxidative damage was observed only with the tethered, intercalating complex. These results, taken together, all support the importance of close association and intercalation for DNA-mediated charge transport. Electronic access to the DNA base pairs, provided by intercalation of the oxidant, is a prerequisite for efficient charge transport through the DNA π-stack.

Introduction

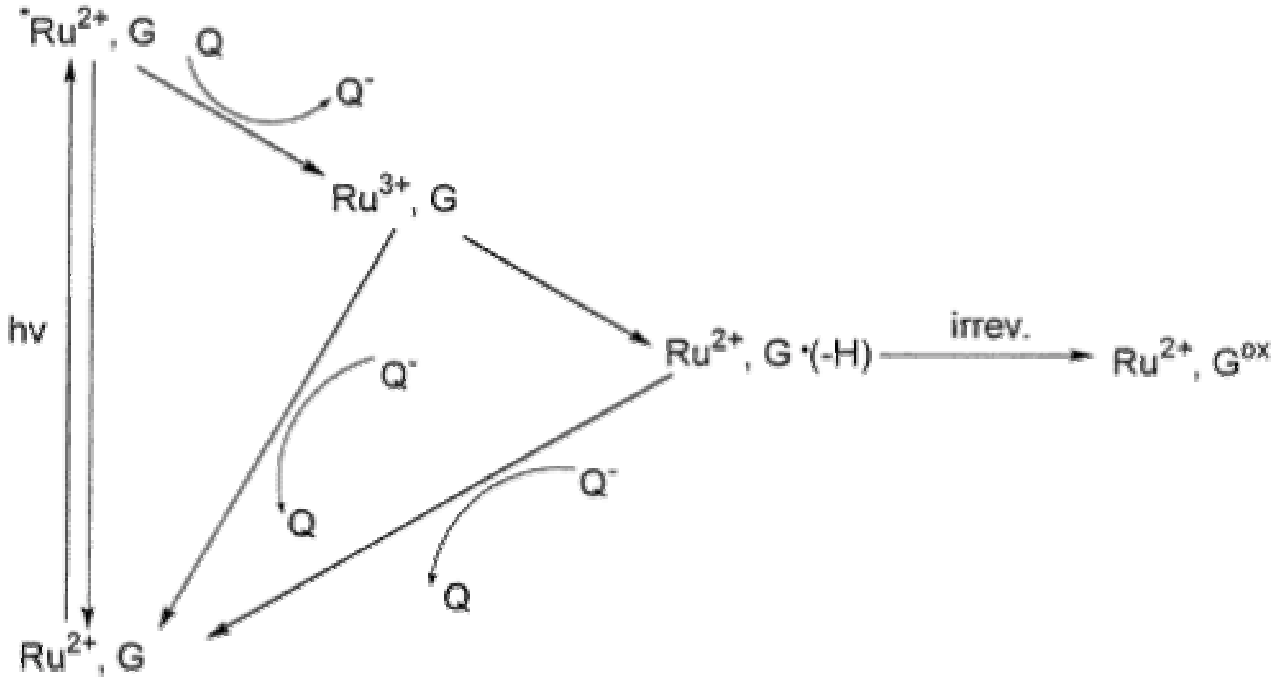
Charge transport through DNA has been shown to require proper stacking of the π-orbitals of the heterocyclic nucleobases (1). When base bulges (2), mismatches (3,4), or nonaromatic residues (5,6) are inserted into the π-stack, charge transport is efficiently shut off. When properly stacked, the DNA π-array has been shown to mediate guanine oxidation at sites 200 Å from a remotely bound oxidant (7, 8). Oxidative damage to DNA from a distance is therefore necessarily sensitive to the intervening DNA sequence and structure (9-12).

Besides proper π-stacking, energetic driving force is a requirement for charge-transfer reactions. Guanine is the easiest nucleobase to oxidize with a potential of 1.29 V versus NHE (13). *Ab initio* molecular orbital calculations predict that in a 5'-GG-3' guanine doublet, the bulk of the HOMO lies on the 5'-G, which has a lower oxidation potential than a single guanine (14, 15).

The work presented here was designed to address a third aspect considered to affect the oxidation of guanine sites in DNA: the ability of the oxidant to intercalate into the π-stack. This intercalation is proposed to result in more effective coupling to the DNA π-array and more efficient hole injection, facilitating long-range charge migration through the π-stack. To probe the importance of intercalation to oxidative damage by long-range charge transport directly, we compared oxidative damage patterns for $Ru(bpy)_2(bpy')^{2+}$ and $Ru(phen)(dppz)(bpy')^{2+}$ (dppz = dipyridophenazine), covalently bound to DNA (Figure 1). The bpy complex shows no intercalative interaction with the duplex while the dppz complex binds avidly by intercalation. Dppz complexes of ruthenium have been extensively studied owing to their unique luminescence properties when bound to DNA (16, 17). These complexes vary both in their ability to stack

intercalatively within the DNA helix and in their efficiency in promoting oxidative DNA damage.

Oxidative damage to DNA is generated with these ruthenium complexes through a flash/quench experiment (18). The flash/quench methodology, originally developed to explore charge transport reactions in proteins (19), has been effectively applied in characterizing transient radical intermediates in the DNA charge transport process (6) and in generating protein/DNA cross-links (20, 21). Scheme 1 illustrates the series of reactions associated with the flash/quench experiment. The cycle is initiated by visible light, which excites the intercalated ruthenium(II) complex. The excited ruthenium(II) complex, *Ru(II), is then quenched by a nonintercalating electron accepting quencher, Q, such as methyl viologen (MV^{2+}), so as to form Ru(III) *in situ*. This species can be reduced back to Ru(II) either through recombination with reduced quencher (Q^{-}) or by electron transfer with guanine (G). The oxidized guanine radical can then return to its resting state by reaction with reduced quencher or undergo further reaction to form a family of oxidative products, G_{ox} (22). In addition to Ru(II) complexes that contain the dppz ligand, $Ru(bpy)_3^{2+}$, which possess the necessary driving force to oxidize DNA but do not intercalate as well as complexes containing the dppz ligand, have been examined.



Scheme 1. Schematic Illustration of the Flash/Quench Methodology

Experimental Section

Materials. $[Ru(bpy)_3]Cl_2$ were purchased from Aldrich and recrystallized from water prior to use. Phosphoramidites were from Glen Research and were used as received.

Metal Complex Synthesis. The three-ligand complex $[Ru(phen)(bpy')(dppz)]Cl_2$ (bpy' = 4-butyric acid-4'-methyl-2,2'-bipyridine) was prepared according to literature procedures (23, 24),and $[Ru(bpy)_2(bpy')]Cl_2$ was prepared from $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (23). For all complexes prepared, ^1H NMR and FAB-MS analyses agreed with values expected.

Oligonucleotide Synthesis. Oligonucleotides were synthesized on an ABI 392 DNA/RNA synthesizer, using standard phosphoramidite chemistry (25). DNA was synthesized with a 5'-dimethoxy trityl (DMT) protecting group and was purified by HPLC using a Dynamax 300 Å C18 reverse-phase column (Rainin) on a Hewlett-Packard 1100 HPLC (95% 30 mM NH_4OAc/5% acetonitrile to 84% 30 mM NH_4OAc/16% acetonitrile over 25 min). The DMT group was removed by incubation with 80% glacial acetic acid for 12 min at ambient temperature and then repurified by HPLC (100% 30 mM NH_4OAc to 75% 30 mM NH_4OAc/25% acetonitrile over 40 min). Quantification was done on a Beckman DU 7400 spectrophotometer using the ε_{260} values estimated for single stranded DNA (26).

Ruthenium-tethered 17-mer oligonucleotides were prepared as described previously (27) and were purified on a Dynamax 300 Å C18 reverse-phase column (Rainin) on a Hewlett-Packard 1050 HPLC (85% 30 mM NH_4OAc/15% acetonitrile to 75% 30 mM NH_4OAc/25% acetonitrile over 40 min). The ruthenium-conjugated oligonucleotides were characterized by mass spectrometry and quantitated using the following extinction coefficients: $Ru(phen)(bpy')(dppz)^{2+}$ modified oligonucleotides ε_{432} = 19000 M^{-1} cm^{-1}; $Ru(bpy)_2(bpy')^{2+}$ modified oligonucleotides ε_{453} = 21000 M^{-1} cm^{-1}.

Assay of Oxidative DNA Damage. Single strand complements to the ruthenium-modified oligonucleotides were 5'-^{32}P end-labeled as described (28) and annealed in an aerated buffer of 35 mM Tris-HCl, 5 mM NaCl, pH 8.0. Oligonucleotide duplexes (2.5 μM) with 25 μM MV^{2+} as an electron accepting quencher were irradiated for 5 min at 432 nm using a 1000 W Hg/Xe lamp equipped with a monochromator. After irradiation, samples were treated with 10% piperidine at 90 °C for 30 min, dried, and electrophoresed through a 20% denaturing polyacrylamide gel. The extent of damage was quantitated by phosphorimagery (ImageQuant).

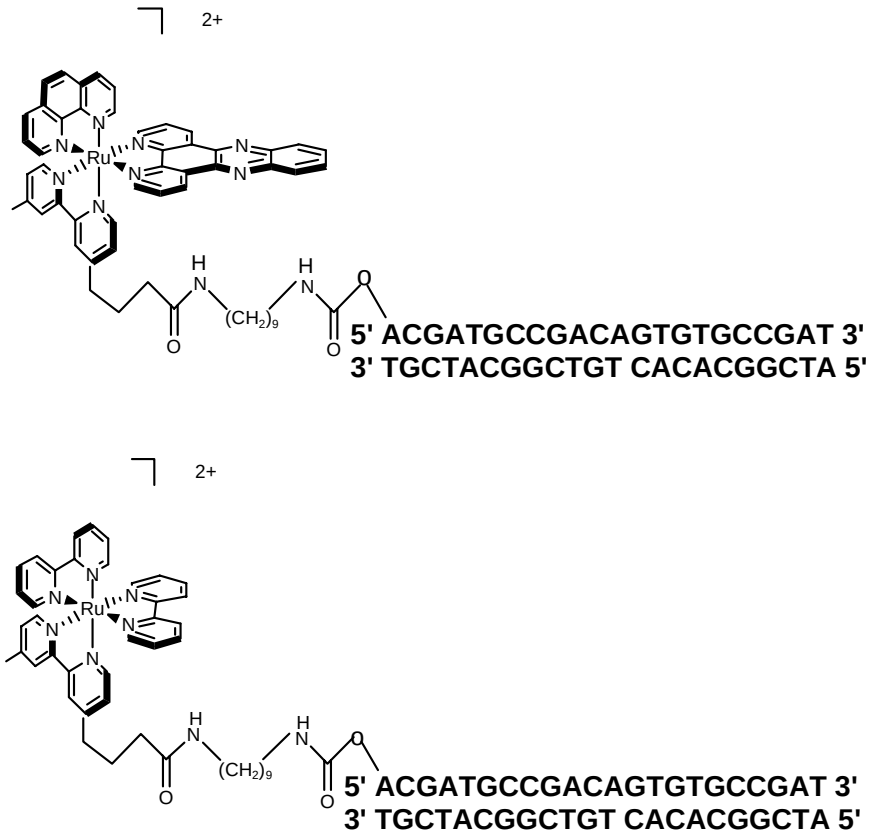


Figure 1. Constructs of metal complex covalently tethered to DNA duplexes employed in the experiment

Results & Discussions

Oxidative Damage by Covalently Bound Ruthenium Complexes. To probe the importance of intercalation to oxidative damage by long-range charge transport most directly, we compared oxidative damage patterns for $Ru(bpy)_2(bpy')^{2+}$ and $Ru(phen)(dppz)(bpy')^{2+}$ covalently bound to DNA (Figure 1). The bpy complex shows no intercalative interaction with the duplex while the dppz complex binds avidly by intercalation. By tethering the two complexes to the DNA duplex, one can therefore distinguish the effects of intercalation from simply a low association with the helix.

Figure 2 shows the results. As is evident, there is no guanine damage on the duplex containing the covalently bound $Ru(bpy)_2(bpy')^{2+}$ (lane 14) just as there is no guanine damage in the case of noncovalently bound $Ru(bpy)_3^{2+}$ when the ratio of oligonucleotide to metal is 1:1 (lane 10). It is noteworthy that significant damage at all guanines is evident at a higher concentration of noncovalent $Ru(bpy)_3^{2+}$ (lane 8). We attribute this oxidation to direct association of the ruthenium complex with the guanine site. Also, for comparison, the expected long-range guanine oxidation is observed with both the covalently (lane 12) and noncovalently bound (lanes 4, 6) dppz derivatives of ruthenium. Interestingly, in the case of noncovalently bound $Ru(bpy)_2(dppz)^{2+}$, damage is also observed at the adenine 5' to the proximal double guanine site. This adenine, as part of a purine tract, may be particularly susceptible to oxidative damage. Control experiments confirm that the long-range guanine damage occurs intraduplex and is not a result of metal intercalation into DNA other than that to which it is covalently tethered (7).

The importance of intercalation is directly illustrated in experiments comparing reactions of covalently bound ruthenium complexes. By tethering the complexes to the DNA duplex, one can distinguish the effects of intercalation from simply a low

association with the helix. When the bpy complex is tethered to DNA, and thus linked to the helix, no DNA charge transfer damage from a distance results; damage is evident only at the site of ruthenium association. We attribute this lack of reactivity at distal positions to the lack of coupling of the ruthenium oxidant into the base pair stack. However, in the case of a tethered dppz complex, extensive damage is observed across the helix and at a site distant from ruthenium intercalation. It is noteworthy that at high enough metal concentrations guanine damage can be observed with noncovalent $Ru(bpy)_3^{2+}$; we attribute this damage to direct contact between guanine and $Ru(bpy)_3^{3+}$ generated in solution. Such reactivity by $Ru(bpy)_3^{3+}$ is precedented (29),although no oxidative damage to DNA *from a distance* has been observed with $Ru(bpy)_3^{3+}$.

It is apparent from this study, that the ability of the metal complex to intercalate into the DNA π-array has been found to affect directly the extent of DNA charge transport and resultant damage. Intercalation can lead to more effective coupling into the π-stack, resulting in more efficient hole injection and charge transport. These results require consideration in comparing reactions on DNA with different photooxidants. The source of charge injection into DNA is therefore a critical parameter in determining the extent of oxidative DNA damage from a distance.

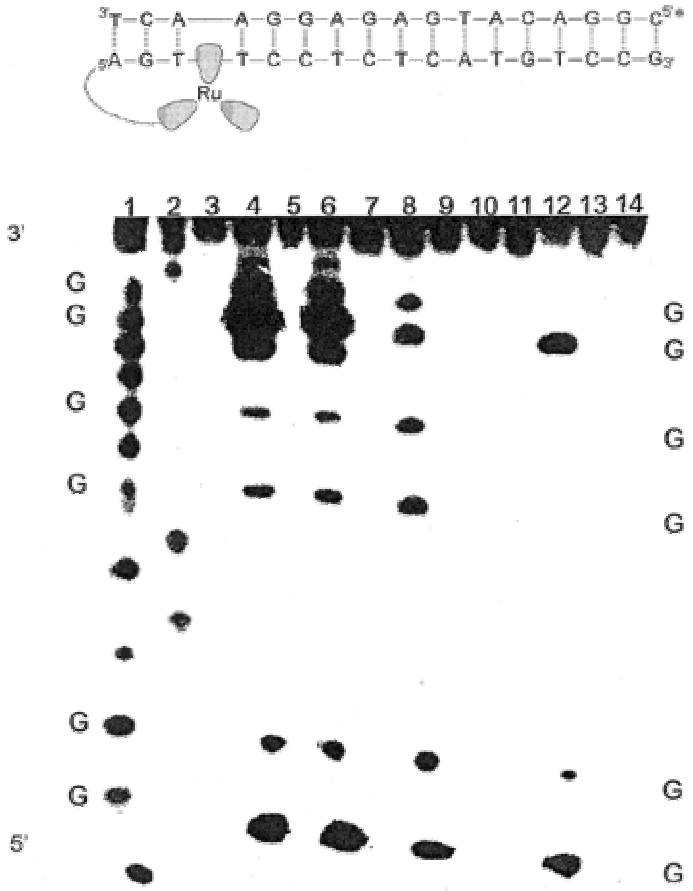


Figure 2. DNA damage by covalent versus noncovalent ruthenium complexes. Shown at the top is the ruthenium-oligonucleotide conjugate used in covalently tethered experiments. Site of ^{32}P labeling is indicated by *. Shown at the bottom is the autoradiogram of the ruthenium-oligonucleotide conjugate after oxidation by noncovalent and covalent bpy and dppz complexes. Samples contain 2.5 μM ruthenium-oligonucleotide and 25 μM MV^{2+} for covalently bound experiments. Noncovalently bound experiments utilized 2.5 μM oligonucleotides, 25 μM MV^{2+}, and indicated metal to DNA ratio. All irradiations were at 432 nm for 5 min. Lanes 1 and 2 show damage pattern after Maxam-Gilbert sequencing reactions A + G and C + T, respectively. Lanes 3, 4 and 5, 6 show damage pattern for oxidation of the oligonucleotide by $Ru(bpy)_2(dppz)^{2+}$, 4:1 and 1:1 metal to DNA, respectively, in absence of light and after irradiation. Lanes 7, 8 and 9, 10 show damage pattern for oxidation by $Ru(bpy)_3^{2+}$, 4:1 and 1:1 metal to DNA, respectively, in absence of light and after irradiation. Lanes 11 and 12 show damage pattern after oxidation by covalently bound $Ru(phen)(dppz)(bpy')^{2+}$, in absence of light and after irradiation, respectively. Lanes 13 and 14 show damage pattern by covalently bound $Ru(bpy)_2(bpy')^{2+}$, in absence of light and after irradiation, respectively.

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