Oxidation of Guanine in DNA by $Ru(phen)_2(dppz)^{3+}$ Using the Flash-Quench Technique

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Abstract: A flash-quench method has been developed to probe oxidative damage to DNA. A photoexcited Ru(II) intercalator is quenched in DNA by a weakly bound, electron-transfer quencher to generate Ru(III), a powerful ground-state oxidant. Once generated, Δ-$Ru(phen)_2(dppz)^{3+}$ bound to poly(dG-dC) rapidly oxidizes guanine within the DNA duplex. Transient absorption spectroscopy indicates rapid formation of the neutral guanine radical within the DNA duplex. Permanent damage resulting from the flash-quench experiment is monitored by gel electrophoresis of synthetic oligonucleotide duplexes. Oxidative damage, visualized by treatment with piperidine, occurs selectively at the 5′-G of 5′-GG-3′ sites and at the 5′- and central G of 5′-GGG-3′ triplets; enzymatic digestion in the absence of piperidine treatment shows formation of 8-oxo-2′-deoxyguanosine with $Ru(NH_3)_6^{3+}$ as quencher. The yield of base damage is, furthermore, modulated by the choice of electron-transfer quencher. Quantum yields for damage vary in the order $Ru(NH_3)_6^{3+}$ < methyl viologen^{2+} < $Co(NH_3)_5Cl^{2+}$ and correlate with the instability of the reduced quencher. The flash-quench method, combining spectroscopy and product analysis, offers a novel and tunable approach to explore electron transfer chemistry on double helical DNA.

Introduction

Understanding the rich electron transfer (ET) chemistry of DNA is essential in characterizing oxidative damage to the double helix.[1] Photoinduced electron transfer reactions with the DNA double helix as a bridge between bound donors and acceptors have been probed via luminescence and transient absorption spectroscopies.[2−5] Studies in our laboratory have indicated that DNA-mediated ET is sensitive to π-stacking,[2] can occur on the picosecond time scale with metallointercalators,[4] and, with tethered intercalators, can result in luminescence quenching over long range.[5] ET chemistry can also occur over a long range with DNA as a reactant.[6,7] Reactions between intercalators and the DNA bases have been probed primarily through the chemical analyses of ET products.[6−11] Here, we apply the flash-quench technique,[9] developed to characterize ET reactions in proteins,[12] to probe damage to DNA both spectroscopically and through analysis of the resultant DNA lesions.

Several laboratories have investigated the oxidative damage of DNA.[6,8−11,14−17] Damage is observed primarily at guanine (G), as predicted by theoretical and experimental studies which have determined that G is the most easily oxidized base. Anthraquinones,[8] napthalimides,[11] riboflavin,[10] and rhodium(III) intercalators[6] have been shown to cause oxidative damage selectively at 5′-GG-3′ sequences. The sites of damage are correlated with the oxidation potentials of G in different sequence contexts;[11] thus, the extended electronic structure of the DNA base stack may determine the extent and sequence-specificity of oxidative damage. Externally bound Ru(III) complexes, generated electrochemically, also have sufficient oxidation potential to react with guanines within the base stack.[14] Recently, in our laboratory, the potent photooxidant and intercalator, $Rh(phi)_2(bpy)^{3+}$, was tethered to a DNA oligonucleotide duplex, and, with this assembly, it was demonstrated that these oxidation reactions could also proceed from a remote position through the DNA π-stack over a distance of ∼37 Å.[6]

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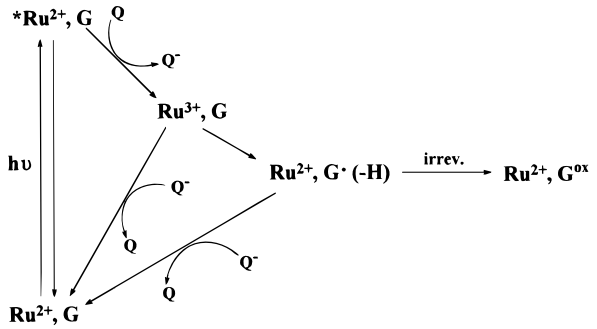
Scheme 1. Flash-Quench Methodology



These studies suggested to us that, by exploiting the characteristics of intercalation, we could oxidize G within the DNA duplex by generating a Ru(III) intercalator *in situ*. Ru(II) polypyridyl chemistry offers the opportunity to tune ET reactions and follow them spectroscopically. An analogous ruthenium flash-quench experiment has been profitably applied in studies of protein-mediated ET.[12] Kochevar and co-workers have used the flash-quench experiment in DNA with ethidium as an intercalator and methyl viologen (MV^{2+}) as a quencher to demonstrate a net reaction at guanines by gel electrophoresis.[9]

Scheme 1 describes the series of ET reactions in our version of the flash-quench experiment. The cycle is initiated by visible light, which excites intercalated $Ru(phen)_2(dppz)^{2+}$ [Ru(II); dppz = dipyridophenazine].[13] The excited ruthenium(II) complex, *Ru(II), is then quenched by a nonintercalating electron acceptor (Q) to form Ru(III); this species can be reduced back to Ru(II) either through bimolecular recombination with reduced quencher Q^- or by electron transfer with a nearby guanine base (G). The oxidized guanine radical can then return to its resting state by reaction with the reduced quencher or undergo further reaction to form the oxidation product(s) G^{ox}.

Here, we describe the oxidation reaction in DNA generated through the flash-quench experiment. Using transient absorption spectroscopy, we characterize the formation and decay of the guanine radical in poly(dG-dC) and in a mixed sequence of DNA. Furthermore, we show that the flash-quench methodology yields permanent damage at 5′-GG-3′ and 5′-GGG-3′ sequences. In this experiment, oxidative damage can be a major pathway for decay of the guanine radical, and the quantum yield can be tuned by the choice of quencher.

Experimental Section

Materials. DNA polymers were purchased from Pharmacia and were dialyzed against a buffer of 5 mM phosphate, 50 mM NaCl, pH 8.5 prior to use. Oligonucleotides were prepared on an Applied Biosystems 394 DNA synthesizer, using standard phosphoramidite chemistry.[18] Duplexes were formed by slow cooling of equal concentrations of complementary strands. $Ru(phen)_2(dppz)^{2+}$ was prepared,[19] and enantiomers were separated[20] as described previously. Racemic metallointercalator was employed for gel electrophoresis measurements of damage yield; pure enantiomers were used for spectroscopic experiments. The quenchers $[Ru(NH_3)_6]Cl_3$, methyl viologen dichloride, and $[Co(NH_3)_5Cl]Cl_2$ were purchased from Aldrich and used as received.

Laser Spectroscopy. Time-resolved emission and transient absorption measurements used an excimer-pumped dye laser (Coumarin 480), as described previously.[2c] Laser powers at $\lambda_{exc} = 480$ nm ranged from 1.0−1.5 mJ @ 10 Hz. To generate the transient absorption spectrum, individual data traces at a given wavelength were fit to an exponential function at times >5 μs, and the absorbance changes were obtained by extrapolation of the fits back to time zero. Samples contained 40 μM Δ-$Ru(phen)_2(dppz)^{2+}$, 0.4 mM $Ru(NH_3)_6^{3+}$, and 4 mM nucleotides poly-(dG-dC) in an aerated, aqueous buffer of 5 mM phosphate, 50 mM NaCl, pH 8.5. Transient absorption measurements of a mixed-sequence of DNA utilized the sequence 5′-TGATCGGTGCGTCTGAGACT-3′ hybridized to its complement. Samples contained 30 μM duplex, 30 μM $Ru(phen)_2(dppz)^{2+}$, and 0.6 mM $Ru(NH_3)_6^{3+}$ in phosphate buffer as above.

Assays of Oxidative Products. Strands were 5′-^{32}P-end-labeled (*) by standard protocols[21] and hybridized to the complementary strands in an aerated buffer of 5 mM phosphate, 50 mM NaCl, pH 8.5. Oligonucleotide duplexes (8 μM) containing 8 μM $Ru(phen)_2(dppz)^{2+}$ and 10−20 equiv of quencher were irradiated at 436 nm with a 1000 W Hg/Xe lamp equipped with a monochrometer (∼6 mW at 442 nm). After irradiation, samples were treated with 100 μL of 1 M 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). Irradiation times varied from 10 s to 60 min.

To characterize the products of oxidative damage, irradiated oligonucleotides were digested (2 h each) with nuclease P_1 (Sigma) and then alkaline phosphatase (Boehringer Mannheim).[6,10] Nucleosides were separated by high performance liquid chromatography [HPLC; Hewlett Packard HP1090, Microsorb MV C18, 100 Å column (Rainin)] and identified by coelution with authentic standards (Caymen Chemicals). HPLC conditions were as follows:[10] oven temperature = 40 °C; solvent A = citric acid, NH_4OAc buffer, pH 5; solvent B = MeOH; gradient = 1−4% B over 40 min.

Quantum Yield Determinations. Samples (20 μL) were irradiated with 436 nm light and analyzed by gel electrophoresis (*vide supra*). The yield of damage was quantitated by phosporimagery and were not corrected for the <1.5% strand scission/G detected in control experiments with piperidine-treated DNA. Using the same geometry as for sample irradiations, ferrioxalate actinometry[22] was conducted to determine light intensity. The quantum yield of damage (Φ_{damage}) was then calculated as mol of strand breaks/mol photons. Care was taken to perform actinometry and cleavage experiments under the same conditions, and several trials were run to ensure precision. Emission quantum yields were measured on an SLM8000 steady-state fluorimeter and were determined relative to $\Phi_{Ru(bpy)3^{2+}} = 0.012$ in aerated acetonitrile.[23]

Results and Discussion

Photoinduced Quenching by Groove-Bound Oxidants. Nonintercalating oxidants such as $Ru(NH_3)_6^{3+}$, methyl viologen, and $Co(NH_3)_5Cl^{2+}$ quench the emission of $*Ru(phen)_2(dppz)^{2+}$ bound to DNA. In contrast to ultrafast quenching with well-intercalated donors and acceptors,[4] the weakly bound oxidants quench $*Ru(phen)_2(dppz)^{2+}$ dynamically on the nanosecond time scale.[2a] The linear Stern−Volmer plots for electron transfer quenching shown in Figure 1 indicate that the reaction occurs by a diffusional mechanism. The kinetics of photoinduced electron transfer with these quenchers are similar when $Ru(phen)_2(dppz)^{2+}$ is intercalated into either poly(dG-dC) or poly(dA-dT).[24]

We can monitor the products of the quenching reaction by transient absorption spectroscopy. When $*\Delta$-$Ru(phen)_2(dppz)^{2+}$ is quenched by $Ru(NH_3)_6^{3+}$ in the presence of poly(dA-dT), we detect a long-lived transient corresponding to the decay of Δ-$Ru(phen)_2(dppz)^{3+}$ on the microsecond time scale. In contrast, when Δ-$Ru(phen)_2(dppz)^{2+}$ is intercalated into poly-(dG-dC) and quenched by $Ru(NH_3)_6^{3+}$ (Figure 2A), we do not observe a long-lived species with the characteristics of Ru(III). Instead, we detect formation of a new species with differential absorption maxima at ∼390 and ∼550 nm.

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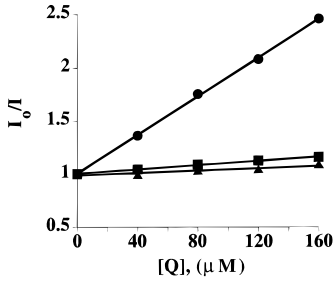

Figure 1. Stern−Volmer plots (I_o/I *vs* [Q]) of quenching of *rac*-Ru(phen)$_2$(dppz)$^{2+}$ intercalated into the oligonucleotide 5′-TGATCGGTGCGTCTGAGACT-3′ hybridized to complement. I_o = intensity of emission in absence of quencher Q; I = intensity of emission in presence of [Q]. Quenchers are $Ru(NH_3)_6^{3+}$ (●), MV^{2+} (■), and $Co(NH_3)_5Cl^{2+}$ (▲). Quenching rate constants extracted from Stern−Volmer plots are given in Table 1.

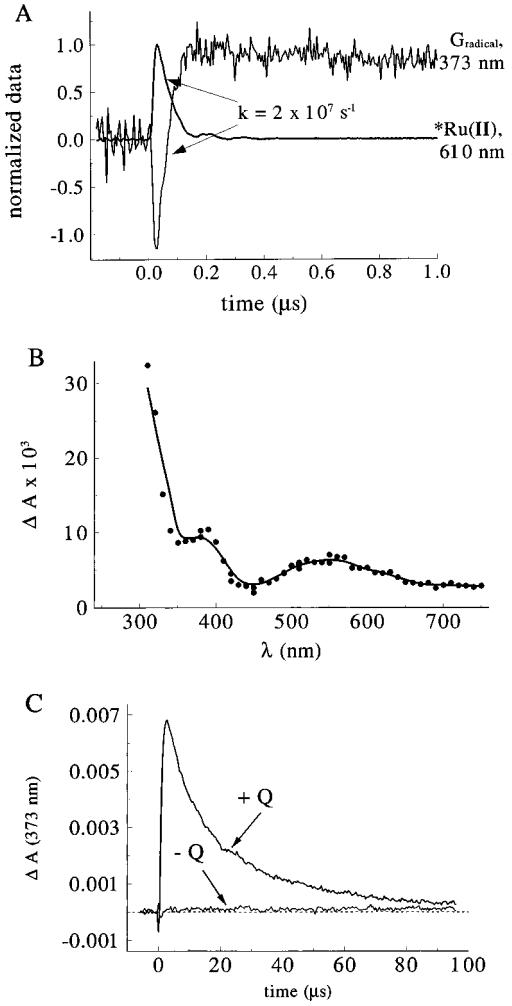

Figure 2. Time-resolved transient absorption data of Δ-Ru(phen)$_2$(dppz)$^{2+}$ (40 μM) bound to poly(dG-dC) (4 mM nucleotides) and quenched by $Ru(NH_3)_6^{3+}$ (0.4 mM). (A) Kinetics of emission decay of *Δ-Ru(phen)$_2$(dppz)$^{2+}$ at 610 nm and the rise of G• (-H) transient at 373 nm, the isobestic point for the *Ru(II)−Ru(II) transient absorption spectrum. The apparent rate constant for the formation of G• (-H) is the same as the rate constant for decay of *Ru(II). (B) Absorption difference spectrum after decay of *Ru(II). This spectrum corresponds closely to that assigned[17] as G• (-H). (C) The decay of the G• (-H) transient at 373 nm.

Photophysical Detection of G• Intermediates. We further characterized the transient formed in poly(dG-dC) as a function of wavelength. Pure enantiomers were used for spectral measurements, since the two intercalated isomers have slightly different absorption spectra; however, both enantiomers gave similar results. The transient spectrum obtained with Δ-Ru(phen)$_2$(dppz)$^{3+}$, formed after quenching by weakly absorbing $Ru(NH_3)_6^{3+}$,[25] is shown in Figure 2B. This UV−visible spectrum corresponds closely to that assigned by Candeias and Steenken[26] as the neutral radical of guanine [G• (-H)] in pulse radiolysis studies with guanosine and guanine monophosphate.[27] Here, the flash-quench method permits the first direct observation of oxidized guanine in duplex DNA by UV−visible absorption spectroscopy. This spectrum indicates that the guanine cation radical, once formed, is rapidly deprotonated in duplex DNA.

Quenching of Δ-Ru(phen)$_2$(dppz)$^{2+}$ by $Ru(NH_3)_6^{3+}$ occurs concomitantly with the formation of G• (-H). The rise of the radical signal is best monitored at 373 nm, the isobestic point for the *Ru(II)−Ru(II) difference spectrum.[2d,24] Time-resolved measurements indicate that both the *Ru(II) emission decay and the rise of G• (-H) absorption occur with $k_{obs} \approx 2 \times 10^7$ s^{-1} (Figure 2A). Thus, oxidation of guanine by intercalated Δ-Ru(phen)$_2$(dppz)$^{3+}$ occurs in less than 200 ns and may in fact be much faster.[2,4,28] Once formed, nearly all of the guanine radical decays within 100 μs (Figure 2C) when $Ru(NH_3)_6^{3+}$ serves as the quencher. With MV^{2+} as quencher, the transient spectrum is complicated by the absorption of reduced $MV^{\bullet+}$. Finally, when $Co(NH_3)_5Cl^{2+}$ serves as a sacrificial quencher, the decay of G• (-H) is much slower (>1 ms).

The formation of G• (-H) by the flash-quench method with different DNAs and intercalators is consistent with the calculated reduction potentials of the reactants. Rapid formation of G• (-H) is observed for Ru(phen)$_2$dppz^{3+} bound to either poly(dG)•poly(dC) or poly (dG-dA)•poly(dC-dT). In the presence of poly(dG-dT)•poly(dC-dA), however, a long-lived Ru(III) signal dominates the transient absorption spectrum, indicating that most of the Ru(III) does not react. This trend may reflect the sequence-dependent redox potential of guanine;[11] additionally, the lack of the guanine radical signal could reflect structural variations within the poly(dG-dT)•(dC-dA) polymer.[29] Moreover, Ru(4,7-dimethylphen)$_2$(dppz)$^{3+}$ and Os(phen)$_2$(dppz)$^{3+}$, which both have lower reduction potentials than Ru(phen)$_2$(dppz)$^{3+}$,[2d,30] do not appear to react with guanine.[2c,30] Quenching of these M(II) intercalators by $Ru(NH_3)_6^{3+}$ in poly(dG-dC) produces long-lived signals characteristic of oxidized metal complexes M(III) and no evidence for G• (-H).

We also examined the formation of the G• (-H) intermediate in a mixed sequence of DNA with $Ru(NH_3)_6^{3+}$ as quencher. At short times, the transient spectrum is dominated by the spectral characteristics of Ru(phen)$_2$dppz^{3+}. After the 100 μs decay of the Ru(III) intermediate, however, a small, long-lived transient consistent with decay of a G• (-H) is detected. This decay occurs on the millisecond time scale and may reflect the reactions which lead to stable oxidized products (*vide infra*). As Scheme 1 indicates, there are several pathways for the decay of G• (-H). Clearly, the relative yields and rate constants are a function of the concentrations of Ru(III), Q^-, and G• (-H), which differ for the mixed-sequence oligomer versus poly(dG-dC).

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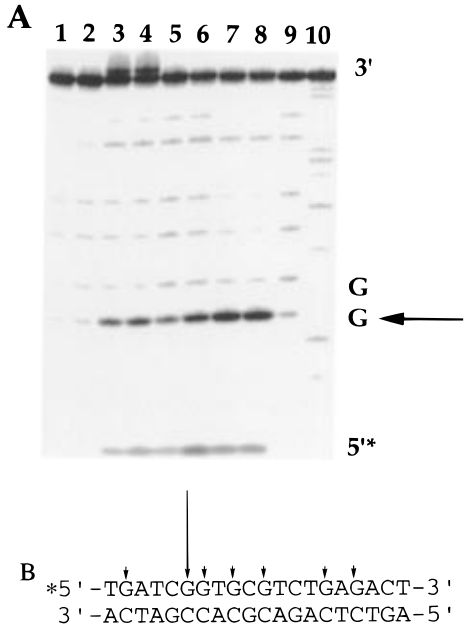

Figure 3. Autoradiograms after denaturing polyacrylamide gel electrophoresis of ^{32}P-5′-TGATCGGTGCGTCTGAGACT-3′ after oxidation of the oligonucleotide duplex by *rac*-Ru(phen)$_2$(dppz)$^{3+}$. (A) Samples shown are as follows: lane 1, DNA + Ru(phen)$_2$(dppz)$^{2+}$ without irradiation; lane 2, DNA + Ru(phen)$_2$(dppz)$^{2+}$ after 60 min irradiation; lanes 3 and 4, DNA + Ru(phen)$_2$(dppz)$^{2+}$ + 20 equiv Ru(NH$_3$)$_6^{3+}$, irradiated for 30 and 60 min, respectively; lanes 5 and 6, DNA + Ru(phen)$_2$(dppz)$^{2+}$ + 10 equiv MV^{2+}, irradiated for 2 and 10 min, respectively; lanes 7 and 8, DNA + Ru(phen)$_2$(dppz)$^{2+}$ + 10 equiv Co(NH$_3$)$_5$Cl^{2+}, irradiated for 2 and 10 min, respectively; lanes 9 and 10, Maxam−Gilbert sequencing reactions for G and C + T, respectively. Note that with Ru(NH$_3$)$_6^{3+}$ as quencher, an additional band with higher molecular weight is evident. (B) Histograms representing oxidative damage of the oligonucleotide duplex by Ru(phen)$_2$(dppz)$^{3+}$.

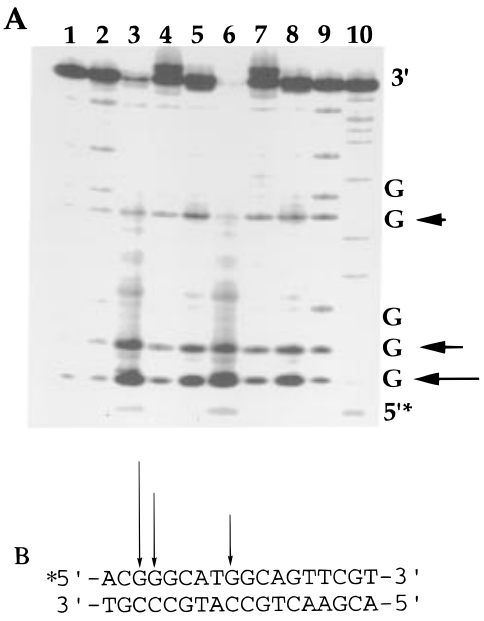

Figure 4. Autoradiograms after denaturing polyacrylamide gel electrophoresis of ^{32}P-5′-ACGGGCATGGCAGTTCGT-3′ after oxidation of the oligonucleotide duplex by *rac*-Ru(phen)$_2$(dppz)$^{3+}$. (A) Samples shown are as follows: lane 1, DNA + 10 equiv Co(NH$_3$)$_5$Cl^{2+} irradiated 60 s; lane 2, DNA + Ru(phen)$_2$(dppz)$^{2+}$ irradiated for 60 min; lanes 3 and 6, DNA + Ru(phen)$_2$(dppz)$^{2+}$ + 10 equiv of methyl viologen, irradiated 10 and 20 min, respectively; lanes 4 and 7, DNA + Ru(phen)$_2$(dppz)$^{2+}$ + 20 equiv of Ru(NH$_3$)$_6^{3+}$, irradiated 10 and 20 min, respectively; lanes 5 and 8, DNA + Ru(phen)$_2$(dppz)$^{2+}$ + 10 equiv of Co(NH$_3$)$_5$Cl^{2+}, irradiated 10 and 20 s, respectively; lanes 9 and 10, Maxam−Gilbert sequencing reactions for G and C + T, respectively. Note that the samples containing Co(NH$_3$)$_5$Cl^{2+} were irradiated for *seconds*, while MV^{2+} and Ru(NH$_3$)$_6^{3+}$ samples were irradiated for several *minutes*. (B) Histograms representing oxidative damage of the oligonucleotide duplex by Ru(phen)$_2$(dppz)$^{3+}$.

Analysis of Oxidative products. The yield of permanent DNA damage which results from the flash-quench experiment is analyzed by gel electrophoresis. Oxidized guanine nucleotides have been shown to be labile upon treatment with piperidine; therefore, the yield and position of guanine oxidation can be revealed by strand scission in a gel electrophoresis experiment.[31] When an oligonucleotide duplex containing a 5′-GG-3′ doublet is irradiated at 436 nm in the presence of both *rac*-Ru(phen)$_2$(dppz)$^{2+}$ and quencher, damage, revealed by treatment with piperidine, is observed selectively at the 5′-G of the GG step, with little damage at other sites (Figure 3). The ET reactions in the flash-quench experiment do lead to significant permanent damage of DNA.

Other characteristics of these gel electrophoresis experiments are noteworthy. When the Δ isomer was employed for *in situ* oxidation, the sites of oxidative damage were the same as for the racemic intercalator, and the quantum yield for damage was slightly higher, consistent with the higher emission quantum yield and more efficient electron transfer observed for the Δ isomer.[4,6] Some damage, albeit at a low level, is also evident at all 5′-GX-3′ sites. This damage is found in all experiments above the control level and likely reflects some oxidation at single G sites. Additionally, Figure 3 indicates a difference in damage yield which depends on quencher. This yield of damage is not directly correlated with the efficiency of quenching, however. Thus, the amount of oxidative damage can be varied not only by the intercalator but also by the quencher as well.

Figure 4 presents data illustrating the damage of an oligonucleotide containing both a 5′-GGG-3′ triplet and 5′-GG-3′ doublet. The yield of oxidized guanine is highest for the 5′-G of the 5′-GGG-3′ triplet, followed by the central G of 5′-GGG-3′ and the 5′-G of 5′-GG-3′, in accordance with other experiments and calculated oxidation potentials.[11] For comparison of Figures 3 and 4, it should be noted that the overall yield of damage is greater for the oligonucleotide containing the 5′-GGG-3′ sequence. Furthermore, samples containing Co(NH$_3$)$_5$Cl^{2+} in Figure 4 are irradiated for only 10 and 20 s, compared to 10 and 20 min for Ru(NH$_3$)$_6^{3+}$and MV^{2+} samples. For each quencher, damage increases with quencher concentration and irradiation time; indeed complete reaction can be observed for MV^{2+} after only 20 min of irradiation. As has been demonstrated with organic intercalators,[8] a high yield of DNA damage by metallointercalators is achieved by this method.

Oxidative damage caused by the flash-quench experiment can be contrasted with DNA damage caused by a ^{1}O$_2$ mechanism. Luminescent diimine complexes of ruthenium(II) do sensitize the formation of singlet oxygen, and this reactive radical species has also been shown to cause piperidine-labile oxidation of guanines in duplex DNA.[32,33] The efficiency of sensitized damage depends directly on the excited-state lifetime[34] and therefore should vary as a function of Ru(II) complex and DNA intercalation site. Lane 2 in Figures 3 and 4 show that ^{1}O$_2$ damage has a relatively low quantum yield in this system and causes DNA damage at all Gs with little sequence-selectivity. This observation is consistent with the low quantum yield[13] of

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Table 1. Parameters for Flash-Quench Cycle with *rac*-Ru(phen)$_2$(dppz)$^{2+}$ + DNA[a]

quencher	Φ_{damage}[b]	damage yield[c]	equiv Q	% quenching[d]	k_q (*Ru^{2+} Q)[e]	k_{obsv} (Ru^{3+} − Ru^{2+})[f]
[Ru(NH$_3$)$_6$]$^{3+}$	2×10^{-5}	1.4×10^{-5}	20	60	10×10^{10} M^{-1} s^{-1}	6×10^4 s^{-1}
methyl viologen^{2+}	5×10^{-4}	0.002	10	8	1×10^{10} M^{-1} s^{-1}	1×10^2 s^{-1}
[Co(NH$_3$)$_5$Cl]$^{2+}$	9×10^{-4}	0.02	10	3	6×10^9 M^{-1} s^{-1}	N/A[g]

[a] Conditions: 8 μM Ru(phen)$_2$(dppz)$^{2+}$, 8 μM DNA duplex (Figure 3B), in an aerated buffer of 5 mM phosphate, 50 mM NaCl, pH 7. [b] Φ_{damage} = quantum yield of piperidine-mediated strand breaks at main damage site. Uncertainty in Φ_{damage} is ∼20%. [c] damage yield = Φ_{damage}/Φ_{ET}; the yield of damage per Ru(III). $\Phi_{ET} = k_q[Q]/k$ where k_q = quenching constant and k = weighted average of the biexponential fit of *Ru(II) emission in the presence of Q. [d] Measured by steady-state emission; uncertainty is ∼5%. [e] $I_o/I = 1 + [Q](k_q/k_o)$; k_o = intrinsic decay constant of *Ru(II) from a weighted average of a biexponential fit of *Ru(II) emission. $k_1 = 4.2 \times 10^6$ s^{-1} (35%), $k_2 = 1.5 \times 10^7$ s^{-1} (65%). Uncertainty is ∼10%. [f] k_{obsv} = observed decay of Ru(III)−Ru(II) transient at 440 nm; uncertainty is ∼10%. [g] Signal too small to determine kinetics accurately.

Ru(phen)$_2$(dppz)$^{2+}$ bound to DNA and the low sequence-selectivity of the metal complex. The slight variations in base damage are likely due to preferences in the sites of Ru(II) intercalation and/or differences in the accessibility of guanine to molecular oxygen. The $^1O_2$-sensitized damage is markedly different, both in position and intensity, from that seen in the flash-quench experiment. By contrast to $^1O_2$-mediated damage, lanes 3−8 in Figures 3 and 4 indicate that the pattern of oxidative damage correlates with the relative reduction potentials of guanine-rich sequences[11] and does not seem to be related to the position of the intercalator on the oligonucleotide duplex. Additionally, no increase in oxidation is observed in D_2O compared to H_2O, despite the longer excited-state lifetimes of both *Ru(II) and $^1O_2$ in D_2O.[2b,34] For a given quencher, the yield of G oxidation increases with the amount of emission quenching; the opposite trend is expected for $^1O_2$-mediated damage. Lastly, the yields of G oxidation far exceed those obtained from $^1O_2$-sensitized cleavage with dppz complexes of Ru(II).

The resultant damage from the flash-quench experiment can also be characterized directly by chemical analysis. Guanine damage was examined by enzymatic digestion[6,10] without piperidine treatment. Separation of the nucleoside products by HPLC and coelution with authentic samples indicated that the major product was 8-oxo-2′-deoxyguanosine (8-oxo-G)[6,8,10,35,36] for samples in which Ru(NH$_3$)$_6^{3+}$ served as a quencher. The yield of piperidine-sensitive damage identified by gel electrophoresis and the amount of 8-oxo-G identified by HPLC were comparable, indicating that piperidine treatment reveals the primary damage. HPLC analysis also indicates several minor products of the reaction; these are likely due to Ru(phen)$_2$(dppz)$^{2+}$ and Ru(NH$_3$)$_6^{3+}$ degradation as well as secondary oxidative products. Interestingly, we do not detect formation of 8-oxo-G in the presence of MV^{2+} and Co(NH$_3$)$_5$Cl^{2+}. Given that complete strand cleavage can be obtained with these quenchers, it is not likely that oxidative damage is left undetected in the gel electrophoresis assay. Additionally, it is possible that 8-oxo-G, which is easily oxidized,[37] reacts further in the presence of these quenchers.

Tuning Reaction with Quencher. The amount of damage incurred at G is clearly modulated by the choice of quencher. As suggested by Scheme 1, the yield for base oxidation is found to depend on the rates of several competing reactions. Table 1 shows the yield of damage as a function of quencher and some of the factors which contribute to these yields. First, some Ru(III) recombines with reduced quencher to give the starting materials Ru(II) and Q. This recombination reaction occurs readily for Ru(NH$_3$)$_6^{3+}$. Thus, even though Ru(phen)$_2$(dppz)$^{2+}$ is highly quenched by Ru(NH$_3$)$_6^{3+}$, the quantum yield for damage (Φ) is low. Second, the yield of damage depends on how rapidly G• (-H) reacts with Q$^-$ and is thus correlated with the instability of the reduced quencher. For example, since MV$^+$ reacts with O_2 on the 100 μs time scale,[38] more damage is observed at 5′-GG-3′ with MV^{2+} as quencher than Ru(NH$_3$)$_6^{3+}$. The superoxide formed in reaction of O_2 and MV$^+$ can also quench the guanine radical,[8a] resulting in an intermediate quantum yield for damage. The lability of Co(II) complexes is exploited by using Co(NH$_3$)$_5$Cl^{2+} as a sacrificial quencher. Since this reduced quencher irreversibly degrades on the microsecond time scale,[39] the highest quantum yield for damage is obtained.

Given that the flash-quench reaction is modulated by the quencher, other observations are understandable in this context. For example, transient absorption spectroscopy indicates that nearly all of the G• (-H) formed in poly(dG-dC) is re-reduced by Ru(NH$_3$)$_6^{3+}$ within 100 μs; in the presence of Co(NH$_3$)$_5$Cl^{2+}, by contrast, the G• signal in poly(dG-dC) does not decay measurably within 1 ms. The persistence of the G• with Co(NH$_3$)$_5$Cl^{2+}and MV^{2+} as quenchers may also contribute to the different oxidative products observed by enzymatic digestion. It is clear, therefore, that the characteristics of the quencher provide a novel means to tune yields of both intermediates and products.

Conclusions

We have demonstrated that a flash-quench methodology can be used to combine spectroscopy and product analysis in the description of ET reactions of DNA. This methodology permits the direct spectroscopic characterization of the neutral guanine radical in duplex DNA. Furthermore, this experiment will also allow us to manipulate reactivity by varying the redox properties of both the quencher and the intercalator. The flash-quench reaction with ruthenium intercalators therefore adds to the growing number of ET reactions involving DNA both as a bridge and as a reactant and permits reaction intermediates and products to be readily identified and compared.

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