Solution Photoreactivity of Phenanthrenequinone Diimine Complexes of Rhodium and Correlations with DNA Photocleavage and Photooxidation

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The transient absorption spectra of Rh(III) complexes containing one or two phi ligands (phi = 9,10-phenanthrenequinone diimine) and various ancillary ligands were measured at pH 5.0 utilizing visible and UV excitation. The spectra were all consistent with a primarily phi ligand-centered (LC) nπ^* transition. The spectral profile obtained with visible excitation of the complexes is slightly different and significantly weaker at pH 8.0, where now $Rh(phi)_2(phen)^{3+}$, $Rh(phi)_2(bpy)^{3+}$, and $Rh(phen)_2(phi)^{3+}$ are deprotonated. Irradiation of these complexes in basic media with 308 nm laser excitation leads to irreversible ligand-loss photochemistry. The LC excited states of $Rh(phi)_2(phen)^{3+}$, $Rh(phi)_2(bpy)^{3+}$, and $Rh(phen)_2(phi)^{3+}$ are reductively quenched by the purine DNA bases, A, dA, dAMP, AMP, G, dG, dGMP, and GMP, with rate constants ranging from 1.4×10^9 M^{-1} s^{-1} to 4.7×10^9 M^{-1} s^{-1} at pH 5.0, but no quenching was observed for dC or dT. Absorbances assigned to the reduced Rh(II) complex and $dG^{\bullet}$ were observed in the transient absorption spectrum. There are some parallels between the observed photochemistry in solution and the DNA photocleavage results, in particular the presence of oxidative damage to the DNA bases.

Introduction

Rhodium(III) complexes possessing phi ligands (phi = 9,-10-phenanthrenequinone diimine; Figure 1) bind to DNA avidly ($K > 10^6$ M^{-1}) and have been shown to undergo a variety of photoinduced reactions with DNA.[1−7] Irradiation at higher energies (λ_{irr} = 313 nm) results in DNA photocleavage at the complex's binding site, a property which has facilitated studies of site-specific DNA recognition.[3] Recently, phi complexes of rhodium have also been utilized to initiate oxidative reactions on DNA. Long-range photooxidative ($\lambda_{irr} \geq 365$ nm) damage to guanine bases at 5′-GG-3′ and 5′-GGG-3′ sites has been observed with $Rh(phi)_2(bpy')^{3+}$ covalently linked to a DNA duplex (bpy′ = 4-butyric acid-4′-dimethyl-2,2′-bipyridine).[1] DNA duplexes containing tethered $Rh(phi)_2(bpy')^{3+}$ have also been prepared in which thymine dimer lesions in DNA could be repaired upon low-energy irradiation (λ_{irr} = 400 nm).[2] Additional interest in intercalating phi complexes of Rh(III) arises from their role as electron acceptors in charge transfer mediated by the DNA base-pair stack.[5−7]

Irradiation of $Rh(phen)_2(phi)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$ (phen = 1,10-phenanthroline, bpy = 2,2′-bipyridine) in the ultraviolet region (λ_{irr} = 313 nm) in the absence of DNA leads to irreversible loss of phi ligand in basic media possibly via a ligand-to-metal charge transfer (LMCT) excited state.[8] It has been shown that irradiation of $Rh(phen)_2(phi)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$ intercalated in DNA leads to direct DNA strand scission with products consistent with 3′-hydrogen abstraction from the deoxyribose backbone of DNA.[9] The mechanism by which the excited rhodium complexes promote direct DNA photocleavage was believed to be associated with the photoinduced ligand-loss reactivity, since the amount of DNA cleavage correlates with the quantum yield of phi ligand loss observed in the absence of DNA. Both processes are substantially favored with high-energy irradiation.

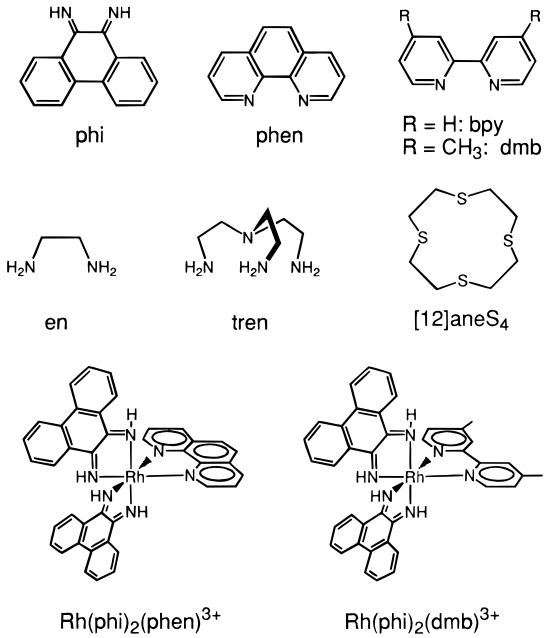

Figure 1. Structures of ligands and the complexes $Rh(phi)_2(phen)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$.

Upon near-UV and visible excitation (λ_{irr} = 355−532 nm) a nonemissive long-lived (∼200 ns) excited state can be accessed

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in complexes of Rh(III) that contain phi in their ligation sphere, such as $Rh(phi)_n(L)_{3-n}^{3+}$ (L = phen, bpy, dmb (dmb = 4,4′-dimethyl-2,2′-bipyridine); $n = 1, 2, 3$), which reversibly returns to the ground state.[10] This state is believed to be mostly LC (ligand-centered) in character arising from an $n\pi^*$ transition within the phi ligand, although the large variations in the lifetimes of the transients in phi complexes of Zn(II),[10] Rh(III), and Ir(III)[11] point to some involvement of the metal in the excited state. It has been demonstrated that at pH 5.0 the LC excited state of $Rh(phi)_2(phen)^{3+}$ is a strong oxidizing agent ($E_{1/2}(Rh^{3+*/2+}) \approx +2.0$ V vs NHE), which is able to produce the radical cations of 9,10-diphenylanthracene ($E_{1/2}(D^{+\bullet}/D) = +1.5$ V vs NHE), *p*-dimethoxybenzene ($E_{1/2}(D^{+\bullet}/D) = +1.6$ V vs NHE), and 9,10-dibromoanthracene ($E_{1/2}(D^{+\bullet}/D) = +1.8$ V vs NHE) following 532 nm excitation (5 mJ/pulse, fwhm = 10 ns).[10]

In the present study, we have focused on the investigation of the excited states of $Rh(L)_n(phi)^{3+}$ (L = NH_3, $n = 4$; L = en (ethylenediamine), phen, $n = 2$; L = tren (tris(2-aminoethyl)-amine), [12]aneS_4 (1,4,7,10-tetrathiacyclododecane), $n = 1$) and $Rh(phi)_2(L)^{3+}$ (L = bpy, dmb (4,4′-dimethyl-2,2′-bipyridine), phen) in solution, as well as their photoreactivity toward nucleotides and nucleosides. The structures of the ligands and complexes are shown in Figure 1. We have utilized nanosecond time-resolved and steady-state spectroscopy to measure the quenching rates and the spectral profiles of the excited states of the metal complexes and the absorption spectra of the redox products. In addition, both the direct photocleavage and photooxidation of duplex DNA by intercalated Rh(III) complexes as a function of pH and irradiation wavelength were compared to the observed excited state reactivity in solution.

Experimental Section

Materials. The ligands 1,10-phenanthroline (phen), 2,2′-bipyridine (bpy), tris(2-aminoethyl)amine (tren), 4,4′-dimethyl-2,2′-bipyridine (dmb), ethylenediamine (en), and 1,4,7,10-tetrathiacyclododecane ([12]aneS_4) were purchased from Aldrich and used without further purification. $Rh(phi)_2(phen)^{3+}$, $Rh(phi)_2(dmb)^{3+}$, and $Rh(phi)_2(bpy)^{3+}$ were prepared from the reaction of $Rh(phi)_2Cl_2$ with the appropriate ligand by a reported method.[8a] The chloride salts of $Rh(phi)(NH_3)_4^{3+}$, $Rh(tren)(phi)^{3+}$, $Rh(en)_2(phi)^{3+}$, and Rh([12]aneS_4)$(phi)^{3+}$, were prepared by reported methods.[8b] Each complex was characterized by comparison of the absorption and NMR spectra to those of complexes reported in the literature.[8,12] All nucleotides, nucleosides, and DNA bases were purchased from Sigma and used without further purification. The 17-mer oligonucleotide 5′-ACGGCACTACGGCTCGT-3′ and its complement were synthesized on an ABI synthesizer and purified by C18 reverse phase HPLC both before and after the removal of the trityl-protecting group. The strand containing the two 5′-GG-3′ sequences was 5′-[^{32}P]-labeled using polynucleotide kinase and purified on a 20% denaturing polyacrylamide gel. After the DNA was extracted from the gel matrix at 37 °C for 4 h with 0.5 mL of TE (10 mM Tris-HCl (pH 7.4), 1 mM EDTA (pH 8.0)), the labeled DNA was desalted on a Nensorb 20 DNA purification cartridge (DuPont). The 140 base-pair *Eco*RI/*Pvu*II restriction fragment from pUC18 was 5′-[^{32}P]-labeled and purified on a 6% nondenaturing polyacrylamide gel.[13]

Methods. Ground state and transient absorption spectra were measured at room temperature in a 1 × 1 cm quartz cuvette equipped with a Teflon stopcock. Typically, the samples were bubbled with argon for ~15 min immediately before each measurement. In all experiments, the concentration of the Rh(III) complex was ~1 mM determined from the absorption spectrum and the reported extinction coefficients of each complex.[8,12] The experiments were performed in water, and the pH was adjusted by addition of 0.18 M HCl or 0.10 M NaOH unless otherwise noted. For experiments in acetonitrile, the protonated form of the Rh(III) complexes was precipitated from a pH 5.0 aqueous solution with ammonium hexafluorophosphate to obtain the PF_6^- salts of the complexes.

A series of phosphate buffers, pH 5−9, were prepared with $HNaPO_4$ and Na_2PO_4. Since sodium phosphate is not an effective buffer above pH 9, a series of NH_4OAc buffers were also made from pH 5 to 10 using ammonium hydroxide and acetic acid. The buffering capabilities were then tested using the experimental calf thymus DNA and metal complex concentrations.

The UV−vis spectra of the metal complexes bound to DNA as a function of pH were measured using 1.5 mL of a solution of 15 μM metal complex and 300 μM calf thymus DNA (base pairs) in 25 mM NH_4OAc, pH 10, and 50 mM NaCl. The pH was adjusted with acetic acid, and measurements were taken approximately every 0.25 pH units. The pK_a was determined from the derivative of a plot of pH vs absorbance at the wavelength of maximum change.

Stock solutions at each pH containing the 17-mer DNA, buffer, and NaCl were annealed by heating at 90 °C for 5 min and then cooling to room temperature over 70 min. DNA cleavage studies were performed by irradiating 20 μL samples containing 200 000 counts of the 5′-[^{32}P]-labeled strand, 2 μM duplex DNA, 5 μM metal complex, 15 mM buffer, and 50 mM NaCl. After irradiation 10 μL was removed and dried under vacuum. To the other 10 μL was added 10 μL of piperidine and 80 μL of water. After 30 min of heating at 90 °C, the samples were dried under vacuum and twice resuspended in 20 μL of water and redried. The samples were resuspended in a 90% formamide denaturing dye, heated to 90 °C, and loaded directly on a 20% denaturing polyacrylamide gel which was visualized by phosphorimagery.

The 140 base-pair restriction fragment was irradiated in the presence of 50 μM calf thymus DNA (base pairs), 2.5 μM metal complex, 15 mM buffer, and 50 mM NaCl. The following irradiation times were used: (1) $Rh(phi)_2dmb^{3+}$, 2 min at 313 nm and 8 min at 365 nm, (2) $Rh(phen)_2phi^{3+}$, 2 min at 313 and 365 nm, (3) $Rh(NH_3)_4phi^{3+}$, 15 min at 365 nm, and (4) Rh([12]ane$S_4)_2phi^{3+}$, 5 min at 313 nm and 45 min at 365 nm. After ethanol precipitation, the 365 nm samples were treated with a 10% piperidine solution as described above. The cleavage bands were resolved on an 8% denaturing polyacrylamide gel and quantitated using ImageQuant software after phosphorimagery.

Instrumentation. Ground state electronic absorption spectra were recorded using a Hewlett Packard diode array spectrometer (HP 8452A) with HP8452Win System software installed in an HP Vectra VL2 4/50 desktop computer or a Cary 2200 spectrometer. The transient absorption instrument has been previously described;[14] spectra were collected following excitation with a doubled (532 nm) or tripled (354.7 nm) Nd:YAG laser (Quanta Ray DCR2(20), 532 nm, fwhm = 8 ns, ~5 mJ/pulse). Alternatively, the output from an excimer (Lambda Physik Lextra (XeCl, 308 nm, fwhm ~10 ns) or excimer-pumped dye laser (Lambda Physik FL 3002 dye laser, ~2 mJ/pulse) with stilbene 420 (420 nm) or coumarin 450 (450 nm) purchased from Exciton was utilized. Irradiations of DNA-containing samples were performed with an Oriel model 6140

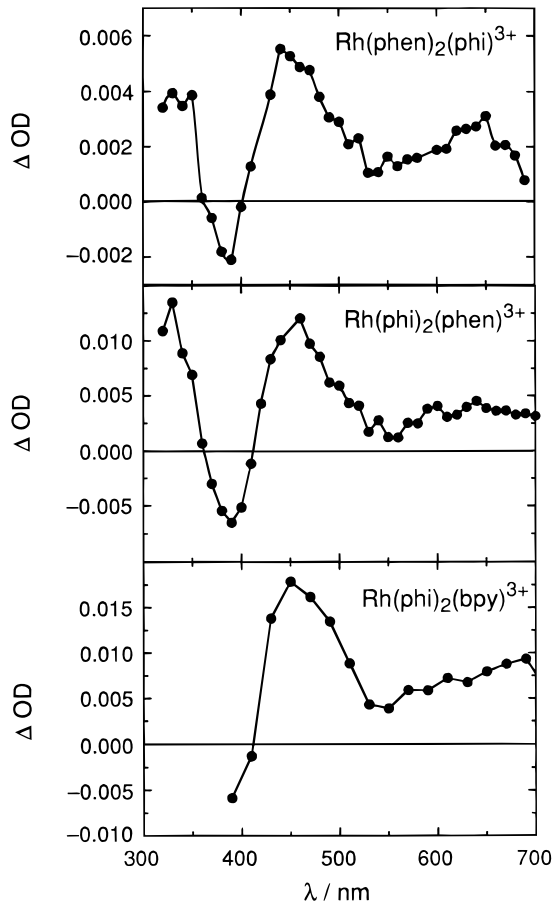

Figure 2. Transient absorption spectra of $Rh(phen)_2(phi)^{3+}$, $Rh(phi)_2(phen)^{3+}$, and $Rh(phi)_2(bpy)^{3+}$ at pH 5.0 collected 20 ns after a 532 nm or 420 nm laser pulse (5 mJ/pulse, fwhm ≈ 10 ns).

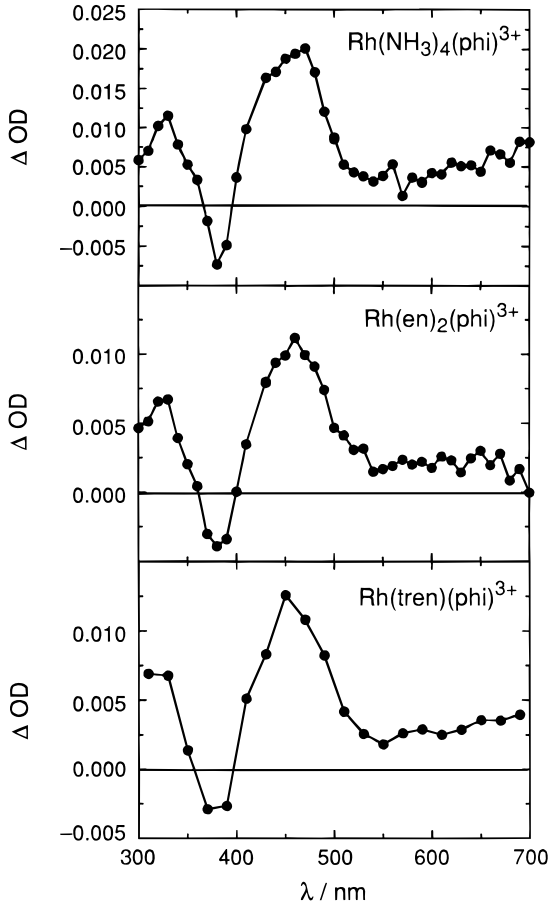

Figure 3. Transient absorption spectra of $Rh(NH_3)_4(phi)^{3+}$, $Rh(en)_2(phi)^{3+}$, and $Rh(tren)_2(phi)^{3+}$ at pH 5.0 collected 20 ns after a 532 nm or 420 nm laser pulse (5 mJ/pulse, fwhm ≈ 10 ns).

1000 W Hg/Xe lamp equipped with an Oriel model 77250 monochromator.

Results

Excited State Absorption Spectra. The transient absorption spectra of $Rh(phen)_2(phi)^{3+}$, $Rh(phi)_2(phen)^{3+}$, and $Rh(phi)_2(bpy)^{3+}$ (structures depicted in Figure 1) following visible excitation at pH 5.0 are shown in Figure 2. All spectra possess a characteristic positive difference absorbance centered at ∼460 nm and broad absorbance in the 600−700 nm region. In addition, bleaching at ∼380 nm is observed, where the ground state of the complexes absorbs strongly. Very similar spectral profiles are observed in the time-resolved absorption spectra of $Rh(NH_3)_4(phi)^{3+}$, $Rh(en)_2(phi)^{3+}$, and $Rh(tren)(phi)^{3+}$ in acidic media (Figure 3). The absorption at 460 nm and the bleaching of the ground state discussed above decay monoexponentially at pH 5.0 with lifetimes listed in Table 1. This transient has been previously assigned to a phi LC excited state $n\pi^*$ in character.[10] The transient is dynamically quenched by O_2, and the measured quenching rate constants are listed in Table 1, along with the pK_a values of each complex. No decomposition was detected by comparison of the electronic absorption spectra of the protonated samples before and immediately following a transient absorption experiment with 420 nm or 532 nm laser excitation. The same LC excited state is observed in the transient absorption spectrum upon 308 nm excitation of $Rh(phen)_2(phi)^{3+}$ at pH 5.0, although there was a ∼5% decrease in the absorption of the complex centered at 380 nm after the experiment.

TABLE 1: Lifetimes of LC Excited State of Rh(III) Complexes[a]

complex	τ/ns[b]	$k_q/\times 10^8$ $M^{-1}\,s^{-1}$ [c]	pK_a (water)	pK_a (DNA)
$Rh(phen)_2(phi)^{3+}$	184	7.9	6.2[d]	7.5
$Rh(phi)_2(phen)^{3+}$	175	5.1	6.7	
$Rh(phi)_2(bpy)^{3+}$	226	6.4	6.8[d]	
$Rh(phi)_2(dmb)^{3+}$	257	12.8	6.8	7.9
$Rh(en)_2(phi)^{3+}$	166	2.4	9.1[d]	
$Rh([12]aneS_4)(phi)^{3+}$	371	12.9	4.7	5.2
$Rh(tren)(phi)^{3+}$	216	8.1	9.1	
$Rh(NH_3)_4(phi)^{3+}$	139	7.8	9.2[d]	>10

[a] Measured under argon at pH 5.0, the quenching rate constants for deactivation of the excited state by O_2, and the pK_a values of each complex in water and bound to DNA. [b] Determined from spectroscopic titrations. [c] Measured utilizing the Stern−Volmer method from the lifetime of the transient at 460 nm. [d] From ref 8.

In contrast to experiments conducted at pH 5, facile irreversible photochemistry of the phi complexes is observed upon 308 nm excitation in basic media as a result of phi ligand loss.[8,12] However, visible excitation (420 nm to 532 nm) at pH 8 leads to the formation of optical transients in all complexes, which reversibly regenerate the starting material. The transient absorption signal obtained for the deprotonated $Rh(phen)_2(phi)^{3+}$ at pH 8.0 (Figure 4) is weaker than that obtained in acidic media. The transient absorption spectra of $Rh(phi)_2(bpy)^{3+}$ and $Rh(phi)_2(phen)^{3+}$ collected at pH 8.0 are approximately 10 times weaker than that of $Rh(phen)_2(phi)^{3+}$. The spectral profile of $Rh(phen)_2(phi)^{3+}$ at pH 8.0 shown in Figure 4 with the bleaching at 520 nm is consistent with greater absorption by the ground state in this spectral region.[8a,12] The decay of the $Rh(phen)_2$-

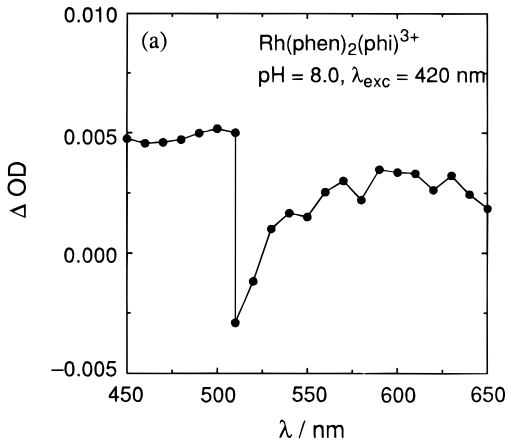

Figure 4. Transient absorption spectra of $Rh(phen)_2(phi)^{3+}$ at pH 8.0 collected 20 ns after a 532 nm laser pulse (5 mJ/pulse, fwhm ≈ 10 ns).

TABLE 2: Quenching Rate Constants of LC Excited State of $Rh(phen)_2(phi)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$ Complexes[a]

		$k_q/\times 10^9$ M^{-1} s^{-1} [b]			
complex	$-\Delta G$/eV[c]	G	dG	GMP	dGMP
$Rh(phen)_2(phi)^{3+}$	0.71	2.4	2.4	3.3	4.4
$Rh(phi)_2(bpy)^{3+}$	0.66	2.6	3.2	4.6	3.4

[a] Measured under argon at pH 5.0 by G, dG, GMP, and dGMP, along with the calculated driving forces for each reaction, ΔG. [b] Measured utilizing the Stern−Volmer method from the lifetime of the transient at 460 nm. [c] Calculated from $-\Delta G = E_{1/2}(Rh^{3+*/2+}) - E_{1/2}(G/G^+)$, with $E_{1/2}(Rh^{3+*/2+}) = E_{00} + E_{1/2}(Rh^{3+/2+})$, where $E_{00} = 2.0$ eV and the $E_{1/2}(Rh^{3+/2+})$ values for $Rh(phen)_2(phi)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$ are 0.0 V and −0.05 V vs NHE, respectively (from ref 5b).

$(phi)^{3+}$ transient in deoxygenated basic media is monoexponential, with a lifetime of 84 ns. It is interesting to note that at neutral pH in water with or without Tris buffer, where both protonated and deprotonated forms of $Rh(phen)_2(phi)^{3+}$ ($pK_a = 6.2$), $Rh(phi)_2(phen)^{3+}$ ($pK_a = 6.7$), and $Rh(phi)_2(bpy)^{3+}$ ($pK_a = 6.8$) are present in solution, irreversible photochemistry takes place with 420 nm and 532 nm excitation. No overall photochemistry is observed at either pH 5 or pH 8 with visible excitation, where all the complexes are either protonated or deprotonated, respectively.

Electron Transfer with DNA Bases. The LC excited state, which has been shown to act as a potent oxidizing agent with organic electron donors, can reversibly undergo one-electron oxidation of the purine DNA bases, A and G, their nucleosides, dA and dG, their nucleotides, dAMP and dGMP, and the ribonucleotides, AMP and GMP. The rate constants at pH 5.0 for the quenching of the lifetime of the nonemissive excited states of $Rh(phen)_2(phi)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$ by these bases are listed in Tables 2 and 3 and were determined utilizing the Stern−Volmer relationship from the measured lifetimes of the transient absorption signal at 460 nm as a function of quencher concentration. In addition, the LC excited state of $Rh(phi)_2(phen)^{3+}$ was quenched by dA and dG with rate constants of 1.7×10^9 M^{-1} s^{-1} and 1.4×10^9 M^{-1} s^{-1}, respectively, at pH 5.0. No quenching of the transient at pH 8.0 was observed for $*Rh(phi)_2(phen)^{3+}$ by dA and dG.

In contrast, there was no apparent quenching of the excited state of these complexes by dC and dT at pH 5.0 (with maximum quencher concentration of 0.1 M), thus imposing a higher limit of $k_q \leq 1 \times 10^7$ M^{-1} s^{-1} for the reaction. Whether the absence of quenching is a result of poor association in solution or of insufficient driving force has not been established.

TABLE 3: Quenching Rate Constants of LC Excited State of $Rh(phen)_2(phi)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$ Complexes[a]

		$k_q/\times 10^9$ M^{-1} s^{-1} [b]			
complex	$-\Delta G$/eV[c]	A	dA	AMP	dAMP
$Rh(phen)_2(phi)^{3+}$	0.63	1.6	2.0	2.0	2.2
$Rh(phi)_2(bpy)^{3+}$	0.58	1.9	2.3	2.6	4.7

[a] Measured under argon at pH 5.0 by A, dA, AMP, and dAMP, along with the calculated driving forces for each reaction, ΔG. [b] Measured utilizing the Stern−Volmer method from the lifetime of the transient at 460 nm. [c] Calculated from $-\Delta G = E_{1/2}(Rh^{3+*/2+}) - E_{1/2}(A/A^+)$, with $E_{1/2}(A/A^+) = 1.42$ V vs NHE (ref 17) and $E_{1/2}(Rh^{3+*/2+}) = E_{00} + E_{1/2}(Rh^{3+/2+})$, where $E_{00} = 2.0$ eV and the $E_{1/2}(Rh^{3+/2+})$ values for $Rh(phen)_2(phi)^{3+}$ and $Rh(phi)_2(bpy)^{3+}$ are 0.0 V and −0.05 V vs NHE, respectively (from ref 5b).

The quenching of the LC excited state of the Rh(III) complexes of phi by A, dA, G, and dG is dynamic, with no overall decrease of the zero-time optical density ($\Delta OD_{t=0}$) as the concentration of the base or of the nucleoside is increased. However, static quenching is observed for the excited state reaction of the cationic Rh(III) complexes by the anionic AMP, GMP, dAMP, and dGMP. This quenching is consistent with preassociation of the reactants, driven by electrostatic and stacking interactions. This association is also evident in the electronic absorption spectra of the complexes, where a small decrease in the 390 nm peak of $Rh(phi)_2(bpy)^{3+}$ is detected upon addition of dGMP. However, the large decrease in $\Delta OD_{t=0}$ for $*Rh(phi)_2(bpy)^{3+}$ measured upon addition of dGMP is due to static quenching and cannot be explained simply by the lower extinction coefficient at the excitation wavelength, since the latter changes are too small to account for the observed decrease in signal intensity.

Excitation of deoxygenated solutions of $Rh(phen)_2(phi)^{3+}$, $Rh(phi)_2(phen)^{3+}$, and $Rh(phi)_2(bpy)^{3+}$ in the presence of dG at pH 5.0 leads to the formation of new transient signals in the microsecond time scale. Sample transient absorption spectra of solutions containing 40 μM $Rh(phen)_2(phi)^{3+}$ in the absence and presence of 3.5 mM dG at pH 5.0 collected 20 ns and 2 μs after a 308 nm laser pulse are shown in Figure 5. For the system containing dG, the excited state of $Rh(phen)_2(phi)^{3+}$ is observed at short times only, but at longer times, absorption features at 340 nm and 440 nm appear (Figure 5b). In addition, a new peak at 540 nm is observed, which possesses decay characteristics similar to those at 340 nm and 440 nm. As shown in Figure 5a, these long-lived transients are not observed in the absence of dG. Similar spectra were collected utilizing visible excitation, although the signals are much weaker owing to the greater extinction coefficient of the Rh(III) complexes in the ultraviolet region. No net photochemistry of the Rh(III) complexes is detected by electronic absorption changes in the presence of dG at pH 5.0.

DNA Duplex Photocleavage. The wavelength dependence of the direct photocleavage of a 17-mer duplex by a variety of metal complexes is shown in Figure 6a. Direct photocleavage of the duplex was observed for all of the complexes upon 313 nm irradiation at pH 8. However, Δ-$Rh(phen)_2(phi)^{3+}$ is distinctive in that 365 and 400 nm light also cause direct cleavage.

The oxidative damage of DNA by the Rh(III) complexes bound to the double helix does not yield direct strand cleavage, but the sites of modified bases can be detected following piperidine treatment, where the oxidative damage is expected to be prominent at the more easily oxidized 5′-G of 5′-GG-3′ sequences.[1,15,16] Following irradiation at pH 8, samples were subjected to piperidine treatment and electrophoresed as shown

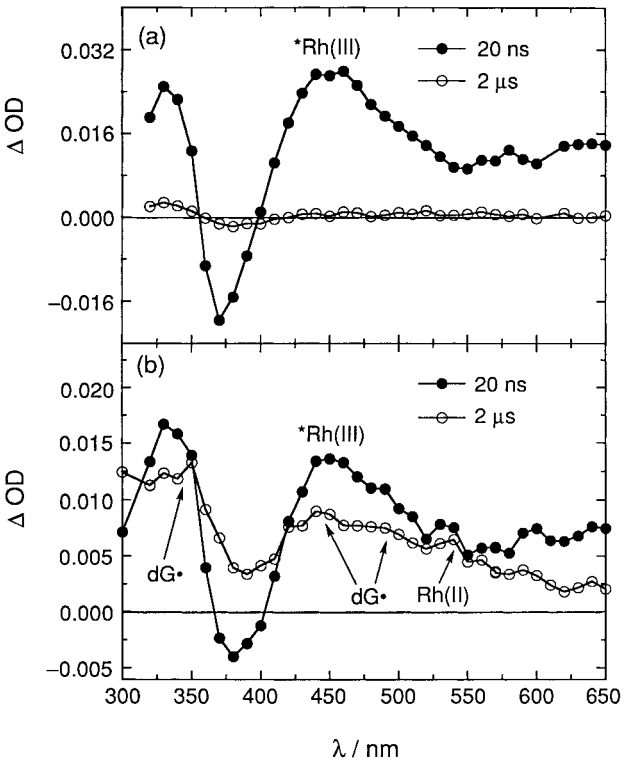

Figure 5. Transient absorption spectra of 40 μM Rh(phen)$_2$(phi)$^{3+}$ in the (a) absence and (b) presence of 3.5 mM dG collected 20 ns and 2 μs after the laser pulse (308 nm, 10 ns) at pH 5.0.

in Figure 6b. The oxidative damage by Rh(phi)$_2$(dmb)$^{3+}$ is most efficient with 365 nm irradiation, and some oxidative damage is also observed upon 400 nm irradiation. Rh(NH$_3$)$_4$(phi)$^{3+}$ also oxidizes DNA with 365 nm light but to a lesser extent than Rh(phi)$_2$(dmb)$^{3+}$. Rh(phi)$_2$(dmb)$^{3+}$ and Rh(NH$_3$)$_4$(phi)$^{3+}$ cause both direct photocleavage and oxidation upon irradiation with 313 nm light. In the case of Δ-Rh(phen)$_2$(phi)$^{3+}$, any oxidative photocleavage is masked by the direct photocleavage observed at all wavelengths. Oxidative damage to DNA by Rh([12]aneS$_4$)(phi)$^{3+}$ does occur, although it is inefficient and observed only after long irradiation times.

The pH dependencies of direct cleavage and photooxidation were studied for metal complexes with a wide range of pK_a's (Table 1). As depicted in Figure 7a, Rh(phi)$_2$(dmb)$^{3+}$ and Rh([12]aneS$_4$)(phi)$^{3+}$ show about 3-fold and 6-fold more direct cleavage at pH 5 than at pH 10, respectively. Rh(phen)$_2$(phi)$^{3+}$ shows the opposite trend upon irradiation with 365 nm light which results in 5-fold more direct cleavage at pH 9 than at pH 5. Irradiation at 313 nm of Rh(phen)$_2$(phi)$^{3+}$ shows a pH dependence more shallow than that of the other complexes, suggesting that two different mechanisms may be operative.

The pH dependence of photooxidation is depicted in Figure 7b. Rh(phi)$_2$(dmb)$^{3+}$ and Rh([12]aneS$_4$)(phi)$^{3+}$ both undergo a modest decrease in oxidation with increasing pH. Oxidation with Rh(NH$_3$)$_4$(phi)$^{3+}$, which is protonated throughout the pH range, increases slightly with pH.

Discussion

Transient Absorption. As reported previously,[10] the observed transient is consistent with a phi-centered intraligand nπ* transition. The quenching rate constants for the deactivation by O_2 of this LC excited state of the Rh(III) complexes listed in Table 1 are of similar magnitude for all of the complexes examined. This finding is consistent with an excited state of

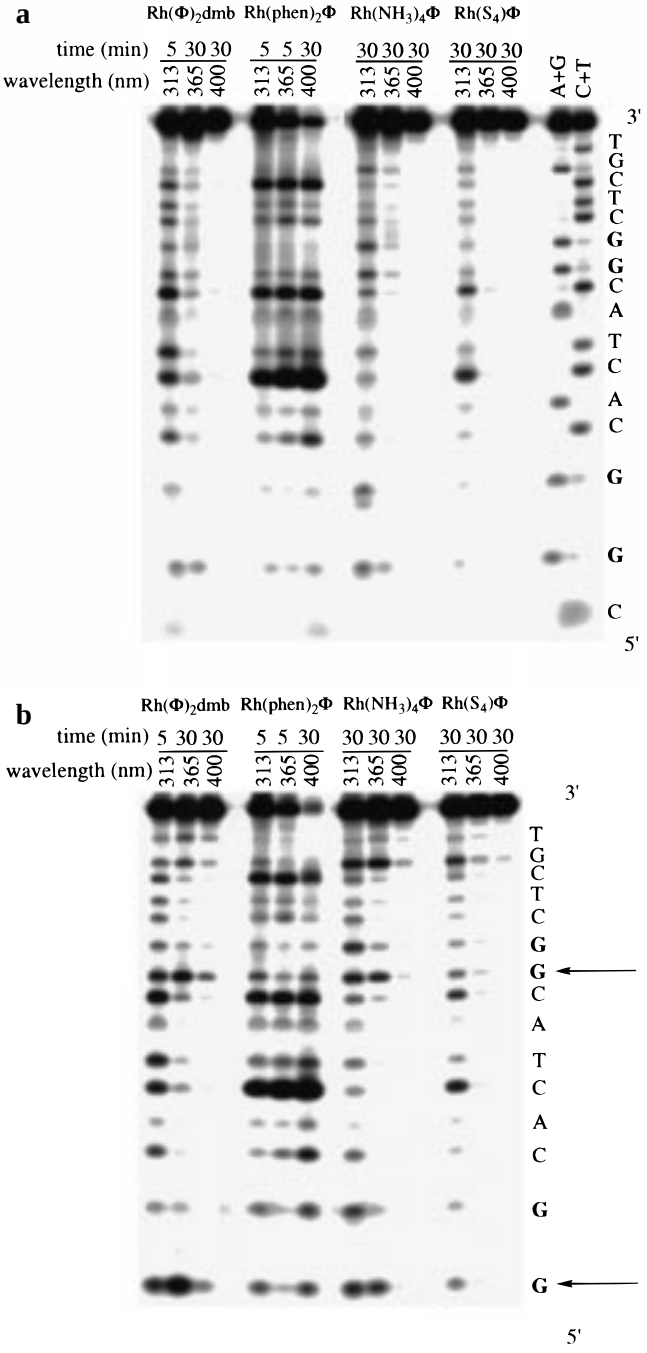

Figure 6. Comparison of the (a) direct photocleavage at pH 8 and (b) oxidative damage after piperidine treatment of a [^{32}P]-labeled 17-mer DNA duplex by various Rh(III) complexes obtained upon irradiation with 313, 365, or 400 nm light for 5 or 30 min as indicated (Φ = phi, S4= [12]aneS4).

similar energy and parentage in all complexes. Indeed, the observation of a similar spectrum for the precursor of the phi ligand, 9,10-diaminophenanthrene (DAP), with 420 nm excitation is in agreement with this assignment. However, the transient formed by DAP in DMSO possesses a lifetime of 2 μs, whereas the lifetimes observed for the Rh(III) complexes range from 100 ns to 400 ns. Since the nitrogen lone pairs on the phi ligand(s) believed to be involved in the transition are coordinated to the metal center in the complexes, it is not surprising that different lifetimes are obtained for phi complexes of Rh(III), compared to the uncoordinated DAP precursor. In addition, there may be some states with metal character mixed with this phi LC state, since the spectral profiles and lifetimes

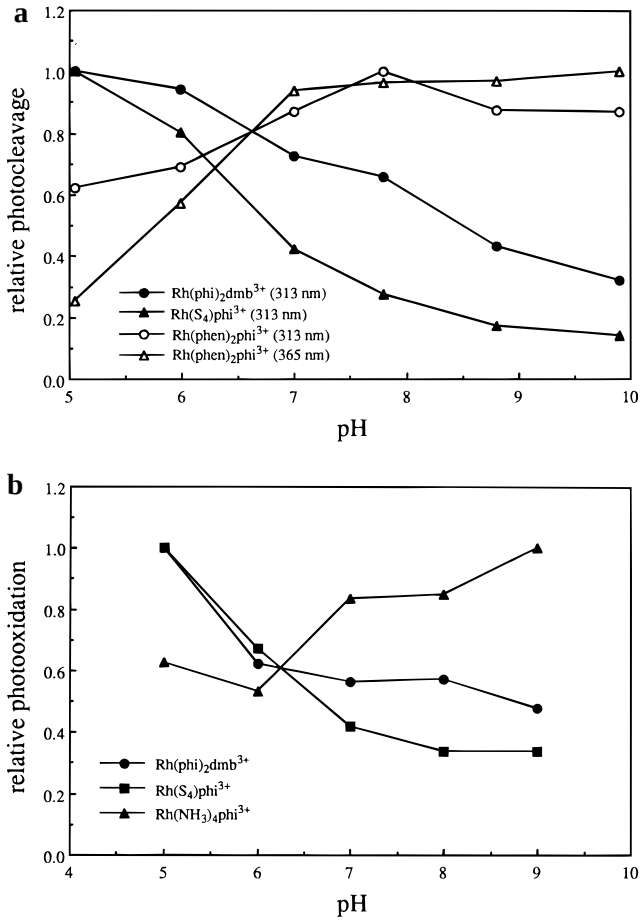

Figure 7. Quantitation of the (a) direct photocleavage and (b) oxidative damage revealed by treatment with piperidine of a 5′-[^{32}P]-labeled 140 base-pair restriction fragment. The intensities of the total cleavage bands were scaled such that the highest value for each metal complex was set to 1.0.

of the DAP transient and that of the phi complexes of Rh(III) are different. Furthermore, a red-shifted transient absorption spectrum was collected for Ir(phi)$_2$(phen)$^{3+}$ with a lifetime of 50 ns at pH 5.0,[11] which is also consistent with the metal center playing a role in the energy and decay rate of the excited state.

All phi complexes of Rh(III) that were investigated possess similar excited state decay lifetimes (Table 1), although the ground state pK_a values vary significantly. Therefore, a ground state transient arising from deprotonation of the phi ligand is not likely. Furthermore, energy transfer experiments from *Rh(phi)$_2$(phen)$^{3+}$ to the $^3\pi\pi^*$ excited state of 9,10-dicyanoanthracene (E_{00} = 1.8 eV),[10] as well as energy transfer quenching by O_2, have shown that the observed transient is an excited electronic state of the complex and not a reactive ground state species.

In basic media, the LC state of Rh(phi)$_2$(L)$^{3+}$ complexes (L = phen, bpy, dmb) is observed with visible excitation (420 nm and 532 nm), but the intensity of the transient is much weaker and the lifetime shorter compared to pH 5. The relatively small changes in the extinction coefficient at the excitation wavelengths upon deprotonation of the complexes do not explain the observed changes in intensity. Instead, the protonated and deprotonated forms of the complexes possess slightly different excited state characteristics, which is not surprising, given the nπ^* character of the transition.

Ligand-loss photochemistry is favored in basic media and takes place effectively under high energy irradiation for Rh(phi)$_2$(bpy)$^{3+}$ and Rh(phi)$_2$(dmb)$^{3+}$.[8,12] However, at pH 5.0, excitation with 308 nm laser light leads to the reversible formation of the low-lying LC excited state (with only a small amount of photodecomposition). Upon visible excitation of Rh(phi)$_2$(bpy)$^{3+}$, no ligand-loss photochemistry is observed at pH 5.0 or pH 8.0, conditions well below or above the pK_a of the complex, respectively. These observations are summarized in eqs 1−3.

$$\mathrm{Rh(phi)_2(bpy)^{3+}} \xrightarrow[\text{pH 5.0}]{308\text{ nm}} \text{LC excited state} \quad (1)$$

$$\mathrm{Rh(phi)_2(bpy)^{3+}} \xrightarrow[\text{pH 8.0}]{308\text{ nm}} \text{ligand-loss photochemistry} \quad (2)$$

$$\mathrm{Rh(phi)_2(bpy)^{3+}} \xrightarrow[\text{pH 5 or 8}]{>350\text{ nm}} \text{LC excited state} \quad (3)$$

The irreversible ligand loss is only accessible in high yield for the deprotonated complex with 308 nm or 313 nm excitation in solution, whereas high-energy irradiation of the protonated phi complexes leads to formation of the lower lying LC state. The observed photochemistry of Rh(phen)$_2$(phi)$^{3+}$, where lower energy excitation at pH 8 also leads to ligand loss, is distinctly different than those of all the other phi complexes of rhodium studied here.

Electron Transfer with DNA Bases. The quenching of the LC excited state of Rh(III) complexes possessing phi ligands by A and G bases in acidic media is thought to involve electron transfer (ET) from the base to the complex. *Rh(phi)$_2$(phen)$^{3+}$ was able to oxidize organic substrates with oxidation potentials up to +1.8 V vs NHE in acetonitrile, but the oxidation of biphenyl ($E_p \approx$ 2.1 V vs NHE) was not observed.[10] Therefore, the LC excited state of Rh(phi)$_2$(phen)$^{3+}$ possesses enough energy to oxidize both A ($E(A^{+\bullet}/A)$ = 1.42 V vs NHE) and G ($E(G^{+\bullet}/G)$ = 1.29 V vs NHE),[17] as well as their nucleosides, nucleotides, and monophosphates. The spectral profiles collected with Rh(phen)$_2$(phi)$^{3+}$ and dG (Figure 5b) are consistent with the formation of the reduced Rh(II) complex[10] and dG$^{\bullet}$, which is known to form via rapid deprotonation of dG$^{+\bullet}$.[16a,18] The reduced Rh(phen)$_2$(phi)$^{2+}$ complex absorbs light at ∼540 nm, consistent with the observed spectrum of Rh(phi)$_2$(phen)$^{2+}$ following excited state ET with anthracene derivatives and 1,4-dimethoxybenzene. Since the radical cations of the donors were readily detected with the additional peak at 540 nm, the latter was assigned to the reduced Rh(II) complex.[10] The remaining features observed in the transient absorption spectrum shown in Figure 5b correspond to those previously reported for dG$^{\bullet}$.[16a,18]

Since the complexes are protonated at pH 5, deactivation of the excited state might be considered to take place via excited state proton transfer from the Rh(III) complexes to the neutral bases. However, the spectral features of the transient absorption spectra are not consistent with this idea. Energy transfer can also be ruled out as a mechanism of excited state deactivation. The energy of the LC excited state energy of Rh(phi)$_2$(phen)$^{3+}$ is ∼2.0 eV,[10] and it is readily quenched by guanine and adenine. However, the excited states of both bases lie well above 2.0 eV,[19] and energy transfer from excited states with energies similar to DNA bases is not observed, such as from the metal-to-ligand charge transfer (MLCT) states of Ru(bpy)$_3^{2+}$ and Ru(phen)$_3^{2+}$ with $E_{00} \approx$ 2.1 eV.[20]

The reduction potentials of the neutral pyrimidine base radicals have been reported to be approximately 1.7 V and 1.6 V vs NHE in neutral aqueous solution for dT and dC, respectively.[17] Although it may be expected that *Rh(phi)$_2$(phen)$^{3+}$ would oxidize dT and dC at pH 5, by comparison to

the organic substrates, no quenching was observed ($k_q \leq 1 \times 10^7$ M^{-1} s^{-1}). The difference can partially be explained in terms of the pH dependence of the oxidation potential of the bases which is expected to increase with decreasing pH. Also, the experiments involving DNA bases were performed in water, whereas oxidation of organic substrates was conducted in acetonitrile. This difference could lead to shifts both in the excited state energy and in the reduction potential, as well as in the solvent's reorganization energy, λ_o.[21] Also, reasonably, the pyrimidine bases offer little surface area for stacking with the metal complexes, and hence, unlike for the purine bases, preassociation in solution is not expected. Thus, while no quenching was observed in solution with the nucleosides, some redox chemistry of pyrimidine bases within the DNA helix, with the rhodium complex intercalated, cannot be ruled out.

DNA Photoreactivity. How do the photophysical properties and excited state reactivities of the Rh(III) complexes observed in solution compare with the DNA photochemistries, both direct cleavage and oxidative damage, that we observe? In order to correlate the photochemistry in homogeneous solution with the reactivity towards DNA, $Rh(phi)_2(dmb)^{3+}$ was irradiated in the presence of DNA at various wavelengths and over a range of pH's. These observations were compared to the solution photochemistry of $Rh(phi)_2(dmb)^{3+}$ and to that of the related $Rh(phi)_2(bpy)^{3+}$ complex, as well as to that of the other phi complexes of Rh(III).

First let us consider direct cleavage. The direct photocleavage clearly is associated with a state or reactive intermediate formed through high-energy excitation, and not from the LC state. Hydrogen atom abstraction at the C3′-position would require these energetics. The direct cleavage reaction has been shown previously to require intimate association between the intercalator and reactive sugar moiety. It is perhaps not surprising, then, that the reactive states involved in this chemistry cannot be readily detected spectroscopically. Certainly, correlations with ligand-loss photochemistry are not complete. At pH 5, where $Rh(phi)_2(dmb)^{3+}$ is fully protonated (Table 1), the direct DNA cleavage at 313 nm is favored and diminishes significantly as the pH is increased. We also tested $Rh([12]aneS_4)(phi)^{3+}$, since it possesses a pK_a value lower than that of $Rh(phi)_2(dmb)^{3+}$, both in water and bound to DNA (Table 1). Direct cleavage by $Rh([12]aneS_4)(phi)^{3+}$ shows a trend similar to that by $Rh(phi)_2(dmb)^{3+}$, except that the cleavage diminishes at a lower pH; this is consistent with the lower pK_a of $Rh([12]aneS_4)(phi)^{3+}$ and confirms that the protonation state of the phi ligand plays a role in the photochemistry. While direct cleavage for the bis(phi) complexes are favored for the protonated species, the ligand-loss photochemistry in the absence of DNA is clearly favored in the deprotonated complex.

Irradiation of $Rh(phen)_2(phi)^{3+}$ at 365 nm shows a pH trend opposite to that observed for the direct cleavage of $Rh(phi)_2(dmb)^{3+}$ and $Rh([12]aneS_4)(phi)^{3+}$; here both ligand loss and direct cleavage are favored for the deprotonated complex. In contrast to 365 nm, 313 nm irradiation at pH 5 leads to significant direct cleavage. Thus, direct cleavage by protonated $Rh(phen)_2(phi)^{3+}$ is highly favored with 313 nm vs 365 nm light, analogous to that by $Rh(phi)_2(dmb)^{3+}$ and $Rh([12]aneS_4)(phi)^{3+}$, whereas deprotonated $Rh(phen)_2(phi)^{3+}$ cleaves DNA efficiently upon 365 nm irradiation and even out into the visible region in a manner consistent with the quantum yield of phi ligand loss in this complex.[11,12] The direct cleavage of DNA by $Rh(phen)_2(phi)^{3+}$ with visible light prevents this complex from being used to study photooxidation, although small amounts can be detected at low pH's (data not shown).

Photocleavage experiments which indicate oxidative damage clearly are associated with redox chemistry of the LC excited state. With low-energy irradiation ($\lambda \geq 365$ nm) of $Rh(phi)_2(dmb)^{3+}$ bound to DNA, predominantly oxidative damage is observed. This observation is consistent with the oxidative reaction taking place from the LC excited state, which is easily accessed with irradiation in the 355 nm to 450 nm range. This result is in agreement with the solution photochemistry, where dG$^\bullet$ is formed from the redox reaction of the LC state excited with dG. The yield of DNA oxidative damage decreases with increasing pH, although the effect is small (50% decrease) and does not reflect the pK_a of $Rh(phi)_2(dmb)^{3+}$. Although the DNA photooxidation by $Rh([12]aneS_4)(phi)^{3+}$ is much less efficient than that of $Rh(phi)_2(dmb)^{3+}$, it demonstrates the same general pH dependence as $Rh(phi)_2(dmb)^{3+}$. It is also informative to examine photooxidation using $Rh(NH_3)_4(phi)^{3+}$, since this complex is predominantly protonated through the pH range studied here. The small increase in photooxidation with increasing pH indicates that there are other factors in addition to the protonation state of the phi ligand important for determining the yield of piperidine-labile lesions. This may partially explain why, for $Rh(phi)_2(dmb)^{3+}$ and $Rh([12]aneS_4)(phi)^{3+}$, the decrease in yield of oxidative damage with increasing pH is not as large as would be expected.

While the enhanced oxidation at low pH is consistent with the observation of the LC transient at pH 5 in homogeneous solution, DNA oxidation is still observed up to pH 10, where the transient is weak and no quenching by guanine is detected. In solution the LC state is also accessed with 308 nm excitation at pH 5, but when the complex is bound to DNA, 313 nm irradiation yields direct photocleavage preferentially to oxidative damage. In this regard, it appears that with rhodium intercalated directly in the stacked base pairs of the DNA helix, the high-energy excitation leads to a photochemical deactivation pathway that is not detected in homogeneous solution, even in the presence of DNA bases.

The differences in the photochemistry of the phi complexes of Rh(III) in solution and in duplex DNA may be partially explained in terms of preassociation of the reactants typically observed in supramolecular photochemistry.[22,23] The photoinduced chemistry in solution results from the bimolecular reactions between the excited state Rh(III) complexes and bases, where the minimum quenching rate constant that can be observed for this system under the experimental conditions is $k_q \leq 1 \times 10^8$ M^{-1} s^{-1}. In the preassociated Rh(III) complex−DNA adduct, diffusion of the reactants is not necessary, making the reaction between *Rh and DNA bases unimolecular. Under such conditions, even a slow reaction may become a competitive means of deactivation of the excited state and an efficient photochemical pathway. Therefore, quenching by guanine in solution may still be operative in basic media, but we do not observe it, owing to the short lifetime of the LC state in basic solutions (84 ns). Similarly, in the absence of an efficient unimolecular photochemical pathway such as direct photocleavage, when the complex is not bound to DNA, excitation with 308 nm in solution results in population of the lower LC excited state or in loss of a phi ligand.

Another important point is that the two techniques are measuring different parameters. Spectroscopy detects all oxidative quenching events; gel electrophoresis detects guanine oxidation events which proceed to yield piperidine-labile lesions or reactions of the sugar which are spectroscopically silent. Guanine oxidation followed by nonproductive back-electron transfer, as an example, will be detected by the former but not

by the latter technique. Therefore, the dependence on pH of the steps leading to product formation following the initial generation of the guanine cation radical will affect the yield of piperidine-labile lesions but not the yield of the LC excited state. Here, by studying DNA oxidation using rhodium complexes with a range of pK_a's, we demonstrate that, while the protonation state of the phi ligand is important in determining the yield of guanine oxidative lesions, the pH affects other parameters as well.

Additional differences in the photophysical properties of the complexes in solution and bound to DNA could be due to the π-stacking of the ligand with the DNA bases, which may shift excited state energies, lifetimes, and redox potentials. The heterogeneous environment of the DNA could also have an effect, such as the shifts in pK_a of the complexes observed upon DNA intercalation (Table 1). Similar effects on the excited state properties may also be operative. It is also interesting to note that $Rh(phi)_2(dmb)^{3+}$ shows less of a pH effect than Rh([12]-aneS$_4$)$(phi)^{3+}$ or $Rh(phen)_2(phi)^{3+}$. This could be due to the different environments of the two phi ligands, since while one phi ligand of each $Rh(phi)_2(dmb)^{3+}$ complex bound to DNA is intercalating, the other is not. Furthermore, although the total absorbance at 365 nm only decreases 5−10% upon deprotonation (and actually increases 20% for Rh([12]aneS$_4$)$(phi)^{3+}$), we cannot rule out the possibility that the absorbance due specifically to the LC nπ* excited state changes significantly at this wavelength.

Nonetheless, these results certainly support the conclusion that the piperidine-labile lesions formed specifically at 5′-guanines in 5′-GG$_n$-3′ ($n \geq 1$) sequences result from oxidative electron transfer chemistry. In addition, because the rate constants are within the same order of magnitude, it is expected that $Rh(phi)_2(dmb)^{3+}$ is able to oxidize all guanines and adenines with similar rates and quantum yields. Thus, the specific damage observed in double-stranded DNA may be due to the equilibration of the electron holes to their thermodynamically most favored sites.

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References and Notes

(1) (a) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731−735. (b) Hall, D. B.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 5045.

(2) Dandliker, P. J.; Holmlin, R. E.; Barton, J. K. *Science* **1997**, *275*, 1465−1468.

(3) (a) Sardesai, N. Y.; Zimmermann, K.; Barton, J. K. *J. Am. Chem. Soc.* **1994**, *116*, 7502−7508. (b) Krotz, A. H.; Hudson, B. P.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 12577−12578. (c) Krotz, A. H.; Kuo, L. Y.; Shields, T. P.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 3877−3882. (d) Terbrueggen, R. H.; Barton, J. K. *Biochemistry* **1995**, *34*, 8227−8234.

(4) (a) David, S. S.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 2984−2985. (b) Sitlani, A.; Dupureur, M.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 12589−12590.

(5) (a) Kelley, S. O.; Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 9861. (b) Arkin, M. R.; Stemp, E. D. A.; Holmlin, R. E.; Barton, J. K.; Hörmann, A.; Olson, E. J. C.; Barbara, P. *F. Science* **1996**, *273*, 475−480. (c) Arkin, M. R.; Stemp, E. D. A.; Turro, C.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 2267−2274. (d) Stemp, E. D. A.; Arkin, M. R.; Barton, J. K. *J. Am. Chem. Soc.* **1995**, *117*, 2375−2376. (e) Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025−1029.

(6) Olson, E. J. C.; Hu, D.; Hörmann, A.; Barbara, P. F. *J. Phys Chem. B* **1997**, *101*, 299−303.

(7) Lincoln, P.; Tuite, E.; Nordén, B. *J. Am. Chem. Soc.* **1997**, *119*, 1454−1455.

(8) (a) Pyle, A. M.; Chiang, M. Y.; Barton, J. K. *Inorg. Chem.* **1990**, *29*, 4487−4495. (b) Krotz, A. H.; Kuo, L. Y.; Barton, J. K. *Inorg. Chem.* **1993**, *32*, 5963−5974.

(9) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303−2312.

(10) Turro, C.; Evenzahav, A.; Bossmann, S. H.; Barton, J. K.; Turro, N. J. *Inorg. Chim. Acta* **1996**, *243*, 101−108.

(11) Erkkilla, K. E.; Chen, W.; Turro, N. J.; Barton, J. K., unpublished results.

(12) (a) Pyle, A. M.; Barton, J. K. *Inorg. Chem.* **1987**, *26*, 3820−3823. (b) Pyle, A. M. Ph.D. Thesis, Columbia University, New York, NY, 1989.

(13) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning*; Cold Spring Harbor Laboratory Press: Plainview, NY, 1982.

(14) Sluggett, G. W.; Turro, C.; George, M. W.; Koptyug, I. V.; Turro, N. J. *J. Am. Chem. Soc.* **1995**, *117*, 5148.

(15) (a) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068. (b) Ito, K.; Kawanishi, S. *Biochemistry* **1997**, *36*, 1774−1781. (c) Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1993**, *268*, 13221−13227. (d) Saito, I.; Takayama, M.; Kawanishi, S. *J. Am. Chem. Soc.* **1995**, *117*, 5590−5591. (e) Saito, I.; Takayama, I.; Sugiyama, H.; Nakuhiko, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406−6407. (f) Breslin, D. T.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 2311−2319. (g) Iverson, B. L. Ph.D. Thesis, California Institute of Technology, Pasadena, CA, 1988.

(16) (a) Stemp, E. D. A; Arkin, M. R.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 2921. (b) Arkin, M. R.; Stemp, E. D. A.; Pulver, S. C.; Barton, J. K. *Chem. Biol.* **1997**, *4*, 389−400.

(17) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618.

(18) (a) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094−1099. (b) Jovanovic, S. L.; Simic, M. G. *Biochim. Biophys. Acta* **1989**, *1008*, 39−44. (c) Candeias, L. P.; Wolf, P.; O'Neill, P.; Steenken, S. *J. Phys. Chem.* **1992**, *96*, 10302−10307. (d) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1992**, *114*, 69−704. (e) Melvin, T.; Botchway, S.; Parker, A. W.; O'Neill, P. *J. Chem. Soc., Chem. Commun.* **1995**, 653−654. (f) Melvin, T.; Plumb, M. A.; Botchway, S. W.; O'Neill, P.; Parker, A. W. *Photochem. Photobiol.* **1995**, *61*, 584−591. (g) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Kelly, J. M.; Tossi, A. B.; Görner, H. *Photochem. Photobiol.* **1992**, *55*, 681−689.

(19) Wood, P. D.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 4256.

(20) K. Kalyanasundaram, *Photochemistry of Polypyridine and Porphyrin Complexes*; Academic Press: San Diego, CA, 1992, and references therein.

(21) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265.

(22) *Molecular and Supramolecular Photochemistry*; Ramamurthy, V., Schanze, K. S., Eds.; Marcel-Dekker: New York, 1997; Vol. 1.

(23) Kalyanasundaran, K. *Photochemistry in Microheterogeneous Systems*; Academic Press: New York, 1987.